Targeted memory reactivation of newly learned words during sleep triggers REM-mediated integration of new memories and existing knowledge

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Abstract

Recent memories are spontaneously reactivated during sleep, leading to their gradual strengthening. Whether reactivation also mediates the integration of new memories with existing knowledge is unknown. We used targeted memory reactivation (TMR) during slow-wave sleep (SWS) to selectively cue reactivation of newly learned spoken words. While integration of new words into their phonological neighbourhood was observed in both cued and uncued words after sleep, TMR-triggered integration was predicted by the time spent in rapid eye movement (REM) sleep. These data support complementary roles for SWS and REM in memory consolidation.

Keywords: Memory; sleep; memory consolidation; targeted memory reactivation
1. Introduction

Sleep may be the optimal brain state for consolidating new information in memory (Diekelmann & Born, 2010). According to the Complementary Learning Systems (CLS) account of memory (McClelland et al., 1995), representations of recent memories are initially mediated by the hippocampus and recalled independently from neocortical memories. Gradually the nature of these representations changes such that the role of the hippocampus decreases and the emerging neocortical representation becomes stronger and allows the new information to be integrated with existing knowledge. This neocortical consolidation relies on neural replay of new memories during sleep (O’Neill et al., 2010). Neural populations or areas of the brain that were active during encoding become spontaneously reactivated during subsequent rest or sleep (Wilson & McNaughton, 1994; Maquet et al., 2000), and the extent of this reactivation predicts overnight improvement in performance (Peigneux et al., 2004).

Reactivation during sleep can also be cued externally. Rasch et al. (2007) created an association between new information and an odour during encoding. Cueing the new memories during slow-wave sleep (SWS) with the odour resulted in enhanced memory performance. While the odour was used to cue all of the new information, cueing can also be targeted to apply to selected memories. Rudoy et al. (2009) carried out targeted memory reactivation (TMR) by having participants learn picture-locations and associating each picture during learning with a unique sound. Playing a targeted set of the sounds was found to selectively benefit the memories associated with those sounds (Antony et al., 2012; van Dongen et al., 2012; Cairney et al., 2014).

While TMR can strengthen declarative memory, little is known about the effects of TMR on other forms of learning postulated by the CLS account. For example, integration of new memories with existing knowledge should occur during consolidation and therefore...
benefit from TMR. Language learning studies have shown that the integration of new words in the mental lexicon involves a central role for sleep. Newly learned spoken words (e.g., *cathedruke*) begin to compete in a word recognition task with similar-sounding existing words (e.g., *cathedral*) after a night of sleep but not after an equivalent period of wake, suggesting that the new words became lexically integrated in their neocortical phonological neighbourhood only after sleep (Dumay & Gaskell, 2007; Davis et al., 2009). Tamminen et al. (2010, 2013) showed that this integration was associated with sleep spindle activity. This suggests that sleep-associated neural replay may be involved in lexical integration, given that sleep spindles are temporally correlated with the occurrence of hippocampal ripples (Siapas & Wilson, 1998; Sirota et al., 2003), which reflect hippocampal replay of newly acquired memories (Girardeau et al., 2009).

Here, we sought to establish the role of TMR on the integration of new words in the mental lexicon. If neural replay during sleep allows the integration of newly acquired information with existing knowledge, we expected TMR to facilitate lexical integration of novel words. TMR however may also enhance the contribution of different sleep stages to consolidation, including stages other than the one in which TMR was applied. Cousins et al. (2016) trained participants on two serial reaction time task sequences and cued one of them during SWS. Cueing-related changes in neural activation were modulated by time spent in SWS and by time spent in rapid eye-movement (REM) sleep, suggesting that cueing using TMR may engage multiple stages of sleep in the consolidation process. We therefore also investigated the association between different sleep stages and behavioural change when cueing was present and not present.
2. Method

2.1 Participants

We trained and tested 20 native English speaking students (4 males, mean age = 19.3 years) on novel words, followed by a nap during which half of the trained words were cued (sleep group). Another group of 20 participants (6 males, mean age = 19.7 years) remained awake and received no TMR (wake group).

2.2 Design and Stimuli

The experiment was run in one continuous session consisting of several phases. Participants were first wired up for the polysomnography (PSG) recording. They were then trained on novel spoken words and their meanings. Immediately after training they carried out the first test session. This started with three tasks measuring learning of word forms: free recall, lexical competition, and old-new categorisation. Tasks measuring learning of word meanings followed. Here we focus on word forms; the meaning tasks did not show effects of sleep vs. wake or of TMR, and are reported in supplementary materials.

At the end of the first test session, participants were told whether they were taking part in the sleep or the wake condition. Wake group watched films with no language input for 90 minutes. Sleep group were asked to take a nap and woken up 90 minutes after lights off time. This sleep/wake period started between 12.30pm and 1.30pm. Once the participant was in SWS, half of the trained novel words were cued by playing them once through loudspeakers located in the bedroom, integrated into unobtrusive background brown noise presented throughout the nap. If the participant woke up during cueing, they were removed from the data analysis.

68 stimulus triplets consisting of a familiar base word (e.g., cathedral), a fictitious novel word (e.g., cathedruke), and a similar-sounding nonword foil to be used in the old-new
categorisation task (e.g., cathe druce) were selected from Tamminen and Gaskell (2008). Base words were bisyllabic or trisyllabic, 6-11 phonemes in length (M= 8.0), and with CELEX frequency in the 2-18 occurrences per million range (M=4.3; Baayen et al., 1995). Triplets were divided into two lists of 34, matched in number of syllables, length, and frequency. For each participant, one of the lists was used for training, and the base words from the other list remained untrained and acted as control words in the lexical competition task. This was counterbalanced. The lexical competition task also required 68 filler words which ensured that only 25% of real words encountered in the task were base words, making it unlikely that participants became aware of the relationship between base words and the phonologically overlapping novel words. The fillers were monosyllabic (N=46), bisyllabic (N=10), or trisyllabic (N=10) and had slightly higher frequency to the base/control words (M=11.0, 11.4, 11.5 respectively). 136 word-like nonwords were created by changing one phoneme of a real word (not used in the other conditions).

2.3 Procedure

2.3.1 Training session. A phoneme monitoring trial started with visual presentation of a target phoneme, followed by auditory presentation of a novel word. Participants indicated with a keypress whether the target was present in the word. In the meaning matching task a novel word was presented visually and auditorily simultaneously. Below, a candidate meaning was presented which was correct 50% of the time. Participants indicated with a keypress whether they thought the meaning was correct. Feedback was given, and the correct meaning was presented. In the cued recall task participants heard a novel word and saw it on the screen. They had to recall the meaning of the word and type it in within 30s. The correct meaning was then presented. The meaning was always unrelated to the meaning of the base word from which the novel word was derived.
Participants first completed one block of phoneme monitoring where each word was encountered six times. One block of the meaning matching task then followed, where each word was encountered twice. Next, a block of cued recall was completed where each novel word was encountered once. This sequence of the three training tasks was then repeated twice, thus giving a total of 27 exposures to each novel word across all tasks. Participants were aware that they would be tested on memory for the novel words.

2.3.2 Test session. After training participants filled in the Stanford Sleepiness Scale and performed the first test session. There were no significant differences in sleepiness between the wake group and the sleep group at the beginning of this (p=.52) or the second test session (p=.14). In the free recall task participants were given three minutes to recall as many novel words as possible. In the lexical competition task participants were presented with a stimulus through headphones; this could be one of the base words (e.g., cathedral), one of the control words (e.g., dolphin), one of the filler words, or one of the nonwords, in random order. Participants made lexical word/nonword decisions with a keypress. In the old-new categorisation task a word was presented over the headphones, and participants indicated whether the word was a trained word (“old”) or a similar-sounding foil (“new”). Stimuli were presented in a pseudorandom order such that at least four trials separated the occurrence of a novel word and its related foil.

An Embla N7000 system recorded PSG. Six scalp electrodes (F3, F4, C3, C4, O1, O2) were used with contralateral mastoid references. Two electro-oculographic channels monitored eye movements and two chin electromyographic channels monitored muscle tone. 30-second epochs of sleep data were scored into different sleep stages following the AASM scoring criteria (Iber et al., 2007). Number of sleep spindles was detected during Stage 2 sleep and SWS using the algorithm developed by Ferrarelli et al. (2007).
3. Results

3.1 Behavioural results

In the free recall task we found a significant interaction between test session and group, $F(1,38)=6.44$, $p=.015$ (Figure 1A). Main effect of group was not significant. In the wake group recall rates declined significantly from the first test to the second test, $t(19)=4.20$, $p<.001$, while in the sleep group there was no significant decline, $t(19)=1.30$, $p=.21$. This suggests that sleep protected memory from forgetting while wake did not, even when the wake contained no interfering language input. Recall levels in the sleep group however were lower than in the wake group in Session 1. To ensure the absence of significant forgetting was not due to this baseline difference, we carried out a reanalysis on groups matched in Session 1 performance. The matched sleep group still showed no forgetting ($p=.21$) while the wake group did ($p=.001$). TMR had no impact on recall (Table 1).

Figure 1. Panel A shows mean percentage of words recalled in the free recall task. Panel B shows the average magnitude of the lexical competition effect, calculated by deducting reaction times (RTs) to control words (e.g., dolphin; words with no new competitor) from
RTs to base words (e.g., cathedral; words with a new competitor). Panel C shows d-prime values and mean RTs in the old-new categorisation task. Error bars indicate standard error. S1 = first test session, S2 = second test session, ns = not significant (p > .05).

In the lexical competition task reaction times (RTs) to base words (familiar words with a novel competitor) and control words (familiar words with no new competitors) showed a significant interaction between this word condition and test session, F(1,38)=5.75, p=.02. (Figure 1B). In the first test session there was no significant difference between the word conditions, t(39)=-1.09, p=.28, but in the second session RTs to base words were significantly slower than RTs to control words, t(39)=2.35, p=.02, indicating the emergence of lexical competition. The absence of any main effect or interaction involving the group factor shows the emergence of the competition effect was statistically similar for the sleep and wake groups. TMR produced no statistically significant changes in the competition effects (Table 1).

No effects of sleep vs. wake group, or TMR were found on RTs in the old-new categorisation task. To take response bias into account, accuracy was analysed by calculating signal detection measures (d’). A significant main effect of test session, F(1,38)=33.57, p<.001, reflected an overall decline in accuracy from the first test to the second (Figure 1C, Table 2). There were no effects of sleep vs. wake group or TMR (Table 1) on accuracy.

3.2 PSG results

To determine whether there was a relationship between time in specific sleep stages (Table 3) and consolidation effects we calculated correlations between percentage of time spent in different sleep stages and the change in performance over the nap (Table 4). The Benjamini-Hochberg (1995) procedure for controlling false discovery rate was used to take
into account multiple comparisons\(^1\). Critically, larger increases in lexical competition were associated with more time spent in REM, \(r=0.47, p=0.037\).

To find out if this REM association, which has not been reported in the lexical competition paradigm before, was a consequence of TMR, we investigated the association separately in cued and uncued words. In cued words, change in lexical competition was strongly correlated with time spent in REM, \(r=0.59, p=0.006\), but in uncued words the change was not correlated with REM, \(r=0.03, p=.89\). Importantly, this correlation was statistically significantly stronger in cued words than in uncued words, \(t(17)=2.19, p=.04\) (Weaver & Wuensch, 2013) (Figure 2). No sleep stage was associated with change in the free recall or old-new categorisation tasks (Table 4). Number of sleep spindles was not significantly correlated with any behavioural measure.

\(A\) Cued words
\(B\) Uncued words

**Figure 2.** Correlation between change in the magnitude of the lexical competition effect and percentage of sleep spent in REM for cued words (A) and uncued words (B).

\(^1\) We acknowledge that the statistically significant correlations reported below would not however survive the more conservative Bonferroni correction.
4. Discussion

While TMR did not significantly affect behaviour, our data suggest that the impact of TMR on lexical integration is mediated by time spent in REM sleep. Although we might intuitively expect TMR to influence only processing in the sleep stage where it is applied, prior studies have shown that cueing in SWS can change the role of REM. For instance, Cousins et al. (2016) found that SWS cueing-related changes in neural activation were modulated by both SWS and subsequent REM. Oudiette et al. (2013) reported that, in the absence of cueing, REM time was associated with declining memory accuracy for object-locations. However, this association was abolished with cueing during SWS, thus cueing may have stabilised the memories and eliminated the processes involved in forgetting during REM sleep. Finally, Hu et al. (2015) had participants undergo two types of counter-bias training one of which was cued during SWS. A larger reduction was seen in the bias that was cued and this cueing-related change was predicted by the product of time spent in SWS and REM.

This literature combines to suggest that cueing in SWS can modulate the role of REM in consolidation. Such findings are in line with several theories proposing that SWS and REM play complementary roles (Giuditta, 2014; Diekelmann & Born, 2010; Ribeiro et al, 2007; Ficca et al., 2004; Stickgold et al., 2000). These theories broadly share the view that SWS strengthens recent memories through spontaneous reactivation, and selecting memories to be retained or forgotten. REM then operates on the memories which were activated during SWS, possibly strengthening their neocortical representations, and integrating them with existing neocortical neural assemblies and semantic knowledge. Our data suggest that cueing of newly learned words during SWS may tag the words for further processing during REM. This likely complements spontaneous reactivation and tagging of the entire set of new words, but the external cueing may result in enhanced SWS reactivation and consequently exaggerate REM-mediated lexical integration. In the absence of cueing, Tamminen et al.
(2010) found no association between any sleep stage and lexical integration but did find a spindle association. We found no spindle association, the current data therefore suggest that cueing in SWS may have triggered a REM-mediated lexical integration that is different from the process which occurs in the absence of cueing.

Although the role of REM in memory consolidation is controversial (Rasch & Born, 2015), REM is a plausible candidate for mediation of lexical integration. There is also a growing literature on the association between REM and declarative memory processes that go beyond strengthening new episodic memories. For instance, REM has been associated with increased semantic priming of distantly related pairs of words (Stickgold, Scott, Rittenhouse & Hobson, 1999; Carr & Nielesen, 2015), problem solving through activation of broad associative semantic networks (Walker, Liston, Hobson & Stickgold, 2002; Cai et al., 2009), and extraction of statistical patterns in learning (Barsky et al., 2015). Our data are consistent with the view that REM activates broad semantic networks and allows the integration of new memories with remotely related existing knowledge.

In addition to the TMR data, we show here for the first time that lexical integration effects can emerge after a brief daytime nap as well as an equivalent time spent awake. This supports the view of Tamminen et al. (2010) that lexical integration occurs offline after encoding, and that this can happen during both sleep and wake. Further research is needed to understand how the mechanisms that support integration during sleep and wake differ, and the task used to measure integration may be critical: both sleep and wake gave rise to integration in the lexical decision task (current data and Tamminen et al, 2010) but only sleep allowed integration in a pause detection task (Dumay & Gaskell, 2007). Systematic research is needed to understand which elements of these tasks are sleep-dependent. Furthermore, we did not observe a correlation between spindles and lexical integration, as reported by Tamminen et al. (2010) in their overnight study. This may be because of the shorter sleep
duration in the current nap study, and the consequently lower spindle counts. This calls for more research into the comparability of nap and overnight studies, at least when interpreting PSG measures.

Finally, we did not detect effects of TMR on behavioural measures of learning. One possible reason is that words were cued only once, and only in SWS, although even this limited cueing was sufficient to bring out an association with REM. While many earlier studies restricted cueing to SWS, a recent study in second language learning cued newly learned words multiple times across SWS and stage 2 (Schreiner & Rasch, 2015) and found that cueing facilitated memory for the words. Future research will show whether altering the amount and timing of cueing might yield more robust effects.

5. Conclusions

Integrating new information with existing knowledge is a key goal of learning. We found that TMR impacted on the association between lexical integration and REM. We tentatively suggest that REM may mediate lexical integration when the new words have been cued during SWS, possibly tagging these memories for later processing during REM. Further work is needed to confirm these hypotheses, especially given the limitations of our correlational design. We also showed that 90 minutes of daytime sleep or wake provides a sufficient delay for lexical integration to emerge, thus extending the existing overnight findings. Finally, we found that sleep protected in free recall of new words against forgetting that occurred during wake even when the wake period contained no interfering language input, thus providing new evidence for how sleep and wake contribute to word learning.
Acknowledgements

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Table 1. Accuracy rates (percent recalled, percent correct, or d-prime values) and mean RTs (in ms) in all tasks for cued stimuli and uncued stimuli in the sleep group. For the lexical competition task the magnitude of the competition effect (difference between RTs to base words for which a new competitor was taught and base words with no new competitors) is given. Standard error shown in parentheses.

<table>
<thead>
<tr>
<th>Test session 1</th>
<th>Test session 2</th>
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<tbody>
<tr>
<td></td>
<td>Cued</td>
</tr>
<tr>
<td>Free recall</td>
<td>% recalled</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Lexical</td>
<td>RT (ms)</td>
</tr>
<tr>
<td>competition</td>
<td></td>
</tr>
<tr>
<td>Old-new</td>
<td>RT (ms)</td>
</tr>
<tr>
<td>categorisation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACC (d’)</td>
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<td></td>
<td></td>
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</tbody>
</table>

*Note:* RT = reaction time, ACC = accuracy
Table 2. Proportions of hits, false alarms, misses, and correct rejections in the old-new categorisation task. Standard error shown in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Wake group</th>
<th>Sleep group (Cued words)</th>
<th>Sleep group (Uncued words)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hits</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>.83 (±2.3)</td>
<td>.85 (±2.1)</td>
<td>.84 (±2.5)</td>
</tr>
<tr>
<td>Session 2</td>
<td>.70 (±3.2)</td>
<td>.76 (±2.3)</td>
<td>.73 (±2.8)</td>
</tr>
<tr>
<td><strong>False alarms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>.09 (±1.4)</td>
<td>.11 (±2.1)</td>
<td>.14 (±2.6)</td>
</tr>
<tr>
<td>Session 2</td>
<td>.13 (±2.2)</td>
<td>.16 (±2.5)</td>
<td>.14 (±2.0)</td>
</tr>
<tr>
<td><strong>Misses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>.17 (±2.3)</td>
<td>.15 (±2.1)</td>
<td>.16 (±2.5)</td>
</tr>
<tr>
<td>Session 2</td>
<td>.30 (±3.2)</td>
<td>.24 (±2.3)</td>
<td>.27 (±2.8)</td>
</tr>
<tr>
<td><strong>Correct rejections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>.91 (±1.4)</td>
<td>.89 (±2.1)</td>
<td>.86 (±2.6)</td>
</tr>
<tr>
<td>Session 2</td>
<td>.87 (±2.2)</td>
<td>.84 (±2.5)</td>
<td>.86 (±2.0)</td>
</tr>
</tbody>
</table>
Table 3. Average time spent in different sleep stages (± standard error).

<table>
<thead>
<tr>
<th>Sleep stage</th>
<th>Time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>6 ± 1 min</td>
</tr>
<tr>
<td>Stage 2</td>
<td>32 ± 3 min</td>
</tr>
<tr>
<td>SWS</td>
<td>23 ± 3 min</td>
</tr>
<tr>
<td>REM</td>
<td>10 ± 2 min</td>
</tr>
<tr>
<td>Total sleep time</td>
<td>71 ± 4 min</td>
</tr>
</tbody>
</table>
Table 4. Correlations (raw, uncorrected figures) between change during the nap in the three test tasks (averaged over cued and uncued TMR conditions) and time spent in different sleep stages.

<table>
<thead>
<tr>
<th>Test Task</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>SWS</th>
<th>REM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free recall</td>
<td>r = -0.22</td>
<td>r = -0.39</td>
<td>r = 0.37</td>
<td>r = -0.004</td>
</tr>
<tr>
<td></td>
<td>p = .34</td>
<td>p = .09</td>
<td>p = .11</td>
<td>p = .99</td>
</tr>
<tr>
<td>Old-new categorisation</td>
<td>r = 0.08</td>
<td>r = -0.22</td>
<td>r = 0.007</td>
<td>r = 0.18</td>
</tr>
<tr>
<td></td>
<td>p = .74</td>
<td>p = .35</td>
<td>p = .98</td>
<td>p = .45</td>
</tr>
<tr>
<td>Lexical competition</td>
<td>r = 0.08</td>
<td>r = -0.35</td>
<td>r = -0.11</td>
<td><strong>r = 0.47</strong></td>
</tr>
<tr>
<td></td>
<td>p = .75</td>
<td>p = .13</td>
<td>p = .64</td>
<td><strong>p = .037</strong></td>
</tr>
</tbody>
</table>