Targeted memory reactivation in human REM sleep elicits recurrent, temporally compressed reactivation

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Abstract

Memories are reactivated during non-rapid eye movement (NREM) sleep, but the question of whether equivalent reactivation also occurs in rapid eye movement (REM) sleep is hotly debated. To examine this, we used a technique called targeted memory reactivation (TMR) in which sounds are paired with learned material in wake, and then re-presented in subsequent sleep to trigger reactivation. We then used machine learning classifiers to identify TMR-induced reactivation in REM. The reactivation we measured was temporally compressed by approximately five times during REM compared to wakeful performance of the task, and often occurred twice after a single TMR cue. Reactivation strength positively predicted overnight performance improvement and was only apparent in trials with high theta activity. These findings provide strong evidence for memory reactivation in human REM sleep after TMR as well as an initial characterisation of this reactivation.

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Introduction

The reactivation of memories in non-REM sleep is widely supported by evidence from humans, rodents, and other animals[1–4], but it is still unclear whether equivalent reactivation occurs in REM sleep. Reactivation in non-REM sleep has been identified using EEG classifiers[3,5–7], with fMRI[8,9], and with intracranial recording[10]. Targeted memory reactivation (TMR), a technique which allows the active triggering of memory reactivation, causes both neural and behavioural plasticity when applied in non-REM sleep, see[11] for a review. However, only a few studies in rodents show evidence for reactivation in REM sleep[12,13]. Furthermore TMR in REM often fails to produce any measurable behavioural impact[9,14], though conditioning seems to be the exception to this rule[15,16]. One prior study elegantly demonstrated that machine learning applied to EEG can be used to distinguish between the REM sleep occurring after training on two different tasks[17]. The authors also found a correlation between the extent to which such patterns were identified in NREM and subsequent behavioural performance, however they did not look for reactivation in terms of wake-like task related activity that reoccurs during REM. In the current study we do just this, and are thus able to establish a direct link between brain activity associated with a memory in wake and the reactivation after a TMR cue in REM. We also explore the temporal characteristics and dynamics of this reactivation.

Memories have a different temporal structure during reactivation compared to encoding. For instance, replay during non-REM sleep has been shown to occur 10 to 20 times faster than in wake[4,18]. On the other hand, wakeful and NREM1 reactivation in humans has also been demonstrated to be both compressed and dilated within the same participant[19]. Given this literature, we looked for evidence of temporal compression or dilation during memory reactivation in human REM sleep. Memory reactivation can reoccur more than once after a TMR cue in non-REM sleep[6], and this may be of functional significance for consolidation from the hippocampus to the cortex[11]. We therefore sought to

determine whether a similar pattern is apparent for REM reactivation. Additionally, theta activity is prominent in REM sleep[20], and human studies suggest a possible relationship between memory and theta[21,22]. Wakeful theta is suggested to provide a preferable window for the encoding of new information[23–25]. Of the few rodent studies that have supported memory reactivation in REM, two showed a link to theta activity[12,26]. We were therefore interested to determine whether theta is associated with TMR cued reactivation in human REM sleep.

We used a serial reaction time task (SRTT), which is known to be sleep sensitive[27,28] to examine these questions. Participants were presented with audio-visual cues and responded by pressing four buttons (two from each hand). Cues were organised in a twelve-item sequence. Sounds were replayed during subsequent REM to trigger the associated memories of left- and right-hand presses (Fig. 1). We used two sequences and replayed only one of them in sleep. For control, we also included an adaptation night in which participants slept in the lab and we played the same tones that would later be played during the experimental night. This provided data in which tones could not have evoked memory reactivation, as participants had not yet learned the behavioural task, see methods for more details about the SRTT.

In summary, we discriminate between neural reactivation of left- and right-hand button presses using linear classification of time domain EEG amplitudes during REM sleep. Our findings demonstrate that such classifiers can detect reactivation after TMR cues in REM sleep, and that classification strength predicts task overnight improvement. We also reveal several key characteristics of this TMR cued reactivation, including recurrence, temporal compression, and an association with high theta activity.

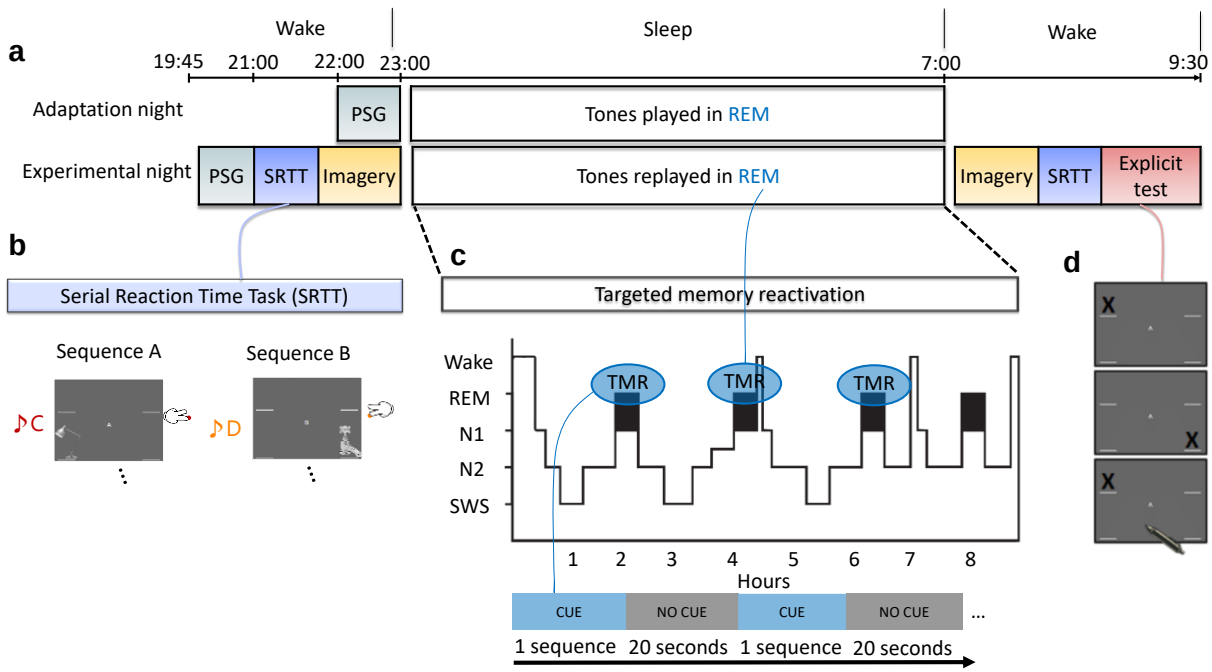

Fig. 1 Experimental design. a The experiment consisted of two nights an adaptation and an experimental night. In the adaptation night, tones were presented to the participants during REM sleep and EEG recordings were acquired. In the experimental night, participants were wired-up for EEG then completed the serial reaction time task (SRTT) and an imagery task in which they were cued with pictures and sounds, but only imagine performing the finger tapping (without movement). Afterwards participants slept in the lab and TMR cues were presented. After waking up, participants completed the motor imagery and SRTT again, and finally they did the explicit recall task. **b** In the SRTT, images are presented in two different sequences each with a different set of tones. Each image is associated with a unique tone and requires a specific button press. In the imagery task, participants hear the tones and see the images as in the SRTT, but only imagine pressing the buttons. **c** The sounds of only one learned sequence (cued sequence) were played during REM sleep, with a 20 second pause between repetitions. **d** Participants were asked to mark the order of each sequence on paper as accurately as they can remember during the explicit recall test after sleep.

Results

Detection of memory reactivation after TMR cues

We trained our classification models using sleep data and then tested them on wake data. This was partly inspired by Loui and Wilson[12] who took a template from sleep data and slid it across wake to detect replay in rodents. Training a classification model on wake could cause the model to weigh features which are dominant in wake very highly even if those features were entirely absent from sleep. By training classification models on sleep data, we ensured that the features associated with reactivation were used by the models, and the models were thus able to look for these in the stronger, less noisy, signals recorded during wake.

We used a linear discriminant analysis (LDA) classifier in a time x time classification procedure[29], see methods for details. We repeated the classification process using the adaptation night to be certain that the classification was not caused by sound induced effect or EEG noise rather than reactivation of the encoded memory. We compared the results from the two nights, both to each other and to chance level using cluster-based permutation. In the adaptation night, no significant classification strength was found compared to chance (area under the receiver operating characteristic curve (AUC) = 0.5), demonstrating that classification of this control condition did not differ from chance level. By contrast, comparison of the experimental night against chance showed a significantly higher AUC for the experimental night (Fig. 2a) which occurred around 1 sec. after the onset of the cue at time 0 (Fig. 2b). Comparison of the experimental night to the adaptation night also showed a significantly higher AUC for the experimental night, described by a cluster in the same timeframe (Fig. 2a). This means that we can detect memory reactivation and discriminate between right- and left-hand movements during REM sleep, and this is significant when evaluated against both control and chance level.

High theta activity mediates reactivation

To test for a relationship between theta power and reactivation we performed a median split on theta power, creating two groups of trials for each participant: those with high theta power and those with low theta power, see methods. This split was performed for both experimental and adaptation nights. We then compared the classification results of each half of the median split (high and low theta power trials) in the experimental night to both chance level and to the equivalent high or low theta power trials in the adaptation night. For high theta trials, this showed a significant effect (Fig. 2c), explained by a cluster occurring around the same time as in the classification result using all trials, (Fig. 2a-b), ($n = 14$, $p = 0.028$), there was also a significant difference against chance level (Fig. 2d), ($n = 14$, $p = 0.001$). The low theta power trials showed no significant effect (Supplementary Fig. 3). These findings demonstrate an association between high theta power and reactivation. To determine whether theta activity offered a preferred window for classification, or it was the actual feature causing the discrimination of classes, we band-pass filtered the signals in the theta band and re-ran our classification analysis. Interestingly, classification did not differ from chance in this filtered data ($p > 0.4$) suggesting that while high theta activity offers a preferred window for reactivation, theta activity itself does not carry the information used to detect reactivation. Time x time classification AUC plots using all trials and also using high theta power trials are shown in (Supplementary Fig. 2).

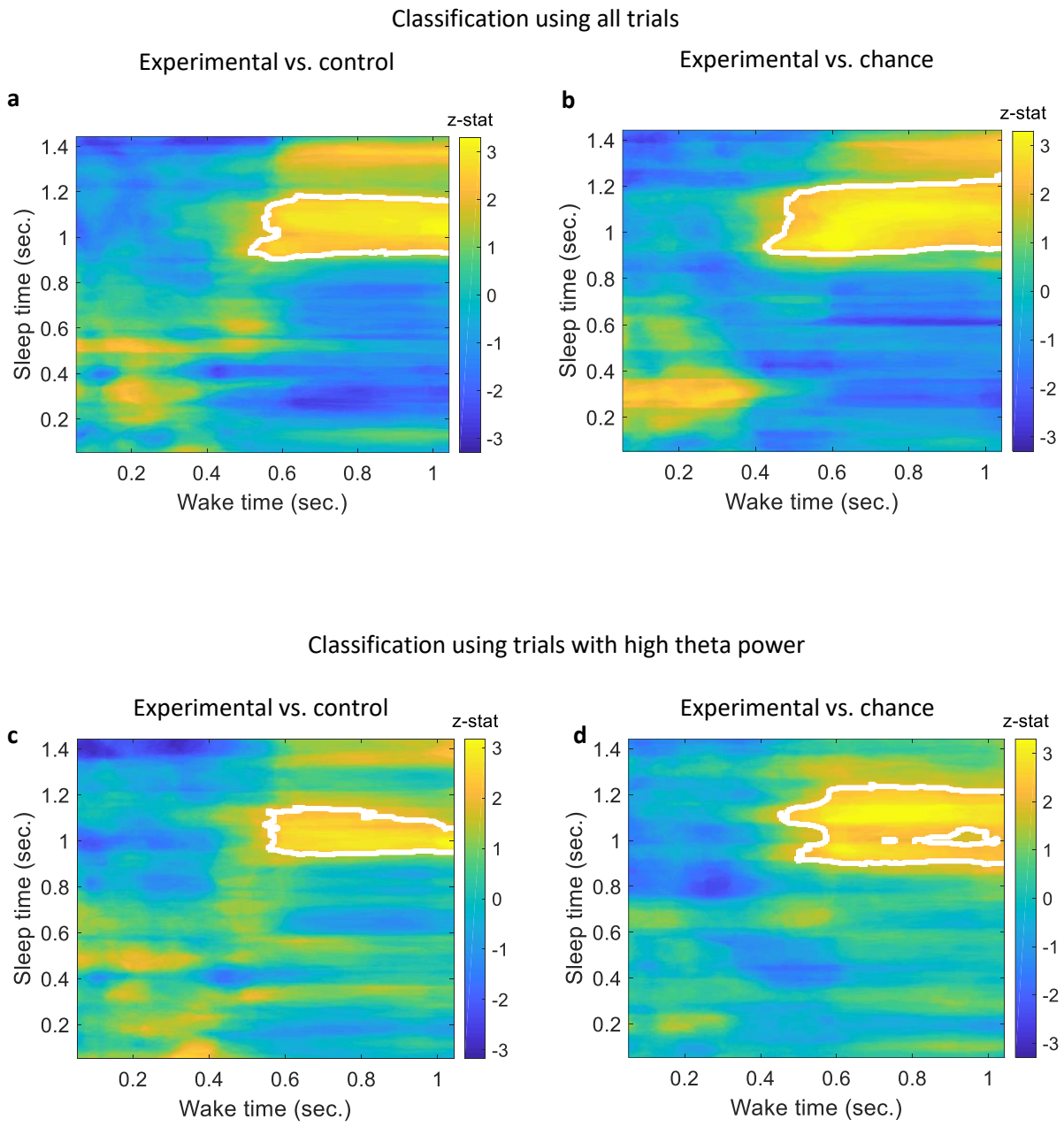

Fig. 2 Classification of left- vs. right-hand. a A comparison between the classification performance of the experimental vs. adaptation night using all trials reveals a significant effect described by a cluster which shows a higher classification performance for the experimental night compared to adaptation (n= 14, p= 0.01), z-statistics values at every point is shown and cluster edges are marked with white after correcting for multiple comparisons with cluster-based permutation (see methods for details). **b** Classification performance for the experimental night was also significantly higher than chance (AUC:

0.5) as shown by the corrected cluster (n= 14, p< 0.0001). **c** A comparison between the experimental and adaptation night classification using trials with high theta power reveals a significant effect described by a cluster that shows a higher classification performance for the experimental night compared to adaptation (n= 14, p= 0.028), z-statistic values at every point is shown and corrected cluster edges are marked with white. **d** A comparison between the classification of experimental night using trials with high theta power and chance level shows a significant effect described by the shown corrected cluster (n= 14, p= 0.001).

Correlation of classification performance with behaviour

We next tested for a relationship between classification performance and improvement on the behavioural task using a spearman correlation, see methods for details. This revealed a positive correlation (Fig. 3a; n = 14, r = 0.67, uncorrected p = 0.01 and Bonferroni corrected p = 0.03). indicating that stronger detected reactivation was associated with greater overnight sequence improvement. Importantly, this correlation only existed for the reactivated sequence (for non-reactivated: n = 14, r = 0.02, p = 0.94). This finding suggests that the degree of detecting reactivation in REM positively predicts the extent to which sequence memory improves overnight. Nevertheless, it is notable that REM TMR did not lead to any overall benefit in performance[30].

Analysis of temporal compression/dilation of reactivation

Prior work has shown that reactivation in non-REM sleep is often temporally compressed with respect to wake[4,18,31]. Recent finding showed that wake and NREM1 reactivation could be sometimes compressed and sometimes dilated within the same data[19]. This motivated us to determine whether reactivation in REM occurs for the same amount of time as the original experience in wake. Thus, we performed an

analysis of temporal compression. First, we applied the time x time classification method on EEG amplitudes without any temporal smoothing, see methods. This analysis on non-smoothed signals revealed that reactivation occurs more than once within the timeframe of the cluster we had originally identified (Fig. 2a). Furthermore, both of the two sub-clusters we identified were temporally compressed during sleep as compared to wake (Fig. 3b), for more details see methods. Specifically, the activation in wake is ~450 ms long and the two reactivations identified by this non-smoothed analysis are ~80 ms long and therefore last approximately 20% of the duration of activation in wake (Fig. 3b). This suggests that reactivation of the memory in REM sleep is approximately five times faster than the activation in wake when the memory was encoded.

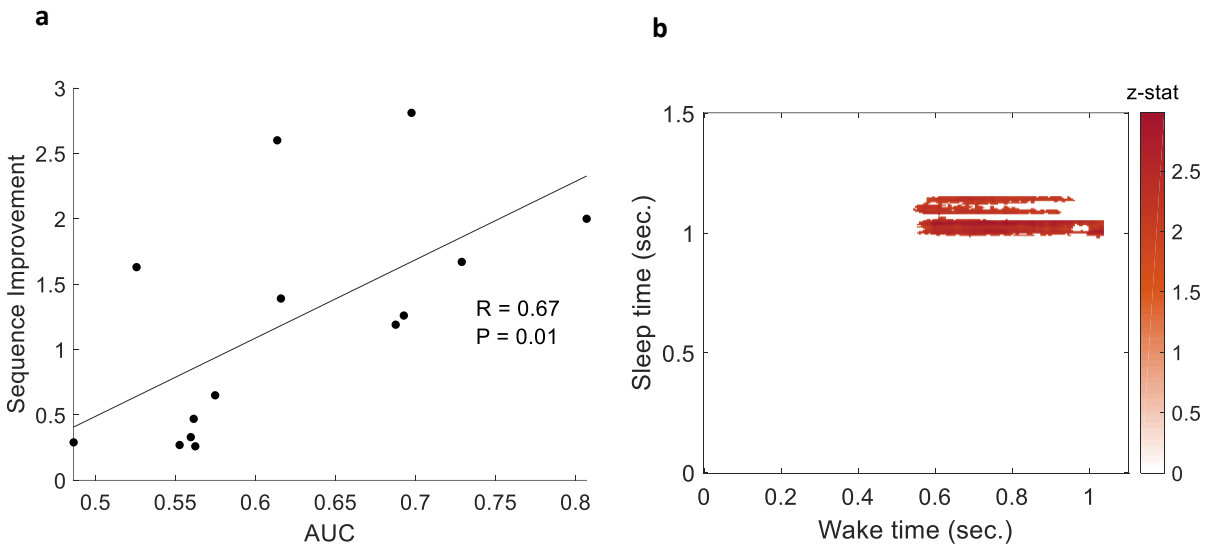

Fig. 3 Characteristics of detected reactivation. a Classification performance positively correlated with behavioural improvement for the reactivated sequence (spearman correlation, n= 14, r= 0.67, uncorrected p= 0.01 and Bonferroni corrected p = 0.03). **b** Classification performance using EEG signals without smoothing showed two significant effects described by two clusters, around 1 sec. after the onset of the cue when compared to the adaptation night (n= 14, p= 0.008 for the earlier and, 0.025 for the later cluster). Z-statistic values are shown and cut with respect to clusters to become visually clearer.

Locations of significant effects and their temporal characteristics reveal that reactivations are approximately five times faster than the activation in wake.

Reoccurrences of reactivation

Finally, we were interested to know if TMR cued reactivation in REM occurs multiple times within each trial, or if the apparent recurrence might instead be due to evaluating performance across multiple trials, with reactivation only happening once during each trial (e.g. at ~1sec. or after 1sec, but not both). To address this, we evaluated the correctness of each sleep trial at the times of each of the two sub-clusters, see methods for details. We then determined whether classification was correct for both sub-clusters or just one sub-cluster for every trial (Fig. 3b). We found that a significantly higher proportion of trials show reactivation at both sub-clusters (early and late), than at just one (Wilcoxon signed-rank test, $n = 14$, $p = 0.001$). This shows that reactivation is recurrent in the majority of trials, and could potentially repeat even more than twice if the duration of trials was longer. This is in keeping with observations from rodent data showing repeated reactivation after a TMR cue in non-REM sleep[2].

Discussion

We have demonstrated that memory reactivation can be detected in human REM sleep using EEG classifiers. Such reactivation appears to be delayed by about one second after the sound cue onset. Reactivation is associated with high theta power, which appears to provide a preferred window for this activity, although it does not carry the discriminative information needed to actually detect the reactivation. Interestingly, reactivations reoccur twice within a single 1,500 ms trial and are temporally compressed approximately five times compared to wake. We also demonstrated that stronger detection

202 of REM reactivation predicts greater overnight sequence improvement on our behavioural task, and this
203 is specific to the reactivated sequence.

204 Because we used a motor task, we used motor channels for classification. This ensured that the
205 reactivation we measure is related to the encoded motor memory and that detected reactivation retains
206 the features of wake. Moreover, comparison of classification results between experimental and control
207 nights allowed us to confirm that classification is not derived by sound induced EEG noise.

208 Reactivation after a reminder TMR cue is delayed during REM sleep as compared to wake. During wake,
209 neural activity associated with our motor imagery task starts around 0.6 seconds after cue onset and lasts
210 until the presentation of the next cue at 1.1 seconds post cue. In REM, on the other hand, reactivation of
211 this task starts around 1 second after the cue and ends at around 1.25 seconds post cue. This delayed
212 start could potentially be due to the brain taking more time to process information and reactivate a
213 memory during REM sleep than during wake. In keeping with this, reactivation of a picture memory task
214 in SWS has also been shown to be delayed, appearing about 2 seconds after cue onset[7].

215 Our results show that classification performance in REM positively predicts overnight sequence
216 improvement. Similar correlations between classification performance and behaviour were found in
217 non-REM TMR[3,8,10] and also spontaneous reactivation[17]. These findings could lead to the speculation that
218 more reactivation means more consolidation, and therefore better post-sleep performance. However
219 the absence of a group-level REM TMR benefit in our behavioural performance (see [30] for a full analysis
220 of the behavioural data), as well as the fact that other studies have observed negative correlations
221 between reactivation evidence and post-sleep improvement[32] mean we must treat such interpretations
222 with caution.

223 REM sleep is dominated by theta activity, which is thought to support the consolidation process[33], and
224 has been linked to reactivation[12]. Theta is linked to attention during wake[34] and is more prominent with

225 higher executive control[35]. Wakeful theta is also associated with the encoding of new information and
226 memory processing[25]. Furthermore, neuronal firing relative to theta phase has been shown to impact
227 upon whether synapses are strengthened or weakened, since stimulation of the positive theta phase
228 induces long-term potentiation and stimulation of the negative theta phase induces depotentiation[36]. A
229 similar pattern of phase dependent potentiation and depotentiation was shown in REM sleep[26],
230 suggesting that REM reactivation and wakeful pattern may be structured in a similar way[23,37]. Theta
231 phase similarity has been shown to be high between SWS and wake, suggesting the importance of theta
232 phase for reactivation[6]. Increased theta power has also been shown to be important for successful
233 cueing during sleep[38]. Our data demonstrated that trials with higher theta power show greater evidence
234 of memory reactivation, but theta band in isolation does not provide sufficient information to detect
235 reactivation. Theta therefore appears to be a marker of reactivation but does not embody the
236 reactivation in and of itself. This pattern of results could indicate that theta provides a timing function
237 for reactivation, while the reactivation itself relies upon other frequencies.

238 Studies in rats have shown that replay is temporally compressed with respect to 'run', or performing the
239 task in question, and the rate of that compression varies between sleep and wake. For instance, replay
240 in both wake and SWS has been shown to occur at a faster rate than in the original task[18,39,40]. However
241 some studies of rodent non-REM sleep showed a compression of 6 to 7 times compared to wake[31], while
242 other studies showed compression rates varying between 10 and 20 times compared to wake[4,18]. Our
243 analysis is in line with this literature, since it suggests that TMR reactivation in human REM sleep is
244 temporally compressed by approximately 5 times compared to wake.

245 The reactivation elicited by TMR in human SWS has been shown to reoccur repeatedly after a cue[6]. A
246 parallel study in rodents[2] showed that TMR cued replay can continue to repeat for up to 10 seconds
247 after the offset of the auditory cue, though this process appears to be interrupted by the presentation of
248 a second stimulus. These observations of repeated replay have been suggested to relate to the

249 suggestion of a cortico–hippocampal–cortical loop[11] since memories are thought to be consolidated via
250 reverberation of replay between the cortex and hippocampus[41], although one might potentially expect
251 such reverberation to occur on a shorter timescale. We tackled the question of whether replay occurs
252 repeatedly after a single TMR cue in REM and found two repetitions following a single TMR cue, which is
253 in good keeping with the results from previous studies[2,6]. Notably, our trials were just 1.5 seconds long,
254 so it is possible that further reoccurrence would have been observed in our paradigm if the cues were
255 spaced farther apart.

256 **Characteristics of reactivations that happen in SWS, and REM sleep**

257 While there is already a growing body of literature about reactivation in non-REM sleep[1–4,7,12,18,42], our
258 findings provide initial information about human memory reactivation using TMR in REM. As such, our
259 data suggests several parallels between reactivation in these two sleep stages. For instance, similar to
260 non-REM[7], reactivation in REM is delayed after cue onset compared to wake. Furthermore, reactivation
261 in REM is related to the oscillatory structure of sleep (e.g. theta activity), which parallels the known
262 relationship between reactivation in non-REM and graphoelements like SOs[43], spindles[7,44], and ripples[10].
263 Importantly, the reactivations we detected are reinstated in the same area of the brain that is used in
264 performing the task and the extracted features of reactivation are similar to those extracted during
265 wake activation which is why it is detectable using our machine learning model. Finally, REM
266 reactivations appear to be temporally compressed by about five times in comparison to wake, which is
267 close to the 6 to 7 times compression observed in non-REM[31].

268 **Summary**

269 The question of whether memories reactivate in REM as well as non-REM sleep has been debated for
270 some years. REM reactivation has been suggested by modelling[45] and has been demonstrated at least
271 once in the rodent literature[12]. Some evidence of learning dependent activation in human REM sleep has

also been observed in humans[46,47]. However, null behavioural findings from human REM TMR studies[1,9] have led to scepticism amongst sleep researchers. Our current findings put such doubts to bed by providing clear evidence that TMR in REM is associated with reactivation, and even predicts overnight improvement on our procedural task, although we found no significant benefit from cueing irrespective of reactivation. Furthermore, our analysis uncovers several important properties of REM reactivation, showing strong parallels with reactivation in non-REM sleep. More work is needed to explore this topic in detail, for instance determining how such reactivation links to behavioural and neural plasticity, and how this differs across a variety of cognitive tasks.

Methods

Participants

EEG and behavioural data were collected from human participants (n = 16) (8 females, 8 males, and age mean: 23.6). All participants had normal or corrected-to-normal vision, normal hearing, and no history of physical, psychological, neurological, or sleep disorders. Responses in a pre-screening questionnaire reported no stressful events and no travel before commencing the study. All participants reported non-familiarity with the SRTT and all of them were right-handed. Participants did not consume alcohol in the 24 hours before the study and caffeine in the 12 hours prior to the study or perform any extreme physical exercise or nap. This study was approved by the School of Psychology, Cardiff University Research Ethics Committee, and all participants gave written informed consents.

Experimental Design

The SRTT was adapted from[48] and participants performed SRTT before and after sleep. Two participants were excluded due to technical problems (n = 14). In SRTT, sounds cued four different finger presses. During wakeful encoding, participants learned two 12-item sequences, A and B, A: 1 2 1 4 2 3 4 1 3 2 4 3

294 and B: 2 4 3 2 3 1 4 2 3 1 4 1. The location indicated which key on the keyboard to press as quickly and
295 accurately as possible afterwards the next image appeared, locations and buttons associations were: 1 –
296 top left corner = left shift key; 2 – bottom left corner = left Ctrl; 3 – top right corner = up arrow; 4 –
297 bottom right corner = down arrow. Sequences had been matched for learning difficulty; both contained
298 each item three times. The blocks were interleaved so that a block of the same sequence was presented
299 no more than twice in a row, and each block contained three repetitions of a sequence. There were 24
300 blocks of each sequence (48 blocks in total), after each block a 15-second pause which could be
301 extended by participants if they wish, during the pause participants were informed with their reaction
302 time and error rate for the last block. The 48 blocks of sequences A and B were followed by four blocks
303 that contained random sequences. Two of these blocks were paired with the tone group of one
304 sequence that was later replayed in REM sleep, and the other two were paired with the tone group of
305 the other non-reactivated sequence. On the centre of the screen 'A' and 'B' appeared while participants
306 performed the task, and they knew that there were two sequences. However, they were not asked to
307 explicitly learn the sequences. The first sequence to present as well as the sequence to reactivate was
308 counterbalanced across participants. A sequence was paired with a group of pure musical tones, either
309 low tones within the 4th octave (C/D/E/F) or high tones within the 5th octave (A/B/C#/D)
310 (counterbalanced). At the beginning of each trial at time 0, a 200 ms tone was played, and at the same
311 time a visual cue appeared in one of the corners of the screen. Participants were instructed to keep
312 individual fingers of their left and right hand on the left and right response keys, respectively. Visual
313 cues were neutral objects or faces, used in previous study[48]. Visual cues appeared in the same position
314 for each sequence (1 = male face, 2 = lamp, 3 = female face, 4 = water tap). Visual cues stayed on the
315 screen until the correct key was pressed, after which an 880 ms inter-trial interval followed. After
316 completing the SRTT, participants performed the imagery task by only imagining pressing the buttons,
317 without movement. This task consisted of 30 interleaved blocks (15 of each sequence) and was

presented in the same order as during the SRTT. Each trial consisted of a tone and a visual stimulus, the latter being shown for 270 ms and followed by an 880 ms inter-trial interval. There were no random blocks during the imagery task and no performance feedback during the pause between blocks. As a control, participants were asked to sleep in the lab before doing the SRTT training. The control night followed the same criteria as the actual experiment with the difference that the sounds were not associated with any task. Participants performed the task again in the morning but this time motor imagery then SRTT. They were then asked to try to remember the locations of the images of the two sequences to test their recall for each of the sequences.

EEG recording and pre-processing

EEG signals were acquired from 21 electrodes, following the 10-20 system. Thirteen electrodes were placed on standard locations: FZ, CZ, PZ, F3, F4, C5, CP3, C6, CP4, P7, P8, O1, and O2. Other electrodes were: left and right EOG, three EMG electrodes on the chin, and the ground electrode was placed on the forehead. Electrodes were referenced to the mean of the left and right mastoid electrodes. The impedance was <5 kΩ for each scalp electrode, and <10 kΩ for each face electrode. Recordings were made with an Embla N7000 amplifier and RemLogic 1.1 PSG Software (Natus Medical Incorporated). PSG recordings were scored by trained sleep scorers and only the segments scored as REM were kept for further analyses.

Data were collected at 200 Hz sampling rate. EEG signals were band pass filtered in the frequency range from (0.1 to 50 HZ). Subsequently, trials were cleaned based on statistical measures consisting of variance and mean. Trials were segmented -0.5 sec. to 3 sec. relative to the onset of the cue. Trials falling two standard deviations higher than the mean were considered outliers and rejected if they show to be outliers for more than 25% of the channels. If trials were bad in less than 25% of the channels, they were interpolated using triangulation of neighbouring channels. Thus, 9.8% and 10.5% of trials

were considered outliers and removed from the experimental night data and the adaptation night, respectively.

Data was subsequently analysed with independent component analysis (ICA), to remove eye movement artifacts which can occur during REM. Components identified by the ICA that were significantly correlated with the signals from the eye electrodes (corrected for multiple comparisons) were removed. All channels for each participant were manually inspected and artifacts were removed. Because TMR will not be effective with all trials, we also rejected trials with low variance that do not differ from their mean across time since they are unlikely to contain a response. The number of clean trials kept after cleaning was consistent among participants such that they contribute equally to the group-level analysis and that number was 366 trials, it was determined according to the participant with the lowest number of such trials. All cleaning was done on all trials irrespective of cue information and stimulation night to avoid bias.

Time x time classification with time domain features

We adopted a time x time classification approach after smoothing the EEG signals of motor imagery using 100 ms window such that every time point is replaced with the average of the 50 ms of both sides around it. Since we know that this task is motor-dependent we focused our classification on the motor area, thus we used four channels around the motor area for classification (C5, CP3, C6, and CP4). In the time x time classification, every time point from sleep was used to train LDA classifier, which was applied to all time points from wake in order to get one row of classification results in the time x time classification plot (e.g., Fig. 2a). The process was repeated for all time points after a sound cue in sleep (trial length in sleep was: 1.5 sec. and 1.1 sec. in wake) (Supplementary Fig. 1). We use the area under the receiver operating characteristic curve (AUC) as the performance measure in our classification. Analyses were done using FieldTrip[49] and customised scripts using Matlab 2018a. The clustering window

used for cluster-based permutation was the whole length of trials (i.e., the whole time x time classification duration) which gave a stringent test. In other words, we did not limit the clustering window to a specific time window.

Theta power calculation

We calculated theta power using band pass filtering in the range (4 to 8 Hz) and Hilbert transform to extract the instantaneous power information of the signals. The power of a trial is calculated as the average power across all time points of that trial and all channels. We then divided the trials of every participant into two groups based on the median power of trials. This gave us trials with high theta power (higher than median) and low theta power (lower than median).

Temporal compression of reactivation

We analysed the temporal compression by applying the time x time classification using the EEG amplitudes without smoothing the EEG signals. We used the cluster we found from the first classification (around 1 sec.) as a clustering window, we used this window as a marker of time of interest because if the reactivation is compressed then it will be temporally short. Duration of sleep reactivation is calculated as the average durations of the two sub-clusters in sleep time. Duration of wake activation was determined by the average durations of the sub-clusters in wake time (Fig. 3b).

Correlation of classification performance with behaviour

Classification performance was averaged inside the cluster. AUC values from the high theta power classification were tested for correlation with the behavioural improvement. The behavioural improvement was calculated as: [(random blocks after sleep - the best 4 blocks after sleep) – (random blocks before sleep – the best 4 blocks before sleep)] / (random blocks before sleep – the best 4 blocks before sleep). The result was corrected for other measures using Bonferroni correction. We extracted

three behavioural measures: early blocks improvement, late blocks improvement, best blocks improvement (described above). Early blocks improvement was defined as: [(random blocks after sleep - the first 4 blocks after sleep) – (random blocks before sleep – the last 4 blocks before sleep)] / (random blocks before sleep – the last 4 blocks before sleep). Late blocks improvement was defined as: [(random blocks after sleep - the last 4 blocks after sleep) – (random blocks before sleep – the last 4 blocks before sleep)] / (random blocks before sleep – the last 4 blocks before sleep).

Reoccurrence

We analysed whether the classification is reoccurring within the same trial or whether it happens once while it only appears to be reoccurring as a result of looking at the performance among multiple trials. For this, we under-sampled wake trials, that is, we used wake time according to the two sub-clusters in (Fig. 3b) and under-sampled the wake trial to match the length of sleep cluster. Then, for every trial of sleep, we performed spearman correlation with all trials from wake such that we ended with one vector of correlation coefficients for every trial from sleep. Subsequently, we counted the number of times a trial from sleep positively correlated with wake trials of the same class, and the other class. A trial from sleep is then considered 'correct' if the number of positive significant correlations is higher for similar class than different class. Afterwards, we determined if reoccurrence is happening in sleep trial if it is correct during both the earlier and later clusters. Then we counted the number of trials with different types: recurrent, only earlier cluster, and later cluster. Afterwards, we normalised by the total number of trials for every participant which gave a datapoint for every participant for every type. Subsequently, the number of trials for every type was compared to chance 0.333 and recurrent reactivation was the only type that showed higher than chance trial count (Wilcoxon signed-rank test, $n = 14$, $p = 0.001$). On the other hand, earlier cluster only: (Wilcoxon signed-rank test, $n = 14$, $p = 0.0009$) was below chance. Also, later cluster: (Wilcoxon signed-rank test, $n = 14$, $p = 0.0015$) was below chance.

Correcting for multiple comparisons

Multiple comparisons correction was done using MVPA-Light toolbox in Matlab[50] and customised scripts. Cluster-based permutation testing was used with sample-specific threshold of 0.05. Permutation test threshold for clusters was 0.05, and 10,000 permutations were calculated.

Data availability

All relevant data generated or analysed are available from the authors upon reasonable request, including the EEG data, behavioural files, all the analyses with MATLAB scripts. Participants private identifications are all anonymized.

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Author contributions

A.C.M.K. and P.A.L. designed the experiment. A.C.M.K collected the data. M.E.A.A analysed the data. M.E.A.A., P.A.L., A.C.M.K. and M.S.T wrote the manuscript.

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