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Rule abstraction is facilitated by auditory cueing in REM sleep

REM sleep and abstract reasoning

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Abstract

Sleep facilitates abstraction, but the exact mechanisms underpinning this are unknown. Here, we aimed to determine whether triggering reactivation in sleep could facilitate this process. We paired abstraction problems with sounds, then replayed these during either slow wave sleep (SWS) or rapid eye movement (REM) sleep to trigger memory reactivation in 27 human participants (19 female). This revealed performance improvements on abstraction problems which were cued in REM, but not problems cued in SWS. Interestingly, the cue-related improvement was not significant until a follow up retest one week after the manipulation, suggesting that REM may initiate a sequence of plasticity events that requires more time to be implemented. Furthermore, memory-linked trigger sounds evoked distinct neural responses in REM, but not SWS. Overall, our findings suggest that targeted memory reactivation in REM can facilitate visual rule abstraction, although this effect takes time to unfold.

Keywords: sleep, rule abstraction, targeted memory reactivation, REM, SWS, synthetic visual reasoning task, event-related potentials, P300.

Significance Statement

The ability to abstract rules from a corpus of experiences is a building block of human reasoning. Sleep is known to facilitate rule abstraction, but it remains unclear whether we can manipulate this process actively and which stage of sleep is most important. Targeted Memory Reactivation (TMR) is a technique which employs re-exposure to learning-related sensory cues during sleep in order to enhance memory consolidation. Here, we show that TMR, when applied during REM sleep, can facilitate the complex recombining of information needed for rule abstraction. Furthermore, we show that this qualitative REM-related benefit emerges over the course of a week after learning, suggesting that memory integration may require a slower form of plasticity.

Introduction

Abstraction, or the process of formulating generalized ideas or concepts by extracting common qualities from specific examples, is a core component of fluid intelligence (Welling, 2007). Sleep has been suggested to play an active role in rule abstraction (for reviews see (Chatburn et al., 2014; Lerner and Gluck, 2019)). For instance, some experimental paradigms which probe rule abstraction such as statistical learning of tone transition patterns have been shown to benefit from slow wave sleep (SWS) (Durrant et al., 2011, 2013, 2016), whereas others, like the weather prediction task, seem to benefit from rapid eye movement sleep (REM)(Barsky et al., 2015). Rule-learning related neural patterns have even been shown to reactivate in the rat medial prefrontal cortex during SWS (Peyrache et al., 2009). However, the mechanisms supporting abstraction in sleep are unknown. It is unclear if one specific sleep stage is more important, and whether the benefit stems from memory reactivation or other types of processing in sleep.

Targeted memory reactivation (TMR) is a method for explicitly controlling memory reactivation in the sleeping brain (Oudiette and Paller, 2013). In TMR, sounds that have been simultaneously paired with recently learned material during wake are softly re-presented during subsequent sleep to trigger reactivation of the associated memories and boost consolidation. TMR is most commonly applied during non-REM (NREM) sleep, where it is known to strengthen memories (Rasch et al., 2007; Rudoy et al., 2009; Antony et al., 2012), but has also been linked to qualitative changes, such as the emergence of explicit knowledge of formerly implicit memories (Cousins et al., 2014). There is currently a debate in the literature regarding whether or not memories can be reactivated during REM sleep using TMR, with some studies reporting null findings (Rasch et al., 2007; Hu et al., 2020), and others reporting significant effects (Sterpenich et al., 2014; Hutchison et al., 2021; Picard-Deland et al., 2021). The present study aims to address this issue within the realm of rule abstraction, since the question of whether TMR can also boost this skill, in addition to memory consolidation, remains to be answered. It is also unclear whether rule abstraction would benefit most from reactivation in SWS or in REM, given the proposed role of these sleep stages in memory restructuring (Landmann et al., 2015) and generalisation (Lewis and Durrant, 2011; Sterpenich et al., 2014; Pereira and Lewis, 2020). One study did apply SWS TMR to an abstraction task and suggest a benefit, but the lack of a non-cued control makes the results difficult to interpret (Batterink and Paller, 2017). Another study showed no effect of SWS TMR on generalisation (Witkowski et al., 2021), while in a third study, such stimulation appeared to produce a deficit in abstraction (Hennies et al., 2017). Nonetheless, SWS has been linked to positive effects in numerous abstraction-related tasks (see (Lerner and Gluck, 2019) for a review).

In the current report, we address the above questions by using TMR to reactivate rule abstraction problems in SWS and REM, with different problems cued in each stage. We used a visual abstraction task called the Synthetic Visual Reasoning Task (SVRT)(Fleuret et al., 2011) which requires participants to abstract rules that define ‘families’ of abstract visual patterns through trial and error exposure. For example, in the problem depicted in Figure 1, the rule is that each image contains two identical shapes. In training, participants are shown a series of images and asked to categorise them as belonging to the family in question or not. They are given feedback on each correct/incorrect categorisation. Each family of shapes is associated with a consistent reference image. At test, participants have to indicate whether or not a given sample image follows the same rule as the reference image for that particular problem. Because the impacts of TMR can last for up to a week(Hu et al., 2015), and may even amplify across this period(Groch et al., 2017), we re-tested our participants one week after the TMR manipulation.

Materials and Methods

Participants

Healthy young adults (mean age 22 years old, range = 19 – 30 years) were recruited online and through advertisements on the university campus to take part in this study. Participants filled out an online screening form and were excluded if: they had any diagnosed sleep, neurological or psychiatric disorders, were taking psychoactive medication, travelled more than two time zones or engaged in regular shift work in the two months prior to the experiment. Participants reported a regular sleep cycle over a four-week period prior to the experiment and were instructed to abstain from alcohol (24h) and caffeine (12h) prior to each visit to the laboratory, as well as daytime napping. Data from 27 individuals (19 females) were collected and used for behavioural analyses. One participant was excluded from the ERP analyses since, due to technical difficulties, no EEG triggers were recorded during TMR (n = 26). All participants signed informed consent and received monetary compensation for their participation. This study was approved by the ethics committee of the School of Psychology of Cardiff University.

Experimental design

The experiment was conducted according to a *within-subject* design (see Figure 1). Participants arrived in the evening (between 6 and 8pm) and were prepared for polysomnography recordings. Subsequently, participants performed a battery of pre-sleep cognitive testing. First, they performed the Image Familiarisation Task, where they passively saw all the images (either faces or landscapes) used in the SVRT. To ensure engagement, participants were instructed to press the space bar whenever a red dot appeared on the screen. After the Image Familiarisation Task, participants performed the Problem-Image Association Task, where they learned to associate each SVRT problem with a particular image of either a face or a landscape. These images were used to group the SVRT problems into 2 categories (category 1: problems paired with faces, category 2: problems paired with landscapes). Next, participants performed the Synthetic Visual Reasoning Test (Fleuret et al., 2011), where they were required to categorize a series of samples from 16 problems as either in-class (following the rule) or out of class (not following the rule) (see Extended Data Figure 1-1). Each problem was always presented in combination with a specific image from one of the two possible categories (faces or landscapes) and with a 200ms sound. During training, participants learned through feedback and trial-and-error until they were able to correctly categorize the samples to 70% accuracy on each problem. During testing, they did not receive any feedback. The last task before sleep was the Problem-Sound Association Task, where participants were trained to recognize which sound had been paired with which problem, until they reached 100% accuracy. This task was introduced to guarantee that the effectiveness of TMR would not be compromised by a weak association between the sounds and their respective problems.

Next, participants went to sleep while non-obtrusive brown noise was continuously played throughout the night. For targeted memory reactivation, each category (sets 1 and 2 of problems paired with faces or problems paired with landscapes) was assigned to a sleep stage (either SWS or REM). Assignment of categories to the sleep stages was counterbalanced across participants. Within each category, half of the problems were cued during sleep and the other half served as a non-cued control (subsets A and B). Assignment of sets 1A, 1B, 2A and 2B to each sleep stage and cueing condition was counterbalanced across participants (see below which SVRT problems were included in each set). The sounds paired with problems assigned to the cued condition were played at the onset of either SWS or REM, as well as new, control sounds, not previously presented to the participant. Upon awakening (day 1), participants performed the Image Familiarisation task again, were wired down, showered, and then were retested on the SVRT. A week later (day 7) participants returned to the lab and were retested once again on the SVRT. Performance on the SVRT was assessed by the accuracy at each time point, and by the accuracy change (difference across time points).

All tasks were implemented in Matlab R2017b using Psychtoolbox 3 and displayed on a 1920 x 1080-pixel computer monitor.

Tasks

Image familiarisation task

This task consisted of 14 blocks of 8 trials (one per problem) for each one of the two categories (i.e. 8 faces and 8 landscapes, for a total of 16 different images), amounting to 112 image presentations per category (224 in total). A variable inter-trial interval was set between 1 and 2 seconds. Participants were asked to press the space bar whenever a red dot appeared on the screen. The red dot was set to appear randomly once every 8 trials. The task was administered in the evening and again in the morning.

Problem-Image Association task

This task was designed to help participants learn to associate each SVRT problem and its corresponding sound with a particular image (either a face or a landscape). It consists of 2 phases: learning and test. For each participant, the images and sounds were randomly assigned to the SVRT problems. During learning, participants performed 3 blocks of 16 trials (one per problem) where they passively viewed the reference representation of any given SVRT problem on the left-hand side of the screen and the image it was paired with (either a face or a landscape) in the centre of the screen, while the 2 second sound paired with that problem-image dyad was played. Participants were instructed to press the space bar if a red dot appeared on the screen. The red dot appeared randomly once per block. In the test phase, participants saw the reference representation in the centre of the screen and heard the same sound that had been paired with it during learning, but now trimmed to only 200 ms. Next, two images appeared on the screen and the participant had to indicate which one had been paired with that particular problem-sound dyad. The test was repeated until participants reached 75% accuracy.

These two tasks, image familiarisation and problem-image association, were added to the experimental design in order to facilitate use of machine learning classification algorithms to detect replay. We performed extra checks to certify that image category was not influencing the SVRT task, see results.

Synthetic Visual Reasoning Test (SVRT)

The SVRT task requires participants to indicate whether or not a given sample image follows the same rule as the reference image for that particular problem (both sample and reference images were displayed simultaneously). The rule governing each problem had to be discovered through trial and error during training. We measured accuracy as the ability to correctly categorize sample images according to whether they followed, or broke, the rule for that problem (Figure 1). Feedback was given after each trial, informing participants whether or not their categorisation of the sample image was correct. For more examples of sample images and rules, please refer to Extended Data Figure 1-1. Each problem was presented in conjunction with a picture of a face or a landscape, to boost the chances of eliciting classifiable EEG patterns, as has been done for objects and scenes (Cairney et al., 2018), and for animals, tools, faces and buildings (Shanahan et al., 2018). Participants were trained on 16 categorization problems, half of which were subsequently used to test the impact of TMR in SWS (4 were cued in SWS and 4 were used as a control), and the other half (4 cued and 4 control) were used to test the impact of TMR in REM.

The test phase consisted of 5 trials for each problem. Out of a pool of 200 images per problem, (100 following the rule and 100 not following the rule), 5 images were randomly selected for each test (pre-sleep, Day 1 and Day 7).

During both training and test phases, a time limit for each response was set to 6 seconds, after which the next trial would start. After each block (i.e. problem) there was a 15 second rest break. The order of problem presentation was randomized for each participant. Each trial began with the presentation of that problem's reference representation on the left-hand side of the screen, the image it had been paired with (either a face or a landscape) in the centre for the screen, and the 200ms sound that these images were associated with. Then, the image to be categorized was displayed on the right-hand side of the screen. Participants were required to press 1 if the image to be categorized was in class (satisfied the rule) or to press 9 if it was out of class (did not satisfy the rule). Performance on the SVRT was assessed by the change in accuracy overnight (post-sleep day 1 – pre-sleep), across the week (post-sleep day 7 – post-sleep day 1). Performance was not affected by the category of the image paired with each problem (i.e. face or landscape(all t-tests $p > 0.4$, uncorrected)).

Problem-Sound Association task

This task was designed to ensure that participants were able to correctly identify all sound-problem dyads introduced while performing the SVRT before sleep, which could otherwise compromise the effectiveness of TMR. Again, the reference representation was presented in combination with its corresponding face or landscape image. Next, two 200ms long sounds were played and the participant indicated which one had been paired with that problem-image dyad. The test was repeated until participants reached 100 % accuracy.

Stimuli

All sounds were obtained from an online repository (www.freesound.org). Initial sounds (2 seconds long; learning phase of the Sound-Problem Association Task) were trimmed into 200ms long sounds using the software Audacity. A pool of sounds was used for each category (faces/landscapes), from which sounds were randomly selected and assigned to a specific SVRT problem. For faces, generic object sounds were used and for landscapes, generic nature sounds were used, such as a bird chirping or the wind blowing. For each category (faces or landscapes) a group of 12 similar but easily distinguishable sounds was selected and from this pool, 8 sounds were randomly paired with an image and used in the SVRT task while the remaining 4 sounds were used as controls during TMR. Sounds for faces and landscapes were matched in duration, and all were played at the same volume within each participant.

The images of faces were obtained from the Karolinska Directed Emotional Faces (KDEF) (Lundqvist et al., 1998). Only faces of females with a neutral facial expression at a straight angle were chosen. The images of landscapes were obtained from an online repository (www.freeimages.com). All images were edited into grayscale and resized (faces: 325 x 435 pixels; landscapes: 435 x 325 pixels) using the software GIMP.

TMR protocol

Audio cues were embedded in brown noise in order to decrease the likelihood that the TMR sounds would elicit an arousal. Brown noise was played throughout the entire night while the cues were only presented when SWS or REM was identified online by the experimenter. Both stimuli (audio cues and brown noise) were played through loud speakers placed behind the participant's bed. The sound volume was manually adjusted for each participant before sleep according to their comfort level. Each cue (either experimental, e.g. paired with a learned rule or control, with no rule associated) was played twice in a row before the next cue was played. All cues were played 4 seconds apart from each other. One loop of cueing consisted of all 8 cues (4 control and 4 experimental) played twice (16 sound presentations). The order of cue presentation was randomized at each iteration of the loop. A total of 14 loops was played in each sleep stage (corresponding to approximately 15 min of cueing), adding up to 28 repetitions of each individual sound and 112 cueing events in each condition (control or experimental). Even though SWS usually occupies a larger proportion of the night than REM (and would thus allow for an extended cueing time), we wanted to ensure that we would be able to deliver the same amount of cueing in both sleep stages, and therefore we opted for limiting cueing to ~ 15 min. Cueing was initiated in the first episode of SWS and REM and was interrupted whenever an arousal or sleep stage transition was identified. In one participant, only 7 out of the 14 loops of REM cueing were completed, due to short sleep duration (n = 1) and in another participant only 8 out of the 14 loops of SWS cueing were completed, due to light sleep throughout the night (n = 1). These participants were not excluded from any analyses. Note that cueing varied between participants, depending on whether or not they obtained ~15 min of uninterrupted SWS and REM, such that for some cueing was finished within the first NREM-REM cycle while for others additional cycles were needed. No significant correlations were found between number of cues delivered in SWS or REM and subsequent performance (all $p > 0.1$). Following offline sleep scoring, cueing accuracy (calculated as the percentage of cues delivered in the intended sleep stage) was determined: 94.44 % for SWS and 93.72 % for REM. Regarding continuity (i.e. whether or not TMR was completed within on sleep cycle, SWS TMR was continuous for 19 participants out of 26 participants and REM TMR was continuous for 1 out of 26 participants only. This is to be expected, since we initiated REM TMR at the onset of the first REM episode, which tends to be very short and our entire cueing procedure required at least 15 min to complete, if uninterrupted. Given this distribution of the data, it is not possible to estimate if the TMR effect differed depending on whether cueing was continuous or discontinuous.

EEG recordings and sleep analysis

EEG was recorded using BrainVision software during the Image Familiarisation task (in the pre-sleep evening and morning of post sleep day 1) and during sleep. Recordings were made at 500 Hz from 22 scalp locations on the standard 10/20 layout (Fz, F3, F4, FC1, FC2, FC5, FC6, Cz, C3, C4, CP5, CP6, Pz, P3, P4, P7, P8, PO3, PO4, Oz, O1 and O2), referenced to the mastoids. Impedances were kept below 5 kΩ. Electrooculogram (EOG) and electromyogram (EMG) signals were also recorded from electrodes next to each eye and 2 electrodes on the chin, respectively. Sleep scoring was accomplished using the guidelines from the American Association of Sleep Medicine (AASM, v. 2.5), within a custom-made script implemented in Matlab. Offline scoring was performed by two independent raters, blind to when cueing occurred, achieving an 88% agreement rate. Discrepancies were resolved by one of the raters.

Spindles and slow oscillations were detected from all channels using the SpiSOP toolbox version 2.3.8.3 (available at https://www.spisop.org/), with the spindle detection algorithm based on (Molle et al., 2002). Centre frequencies of fast and slow spindles were visually determined for each participant and used to define the finite impulse response (FIR) filter (center frequency 13.29Hz (std: 0.69)). The root mean square (RMS) of the filtered signal was computed using a 0.2s time window and smoothed by a moving average of another 0.2s window. Any event that surpassed the 1.5 SD of the RMS signal was considered a candidate spindle. To fit the spindle detection criteria, the candidate events had to last between 0.5s and 3s. Because we had no *apriori* hypothesis about specific channels, all correlations were made with the average across channels.

Similarly slow oscillation detection is based on (Mölle et al. 2002) but also see (Ngo et al. 2013). Prior to the actual detection, the signal is high pass filtered (IIR by default) then low pass filtered (FIR) to contain frequency components observed in slow oscillations in a specified band (0.3 to 3.5 Hz). Then all the time intervals with consecutive positive-to-negative zero crossings are marked. Only intervals with durations corresponding to a minimum (set to 0.5Hz) and maximum (set to 1.11Hz) slow oscillation frequency are considered as putative slow oscillations. The threshold for negative peaks is set to 1.25 and for negative to positive peaks amplitude was also set to 1.25 (default parameters).

EEG pre-processing

First, the data was high-pass filtered at 0.3 Hz and low-pass filtered at 35 Hz. Then, the continuous EEG was epoched into trials from 1 s before to 3 s after sound cue onset (since the cues were 4 s apart). Noisy channels were repaired by interpolating data from neighbouring electrodes and trials containing arousals or movement artefacts (as determined during sleep scoring) were removed. Finally, any remaining noisy trials were manually removed following visual inspection. The number of trials included in the final analysis for each participant, sleep stage and condition are presented in Extended Data Figure 2-3.

Baseline correction was performed on the single trial level using the entire trial length [-1 3] (Grandchamp and Delorme, 2011). Trials were then separated into conditions (control and experimental) and sleep stages (SWS and REM). One participant was excluded from all analyses, since they did not have EEG triggers during TMR (final n = 26).

EEG analysis

Event-related potentials (ERPs) analyses were carried out in Fieldtrip (Oostenveld et al., 2011) (available at: http://www.fieldtriptoolbox.org/). ERPs were calculated for each condition and sleep stage, and compared within subjects and between conditions, across all channels, within a time window from 0 to 2000ms (not averaged).

ERPs of control and experimental sounds were compared using Monte-Carlo cluster permutation tests, corrected for multiple comparisons (Maris and Oostenveld, 2007). The cluster alpha was set to 0.05 and 150000 randomizations were carried out for every test. Clusters were considered significant at $p < 0.025$ (two tailed). Similar parameters were set-up for time-frequency analysis for each frequency band of interest: theta (4 to 8Hz), spindles (9 to 15Hz) and low-beta (12.5 to 16Hz). More specifically, the time-frequency cluster permutation analysis was calculated using the average across trials for each participant in the window of interest (0 to 2s). The statistical analysis was performed for experimental vs control sounds in SWS, REM and also for their interaction (SWS difference vs REM difference, where difference was calculated as experimental minus control sounds) for each frequency band. The minimum number of channels to form a cluster was set to 2, the number of randomisations set to 250000 and the cluster alpha at $p=0.025$ (two-tailed).

To determine whether stimulation lead to a change in spindles or slow oscillations, we calculated the number and duration of spindles and slow oscillations per condition (experimental and control sounds). We then compared these between conditions using a cluster permutation analysis. The cluster alpha was set to 0.05 and 250000 randomizations were carried out for every test. Clusters were considered significant at $p < 0.025$ (two tailed).

Finally, we sought to detect memory reactivation after our TMR cues using an EEG classifier. Thus, ERP values were used as features to feed a linear Support Vector Machine (SVM). To avoid overfitting, we used 5-fold validation repeated twice. As a performance metric we used the traditional accuracy but also area under the curve. The classification was performed separately for SWS and REM stages for each participant. Statistics were performed at a group level to check if for any above-chance time-cluster. No significant cluster was found for either of the performance metrics or for either sleep stage.

Statistical analyses

Performance change on the SVRT was compared using a repeated measures ANOVA with between-subjects factors sleep stage (SWS/REM), cueing condition (cued/non-cued) and session (overnight/across the week) as repeated factor. We ran an outlier analysis using the ROUT method (Q = 1%) and identified two outliers on the SWS cued group. Upon removal of these outliers, the results remained the same as those in Figure 2A, where no significant differences were found between overall performance change on SWS cued and non-cued problems ($t(1,24) = 1.132$, $p = 0.269$).

Descriptive statistics (mean, standard deviation, standard error of the mean and confidence intervals) are presented in Figure Extended Data Figure 2-4 The combined performance change was compared between non-cued and cued conditions using paired t-tests. Pearson’s correlations were calculated between the combined performance change and the average number of slow oscillations and spindles in frontal, central and parietal derivations.

Data are presented as mean ± SEM and we report eta squared (η^2) and Cohen's *d* as effect size estimates for significant findings.

Statistical analyses of the behavioural data were conducted on JASP 0.10.2.0 while statistical analyses of EEG data were conducted on Matlab R2017b using the Fieldtrip toolbox (version 20190904).

Results

TMR in REM improves rule abstraction

We examined baseline performance (pre-sleep) using an ANOVA with the factors cueing condition (cued/non-cued) and Sleep stage (SWS/REM). No differences or interaction were found (smallest *p*=0.666). Refer to Figure 2.B and Extended Data Figure 2-1a for full statistical details.

To assess the impact of cueing, upon consolidation across a retention interval, we compared SVRT performance change (overnight accuracy change: post-sleep day 1 - pre-sleep; and across a week: post-sleep day 7 - post-sleep day 1) using a repeated measures ANOVA with factors sleep stage (SWS and REM), cueing condition (cued and non-cued), and retention interval (overnight and across a week post-sleep) as repeated measure. This showed a significant sleep stage*cueing condition interaction ($F_{(1,26)} = 6.091$, $p = 0.020$, $\eta^2 = 0.013$), with no other factor or interaction being significant (smallest *p*=0.128, Figure 2A, Extended Data Figure 2-1b). This indicates that cueing had different effects when applied in SWS and REM. To investigate this, we conducted a simple main effects test (sleep stages x cueing), which revealed better performance in the cued condition for REM than SWS ($F_{(1,26)} = 4.463$, $p = 0.044$), with no differences between SWS and REM in the non-cued control condition ($F_{(1,26)} = 0.774$, $p = 0.387$; Figure 2A). This result could suggest that cueing benefited rule abstraction when delivered during REM sleep, but not SWS.

To better understand this pattern of results, and also to gain statistical power, we next analysed each sleep stage separately using a 2-way ANOVA with factors cueing condition (cued and non-cued) and retention interval (overnight and across a week post-sleep). For SVRT problems cued in SWS, there was no effect of cueing, session or interaction between these (smallest p=0.198). For problems cued in REM sleep however, we found a significant cueing effect ($F_{(1,26)} = 7.930$, $p = 0.009$, $\eta^2 = 0.019$), indicating that performance improvements were superior for cued problems, compared to non-cued problems. There was no effect of session or cueing*session interaction (*smallest* $p=0.231$). To further understand the origin of the cueing effect in REM sleep we performed a paired t-test (cued vs non-cued) on accuracy at each session (Pre-sleep, post-sleep day1 and post-sleep day 7), Figure 2B and Extended Data Figure 2-3 for full statistical results. Accuracy was superior for REM cued problems, as compared to non-cued ($t_{(26)} = 3.357$, $p = 0.002$, Cohen's $d = 0.646$) only at Post-sleep day 7.

Overall, these findings suggest that reactivating problems during REM leads to a significant advantage in rule knowledge after seven days and nights.

Event-related potentials in REM differ between control and experimental sounds

To examine neural processing associated with TMR cues, we plotted sound-evoked ERPs for each sleep stage of cueing (SWS and REM) and sound category (control and experimental) at Cz for illustration purposes, see Figure 3. Topographies showing the spatial distribution of significant channels over time are available in the (Figure 4 for all EEG channels). We analysed a large time window (0–2000ms), which includes all known auditory event-related potentials (Winkler et al., n.d.) and has previously been associated with processing auditory stimuli in both NREM and REM sleep (Campbell and Muller-Gass, 2011). To determine whether the response to control and experimental sounds differed in each sleep stage, we performed a cluster analysis on the ERP amplitudes (all channels, not averaged). This revealed a significant difference between experimental (familiar) and control (new) sounds in REM sleep (cluster corrected for multiple comparisons, p=0.048), but not in the SWS (all $p > 0.05$). This negative cluster ranges from 228ms to 400ms. The elicitation of a larger ERP amplitude for new sounds than for familiar sounds demonstrates an ability to detect novelty. Our observation of this response in REM but not SWS is in keeping with prior literature showing greater responsivity in REM compared to SWS (see(Ibáñez et al., 2009) for a review).

To probe the data further, we performed a time-frequency analysis per sleep stage in the same time window (0-2000ms) choosing relevant frequency bands based on previous work on SWS: theta-band (4-8Hz) and spindle band (9-15Hz), and lower beta band (13-16Hz) for REM sleep. Cluster statistics revealed nothing significant for either frequency band or sleep stage (smallest *p*-value 0.052). Full list of results in Extended data Figure 4-1.

Does cueing in each sleep stage interfere with consolidation of cueing in the other?

Because we applied TMR in both SWS and REM (though stimulating different problems in each stage) we were interested to know whether TMR in REM might have obscured or interfered with the effects of TMR in SWS. In the case of direct interference, we might expect a negative correlation between the extent to which participants benefit from REM TMR and the extent to which they benefit from SWS TMR. To test for this, we looked for a relationship between performance on problems cued in SWS and REM in two different ways, using overnight gain and using TMR cueing benefit. Thus, we ran a correlation between overnight performance change (difference between post-sleep and pre-sleep) for problems cued in SWS and overnight performance change for problems cued in REM. This showed no correlation ($r = -0.162$, $p = 0.420$). Next, we calculated the cueing benefit (difference between performance on cued and non-cued problems) for SWS-related problems and REM-related problems at each session and across sessions, to check if TMR-related improvements in REM problems were obtained at the expense of cueing benefit in problems cued in SWS. This showed no significant relationships ($p > 0.05$, uncorrected; Table 2). These results show that the extent of TMR related consolidation in REM doesn't predict any specific deficit in the benefit accrued from equivalent cues in SWS.

There is no relationship between time spent in non-manipulated REM sleep and performance on problems cued in SWS

It could be argued that successive TMR in SWS and REM might have curtailed the amount of non-manipulated REM available to further advance any consolidation processes initiated by TMR in SWS, thus disrupting any potential benefits from this manipulation. We inspected sleep architecture in relation to TMR and found that 25 out of 26 participants had a period of non-manipulated REM sleep after REM cueing had terminated: an average of 65.9 min (ranging from 24 min to 117.5 min). Furthermore, the amount of non-manipulated REM sleep in each participant was not correlated with performance on SWS cued problems on either post-sleep day 1 ($r = 0.284$, $p = 0.160$) or post-sleep day 7 ($r = 0.166$, $p = 0.419$).

Relation between rule abstraction and NREM graphoelements

Sleep architecture data from all 27 participants is presented in Table 1.

Slow oscillations and sleep spindles are thought to mediate TMR-related benefits to memory consolidation(Schouten et al., 2017; Cairney et al., 2018; Göldi et al., 2019). In order to determine if the same was true for rule abstraction, we counted the number of slow oscillations and sleep spindles in NREM sleep for each participant and checked for correlations between each of these and the SVRT performance change for problems cued in SWS and REM, as well as the control non-cued problems for each sleep stage. In line with the observation that TMR in SWS did not improve rule abstraction, we found no correlation between performance on the SVRT task and either spindles or slow oscillations (all $p >= 0.1$, uncorrected, Table 4).

Next, we wanted to determine whether TMR cueing altered spindles or slow oscillations in a way that related to subsequent changes in performance on our task. We thus calculated the number and duration (samples) of spindles and slow oscillation in the 3 second epoch following TMR stimulation for each condition (experimental and control). No significant results were found for spindles (smallest p value=0.06, see topography in Figure 5). But two significant clusters were found for the number of SOs. One in the left hemisphere, $t=-9.08$ p value=0.007, and one on the right hemisphere ($t=-6.50$, $p=0.012$), see Figure 5. Both indicated a higher number of SO after control than experimental sounds. We then correlated the mean number of SOs detected in each cluster with behavioural performance change for items (cued in REM/SWS and non-cued for both stages) both overnight and over the subsequent week and for both cued and non-cued items. This revealed a significant positive relationship between both the right hemispheric cluster (Rho = .44, p=0.03) and the left hemispheric cluster (Rho = .42, p= 0.04), uncorrected. Overall, these data appear to suggest that cueing with the experimental TMR tone lead to a reduction in SOs over these electrodes and this seems to be associated with TMR benefit, although the correlations do not survive correction for multiple comparisons. However, because we had no *apriori* hypothesis to this effect, and the correlations do not survive correction for multiple comparisons, we feel this should be treated with caution.

Image category did not affect SVRT performance

To determine whether being associated with the face/object sounds versus the landscape/nature sounds had any impact on behaviour, we directly compared performance on problems associated with faces and landscapes, irrespective of sleep stage or cueing condition. There were no differences in performance between the two. We conducted a two-way repeated measures ANOVA on the raw accuracy values with the factors category: (faces and landscapes) and session: (pre-sleep, post-sleep day 1 and post-sleep day 7). There was no effect of category ($F_{(1,26)}$ = 0.362; p = 0.553; η^2 = 0.003) or session ($F_{(1,26)}$ = 2.054; p = 0.139; η^2 = 0.007) , and no interaction ($F_{(1,26)}$ = 0.253 ; p = 0.778; η^2 = 0.001). The same analysis was conducted on the performance changes (overnight, over a week and overall change), with Greenhouse-Geisser sphericity correction. Similarly, no effect of category ($F_{(1,26)}$ = 0.365; p = 0.551; η^2 = 0.004) or session ($F_{(1,26)}$ = 0.610; p = 0.480; η^2 = 0.004) was found, and there was no interaction ($F(_{1,26})$ = 0.165; p = 0.729; η^2 = 0.002). We ran paired t-tests between the same time points in each category (e.g. Faces at pre-sleep vs Landscapes at pre-sleep). No differences were found (all p > 0.4, uncorrected).

Discussion

This study shows that rule abstraction, one of the building blocks of human reasoning, can be facilitated by applying targeted memory reactivation during sleep. Interestingly, when different problems were cued in SWS and REM within the same night, the problems cued in REM benefitted from offline rehearsal, shedding light on a possible role for previously detected reactivation during REM (Maquet et al., 2000; Louie and Wilson, 2001; Mainieri et al., 2019). Furthermore, we found that REM TMR mediated facilitation of abstraction requires time to emerge, since cued problems have a significant advantage over non-cued problems one week after the manipulation. This is important, because it joins a small but growing literature suggesting that some sleep-related memory benefits may require more than just one episode of sleep to emerge (Groch et al., 2017; Cairney et al., 2018).

Abstraction underpins the ability to categorise items and generalize rules to new, never before seen exemplars. This is a core component of fluid intelligence(Otero, 2017), and is particularly important when one is faced with a new problem that cannot be solved exclusively by prior knowledge. Our data appear to show a dissociation between REM and SWS, with TMR in the former but not the latter facilitating performance on a complex task requiring rule abstraction and pattern categorization. Un-manipulated SWS has been shown to be involved in both quantitative (Rasch and Born, 2013) and qualitative changes to recently encoded memories (Wagner et al., 2004; Lau et al., 2010; Durrant et al., 2011, 2013; Wilhelm et al., 2013; Kirov et al., 2015), while REM has been suggested to be more involved with qualitative changes, such as forming unexpected links between different memories or concepts (Lewis et al., 2018). This possibility is supported by studies showing that REM duration predicts visual abstraction (Lutz et al., 2017), category learning (Djonlagic et al., 2009), lexical integration (Tamminen et al., 2017) and grammar learning (Batterink and Paller, 2017), all of which are highly integrative forms of memory. Our finding with respect to REM is also in line with a recent review suggesting that abstraction of explicit rules based on prior knowledge is often linked to REM sleep (Lerner and Gluck, 2019), and extends these ideas by providing clues to the underlying mechanisms of REM-dependent rule abstraction. In addition, one study demonstrated that TMR in SWS can actually impair the abstraction of grammar-like transition statistics(Hennies et al., 2017), suggesting that promotion of memory for specific episodes through reactivation in SWS may disrupt the abstraction of generalised statistics. Taken together with this literature, our findings suggest that REM TMR may have the capacity to directly promote abstraction. Supporting this, studies using REM TMR to investigate qualitative changes, such as the affective tone of emotional memories (Rihm and Rasch, 2015; Lehmann et al., 2016) and the generalization/integration of pictures with emotional content (Sterpenich et al., 2014), typically do find a benefit from REM TMR, as did our current study. If abstraction-like processing turns out to be the main function of REM for memory, that could explain why most REM TMR studies have shown little or no benefit to memory consolidation (for a meta-analysis see (Hu et al., 2019)), since such studies typically assessed quantitative, rather than qualitative changes, and thus do not test abstraction.

In the current study, while TMR in REM facilitated rule abstraction, TMR in SWS did not. Given this result, it might be tempting to conclude that TMR in SWS does not facilitate this kind of abstraction. However, we cannot exclude the possibility that cueing problems in SWS triggered a consolidation process which would have facilitated abstraction, but which was disrupted by subsequent cueing in REM. We ran several analyses to investigate this possibility and found that there is no relationship between the extent to which SVRT performance benefitted from cueing in REM and cueing in SWS. We also found that the vast majority of participants had epochs of non-manipulated REM sleep after REM cueing had ceased, which presumably provided an opportunity for items that had been cued in SWS to continue their consolidation in REM as needed. Nonetheless, we still cannot rule out some kind of interference and thus remain cautious in our interpretation. We therefore conclude only that REM TMR is sufficient to start a consolidation process which facilitates rule abstraction and cannot draw conclusions about the impacts of SWS TMR on this process based on the current data alone.

Regarding the timing of the TMR effects, our data suggest that the impact of TMR may continue to unfold for at least a week, with performance on cued and non-cued problems only becoming significantly different after that temporal delay. Notably, we did not test performance between days one and seven, so we do not know how quickly this process unfolds. If qualitative changes in memory representations, such as abstraction, require longer periods of time to evolve (Sterpenich et al., 2014; Lutz et al., 2017), then they may escape detection by the commonly used 12 hour test-retest paradigm. Prior studies have considered longer test periods and have shown that TMR-related benefits sometimes disappear over a week (Shanahan et al., 2018), but can also persist over this period (Hu et al., 2015; Groch et al., 2017; Simon et al., 2018). Our current study builds on these reports by showing that the benefit to abstraction which was not significant at day one post-sleep became significant by day seven. This is in keeping with a study of emotional processing, which showed that the impact of NREM TMR on emotional content was amplified across a week (Groch et al., 2017), and also with our own work on the serial reaction time task which shows that benefit from TMR can emerge after 10 days or more (Rakowska et al., 2021).

Building on a model of synaptic plasticity across brain states (Redondo and Morris, 2011; Seibt and Frank, 2019), we have recently proposed a series of plasticity-related events that take place in both NREM and REM which could explain why the effect of sleep on memory consolidation may require extended periods of time before it becomes detectable (Pereira and Lewis, 2020). According to a recent framework (Seibt and Frank, 2019), neuronal ensembles associated with the task are tagged during wakeful encoding. During subsequent NREM reactivation, mRNAs or other Plasticity-Related Products (PRPs) are captured by these tagged synapses. Finally, in subsequent REM, these PRPs are translated into proteins which enable synapses to undergo intense remodelling. In light of our current results, we speculate that applying TMR in REM might potentially bypass the need for PRP capture in

NREM, instead promoting PRP capture and translation at task-related synapses. Given the time-consuming nature of these processes, multiple nights of sleep could be required before measurable behavioural effects emerge. Of course, this does not explain why TMR cueing in SWS, which might reasonably be expected to result in extra PRP capture by task-related synapses, did not result in a behavioural benefit. We can only speculate that such PRP capture is not sufficient in the case of our abstraction task. Alternatively, it is also possible that cueing in REM subsequent to SWS somehow interfered with consolidation such that PRPs capture during SWS cueing were not subsequently translated. More work will be needed to disentangle such effects.

Our ERP analysis complements our behavioural findings by revealing differential neural responses to experimental and control stimuli in REM, but not SWS. These differential responses were found between 228 to 400ms post cue onset, a time window during which auditory stimuli are known to be extensively processed in both NREM and REM sleep (Campbell and Muller-Gass, 2011) and which is also associated with the P300 component (Picton, 1992). The P300 is thought to reflect higher order cognitive processing related to selective attention and resource allocation, with its amplitude proportional to the amount of attentional resource recruited for scrutiny of a given stimulus (Ibáñez et al., 2009). The P300 has also been detected during REM, with larger peak amplitudes occurring for rare sounds in the oddball paradigm (Cote and Campbell, 1999). Our data mirror this result by showing that ‘new’ control sounds elicited greater P300 waves than ‘familiar’ task-related sounds. Interestingly, the P300 has been found in response to hearing one’s own name in REM sleep, but not in response to hearing another name. This could indicate that some level of cognitive processing persists during REM (Bastuji et al., 2002). The fact that we observed a difference between familiar and unfamiliar P300 responses in REM but not in SWS, is therefore in keeping with the literature. Other authors have interpreted such results as suggesting that stimuli are processed at a deeper, more cognitive, level during REM (see (Ibáñez et al., 2009) for a review).

Conclusion

In sum, we found that TMR in REM is sufficient to benefit a visual reasoning task commonly used in the field of Artificial Intelligence (Fleuret et al., 2011; Ellis et al., 2015), but never before tested in a sleep study. Furthermore, ERPs suggested a deeper level of processing in REM than SWS, and behavioural findings suggest that the process started by TMR in REM requires more than one night of sleep to unfold. These findings open exciting new avenues for exploring TMR as a tool to enhance higher order cognitive functions such as abstraction, a core component of fluid intelligence and creativity.

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Author Contributions

SIRP, PL and MVR designed the experiments, SIRP, RA and ES collected the data, SIRP and LS analysed the data and all authors wrote the manuscript**.**

Declaration of Interests

The authors declare no competing interests.

Source data

The full dataset presented here, including demographics, behavioural and EEG data, as well as the Matlab scripts used in the ERP analyses, is available at 10.5281/zenodo.7215812.

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Table 1. Sleep architecture (n = 27)

Sleep variable	Mean	SEM
TST (min)	490.3	10.5
Sleep latency (min)	20.6	2.9
WASO (min)	15.9	3.8
Micro-arousals (#)	39.3	4.6
NREM 1 (min)	33.5	3.0
NREM 2 (min)	254.9	7.9
SWS (min)	85.7	4.2
REM (min)	100.3	4.9
WASO (%)	3.2	3.2
NREM 1 (%)	6.7	0.5
NREM 2 (%)	52.0	1.2
SWS (%)	17.7	0.9
REM (%)	20.3	0.9

Total sleep time (TST); Wake after sleep onset (WASO).

Table 2 – Correlations between Cueing Benefit* in REM and SWS

		Pearson's r	p†
SWS Pre-sleep	with REM Pre-sleep	-0.205	0.304
SWS Day 1	with REM Day1	-0.003	0.987
SWS Day 7	with REM Day7	-0.147	0.465
SWS Overnight	with REM Overnight	-0.086	0.669
SWS Week	with REM Week	-0.207	0.300
SWS Total	with REM Total	-0.338	0.085

*cueing benefit = cued – non-cued; Overnight = Day 1 – Pre-sleep; Week = Day 7 – Day 1; Total = Day 7 – Pre-sleep; uncorrected †*p*-value.

Table 3. Spindles and Slow Oscillations identified in epochs after control and experimental sounds

	Number	Duration (samples)
Spindles		
Control	43.75 (2.01)	78.16 (0.28)
Experimental	43.63 (2.02)	77.20 (0.29)
Slow Oscillations		
Control	59.96 (2.17)	215.46 (1.64)
Experimental	56.39 (2.03)	221.03 (1.65)

Table 3: Spindles and slow oscillations summary, averaged across participants and channels separately for control and experimental epochs. Values within brackets indicates SEM.

Table 4: Spindles and slow oscillations summary, averaged across participants and channels separately for control and experimental epochs. Values within brackets indicates SEM error.

Sleep Stage	Cueing Condition	Oscillation	Pearson's *r*	*p*
SWS	Non-cued	Spindles (#)	0.008	0.97
		Sos (#)	-0.015	0.94
	Cued	Spindles (#)	0.118	0.56
		Sos (#)	0.148	0.46
REM	Non-cued	Spindles (#)	0.324	0.10
		Sos (#)	0.231	0.25
	Cued	Spindles (#)	-0.114	0.57
		Sos (#)	0.016	0.94

Slow Oscillations (Sos); Number (#). N = 27

Figure 1. Experimental design. A) Before sleep, participants learned to pair each image (a face or a landscape) with an SVRT problem and its associated sound (Problem-Image Association task). Next, they were trained and tested on the SVRT task, where they had to decide whether or not the test image followed the same rule as the reference image for any given problem, as shown in the upper panel in A. For example, in the problem shown here the rule is: each image contains two identical shapes(Fleuret et al., 2011), see Extended Data Figure 1-1 for another example. Immediately before sleep, participants were probed on their ability to recall which sound (speaker symbols) had been paired to which SVRT problem (Problem-Sound Association task). TMR was applied to different problems during REM and SWS during the night (see B). Finally, participants were retested on the SVRT both next morning (post-sleep day 1) and a week later (post-sleep day 7). B) Representative hypnogram depicting the TMR protocol. During TMR in the night, sounds associated with four problems were replayed in SWS and sounds associated with four other problems were replayed in REM. Control sounds that had not been associated with any problems (new sounds) but instead served as controls for auditory responses were also replayed in both sleep stages. Cueing started with the first instance of SWS and REM and terminated once control and experimental sounds had been presented 28 times each (twice per loop, 14 loops).

Extended Data Figure 1-1 SVRT stimuli examples. Sample images from problem 1 (top panel) and problem 2 (bottom panel), that either follow the rule (on the left) or break the rule (on the right)(Fleuret et al., 2011). For problem 1 the rule is that: each picture contains two identical shapes. The squiggly lines were introduced as distractors (not a part of the rule), to increase the difficulty level. For problem 2 the rule is each image contains two shapes of different sizes, the smaller one inside the larger one, roughly centred. The black filling of the smaller shaped was added in some images as a distractor to increase the difficulty level. Other problems had rules relating, for example, to the number of identical shapes (pairs or triplets), their position (mirrored or translated, touching or not touching, inside or outside one another, aligned or not aligned, etc.) or their arrangement (odd shape in the middle, bigger shape at the edge, etc.).

Figure 2 –TMR in REM improves rule abstraction.

A) SVRT accuracy change overnight (post-sleep day 1 – pre-sleep) and across the week (post-sleep day 7 – post-sleep day 1) is plotted for each sleep stage (SWS and REM) and cueing condition (non-cued and cued). A repeated measures ANOVA revealed a significant sleep stage*cueing condition interaction (*p* = 0.013) and a simple main-effects analysis showed better performance for problems cued in REM, as compared to problems cued in SWS (*p* = 0.044). See Extended Data Figure 2-1. **B)** In SWS problems (left), there was no difference between cued and non-cued accuracy in any individual session (p > 0.3). In REM problems (right) there was no difference between cued and non-cued conditions on day 1 (*p* = 0.550), but at day 7, accuracy was higher on cued compared to non-cued problems (*p* = 0.002). Mean and SEM are depicted, see also Extended Data Figure 2-2. See Extended Data Figure 2-3 for numbers of trials.

Extended Data Figure 2-1a – SVRT accuracy at baseline (pre-sleep). ANOVA with Cueing (cued/non-cued) and Sleep stage (REM/SWS) as factors.

Extended Data Figure 2-1b – TMR benefit. Repeated measures ANOVA on retention interval (overnight/week) and Cueing (cued/non-cued) and Sleep stages (SWS/REM). Shaded areas highlight significant results. Overnight benefit is calculated as the difference between Post-sleep day 1 and pre sleep and the week performance is calculated as the difference between both post sleep sessions (Day 7 – Day 1).

Extended Data Figure 2-1c – TMR benefit post-hoc analysis. Paired t-test for REM conditions to understand the differences between cued and non-cued problems per session (Post-sleep Day1 and Day 7) and also the cueing benefit overnight (difference between Post sleep Day1 and Pre-sleep), a week after (Post-sleep Day7 vs. Pre-sleep) and also the difference between Day 7 and Pre-sleep.

Extended Data Figure 2-2: Accuracy on the SVRT per group and session

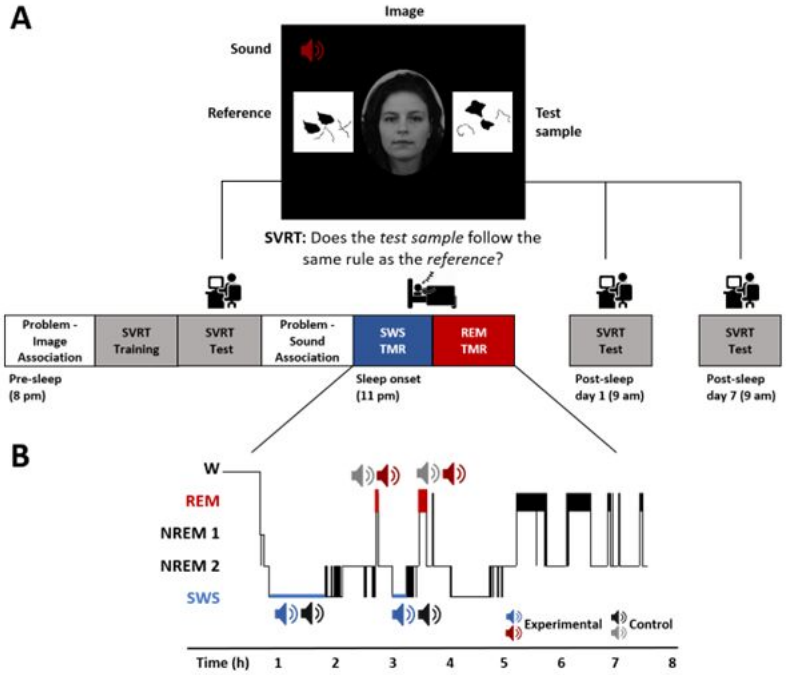
Extended Data Figure 2-3. Number of trials used per participant and condition

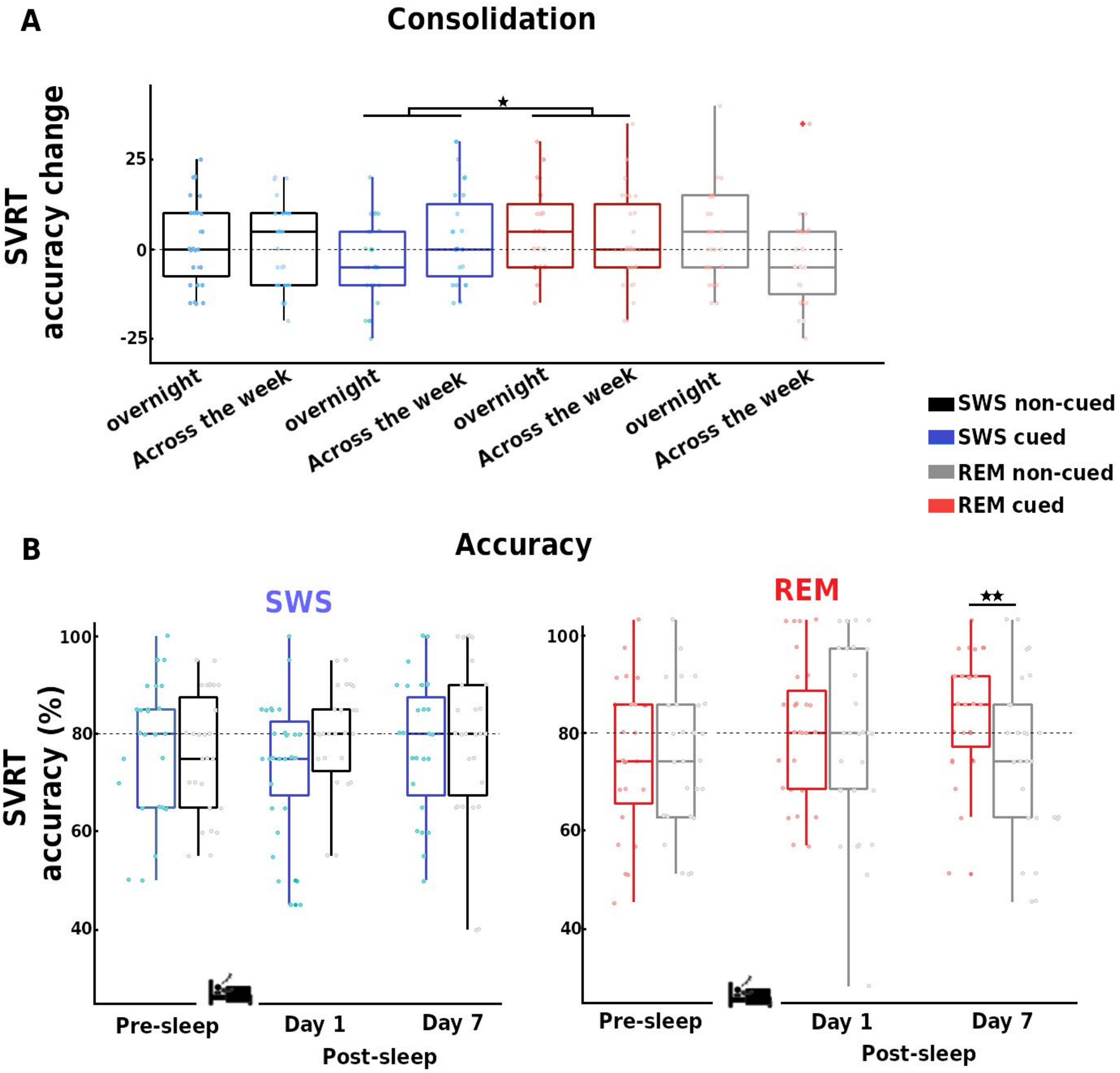
Figure 3 – Event-related Potentials at Cz during Targeted Memory Reactivation. Cz ERPs in SWS (blue top panel) and REM (red bottom panel) elicited by control (new) and experimental (task-related) sounds. The vertical dashed line at 0 indicates cue onset (200ms long). A cluster analysis revealed a significant difference between ERPs in response to control and experimental sound in REM between 228ms and 400ms (cluster corrected **p* =0.048). Data are depicted as mean ± SEM (n = 26).

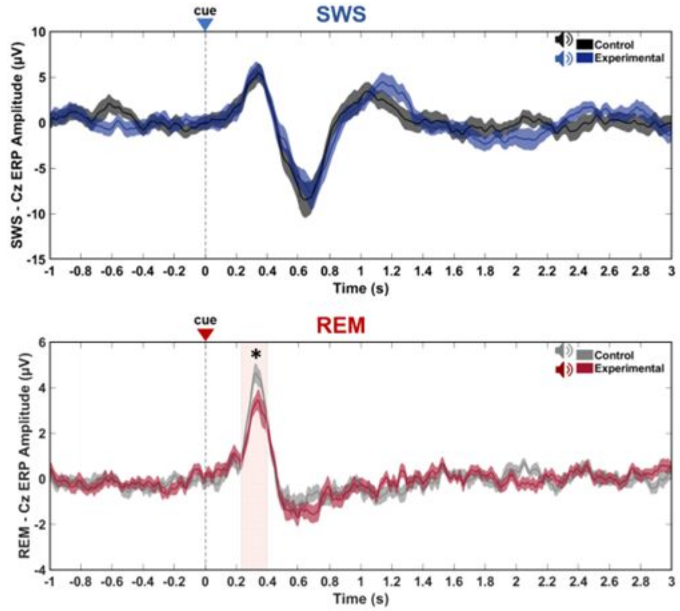
Figure 4 – Spatial distribution of channels with a statistically significant difference between experimental and control sounds during REM. Data is displayed as the averaged difference (n=26) between experimental and control sounds ERPs in 20ms time bins. * Indicates the position of a significant channel. The time-frequency cluster permutation analysis for these data is shown in Extended Data Figure 4-1.

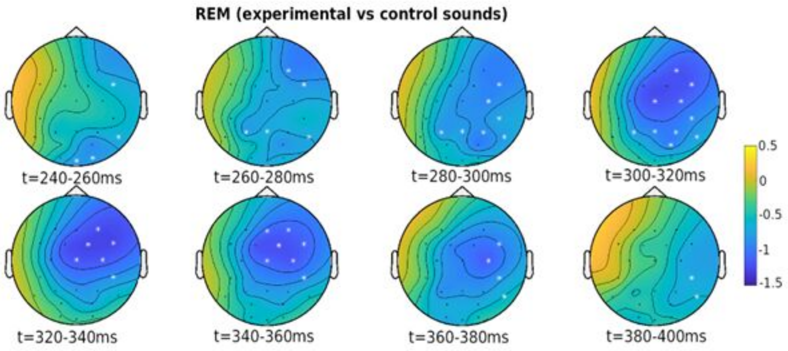
Extended Data Figure 4-1. Time-frequency cluster permutation analysis. When more than one cluster is present, the lowest p-value was selected. When no clusters are found is indicated by (-). No statistically significant clusters were found.

Figure 5: Spindles and slow oscillations evoked by TMR. Top row shows the average of differences in spindles following experimental and control TMR cues, while the bottom line shows the same for slow oscillations. Durations are shown on the left and count is shown on the right. Blue colours indicating higher spindle duration/count for control than experimental. Significant clusters are highlighted with a white star.

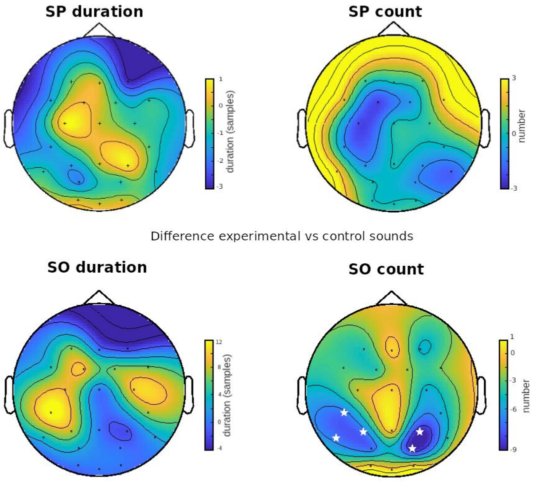

A
Image
Sound
Reference
Test sample
SVRT: Does the test sample follow the same rule as the reference?
Problem - Image Association
SVRT Training
SVRT Test
Problem - Sound Association
SWS TMR
REM TMR
SVRT Test
SVRT Test
Pre-sleep (8 pm)
Sleep onset (11 pm)
Post-sleep day 1 (9 am)
Post-sleep day 7 (9 am)
B
W
REM
NREM 1
NREM 2
SWS
Experimental
Control
Time (h)
1
2
3
4
5
6
7
8


A
Consolidation
SVRT accuracy change
25
0
-25
★
overnight
Across the week
overnight
Across the week
overnight
Across the week
overnight
Across the week
SWS non-cued
SWS cued
REM non-cued
REM cued
B
Accuracy
SWS
REM
★★
SVRT accuracy (%)
100
80
60
40
Pre-sleep
Day 1
Day 7
Post-sleep
Pre-sleep
Day 1
Day 7
Post-sleep


cue
SWS
Control
Experimental
SWS - Cz ERP Amplitude (µV)
10
5
0
-5
-10
-15
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
2.4
2.6
2.8
3
Time (s)
cue
REM
*
Control
Experimental
REM - Cz ERP Amplitude (µV)
6
4
2
0
-2
-4
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
2.4
2.6
2.8
3
Time (s)



REM (experimental vs control sounds)


SP duration
1
0
-1
-2
-3
duration (samples)
SP count
3
0
-3
number
Difference experimental vs control sounds
SO duration
12
8
4
0
-4
duration (samples)
SO count
1
0
-3
-6
-9
number