Sleep's role in the reconsolidation of declarative memories

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ABSTRACT

Sleep is known to support the consolidation of newly encoded and initially labile memories. Once consolidated, remote memories can return to a labile state upon reactivation and need to become reconsolidated in order to persist. Here we asked whether sleep also benefits the reconsolidation of remote memories after their reactivation and how reconsolidation during sleep compares to sleep-dependent consolidation processes. In three groups, participants were trained on a visuo-spatial learning task in the presence of a contextual odor. Participants in the 'reconsolidation' group learned the task on day 1. On day 2, they were subjected to a reactivation procedure by presenting the odor cue and a mock recall test in the learning context before a 40-min sleep or wake period. Participants in the 'remote consolidation' group followed the same procedure but did not receive reactivation on day 2. Participants in the 'recent consolidation' group skipped the procedure on day 1 and learned the task immediately before the sleep or wake period. After the sleep or wake interval, memory stability was tested in all subjects. The results show that this short 40-min sleep period significantly facilitated the reconsolidation of reactivated memories, whereas the consolidation of non-reactivated remote memories was less affected and recently encoded memories did not benefit at all. These findings tentatively suggest that sleep has a beneficial effect on the reconsolidation of remote memories, acting at a faster rate than sleep-associated consolidation.

1. Introduction

Recently acquired memories are initially labile and susceptible to interference and forgetting. They require consolidation, a process in which they are qualitatively transformed and stabilized into long-term memory (Dudai, Karni, & Born, 2015; Kandel, Dudai, & Mayford, 2014). Once consolidated, memories are relatively resistant to alteration or disruption by amnesic treatments (Nader & Hardt, 2009). When such remote memories are reactivated by active recall or reminders they can again become labile and require reconsolidation to persist, which has been demonstrated in different organisms (from sea slugs to humans) and different memory types (from fear conditioning to spatial memory) (Nader & Einarsson, 2010; Nader & Hardt, 2009). In humans, consolidated memories have been shown to have returned to a labile state by reactivation in procedural learning (Walker, Brakefield, Hobson, & Stickgold, 2003), declarative syllable-pair associations (Forcato et al., 2007), episodic memories of real-world items (Hupbach, Gomez, Hardt, & Nadel, 2007; Hupbach, Gomez, & Nadel, 2011; Hupbach, Hardt, Gomez, & Nadel, 2008), and even fear responses (Schiller et al., 2010). While the precise timescale on which reconsolidation operates is still unknown, there is evidence suggesting that reconsolidation processes act faster than original consolidation processes (Debiec, LeDoux, & Nader, 2002; Gordon, 1977).

Sleep is known to facilitate the consolidation and stabilization of new memories. A sleep period following new learning improves subsequent memory retrieval as well as memory stability when compared to a wake retention interval of equal length (Diekelmann & Born, 2010; Rasch & Born, 2013). Specifically slow wave sleep (SWS) has been linked to the consolidation of declarative memories (Alger, Lau, & Fishbein, 2012; Plihal & Born, 1997; Yaroush, Sullivan, & Ekstrand, 1971) with some evidence pointing to a particularly important role of sleep stage 4, i.e. deepest SWS (Barrett & Ekstrand, 1972; Fowler, Sullivan, & Ekstrand, 1973;

Wilhelm et al., 2011). For sleep to benefit the consolidation of new declarative memories, a certain minimal amount of sleep seems to be necessary. A previous study found that a sleep period of 90 min successfully stabilized memories against interference, with this effect being associated with the amount of SWS obtained, whereas a 40 min sleep period was not sufficient for memory consolidation (Diekelmann, Biggel, Rasch, & Born, 2012).

Despite our increasing knowledge about consolidation processes during sleep, the role of sleep in the reconsolidation of declarative memories is unclear. Here we asked whether sleep facilitates the reconsolidation of declarative memories and how the effect of sleep on reconsolidation compares to the effect on consolidation. To study reconsolidation, we applied a reminder approach that combined learning-associated odor cues, the same learning environment (i.e. same room and experimenter), and a staged recall procedure to reactivate visuo-spatial memories that had been learned 24 h before. Following the reactivation session, subjects either were allowed to sleep for 40 min or stayed awake for an equivalent time interval before retrieval was tested. The 40 min sleep duration was chosen based on the idea that (i) reconsolidation processes act at a faster rate than consolidation processes (Debiec et al., 2002; Gordon, 1977) and (ii) that 40 min of sleep are not sufficient to consolidate new memories (Diekelmann et al., 2012). Accordingly, we hypothesized that 40 min of sleep following reminder presentation rapidly restabilize (reconsolidate) the labilized memory traces and thereby improve resistance to subsequent interference learning. In contrast to reconsolidation, we expected no effect of the 40-min sleep period on the consolidation of either remote memories that are not reactivated before sleep (remote consolidation) or recent memories that are encoded immediately before sleep (recent consolidation).

2. Methods

2.1. Participants

A total of 104 subjects (53 females) between 18 and 30 years (mean age ± SD: 22.92 ± 2.62 years) were allocated to one of three groups: the 'reconsolidation' group (n = 34), the 'remote consolidation' group (n = 40) or the 'recent consolidation' group (n = 30). In each of these groups, subjects were equally distributed into sleep and wake conditions. None of the participants reported ongoing medication, health problems, medical interventions, or a history of psychiatric, neurological, or sleep disorders. Further exclusion criteria were night or shift work as well as exam periods and other learning- or stress-intense occupations for at least three weeks prior to the experiment. On experimental days, daytime naps, extensive physical exercise as well as the intake of alcohol or caffeine were prohibited. Subjects in the sleep conditions spent an adaptation night in the sleep laboratory to habituate to sleeping under the experimental conditions. The study was approved by the local ethics committee of the medical faculty of the University Tübingen and all subjects gave written informed consent.

2.2. Experimental procedure

The experimental procedures are outlined in Fig. 1. In the reconsolidation and remote consolidation groups subjects learned an object-location task in the evening of day 1 between 20:30 and 21:30. The task was performed in the presence of an odor, linking this odor to the content and context of the learning task. An odor detection test (10 trials) that was performed before and after learning was designed to further strengthen the association between task and learning context. Following learning, participants left the laboratory for a full night of sleep at home.

On day 2 (around 24 h after learning), subjects in the reconsolidation group returned to the lab at around 21:00 for the reminder session. Participants in the sleep condition had EEG electrodes attached for later polysomnographic recordings before the start of the reminder session. The reminder session took place in the same learning context, i.e. the same building and same room, and included a staged testing procedure that was set up to act as a strong reminder of the initial learning situation. Subjects were told that they would be tested for their memory of the object-location task that they had learned the day before. However, during the first trial of the recall test, right before an answer could be given for the first cue, the program appeared to crash. Subjects were told that it had been a mistake to start the testing procedure at this point of the experiment and that the actual testing would take place later on. An extended odor detection test (with 30 instead of 10 trials) was performed before and after the staged recall, utilizing the property of odors to act as contextual reminders. After the reminder session, participants either went to bed at 23:00 ('sleep condition', n = 16) or stayed awake and watched a movie ('wake condition', n = 18). After about 40 min of sleep or wakefulness and another 30 min break, participants in both conditions learned an interference task. About 30 min after the end of interference learning, memory for the original object-location task was tested.

In the remote consolidation group ('sleep condition' n = 18, 'wake condition' n = 22), subjects followed the same procedure as in the reconsolidation group, with the important difference that on day 2 (a) the experiment was conducted at a different site (i.e. a different building and room) to reduce potential context reminders of the learning environment, and (b) they did not receive the reminder procedure (i.e. the staged recall and odor cues). Subjects arrived at the lab between 21:45 and 22:15 and in the sleep condition went to bed at around 23:00 after attachment of the EEG electrodes.

In the recent consolidation group, subjects arrived at the laboratory at 21:00 and learned the object-location task at 22:00, after attachment of the EEG electrodes for subjects in the sleep condition. Following the learning procedure, participants either slept (n = 16) or stayed awake (n = 14) like in the other groups. The remaining procedure was identical to the reconsolidation and remote consolidation groups.

2.3. Object-location task

Subjects performed a two-dimensional object-location memory task, in which locations of card pairs were learned, resembling the game 'concentration' (Diekelmann, Büchel, Born, & Rasch, 2011; Diekelmann et al., 2012; Rasch, Büchel, Gais, & Born, 2007). The task involves associative visuo-spatial memory and relies on structures in the medial temporal lobe (Sommer, Rose, & Gläscher, 2005). 15 card pairs, which depicted animals and everyday objects in full color, were shown on a computer screen in a 5×6 matrix. During learning, the locations of all 15 card pairs were presented twice. For each card pair, the first card was presented for one second, followed by the presentation of both cards for three seconds, with an inter-trial interval of three seconds until the next card pair appeared. The experimental odor was administered during the four seconds of stimulus presentation. After completing the two learning runs, immediate recall was tested. For this, the first card of each pair was presented and subjects were asked to indicate the location of the second card with the computer mouse. Visual feedback was given for each decision (either a green checkmark for 'correct' or a red cross for 'incorrect') and the cards were shown in their correct positions for two seconds. After recall of all card pairs was finished, visual feedback on the percentage of correct responses was given to the participant. The test was repeated until a criterion of
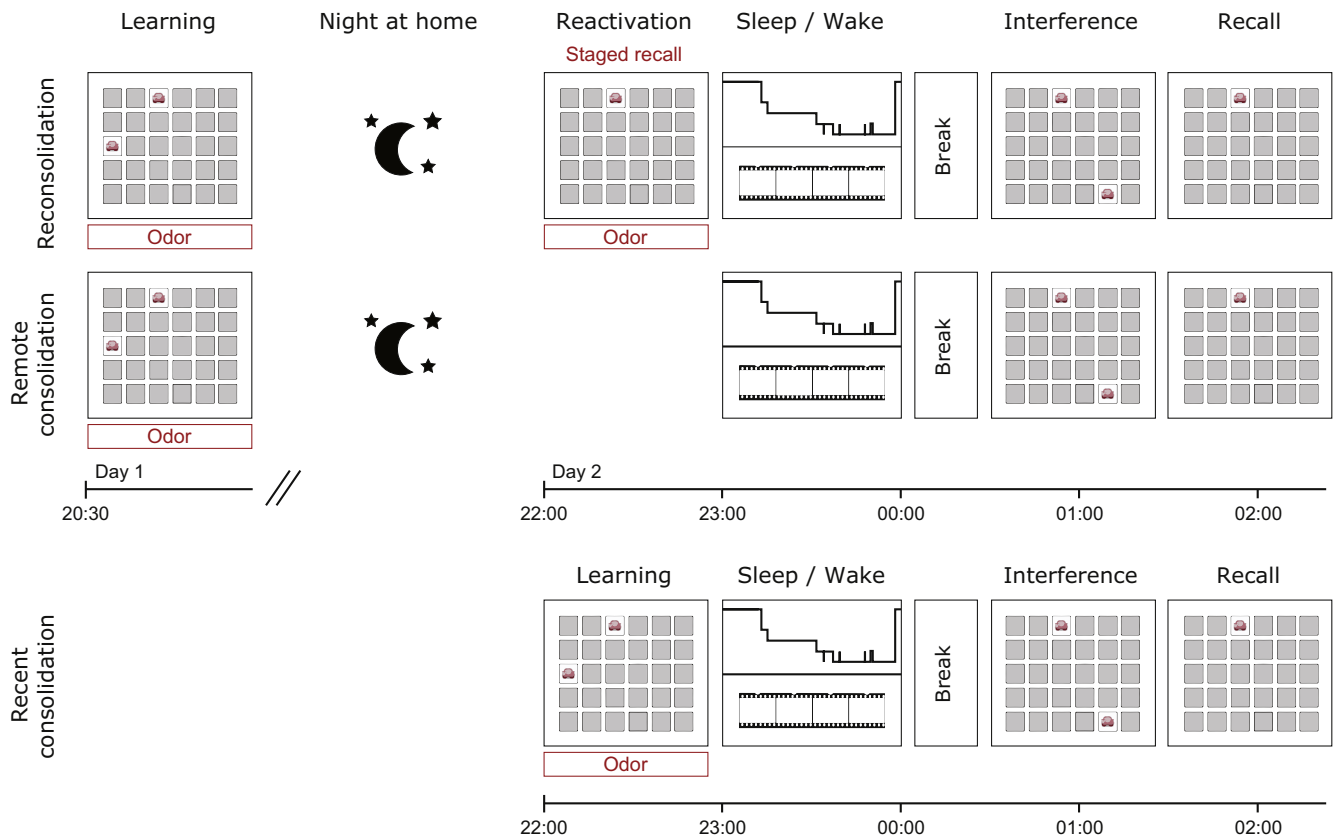
Fig. 1. Experimental design. In each of three separate groups subjects participated either in a sleep or a wake condition. Subjects in the reconsolidation and remote consolidation groups learned a two-dimensional (2D) object location task under the presence of an odor on day 1 until they reached a criterion of 60% correct responses. On day 2, in the reconsolidation group, the same learning context, the odor cues and a mock recall test were used to reactivate the memories before a 40-min period of sleep or wakefulness (watching a movie). In the remote consolidation group, subjects followed the same procedure but did not receive reactivation on day 2. In the recent consolidation group, subjects learned the 2D object location task right before going to sleep or staying awake. Because of the shorter retention interval in the recent consolidation group, the learning criterion was reduced to 40% in order to obtain roughly comparable encoding and absolute performance levels as the other groups for which recall took place about 24 h after encoding. About 30 min after awakening, all subjects learned an interference task and were tested on the original task.

60% correct responses was reached. In the recent consolidation group, a lower learning criterion of 40% was applied in order to decrease the subjects' exposure to the learned material with the goal to reduce the overall encoding level in this group, and thereby roughly match second-day memory strength and absolute recall performance with the reconsolidation and remote consolidation groups. In all groups, subjects that did not reach the criterion after a maximum of five immediate recall runs were dismissed from the study (5 subjects in the reconsolidation group, 8 subjects in the remote consolidation group, and 3 subjects in the recent consolidation group).

The interference task was administered to test the stability of the original memories against retroactive interference. The task was similar to the original learning task in that the same 15 card pairs were used as during initial learning and the location of the first card was identical for the original task and the interference task. The second card of each pair was placed at a different position, implementing an A–B, A–C interference paradigm (A, B, and C being sets of card positions). The immediate recall of the interference task was completed only once without a learning criterion to ensure comparable interference levels across participants (Diekelmann et al., 2011, 2012). No odor was presented during the interference task.

The final recall after the sleep/wake interval consisted of a single run in which the first card of each card pair was presented and subjects had to indicate the location of the second card of the originally learned task. Memory performance was measured by the ratio of correctly indicated card locations at final recall with the number of correct card locations during the last immediate recall trial (i.e. criterion trial) of original learning set to 100%. Additionally, interference errors were analyzed as the ratio of interference task card positions that were falsely indicated during final recall of the original learning task relative to the number of positions correctly remembered during immediate recall of the interference task (i.e. with interference learning performance set to 100%).

2.4. Control variables

Before learning, before reactivation, after interference learning, and after final recall subjects rated their sleepiness on the Stanford Sleepiness Scale (SSS, 7-point scale, ranging from 1 "feeling active, vital, alert, or wide awake" to 7 "no longer fighting sleep, sleep onset soon, having dream-like thoughts"). Before learning and after final recall, vigilance was assessed by a computer-based test. With intervals of 2–10 s a red circle was presented at either the left or right side of the screen and subjects were asked to respond as quick and precise as possible by pressing the left or right response button. The vigilance test took about 10 min in which a total of 160 trials were completed and the average reaction time was analyzed.

In the reconsolidation and the remote consolidation groups, participants additionally filled in two questionnaires to rate their subjective sleep quality for the night of sleep at home after the learning session and to log their activities between leaving the lab on day 1 and returning on day 2. An odor detection test was performed before and after learning and, in the reconsolidation group, before and after the reminder procedure on day 2. Each

run consisted of 10 trials (30 trials for the reminder procedure) in which participants were asked to indicate whether or not the odor was presented. Subsequently they rated on a scale from 0 to 9 the odor's valence and how familiar, exciting, intense, and pungent they had experienced the odor.

2.5. Polysomnographic recordings

Standard polysomnographic recordings were obtained to assess sleep, including electroencephalography (EEG), electromyography (EMG), and electrooculography (EOG). Two electrodes were used for EEG, placed at C3 and C4, according to the International 10–20 system, referenced to electrodes attached to the mastoids (M1, M2). Data were recorded at a sampling frequency of 200 Hz and bandpass filtered between 0.16 and 35 Hz. Recordings were scored by two experienced researchers according to standard criteria (Rechtschaffen & Kales, 1968) into wake, sleep stages 1–4 (3 and 4 representing SWS), and rapid eye movement (REM) sleep.

2.6. Statistical analyses

The three groups were initially analyzed in a 3×2 between-subjects factorial design (group $\times$ sleep/wake). Because the recent consolidation group differed from the other groups in several respects, the reconsolidation group and the remote consolidation group were then additionally analyzed separately in a 2×2 between-subjects factorial design (group $\times$ sleep/wake). To analyze memory performance, the number of learning trials needed to reach the criterion was introduced as a covariate. Post-hoc tests were performed using univariate ANCOVAS (with the between-subjects factor sleep/wake) for each group, again incorporating the number of learning trials as a covariate. Further learning and memory parameters (absolute learning performance, learning trials, absolute recall performance, interference learning performance, and interference errors) were analyzed using the same design but without covariates. Control variables were analyzed using ANOVAs for each time point with the between-subjects factors 'group' and 'sleep/wake'. Pairwise post-hoc comparisons were performed in case of significant effects.

Effect sizes are provided as partial eta-squared η_p^2. Correlations were calculated with Pearson's product-moment correlation coefficient r. Breusch-Pagan tests were performed to test for heteroscedasticity. P-values <0.05 were considered significant, all p-values are reported two-sided. Greenhouse-Geisser-correction was applied in cases in which the sphericity assumption was violated. Significances of the control variables are reported Bonferroni-corrected for the number of tests. All post-hoc comparisons are reported uncorrected.

3. Results

3.1. Memory performance

Analyses of memory performance (i.e., recall performance relative to learning performance with learning performance set to 100%) revealed a beneficial effect of sleep for the reconsolidation of reactivated memories but not for the consolidation of remote non-reactivated memories or recent memories. When analyzing all three groups collectively, memory performance did not differ between sleep and wake conditions (interaction 'group' $\times$ 'sleep/wake': F(2, 97) = 1.81, p = 0.17; main effect 'sleep/wake': F(1, 97) = 1.04, p = 0.31). Because of the shorter retention interval, subjects in the recent consolidation group performed on an overall higher level than both other groups, despite our efforts to reduce the learning criterion in the recent consolidation group (Fig. 2, main
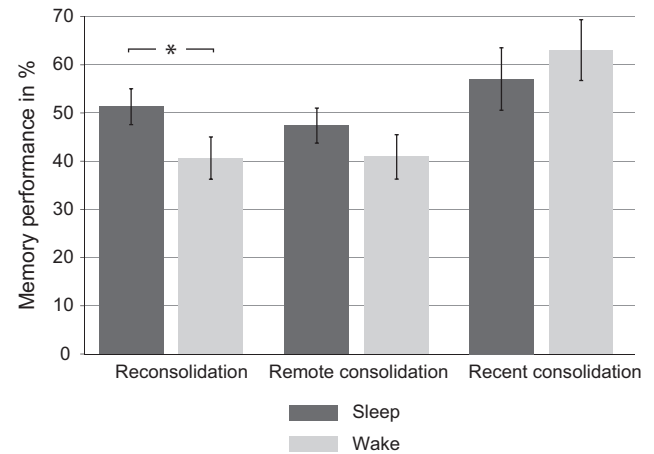

Fig. 2. Effects of sleep on reconsolidation and consolidation. In the reconsolidation group, memory performance was significantly improved after sleep compared to wakefulness (p = 0.046). There was no significant difference between sleep and wake conditions in the remote consolidation group, in which no reactivation procedure was performed before sleep (p = 0.31), or the recent consolidation group, in which subjects slept immediately after learning (p = 0.47). Memory performance is given relative to learning, with performance at learning set to 100% (means ± SEM are indicated; *p < 0.05).

effect 'group': F(2, 97) = 6.81, p = 0.002, η_p^2 = 0.12; pairwise post-hoc comparisons with recent consolidation group: both p < 0.01). To control for this factor, we equalized general memory performance by excluding subjects from the recent consolidation group who exceeded the highest memory performance levels in both other groups (n = 6 with a memory performance >82%; Supplementary Fig. 1). This procedure diminished the difference in general performance level between groups (main effect 'group': F(2, 91) = 1.67, p = 0.19) and unmasked a differential effect of sleep for reconsolidation and consolidation (interaction 'group' - $\times$ 'sleep/wake': F(2, 91) = 3.57, p = 0.03, η_p^2 = 0.07, without covariate: p = 0.06). Subsequent whole-sample post-hoc tests revealed that sleep as compared with the wake retention interval significantly improved memory performance only in the reconsolidation group (Fig. 2). Subjects who had slept after reactivation indicated more correct card locations relative to their learning level than subjects that had stayed awake (sleep: 51.31 ± 3.73%, wake: 40.64 ± 4.39%; F(1, 31) = 4.34, p = 0.046, η_p^2 = 0.12; without covariate p = 0.077), whereas no significant difference between the sleep and wake conditions was evident for remote consolidation (sleep: 47.39 ± 3.63%, wake: 40.89 ± 4.61%; F(1, 37) = 1.07, p = 0.31) and recent consolidation (sleep: 57.05 ± 6.48%, wake: 63.05 ± 6.32%; F (1, 27) = 0.53, p = 0.47).

When analyzing the reconsolidation group and the remote consolidation group separately, both groups showed a beneficial effect of sleep (main effect 'sleep/wake': F(1, 69) = 4.84, p = 0.031, η_p^2 = 0.07) independent of the reactivation procedure (main effect 'group': F(1, 69) = 0.22, p = 0.64; interaction 'group' $\times$ 'sleep/wake': F(1, 69) = 0.53, p = 0.47). However, as mentioned in the previous analysis, post-hoc tests revealed a significant benefit of sleep only in the reconsolidation group (p = 0.046) but not in the remote consolidation group (p = 0.31).

Relative interference errors did not differ across experimental groups and sleep/wake conditions (reconsolidation: sleep 13.65 ± 4.01%, wake 21.90 ± 5.92%; remote consolidation: sleep 14.40 ± 5.59%, wake 31.09 ± 6.97%; recent consolidation: sleep 20.59 ± 5.85%, wake 21.75 ± 6.44; main effect 'group': F(2, 98) = 0.36, p = 0.70; main effect 'sleep/wake': F(1, 98) = 3.03, p = 0.08, η_p^2 = 0.03; interaction 'group' $\times$ 'sleep/wake': F(2, 98) = 0.82, p = 0.44). As intended, absolute learning performance (number of

cards correctly recalled at the criterion learning trial) and the number of trials needed to reach the criterion were significantly lower in the recent consolidation group than in the other two groups, confirming that the manipulation of a lower learning criterion to reduce encoding strength in the recent consolidation group was at least partly successful (main effect 'group': learning performance F(2, 98) = 25.53, p < 0.01, $\eta_p^2 = 0.34$; learning trials F(2, 98) = 3.90, p = 0.02, $\eta_p^2 = 0.07$; recent consolidation group vs. both other groups: all p < 0.02, all $\eta_p^2 > 0.09$). There were no significant differences between groups and sleep/wake conditions in absolute recall performance or interference learning (all p > 0.05, all $\eta_p^2 < 0.06$). For a comprehensive listing of the results see Table 1.

3.2. Sleep parameters

Subjects slept for an average of 44.96 min (reconsolidation: 49.16 ± 1.88 min, remote consolidation: 46.44 ± 2.38 min, recent consolidation: 39.19 ± 2.06 min; Table 2). Total sleep time in the recent consolidation group was slightly shorter than in the two other groups (main effect 'group': F(2, 46) = 5.77, p < 0.01, $\eta_p^2 = 0.20$; post-hoc comparisons with recent consolidation group: both p < 0.03, both $\eta_p^2 > 0.14$).

Memory performance was correlated with time spent in sleep stage 4, i.e. deepest slow wave sleep, in the reconsolidation group (r = 0.58, p = 0.019) but not in the other groups (remote consolidation: r = 0.01, p = 0.98; recent consolidation: r = −0.15, p = 0.59; Breusch-Pagan tests, all p > 0.18; Fig. 3). Comparing these correlations directly, the correlation in the reconsolidation group was significantly different from the recent consolidation group (p = 0.04) and tended to be different from the remote consolidation group (p = 0.09). These findings remained essentially the same after excluding single outliers from the analysis (results after exclusion of one subject per group with more than 2 standard deviations above the group mean: reconsolidation: r = 0.54, p = 0.036; remote consolidation: r = −0.02, p = 0.94; recent consolidation: r = −0.13,

Table 1
Memory task performance.

	Reconsolidation	Remote consolidation	Recent consolidation
Learning performance[†]			
Sleep	10.69 ± 0.46	10.44 ± 0.33	8.00 ± 0.49[**]
Wake	10.17 ± 0.34	10.09 ± 0.24	7.93 ± 0.40[**]
Learning trials[†]			
Sleep	2.00 ± 0.30	2.61 ± 0.38	1.88 ± 0.30
Wake	2.94 ± 0.31	2.50 ± 0.29	1.57 ± 0.27[*]
Recall performance			
Sleep	5.56 ± 0.54	4.89 ± 0.36	4.56 ± 0.60
Wake	4.11 ± 0.46	4.14 ± 0.49	5.00 ± 0.54
Interference learning			
Sleep	9.94 ± 0.77	8.61 ± 0.80	7.25 ± 0.72
Wake	8.72 ± 0.71	8.50 ± 0.64	7.64 ± 0.79
Absolute interference errors[#]			
Sleep	1.06 ± 0.27	0.94 ± 0.24	1.25 ± 0.31
Wake	1.56 ± 0.28	2.09 ± 0.34	1.21 ± 0.26

Learning performance (absolute number of cards correctly recalled in the criterion trial), learning trials (repetitions needed to reach the learning criterion), recall performance (absolute number of cards correctly indicated at final recall), interference learning performance (cards correctly indicated during immediate recall of the interference version of the task), and absolute interference errors (absolute number of interference card locations falsely indicated during final recall of the original task) for each experimental group (means ± SEM). Due to the reduced learning criterion of 40% in the recent consolidation group participants needed less learning trials and exhibited lower learning performance.
[†] p < 0.05, main effect 'group'.
[#] p = 0.03, main effect 'sleep/wake'.
[*] p < 0.05, post-hoc pairwise comparisons with both other groups.
[**] p < 0.001, post-hoc pairwise comparisons with both other groups.

Table 2
Time spent in different sleep stages.

	Reconsolidation	Remote consolidation	Recent consolidation
TST[†]	49.16 ± 1.88	46.44 ± 2.38	39.19 ± 2.06[*]
Wake	2.81 ± 1.40	0.85 ± 0.51	0.75 ± 0.56
S1	5.69 ± 0.94	5.47 ± 0.76	4.50 ± 0.58
S2	19.75 ± 1.65	19.79 ± 2.57	16.38 ± 1.33
S3	16.78 ± 1.58	18.00 ± 1.47	14.16 ± 1.13
S4	4.06 ± 1.43	2.24 ± 0.84	3.38 ± 1.16
SWS	20.84 ± 1.67	20.24 ± 1.73	17.53 ± 1.75
REM	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Total sleep time (TST), time awake after sleep onset (Wake), sleep stage 1–4 (S1–S4), slow wave sleep (SWS, i.e. the sum of S3 and S4), and rapid eye movement sleep (REM) in minutes are presented (means ± SEM).
[†] p < 0.01, main effect 'group'.
[*] p < 0.03, post-hoc comparison with both other groups. No other significant main effects.

p = 0.65; Breusch-Pagan tests, all p > 0.20; reconsolidation vs. recent consolidation: p = 0.07; reconsolidation vs. remote consolidation: p = 0.12). Time spent in other sleep stages (S2 and S3) as well as total sleep time were not correlated with memory performance (all p > 0.19).

3.3. Control variables

Subjective sleepiness and vigilance did not significantly differ between participants in the sleep and wake conditions (main effect 'sleep/wake': p > 1 for all time points). However, subjective sleepiness was slightly different between experimental groups (p < 0.01 for learning, interference and recall). Vigilance differed at learning and recall (both p < 0.01). Importantly, this difference did not interact with the factor 'sleep/wake' (all p > 0.51) and at no time point did any of these control parameters correlate with memory performance (all p > 0.12 for correlations within each group and across all groups), indicating that the observed differences in memory performance were not affected by general alertness levels. Results of the subjective sleepiness and vigilance tests are comprehensively listed in Table 3. Sleep duration, tiredness, and sleep quality for the first night after learning spent at home in the reconsolidation and the remote consolidation groups were comparable between groups and sleep/wake conditions (all p > 0.26). Subjects' odor detection accuracy was close to perfect in all subjects, with an average of 95.91 ± 0.55% correct responses in the odor detection test. The odor was assessed as neither positive nor negative (4.56 ± 0.19), medium familiar (4.99 ± 0.24), rather unexciting (3.51 ± 0.22), slightly intense (5.92 ± 0.17), and medium pungent (4.54 ± 0.22).

4. Discussion

While it is widely accepted that sleep facilitates the consolidation of newly acquired memories, the contribution of sleep for the reconsolidation of declarative memories remains largely unknown. Here we show that a short 40-min period of night sleep facilitates the re-stabilization of reactivated declarative memories. Only to a lesser extent did this short sleep period affect second-night consolidation of non-reactivated memories and first-night consolidation of recently acquired memories did not benefit at all.

The finding that sleep strengthens remote memories after their reactivation is in accordance with the hypothesis that sleep facilitates memory reconsolidation (Stickgold & Walker, 2007; Walker & Stickgold, 2006). Reconsolidation typically refers to the process of re-stabilization of memories after their reactivation or retrieval from long-term memory, with this re-stabilization being required for the memories to persist (Nader & Hardt, 2009). However, the
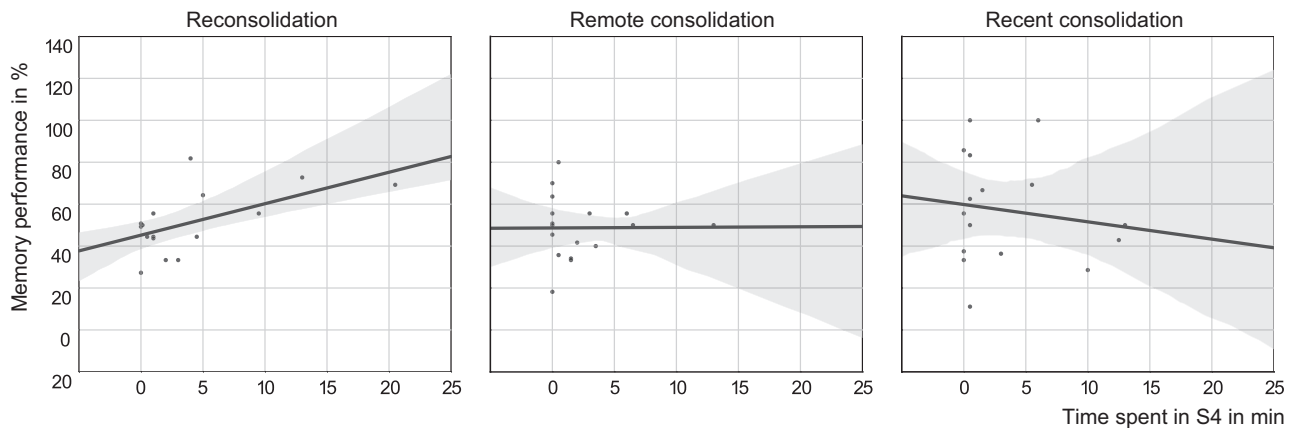
Fig. 3. Associations between memory performance and stage 4 sleep. Correlations between memory performance and time spent in sleep stage 4 (S4) are shown with lines indicating a first-order model prediction (linear regression). Bands around the regression lines indicate the standard error of the estimates (68% confidence interval). The correlation is significant only in the reconsolidation group (r = 0.58, p = 0.019) but not in the remote consolidation (r = 0.01, p = 0.98) or recent consolidation group (r = −0.15, p = 0.59). Note that the number of subjects that did not reach S4 is slightly lower in the reconsolidation group than in the other two groups.

Table 3
Control variables.

		Reconsolidation	Remote consolidation	Recent consolidation
Sleepiness				
Learning[†]	Sleep	2.31 ± 0.15	2.44 ± 0.23	3.19 ± 0.23[*]
	Wake	2.17 ± 0.15	2.24 ± 0.14	3.29 ± 0.24[**]
Reactivation	Sleep	2.69 ± 0.25	3.44 ± 0.35	
	Wake	2.56 ± 0.23	3.05 ± 0.18	
Interference[†]	Sleep	3.31 ± 0.35[#]	4.06 ± 0.37	4.47 ± 0.32[#]
	Wake	3.61 ± 0.23	3.73 ± 0.21	4.71 ± 0.30[**]
Recall[†]	Sleep	3.88 ± 0.30[*]	5.06 ± 0.29	4.94 ± 0.32
	Wake	4.11 ± 0.25	4.59 ± 0.21	5.71 ± 0.24[**]
Vigilance				
Learning[†]	Sleep	385.49 ± 16.31[**]	331.11 ± 7.61[**]	452.35 ± 17.40[**]
	Wake	375.43 ± 10.54	351.57 ± 10.07	439.73 ± 13.21[**]
Recall[†]	Sleep	411.05 ± 19.42[*]	466.08 ± 8.15	464.77 ± 15.09
	Wake	410.32 ± 22.51[*]	465.94 ± 10.73	478.24 ± 15.56

Sleepiness is indicated as ratings on the Stanford Sleepiness Scale and vigilance refers to reaction times in ms in the vigilance task (means ± SEM). While sleepiness and vigilance did not differ between subjects in the sleep and wake conditions, some variables differed between the Reconsolidation, Remote, and Recent consolidation groups.
[†] p < 0.01, main effect 'group'.
[#] p < 0.05 post-hoc comparison with one of the other groups (uncorr.).
[*] p < 0.05 post-hoc comparison with both other groups (uncorr.).
[**] p < 0.01 post-hoc comparison with both other groups (uncorr.).

term 'reconsolidation' is not uncontroversial in the literature and it is a matter of debate whether initial consolidation and reconsolidation refer to the same or different processes and whether different or similar mechanisms are involved. Despite certain differences that have been discussed, consolidation of new memories and reconsolidation of reactivated memories share a number of similarities in their underlying plastic processes (e.g., Alberini, 2005; Nader, 2015). There is mounting evidence that the consolidation of declarative memories particularly depends on slow wave sleep and its associated processes such as slow oscillations, spindles, and ripples (Rasch & Born, 2013; Watson & Buzsáki, 2015). The finding that in the present study – and selectively after reactivation – memory recall was associated with time spent in stage 4 sleep, i.e. deepest slow wave sleep, indicates that similar SWS-associated processes may be involved in declarative memory reconsolidation. This is in line with a previous electrophysiological study in rats, which observed a short-lasting increase in hippocampal ripples – a marker of memory 'replay' – during SWS after retrieval of a previously trained odor-reward association (Eschenko, Ramadan, Mölle, Born, & Sara, 2008). This observation indicates that reconsolidation, like consolidation, may be supported by SWS-related replay events.

The finding that the short sleep period of only 40 min facilitated reconsolidation but not consolidation is in keeping with prior evidence from experiments in animals suggesting that reconsolidation processes after reactivation operate on shorter timescales than consolidation after new learning (Debiec et al., 2002; Gordon, 1977). Importantly, a 40-min sleep period has previously been shown to be insufficient to consolidate memories immediately after acquisition in an identical learning paradigm (Diekelmann et al., 2012). In that study, a sleep benefit was only observed when the sleep duration was prolonged to 90 min or when consolidation processes were accelerated by presenting learning-associated olfactory reminder cues during SWS. Our present study replicates and extends these findings, by showing that 40 min of sleep are sufficient to restabilize remote memories after their labilization, suggesting that sleep-dependent reconsolidation acts at a faster rate than sleep-dependent consolidation.

It is to note that the differential effect of sleep for reconsolidation and consolidation reached significance only after controlling for memory strength in the recent consolidation group by introducing a lower learning criterion and excluding the highest performing subjects from the analysis. This may suggest that the efficacy of sleep for consolidation and reconsolidation processes

depends on the strength of the memory trace when entering the sleep state. It has been suggested that sleep benefits memory optimally in the low to medium range of memory strength as compared with memory traces that are already strong after learning (Creery, Oudiette, Antony, & Paller, 2015; Drosopoulos, Schulze, Fischer, & Born, 2007; Stickgold, 2009). Likewise, reconsolidation is assumed to depend on the age and the strength of the memory representation, with older and stronger memories being less sensitive to reactivation and reconsolidation processes (Alberini, 2011; Suzuki et al., 2004). Future studies will have to test directly for the role of memory strength in reconsolidation during sleep.

In an alternative analysis, we directly compared the reconsolidation and remote consolidation groups, leaving out the recent consolidation group, which differed from the other groups in the experimental procedure as well as in overall memory performance. Interestingly, this alternative analysis revealed a beneficial (main) effect of sleep on memory across both groups, irrespective of whether subjects underwent the reactivation procedure or not. This analysis could lead to the conclusion that the reconsolidation and remote consolidation groups benefitted in a similar way from the sleep manipulation. This would indicate that memories that are not affected by a short period of sleep right after acquisition can still benefit from the same sleep duration 24 h later, even without prior reactivation. However, to the best of our knowledge, such an accelerated second-night sleep effect has never been demonstrated before. Alternatively, a certain degree of reactivation and reconsolidation may also have occurred in the remote consolidation condition. The employed reactivation procedure in the reconsolidation group was optimized to provide strong multisensoric contextual and behavioral reminder cues, including the same learning context, exposure to the learning-associated odor, and a staged recall test, with all of these factors having previously been shown to effectively trigger memory labilization (Diekelmann et al., 2011; Forcato, Argibay, Pedreira, & Maldonado, 2009; Hupbach et al., 2008). However, there is little insight into the specific boundary conditions for consolidated declarative memories to re-enter a labile state (Forcato, Fernandez, & Pedreira, 2014; Schiller & Phelps, 2011). Although we tried to avoid advertent reactivation in the remote consolidation group by omitting the reactivation procedure and having subjects spend the second part of the experiment (i.e. day 2) in a different room and a different building, relatively abstract behavioral and contextual similarities (e.g., encountering a similar lab environment at the same university campus) may have sufficed to labilize memory traces to some, although lower, extent.

A limitation of the present study design is that memory stability was tested shortly after interference learning, whereas classical reconsolidation studies typically test memory stability after longer restabilization periods. Some studies have demonstrated an impairing effect of interference learning only if the original material was tested 24 h after the interference task (Hupbach et al., 2007; Walker et al., 2003). If the impact of interference learning increases over the timespan of the reconsolidation process, testing memory stability after a longer delay may yield larger differences between the reconsolidation and consolidation conditions. This factor should be systematically tested in further studies. To some degree, the present study also leaves open the question whether the benefit of sleep was specific for the reconsolidation of memories or whether an enhanced fast-acting second-night effect of sleep on consolidation contributed to the observed performance differences. Moreover, the present study cannot differentiate whether sleep benefits declarative memory reconsolidation by strengthening reactivated memories, by increasing their resistance to interference, or by a combination of both processes. To test for the exact nature of sleep's effect on reconsolidation, future studies will have to include additional experimental groups without inter-ference learning before the retrieval test. If sleep exclusively increases the stability of reactivated memories against interference, no sleep effect would be expected in these groups.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nlm.2016.10.004.

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