nature portfolio

Peer Review File

Both Slow Wave and Rapid Eye Movement Sleep Contribute to Emotional Memory Consolidation

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This is a study investigating targeted memory reactivation in human. The experiments included emotional or neutral memory. Results showed that reactivation of emotional memory during REM increased the percentage of error (detrimental to memory). Emotional memory reactivation during SWS lead to a larger memory benefit than reactivation of neutral memories. The memory benefit was also strongly correlated with the SWS x REM product. The modulation in the slow wave and spindles frequency range was modulated by the emotional charge of memories.

The data and analyses appear solid and convincing. I have one minor comment regarding the last conclusion that slow wave (or delta/theta as named by authors) was modulated by the emotional charge of memories. My understanding is that the sound played for memory reactivation triggers slow wave at the cortical level which then creates conditions for spindles to occur in the thalamus (and then in cortex). Figure S2 shows an increase in the slow wave range around 400-600 ms (for the N-SWS group), similar to the increase seen in the E-SWS group (figure 4A), although it didn't reach statistical significance for the N-SWS group. If the stimulus is less efficient at triggering slow waves, one would expect that spindles will also be less triggered. Hence, I have no issues with the figure 4C (right panel) showing increased spindle power in E-SWS compared to N-SWS, and based on that, the conclusion is correct. However, my question would be: is the intensity, the frequency of the sound very similar for emotional sound vs neutral sound? In simpler word, if a sound played to cue an emotional memory was played to a naïve person (for which there would be no prior association to emotional memory), would this sound stimuli be more efficient in triggering slow wave activity than neutral memory sounds? If the answer would be yes, then the modulation of slow wave and spindle frequencies would not necessarily be due to the emotional charge, but rather to the stimulus itself being more or less efficient at triggering slow wave. I understand this cannot be tested for this paper and I am not asking this to be done. But perhaps, authors could briefly discuss whether the emotional sound stimuli are fully equivalent to the sound stimuli of neutral memory. And of course, if the mechanism is indeed related to triggering slow waves, then reactivation during REM sleep would not be efficient to improve memory as it is the case in this study.

Reviewer #2

(Remarks to the Author)
This study is the first to systematically investigate the effect for targeted memory reactivation (TMR) of emotional versus neutral episodic memories across different sleep stages, namely slow-wave sleep (SWS) and REM sleep (REM). In a between-subjects design, participants learnt picture-location associations (with the pictures presented together with a specific sound) for either neutral or emotional stimuli. The learning and immediate retrieval sessions were followed by a nap and two delayed recalls of the learned locations for each picture. During the nap, sounds were played either during REM or SWS, which had been associated with the images during learning. Surprisingly, emotional memory reactivation during REM impaired performance after the nap, whereas the cueing benefit was higher for emotional compared to neutral stimuli during SWS reactivations (E-SWS vs. N-SWS). The cuing benefit during E-SWS correlated with the interaction of SWS and REM sleep. With these main findings, the study aims to shed light on a conflicting body of TMR research with altogether mixed results as to the benefits of REM and SWS on episodic memories.
Although the design was not fully controlled (N-REM group is missing) and the group sizes are rather small, this study provides very valuable insights into the combined relevance of SWS and REM sleep for emotional memory formation during sleep.

Major Comments:

1. Some results are missing in the text. For instance:
a. In table S2 a significant three-way interaction (Reactivation*Group*SWSxREM) is shown but I did not find it mentioned in the results. If the authors wish to include this factor in a model, it should be reported. (Similarly, the other effects in Table S2 that were significant).
b. Section “Correlations with Sleep Measures”: the authors report on the correlation between CB and SWSxREM for the E-SWS group. Were these correlations also computed for the E-REM and N-SWS groups? Please, add these to the text (and perhaps also to the figure) to help the reader put the findings into context. Looking at the three-way interactions in Table S2 it looks like there is a difference in CB between the groups.
c. Figure 4: Was there a correlation between spindle power and cueing benefit in the E-SWS condition? - If it was significant you may add the plot to Figure 4. If not, please at least report it in the text.
2. It is stated in the Methods that participants filled in the Epworth Sleepiness Scale upon arrival at the lab. Did they also do this at T1 and T2? If so, please show the results of the ratings to exclude that performance differences between groups were due to differences in sleepiness. If there were no control measures this should be mentioned as a limitation in the discussion section.
3. In Fig. 4B, there is a correlation between emotional sound valence and theta power, as well as a correlation between theta and CB for the E-SWS group. Is there also a correlation between valence and CB? In this case, the authors could perform a mediation analysis on “valence → theta → CB” to make a stronger point for the role of theta in emotional memory processing.

Minor Comments:

1. Please, provide a more comprehensive reasoning on why you did not include an N-REM group (see “Although a neutral REM group was also planned, this was abandoned due to the limitations in resources.”). Considering that data had already been collected from over 120 participants, recruiting another 20-25 participants seems feasible.
2. Intro, 3rd paragraph, reference: TMR studies on emotional memories have recently been reviewed by Carbone & Diekelmann (2024, npj Sci. Learn) which might be added.
3. Section 2E, last sentence: It is stated that “[...] we focused time-frequency analyses on electrode Cz as this site has previously shown to be sensitive to TMR-related effects.”- Please include a reference.
4. Methods, Design & Procedure: Were sleep stages detected and sounds played during the nap automatically or manually? Please, add this information.
5. Methods, Statistical Analysis: “A one-way MANOVA was used to compare the sleep stage compositions between groups”. Which parameters were included in the “sleep stage composition”?.
6. The fact that the E-REM had significantly more REM than the other groups is interesting. The authors suggest this could be because only subjects with stable REM were included in this group. Could that also be a consequence of TMR? .
7. Given the often modest size of TMR effects (and that many studies report conflicting findings), it would be helpful to add effect sizes to the results sections of the LMM analyses.
8. Correlation analyses: Please, include the correlation coefficient and (corrected) p-values in the figures. It is easier for the reader to see the results already in the figure.
9. Figure 4A: Since you are showing a difference in 12-19 Hz power in the E-SWS compared to N-SWS group, it would be interesting to also see a Time-Frequency map for the N-SWS condition in panel A.
10. The 7th paragraph of the discussion states “ Cueing emotional stimuli during SWS generated EEG responses first in the delta/theta band and then at spindle frequencies (Forcato et al., 2020; Schechtman et al., 2021).” Those studies did not cue emotional memory, and the pattern of a theta increase followed by an increase in spindle frequencies is typically observed in TMR studies reflecting a positive effect of TMR, but has not been linked to emotional memories. Please, rephrase more accurately.
11. Typos: 2nd. paragraph of discussion, a dot is missing: “...declarative memories Our findings…”, and last sentence of the discussion: “SWS” instead of “SW”

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This is my second review of the manuscript. I had only a minor comment on the previous version, which is fully addressed in the revised version.

Reviewer #2

(Remarks to the Author)
The authors have very carefully addressed all of my comments. I think this ms is ready for publication!

12.1.2024

Response to reviews for the manuscript entitled “Both Slow Wave and Rapid Eye Movement Sleep Contribute to Emotional Memory Consolidation ”, submitted to Communications Biology.

We sincerely appreciate the reviewers' thoughtful comments and suggestions. We have thoroughly addressed each point as detailed below. Where corresponding changes have been made in the revised manuscript, we have indicated the specific pages in our responses. Whenever possible, we have also included the modifications directly in this rebuttal letter.

Reviewer #1 (Remarks to the Author):

This is a study investigating targeted memory reactivation in human. The experiments included emotional or neutral memory. Results showed that reactivation of emotional memory during REM increased the percentage of error (detrimental to memory). Emotional memory reactivation during SWS lead to a larger memory benefit than reactivation of neutral memories. The memory benefit was also strongly correlated with the SWS x REM product. The modulation in the slow wave and spindles frequency range was modulated by the emotional charge of memories.

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The reviewer is correct in pointing out that sound characteristics could theoretically be responsible for the differential cue-evoked response between groups. The sound intensity

(volume) was kept equal between groups. However, it was not possible to achieve equal frequencies between stimulus sets (emotional and neutral) because each sound stimulus had its own unique profile that was composed of a range of frequencies, and there were 50 different sounds within each stimulus set. In other words, we did not use a single sound with a single frequency, such as the note "A", to reactivate all emotional or neutral items, but used individual complex sounds (e.g., a cat's meow or a human sneezing) that consisted of a heterogenous frequency profile, for each item. Therefore, it is unlikely that a systematic difference in sound frequencies between stimulus sets can explain the differences in the cue-evoked responses between groups. More importantly, the correlation of both delta/theta and spindle activity with average valence ratings for the replayed stimulus -and specifically with valence but not arousal ratings- further argues against this possibility. In response to the reviewer's comment, we now added the latter point to the Discussion (page 20, line 417 [clean version of the manuscript]):

"The correlation of cue-evoked spindle activity with average valence ratings for the replayed stimulus -and specifically with valence but not arousal ratings- argues against the possibility that the difference in spindle power may be due to any systematic differences in sound characteristics between the stimulus sets."

Reviewer #2 (Remarks to the Author):

This study is the first to systematically investigate the effect for targeted memory reactivation (TMR) of emotional versus neutral episodic memories across different sleep stages, namely slow-wave sleep (SWS) and REM sleep (REM). In a between-subjects design, participants learnt picture-location associations (with the pictures presented together with a specific sound) for either neutral or emotional stimuli. The learning and immediate retrieval sessions were followed by a nap and two delayed recalls of the learned locations for each picture. During the nap, sounds were played either during REM or SWS, which had been associated with the images during learning. Surprisingly, emotional memory reactivation during REM impaired performance after the nap, whereas the cueing benefit was higher for emotional compared to neutral stimuli during SWS reactivations (E-SWS vs. N-SWS). The cuing benefit during E-SWS correlated with the interaction of SWS and REM sleep. With these main findings, the study aims to shed light on a conflicting body of TMR research with altogether mixed results as to the benefits of REM and SWS on episodic memories.
Although the design was not fully controlled (N-REM group is missing) and the group sizes are rather small, this study provides very valuable insights into the combined relevance of SWS and REM sleep for emotional memory formation during sleep.

Major Comments:

1. Some results are missing in the text. For instance:
a. In table S2 a significant three-way interaction (Reactivation*Group*SWSxREM) is shown but I did not find it mentioned in the results. If the authors wish to include this factor in a model, it should be reported. (Similarly, the other effects in Table S2 that were significant).

We thank the reviewer for pointing out this oversight. Significant three-way interactions are now reported in the Results>Memory Performance> Comparison of TMR Effects on Memory Between Groups (page 12, line 245 [clean version of the manuscript]):

"In the analysis that included E-SWS vs. E-REM, there was no 'Group × Reactivation' interaction (β [SE] = .16 [.14], t(45)=1.12, p=.27), but a significant 'Reactivation × Group × SWS×REM product' interaction **(supplementary Table S2)**. On the other hand, in the analysis that included E-SWS with N-SWS, 'Group × Reactivation' interaction was significant (in addition to a significant three-way interaction), with reactivation of emotional items showing a larger memory

benefit compared to neutral items (β [SE] = .39[.12], t(49)=3.36, p=.002) **(Figure 2.B, supplementary Table S2)**."

We also further clarified our statistical approach in Methods>Statistical Analyses (page 10, line 204 [clean version of the manuscript] :

"Our primary interest was in the two-way interaction between 'Group' and 'Reactivation'; however, we used full-factorial models, which also included the three-way interaction ('Reactivation × Group × SWS×REM product') for completeness."

b. Section "Correlations with Sleep Measures": the authors report on the correlation between CB and SWSxREM for the E-SWS group. Were these correlations also computed for the E-REM and N-SWS groups? Please, add these to the text (and perhaps also to the figure) to help the reader put the findings into context. Looking at the three-way interactions in Table S2 it looks like there is a difference in CB between the groups.

These correlations were computed for E-REM and N-SWS groups too; however, they were not significant. We now added this information to the relevant section (Results>Correlations with Sleep Measures; page 13, line 265 [clean version of the manuscript]):

"No such significant correlation was found in E-REM (r=-.04, p=.87) or N-SWS (r=-.29, p=.14)."

c. Figure 4: Was there a correlation between spindle power and cueing benefit in the E-SWS condition? - If it was significant you may add the plot to Figure 4. If not, please at least report it in the text.

There was no significant correlation between spindle power and cueing benefit in any of the groups. This information is now added to the relevant section (Results>Cue-evoked EEG activity; page 14, line 300 [clean version of the manuscript]):

"Spindle power was not significantly correlated with CB in any of the groups."

2. It is stated in the Methods that participants filled in the Epworth Sleepiness Scale upon arrival at the lab. Did they also do this at T1 and T2? If so, please show the results of the ratings to exclude that performance differences between groups were due to differences in sleepiness. If there were no control measures this should be mentioned as a limitation in the discussion section.

Epworth sleepiness scale, to evaluate the sleep propensity in certain situations, was filled out by the participants only once. However, we evaluated participants' alertness using the Stanford Sleepiness Scale (SSS) at baseline, T1, T2, and T3. Kruskal-Wallis test indicated that there was no difference in SSS scores between groups at any time points (baseline: $\chi^2(4) = 1.34$, $p = .86$; T1: $\chi^2(4) = 1.34$, $p = .86$; T2: $\chi^2(4) = 2.60$, $p = .63$; T3: $\chi^2(4) = 1.80$, $p = .77$). The medians of SSS and test statistics are now included in supplementary materials (Table S1).

3. In Fig. 4B, there is a correlation between emotional sound valence and theta power, as well as a correlation between theta and CB for the E-SWS group. Is there also a correlation between valence and CB? In this case, the authors could perform a mediation analysis on "valence → theta → CB" to make a stronger point for the role of theta in emotional memory processing.

We thank the reviewer for this suggestion. However, there was no significant correlation between the valence of replayed sounds and CB in E-SWS (r=0.182, p=0.395).

We did not include this information in the manuscript, but we would be happy to do so if the reviewer recommends that we do.

Minor Comments:

1. Please, provide a more comprehensive reasoning on why you did not include an N-REM group (see "Although a neutral REM group was also planned, this was abandoned due to the limitations in resources."). Considering that data had already been collected from over 120 participants, recruiting another 20-25 participants seems feasible.

Our laboratory operations were anticipated to stop because of the PI's (Dr. Stickgold) upcoming retirement. Given this time constraint, we had to limit the number of groups that were included in the study. We now changed this sentence to (line 101 [clean version of the manuscript]):

"Although a neutral REM group was also planned, this was abandoned due to the time constraint imposed by an upcoming halt in laboratory operations."

2. Intro, 3rd paragraph, reference: TMR studies on emotional memories have recently been reviewed by Carbone & Diekelmann (2024, npj Sci. Learn) which might be added.

This paper is now cited in the Introduction, paragraph 3 (line 71), as the reviewer suggested.

3. Section 2E, last sentence: It is stated that "[…] we focused time-frequency analyses on electrode Cz as this site has previously shown to be sensitive to TMR-related effects."- Please include a reference.

We have added two recent citations that focused on Cz for their time-frequency analysis of TMR-related effects. We now write (line 182 [clean version of the manuscript]):

"To reduce EEG data complexity, we focused time-frequency analyses on electrode Cz as this site has previously shown to be sensitive to TMR-related effects (Denis & Payne, 2024; Schechtman et al, 2023)."

Denis, D., & Payne, J.D. (2024). Targeted memory reactivation during nonrapid eye movement sleep enhances neutral, but not negative, components of memory. *eNeuro*. 11 (5).

Schechtman, E., Heilberg, J., and Paller, K.A. (2023). Memory consolidation during sleep involves context reinstatement in humans. *Cell Reports*. 42. 112331.

4. Methods, Design & Procedure: Were sleep stages detected and sounds played during the nap automatically or manually? Please, add this information.

Cue presentations were started manually. This information is now added in Methods>Design and Procedures, page 6, line 129.

5. Methods, Statistical Analysis: "A one-way MANOVA was used to compare the sleep stage compositions between groups". Which parameters were included in the "sleep stage composition"?

The sleep measures that were compared between groups are now explicitly stated (Methods>Statistical Analyses; page 10, line 209 [clean version of the manuscript]):

"A one-way MANOVA was used to compare the sleep variables (total sleep time [TST], wake after sleep onset [WASO], %N1, %N2, %SWS, %REM and SWS×REM product) between the reactivation groups, with Tukey's test for post-hoc analyses."

6. The fact that the E-REM had significantly more REM than the other groups is interesting. The authors suggest this could be because only subjects with stable REM were included in this group. Could that also be a consequence of TMR?

The reviewer is correct in pointing out that TMR during REM may also have induced an adaptive increase in REM to process the emotional stimuli. This point is now added to the relevant section (Results>Baseline Memory, Sleep Architecture, and Recall in Nap and Wake Groups; page 14, line 279 [clean version of the manuscript]):

"It is also possible that the reactivation of emotional memories during REM may have further increased the time spent in this sleep stage (Kim and Dimsdale, 2007; Suchecki et al., 2012)."

7. Given the often modest size of TMR effects (and that many studies report conflicting findings), it would be helpful to add effect sizes to the results sections of the LMM analyses.

We have now added the effect sizes (Cohen's d) for the TMR effect in each group in supplementary table S2 (previously table S1).

8. Correlation analyses: Please, include the correlation coefficient and (corrected) p-values in the figures. It is easier for the reader to see the results already in the figure.

We now included the correlation coefficients and p values in all the figures, as the reviewer suggested.

9. Figure 4A: Since you are showing a difference in 12-19 Hz power in the E-SWS compared to N-SWS group, it would be interesting to also see a Time-Frequency map for the N-SWS condition in panel A.

We now moved the time-frequency map for N-SWS to Figure 4A, as the reviewer suggested, and modified the figure legend accordingly.

10. The 7th paragraph of the discussion states " Cueing emotional stimuli during SWS generated EEG responses first in the delta/theta band and then at spindle frequencies (Forcato et al., 2020; Schechtman et al., 2021)." Those studies did not cue emotional memory, and the pattern of a theta increase followed by an increase in spindle frequencies is typically observed in TMR studies reflecting a positive effect of TMR, but has not been linked to emotional memories. Please, rephrase more accurately.

That sentence aimed to simply summarize the observed cue-evoked response, to be followed by the discussion of the association of activity in each band with cueing benefit and the stimulus valence. We now removed the references, to avoid any misunderstanding that the references were related to emotional cueing.

11. Typos: 2nd. paragraph of discussion, a dot is missing: "…declarative memories Our findings…", and last sentence of the discussion: "SWS" instead of "SW".

We have now corrected these typos.