A complementary contribution of REM sleep after targeted memory reactivation during NREM sleep for memory consolidation

Rebeca Sifuentes Ortega[1], Péter Simor[1,2,3], Philippe Peigneux[1]

[1] UR2NF, Neuropsychology and Functional Neuroimaging Research Unit at CRCN affiliated at Center for Research in Cognition and Neurosciences and UNI – ULB Neurosciences Institute, Université Libre de Bruxelles (ULB), Belgium.

[2] Institute of Psychology, ELTE, Eötvös Loránd University, Budapest, Hungary.

[3]Institute of Behavioural Sciences, Semmelweis University, Budapest, Hungary.

Abstract

Presentation of learning-related cues during NREM sleep has been shown to improve memory consolidation. Past studies suggest that REM sleep may contribute to the beneficial effect of reactivating memories during NREM sleep, but the relationship between REM sleep and induced reactivations in NREM remains unclear. We investigated whether a naturally ensuing REM sleep episode is necessary for prior NREM targeted memory reactivation (TMR) to exert a beneficial effect on memory consolidation. Results disclosed better name recognition for words reactivated during NREM TMR followed by REM sleep, as compared to NREM-TMR without subsequent REM sleep. Sleep spindle activity increased only when NREM TMR preceded REM sleep, and associations with subsequent REM theta activity and memory benefits were contingent upon the level of relatedness with prior knowledge for the learned material. Our findings suggest a complex interplay between NREM and REM sleep subtending memory reactivation and consolidation processes.

Introduction

Targeted memory reactivation (TMR) in sleep, i.e., the external reactivation of memory traces through the presentation of learning-related cues, has proven useful to understand post-learning memory consolidation processes taking place during sleep. A growing body of evidence has shown that TMR, especially when applied during non-rapid eye movement sleep (NREM), can result in improved performance in various memory domains (Hu et al. 2020). It was proposed that this improvement is facilitated by the coordination of oscillatory phenomena characterising NREM sleep, namely the hippocampal sharp-wave ripples (SWR) associated to memory reactivations (Buzsáki 2015), nested within thalamic spindles (Siapas and Wilson 1998; Staresina et al. 2015) that in turn are coupled within the up-states of neocortical slow oscillations (Sirota et al. 2003; Latchoumane et al. 2017). This integrated mechanism would subtend the consolidation of hippocampus-dependent memories and their integration into neocortical long-term stores (Buzsáki 1996; Diekelmann and Born 2010; Klinzing et al. 2019).

Recently acquired memories undergo continuous transformations throughout post-encoding sleep, characterized by alternating NREM-REM cycles. Several accounts assign complementary roles to these sleep stages in the consolidation and transformation of memories (Giuditta et al. 1995; Ambrosini and Giuditta 2001; Diekelmann and Born 2010). In the framework of the active systems consolidation model, recent memories are first reorganized by coordinated oscillatory activities in NREM slow wave sleep (SWS), then strengthened at the synaptic level during subsequent REM sleep (Diekelmann and Born 2010; Klinzing et al. 2019). Alternatively, the sequential hypothesis account proposes that it is the physiologically ordered NREM-REM sleep sequence that plays a key role for memory consolidation (Giuditta et al. 1995). In SWS, non-adaptive memory traces would be weakened or eliminated, leading to a relative strengthening of the adaptive traces, and then these traces would be consolidated and integrated into long-term stores during a following episode of REM sleep. Evidence supporting the sequential hypothesis in humans showed that ordered NREM-REM sequences are relevant for declarative memory (Ficca et al. 2000) and visual perceptual skill learning (Strauss et al. 2022). Even though these models consider that consolidation processes continue throughout both sleep stages, little is known about the role played by REM sleep after TMR-triggered memory reactivations during prior NREM sleep.

In two novel word learning studies, REM sleep was found to exert an influence on the outcome of prior NREM-TMR. Batterink et al. (2017) found that NREM sleep TMR during a nap did not benefit memory performance for cued words (i.e., pseudowords associated with pictures of known objects at learning) at the group level, at variance with previous studies disclosing an advantage for cued foreign language word pairs (Schreiner and Rasch 2015;

Schreiner et al. 2015). Notwithstanding, Batterink et al. (2017) observed the expected TMR benefit in participants who obtained the greatest amounts of REM sleep, whereas those with little to no REM sleep paradoxically exhibited worse memory performance for cued than uncued associations. Similarly, Tamminen et al. (2017) found no NREM-TMR effect on the retrieval of novel words (e.g., *cathedruke* derived from base word *cathedral*), but found that the change in lexical competition (a measure of lexical integration) was associated with the amount of time spent in REM sleep during the afternoon nap. Words cued during NREM sleep benefited from subsequent REM sleep and exhibited increased lexical competition, whereas such relationship was not observed for uncued words. These results suggest that REM sleep following the reactivation of new memories may be particularly beneficial when the learned associations require further integration within pre-existing knowledge networks, as is often the case in novel vocabulary learning (Batterink et al. 2017). Conversely, it was suggested that NREM reactivation of novel memories highly linked to well established knowledge networks or schemas may lead to their destabilization in the absence of subsequent REM sleep, possibly due to the interference from older memories (Batterink et al. 2017). Hence, REM sleep after NREM TMR might play a key role in modulating its effects for novel word learning because vocabulary learning requires integration with previous knowledge (Tamminen et al. 2017), as opposed to simpler associations that could still benefit from TMR in the absence of REM sleep (Hu et al. 2020). Another line of evidence has shown that offline periods filled with sleep facilitate novel word integration (Gais et al. 2006; Dumay and Gaskell 2007; Tamminen et al. 2010; Schimke et al. 2021). In particular, sleep spindles and SWS have been associated with overnight improvements in novel word recognition and lexical competition (Tamminen et al. 2010). Moreover, NREM-TMR studies showing a benefit in the recall of foreign language word pairs (Schreiner and Rasch 2015; Schreiner et al. 2015) or pseudoword-picture associations (Groch et al. 2017) also featured increased theta and spindle activity after the onset of the learning-related cues. It is worth noticing that in these studies, TMR was performed during the first half of the night, allowing participants to spend at least some time in REM sleep (Schreiner and Rasch 2015; Schreiner et al. 2015; Groch et al. 2017). Still, it remains to be systematically tested whether a period of REM sleep *after* NREM-TMR is relevant for strengthening and integrating new memories depending on their level of relatedness with prior knowledge.

As stated above, acquisition of information that fits with pre-existing schematic representations is thought to benefit from sleep-related consolidation processes to a greater extent than unfamiliar, or schema non-conformant information (Durrant et al. 2015; Hennies et al. 2016). In line with these findings, Groch et al. (2017) found that NREM-TMR of pseudowords during nocturnal sleep led to a selective memory recall improvement for cued

pseudowords previously associated with pictures of familiar objects, as opposed to pseudowords paired with unfamiliar objects. Furthermore, presentation of prior known cues elicited higher oscillatory activity responses in the spindle and theta bands. Increased spindle activity in turn predicted a cueing benefit for prior known associations only, supporting the notion that prior knowledge is an essential element for the beneficial effects of TMR to unfold (Groch et al. 2017). Thus, the processing trajectory of sleep-reactivated memories may depend on the nature of the learned material, and continue throughout subsequent sleep states (for a challenging view, see Ashton et al. 2022; Cordi et al. 2023).

In this context, we aimed at investigating whether NREM-cued memories benefit from a subsequent episode of REM sleep, and if this potential benefit is contingent upon prior knowledge. To this end, we performed a within-subject study in which TMR took place either before or after a final episode of REM sleep, controlling for a similar sleep duration. In line with the Sequential Hypothesis (Giuditta et al. 1995; Ambrosini and Giuditta 2001) and studies showing an influence of REM sleep on the beneficial effects of NREM-TMR for highly integrative stimuli such as novel words (Batterink et al. 2017; Tamminen et al. 2017), we hypothesized that memory performance would benefit from NREM cueing only when followed by a period of REM sleep (Pre-REM TMR condition). In particular, we predicted that cued associations that involve prior knowledge would benefit to a greater extent from TMR, given their consistent links with pre-existing knowledge networks, and from subsequent REM sleep proposed to integrate previously reactivated memories (Batterink et al. 2017; Tamminen et al. 2017). On the other hand, we hypothesized that cueing memories during NREM sleep without a subsequent period of REM sleep (Post-REM TMR condition) may lead to the destabilization of the memory traces, and consequently a decrease in memory performance for cued associations. This decrease would be especially prominent for associations involving less connections with prior knowledge, since they would benefit less from the reactivation processes promoting integration into pre-existing networks. Additionally, we investigated the oscillatory activity related to cue presentation in both TMR conditions, and the potential role of REM theta activity following a period of NREM TMR. We focused on REM sleep theta activity given its role in memory consolidation processes (Boyce et al. 2016, 2017) and its observed role in modulating hippocampal SWR activity, likely associated to memory reactivations, over the course of NREM-REM cycles (Grosmark et al. 2012).

Materials and Methods

Participants

Twenty-two healthy participants (10 males, mean age 24.6 ± 3.5 years, range 20-33) gave written informed consent to take part in this study approved by the ULB-Erasme Hospital Ethics Committee (Ref. P2019/332). They were French-native speakers, with no history of neurological or psychiatric disorders, no signs of depressive symptoms (short form of the Beck Depression Inventory BDI-13; Beck and Beck 1972; mean score = 1.2 ± 1.9), free of medication, with reported good sleep quality (Pittsburgh Sleep Quality Index PSQI; Buysse et al., 1989; mean score = 2.6 ± 1.2), and neutral to moderate chronotype (Morningness-Eveningness Questionnaire; Horne and Östberg, 1976; mean score = 56.5 ± 5.2). Three participants were excluded from the analyses due to poor sleep during the experimental nights and REM sleep intrusion in the Post-REM cueing condition (n= 1), dropping out of the study (n=1), or poor performance at the immediate recall test (i.e., scoring 3 times below the median absolute deviation; n = 1). Data are thus analysed on 19 participants (8 males, mean age 24.4 ± 3.6 years). All participants were instructed to refrain from consuming alcohol, taking caffeine or stimulant drinks after noon, or napping on the experimental days. After completion of the experiment, they received a monetary compensation.

Materials

One hundred and fifty 2D pictures of known and unknown objects, adapted from a previous study (Urbain et al. 2013) and pre-existing databases (Kroll and Potter 1984; Rossion and Pourtois 2004), were assigned to three different lists consisting each of 25 prior known and 25 non-prior known objects. Each picture was paired with a pseudoword and a “magical” function or property of the object (Fig. 1). Pseudowords were generated using the Lexique toolbox (New et al. 2001) according to French language rules, and balanced across lists in syllable number (mean syllable length = 2.46, range 1-3) and word duration (mean = 794.9 ± 172.9 ms). Picture definitions were 5 to 13 words long. Pseudowords and definitions were recorded by a female French native speaker. Stimuli were presented using PsychoPy 3 (Peirce et al. 2019).

General procedure

In total, participants took part in 4 experimental sessions, each separated by at least one week (for an overview, see Fig.1). The first session was the habituation night to familiarize participants with the polysomnography (PSG) setup and the sleep lab environment. For the three other sessions, the experimental night started at 20:30 with the setup of the electrodes, followed by a learning and immediate recall phase, then nocturnal sleep in one of the 3

experimental conditions (see below), and delayed recall in the morning. At each experimental night, participants learned a different list of 50 pseudoword-picture associations. List order was counterbalanced across conditions. According to the experimental condition, TMR of previously learned words was performed during the second half of the night during NREM sleep either (a) before the last expected REM episode in the night (Pre-REM TMR) or (b) after the last REM sleep episode (Post-REM TMR), or (c) no TMR was performed (Sleep Control). The order of the experimental conditions was counterbalanced across participants. Thirty minutes after participants were woken up, the delayed recall test was administered.

Pseudoword-picture association learning

In the initial learning phase, participants had to memorize the association between 50 pictures of prior and non-prior known objects, their names consisting of pseudowords, and their functions. Each object picture was displayed on a computer screen for 7 s together with its name and function which were presented auditorily through earphones. After the presentation of each association, a question mark replaced the object picture, and the participant was asked to repeat verbally the name and function of the object. Associations were presented in blocks of five, after which participants were presented again with each of the last five objects and their names (1.8 s) and were asked to recall once again the complete association when the question mark appeared. After completing the learning procedure with all 50 associations, a practice round followed.

For the practice round, each object picture was presented (1.8 s) either with its corresponding name or an incorrect one (targets and lures respectively, presented 50% of the time for each knowledge category). Next, a question mark appeared on the screen and participants had to judge whether the name corresponded or not to the object (i.e., by choosing between “correct” or “incorrect”), and to recall the object function. Participants had to reach 60% retrieval accuracy for full associations (i.e., correctly retrieved name and function). If the criterion was not reached, participants went through a new learning and practice round including only incorrectly remembered associations. Once participants reached the learning criterion, they were administered an immediate recall test as explained below.

Immediate and delayed recall tests

Each of the 50 object pictures was presented with an associated name, and participants had then to judge whether it was “correct” or “incorrect” and recall its function. Immediately after each object presentation, they rated their confidence in their name recognition response on a scale ranging from 1 “not sure at all” to 4 “completely sure” (see Fig. 1).

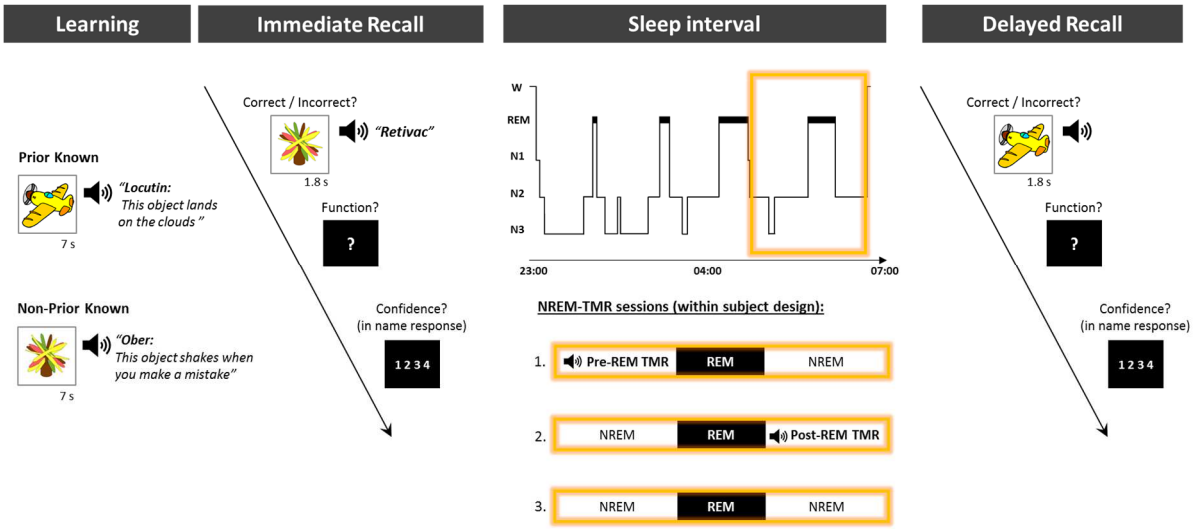

Figure 1. Experimental design. After a habituation night, participants took part in 3 experimental sessions. In each session, three different lists of word-picture associations were presented. During the encoding phase, participants learned 50 new pseudowords associated to a prior known or non-prior known object and their "magical" function. After reaching a 60% learning criterion, immediate recall was tested. In this phase, participants were asked to rate their confidence on the correctness of their name recognition responses. During the sleep interval, sleep signals were monitored and TMR was performed during NREM sleep either before a REM sleep episode (Pre-REM cueing) or after a REM sleep episode (Post-REM cueing), or participants slept undisturbed (Control). After the sleep interval, a delayed recall test took place. REM; rapid-eye movement sleep, NREM; non-rapid eye movement sleep.

Targeted Memory Reactivation

After completion of the immediate recall test, participants went to sleep. In both the Pre-REM and Post-REM TMR conditions, half of the prior known and non-prior known associations fully remembered at the immediate recall test were randomly assigned either to a "cueing" or "no cueing" word subset using an algorithm implemented in Matlab R2017b (https://www.mathworks.com). The cueing subset word list was repeatedly played during the selected NREM sleep episode at ~40 dB via insert earphones. The inter-cue interval was of 9 s. The subset of uncued words served as a reference to compare the effects of TMR on the next day's memory performance at delayed recall. The total number of words selected for cueing (Pre-REM TMR = 17.79 ± 2.8; Post-REM TMR = 17.42 ± 2.3), and single word repetition (Pre-REM TMR = 5.7 ± 2.1; Post-REM TMR = 7.2 ± 3.9) did not differ significantly between TMR conditions (*ps* > 0.17). TMR was performed when stable NREM sleep was observed during online monitoring of the PSG signals, and paused whenever signs of arousal appeared. In the Pre-REM TMR condition, cueing was performed within the NREM period preceding the last REM episode expected in the night, starting approximately during the 4th sleep cycle. After cueing, participants were allowed to transition to the following REM episode

and then awoken at the end of the subsequent NREM episode. In the Post-REM TMR condition, NREM cueing started approximately on the 5th sleep cycle, right after the last REM episode of the night was over. For this condition, participants were awoken towards the end of the cued NREM episode, hence avoiding the transition to a subsequent REM episode.

Polysomnography

Continuous EEG recordings were conducted using an Active-Two Biosemi system (Biosemi, The Netherlands) with active Ag-AgCl electrodes at the 19 scalp locations of the 10-20 international system, as well as left and right mastoids, and standard electrooculography (EOG) and electromyography (EMG) derivations (Berry et al. 2017). Signals were referenced to the Common Mode Sense (CMS) electrode and digitized at 2048 Hz sampling rate. Exported signals were downsampled to 512 Hz and EEG channels were re-referenced to averaged mastoids (M1 and M2). EEG and EOG channels were high-passed filtered at 0.3 Hz and low-passed filtered at 45 Hz, and EMG was high- and low-passed filtered at 10 and 50 Hz respectively. Offline sleep scoring was performed on 30-s epochs according to standard criteria (Berry et al. 2017) by an experienced researcher blind to stimulation onsets.

Statistics and Reproducibility of Behavioural Data

Behavioural data were analysed in JASP (JASP Team 2023). Repeated-measures analysis of variance (ANOVA) were performed as indicated below. Post-hoc t-tests were two-sided. Significance level was set at $p < 0.05$ and effect sizes are reported as partial eta squared or Cohen's d where appropriate.

We first investigated overall changes in memory performance by comparing the number of fully remembered associations, the number of remembered functions, and the recognition accuracy for object names. Fully remembered associations were defined as correctly retrieving both the name and function associated to an object, whereas name recognition accuracy was assessed with the *d'* sensitivity index, calculated as: z(*p*[hit]) - z(*p*[false alarms]) according to signal detection theory (Stanislaw and Todorov 1999). To avoid undefined values of *d'* due to extreme proportions of hits and false alarms, we applied the log-linear rule (Hautus 1995) before calculating response proportions. The log-linear approach consists in adding 0.5 to the total number of hits and false alarms and adding 1 to the number of trials before calculating each proportion of responses. Overall change in memory performance was compared across the three experimental conditions (Pre-REM, Post-REM, and Control), recall sessions (Immediate vs. Delayed), and type of knowledge involved in the learned associations (Prior vs. Non-Prior) by means of repeated-measures ANOVAs.

In a second step we investigated the cueing effects of TMR by focusing on the relative change in memory performance measures within the subset of fully remembered associations at immediate recall. In the TMR conditions, half of the previously remembered words were randomly assigned to a “cueing” condition, while the other half was allocated to an “uncued” subset of words. In this way, performance was matched between cued and uncued conditions prior to sleep, enabling us to investigate whether TMR elicited any change in memory performance after sleep. Changes in memory for full associations, functions, and name recognition were investigated across TMR conditions (Pre-REM vs. Post-REM), cueing (Cued vs. Uncued) and knowledge (Prior vs. Non-prior known) by means of repeated-measures ANOVAs.

Name recognition based on confidence ratings

We further assessed the participants’ ability to discriminate correct from incorrect object names after TMR by computing Receiver Operating Characteristic (ROC) curves for each individual and condition by using the confidence ratings provided after each recognition trial (Weidemann and Kahana 2016). Binary responses (“correct” / “incorrect”) to target and lure trials were classified according to confidence ratings in an 8-bin scale ranging from “correct” responses with the highest level of confidence (4) to “incorrect” responses with the highest level of confidence (4). Hit and false alarm proportions were calculated at each bin. ROC curves were constructed by plotting the cumulative hit rate (i.e., “correct” responses to targets, and “incorrect” responses to lures) against the false alarm rate (i.e., “incorrect” responses to targets and “correct” responses to lures) for each condition. We next calculated the area under the curve (AUC) for each individual ROC curve by using the trapezoidal rule in order to compare name discrimination across TMR, cueing and prior knowledge conditions by means of a repeated-measures ANOVA.

Statistics and Reproducibility of EEG Data

Pre-processing and analysis of EEG data were performed with the Fieldtrip toolbox (Oostenveld et al. 2011). Changes in time-frequency representations of power (TFRs) and Pearson correlations between EEG parameters and the TMR-benefit index were statistically tested with non-parametric cluster-based permutation testing as implemented in Fieldtrip (Maris and Oostenveld 2007). Correlations were computed over six electrode clusters as described below, and the false discovery rate (FDR) correction was applied for multiple comparisons (Benjamini and Hochberg 1995). Differences between correlations were tested using Steiger’s Z-test as implemented in the *cocor* package (Diedenhofen and Musch 2015) in R (R Core Team, 2019). An overall threshold of $p < 0.05$ was used for statistical significance across EEG analyses.

Time-frequency analysis of cue-related responses

To investigate the changes in spectral power over time in response to cue presentation (prior and non-prior known words) during both TMR conditions (Pre-REM and Post-REM), TFRs were computed by using the Fieldtrip 'mtmconvol' function. To this end, pre-processed continuous signals from sleep were segmented into epochs from -3 to 6 s relative to cue onset, that were next sorted according to the type of cue (prior known or non-prior known), and subsequent memory performance (remembered or forgotten at delayed recall). After visual inspection of trials for artefact removal, TFRs were computed by implementing a Fast Fourier Transform analysis on a Hanning window of 2 s duration sliding in increments of 50 ms over trials of -3 to 6 s window length. Frequencies of interest ranged from 0.5-25 Hz, and a relative baseline correction was performed on the TFRs with respect to the pre-stimulus onset period between -2 to -1 s. Next, TFRs from subsequently remembered cued associations were compared across TMR and type of knowledge conditions. Correlations between mean power changes found in the TFR analysis and the TMR benefit index for prior known and non-prior known associations were computed.

The TMR benefit index was calculated as the relative change in memory performance (from immediate to delayed recall) for fully remembered cued associations minus the relative change for uncued ones (e.g., Prior Known TMR benefit = %change cued prior known - %change uncued prior known). We chose to calculate the TMR benefit index on fully remembered associations since this is the outcome upon which cued and uncued word subsets were assigned after immediate recall testing to measure cueing effects.

Spectral power analysis of REM sleep after TMR

We further investigated the relation between theta power (4-8 Hz) in the subsequent REM sleep period and the Pre-REM TMR benefit index for both prior and non-prior known associations. Sleep epochs of the subsequent REM sleep episode in the Pre-REM TMR condition were segmented into consecutive 5-sec epochs with a 50% overlap and tapered with a Hanning window prior to spectral analysis. Power spectra were obtained by means of the Fast Fourier transform at all electrodes and averaged according to the following locations: frontopolar (Fp1, Fp2), frontal (F3, Fz, F4, F7, F8), central (C3, Cz, C4), temporal (T3, T4, T5, T6), parietal (P3, Pz, P4) and occipital (O1, O2). Correlations between REM theta power and the TMR benefit index were calculated over the six electrode clusters.

Results

Sleep parameters

Total sleep time, wake after sleep onset (WASO), and sleep stage duration did not differ significantly between control and TMR Pre- and Post-REM conditions, except for stages 2 and 3 of NREM sleep, where stage 2 duration was longer in both TMR conditions as compared to the control night, whereas stage 3 duration was longer in the control night as compared to both TMR conditions probably due to the TMR intervention lightening NREM sleep (Table 1). Critically, REM duration after NREM-TMR significantly differed between the Pre-REM (mean duration = 30.29 ± 12.9 min) and Post-REM (mean duration = 0.18 ± 0.3 min) TMR conditions ($t_{1,18}$ = 10.09, p < 0.0001, d = 2.34).

Table 1. Sleep parameters

Duration (min)	Control	Pre-REM	Post-REM	*F*	*p*	*Control vs. Pre-REM*	*Control vs. Post-REM*	*Pre-REM vs. Post-REM*
N1	18.2 ± 2.5	14.8 ± 1.3	16.0 ± 2.4	1.06	0.36			
N2	203.6 ± 8.2	232.1 ± 7.1	241.9 ± 7.4	9.23	0.001*	t = -3.08, p < 0.01*	t = -4.14, p < 0.001*	t = 1.06, p = 0.3
N3	119.6 ± 7.5	96.0 ± 6.3	94.7 ± 8.5	12.72	0.0001*	t = 4.24, p < 0.001*	t = 4.48, p < 0.001*	t = -0.24, p = 0.8
REM	94.5 ± 6.4	96.3 ± 6.4	91.6 ± 5.0	0.23	0.79			
WASO	31.16 ± 6.7	19.18 ± 1.2	18.90 ± 2.5	2.8	0.1			
TST	435.97 ± 14.9	443.16 ± 9.5	444.37 ± 8.2	0.27	0.77			

Sleep architecture parameters (mean ± SEM). N1, N2, N3: NREM sleep stages 1, 2 and 3; REM, rapid eye movement sleep; WASO, wake after sleep onset; TST, total sleep time. *P < .05.

Change in overall memory performance

Overall memory performance in the pseudoword-picture association task was compared across conditions (see Supplementary Information Table S1). Comparison of fully remembered associations across the three experimental conditions (Pre-REM, Post-REM, and Control) with factors Recall (Immediate vs. Delayed) and Knowledge (Prior vs, Non-Prior) revealed a significant main effect of knowledge ($F_{1,18}$ = 44.12, p < 0.0001 , η_p^2 = 0.71) with better memory performance for prior known associations (Fig. 2). There were no main effects

of recall ($p = 0.12$, $\eta_p^2 = 0.12$), TMR condition ($p = 0.27$, $\eta_p^2 = 0.07$), nor significant interactions (*ps* > 0.12). A similar pattern of results emerged for overall function recall, with a significant main effect of knowledge ($F_{1,18} = 23.32$, $p = 0.0001$, $\eta_p^2 = 0.56$), and non-significant main effects of recall ($p = 0.25$, $\eta_p^2 = 0.07$), TMR condition ($p = 0.65$, $\eta_p^2 = 0.01$), nor interactions between factors (*ps* > 0.08) (Fig. 2). Overall accuracy in name recognition was again better for prior known as compared to non-prior associations ($F_{1,18} = 22.72$, $p = 0.0002$, $\eta_p^2 = 0.56$), with no main effects of recall ($p = 0.23$, $\eta_p^2 = 0.08$), condition ($p = 0.47$, $\eta_p^2 = 0.04$), nor significant interactions (ps > 0.25; Fig. 2).

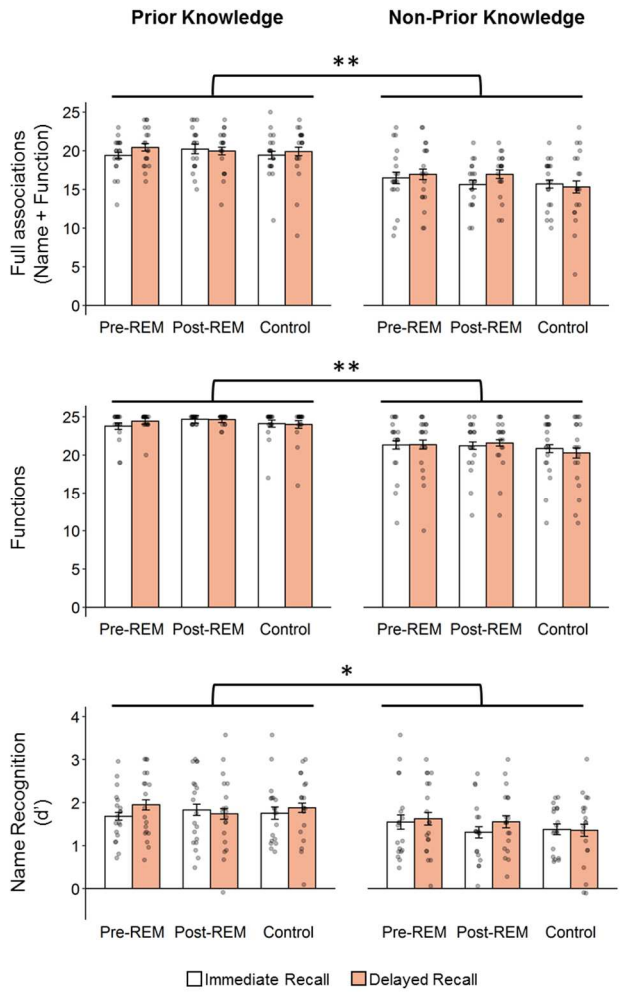

Figure 2. Overall performance. Prior known associations are better remembered than non-prior known associations regardless of session (immediate vs. delayed recall), and condition (Pre-REM TMR, Post-REM TMR or Control), in all types of memory retrieval, i.e., fully remembered associations (top), functions (middle), and name recognition (bottom). Data are shown as mean ± SEM; ** $p < 0.0001$, * $p < 0.001$.

Cueing effects on memory performance

We then compared the relative change in memory performance after TMR conditions (see Supplementary Information Table S2). The change for fully remembered associations at immediate recall, expressed as percentage change, did not differ between Pre-REM and Post-

REM TMR conditions ($p = 0.13$, $\eta_p^2 = 0.12$), between cued and uncued subsets ($p = 0.39$, $\eta_p^2 = 0.04$), nor between prior and non-prior knowledge ($p = 0.13$, $\eta_p^2 = 0.12$). There were also no significant interactions (ps > 0.18) (Fig. 3a). Similarly, change in function recall did not differ significantly between TMR ($p = 0.91$, $\eta_p^2 = 0.001$), cueing ($p = 0.2$, $\eta_p^2 = 0.09$), and knowledge ($p = 0.24$, $\eta_p^2 = 0.08$) conditions, and no significant interactions emerged (all ps > 0.33) (Fig. 3a). In contrast, analysis of cueing effects on the relative change in name recognition accuracy (i.e., d' change = delayed recall d' – immediate recall d') revealed a significant main effect of TMR condition, with better performance in the Pre-REM TMR condition as compared to the Post-REM TMR condition ($F_{1,18} = 4.51$, $p = 0.048$, $\eta_p^2 = 0.2$), regardless of cueing ($p = 0.36$, $\eta_p^2 = 0.05$) or knowledge ($p = 0.49$, $\eta_p^2 = 0.03$). There were no significant interaction effects (ps > 0.28) (Fig. 3b).

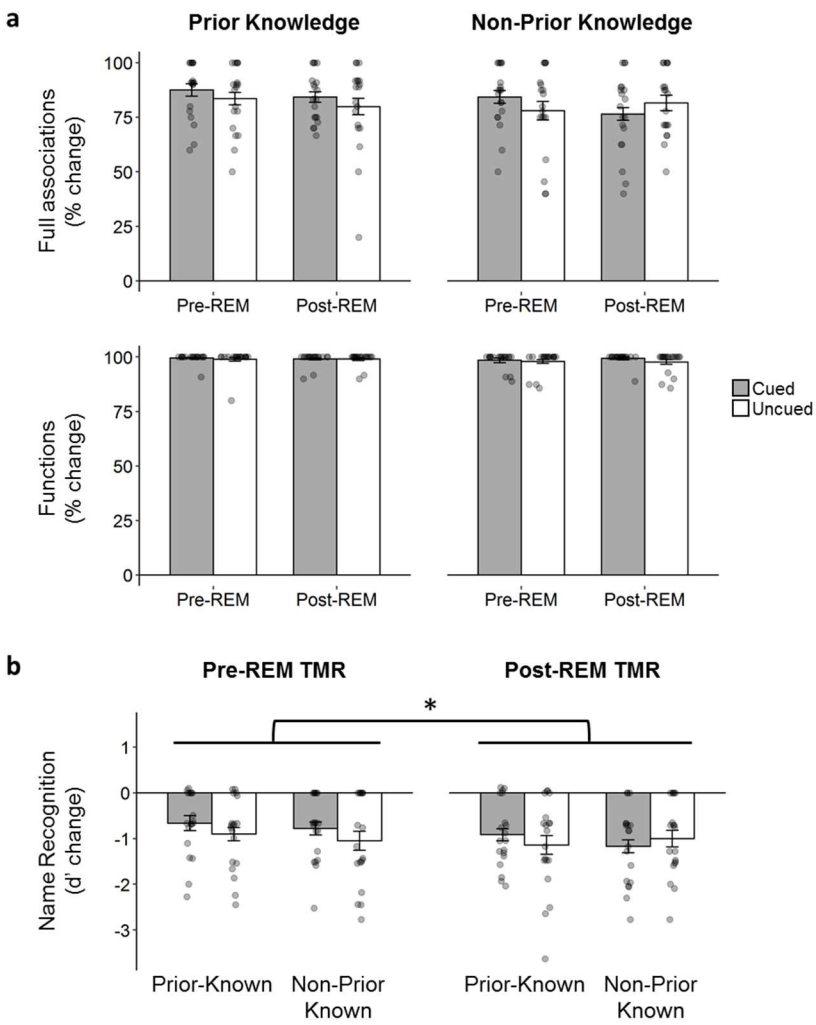

Figure 3. Cueing effects. **a** No significant differences in the relative change in memory performance for full associations and function recall across TMR (Pre-REM vs. Post-REM), cueing (cued vs. uncued words) and knowledge (prior vs. non-prior known). **b** Relative change in name recognition is significantly better in the Pre-REM TMR than in the Post-REM TMR condition, regardless of cueing or type of knowledge. Data are shown as mean ± SEM; * $p < 0.05$.

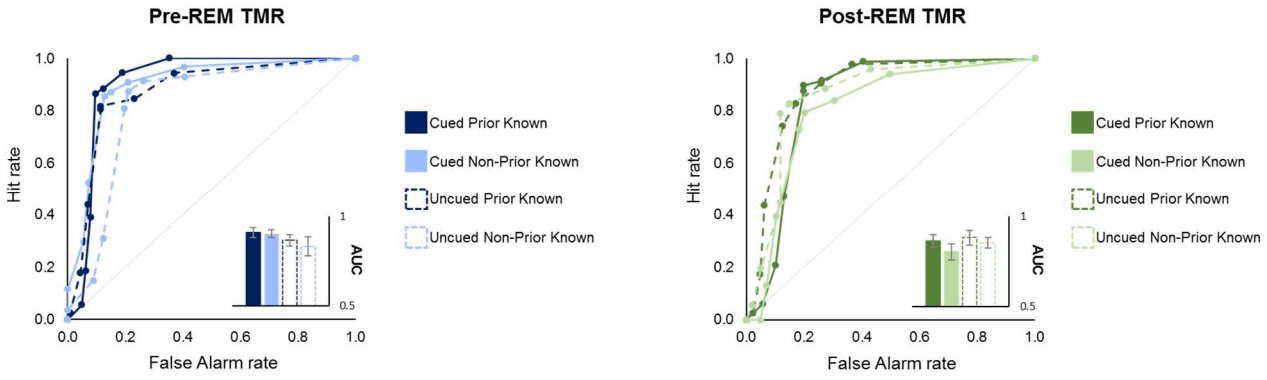

Figure 4. Cueing effects on confidence ratings for name recognition. ROC functions are computed for each cueing (cued, uncued words), and type of knowledge (prior known, non-prior known) condition for both Pre-REM (left) and Post-REM TMR conditions (right). Inset plots show the corresponding ROC AUCs. Data are shown as mean ± SEM.

Cueing effects on name recognition based on confidence ratings

To further understand the effect of TMR on name recognition, individual ROC curves were obtained for each condition at the delayed recall test, and their respective AUCs were calculated and compared. ROC curves averaged across participants per condition are shown (Fig. 4). Comparison of the ROC AUCs revealed no main effects of TMR condition ($p = 0.24$, $\eta_p^2 = 0.08$), cueing ($p = 0.7$, $\eta_p^2 = 0.01$), or knowledge ($p = 0.11$, $\eta_p^2 = 0.14$) on name discrimination. The condition by cueing interaction was significant ($F_{1,18} = 5.39$, $p = 0.03$, $\eta_p^2 = 0.23$), showing better name discrimination for cued associations in the Pre-REM TMR condition as compared to cued associations in the Post-REM TMR condition ($t_{1,18} = 2.3$, $p = 0.03$, $d = 0.53$). However, differences between cued and uncued associations in the Pre-REM TMR ($t_{1,18} = 1.67$, $p = 0.11$, $d = 0.38$) and Post-REM TMR conditions ($t_{1,18} = -0.98$, $p = 0.34$, $d = -0.23$) were not significant (see Figure 5**)**. No further significant interactions between condition, cueing and knowledge factors were found (ps > 0.54).

Time-frequency analysis of cue-related activity

Changes in spectral power in response to cue presentation in Pre- and Post-REM TMR conditions are shown in Fig. 6. TFRs of cue-related activity were compared across TMR conditions and type of knowledge. Comparison of TFRs between Pre-REM and Post-REM TMR conditions revealed a significant increase in spindle power, between 11-16 Hz, lasting approximately from 1.4 – 4.1 sec after cue presentation in the Pre-REM TMR as compared to the Post-REM TMR condition ($p = 0.02$, cluster-based permutation test; Fig. 6). For a detailed characterisation of the cluster over each electrode, see Supplementary Fig. S1. There were no significant differences in relative power changes between prior and non-prior knowledge types of cues (ps > 0.15). Comparison of the mean difference between responses to prior

known versus non-prior known cues in each TMR condition did not reveal significant interaction effects between these factors (ps > 0.4).

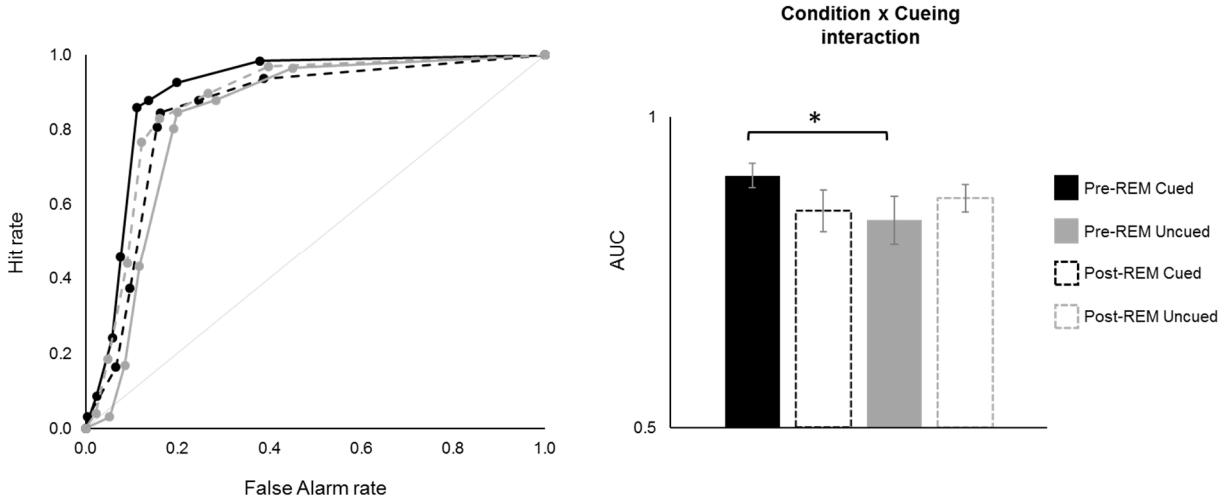

Figure 5. Condition by cueing interaction effects on confidence ratings for name recognition. ROC functions for cueing and TMR conditions were collapsed across types of knowledge (left). The ROC AUCs for cued associations in the Pre-REM TMR condition are significantly larger than ROC AUCs for cued items in the Post-REM TMR condition (right), suggesting an advantage for NREM-TMR cued associations when followed by REM sleep. Data are shown as mean ± SEM; * p < 0.05.

Analysis of spindle activity after cue presentation

To further investigate the cue-related changes in spindle power and the behavioural outcomes of TMR, we analysed the correlation between spindle power (11-16 Hz) in response to cues within the identified cluster time window and the TMR benefit on memory performance for each condition and type of knowledge. We found that spindle power changes over frontopolar (r = 0.35, p = 0.079) and frontal (r = 0.38, p = 0.049) derivations exhibited a trend for a positive correlation with the TMR benefit for prior known associations cued in the Pre-REM TMR condition. A similar pattern of correlational trends emerged for the Post-REM TMR condition where spindle power over frontal (r = 0.36, p = 0.067), central (r = 0.50, p = 0.011) and parietal (r = 0.47, p = 0.021) channels correlated positively with the TMR benefit for prior known associations. However, significant correlations did not survive FDR correction for multiple comparisons. These results are thus reported for the sake of completeness, see Supplementary Information Table S3. No correlations between the change in spindle power after cue presentation and the TMR benefit for non-prior known associations were found in neither of the TMR conditions (ps > 0.24). Comparison between prior and non-prior known correlations did not reveal significant differences in the Pre-REM TMR condition (ps > 0.11) but revealed a significant difference at central channels (Z = 2.06; p = 0.04) in the Post-REM

TMR condition (Fig. 7a). Significant differences between correlations were not revealed in none of the other locations (ps > 0.06; see Supplementary Information Table S3 for correlations).

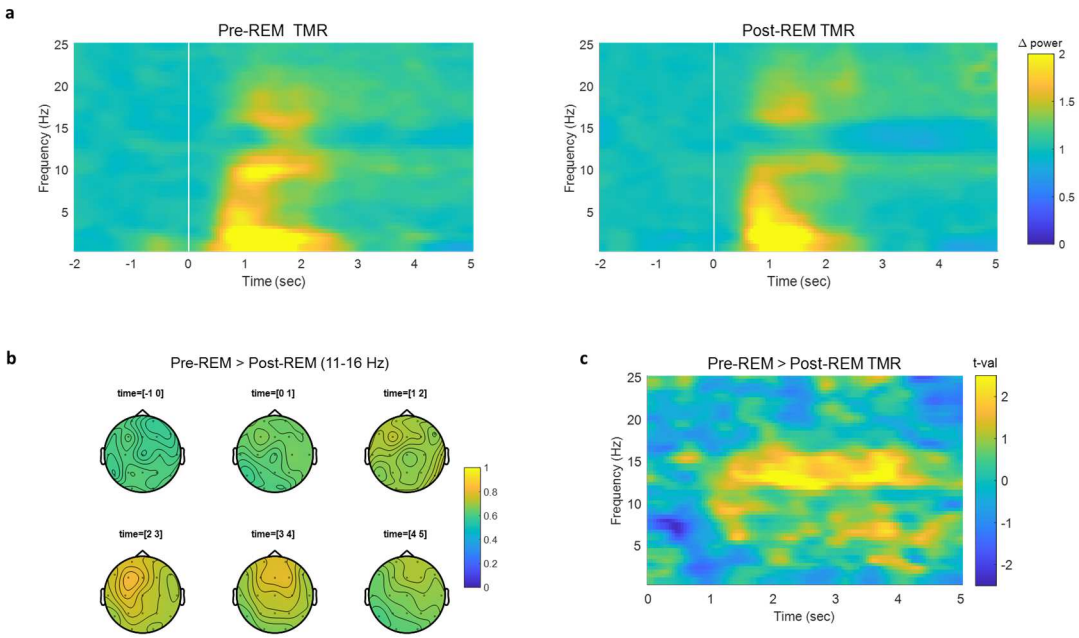

Figure 6. Time-frequency power changes during TMR. **a** EEG power changes after presentation of subsequently remembered cues are shown for Pre-REM and Post-REM TMR conditions (cue onset = 0 s). **b** Topographical distribution of the cluster of activity differentiating Pre-REM versus Post-REM TMR responses to cues within the spindle frequency range (11-16 Hz). **c** Power differences between Pre-REM and Post-REM TMR conditions indicated by T-values. Responses to cues are collapsed across prior and non-prior types of knowledge.

Analysis of REM sleep theta activity after NREM-TMR

Given the role of REM sleep theta activity in memory consolidation processes (Boyce et al. 2016, 2017), we investigated REM sleep theta power following Pre-REM TMR. We ran correlation analyses between REM sleep theta power (4-8 Hz) and the TMR benefit index of both, prior known and non-prior known associations. We did not find significant correlations between theta power and the TMR benefit for prior known associations (Table 2). Interestingly, significant negative correlations emerged between theta power and the TMR benefit index for non-prior known associations over all electrode clusters, with higher correlations (r > - 0.53) over posterior regions (Table 2). Correlations between Pre-REM TMR benefit for prior known associations showed trends towards a negative association with subsequent REM theta power as well. Comparison between non-prior and prior known associations revealed a trend towards significance between correlations in the occipital electrode cluster (Z = 1.89, p-value = 0.057; Fig. 7b). No other correlations differed between other locations (*ps* > 0.16).

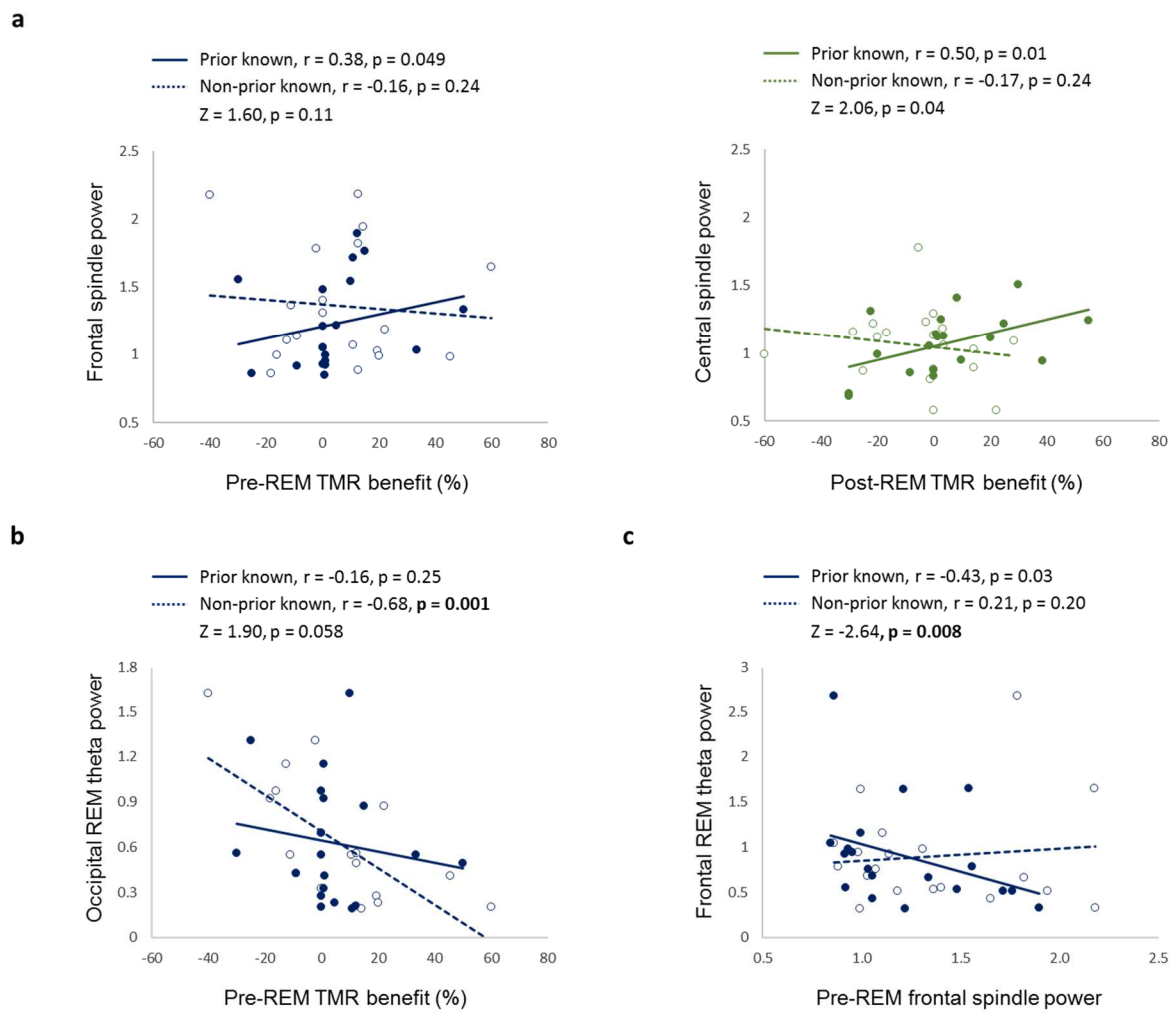

Figure 7. Correlation analyses results showing the relation between cue-evoked spindle activity, subsequent REM theta power and the TMR benefit index across conditions. **a** Positive associations between evoked spindle power and the TMR memory benefit for prior known associations emerged in both Pre-REM and Post-REM TMR conditions. This was significantly different from the non-prior known correlation in the Post-REM TMR condition. **b** The Pre-REM TMR benefit index for non-prior known associations negatively correlated with subsequent REM theta power over posterior regions. **c** Exploratory correlation analysis showed a negative association between spindle power evoked by reactivation of prior known associations and subsequent REM theta power over frontal regions. This correlation significantly differed from the non-prior known reactivation condition. Significant p-values after FDR correction are printed in bold.

Finally, we conducted an exploratory analysis on the association between spindle power (11-16 Hz) in response to both types of cues in the Pre-REM TMR condition, and subsequent REM theta power. There was a significant negative correlation between spindle power changes in response to prior known cues and subsequent REM sleep theta power over frontal electrodes (r = - 0.43; p = 0.03, uncorrected). Frontopolar and central electrode clusters showed a similar pattern of results, but these did not reach significance (ps > 0.05). As for non-prior known evoked spindle activity, no correlations with subsequent REM theta activity were found. Further comparison between correlations revealed significant differences

between prior and non-prior knowledge cueing over frontopolar (Z = 2.55; p = 0.01), and frontal electrodes (Z = 2.64; p = 0.008) (Fig. 7c). No other correlations differed significantly over other electrode locations (*ps* > 0.09; see Supplementary Information Table S4 for correlations).

Table 2. Correlations between Pre-REM TMR benefit index and subsequent REM theta power (4-8 Hz).

	Prior known		Non-prior known		Correlation comparison (Steiger's Z-test)	
	r	p	r	p	Z	p
Frontopolar	-0.37	0.055	-0.48	**0.014**	0.38	0.71
Frontal	-0.38	0.046	-0.49	**0.014**	0.39	0.70
Central	-0.32	0.086	-0.61	**0.003**	1.06	0.29
Temporal	-0.31	0.103	-0.53	**0.007**	0.79	0.43
Parietal	-0.33	0.071	-0.67	**0.001**	1.31	0.19
Occipital	-0.16	0.251	-0.68	**0.001**	1.90	0.06

Significant p-values after FDR correction are printed in bold.

Discussion

The present study aimed at investigating whether a naturally following REM sleep episode is necessary for prior TMR during NREM sleep to exert a beneficial effect on the consolidation of novel word associations. In addition, we investigated whether this potential benefit interacts with the level of integration with prior knowledge that the newly learned associations require. We performed a within-subject study in which TMR was delivered in the second half of the night either during NREM sleep followed by REM sleep, or during NREM after the last episode of REM sleep. Targeted memories included both prior known and non-prior known associations (i.e., pseudowords associated with familiar or novel object pictures). In line with sequential accounts of sleep for memory consolidation (Giuditta et al. 1995; Ambrosini and Giuditta 2001) and TMR studies pointing to a benefit of REM sleep after NREM-TMR (Batterink et al. 2017; Tamminen et al. 2017), we predicted that NREM-TMR would be beneficial for memory performance when followed by a period of REM sleep (Pre-REM TMR) as compared to a period of NREM-TMR not completed by REM sleep. In particular, we hypothesized that cued associations involving a higher degree of prior knowledge would benefit to a greater extent from the Pre-REM TMR condition, due to their higher connections with pre-existing knowledge networks and to the effect of REM sleep in integrating those associations (Walker and Stickgold 2010). On the other hand, we hypothesized that cueing memories during NREM sleep without a following period of REM sleep (Post-REM TMR) may lead to destabilization of memory traces, and consequently a decrease in memory performance for cued associations, particularly in those involving less connections with pre-existing networks.

Our results are only partially in line with these predictions. At the behavioral level, we compared the relative change in memory performance for cued vs uncued associations fully remembered at immediate recall. Contrary to our predictions, there were no differences between TMR (Pre-REM vs. Post-REM), cueing (cued vs. uncued), and knowledge (prior known vs. non-prior known) for full association and function recall. However, accuracy in name recognition was significantly better in the Pre-REM TMR than the Post-REM TMR condition, regardless of cueing or knowledge type. A further analysis conducted on confidence ratings for name recognition highlighted a significant interaction between TMR and cueing factors, indicating larger ROC AUCs for cued words in the Pre-REM TMR condition relative to Post-REM TMR cued words, regardless of knowledge. Still, differences between cued and uncued associations within each of the TMR conditions were not disclosed. These results partially support our hypothesis that REM sleep after NREM TMR cueing may be relevant for the beneficial effects of TMR to unfold. On the other hand, prior knowledge did not seem to benefit to a greater extent from Pre-REM TMR.

At the neural level, comparison of time-frequency representations of power after cue presentation across TMR and knowledge conditions revealed increased spindle activity for later remembered cues in Pre-REM TMR as compared to later remembered cues in the Post-REM TMR condition. In contrast with a previous study (Groch et al. 2017), we found no differences in power changes between prior known and non-prior known associations, nor interactions between TMR condition and type of knowledge. Still, correlation analyses between evoked spindle power and the TMR benefit index for prior known associations disclosed positive correlations in fronto-central and parietal regions in the Pre-REM and Post-REM TMR conditions, respectively. These associations were not observed for non-prior known associations. These results are in line with previous work suggesting that prior knowledge is relevant for TMR to succeed, and that successful cueing of prior knowledge is associated with increased spindle activity (Groch et al. 2017). These results should be considered with caution, and independently confirmed, since significant correlations between spindle activity and the TMR benefit for prior known associations did not survive correction for multiple comparisons.

Finally, we investigated whether subsequent REM theta activity had a potential role in supporting the memory reactivation processes induced during a preceding period of NREM-TMR. We found significant negative correlations between REM sleep theta activity and the TMR benefit index of non-prior known associations from the Pre-REM TMR condition. Trends for a similar pattern of negative correlations were observed for prior known associations. Even though these correlations only marginally differed, the negative correlations between theta power and the TMR benefit index for non-prior associations exhibited higher values over

posterior regions. Furthermore, we observed negative correlations between spindle activity evoked by prior known cues and subsequent REM theta activity over frontal regions, but again these did not survive correction for multiple comparisons. However, correlation comparison between prior and non-prior knowledge conditions revealed significant differences over frontopolar and frontal electrode clusters, suggesting that differences in spindle activity elicited by both types of cues may become relevant in relation to memory processes if REM sleep follows. Taken together, our findings suggest a complex interplay in the processing of reactivated memories within NREM-REM sleep sequences, and a diversity of memory processing trajectories depending on the type of knowledge that has been learned.

Effects of subsequent REM sleep after NREM TMR on novel word recall

When investigating overall performance for learned associations before and after sleep, and across TMR and control conditions, we found an advantage in memory performance for prior known associations regardless of sleep. This is in line with a well-established effect of prior knowledge in facilitating learning of new information consistent with existing schemata (Tse et al. 2007; Van Kesteren et al. 2012). Additionally, the acquisition of familiar or schema conformant information was proposed to further benefit from sleep-mediated consolidation processes (Durrant et al. 2015; Hennies et al. 2016), although recent studies have challenged this view (Ashton et al. 2022; Cordi et al. 2023). Indeed, it was suggested that sleep-mediated benefits may stem from a difference in memory strength at encoding, that can be compensated by giving additional practice rounds for more difficult, unfamiliar associations (Cordi et al. 2023). In our study, we did not find an advantage for prior known over non-prior known associations after the sleep interval. By design, it was expected that during the learning and practice rounds, participants would initially retrieve a higher number of associations from the prior knowledge category, as the 60% performance criterion included both types of associations. In average, participants required at least 2 practice rounds to achieve this criterion, resulting in more repetitions of non-prior known associations (successfully retrieved associations were discarded in subsequent practice rounds). It is possible that weaker encoding of non-prior known associations was compensated through re-exposure and practice, leading to similar effects of overnight sleep for both types of knowledge, but with generally better memory for prior knowledge associations. To better understand the effects of sleep and cueing on memory, future studies should consider setting separate learning criteria for prior and non-prior knowledge material, with additional practice rounds to avoid encoding strength differences.

To investigate the effects of cueing in the Pre- and Post-REM TMR nights, we computed the relative change in memory performance from pre- to post-sleep recall for cued

and uncued associations. The previously observed main effect of knowledge for overall performance was no longer significant, and no further differences between TMR, cueing and knowledge factors were found for neither full association nor function recall. However, name recognition was better in the Pre-REM TMR condition, regardless of cueing and knowledge factors. Further analysis conducted on confidence ratings for name recognition showed that the advantage in the Pre-REM TMR condition was specific to the cued associations regardless of the type of knowledge, as revealed by a significant interaction between TMR and cueing. The advantage for Pre-REM cued associations was only in comparison with cued associations in the Post-REM TMR condition, and no selective advantage of cued versus uncued items emerged in any of the TMR conditions. The lack of a selective enhancement for cued associations is in contrast with previous novel word learning TMR studies, in which memory for cued foreign language word pairs (Schreiner and Rasch 2015; Schreiner et al. 2015) or pseudowords associated with familiar objects (Groch et al. 2017) significantly improved as compared to uncued pairs. Nonetheless, our results partially align with the prediction that reactivated memories continue to be processed in REM sleep. The observed advantage for Pre-REM cued over Post-REM cued associations suggests that reactivating certain memories without further REM sleep may indeed lead to the destabilization of those memories. This pattern of results is similar to two previous studies showing no cueing effects of TMR over an afternoon nap for pseudoword-picture learning (Batterink et al. 2017) and lexical competition (Tamminen et al. 2017), but an influence of REM sleep duration on the effect of TMR. While participants who spent more time in REM sleep exhibited a memory performance benefit for cued words, participants who spent little or no time in REM sleep had worse memory for cued words (Batterink et al. 2017). Such changes in the direction of the TMR effect mediated by REM sleep were not observed for uncued associations. As suggested by Batterink et al. (2017), reactivation of memories such as novel words which are usually linked to pre-existing knowledge networks may lead to memory destabilization in the absence of subsequent REM sleep. This is indeed what we observe in the present study, although memory for Post-REM cued words did not differ between types of knowledge.

One factor that could explain the lack of enhancement between cued and uncued associations in our study is the time of the night that we chose to deliver TMR (i.e., either before or after the last episode of REM sleep in the second half of the night). After learning and immediate recall, participants were allowed to sleep without any stimulation until the last two cycles of the night, targeted because REM episodes are longer and richer in phasic events at that time (Simor et al. 2020). Spontaneous reactivations taking place during the first sleep cycles, richer in SWS, are likely to have triggered the consolidation of the newly learned material, eventually resulting in a less effective impact of TMR performed later in the night.

This interpretation is supported by the advantage we found for Pre-REM over Post-REM TMR and the increased activity in the spindle band in response to cue presentation shown in the TFR analysis, as well as by numerous studies showing cueing effects when TMR is performed during the first episodes of NREM sleep (Rasch et al. 2007; Rudoy et al. 2009; Schreiner and Rasch 2015; Groch et al. 2017) and associated increases in spindle activity after cue presentation (Schreiner et al. 2015; Cairney et al. 2017; Farthouat et al. 2017). We do not discard either possible neuromodulatory or circadian influences on cueing effects. For instance, it has been found that pharmacologically blocking the early morning (4 a.m.) cortisol rise right after reactivating previously consolidated stories boosts memory performance for reactivated memories relative to non-reactivated ones (Antypa et al. 2021). On the other hand, we think unlikely that factors such as arousal, or limited cue repetitions due to lighter sleep may have negatively impacted Post-REM TMR given that cue repetition was similar between TMR nights, and WASO duration, an indirect measure of arousal, did not differ across control and TMR conditions.

Neural responses to NREM TMR, subsequent REM sleep, and their association with TMR effects on prior knowledge

Despite no observed differences in spectral power between prior known and non-prior known TFRs, we found that the TMR benefit index for prior known associations positively correlated with evoked spindle power at fronto-central and parietal regions in Pre- and Post-REM TMR conditions respectively, whereas such pattern of associations was not observed for non-prior known associations in neither of the TMR conditions. These results are in line with previous work showing that increased spindle activity elicited by cueing of prior known associations is predictive of post-sleep TMR benefits for memory performance (Groch et al. 2017). These results are in line with the proposal that spindle activity mediates the integration of novel information into pre-existing knowledge networks (Tamminen et al. 2010, 2013; Hennies et al. 2016).

We also found significant negative associations between REM sleep theta power and the Pre-REM TMR benefit index of non-prior known associations. A similar pattern (trends) for negative associations emerged for the TMR benefit index of prior known associations. These results are surprising given the proposed role of REM theta activity for memory consolidation (Boyce et al. 2016, 2017). Notwithstanding, REM sleep was also found to be implicated in synaptic depotentiation, or forgetting (Poe 2017), with support for this notion coming from studies showing a role of REM sleep in selective pruning of synapses (Li et al. 2017), active forgetting of hippocampus-dependent memories (Izawa et al. 2019) and downscaling of hippocampal and neocortical excitability (Grosmark et al. 2012; Miyawaki and Diba 2016; Watson et al. 2016). In humans, studies found negative associations between REM sleep and

perceptual (Strauss et al. 2022) and motor skills (Rasch et al. 2009). Since TMR benefits for both types of knowledge showed negative associations, yet with stronger correlations for non-prior known cueing, we may speculate that cueing in the Pre-REM TMR condition not only led to differential processing of cues during NREM sleep, as reflected by positive associations between evoked spindle power and prior knowledge cueing benefits, but also to further processing of targeted memories during subsequent REM sleep. While reactivation of prior knowledge associations may have promoted their stabilization and integration into pre-existing networks, reactivation of non-prior known associations involving less overlap with pre-existing networks may have even been weakened (Lewis and Durrant 2011). Furthermore, in a second step, potential downscaling taking place during REM sleep may have been more deleterious for non-prior known cued memories, as reflected by the negative association between REM theta power and non-prior knowledge cueing benefits. The fact that these correlations were stronger in posterior areas is intriguing and deserves further investigation. One possibility is that the observed pattern of correlations was related to the kind of learned associations, as it has been shown that posterior temporal and parietal cortical areas are involved in the integration of visual representations of objects, phonological presentation of novel words, and related semantic information (Takashima et al. 2017).

The hypothesis that NREM TMR and REM sleep act synergistically in the processing of reactivated memories was further supported by an exploratory analysis disclosing a negative correlation between spindle activity evoked by prior known cues at frontal regions and subsequent REM theta activity. These results are in agreement with previous studies showing a complex interplay between sequential NREM and REM sleep, and related oscillatory phenomena for memory processing. In a rare opportunity to directly test the role of ordered NREM-REM sequences in memory consolidation, Strauss et al. (2022) tested patients with narcolepsy (who may often start sleeping directly in REM) on a visual perceptual skills task before and after a one-cycle daytime nap comprising either a REM-NREM or NREM-REM sleep sequence. Even though performance was similar and remained stable after both types of naps, the authors found that spindle duration positively correlated with post-sleep performance only when NREM sleep was followed by REM sleep, and that REM sleep phasic events (rapid eye movements) negatively correlated with skill performance. In another human study using magnetic resonance spectroscopy (Tamaki et al. 2020), the excitatory and inhibitory (E/I) balance (i.e., an index of plasticity) was studied during sleep following training on a visual skills task. The authors found that in visual areas, the increased E/I balance during NREM sleep, observed regardless of pre-sleep training, was associated with post-sleep performance gains, whereas pre-sleep training associated E/I decreases during REM sleep were associated with learning stabilization of the trained skill. Interestingly, and similar to our

pattern of results, they also found that NREM spindle and REM theta power in task-related areas positively correlated with performance gains and stabilization of the skill respectively, whereas REM theta power also negatively correlated with the E/I balance. As for declarative memory tasks, Blaskovich et al. (2017) showed that recall of relevant words in a directed forgetting paradigm was better after an afternoon nap only when participants reached REM sleep. Moreover, spindle activity and subsequent REM duration positively correlated with recall of relevant words, suggesting that besides a sequential role of NREM spindle activity and REM sleep for consolidation processes, memories may be selected and differentially processed depending on their future relevance. Further evidence has shown that the interplay between sequential NREM and REM sleep oscillatory patterns may also impact the homeostatic regulation of neuronal activity. For instance, down-regulation of neocortical and hippocampal firing rates during sleep was observed to decrease over interleaving bouts of REM sleep (Grosmark et al. 2012; Miyawaki and Diba 2016), and this decrease in firing was predicted by spindles and SWRs only when NREM episodes were separated by REM sleep. Moreover, spindle rates from preceding NREM epochs predicted subsequent REM sleep theta power (Miyawaki and Diba 2016).

To sum up, the relation between REM sleep and memory reactivation processes triggered by TMR during prior NREM sleep is complex. Our study suggests that the fate of reactivated memories may depend not only on the sequence of sleep stages but also on the type of associations that are reactivated. The oscillatory hallmarks of NREM and REM sleep reflect a precisely timed coordination of neural circuits (Girardeau and Lopes-Dos-Santos 2021) that may selectively promote memory consolidation and homeostatic processes. How this selection is implemented along sleep stages and how it can be influenced by TMR, requires further investigation.

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