Low Frequency Effects of Targeted Memory Reactivations on Subsequent Recall Processes

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There is an unmistakable link between the sleeping state and memory. The leading theory to explain this effect is the systems consolidation theory (Rasch & Born, 2013): a memory is consolidated through repeated, unconscious reactivations of prior learned memories. This project furthers the research into this theory by studying both the behavioural and oscillatory effects of these reactivations through targeted memory reactivations. Participants learned word-image pairs, of which half were then reactivated during a period of sleep by playing back the words in the pair. The long-term effects of this manipulation were then studied during subsequent cued recall. We clearly observed that behavioural performance increased as a result of cueing, as well as alpha band desynchronisation related to successful recall and stronger lateralised activity. Our results suggest that memory replay helps preserve the memory trace and, thus, makes the memory more accessible during recall.

Keywords: sleep, memory, consolidation, systems consolidation theory, alpha desynchronisation, recall

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Everyone knows about the restorative effects of a good night of rest, and many of us have observed the negative cognitive effects of not sleeping enough; sleep is essential. While it is generally a critical part of our lives, it is less known that sleep is also paramount in the formation of new memories. An example of this is the necessity of a night of rest for studying to actually take effect, the memories being more easily accessible the following day. In this project we will explore the biological underpinnings of this memory strengthening effect. Firstly, I will address our knowledge to date regarding this link.

Memory

Memory is generally thought to be comprised of three main processes: encoding, consolidation, and recall. Encoding is the process during which stimuli are perceived and eventually represented in the brain. This process depends on executive control and requires attention to the stimulus to function (Chun & Turk-Browne, 2007; Craik, Govoni, Naveh-Benjamin, & Anderson, 1996; Fernandes & Moscovitch, 2000). The representations created by this process are cortically distributed (Ahmad & Hawkins, 2015), yet depend on hippocampal activation to be accessed; the hippocampus in this case acting as an index of those representations (Moscovitch, Nadel, Winocur, Gilboa, & Rosenbaum, 2006).

Consolidation is the process through which encoded memories are stored and either further integrated into our current knowledge, or discarded. We currently do not know all the factors that influence if a certain memory is discarded or integrated, but reactivations (replay of the neural activity observed during learning) when awake could play a role, by increasing the likelihood of the reactivated memories to be replayed during sleep (Carr, Jadhav, & Frank, 2011; Diekelmann & Born, 2010; Diekelmann, Büchel, Born, & Rasch, 2011; Jafarpour, Horner, Fuentemilla, Penny, & Duzel, 2013; O'Neill, Pleydell-Bouverie, Dupret, & Csicsvari, 2010). Consolidation begins immediately after encoding and can take various amounts of time, but most evidence points to the fact that sleep plays a pivotal role in this regard (Buzsáki, 1998; Fenn, Nusbaum, & Margoliash, 2003; Lee & Wilson, 2002; Vertes, 2004). Consolidation is thought to be produced mainly by spurious memory reactivations that occur during the sleeping state (Born & Wilhelm, 2012; Dudai, Karni, & Born, 2015; Lewis & Durrant, 2011; Stickgold, 2005). This hypothesis has been previously tested by provoking memory reactivations through playing back a part of a learned stimulus (one word of a pair) and measuring the effect this has on the recall of the entire memory. The beneficial effect of memory cueing during sleep, as well as replay of previously encoded memories, has been observed both in humans and rodents (Peigneux et al., 2004; Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009; Schreiner & Rasch, 2015; Wilson & McNaughton, 1994).

The third process involved in the memory system is retrieval, through which a stored memory is reactivated and made consciously available. The ease of retrieval is dependent on the integration of the memory and, therefore, dependent on how much the memory has been consolidated. Another phenomenon that has been observed in relation to retrieval that may hold some functional significance is the encoding-retrieval match, activity present during encoding of a piece of information closely resembles activity present during recall (Reijmers, Perkins, Matsuo, & Mayford, 2007).

This study focuses on the consolidation and retrieval aspects of memory, as our manipulation has effects on the consolidation processes and the outcome measures can only be related to recall (as the study of consolidation on its own is not easily achieved). By targeting our manipulation either in sleep or waking states, and measuring retrieval processes multiple times, we may also gain a more complex understanding of when different consolidation processes take place, and what effect these have on the memory trace.

Sleep and memory – Systems consolidation theory

Sleep is essential for many processes that happen in the brain, as well as the rest of the body. The restorative effects of sleep are well known to everyone, and the cognitive toll that comes with sleep deprivation has not only been felt by most, but also tested and measured in laboratory settings (Pilcher & Huffcutt, 1996). Sleep deprivation has been proven to cause a marked decline in executive functioning, and can lead to serious cognitive symptoms such as confusion, hallucinations and delirium (Samkoff & Jacques, 1991). From a physical health perspective, Samkoff and Jacques also noted that sleep deprivation can lead to irregular heart rhythms, irregularities in body temperature, and has a negative impact on the immune system and can even lead to death if the deprivation is extended.

Aside from the restorative biological and cognitive roles of sleep, it also seems to play an important part in memory.

One study on the link between sleep and memory selectively deprived people of a single stage of their sleep and examined what effects the loss of that stage had on different types of memory (Rauchs, Desgranges, Foret, & Eustache, 2005; Rotenberg, 1992). Through these approaches, it was discovered that Rapid Eye Movement (REM) sleep was more important for procedural memories (how to do things), as well as memories with high emotional content, than for episodic (personal events) and declarative memories (common knowledge, facts, not tied to personal self). Rauchs et al. (2005) also noted in their review that non-REM, and more specifically, in most cases slow-wave sleep (SWS) has been found to be important for the consolidation of associative and episodic memories.

The current leading theory to explain how consolidation happens during sleep is the systems consolidation theory (Born & Wilhelm, 2012; Frankland & Bontempi, 2005; Pavlides & Winson, 1989). This theory states that memories are consolidated through multiple unconscious reactivations of prior learned memories. The first evidence of replay was found in rodents (Pavlides, 1989): Place cells that were active during waking periods were more likely to become active during subsequent sleep. Replay of more complex memories was observed, using single unit recordings of place cells, cells that specifically record an animal's position in a certain environment, during learning of a path and subsequent sleep (Wilson & McNaughton, 1994). Wilson and McNaughton (1994) observed that the place ensembles, being more complex representations of the followed path, that were active in the hippocampus during the exploration of a certain maze, reactivate during sleep.

The underlying neural activity behind this replay seems to be a case of first synchronisation, then communication between the hippocampus and neocortical areas (Girardeau, Benchenane, Wiener, Buzsáki, & Zugaro, 2009; Girardeau & Zugaro, 2011). Sharp wave ripples (SWR) in the hippocampus seem to be the events during which replay occurs most often, and blocking these events has been shown to cause the consolidation effect to disappear completely. These ripples travel over the brain, acting as carriers of the memory information. Slow wave sleep represents a state where large populations of neurons are either active or inactive at the same time. This is then a prime example of a period when neocortical areas and subcortical areas are synchronised. During these synchronised states, the memory information can be transmitted in the form of thalamo-cortical spindles which spread across the neocortex. Nested in these thalamo-cortical spindles, SWR form in the hippocampus, in which the actual memory trace is encoded. By repeating and spreading the activation related to a memory trace during synchronised periods of SWS, the memory is transformed from a temporary form stored in the hippocampus, to an integrated form stored in the neocortex (Rasch & Born, 2013).

The research in this field relies on being able to provoke memory reactivations in a reliable way, in order to study their functional significance. It has been observed that these reactivations can be provoked by presenting one of a pair of associated stimuli (Oudiette, Antony, Creery, & Paller, 2013; Oudiette & Paller, 2013; Rasch & Born, 2007; Rasch, Büchel, Gais, & Born, 2007; Rudoy, Voss, Westerberg, & Paller, 2009; Schönauer, Geisler, & Gais, 2014). Participants would learn pairs of stimuli (sounds paired with targets which could be other sounds or images), and then to unobtrusively stimulate the replay of the full association, those sounds are presented during non-REM. By presenting these cues during sleep, it has been found that the associated memories become stronger (have a higher chance to be recalled) than the memories for which cueing was not performed.

Importantly, this cueing effect appears to be hippocampus-dependent; this can be one of the reasons why the hippocampus is essential for the formation of long-term memories. An experiment conducted by Fuentemilla and colleagues (2013) has shown a strong negative correlation between the amount of hippocampal damage and the strength of the cueing effect in epilepsy patients. They showed that the effect is hippocampus-dependent, as patients with bilaterally removed hippocampi did not benefit from cueing at all (Fuentemilla et al., 2013).

Oscillatory recall effects

As previously mentioned, recall is the process through which memories become active and consciously available, and has been associated with a marked desynchronisation in the alpha and beta frequency ranges, reflecting reactivation of the memory trace (Dujardin, Bourriez, & Guieu, 1994; Klimesch, 1999). The role of alpha oscillations has been proposed to be one of targeted inhibition of task-irrelevant information, as increases in alpha power have been observed in relation to the number of distractors involved in visual processing tasks (Bonnefond & Jensen, 2012). The alpha power increase also seemed to be lateralised: if task irrelevant information was presented in the right visual field, alpha power increased in the contralateral occipital cortex, and vice-versa. In the case of memory retrieval, a review from Hanslmayr, Staudigl and Fellner (2012) proposed the information through desynchronisation hypothesis, which explains how desynchronisation in the alpha and beta ranges could reflect the richness of information carried by those oscillations, and therefore, aid in the recall of memories. An example of this effect can be seen in the study of Waldhauser et al. (2012), in which participants learned two separate memories, stored either in the left or right side of the visual cortex, associated with one cue. One of the memories was the target, the other a distractor. During recall, they observed increased alpha and beta activity in the hemisphere with the distractor memory, and a marked alpha desynchronisation in the side where the target was stored. This indicates that there was increased inhibition in the areas related to distractor information, while the target areas were more accessible (through the alpha desynchronisation) for recall.

Another phenomenon that has been observed in relation to recall is epochry: the reactivation of activity present during encoding. Another study by Waldhauser and colleagues (2016), demonstrated that episodic memory relies on reactivation of sensory information. By presenting stimuli in a lateralised manner during encoding, followed by retrieval using centrally presented retrieval cues, they were able to find that successful retrieval was associated with alpha and beta decreases in the visual cortex contralateral to the visual field at encoding. In another experiment Waldhauser et al., 2016), they tried disrupting this activation using transcranial magnetic stimulation (TMS), and found significant memory performance decreases only for memories stored in the disrupted visual field.

In this study, we focus on tracking the evolution of memories, more specifically related to memory reactivations during sleep, as well as the oscillatory effects of cueing during sleep on subsequent recall processes. In this regard, we asked participants to learn a list of associated word-picture pairs, use targeted memory cueing (presenting the words at low volume) as a way to provoke memory reactivations during sleep, and study the oscillatory and behavioural effects of this manipulation during recall. We expected to first reproduce the memory effect of desynchronisation in the alpha and beta ranges as well as find differences between cued and uncued words in this regard. As alpha desynchronisation has been associated with better memory, we propose that cued words would be remembered better and would have a greater associated alpha power decrease. As we present the pictures in a lateralised manner during encoding, we also hypothesise there to be significant lateralisation effects and we expect these effects to vary between cued and uncued words. More specifically we expect to find stronger lateralisation in cued words, as these would have a better-preserved memory trace.

Method

As this project is a part of a larger experiment meant to measure the effects of wakeful reactivations in comparison to sleep reactivations, here I will present the entire experimental protocol for reproducibility. This project strictly focuses on the final recall phase of the experiment, as well as the sleep cueing condition of the study.

Participants

Data from 15 participants, aged between 19 and 35 (*M* = 24, *SD* = 4.26; 8 female, 7 male), were collected. The participants were all Dutch native speakers in order to minimize any possible language effects associated with the task. None of the participants reported taking any medication at the time of the experiment nor any chronic medication and none had a history of neurological or psychiatric disorders. Participants who had any metal implants (including dental wires) were also excluded from the experiment to reduce noise in the Magnetoencephalogram (MEG) signal. Participants were also informed of the sleeping conditions and were required to consider themselves “good sleepers”, in order to ensure that they would be able to sleep in the lab. Also in this regard, on experimental dates, subjects were instructed to restrict their sleep to six hours. This study was approved by the ethics board of the Radboud University, and all participants gave informed consent to the experiment.

General procedure and task design

The experiment was performed in the MEG laboratory of the Donders Institute, Radboud University, Nijmegen. Participants came in for a total of four sessions during the experiment: one adaptation evening, two experimental evenings, and

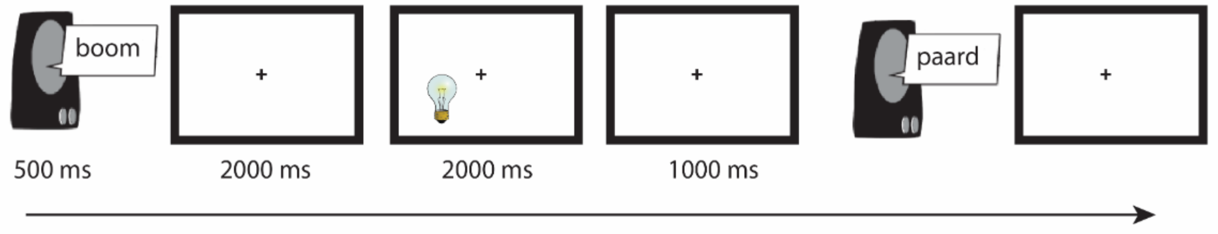

Fig. 1. Learning form of the task, adapted from Schreiner & Rasch (2015).

one Magnetic Resonance Imaging (MRI) session, with the two experimental conditions being separated by one week, creating a repeated measures design, where we compared the performance and neural underpinnings between conditions. During the first adaptation evening, participants were exposed to the MEG environment and allowed to sleep for a total of 60 minutes. The role of this initial meeting was to get participants habituated to the novel environment and to ensure that they would be able to sleep in the MEG. The second and third nights were the experimental evenings, during which a memory task was learned and tested repeatedly after a period of active wakefulness (60 minutes) and one of sleep (120 minutes). The memory task had three forms: learning, cued recall with feedback, and cued recall without feedback. In the learning form, participants were instructed to learn as many word picture associations as they could, with the words being presented in an auditory manner (500 ms), followed (after a fixation cross of 2000 ms) by a lateralised presentation of the picture stimulus (2000 ms, and finally another fixation for 1000 ms; see Figure 1). A total of 140 word-picture pairs were presented, with 70 pictures appearing in the right visual field and the other 70 in the left. The entire task was presented and solved in Dutch.

In the feedback form of the task, after the word was presented, a question mark appeared, during which participants had to try to name the object previously presented in the picture associated with the word (see Figure 2). After their response was recorded, participants were presented with the associated picture again, in order to strengthen the memory. The recall version of the task was the same as the feedback version, except for the fact that participants no longer received feedback (the correct image) after their response, and its role was to measure the strength of the memory at different steps during the experimental design.

In order to have a more robust measure of memory, and the role of waking and sleeping reactivations, multiple measures of recall were needed. During experimental dates, participants first completed the initial learning and feedback learning versions of the task, after which a first recall was measured. After this, a period of relaxed wakefulness ensued (of 60 minutes) during which participants had to complete a number sorting task (odd-even), in order to ensure vigilance. Recall was then measured again, before allowing participants to sleep for 120 minutes. Finally, recall was measured one last time. During one of the experimental nights, cueing was applied during the waking phase, while in the other, cueing was applied during the sleeping phase (see Figure 3 for full experimental design). Different word lists were used for the experimental evenings, to avoid learning effects. The order of the word lists, as well as the order of the experimental evenings were counterbalanced across participants.

Of the 140 words on the list, half of the remembered pairs and half of the forgotten pairs were randomly chosen as cue targets. Whether a pair was marked as remembered or not remembered, was decided by the performance on the recall, immediately prior to the cueing phase. As such, half of the remembered words associated with left or right lateralised pictures were cued or not cued

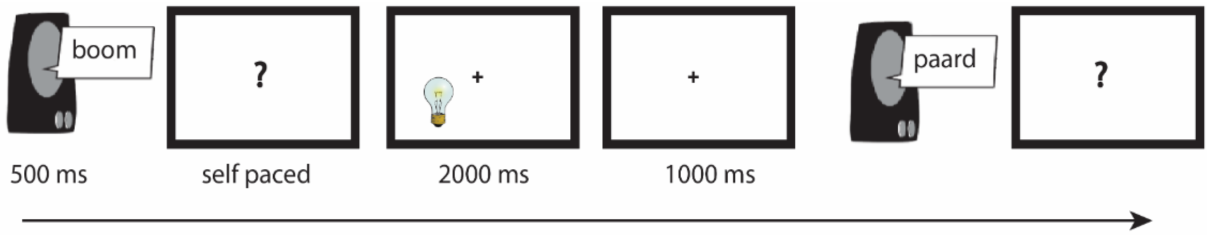

Fig. 2. Cued recall form of the task, adapted from Schreiner & Rasch (2015).

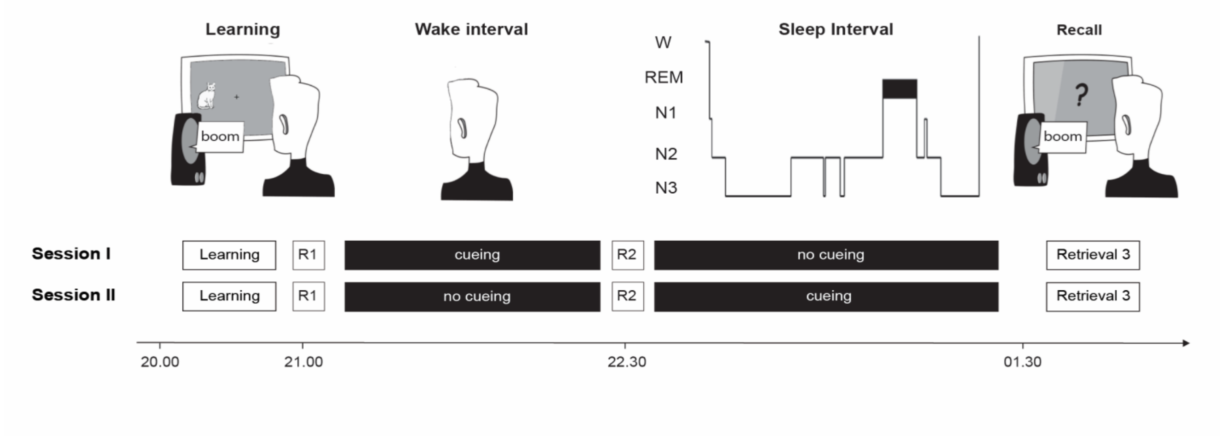

Fig. 3. Experimental design, figure adapted from Schreiner & Rasch (2015).

respectively. Cueing was realised by playing back the previously associated words at a low volume level (approximately 50dB), either during the number sorting task, or during non-REM sleep, depending on the experimental condition. Presentation occurred every 5800 - 6200 ms in a randomized order for a total of 60 minutes, resulting in ~10 exposures to each word. Participants' sleep was constantly monitored to ensure that the cueing did not wake them up.

Recording, software, pre-processing

Electroencephalogram (EEG) data were collected from four electrodes (F3, F4, C3, C4) with a left mastoid reference (A1) placed according to the 10-20 system, at a sampling rate of 600 Hz. The MEG recorded data from 275 axial gradiometers at a sampling rate of 600 Hz (VSM/CTF Systems, Port Coquitlam, British Columbia, Canada). Head position relative to the helmet was monitored with the use of three head-localisation coils (placed in the ears and on the nasion). The data from these coils were analysed using a real-time head localiser (Stolk, Todorovic, Schoffelen, & Oostenveld, 2013). Four electrodes were also placed on the participants' faces for the collection of vertical and horizontal Electrooculogram (EOG).

The stimuli were presented using presentation software and all analyses were conducted in Matlab. The fieldtrip toolbox created for Matlab (Oostenveld, Fries, Maris, & Schoffelen, 2011) was also used in the oscillatory analyses. Data were divided into single trials of 3 seconds after the auditory stimulus presentation. Trials were corrected for cardiac and eye movement artifacts using independent component analysis (Makeig, Bell, Jung, & Sejnowski, 1996) after manual artefact rejection. The trials were sorted into conditions as a function of final recall performance (remembered versus forgotten), cueing condition (cued versus uncued) and initial stimulus presentation (left versus right). Frequency decomposition of the data was achieved via Fourier analysis based on sliding time windows (moving forward in 10 ms increments). The settings were optimised for low frequency ranges (2-29 Hz, 1-Hz steps), the window length was set to five cycles of a given frequency (for example, 500 ms for 10 Hz; 250 ms for 20 Hz), and the windowed data segments were multiplied with a Hanning taper before Fourier analysis. The resulting power maps were normalised by dividing over the averaged −1 s pre-stimulus baseline window and subjected to direct comparison between conditions of interest.

A lateralisation index was computed, adapted from D'arcy et al. (2013), by subtracting the activity observed in the left condition from activity observed in the right condition and dividing by the sum of these. In this way, we can see which activity is specific to processing information presented in one visual field.

Results

Behavioural results

The behavioural data were analysed using IBM SPSS Statistics 20, using the standard settings. We used repeated measures two-way Analysis of Variance (ANOVA) for factorial analysis of effects, and post-hoc t-tests for confirmation and detailing. Repeated measures t-tests were also used to exclude certain possible confound effects (equivalency between pre-cue periods was assured).

Already from the behavioural results we can deduce that cueing words led to an increase in performance, independent of whether cueing was performed during the waking or sleeping state. We

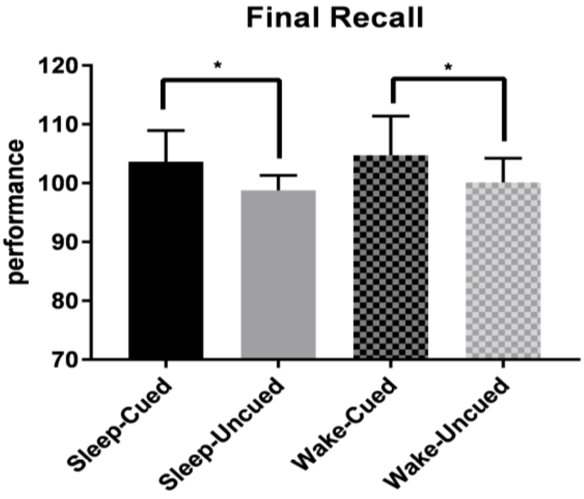

Fig. 4. Hit increases between second recall and final recall expressed in percentage; * denotes significant differences at $p < .05$.

used a repeated measures ANOVA to measure the comparative effects of cue presence (cued versus uncued), cue time (wake versus sleep cueing) and lateralisation (words presented on the left versus right during encoding), as well as any interactions between these effects (see Figure 4). Firstly, cueing has an effect independent of the cue time on the performance during the final recall, with cued words being better remembered than uncued ones (main effect of cue presence: $F(1,14) = 11.89$, $p = .004$). Secondly, this cueing effect is not different between the conditions (cue time effect: $F(1, 14) = .6$, $p =.44$, interaction cue time X cue presence: $F(1, 14) = .13$, $p = .91$). Post-hoc repeated measures t-tests between cued and uncued words revealed this effect in both the sleep ($t(14) = 2.85$, $p = .01$) and wake ($t(14) = 2.51$, $p = .02$) conditions.

When comparing performance in the recalls immediately pre and post cueing using paired samples t-tests, we observed that only sleep cueing has an immediate effect ($t(14) = -2.35$, $p = .03$), while wake cueing does not ($t(14) = -1.9$, $p = .07$). This suggests that wake cueing requires subsequent sleep in order to have an effect on performance. Thus, when comparing the effects of cueing, lateralisation, and time of cueing, the only significant factor is whether the pairs were cued or not. An interesting fact that needs to be pointed out is that performance during the final recall was almost always at least as good as the performance in the previous recall stage (the performance not dropping below 100% of the previous round). This could be due to the fact that even uncued words may be reactivated without the aid of cueing.

Furthermore, we used paired samples t-tests to check whether the pre-cue periods were equivalent between conditions. This is necessary due to the fact that in the sleep cueing condition, the participant goes through two recall sessions before cueing takes place (and one after), while in the wake cueing condition, there is one recall before cueing and two afterwards. This analysis proved, as expected, that before cueing the participants had the same performance in the wake and sleep cueing conditions, meaning that the differences observed during the final recall were due to the cueing manipulation ($t\ (14) = .5$, $p = .5$).

Finally, to assure that there were no immediate effects of cueing in the wake condition, we ran another paired samples t-test between the pre-cue and post-cue interval in the wake condition. This analysis showed that sleep was necessary for the wake cueing to function by showing that wake cueing on its own did not produce the observed final effects ($t\ (14) = -1.9$, $p = .07$).

Oscillatory results

Seeing that there is a behavioural effect of cueing, the next step in the analysis was to look for the neural underpinnings of this effect. In other words, what changes does cueing provoke from an oscillatory perspective that correlate with our behavioural results? Before looking into more detailed, fine-grained cueing effects, we first searched for the oscillatory underpinnings of successful retrieval in order to further orient our search. In this regard, we ran a memory contrast, between remembered and forgotten words across the entire epoch and with the frequency range between 3 and 20 Hz, as most memory effects are found within this range. A single negative cluster was observed in the alpha range, between 0.6 and 1.7 s, not only confirming the main memory effect, but also informing the following analyses (see Figure 5). We used the time and frequency ranges found in the first analysis to inform the following analyses, as cueing directly impacts the strength of the memory, and as such we expected the effects to be similar. Also important to note is the localization of this effect, the topography suggesting the source of the activity being localised in the parietal region, which is in line with many recent memory studies (Gluth, Sommer, Rieskamp, & Büchel, 2015; Tanaka et al., 2014).

The next step in searching for the oscillatory underpinnings was to look for differences between the cued and uncued words, as this was the main effect found in the behavioural analysis. Running

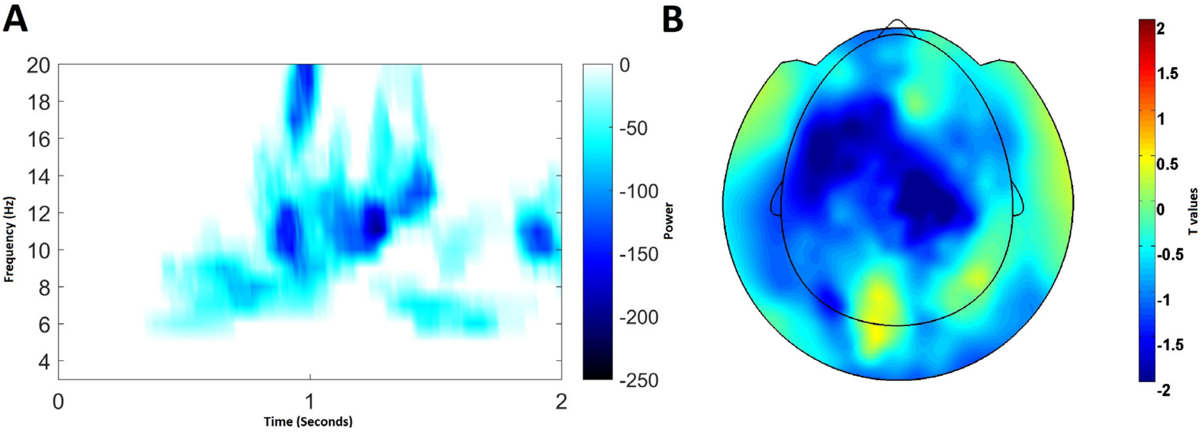

Fig. 5A. Power contrast between remembered and forgotten words during the final recall. **B.** Localisation of effect found in A in the form of t-values, taking into account all sensors.

a contrast between cued and uncued words that were remembered yielded no significant clusters, nor did the same contrast for words that were forgotten (neither for the full time interval and over all frequencies, nor for specifically 0.6-1.6s, 10-14 Hz). These results suggest that there is no combined effect of cueing and memory, yet this clashes with the behavioural results. While not finding an effect in the case of forgotten words can be expected, in this case, the memory is not accessible, and thus, any memory effects would disappear. Not finding a cueing effect in the remembered words contradicts the systems consolidation theory.

Contrasts between remembered and forgotten words between 10-14 Hz from 0.6-1.7 s post stimulus in the cued and uncued conditions, on the other hand, revealed a negative cluster in the uncued conditions ($p = .05$) and a negative trend ($p = .06$) in the cued condition (see Figure 6). These results, while being more in line with previous findings revealing the previous memory effect, still do not explain the difference between cued and uncued words, as the effects are in the same direction.

As these effects do not fully explain our behavioural results, we decided to look more closely at the phenomenon of epochry and how it behaves as a function of cueing. As such, we prepared a lateralisation index, by subtracting the activity observed when recalling images presented on the right side of the screen from the activity when recalling

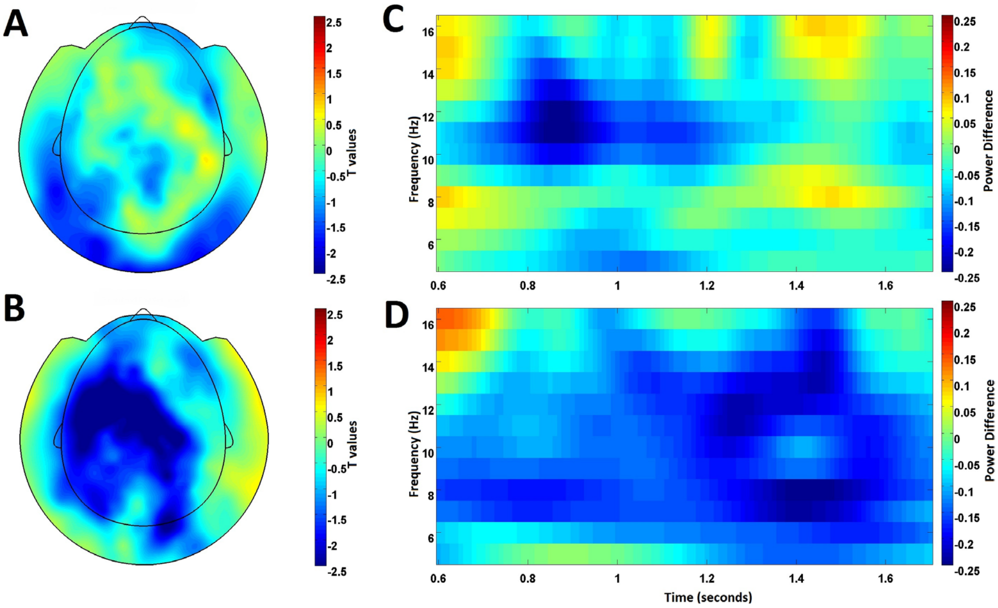

Fig. 6. Contrast between remembered and forgotten words in the cued (**A** and **C**) and uncued (**B** and **D**) conditions. **C** and **D** represent the average power values of the difference between all significant sensors observed in **A** and **B**.

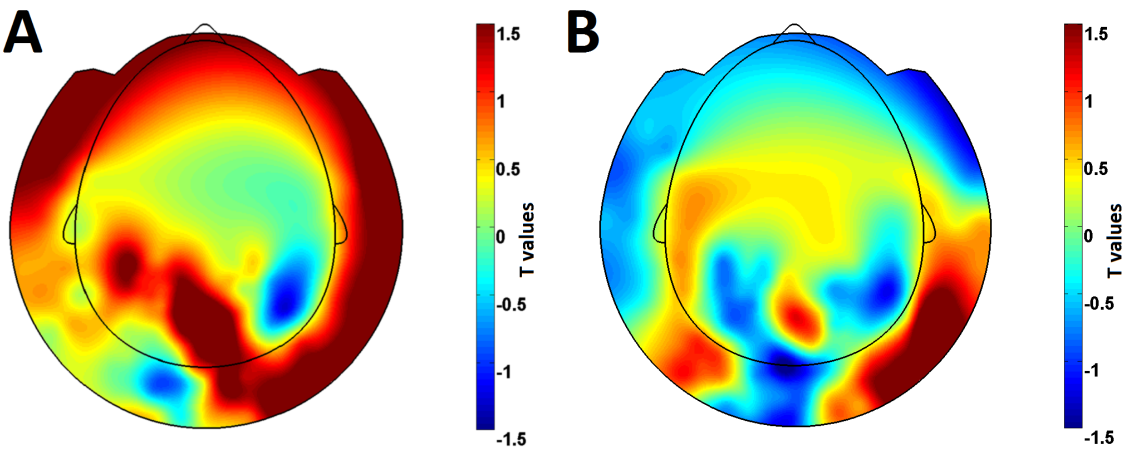

Fig. 7. Topography of the difference between the left and right lateralisation indices in the cued (**A**) and uncued (**B**) conditions, excluding frontal sensors. Time range 0.7-1 seconds, Frequency range 10-14 Hz.

images on the left side of the screen and divided by their sum (left-right/left+right). By contrasting the lateralisation indices for remembered, cued words, and excluding frontal sensors, a significant cluster was observed in the alpha range (*p* = .04, from 0.7 to 1 s and between 10-14 Hz). Applying this same contrast to uncued words, on the other hand, yielded no significant clusters (see Figure 7).

This result is pivotal, as it shows a clear effect of cueing that is not present in the uncued condition, and as such could underlie our behavioural effects. The fact that there is a difference between words encoded on the left side and words encoded on the right side simply confirms the phenomenon of epochry. The fact that this effect changes as a function of cueing shows that cueing words helps preserve the memory trace, the reactivation of the memories being stronger in the cued condition. The fact that this is a lateralised effect could also explain why differences were not found earlier in the analysis, as it may act as a confound in the other analyses.

Discussion

This study aimed to take a look at the effects of memory reactivations during sleep on subsequent recall, by comparing the neural activity observed when recalling words that were cued during prior sleep with those which were not. In this regard, we asked participants to learn word-picture pairs, with the words being presented in an auditory manner. Then, during a period of subsequent sleep we cued half of the words (by replaying at a low volume), while leaving the rest to be consolidated under normal conditions. Finally, we observed the differences between these words during the final recall period from behavioural and oscillatory points of view.

Our behavioural results provide evidence sustaining the systems consolidation theory, and offer a good starting point to look for the oscillatory mechanisms that sustain this cueing effect. Firstly, and most clear of all, there is a behavioural effect of cueing on memory. This provides evidence for the fact that memory reactivations strengthen memories. This result also fits with previous cueing studies which have found similar effects when cueing pairs of associated stimuli (Rasch, Büchel, Gais, & Born, 2007; Schreiner, Göldi, & Rasch, 2015; Schreiner & Rasch, 2015).

The neural underpinnings of this behavioural effect were more difficult to isolate, yet the effects that we found fall in line with previous literature about systems consolidation theory, as well as epochry. The initial memory contrast provided data that confirms previous work on memory-related effects (Dujardin et al., 1994; Hanslmayr, Staudigl, & Fellner, 2012) and offered us a starting point for the rest of our analysis, narrowing down both the time and frequency windows to memory-related activity.

In accordance with previous findings, we observed desynchronisation in the alpha band related to successful retrieval of memories. This effect has been previously described in multiple other studies and is thought to reflect the memory trace becoming active, a greater desynchronisation in the alpha band allowing for easier access to the memory through lowered inhibition (Hanslmayr et al., 2012). Furthermore, this activity seemed to not differ significantly between cued and uncued conditions, which is a relatively surprising result taking into account that cued memories were easier to recall than uncued ones, from a behavioural perspective. This apparently indicates that there is no combined effect of cueing and memory, yet this does not fall in line with our behavioural results.

As such we decided to look more closely at how the memory itself changes as a function of cueing. Words presented on the left side of the screen during the learning period cause an increase in the activity present in the contralateral (right) occipital cortex. Because we expect this activity to correlate with the activity present during retrieval (leading to contralateral increases during retrieval), we used this as a marker of the strength of the memory. In this way, we found clear effects in the cued condition that were not found in the uncued condition. In other words, the activity present during retrieval was more lateralised in the cued condition, leading to the conclusion that cueing increases epochry.

This lateralisation effect could also be a possible explanation for the lack of direct differences between cued and uncued conditions when looking at alpha desynchronisation. Because the desynchronisation is lateralised, by averaging over conditions the activity related to left and right words would cancel each other out, and this canceling would be more powerful in the cued condition since all words would present a stronger lateralisation. Meanwhile in the uncued condition, some words would be spontaneously replayed, leading to a less lateralised trace that would not cancel out as strongly through averaging.

In the context of systems consolidation theory, our data suggest that memory reactivations during sleep cause the engram of the memory to be more accessible during recall, preventing the deterioration of the memory trace. The observed lateralised activity in the cueing condition suggests that cueing helps preserve the engram. Meanwhile, the lack of these reactivations results in a weaker memory trace, and as such does not lead to any increases. The fact that cueing during sleep has this effect is in accordance with the systems consolidation theory, which states that reactivations during sleep cause memories to be transformed from a labile short term state to a more stable long term representation (Rasch & Born, 2013). The fact that we cannot be sure that uncued words are not replayed automatically during sleep can explain why we did not observe drops in performance for uncued words, and can also be one of the reasons why the neural effects were more difficult to see.

In order to continue this line of research and build on these results, the next logical step would be to follow this lateralisation effect and its lateralisation across the rest of the recall periods. In this way, we would be able to quantify the memory losses due to the passage of time, as well as see how this degradation is affected by cueing in the short term (immediate effects), as well as in the long term (post-sleep effects). By following the evolution of this effect in the wake cueing condition as well as the sleep cueing one, we would be able to have a clearer, larger picture, which has better ecological validity, as memory reactivations in humans are almost never cued (in a way similar to the one we tested), and happen spuriously throughout the day and during sleep (Atherton, Dupret, & Mellor, 2015). For example, in the wake cueing condition, we would expect that the memory trace becomes weaker up to sleep, after which it would bounce back to the original levels.

Another analysis that could strengthen our argument and could be further applied to the data would be to use a more specific measure of the memory trace such as Representational Similarity Analysis (RSA) in order to capture the reactivations occurring during sleep. RSA could be used to compare the activity observed during encoding with the activity observed immediately post-cue, which, if positive, would be the first direct evidence of human memory reactivations during sleep. The functional relevance of these reactivations could then be studied by comparing these to memory performance. Furthermore, this type of analysis could also yield insights into how wake reactivations work and what their role is, by searching through the sleeping period for the activity of previously cued words.

Using a different experimental protocol, not dependent on cueing, with this kind of analysis, it might also be possible to capture and study natural memory reactivations, by provoking them through different means. For example, by taking advantage of the directed attention effect on memory (Dulas & Duarte, 2013) we can increase the likelihood of certain words to be replayed. We can then search for the representations of those specific words during subsequent waking or sleeping periods and thus have a model for natural memory formation and integration.

Conclusion

This thesis is part of a larger project with the aim of disentangling the effects of wake reactivations of memories from the effects of reactivations occurring during sleep. This thesis aids in reaching that goal, not only by demonstrating the effects of cueing during sleep, but also by outlining a way to measure the effects of cueing directly on the memory trace. We have found both behavioural and oscillatory effects of cueing during sleep. Starting with demonstrating the alpha desynchronisation related to memory and building up to demonstrating that sleep cueing directly increases the accessibility to the memories that were cued, this project adds to the current knowledge in the field of sleep and memory research. By design, these findings provide clear evidence that cueing during sleep has a positive effect on memory. Furthermore, this project adds to the current understanding of how memory reactivations strengthen memories by demonstrating that the original memory is more active after cueing during sleep. From a methodological perspective, this project adds to the field by outlining a way to search for reactivations of encoded memories. To be fair, the reactivations that were described in this thesis are related to recall, but there is no reason that the same statistical methods could not be used to study reactivations pertaining to the integration of memories. By applying these methods to other parts of the experiment, we could glean knowledge about the differentiated effects of sleep and wake cueing. In conclusion, this project adds both to the field of sleep research as a whole, and to the larger project that it is a part of.

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