Title: Emotional memories are enhanced when reactivated in slow wave sleep, but impaired when reactivated in REM

Abbreviated Title: Sleep can enhance or impair emotional memories.

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Abstract

Sleep supports memory consolidation. However, it is not completely clear how different sleep stages contribute to this process. While rapid eye movement sleep (REM) has been traditionally implicated in the processing of emotionally charged material, recent studies indicate a role for slow wave sleep (SWS) in strengthening the memories of emotional stimuli. Here, to directly examine which sleep stage is primarily involved in emotional memory consolidation, we used targeted memory reactivation (TMR) in REM and SWS during a daytime nap. We also examined neural oscillations associated with TMR-related changes in memory. Contrary to our hypothesis, reactivation of emotional stimuli during REM led to impaired memory. Meanwhile, reactivation of emotional stimuli in SWS improved memory and was strongly correlated with the product of times spent in REM and SWS (%SWS × %REM). When this variable was taken into account, reactivation significantly enhanced memory, with larger reactivation benefits compared to reactivation in REM. Notably, sleep spindle activity was modulated by emotional valence, and delta/theta activity was correlated with the memory benefit for both emotional and neutral items. Finally, we found no evidence that emotional memories benefited from TMR more than did neutral ones. Our results provide direct evidence for a complementary role of both REM and SWS in emotional memory consolidation, and suggest that REM may separately facilitate forgetting. In addition, our findings expand upon recent evidence indicating a link between sleep spindles and emotional processing.

1. INTRODUCTION

Sleep supports memory consolidation, which allows the stabilization and integration of recently formed labile memories (Stickgold, 2005). However, it is unclear how different sleep stages, with their distinct physiology, contribute to this process. For declarative memories, the predominant model, the active system consolidation theory, posits that memory consolidation is achieved by reactivation of recently encoded, hippocampus-dependent memories during slow-wave sleep (SWS), when the coordinated activity of hippocampal ripples, thalamocortical spindles and cortical slow oscillations distribute these memories to long-term stores in neocortical networks (Klinzing et al., 2019). While this framework is constructed around non-REM sleep (NREM) physiology, and SWS in particular, there is strong evidence that rapid eye movement sleep (REM) is also involved in the consolidation of declarative memories (Boyce et al., 2017). Instead of a NREM-REM dichotomy, alternative but compatible accounts, such as the sequential hypothesis (Giuditta, 2014) and others (Singh et al., 2022), attribute the memory benefit of sleep to successive NREM-REM episodes.

Processing of emotional material during sleep, including emotional memory consolidation, has been traditionally attributed to REM (Davidson et al., 2021). Some studies supported this view, and showed higher retention of emotional content following REM-rich late night sleep but not after early sleep (Groch et al., 2013) or in SWS deprived participants but not in those deprived of REM (Wiesner et al., 2015). In addition, REM measures were correlated with increased retention of emotional memories (Nishida et al., 2009). Based on these findings, it was hypothesized that REM provides a unique milieu which facilitates the consolidation of memory of emotional experiences (Goldstein and Walker, 2014). However, this hypothesis is challenged by an accumulating number of studies which found no association of REM measures with emotional

memory performance (Baran et al., 2012; Kaestner et al., 2013; Cairney et al., 2014a; Ackermann et al., 2015; Alger et al., 2018; Sopp et al., 2018; see Davidson et al., 2021 for a review). More importantly, increasing evidence suggests that NREM is involved in emotional memory consolidation. Two studies found NREM to be sufficient for emotional memory consolidation, regardless of the presence of REM (Morgenthaler et al., 2014; Cellini et al., 2016). Furthermore, several studies showed correlations of emotional memory benefit with time spent in NREM (Wagner et al., 2007; Cairney et al., 2015; Payne et al., 2015; Alger et al., 2018) and spectral power in the delta band (Payne et al., 2015). Beyond correlations, pharmacologically increasing slow wave activity (Benedict et al., 2009) and sleep spindle density (Kaestner et al., 2013) enhances memory for emotional items.

Another line of direct evidence for a role of NREM in emotional memory consolidation comes from targeted memory reactivation (TMR) studies. TMR relies on replaying cues that are associated with recently encoded memories and has been shown to enhance declarative memory retention (Hu et al., 2020). In one TMR study, emotional and neutral memories were cued during SWS (Cairney et al., 2014b). While there were no memory benefits, SWS duration and number of spindles were associated with shorter reaction times for emotional items. In another study, which cued emotional and neutral items in NREM or REM, memory for emotional items was improved after cueing in NREM but not REM (Lehmann et al., 2016). Furthermore, successful cueing was associated with increases in spindle and theta power. However, another study found no effect of TMR in NREM on emotional memory (Ashton et al., 2018).

In this study, we used TMR to examine which sleep stage is primarily involved in emotional memory consolidation. Consistent with the prevailing view in the literature on the role of REM in emotional memory consolidation, we hypothesized that replay of cues for emotional items during

REM would lead to enhanced retention compared to replay in SWS. We also explored whether emotional memories are retained better than neutral memories. We hypothesized that replay of cues for emotional items would lead to greater memory improvements than the cueing of neutral items.

METHODS

Participants

Study sample included healthy young participants recruited from colleges in the greater Boston area. A total of 125 participants consented to the experiment, of which 81 provided usable datasets for analysis (age: 21.6±2.6; 67.1% female; see below for details). Participants reported no abnormal sleep patterns, history of psychiatric or neurological disorders, or current medication use. They were instructed to keep a regular sleep schedule for the 3 nights preceding the experiment and were asked to refrain from recreational drugs and alcohol for 48 hours, and caffeine in the morning, before their visits. All participants provided written consent approved by the Institutional Review Board of Beth Israel Deaconess Medical Center.

Participants were randomly assigned to one of 3 groups. Emotional SWS (E-SWS) and emotional REM (E-REM) groups learned emotional items, and were then exposed to reactivations during SWS or REM, respectively. Similarly, a neutral SWS (N-SWS) group learned neutral items with reactivations in SWS. Groups were matched in age ($F=1.05$, $p=.36$) and sex ($X^2=1.96$, $p=.38$). We also included emotional nap (E-Nap; n=21) and emotional wake (E-Wake; n=20) groups, which learned emotional items and took a nap without any reactivations or did not take a nap but rested quietly for a similar period before testing. E-Wake and E-Nap groups did not differ in % change

in recall accuracy at T2 or T3 (t= 1.29, p= 0.20 and t= 1.32, p=.20, respectively) and results for these groups are not further discussed.

Design and Procedures

The protocol included 2 visits (**Figure 1.A**). On the first visit, participants arrived at the laboratory at around 11:00 AM. After consenting, they filled out the Epworth Sleepiness Scale (Johns, 1991) and questionnaires about their sleep patterns and quality in the preceding 3 days. They were then wired for EEG (detailed below) and a brief (7 minutes) resting state EEG was recorded with eyes closed. We do not report EEG data from the rest periods in this paper.

We used a modified version of the TMR task described in previous publications (Rudoy et al., 2009; Creery et al., 2015; **Figure 1.B**). Learning included two successive phases, training and practice. During training, participants viewed 50 neutral or negative pictures appearing in different locations on a grid, in random order, while simultaneously hearing a 1-second sound that was naturally linked with that object. During practice, pictures appeared in the center of the screen while their corresponding sound played. Participants were instructed to move the objects to their original location and press the mouse button. After the mouse was clicked, the picture was moved to its correct location, which provided feedback. In the first two runs of the practice phase, all 50 objects were tested. In subsequent rounds, items that had been placed within 150 pixels of their correct locations in two successive rounds no longer appeared. The practice phase continued until all objects had been removed from the testing pool. The baseline test (T1) followed immediately after learning. This phase was similar to practice, except participants placed each picture only once and no feedback was provided. Following test 1, another 7-minutes resting state EEG was collected.

For participants in the sleep group, lights were turned off shortly after the baseline test, usually around 2 PM. Sleep was allowed up to 2 hours. For all sleep participants, white noise was played through bedside speakers (~39 dB on the pillow) starting at lights off and continuing until lights on. In the reactivation groups, half of the sounds (n=25) were presented in a random order, with 5-second interstimulus intervals. Sound presentations began approximately 1.5 minutes after SWS or REM onset. Replayed sounds were selected by a computer algorithm so that memory accuracy at T1 for the replayed and non-replayed sounds were similar. Sounds were presented until the specific sleep stage (SWS or REM) ended. If the participant entered the same sleep stage again, reactivation was resumed. Data from a participant were included in analyses if all 25 sounds were played at least once. After lights on, a final resting state EEG data was collected. Wake participants were also wired for EEG and spent an equal amount of time in the bedroom, doing relaxing activities such as reading, while they were observed to ensure wakefulness.

Retests were carried out the same way as Test 1. The first retest (T2) took place approximately 45 minutes after lights on, or at the end of the rest period for the wake group. Before they left the laboratory, participants were instructed that their memory would be tested, in the same way, at their second visit. The second visit was approximately one week later and included only the delayed retest (T3), which took place at 4:45 PM, to match the timing of Test 2. EEG was not monitored at this visit.

Before training and each retest, participants filled out the Stanford Sleepiness Scale (Hoddes et al., 1972) and a two-item questionnaire about their ability to concentrate and their level of “feeling refreshed”. After the tests, they were asked to report how difficult or easy and how boring or interesting the tests were, and the strongest emotion they felt.

Stimuli

50 emotional and 50 neutral images from online picture databases, the International Affective Pictures System (Lang et al., 2008) and the Geneva Affective Picture Database (Dan-Glauser and Scherer, 2011), and Google image search, were used. Images were 150 x 150 pixels and were displayed on a 67.5 cm x 57.25 cm monitor with a viewing resolution at 1440 x 900 pixels. Emotional images were only negative. 1-second sounds that were naturally linked to each of these images were then taken from the database Pond5 (www.pond5.com) and were paired with each image.

Prior to the main study, a pilot study was carried out to confirm that the emotional and neutral stimulus sets were significantly different in emotion ratings. This study confirmed that both emotional sounds and emotional sound-picture pairs were significantly more negative than their neutral counterparts (See Supplementary material).

EEG Acquisition and preprocessing

EEG data was acquired from 57 channels (positioned according to the 10-20 system). Additional electrodes were placed on the left and right mastoids, above the right eye and below the left eye (for EOG), two placed on the chin (for EMG), one on the forehead (recording reference), and one on the collarbone (ground). Data were collected with an Aura-LTM64 amplifier and TWin software (Grass Technologies). All impedances were kept to <25 kOhm. The sampling rate was 400 Hz.

Sleep scoring was performed using TWin software and MATLAB (The MathWorks, Natick, MA)) according to AASM criteria (Berry RB, 2020). Subsequent EEG analyses were performed in

MATLAB using custom scripts. First, all EEG channels were re-referenced to the average of the two mastoids, high-pass filtered at 0.3Hz, and notch filtered at 60Hz. Data were then artifact rejected based on visual inspection, with bad segments of data being marked and removed from subsequent analyses. Bad channels were identified by visual inspection and interpolated using a spherical spline algorithm. All artifact-free data were then subjected to further analysis.

EEG data analysis

Clean, artifact-free data were segmented into epochs that extended from 1 second before stimulus-onset to 3 seconds after and baseline adjusted to the mean voltage during the 1-second before cue onset. Complex Morlet wavelets were used to decompose the epoched and baseline adjusted time series data into time-frequency representations (Cohen, 2014), with spectral power being extracted at 30 logarithmically spaced frequencies from 2-40Hz and the number of wavelet cycles increasing from 3 to 10 in 30 logarithmically spaced steps to match the number of frequency bins. For analysis, power was decibel-normalized within-subject (10 x log10(power/baseline), where the baseline was mean power in the 200-500 milliseconds prior to cue-onset. This baseline period was chosen to mitigate contamination of the baseline period by post-stimulus activity. As such, positive values reflect relative increases in power following sound cues compared to the pre-cue baseline, whereas negative values reflect decreases. All time-frequency analyses were conducted at electrode site Cz only.

Data reduction

Some participants' data were excluded, because of achieving less than one full round of reactivation (n=32), having a full round of reactivation carried out in the wrong sleep stage (n=4),

or due to equipment failure (n=5), failing to complete the experiment (n=2), or not meeting healthy control criteria (n=1). In addition, 2 participants from the N-SWS group were removed because their error at T1 and T2 (first participant) or cueing benefit at T2 (second participant) were more than 3 standard deviations from the mean. The final sample included 24 participants in E-SWS, 26 in E-REM and 29 in N-SWS.

Statistical Analyses

Recall accuracy was measured as the distance between where participants placed the pictures and their correct locations, measured in pixels. Because picture recall accuracy was not normally distributed within subjects, we used medians in all analyses. The effect of reactivation was examined using ANOVAs with % change in recall accuracy from T1 to T2 or to T3 as the dependent variable. Covariates were added later when appropriate. A one-way MANOVA was used to compare the sleep stage compositions between groups, with Tukey's test for post-hoc analyses. Cueing benefit (% change in error for the non-reactivated items - % change in error for the reactivated items; (Creery et al., 2015)) was used for correlations with sleep and EEG measures. Pearson's correlation was used for correlations between normally distributed variables. Spearman's correlation was used if variables were not normally distributed.

To analyze cue-evoked EEG data, we first sought to identify clusters of post-cue activity that were significantly different from zero. For each group, we conducted one-sample t-tests across participants to detect points in the time-frequency space at which spectral power was systematically different from zero ($p < .05$, false discovery rate (FDR) adjusted) (see Schechtman et al., 2021 for a similar approach). Clusters of significant activity were identified using the *bwlabeln* function in MATLAB (Cohen 2014). Within each cluster, spectral power was averaged

across all time-frequency points in that cluster, producing for each participant a single spectral power value for each cluster. These values were then used in analyses correlating cue-evoked spectral power with cueing benefit.

2. RESULTS

Memory Performance

In the E-SWS group, cueing benefits at T2 were significantly correlated with %SWS (r=.46, p=.03). Because there is evidence suggesting that NREM and REM may have complimentary roles in memory consolidation, we also examined the correlation of the product of %SWS and %REM (%SWS × %REM) (Stickgold, 2000; Mednick, 2003; Hu, 2015). This revealed a stronger correlation (spearman's r_s=.63, p=9.5x10^{-4}; **Figure 2.A**). %SWS × %REM was also significantly correlated with cueing benefit at T3 in the N-SWS group (r=.46, p=.02). There were no other correlations of sleep parameters with cueing benefit at T2 or T3, in any of the groups.

To test our primary hypothesis, we built 2 (reactivation; reactivated vs. non-reactivated) x 2 (group; E-REM vs. E-SWS) mixed ANCOVAs, with % change in error from T1 to T2 as the dependent variable. %SWS × %REM was added as a covariate because it was correlated with cueing benefit in E-SWS and was significantly higher in E-REM compared to E-SWS (t=3.30, p=.002). The analysis showed a significant reactivation x group interaction, with a larger memory benefit at T2 for reactivated items in E-SWS than in E-REM, (F = 4.64, p=.04; **Figure 2.B**). In the follow-up analysis for simple main effects, adjusted for %SWS × %REM, reactivation was associated with significantly smaller increase in error within E-SWS group (F=12.81, p=.002). In contrast, there was a significantly larger increase in error for reactivated items within E-REM

(F=4.64, p=.04). A similar analysis of residual effects of reactivation at one week did not reveal any main effects or interaction for change in memory at T3 (F=.03, p=.87).

In a secondary analysis, we tested the hypothesis that reactivation of emotional items during SWS would show a larger cueing benefit at T2 than reactivation of neutral items using 2 (reactivation; reactivated vs. non-reactivated) x 2 (group; E-SWS vs. N-SWS) mixed ANCOVAs. These analyses did not reveal any interaction effects at T2 (F=.02, p=.88) or T3 (F=.68, p=.41), indicating that reactivation of emotional and neutral stimuli in SWS produced similar cuing benefits. Change in error for all groups at T2 and T3 can be found in supplementary figures S1 and S2.

As expected, recall immediately after training, at T1, for items that would subsequently be reactivated did not differ significantly from recall of those that would not be reactivated in any of the groups (E-SWS: t=1.14, p=.27; E-REM: t=-.53, p=.60; N-SWS: t=.45, p=.66) **(Supplementary Table S1)**. There was also no difference in recall at T1 between groups (F=1.66, p=.20). Finally, we compared the sleep architecture between groups included in the ANCOVAs (E-SWS vs. E-REM; E-SWS vs. N-SWS) and found a higher %REM in E-REM than in E-SWS (p<.001) and N-SWS (p=.01) **(Supplementary Table S2)**.

Cue-evoked activity

To investigate cue-related modulation of EEG activity during sleep, we first identified clusters of cue-induced activity that were different significantly from zero (see Methods). In the E-SWS group, we identified two time-frequency clusters in which the cues induced increased activity ($p < .05$, FDR adjusted; **Figure 3A)**. The first cluster (2.0 - 8.5Hz), extending from 235-975 ms after cue onset, comprises activity in the canonical delta and theta bands. The second cluster (11.6 -

19.1Hz), occurring 638-1,475 ms following cue onset, corresponds broadly with the sigma band. This pattern of increased delta/theta and spindle band activity following the replay of emotional sounds during SWS replicates findings in other studies of cueing during SWS (Lehmann et al., 2016; Forcato et al., 2020; Schechtman et al., 2021).

Interestingly, power in the sigma cluster was significantly higher in the E-SWS group compared to the N-SWS group (t (41.9) = 2.99, p = .005; **Figure 3B, left**). The two groups were equivalent in terms of power in the delta-theta cluster (t (41.6) = 0.93, p = .36; **Figure 3C, left**), suggesting a difference between emotional and neutral sounds in terms of their modulation of the EEG signal. This was further suggested by a significant correlation between the magnitude of the sigma band response and the subjectively rated valence (derived from our pilot study; see supplementary methods) of the emotional sounds cued during sleep (r = .55, p = .008; **Figure 3B, right)**. In both groups, power in the delta-theta cluster was positively correlated with the T2 cueing benefit (E-SWS: r = .52, p = .014; N-SWS: r = .53, p = .025; **Figure 3C, right**. Notably, when re-ran the cluster analysis on only the N-SWS group, no significant clusters emerged at the < .05 (FDR corrected) level (**Supplementary Figure S3).**

With regards to cueing during REM sleep, we observed an increase in power across a broad frequency range throughout the entire post-cue period, but the increase was only significant in alpha-beta frequencies (9.4 - 26.5Hz) from 1,375 to 1,725 ms following cue onset (**Figure 4A**). Power in the identified cluster did not correlate with the cueing benefit at either T2 (**Figure 4B, left**) or T3 (all p > .43). Similarly, post-cue EEG activity following REM cueing was not related to stimulus valence (r = .12, p = .61; **Figure 4B, right**).

Correlations with Measures of Sleep Fragmentation and Arousal

A recent study showed that participants who were awakened by reactivations showed memory impairment for cued items (Goldi and Rasch, 2019). We asked whether the memory impairment in E-REM group was associated with sleep disruptions, carrying out correlations between cueing benefit at T2 and arousal index (AI; number of arousals/minute; (Berry et al., 2012)), sleep fragmentation index (SFI; number of sleep stage transitions/minute; (Haba-Rubio et al., 2004)) and the duration of wake time after sleep onset (WASO). None of these revealed a significant association (AI: r=-.27, p=.19; SFI: r=.14, p=.50; WASO: r=.32, p=.11). Because arousals could limit the number of reactivations, we also examined the correlation between cueing benefit and number of reactivations, but this also was not significant (r=-0.06, p=.77). No significant associations were observed when the same analyses were repeated in the other reactivation groups.

Another recent TMR study found an inverse correlation of cueing benefit with cue-evoked power in beta band, and a strong trend for a similar correlation with power in alpha band (Whitmore et al., 2022). To examine if a similar mechanism explained the memory impairment for the reactivated items in E-REM and the negative cueing benefits in some participants in the other reactivation groups, we took the absolute difference in spectral power between the pre- and post-cue periods in the alpha (8-11 Hz) and beta (17-21 Hz) bands as an EEG-measure of cue-related arousal (Whitmore et al., 2022). While there were no correlations between these measures and the cueing benefit in the E-REM group, a significant negative correlation was observed between alpha power and the T2 cueing benefit in the E-SWS group (r = -.50, p = .02), similar to that seen by Whitmore et al. (2022). This suggests that higher cue-evoked alpha activity might contribute to the negative effect of SWS-TMR on emotional memory.

3. DISCUSSION

We applied TMR in SWS and REM to test the hypothesis that consolidation of emotional memories occurs primarily in REM. Contrary to our expectations, reactivation of emotional stimuli during REM led to poorer recall. On the other hand, the memory benefit of emotional memory reactivation during SWS was strongly correlated with the product of SWS and REM times, and when this variable was taken into account, reactivation significantly enhanced memory, with larger cueing benefit than REM reactivation. We also tested the hypothesis that reactivation of emotional items would be associated with enhanced memory compared to neutral items. However, for reactivation in SWS, we did not find any difference in cueing benefit for emotional and neutral items.

The strong correlation of cueing benefit in the E-SWS group with the product of times spent in SWS and REM suggests a complementary role for these sleep stages. A significant correlation between this product and cueing benefit was also observed at delayed retest in the N-SWS group. These findings are in agreement with accounts that propose that both sleep stages are required for some forms of sleep-dependent memory consolidation (Giuditta et al., 1995; Ficca and Salzarulo, 2004; Diekelmann and Born, 2010; Walker and Stickgold, 2010; Langille, 2019) and the expanding literature which provides supporting evidence in humans. Studies using a visual discrimination task found that improvement was correlated with the product of times spent in SWS and REM (Stickgold et al., 2000), largest after a full night of sleep compared to early or late night-half sleep (Gais et al., 2000), and present after a nap only if it contained both NREM and REMs (Mednick et al., 2003). In studies that used verbal memory tasks, recall was positively correlated with the duration of NREM/REM cycles (Mazzoni et al., 1999) and was impaired when sleep cycles were interrupted, but not after sleep fragmentation with intact sleep cycles (Ficca et al.,

2000). Finally, similar to our study, several TMR studies that carried out reactivations in SWS found that REM or both REM and SWS were associated with outcomes. REM duration was the only measure correlated with enhanced memory for the meaning of novel words (Batterink et al. 2017) and lexical integration (Tamminen et al. 2017) after TMR. In another study, TMR related reduction in social bias was correlated with %SWS × %REM (Hu et al. 2015). Besides behavioral effects, other TMR studies with reactivation in SWS, found neurobiological alterations associated with REM (Cairney et al. 2015, Cousins et al. 2016). In summary, there is now a substantial body of evidence which suggests that memory consolidation is attained via processes that take place across NREM and REM sleep. Further studies are needed to directly examine the dynamics of this coordination, such as how brain oscillations interact across sleep stages.

Cueing emotional stimuli during SWS generated EEG responses in the delta/theta band and then at sigma frequencies, similar to previous studies (Lehmann et al., 2016; Forcato et al., 2020; Schechtman et al., 2021). Notably, the extent of delta/theta activity following reactivation was correlated with memory benefits in two independent groups (E-SWS and N-SWS) exposed to different sets of stimuli. Enhanced memory being present only for the group with a net increase in this frequency range (*i.e.*, E-SWS) further supports this association. These results suggest that delta/theta activity enhances memories independent of their emotional charge. Lack of increased activity in this frequency in N-SWS could be due to neutral sounds not being sufficiently discernible or failure to establish strong sound-picture associations.

Spindle activity was modulated by emotional valence, as indicated by two complementary findings. First, power in the sigma band was the only difference in cue-evoked activity between E-SWS and N-SWS groups, with significantly higher sigma power in E-SWS. Second, within E-SWS, sigma power was significantly and positively correlated with the average of the valence of

the reactivated stimuli. A role for spindles in emotional processing has been suggested in recent studies that showed an association of spindle activity with enhanced consolidation of emotional memories (Cairney et al., 2014a; Lehmann et al., 2016; Alger et al., 2018) and with reduction in reactivity to negative memories after sleep (Azza et al., 2022). In addition to correlations, another study (Kaestner et al., 2013) found that increasing spindle activity pharmacologically significantly improved memory for negative and high-arousal items. Aside from experimental studies, spindles are also implicated in the pathophysiology of psychiatric disorders including social anxiety disorder (Wilhelm et al., 2017) and major depressive disorder (Lopez et al., 2010; Plante et al., 2013; Nishida et al., 2014; Sesso et al., 2017). Furthermore, altered spindle activity is associated with specific emotional symptoms, including worry (Hamann et al., 2019), internalizing problems (Kathrin et al., 2021), intrusive memories (van der Heijden et al., 2022), and frequent nightmares (Picard-Deland et al., 2018). Our results lend further support to the burgeoning evidence suggesting that spindles may be involved in emotional processing.

The decrease in recall after reactivation during REM was unexpected. To the best of our knowledge, this is the first TMR study to show such an effect associated with REM. A role for REM in eliminating specific memories was first proposed by Crick and Mitchison (Crick and Mitchison, 1983). Later, different lines of research provided evidence suggesting that REM may be involved in selective removal of memory representations (Feld and Born, 2017; Poe, 2017; Langille, 2019), however, a direct link with forgetting in humans has been missing. While theta peak activity is associated with long-term potentiation in the hippocampus, several studies found that activity in the troughs causes depotentiation (Huerta and Lisman, 1995; Holscher et al., 1997). In one study, hippocampal place cells reversed their discharge phase to theta troughs during REM once the exposed environment became familiar, suggesting that REM may serve to “refresh”

synapses for future use in encoding new memories (Poe et al., 2000). Furthermore, two recent studies showed direct evidence of synaptic pruning during REM after learning (Li et al., 2017; Zhou et al., 2020). Finally, direct behavioral evidence for REM related memory decay was found in a recent study (Izawa et al., 2019), which showed that a subset of melanin-concentrating-hormone-producing neurons were active specifically in REM and their activation was associated with impairment in memory. In light of this evidence, we can speculate that reactivations in REM may have led to the weakening of synapses that represent the cued items. The study by Li and colleagues suggest that a corollary of such synaptic weakening could be strengthening of the memory for the remaining items (Li et al., 2017). Indeed, the increase in error for the non-reactivated items in the E-REM group was 55% less than that in E-SWS.

Memory transformation might also have contributed to the poorer memory for reactivated items in E-REM, although we did not use any measures to examine this possibility. REM is particularly conducive to forming new associations and reactivations during REM might have facilitated extraction of common elements across stimuli (Lewis, 2018), such as bodily harm, at the expense of information specific to individual stimulus (spatial location) (Langille, 2019). Another possibility is that we are seeing the effects of an "emotional memory trade-off", characterized by sleep selectively enhancing emotional aspects of memory while memory for less salient elements weakens (Denis et al., 2022). This trade-off might have been magnified for the reactivated stimuli with the contextual information (spatial location) decaying to promote the emotional material (picture content). Finally, decline in memory might be related to the TMR method itself. In recent TMR studies, decreased recall was associated with cueing related increase in power in the alpha and beta bands (Whitmore et al., 2022) and memory for new vocabulary was impaired for the cued items, when the sound cues resulted in awakenings (Goldi and Rasch, 2019). We did not find any

association of change in memory with arousals (AI or SI) but found increased alpha/beta activity. While this increase was not correlated with change in memory, we cannot exclude the possibility that arousal contributed to the memory decline. However, additional mechanisms can explain the oscillatory activity we observed. A recent study which carried out intracranial recordings found that anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC) showed bursts of beta activity during REM, in addition to the expected theta activity (Vijayan et al., 2017). Furthermore, another study which used auditory stimulation to enhance theta activity, observed increased power in the alpha/beta band similar to our study (Harrington et al., 2021). While we did not observe an increase in theta oscillations, increased beta activity may be related to activation in regions involved in emotional processing, such as ACC.

In summary, our results strongly suggest that memory consolidation, including of emotional memories, requires reactivation in SWS, and is dependent on processes that take place in both SWS and REM. Future studies which examine the role of different sleep stages in memory consolidation, should take this interaction into account, as well as the possibility that memory reactivation in REM leads to the forgetting of emotional memories or weakens aspects of them.

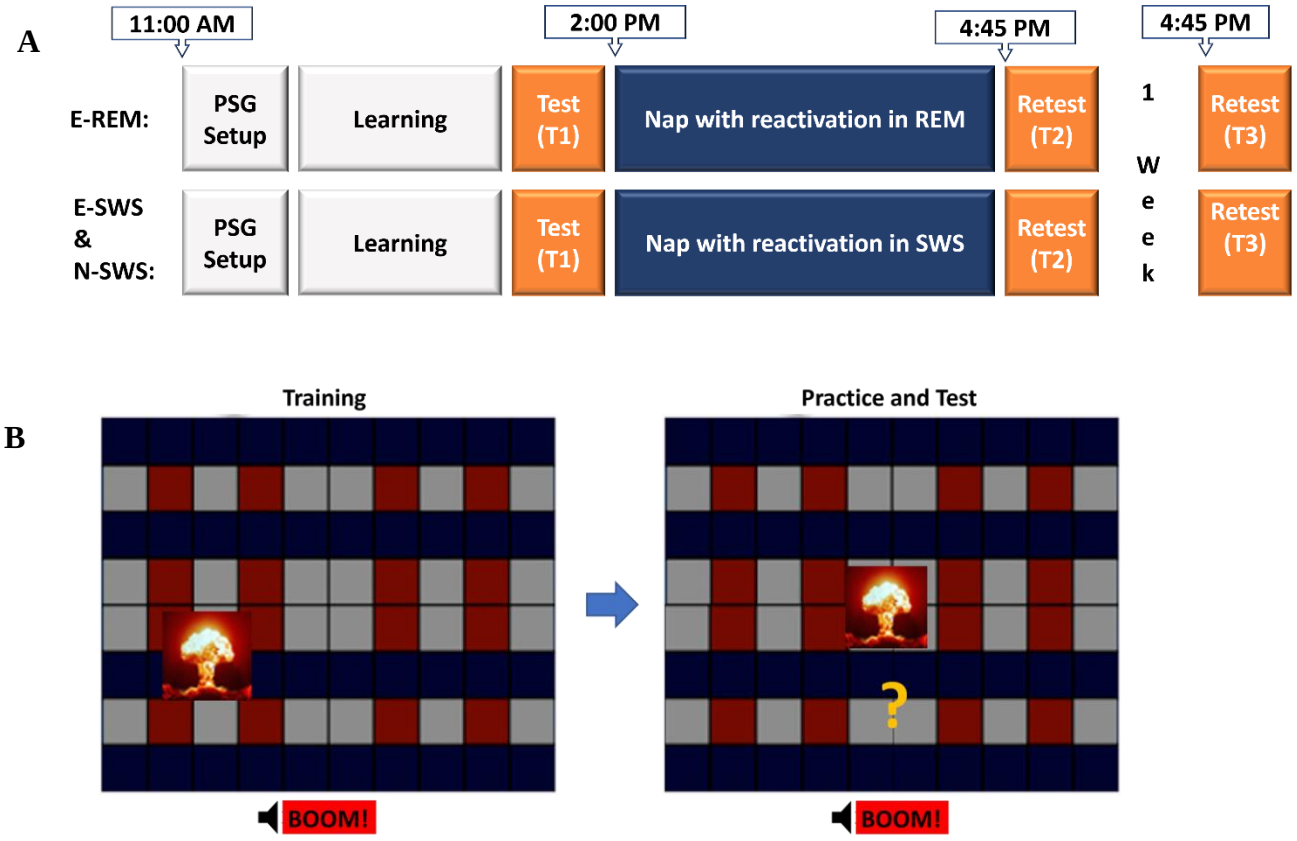

Figure 1. A - Timeline of the study procedures for different groups are depicted. In all groups, the first visit started at 11:00 AM. After training and practice, a baseline test (T1) was carried out immediately before the nap. The first retest (T2) was approximately 45 minutes after waking up. The second retest (T3) was approximately 1 week after the first visit. **B –** During training, participants passively viewed emotional or neutral pictures appearing at different locations on a grid, while a paired sound was played. During practice and test, pictures appeared in the center of the screen, and participants were asked to move them to their correct location.

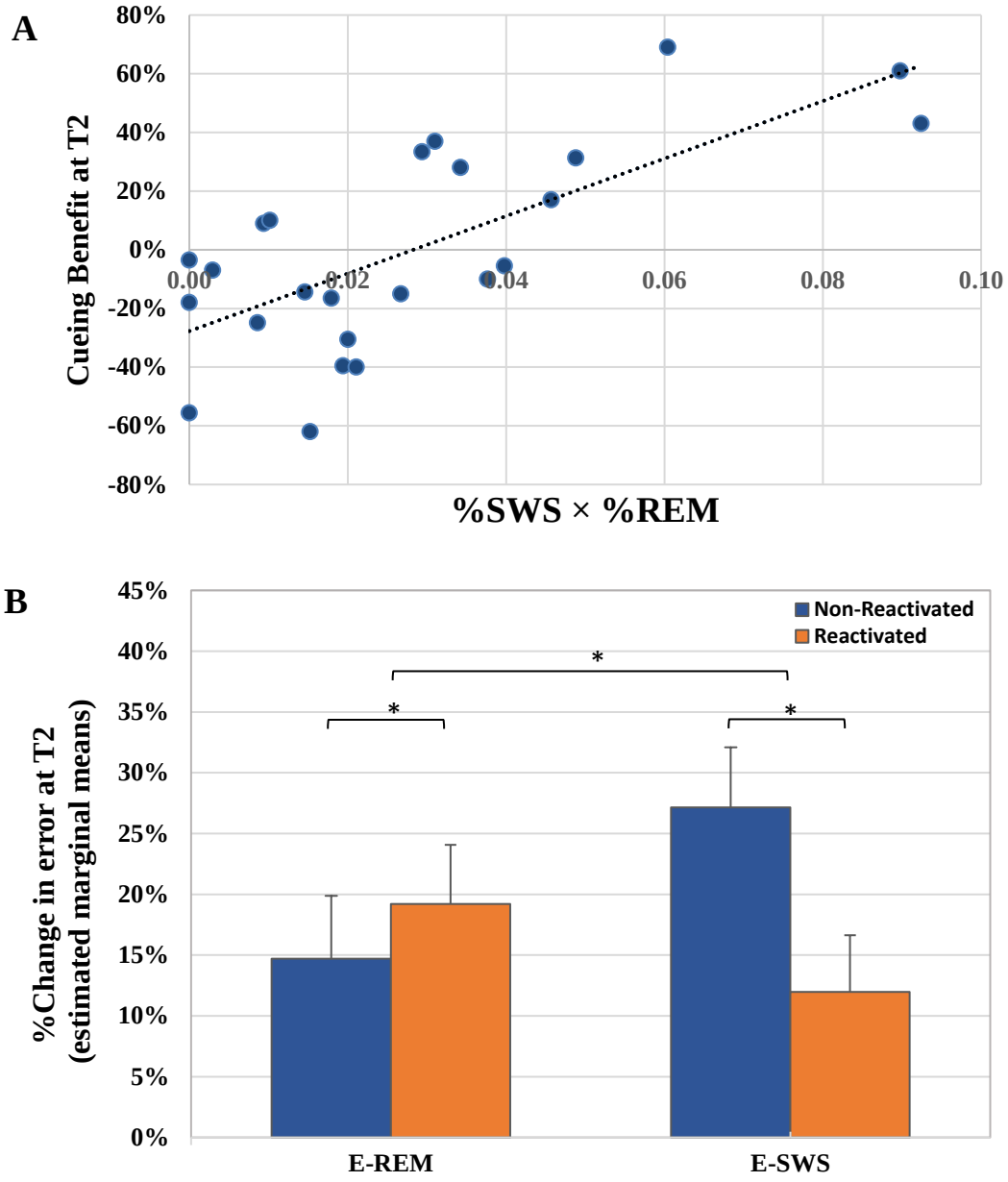

Figure 2. A - %SWS × %REM is strongly correlated with cueing benefit at T2, in the E-SWS group. **B** - Estimated marginal means of change in error at T2 adjusted for %SWS × %REM from the ANCOVA is shown. There was a significant group x reactivation interaction, with a significantly larger memory benefit for reactivated items in E-SWS compared to E-REM. In post-hoc tests, recall was significantly improved for reactivated items in E-SWS, when %SWS × %REM was taken into account, whereas memory was significantly impaired for cued items in E-REM. Error bars indicate the standard error. *: $p<0.05$

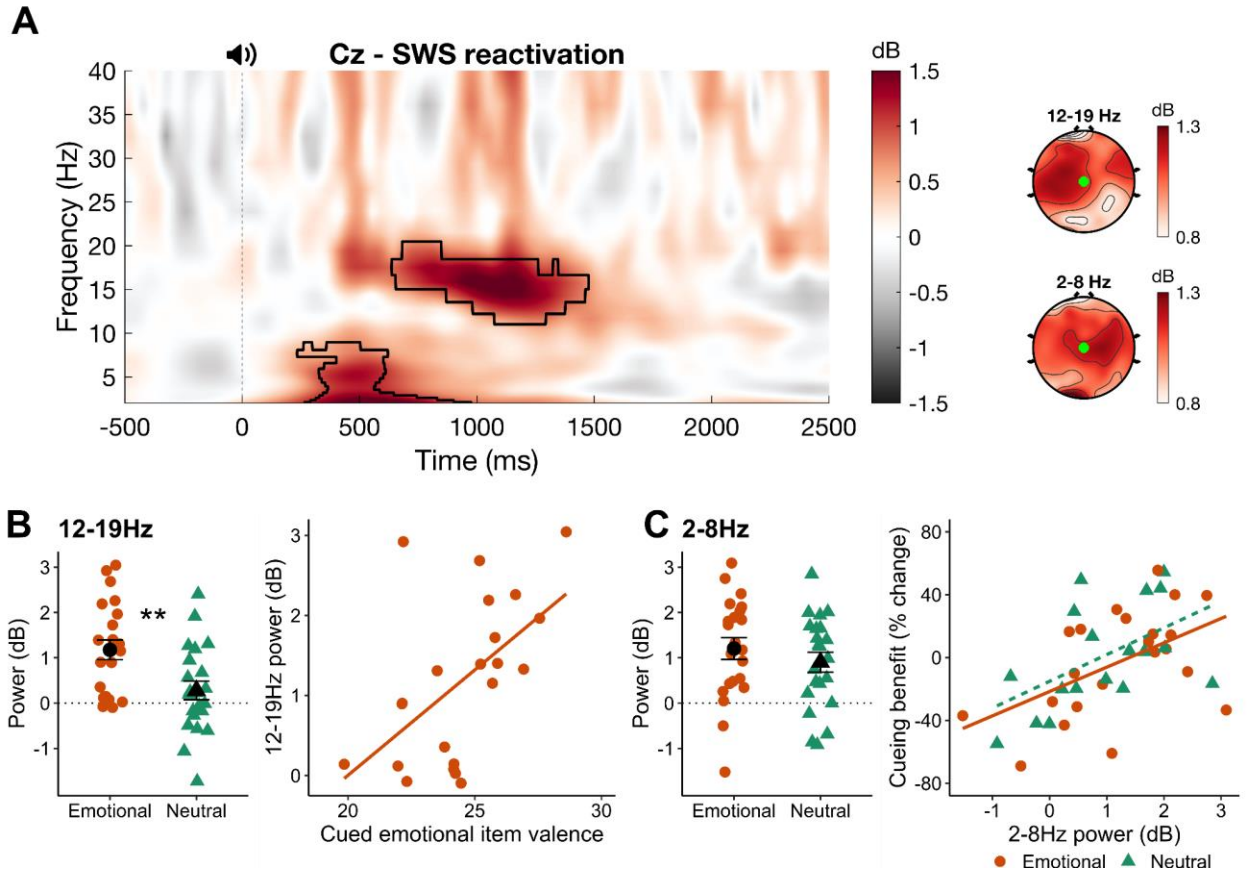

Figure 3. Effect of cueing during slow wave sleep. **A -** Left: Time frequency response to emotional cue presentation during slow wave sleep at electrode Cz. Time zero represents the initiation of sound presentation during sleep. Black contour lines highlight significant clusters of activation ($p < .05$, FDR adjusted). Right: Topographical visualizations of cluster-averaged activity. **B -** 12-19 Hz response is associated with the valence of cued sounds. Left: Cluster-averaged time frequency activity in the 12-19 Hz response in response to either emotional or neutral sound cues. **C -** 2-8 Hz response is associated with cueing benefit. Left: Cluster-averaged time frequency activity in the 2-8Hz cluster in response to either emotional or neutral sound cues. Error bars indicate the standard error. Right: Scatterplot showing a significant positive correlation between power in the 2-8Hz cluster and the magnitude of the cueing benefit. Error bars indicate the standard error. ** = $p < .01$. Right: Scatterplot showing a significant positive correlation between power in the 12-19Hz cluster and the valence of cued negative sounds.

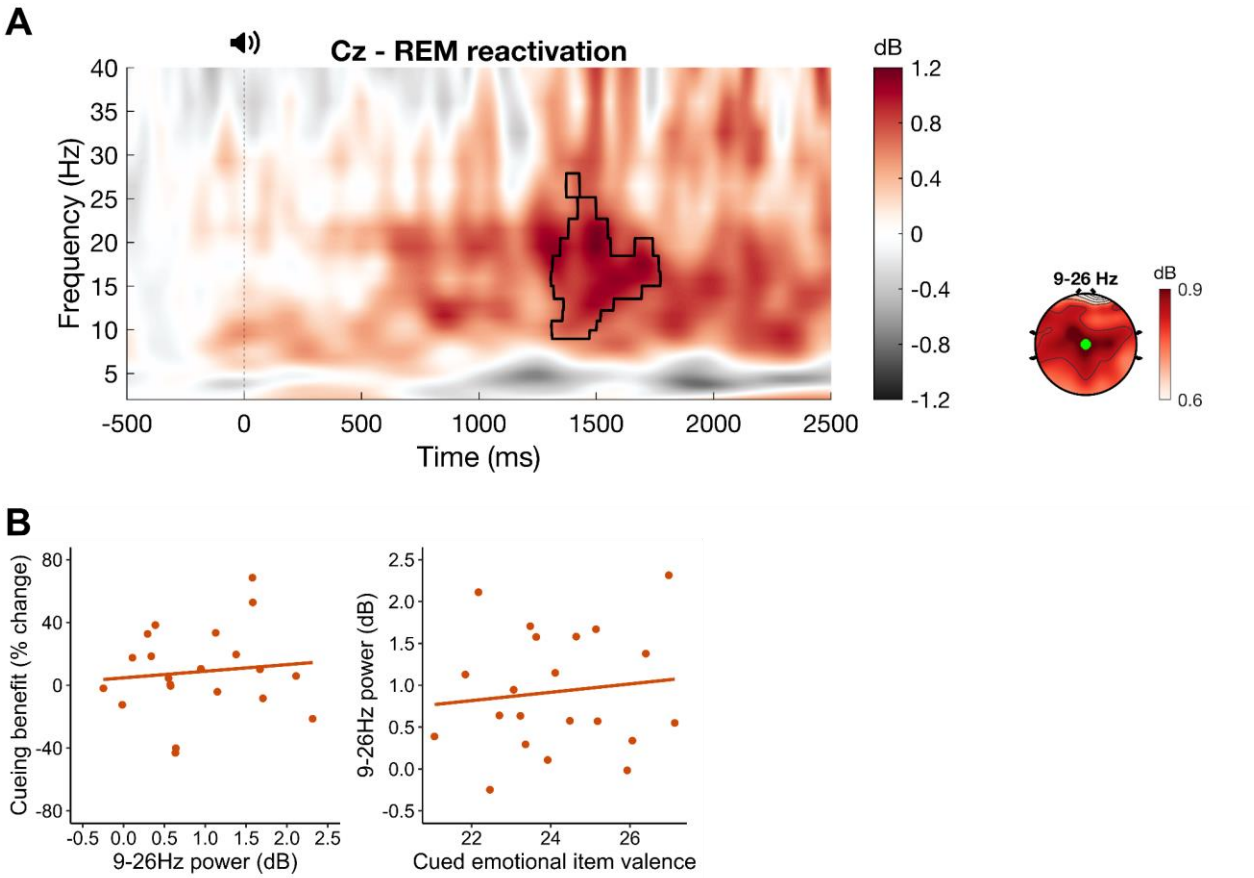

Figure 4. Effect of cueing during rapid eye movement sleep. **A -** Left: Time frequency response to emotional cue presentation during rapid eye movement sleep at electrode Cz. Time zero represents the initiation of sound presentation during sleep. Black contour line highlights the significant cluster of activation ($p < .05$, FDR adjusted). Right: Topographical visualizations of cluster-averaged activity. **B** - Left: Scatterplot showing a lack of relationship between 9-26Hz cluster power and cueing benefit. Right: Scatterplot showing a lack of relationship between cued emotional item valence and 9-26Hz cluster power.

References

Ackermann S, Hartmann F, Papassotiropoulos A, de Quervain DJ, Rasch B (2015) No Associations between Interindividual Differences in Sleep Parameters and Episodic Memory Consolidation. Sleep 38:951-959.

Alger SE, Kensinger EA, Payne JD (2018) Preferential consolidation of emotionally salient information during a nap is preserved in middle age. Neurobiol Aging 68:34-47.

Ashton JE, Cairney SA, Gaskell MG (2018) No effect of targeted memory reactivation during slow-wave sleep on emotional recognition memory. J Sleep Res 27:129-137.

Azza Y, Wilhelm FH, Seifritz E, Junghanns K, Kleim B, Wilhelm I (2022) Sleep's role in updating aversive autobiographical memories. Transl Psychiatry 12:117.

Baran B, Pace-Schott EF, Ericson C, Spencer RM (2012) Processing of emotional reactivity and emotional memory over sleep. J Neurosci 32:1035-1042.

Batterink LJ, Westerberg CE, Paller KA (2017) Vocabulary learning benefits from REM after slow-wave sleep. Neurobiol Learn Mem 144:102-113.

Benedict C, Scheller J, Rose-John S, Born J, Marshall L (2009) Enhancing influence of intranasal interleukin-6 on slow-wave activity and memory consolidation during sleep. FASEB J 23:3629-3636.

Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, Marcus CL, Mehra R, Parthasarathy S, Quan SF, Redline S, Strohl KP, Davidson Ward SL, Tangredi MM, American Academy of Sleep M (2012) Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. J Clin Sleep Med 8:597-619.

Berry RB QS, Abreu AR, et al.; for the American Academy of Sleep Medicine (2020) The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications, Version 2.6. Darien, Illinois: American Academy of Sleep Medicine.

Boyce R, Williams S, Adamantidis A (2017) REM sleep and memory. Curr Opin Neurobiol 44:167-177.

Cairney SA, Durrant SJ, Jackson R, Lewis PA (2014a) Sleep spindles provide indirect support to the consolidation of emotional encoding contexts. Neuropsychologia 63:285-292.

Cairney SA, Durrant SJ, Hulleman J, Lewis PA (2014b) Targeted memory reactivation during slow wave sleep facilitates emotional memory consolidation. Sleep 37:701-707, 707A.

Cairney SA, Durrant SJ, Power R, Lewis PA (2015) Complementary roles of slow-wave sleep and rapid eye movement sleep in emotional memory consolidation. Cereb Cortex 25:1565-1575.

Cellini N, Torre J, Stegagno L, Sarlo M (2016) Sleep before and after learning promotes the consolidation of both neutral and emotional information regardless of REM presence. Neurobiol Learn Mem 133:136-144.

Cohen X (2014) Analyzing Neural Time Series Data Theory and Practice. Cambridge, MA: Massachusetts Institute of Technology (MIT) Press.

Creery JD, Oudiette D, Antony JW, Paller KA (2015) Targeted Memory Reactivation during Sleep Depends on Prior Learning. Sleep 38:755-763.

Crick F, Mitchison G (1983) The function of dream sleep. Nature 304:111-114.

Dan-Glauser ES, Scherer KR (2011) The Geneva affective picture database (GAPED): a new 730-picture database focusing on valence and normative significance. Behav Res Methods 43:468-477.

Davidson P, Jonsson P, Carlsson I, Pace-Schott E (2021) Does Sleep Selectively Strengthen Certain Memories Over Others Based on Emotion and Perceived Future Relevance? Nat Sci Sleep 13:1257-1306.

Denis D, Sanders KEG, Kensinger EA, Payne JD (2022) Sleep preferentially consolidates negative aspects of human memory: Well-powered evidence from two large online experiments. Proc Natl Acad Sci U S A 119:e2202657119.

Diekelmann S, Born J (2010) The memory function of sleep. Nat Rev Neurosci 11:114-126.

Feld GB, Born J (2017) Sculpting memory during sleep: concurrent consolidation and forgetting. Curr Opin Neurobiol 44:20-27.

Ficca G, Salzarulo P (2004) What in sleep is for memory. Sleep Med 5:225-230.

Ficca G, Lombardo P, Rossi L, Salzarulo P (2000) Morning recall of verbal material depends on prior sleep organization. Behav Brain Res 112:159-163.

Forcato C, Klinzing JG, Carbone J, Radloff M, Weber FD, Born J, Diekelmann S (2020) Reactivation during sleep with incomplete reminder cues rather than complete ones stabilizes long-term memory in humans. Commun Biol 3:733.

Gais S, Plihal W, Wagner U, Born J (2000) Early sleep triggers memory for early visual discrimination skills. Nat Neurosci 3:1335-1339.

Giuditta A (2014) Sleep memory processing: the sequential hypothesis. Front Syst Neurosci 8:219.

Giuditta A, Ambrosini MV, Montagnese P, Mandile P, Cotugno M, Grassi Zucconi G, Vescia S (1995) The sequential hypothesis of the function of sleep. Behav Brain Res 69:157-166.

Goldi M, Rasch B (2019) Effects of targeted memory reactivation during sleep at home depend on sleep disturbances and habituation. NPJ Sci Learn 4:5.

Goldi M, van Poppel EAM, Rasch B, Schreiner T (2019) Increased neuronal signatures of targeted memory reactivation during slow-wave up states. Sci Rep 9:2715.

Goldstein AN, Walker MP (2014) The role of sleep in emotional brain function. Annu Rev Clin Psychol 10:679-708.

Groch S, Wilhelm I, Diekelmann S, Born J (2013) The role of REM sleep in the processing of emotional memories: evidence from behavior and event-related potentials. Neurobiol Learn Mem 99:1-9.

Haba-Rubio J, Ibanez V, Sforza E (2004) An alternative measure of sleep fragmentation in clinical practice: the sleep fragmentation index. Sleep Med 5:577-581.

Hamann C, Rusterholz T, Studer M, Kaess M, Tarokh L (2019) Association between depressive symptoms and sleep neurophysiology in early adolescence. J Child Psychol Psychiatry 60:1334-1342.

Harrington MO, Ashton JE, Ngo HV, Cairney SA (2021) Phase-locked auditory stimulation of theta oscillations during rapid eye movement sleep. Sleep 44.

Hoddes E, Dement W, Zarcone V (1972) The development and use of the Stanford Sleepiness Scale (SSS). Psychophysiology 9:150.

Holscher C, Anwyl R, Rowan MJ (1997) Stimulation on the positive phase of hippocampal theta rhythm induces long-term potentiation that can Be depotentiated by stimulation on the negative phase in area CA1 in vivo. J Neurosci 17:6470-6477.

Hu X, Cheng LY, Chiu MH, Paller KA (2020) Promoting memory consolidation during sleep: A meta-analysis of targeted memory reactivation. Psychol Bull 146:218-244.

Hu X, Antony JW, Creery JD, Vargas IM, Bodenhausen GV, Paller KA (2015) Cognitive neuroscience. Unlearning implicit social biases during sleep. Science 348:1013-1015.

Huerta PT, Lisman JE (1995) Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 in vitro. Neuron 15:1053-1063.

Izawa S, Chowdhury S, Miyazaki T, Mukai Y, Ono D, Inoue R, Ohmura Y, Mizoguchi H, Kimura K, Yoshioka M, Terao A, Kilduff TS, Yamanaka A (2019) REM sleep-active MCH neurons are involved in forgetting hippocampus-dependent memories. Science 365:1308-1313.

Johns MW (1991) A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14:540-545.

Kaestner EJ, Wixted JT, Mednick SC (2013) Pharmacologically increasing sleep spindles enhances recognition for negative and high-arousal memories. J Cogn Neurosci 25:1597-1610.

Kathrin B, Michael AH, Ines W, Kerstin H (2021) The relation between sigma power and internalizing problems across development. J Psychiatr Res 135:302-310.

Klinzing JG, Niethard N, Born J (2019) Mechanisms of systems memory consolidation during sleep. Nat Neurosci 22:1598-1610.

Lang PJ, Bradley MM, Cuthbert BN (2008) International Affective Picture System (IAPS): Instruction manual and affective ratings, Technical Report A-8. Gainesville: The Center for Research in Psychophysiology, University of Florida.

Langille JJ (2019) Remembering to Forget: A Dual Role for Sleep Oscillations in Memory Consolidation and Forgetting. Front Cell Neurosci 13:71.

Lehmann M, Schreiner T, Seifritz E, Rasch B (2016) Emotional arousal modulates oscillatory correlates of targeted memory reactivation during NREM, but not REM sleep. Sci Rep 6:39229.

Li W, Ma L, Yang G, Gan WB (2017) REM sleep selectively prunes and maintains new synapses in development and learning. Nat Neurosci 20:427-437.

Lopez J, Hoffmann R, Armitage R (2010) Reduced sleep spindle activity in early-onset and elevated risk for depression. J Am Acad Child Adolesc Psychiatry 49:934-943.

Mazzoni G, Gori S, Formicola G, Gneri C, Massetani R, Murri L, Salzarulo P (1999) Word recall correlates with sleep cycles in elderly subjects. J Sleep Res 8:185-188.

Mednick S, Nakayama K, Stickgold R (2003) Sleep-dependent learning: a nap is as good as a night. Nat Neurosci 6:697-698.

Morgenthaler J, Wiesner CD, Hinze K, Abels LC, Prehn-Kristensen A, Goder R (2014) Selective REM-sleep deprivation does not diminish emotional memory consolidation in young healthy subjects. PLoS One 9:e89849.

Nishida M, Nakashima Y, Nishikawa T (2014) Topographical distribution of fast and slow sleep spindles in medicated depressive patients. J Clin Neurophysiol 31:402-408.

Nishida M, Pearsall J, Buckner RL, Walker MP (2009) REM sleep, prefrontal theta, and the consolidation of human emotional memory. Cereb Cortex 19:1158-1166.

Payne JD, Kensinger EA, Wamsley EJ, Spreng RN, Alger SE, Gibler K, Schacter DL, Stickgold R (2015) Napping and the selective consolidation of negative aspects of scenes. Emotion 15:176-186.

Picard-Deland C, Carr M, Paquette T, Saint-Onge K, Nielsen T (2018) Sleep spindle and psychopathology characteristics of frequent nightmare recallers. Sleep Med 50:113-131.

Plante DT, Goldstein MR, Landsness EC, Peterson MJ, Riedner BA, Ferrarelli F, Wanger T, Guokas JJ, Tononi G, Benca RM (2013) Topographic and sex-related differences in sleep spindles in major depressive disorder: a high-density EEG investigation. J Affect Disord 146:120-125.

Poe GR (2017) Sleep Is for Forgetting. J Neurosci 37:464-473.

Poe GR, Nitz DA, McNaughton BL, Barnes CA (2000) Experience-dependent phase-reversal of hippocampal neuron firing during REM sleep. Brain Res 855:176-180.

Rudoy JD, Voss JL, Westerberg CE, Paller KA (2009) Strengthening individual memories by reactivating them during sleep. Science 326:1079.

Schechtman E, Antony JW, Lampe A, Wilson BJ, Norman KA, Paller KA (2021) Multiple memories can be simultaneously reactivated during sleep as effectively as a single memory. Commun Biol 4:25.

Sesso G, Bat-Pitault F, Guyon A, Plancoulaine S, Banfi T, Milioli G, Parrino L, Faraguna U, Franco P (2017) Electrophysiological and microstructural features of sleep in children at high risk for depression: a preliminary study. Sleep Med 36:95-103.

Singh D, Norman KA, Schapiro AC (2022) A model of autonomous interactions between hippocampus and neocortex driving sleep-dependent memory consolidation. Proc Natl Acad Sci U S A 119:e2123432119.

Sopp MR, Michael T, Mecklinger A (2018) Effects of early morning nap sleep on associative memory for neutral and emotional stimuli. Brain Res 1698:29-42.

Stickgold R (2005) Sleep-dependent memory consolidation. Nature 437:1272-1278.

Stickgold R, Whidbee D, Schirmer B, Patel V, Hobson JA (2000) Visual discrimination task improvement: A multi-step process occurring during sleep. J Cogn Neurosci 12:246-254.

Tamminen J, Lambon Ralph MA, Lewis PA (2017) Targeted memory reactivation of newly learned words during sleep triggers REM-mediated integration of new memories and existing knowledge. Neurobiol Learn Mem 137:77-82.

van der Heijden AC, Hofman WF, de Boer M, Nijdam MJ, van Marle HJF, Jongedijk RA, Olff M, Talamini LM (2022) Sleep spindle dynamics suggest over-consolidation in post-traumatic stress disorder. Sleep 45.

Vijayan S, Lepage KQ, Kopell NJ, Cash SS (2017) Frontal beta-theta network during REM sleep. Elife 6.

Wagner U, Kashyap N, Diekelmann S, Born J (2007) The impact of post-learning sleep vs. wakefulness on recognition memory for faces with different facial expressions. Neurobiol Learn Mem 87:679-687.

Walker MP, Stickgold R (2010) Overnight alchemy: sleep-dependent memory evolution. Nat Rev Neurosci 11:218; author reply 218.

Wang JY, Heck KL, Born J, Ngo HV, Diekelmann S (2022) No difference between slow oscillation up- and down-state cueing for memory consolidation during sleep. J Sleep Res 31:e13562.

Whitmore NW, Bassard AM, Paller KA (2022) Targeted memory reactivation of face-name learning depends on ample and undisturbed slow-wave sleep. NPJ Sci Learn 7:1.

Wiesner CD, Pulst J, Krause F, Elsner M, Baving L, Pedersen A, Prehn-Kristensen A, Goder R (2015) The effect of selective REM-sleep deprivation on the consolidation and affective evaluation of emotional memories. Neurobiol Learn Mem 122:131-141.

Wilhelm I, Groch S, Preiss A, Walitza S, Huber R (2017) Widespread reduction in sleep spindle activity in socially anxious children and adolescents. J Psychiatr Res 88:47-55.

Zhou Y, Lai CSW, Bai Y, Li W, Zhao R, Yang G, Frank MG, Gan WB (2020) REM sleep promotes experience-dependent dendritic spine elimination in the mouse cortex. Nat Commun 11:4819.