Cueing emotional memories during slow wave sleep modulates next-day activity in the orbitofrontal cortex and the amygdala

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A B S T R A C T

Emotional memories are preferentially consolidated during sleep, through the process of memory reactivation. Targeted memory reactivation (TMR) has been shown to boost memory consolidation during sleep, but its neural correlates remain unclear, particularly for emotional memories. Here, we aimed to examine how TMR of emotional material during slow wave sleep (SWS) impacts upon neural processing during a subsequent arousal rating task. Participants were trained on a spatial memory task including negative and neutral pictures paired with semantically matching sounds. The picture-sound pairs were rated for emotional arousal before and after the spatial memory task. Then, half of the sounds from each emotional category (negative and neutral) were cued during SWS. The next day, participants were retested on both the arousal rating and the spatial memory task inside an MRI scanner, followed by another retest session a week later. Memory consolidation and arousal processing did not differ between cued and non-cued items of either emotional category. We found increased responses to emotional stimuli in the amygdala and orbitofrontal cortex (OFC), and a cueing versus emotion interaction in the OFC, whereby cueing neutral stimuli led to an increase in OFC activity, while cueing negative stimuli led to decreased OFC activation. Interestingly, the effect of cueing on amygdala activation was modulated by time spent in REM sleep. We conclude that SWS TMR impacts OFC activity, while REM sleep plays a role in mediating the effect of such cueing on amygdala.

1. Introduction

Emotional memories are better consolidated than neutral memories, and have been shown to be preferentially consolidated across sleep in some studies (Hu et al., 2006; Groch et al., 2013; Cairney et al., 2014; Nishida et al., 2009; Tempesta et al., 2015; Wagner et al., 2006), but not all (Groch et al., 2013; Ashton et al., 2019; Bolinger et al., 2019; Jones and Spencer, 2019; Pace-Schott et al., 2011; Wiesner et al., 2015). There is also conflicting evidence around which sleep stage plays the main role in this process: while many studies suggest that rapid-eye movement sleep (REM) is important for the consolidation of emotional memories over sleep (Hu et al., 2006; Groch et al., 2013; Nishida et al., 2009; Groch et al., 2015; Menz et al., 2013; Menz et al., 2016; Payne et al., 2012; Wagner et al., 2001), others have implicated slow wave sleep (SWS) (Lehmann et al., 2016), and a synergistic interplay between SWS and REM sleep has also been proposed (Cairney et al., 2014). Given the frequently observed co-morbidity of sleep and affective disorders in humans and rodents (Armitage, 2007; Mellman et al., 2007; Fuller et al., 1997; Kimura et al., 2014), understanding this relationship is of clinical relevance.

The offline neural reactivation of memories is important for consolidation across sleep (Born et al., 2006; Walker, 2009; Bendor and Wilson, 2012), and targeted memory reactivation (TMR) is an elegant tool for exploring the mechanisms of sleep-dependant memory consolidation (Rasch et al., 2007; JD Rudoy et al., 2009; Oudiette and Paller, 2013; Schouten et al., 2017; Hu et al., 2020). TMR involves training individuals to associate specific memories with auditory or olfactory cues and presenting these cues during subsequent sleep with the intention of eliciting memory replay. TMR can be used both in SWS (Rasch et al., 2007) and REM (Rihm and Rasch, April 2015; Sterpenich et al., 2014; Hutchison et al., 2021) to manipulate both neutral (Rasch et al., 2007; JD Rudoy et al., 2009) and emotional memories (Lehmann et al., 2016; Rihm and Rasch, April 2015; Sterpenich et al., 2014; Hutchison et al., 2021; SA Cairney et al., 2014; Groch et al., 2017), however the literature

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is sparse and inconsistent with respect to impacts of TMR on emotional memory. For instance, in one study, TMR during SWS sped up the recall of negative stimuli (SA Cairney et al., 2014), while in another, cueing during non-REM (NREM), but not REM sleep, improved emotional memory consolidation (Lehmann et al., 2016). However, TMR did not impact on fear extinction when applied in either REM or NREM. Instead stimulation decreased subjective arousal ratings for both conditioned and non-conditioned stimuli (Rihm and Rasch, April 2015). More recently, our own report showed that TMR in REM but not SWS leads to a reduction in the subjective arousal ratings of emotional images (Hutchison et al., 2021). The lack of consistency in the literature suggests that further studies are needed to elucidate the effect of TMR during SWS on emotional memory consolidation and arousal processing.

Regions known to be important for emotional memory include amygdala, hippocampus and parahippocampus (Cairney et al., 2014; Rasch et al., 2007; Murty et al., 2010; Van Der Helm and Walker, 2011), insula (Gu et al., 2013; Gasquoine, 2014) and orbitofrontal cortex (OFC) (Rolls, 2019). Activity in the amygdala (Van der Helm et al., 2011) and the hippocampus (Cairney et al., 2014) elicited by emotional experiences has been shown to be modulated by REM sleep, and memory activations experimentally induced using TMR during SWS have been shown to trigger hippocampal activation (Rasch et al., 2007). On the other hand, the insula may represent a key node in the integration of cognition and emotion (Gu et al., 2013; Gasquoine, 2014). Similarly, OFC is thought to play a key role in emotion by representing reward or affective (Rolls, 2019).

To investigate the impact of TMR on neural correlates of memory and arousal, we chose to modify the picture location task that Rudoy and colleagues used in their seminal demonstration that TMR leads to superior retrieval of spatial locations after a nap (JD Rudoy et al., 2009). We previously modified this task to include emotional memory (SA Cairney et al., 2014), showing that SWS duration and spindles predict faster memory judgments for negative, but not neutral, picture locations after TMR. In the current report, we set out to extend this work by examining the neural correlates of these effects as well as any impacts of TMR on arousal ratings (Fig. 1). We chose a 12-hour time interval in keeping with much of the existing literature. We also included a one-week follow-up because we have found that TMR effects often evolve over time (Rakowska et al., 2021). We hypothesized there would be a stronger overnight decrease in arousal and a concomitant increase in
consolidation following TMR for negative compared to neutral memories, and furthermore that the amygdala, hippocampus, parahippocampus, OFC and insula would show stronger activation for negative items when compared to neutral ones both in the arousal and the memory tasks.

2. Methods

2.1. Participants

Thirty healthy participants aged 18–37 years (mean age: 24.43 (SD ± 5.69)) took part in this study. All participants were female, right-handed, reported consistent sleep/wake cycles for a month prior to the study, had no history of any neurological, psychiatric or sleep disorders, and abstained from caffeine and alcohol for 24 h prior to and during each study session. The choice of only female participants was based on previous research suggesting that women, compared to men, find negative content to be more arousing, remain engaged with it for a longer period, and use different strategies to regulate emotions even at a neural level (Gard and Kring, 2007; Moriguchi et al., 2014; Spalek et al., 2015; Whittle et al., 2011). Seven participants were excluded due to technical errors during the targeted memory reactivation (TMR) procedure, three participants were excluded due to missing fMRI data (arousal rating task n = 20) and another two were excluded from the memory task due to multiple movement artifacts in the MRI scans (memory task n = 18). All participants gave written informed consent and were compensated for their participation. This study was approved by the University of Manchester research ethics committee.

2.2. Stimuli

The stimuli, 72 pictures and 72 sounds, were the same as in one of our group’s previous studies on emotional memory (SA Cairney et al., 2014). Half of the pictures were of negative valence and the other half neutral. The sounds were matched semantically to the content of each picture (SA Cairney et al., 2014), for example, the sound car crash was paired with a picture of a wrecked car, and both elicited equivalent arousal levels. All of the pictures were selected from the International Affective Pictures System (IAPS) (Bradley and Lang, 2017) and all of the sounds from the International Affective Digitized Sounds (IADS) battery (Bradley and Lang, 2009). Each sound was 6 s long. Both sets of stimuli differed significantly on valence and arousal ratings, with negative stimuli being more arousing.

Before starting the experimental tasks, a separate neutral noise was played to determine the appropriate sound volume level. Sounds were delivered through over ear noise cancelling headphones (Sony MDR-ZX110NA) during the experimental tasks in the sleep lab, through PC speakers (Dell A425) during sleep, and through an MR compatible noise-cancelling headphone unit developed by MR Confon (http://www.mr-confon.de/) inside the MR scanner. Brown noise was played throughout the night to minimize noise-induced arousals. To ensure that the sound stimuli would not arouse participants, they were further modified using the program Audacity in order to fade in and out for the first and last two seconds, respectively. Additionally, right before sleep, participants were asked to adjust the volume of a sample sound to a level that they believed would not arouse them during sleep, but would still be audible above the brown noise.

2.3. Questionnaires

All participants answered the Karolinska Sleepiness Scale (KSS (Åkerstedt and Gillberg, 1990)) and the 20-items Positive and Negative Affect Schedule (PANAS (Watson et al., 1988)) four times: at the beginning of the experiment (session 1), after the PSG wire-up (session 2), before entering the scanner (session 3), and before starting the final follow-up session (session 4). Participants also answered the Depression, Anxiety and Stress Scale – 21 items (DASS - 21 Lovibond and Lovibond, 1995), the Edinburgh Handedness Inventory (Oldfield, 1971), and the Morningness-Eveningness Questionnaire (MEQ) at the beginning of the experiment.

2.4. Tasks

For both tasks, the picture-sound paired stimuli were presented in a pseudorandom order, with no more than two stimuli of the same category being presented after each other. Participants had the chance to perform at least one practice round before each task, to learn how to use four keys in order to move a cursor for the arousal ratings or to place the pictures for the memory task. Learning how to move the objects on the screen using four keys added a procedural learning component to both tasks.

All stimuli were presented using E-prime 1.0, on a computer screen with a resolution of 1024 × 768 pixels.

2.4.1. Arousal rating task

Participants were instructed to rate each picture – sound pair using a 9 items Likert scale. A self-assessment manikin (SAM) for arousal (Bradley and Lang, 1994) was presented above the scale. Participants were instructed to decide what rating to give according to their first impression while the stimuli were being presented. The picture was displayed on full screen for as long as the sound duration. They then had a period of 5 s to give their rating (see Fig. 1A). The ratings given at each one of the 4 sessions were used as the outcome measure used to assess arousal.

2.4.2. Spatial memory task

The spatial memory task was adapted from (JD Rudoy et al., 2009). During the learning session, participants were instructed to memorise the location of each picture that was presented at a random screen location, having a grid background as reference, while listening simultaneously to its paired sound. For the training session, the picture would appear in the centre of the screen and its corresponding sound would play. Then, the participants were instructed to move the picture to the location they thought to be correct within 8 s. After 8 s, feedback was provided as the picture moved to its correct location and its associated sound was played again. Participants completed several rounds of training up to a criterion threshold through repeated testing with feedback, following the same protocol used in (JD Rudoy et al., 2009). The testing session was similar to the training session, with the difference of having only one round with all stimuli and no feedback was provided (see Fig. 1B). The error, defined as the distance in pixels from each picture’s correct location to the location assigned by the participant at testing, was taken as the outcome measure used to assess spatial memory.

2.5. Experimental design

The experimental design is depicted in Fig. 1C. Participants gave written informed consent upon arrival to the sleep lab. At least one training round to get acquainted with how to use the keys to move the cursor, they performed the first session of the arousal rating task (t = 0 h). Next, they performed the learning session of the memory task, followed by at least one practice round to learn how to move the picture within the grid, and then they performed the training session of the memory task. Once the training session was finished, the electrodes for the overnight polysomnography (PSG) were placed. Finally, participants performed the first memory testing session and the second sessions of the arousal rating task (t = 3 h).

Participants were then instructed to go to sleep while brown noise was delivered through a pair of speakers. During the night, TMR was applied in SWS, according to the protocol described below. The next morning, participants were woken up after approximately 7 h of sleep,
the PSG electrodes were removed, participants given the opportunity to shower and had a light breakfast before the MRI procedure.

After a short structural scan and a practice round to refresh their memory on how to use the four keys, they performed the arousal rating task a third time, followed by the second retest of the memory task (t = 12 h). Approximately one week later, participants were asked to return to the sleep lab in the morning for a follow-up session. Once again, participants completed the arousal rating task and were retested on the memory task (t = 1 week).

2.6. Targeted memory reactivation protocol

The 36 sounds from each emotion category (negative and neutral, total = 72 sounds) were split into two sets of equal mean valence and arousal, and half of them were cued during sleep (n = 18) while the other half was used as the non-cued control (n = 18). Once the participant had entered SWS for at least one minute, 6 sounds (3 neutral and 3 negative) were played, with 2 s gaps between them. Another 6 sounds would be played after another 2 s, unless there was a sleep arousal or disturbance, until all 18 sounds had been played. In case of an arousal during a cueing period or a sleep stage change, sound playing was stopped and was resumed once the participant was back in SWS. Each individual sound was played 5 times.

2.7. PSG data acquisition and analysis

Polysonmography activity was recorded through an EmblaR N7000™ PSG amplifier using the EmblaR RemLogic™ PSG software version 1.1.0.2057 (Natus Neurology Inc., Middleton, Canada). Silver-silver chloride electrodes were attached to the scalp and face of the participants according to the international 10–20 rule. The scalp electrodes were placed on the standardised locations F3, F4, C3, C4, O1, and O2, and referenced to the contralateral mastoid. The connection impedance was kept under 5 kOhms and the sample rate was 200 Hz. All PSG recordings were scored by two experimenters who were blind to whether and when cueing took place, according to The AASM Manual for the Scoring of Sleep and Associated Events (Iber et al., 2007). All scoring differences were resolved by the one of the experimenters, who directly compared the different scorings given by each experimenter, blind to their authorship, and decided on a final score.

2.8. fMRI data acquisition and analysis

Imaging data were acquired on a 3T Philips Achieva scanner using an eight-element SENSE head coil with a SENSE factor of 2.5. We used a dual-echo sequence with a short TE and long TE of 12 and 35 ms, respectively, and a TR of 2800 ms. The short TE was optimal for reduced signal loss and sufficient contrast sensitivity and the long TE provided whole brain sensitivity (Posner et al., 2006). The functional parameters were: 31 slices, 80 × 80 acquisition matrix, 240 × 124 × 240 mm FOV, in-plane resolution of 3 × 3 mm², slice thickness of 4 mm (no gap) and an anterior-posterior (A-P) phase encoding direction. For the arousal task, 200 vol were collected per scanning session and 244 for the memory task.

A high-resolution T1-weighted structural scan was also acquired using a 3D MP-RAGE pulse sequence, with in-plane resolution of 0.94 mm, slice thickness 0.9 mm, TR = 8 ms, TE = 3.9 ms, and 188 slices. The high-resolution T1-weighted structural image served for co-registration purposes. Each task was split into two functional scans. Each scan had 5 null events, 12 s long for the arousal sessions and 15 s for the memory sessions. Before each picture, a black screen with a fixation cross in its centre was presented for a mean jittered time of 1 second.

Functional volumes were pre-processed and analysed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/; Welcome Department of Imaging Neuroscience, London, UK). First, we merged the data from both echoes by extracting an image volume for each echo and subsequently averaging the short and long echo for each TR. The resulting images were realigned to the first volume and the structural was co-registered to the mean functional image. The structural image was then segmented and the estimated transformations to MNI space were applied to all functional scans. All functional images were smoothed using a Gaussian kernel with a full-width half maximum (FWHM) of 8 × 8 × 8 mm and re-sampled to MNI space, retaining the original voxel dimension of 3 × 3 × 4 mm using 4th degree B-spline interpolation. After re-alignment, co-registration and smoothing, we proceeded with artefact detection. The artefact Detection Toolbox (ART, http://www.nitrc.org/projects/artefact_detect/) was used to estimate per-volume movement outliers using > 3 standard deviations from the mean signal intensity and volume-to-volume movement of 1.5 mm. We excluded a scan if >15% of the number of volumes were classified as outliers. The remaining scans were scrubbed, as a separate regressor for each outlying volume was included in the first-level design matrix, concatenating the resultant time-series (Power et al., 2012).

2.8.1. First-level analysis

The first-level models of the individual task conditions were fit using SPM12. The jittered time between blocks, as well as the null events, served as the baseline condition modelled implicitly in the block design. High-pass filtering was implemented in the design matrix using a cut-off period of 128 s to remove slow drifts from the time series. Movement parameters derived from the realignment of the functional volumes were also included as covariates of no interest. Each condition was modelled using a double-gamma haemodynamic response function (HRF) with time and dispersion derivatives. For each task, we modelled the following blocks: negative cued viewing, neutral cued viewing, negative uncued viewing, neutral uncued viewing, negative cued responding, neutral cued responding, negative uncued responding, and neutral uncued responding. We also modelled all key-presses as events of no interest.

2.8.2. Second-level analysis

For the second-level models, we used the MRM toolbox (McFarquhar et al., 2016) to specify a repeated measures model with within-subject factors of cueing (cued and non-cued levels) and emotion (negative and neutral levels). This technique allows for the unconstrained modelling of the variance-covariance structure of the data per-voxel, unlike SPM which assumes a single structure for the whole brain. Therefore, MRM should allow for more valid inference. The analysis was done voxel-by-voxel, applying a multivariate general linear model (GLM). For the arousal task, we used the first-level contrasts from the viewing blocks and for the memory task from the responding blocks, to build the repeated-measure contrasts of main effects and interactions. Thresholding was performed at the voxel level using 5000 permutations to generate p-values that were corrected using the false discovery rate (FDR) procedure at p < 0.05. The multivariate test statistic used was Wilk’s lambda. Further analyses were run for each task using as covariates the mean centred duration of SWS and REM in minutes. We did not use both stages as covariates in the same model to avoid collinearity issues (Mumford et al., 2015).

For both tasks, we conducted 2nd level inference restricted by a number of a priori defined regions of interest (ROI) using small volume correction. Masks were created for each individual ROI using the integrated AAL atlas (Tzourio-Mazoyer et al., 2002) of the Wake Forest University Pick Atlas toolbox (http://fmr.i.wfu.edu/software/PickAtlas). The ROIs included in this study consisted of the bilateral OFC, insula, hippocampus, parahippocampus and amygdala. These regions were selected from previous studies that have reported activations due to sleep, memory consolidation or emotional reactivity (Rasch et al., 2007; Van Der Helm and Walker, 2011; Gu et al., 2013; Gasquione, 2014; Rolls, 2019; Aguirre et al., 1996). For whole brain results, please check the supplement (Table S3). Significant clusters were subsequently manually thresholded at a minimum of 10 voxels, which has been shown to
provide an optimal balance between sensitivity and false-positive rate (Radua et al., 2012).

2.9. Statistical analyses

All statistical analyses of the behavioural data were done in JASP 0.10.2.0 (https://jasp-stats.org/) and GraphPad Prism 9 (https://www.graphpad.com/scientific-software/prism/). Mixed analyses were conducted to assess any session, cueing or emotion effects on arousal and on performance in the spatial memory task. The fixed effects were cueing (cued or non-cued), emotion (negative or neutral), and session (0 h, 3 h, 12 h and 1 week for the arousal ratings and 3 h, 12 h and 1 week for the spatial memory task, included as a factor, not as a continuous variable) and the subjects were defined as the random effects. Significant main effects were further investigated with post-hoc tests, corrected for multiple comparisons by controlling the FDR with the two stage step up method of Benjamini, Krieger and Yekutieli (Benjamini et al., 2006). Statistical analyses of the fMRI data were conducted using the MRM toolbox as described above in the second-level analysis subsection of the fMRI data acquisition and analysis heading. All analyses were conducted at a 0.05 significance level.

3. Data availability statement

The raw behavioural data and the fMRI data used for the statistical analysis in MRM are available at: https://tinyurl.com/PereiraNeuroimage (password: @PEREIRA2021).

4. Results

4.1. Arousal ratings

A mixed effects model was used to analyse the arousal ratings, with fixed effects session (0 h, 3 h, 12 h and 1 week), cueing (cued and non-cued) and emotion (negative and neutral), and with the subjects as the random effect (see Fig. 2). There was no cueing effect ($F_{(0.8, 30.84)} = 1.068; p = 0.293$), and no interactions: session x cueing ($F_{(2.7, 100.9)} = 0.845; p = 0.461$), session x emotion ($F_{(3, 114)} = 1.018; p = 0.388$), cueing x emotion ($F_{(1, 38)} = 0.305; p = 0.584$) and session x cueing x emotion ($F_{(3, 114)} = 0.176; p = 0.913$). The results showed effects of session ($F_{(3, 114)} = 2.824; p = 0.042$) and emotion ($F_{(1, 38)} = 131.6; p < 0.0001$). Post-hoc tests revealed a significant decrease in arousal across the learning period ($t = 3.720; P_{FDR} = 0.002$), i.e. from session 1 (0 h; mean = 4.889) to session 2 (3 h; mean = 4.592).

There was a trend towards a significant increase in arousal overnight (3 h; mean = 4.592; 12 h; mean = 4.710; $t = 1.951; P_{FDR} = 0.08$) and from session 2 (3 h mean = 4.592) to session 4 (1 week; mean = 4.754; $t = 1.894; P_{FDR} = 0.081$).

4.2. Functional imaging of the arousal rating task

We used a repeated measures ANOVA with factors cueing (cued and non-cued) and emotion (negative and neutral) to examine the neuroimaging data in our ROIs (amygdala, hippocampus, parahippocampal, insula, and OFC). This showed a main effect of emotion in the amygdala and OFC (Table 1 and Fig. 3). In the amygdala, negative items elicited greater activation than neutral items, while in the OFC, the opposite was true.

We also found a cueing x emotion interaction in the OFC (Table 2 and Fig. 4).

To further investigate this interaction, we created a mask using only the significant clusters listed in Table 2 and ran a simple main effect analysis of cueing for the neutral and negative items. Significant clusters are listed in Table 3 and depicted in Fig. 4.

Parameter estimates from the peak clusters on each side of the OFC were plotted for the two valence categories in both the cued and non-cued conditions (Fig. 4). Note that responses for negative items were plotted in red-orange, while responses for neutral items were plotted in blue-green, and parameter estimates are shown on the right. Interestingly, cueing decreased activity in the OFC in response to negative items and increased it in response to neutral items.

Next, we investigated whether cueing effects were modulated by the duration of SWS or REM, as shown in other studies (Cairney et al., 2014; Tamminen et al., 2017). To this end, we added the amount of time spent in each sleep stage as a covariate and re-ran the second-level analysis. This revealed a significant interaction between cueing...
Table 1
Main effect of emotion (negative > neutral) in the amygdala and OFC.

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Side</th>
<th>Cluster size</th>
<th>F-value</th>
<th>q-value (FDR - voxel)</th>
<th>MNI (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala Right</td>
<td>24</td>
<td>27.671</td>
<td>0.012</td>
<td>24 -4 -18</td>
<td></td>
</tr>
<tr>
<td>Amygdala Left</td>
<td>10</td>
<td>11.243</td>
<td>0.028</td>
<td>-18 -4 -18</td>
<td></td>
</tr>
<tr>
<td>OFC Left</td>
<td>25</td>
<td>24.027</td>
<td>0.034</td>
<td>-36 41 -6</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Main effect of emotion in the amygdala and OFC. On the left, activity in the orbitofrontal cortex is depicted in green in an axial slice at z = −10. In the middle, activity in the amygdala in depicted in blue in a coronal slice at y = −4. Similarly, on the right, activity in the amygdala is depicted on a sagittal slice at x = 21. Coordinates are in MNI space. p < 0.05, FDR-corrected.

Table 2
Cueing x Emotion interaction (cued negative – cued neutral) – (non-cued negative- non-cued neutral) in the OFC.

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Side</th>
<th>Cluster size</th>
<th>F-value</th>
<th>q-value (FDR - voxel)</th>
<th>MNI (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFC Left</td>
<td>95</td>
<td>20.966</td>
<td>0.024</td>
<td>-51 23 -6</td>
<td></td>
</tr>
<tr>
<td>OFC Right</td>
<td>84</td>
<td>22.893</td>
<td>0.024</td>
<td>36 29 -18</td>
<td></td>
</tr>
<tr>
<td>OFC Right</td>
<td>19</td>
<td>18.626</td>
<td>0.024</td>
<td>24 50 -14</td>
<td></td>
</tr>
<tr>
<td>OFC Right</td>
<td>19</td>
<td>14.391</td>
<td>0.024</td>
<td>21 53 -10</td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Simple main effect of cueing on Neutral and Negative items.

<table>
<thead>
<tr>
<th>Side</th>
<th>Cluster size</th>
<th>F-value</th>
<th>q-value (FDR - voxel)</th>
<th>MNI (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral OFC Right</td>
<td>48</td>
<td>18.093</td>
<td>0.032</td>
<td>30 26 -22</td>
</tr>
<tr>
<td>OFC Left</td>
<td>30</td>
<td>14.275</td>
<td>0.032</td>
<td>-30 26 -18</td>
</tr>
<tr>
<td>OFC Left</td>
<td>21</td>
<td>10.83</td>
<td>0.033</td>
<td>-42 17 -6</td>
</tr>
<tr>
<td>OFC Right</td>
<td>12</td>
<td>11.25</td>
<td>0.032</td>
<td>21 53 -14</td>
</tr>
<tr>
<td>OFC Right</td>
<td>12</td>
<td>7.206</td>
<td>0.041</td>
<td>30 44 -14</td>
</tr>
<tr>
<td>Negative OFC Left</td>
<td>61</td>
<td>18.734</td>
<td>0.043</td>
<td>-39 29 -10</td>
</tr>
<tr>
<td>OFC Right</td>
<td>48</td>
<td>9.964</td>
<td>0.046</td>
<td>39 32 -18</td>
</tr>
</tbody>
</table>
and time spent in REM sleep in the left amygdala, \(F = 16.857\), q-value (FDR-voxel) = 0.028, cluster size = 12, \([x y z] = -18 2-18\); Fig. 5), suggesting that time spent in REM sleep modulated the effect of cueing in this structure. No effect was found when taking into account time spent in SWS.

4.3. Spatial memory performance

We used a mixed effects model to analyse the error rate on the spatial memory task, with fixed effects session (3 h, 12 h and 1 week), cueing (cued and non-cued) and emotion (negative and neutral), and with the subjects as the random effect (see Fig. 6). This showed a significant session effect \((F_{[2, 60]} = 60.04, p < 0.001)\), with post-hoc tests revealing a significant increase in the error rate (i.e., more forgetting, as expected) across the night (3 h vs 12 h, \(t = 6.626, q < 0.0001; p < 0.0001\)), during the week (12 h vs 1 week, \(t = 10.96, q < 0.0001; p < 0.0001\)) and across the entire experiment (3 h vs 1 week, \(t = 8.302, q < 0.0001; p < 0.0001\)). No other significant effects or interactions were found (all \(p > 0.1\)).

4.4. Functional imaging of the spatial memory task

We analysed the imaging data from the spatial memory task with the exact same procedure used for the arousal rating task. This showed no main effect of cueing, emotion or interaction between these in any of the ROIs investigated, with or without sleep parameters as co-variates (time in SWS and REM).

5. Discussion

In line with our hypotheses, this study shows changes in brain activity in the OFC and amygdala during an arousal rating task as a function of targeted memory reactivation during SWS. Interestingly, TMR modulated OFC activity in a valence-specific manner: cueing neutral items increased OFC activity, while cueing negative items decreased it. In addition, the effect of cueing on amygdalar activation was greater when more time was spent in REM sleep. Despite these differences in brain activity, and contrary to our predictions, no change was found in subjective measures of arousal in response to re-exposure to auditory cues during sleep. Similarly, neither brain activity nor accuracy on the spatial memory task was influenced by either stimulus valence or TMR. We discuss these findings below.

5.1. Sleep, TMR and subjective arousal ratings

We found no effect of overnight sleep, with or without TMR, on subjective arousal ratings either 12 h post-learning or 1 week later. This is in line with a number of studies which have shown that sleep has no effect on arousal (Groch et al., 2013; Ashton et al., 2019; Bolinger et al., 2019; Jones and Spencer, 2019; Pace-Schott et al., 2011; Wieser et al., 2015). For example, no differences were found in subjective arousal ratings of negative and neutral pictures over a 2 h nap, over a period of wakefulness (Pace-Schott et al., 2011), or between groups (nap vs wake)
ings of negative and neutral stimuli (Lehmann et al., 2016). Furthermore, our own recent work shows unchanged arousal ratings following SWS TMR (Hutchison et al., 2021). Interestingly, however, SWS TMR has been shown to promote both decreased (Hauner et al., 2013; He et al., 2015), and increased fear expression (Ai et al., 2015) in fear conditioning paradigms, as assessed by skin conductance response (SCR). Furthermore, TMR in both NREM and REM sleep led to an unspecified decrease in arousal for both negative and neutral sounds in a Pavlovian conditioning study (Rihm and Rasch, April 2015). Raises the question of whether the effects of TMR on arousal are task-dependent (conditioning vs arousal rating and emotional memory), sleep stage dependent (NREM/SWS or REM), or both.

Notably, autonomic measures of arousal and habituation, skin conductance response (SCR), heart rate deceleration (HRD) and corrugator superciliar electromyography response (EMG) have all been shown to be influenced by sleep (Ashton et al., 2019; Bolinger et al., 2019; Jones and Spencer, 2019; Pace-Schott et al., 2011; Hauner et al., 2013; He et al., 2015). Therefore, we cannot exclude the possibility that even though it does not appear to impact upon subjective arousal ratings, TMR may still have had an impact on autonomic markers of arousal, given that our experimental design did not address this question.

5.2. Sleep, TMR and spatial memory

As expected, performance on the spatial memory task employed in this study deteriorated across sessions. This effect was not modulated by stimulus valence (negative or neutral), in line with results from a meta-analysis of 20 datasets and 785 participants showing that there is no overall effect for preferential sleep-dependent consolidation of emotional over neutral material (Lipinska et al., 2019). Surprisingly, direct comparison of cued and non-cued items revealed no differences. This null finding is out of line with prior work on neutral memory, where TMR has consistently been shown to be beneficial (Hu et al., 2020; JD Rudoy et al., 2009). However, our null finding could be driven by the fact that we included emotional stimuli. Prior examinations of TMR on emotional memories have frequently shown null effects (Rihm and Rasch, April 2015; SA Cairney et al., 2014; Ashton et al., 2017), a result supported by a meta-analysis showing no effect of TMR on emotional memories when analysing data from 5 separate studies and 97 participants (Hu et al., 2020). For example, one report found no effect of SWS TMR on emotional memory recognition (Ashton et al., 2017), and another showed that TMR in SWS improved reaction times but not memory accuracy for the spatial locations of emotional pictures (SA Cairney et al., 2017).

![Fig. 5. Activity in the left amygdala in the cueing x REM contrast. One significant cluster was found in the left amygdala. Frontal view of a coronal MRI slice is depicted. Coordinates are in MNI space. p < 0.05, FDR-corrected.

![Fig. 6. Performance on the spatial memory task in each group across time, as assessed by the error rate; Violin plots in blue depict ratings for the negative items, while grey plots depict ratings for the neutral items. Colour-filled plots depict data from the cued items and white-filled plots depict the non-cued items. Black dots represent data from individual subjects. As expected, memory for the exact location of each item deteriorated across time, resulting in increased error rates (session effect: F(2, 80) = 60.04, p < 0.001).](image-url)
et al., 2014). Thus, the inclusion of emotional memories may somehow inhibit the usual benefit of TMR even for neutral memories. The fact that our participants were performing the task in the MRI scanner, and using a button box to move the images on-screen, as opposed to the joystick used in prior studies, could also have muted the expected memory effects. Our use of only female participants may also have been a factor, since the effect of sleep on the encoding of emotional material has been shown to be more pronounced in studies using men-only samples (Lipinska et al., 2019).

5.3. Functional imaging during the arousal rating task

5.3.1. Main effect of emotion

We found a main effect of emotion in the OFC and amygdala. This is in line with extensive literature implicating the amygdala in emotional processing (Murty et al., 2010; Gallagher and Chiba, 1996; LeDoux, 2000; Phelps and LeDoux, 2005; Buchanan, 2007) and the OFC in the subjective emotional experience of affective stimuli (Bechara et al., 2000; Rolls and Grabenhorst, 2008). We did not, however, find activation in the insula, hippocampus or parahippocampus. This is consistent with a study of the neural correlates of arousal and valence in panic disorder, where, no significant results were found in the healthy control group when correcting for multiple comparisons at the group level (Wade-Bohleber et al., 2020). These regions may be more involved in processes not specifically targeted by the task we applied.

5.3.2. Cueing and emotion interaction

We found a main effect of emotion and a cue x emotion interaction in the OFC. Interestingly, cueing had opposite effects on OFC activity depending on stimulus valence: while negative cued items saw a reduction in OFC activity, neutral cued items saw an increase. A similar result has been reported in the amygdala, where the effect of arousal depended on stimuli valence (Mickley Steinmetz et al., 2010). In that study, participants were exposed to negative and positively valenced stimuli of both high and low arousal levels in the fMRI scanner. In high arousal negative items, the strength of amygdala projections to the inferior frontal gyrus (IFG) and the middle occipital gyrus (MOG) increased when compared to the low arousal negative items; whereas the strength of these same projections was decreased for high arousal, compared to low arousal, positive stimuli (Mickley Steinmetz et al., 2010). These authors also show that arousal had a more widespread effect for negative items, modulating connectivity between other nodes in the emotional memory network such as the hippocampus and fusiform (Mickley Steinmetz et al., 2010). Amygdala and OFC communicate through bilateral projections (Frank et al., 2019), so it is possible that connectivity between these two areas was also differently affected by stimulus valence in our study.

It is unclear why reactivating neutral items would cause an increase in OFC activation, but one potential explanation relates to generalization. TMR has been shown to promote generalization and integration of information (Sterpenich et al., 2009; Schechtman et al., 2021; Oudiette et al., 2013), so by mixing neutral and negative items within the same learning context we may have facilitated the formation of a schema including both categories of affective stimuli. Thus, cueing may have promoted generalization of emotional tone across categories that decreased arousal in negative items and increased it in neutral items, as our amygdala imaging data seems to suggest. However, given that our behavioural results failed to show an effect of cueing on subjective arousal, this proposal remains speculative. Including autonomic measures of arousal in future TMR studies could shed further light on this issue.

5.3.3. Cueing and REM sleep

Cueing during SWS modulated activity in the amygdala when controlling for time spent in REM sleep. This finding is in line with the sequential hypothesis (Giuditta, 2014) and with studies showing that SWS and REM play complementary roles in an intricately orchestrated series of events leading up to memory consolidation, generalization and integration (Cairney et al., 2014; Groch et al., 2015; Tamminen et al., 2017; Lewis et al., 2018; Pereira and Lewis, 2020; Ackermann and Rasch, 2014). Our own work has shown that TMR of procedural information in SWS can impact functional responses in a manner that is modulated by time spent in REM (Cousins et al., 2016). We have similarly shown that TMR of lexical information in SWS can impact on a behavioural task in a manner that is modulated by REM (Tamminen et al., 2017). It is still unclear how SWS and REM collaborate to tackle the challenge of emotional memory consolidation, in which emotional tone and is decreased, and memory is simultaneously strengthened (Van Der Helm and Walker, 2011). However, our current observation that TMR of an emotional task in SWS impacts on amygdala activity in a manner that is modulated by time in REM once again demonstrates that there is an interaction between the two stages, and extends this finding to the domain of emotional memory.

Despite the apparent involvement of SWS in emotional memory consolidation (Lehmann et al., 2016; SA Cairney et al., 2014), there is also extensive evidence for a role of REM in such processing emotional (Wiesner et al., 2015; Lipinska et al., 2019; Walker and van der Helm, 2009; Wassing et al., 2019; Marquis et al., 2017). Indeed, the involvement of REM in emotional processing is the central tenet of the Sleep to Forget Sleep to Remember (SFSR) hypothesis (Van Der Helm and Walker, 2011). Furthermore, an elegant study of self-conscious emotional experience recently found a direct link between REM sleep integrity and amygdala reactivity (Wassing et al., 2019). Thus, amygdala reactivity decreased overnight in proportion to the duration of consolidated REM sleep. In contrast, restless REM sleep prevented amygdala adaptation. The authors replicated this finding by using TMR to enhance both the beneficial effect of uninterrupted REM and the detrimental effect of restless REM sleep (Wassing et al., 2019). Taken together with our current result, these findings suggest that the effect of TMR on amygdala reactivity depends on not only REM time, but also the specific architecture of REM sleep.

5.4. Functional imaging during the spatial memory task

We found no effect of either emotion or cueing on brain activity during the spatial memory task in our chosen regions of interest, namely the amygdala, hippocampus, parahippocampus, OFC and insula. This could be due to the timing at which scanning took place: 12 h after learning, since recent studies suggest that the effects of TMR during sleep may take several days to emerge (Bolinger et al., 2019; Groch et al., 2017; Koopman et al., 2020; Cairney et al., 2018). Similarly, the Sleep to Forget Sleep to Remember hypothesis posits that the process of de potentiation of the emotional tone while preserving the memory trace may require multiple nights of sleep to complete (Van Der Helm and Walker, 2011). Future studies on the neural correlates of emotional memory retrieval include follow up functional scans at different time points after learning.

5.5. Limitations

This study has a few limitations worth bearing in mind: the high number of datasets that needed to be excluded from analysis rendered our final sample size small (n = 20 for the arousal task and n = 18 for the memory task). In addition, due to the fact that males rarely rated our stimuli as highly arousing, we included only women in the study so it is unclear whether these findings can be extrapolated to men. Finally, only subjective, self-reported measures of arousal were assessed, so the fate of autonomic measure of arousal following SWS TMR remains to be determined.

6. Conclusion

In conclusion, this study shows that targeted memory reactivation during SWS results in altered OFC and amygdala activity during subse-
quent arousal ratings 12 h after encoding. This effect is modulated by time spent in REM sleep and stimuli valence. These findings begin to unravel the neural correlates of experimentally induced reactivation of emotional memories during sleep. In light of the worldwide high prevalence of mental health disorders characterized by dysfunctional emotional processing such as PTSD, depression, generalized anxiety disorder and panic disorder, studies looking at non-invasive, relatively simple, and safe interventions such as this one pave the way towards the development of new strategies to promote wellbeing.

Credit authorship contribution statement

Sofia Isabel Ribeiro Pereira: Formal analysis, Visualization, Writing – original draft, Writing – review & editing. Maria-Efstratia Tsimpanouli: Conceptualization, Investigation, Formal analysis, Writing – original draft. Isabel Hutchison: Investigation, Writing – review & editing. Jules Schneider: Investigation, Writing – review & editing. Ian M. Anderson: Conceptualization, Supervision, Writing – review & editing. Martyn McFarquhar: Conceptualization, Supervision, Formal analysis, Writing – review & editing. Rebecca Elliott: Conceptualization, Supervision, Writing – review & editing. Penelope A. Lewis: Project administration, Funding acquisition, Conceptualization, Supervision, Writing – review & editing.

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Data availability statement

The raw behavioural data and the fMRI data used for the statistical analysis in MRM are available at: https://tinyurl.com/PereiraNeuroimage (password: @PEREIRA2021).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2022.119120.

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