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Improving Human Memory by Manipulating Consolidation During Sleep

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Nathan Whitmore

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Abstract

Research over the past several decades has revealed that memory reactivation in sleep contributes to the formation of long-lasting memories. Among the most recent developments in this field is the widespread use of the technique of targeted memory reactivation (TMR), which allows researchers to induce reactivation of specific memories during sleep. TMR has been demonstrated to enhance consolidation of memories in a wide range of tasks, raising the possibility that it could be useful as a general intervention to improve memory. In this thesis, I describe several experiments aimed at using TMR to improve human memory.

In chapter 2, I describe an experiment aimed at testing whether TMR could improve performance in a naturalistic face-name associative memory task. This experiment led to a surprising novel finding that sleep quality is a critical factor that determines whether TMR enhances or worsens memory. Improvement in memory was found when sleep was not disrupted by sound presentations.

In chapter 3, I describe an additional study on this effect, combining TMR with deliberate sleep disruption. This study confirmed results from the previous study and found that when TMR was paired with sleep disruption, the effects were reversed such that reactivated memories were degraded rather than strengthened.

In chapter 4, I describe the design and testing of a system based on smartphones and consumer smartwatches for performing TMR automatically outside of the sleep lab. Our results suggest that this system can replicate the effects of TMR on memory observed in laboratory studies.

Overall, the experiments described here demonstrated a novel finding that sleep disruption shapes the effects of TMR, and when sleep disruption is controlled TMR can be used to improve human memory. I also demonstrated that a new system for automated TMR using consumer wearables can be used for TMR outside the sleep lab, expanding the possibilities achievable with TMR.

Acknowledgements

First, my parents, who not only pushed me academically but encouraged a sense of curiosity. I'm convinced that a not a small part of my decision to become a scientist came down to the way they nurtured curiosity and wonder, from dinners where literally everything was up for discussion, to the electronics lab they set up in the laundry room, to the nearly endless patience every time I broke the family computer changing all the settings to see what would happen. My parents also instilled their core belief that we have a duty to make the world better, something I now strive to do through science.

Next my early research mentors—Paul Hagerman, Leslie Lyons, Simona Ghetti, and Marjorie Solomon, and their lab stuff, who let me mess about in their labs and see what doing science was really all about. Along with them I'd also mention the Davis High robotics club where I got my first taste of working on a big, collaborative tech project.

At Hampshire there are almost too many people to thank—it's an amazing place where I blossomed and learned to think deeply about things. But two particular people were Professors Joanna Morris and Neil Stillings. I still remember my sense of giddy excitement watching components average out in Joanna's ERP class, and I can credit both of them for my decision to go into cognitive neuroscience. Thanks are also due to the Lemelson Center, a wonderful engineering playground, and the Mad Science club.

Also to Shih-Chieh Lin and my lab at NIH, who taught me I could learn to do anything. I still remember Dr. Lin explaining that I should implant a surgical probe in some rats, to which I responded that I didn't know how to do brain surgery. His response--basically "well, learn brain

surgery and then implant the probe", is the attitude that I've tried to carry with me through my PhD.

Doing TMR experiments is really, really hard, and it wouldn't have been possible without a bunch of people. I'm incredibly grateful to Ken Paller, who has been the most supportive and kindest advisor I could have asked for. I'm also grateful to Susan Florczak and Bruce Caughran, our lab managers kept everything running through the literal apocalypse, and a whole host of people I worked with on research projects-- Adrianna Bassard, Carmen Westerberg, Donna Bridge, Eitan Schechtman-Drayman, Jessica Creery, Joel Voss, Karen Konkoly, Kristin Sanders, Laura Batterink, Marcia Grabowecky, Remy Mallet,,Sadie Witkowski, Shani Kaplan-Golan ,and Torin Kovach.

And finally, to all the friends I made along the way. I couldn't have imagined I would have such an amazing community of supportive people.

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Chapter 1—General Introduction

Of all the things that our brain does, memory is perhaps the most personal and intimate. The patterns of connections in our brains created through experience define how we understand and interact with the world, and fundamentally who we are.

It is not surprising then, that we often find it distressing when our memory systems fail us. These failures include simple failures to encode and recall information, but can also be thought of more broadly, to include things like the difficulty of establishing a new habit or breaking out of dysfunctional patterns of thinking.

Given the importance of memory, can we perhaps engineer our memory systems to work better, to help us remember the things we want and forget the things we don't? In this thesis, I suggest that manipulating memory consolidation during sleep can offer tools to improve our memory function and describe three experiments aimed at this goal.

1.1 Role of Memory Replay in Sleep

Psychologists have recognized the importance of sleep for memory for more than a hundred years (Patrick & Gilbert, 1896). Sleep probably contributes to memory function in several ways, including by promoting homeostasis in the brain (González-Rueda et al., 2018), and by reducing interference to recently acquired memories (Ellenbogen et al., 2006). In addition to these mechanisms, sleep appear to be a period where memories are actively transformed and

stabilized though a “replay” process in which neural systems that were active at the time of the experience exhibit the same patterns of activity during sleep s

Replay was first described by Pavlides & Winson (1989) who observed that after when rats slept after exploring an area, place cells corresponding to the explored area (but not those corresponding to an unexplored area) fired during sleep, and they proposed that this firing represented processing of information during sleep. This effect was widely replicated by other researchers, who found that entire trajectories taken by the animal could be decoded during sleep (Foster, 2017).

Further studies have also shed light on the nature of this replay process. Place-cell replay is accompanied by a characteristic pattern recorded in the hippocampal LFP called a sharp-wave ripple, which is also observed during waking recall (Buzsáki, 2015). In human sleep, sharp-wave ripples in the hippocampus tend to occur with cortical sleep spindles, stereotyped oscillations which occurs at 12-15 Hz at multiple locations across the cortex during non-REM sleep. (Clemens et al., 2007).Spindles and ripples further appear to be orchestrated by cortical slow waves, very large oscillation in cortical LFP that occur during sleep stages N2 and N3. Spindles and ripples occur more frequently when slow waves are present, and preferentially occur in the depolarized phase of the cortical slow wave (Clemens et al., 2007).

Studies examining phase coupling between spindles and ripples have suggested that these spindle-ripple events are windows of communication between the hippocampus and neocortex during sleep (Ngo et al., 2020). Intriguingly, spindles appear to also be periods of memory reactivation in the cortex; when memory reactivation is induced using targeted memory reactivation, information about the reactivated memory can be decoded from the cortical EEG during spindle periods (Cairney et al., 2018). Similarly, Wang et al., (2019) found that

decodability was correlated with spindle-band power. Schönauer et al. (2017) also found that patterns of spindle power during sleep could be used to decode what type of information was learned before sleep, much like place cell replay can be used to decode which locations an animal visited before sleep.

Thus, sleep appears to be a period in which the hippocampus and neocortex communicate while both process information about recent events. Why might this occur? An influential theory is that memory processing during sleep promotes “systems consolidation”, the transfer of memories from short-term storage which depends on the hippocampus to long-term storage which does not. Systems consolidation is a well-established theory in cognitive neuroscience; it is best demonstrated in patients with hippocampal injuries, who often lose memory for events occurring shortly before the injury, but retain their memory for events that occurred many years prior (Squire & Alvarez, 1995). While there is considerable controversy about what memory processes the hippocampus is required for (Moscovitch & Gilboa, 2021) these experiments demonstrate that at a minimum memories undergo some type of transformation that makes them less vulnerable to hippocampal disruption over time.

Why do memories undergo this transformation? A leading hypothesis is that it enables us to retain important information over time while forgetting unimportant information. In this hypothesis, information which is not consolidated is forgotten quickly, possibly because hippocampal neurons are frequently remapped for other uses. Systems consolidation results in the formation of longer-lasting engrams in cortex which allow information to be retained long periods of time (Alvarez & Squire, 1994; Marr & Brindley, 1970; Squire & Zola-Morgan, 1991). Systems consolidation not only allows for long-term memory, but also allows for memories to be filtered based on usefulness. For example, memories which are retrieved and practiced

frequently after they are initially learned are forgotten less than memories which are rarely retrieved (van den Broek et al., 2014). Thus, systems consolidation allows us to form a lasting representation of useful information (the route to a friend's house) while forgetting potentially confusing or irrelevant information (such as the incorrect directions given by a confused stranger).

Mechanistically, reactivation during sleep could be conceptualized to improve memory through practice effects. It is well understood that voluntary retrieval practice aids memory retention (Roediger & Butler, 2011); a similar effect may occur during reactivation in sleep. A popular, but speculative model hypothesizes that reactivation in sleep might produce consolidation through Hebbian plasticity in the cortex; in this model hippocampal input during reactivation drives cortical neurons, and Hebbian plasticity between co-activated neocortical neurons produces a neocortical engram (Alvarez & Squire, 1994).

Given this, what evidence suggests memory reactivation might promote consolidation? Multiple studies have shown that the rate of forgetting is modulated by with sleep quality and quantity, with a typical finding that poor sleep results in a greater decline of memory overnight (Walker & Stickgold, 2004). However, these studies cannot specifically address the role of reactivation processes.

A more specific experiment by Ego-Stengel & Wilson (2010) used electrical stimulation to disrupt hippocampal activity during sharp-wave ripples, without affecting overall sleep patterns, with the goal of specifically disrupting the memory reactivation process. In this experiment, rats learned the layout of a maze more slowly when they received ripple-targeted stimulation, suggesting that disrupting memory reactivation could impair learning.

Replay in rodents has also been observed to prioritize important information such as locations associated with reward over those with no reward (Belal et al., 2018), an effect which parallels the theoretical role of systems consolidation in selecting important information.

1.2 Targeted Memory Reactivation

The best evidence for reactivation as a consolidation mechanism has come from targeted memory reactivation (TMR) studies, which use sensory cues to induce reactivation of a specific memory during sleep.

In the first experiment to use this approach, Rasch et al., (2007) demonstrated that a sensory cue linked to a specific memories in slow-wave sleep could improve recall performance after sleep. In this experiment, participants learned the locations of objects in a spatial memory task while being exposed to rose odor. Participants then slept overnight in the lab, where half received rose odor during non-REM stage 3 (N3) and half received no odor during sleep. After waking, participants who received odor remembered the objects more accurately than those who did not. A subsequent experiment found no effect on memory when the odor was presented during sleep but not during learning, demonstrating that the odor in N3 improved memory only because the sensory cue was linked to the learning.

A second experiment by Rudoy et al. (2009) found that sensory cues could be used to reactivate individual object locations. In this experiment, participants learned locations of objects on a grid, and during the learning each object was associated with a unique sound. Half of the

sounds were played very softly during N3 sleep and recall accuracy after awakening was better for the objects cued during sleep than the objects not cued.

In these and other early TMR experiments, reactivation of the memory during sleep was inferred by the observation that the memory was modified by sensory cues in sleep. More recently, studies have directly demonstrated reactivation evoked by TMR in humans and in rodents. Cairney et al. (2018) demonstrated that after a TMR cue it is possible to decode the information associated with that cue from the EEG, implying that the information was actively processed following the cue. Bendor & Wilson, (2012) trained rats to associate sounds with reward locations and presented sounds in sleep while recording hippocampal place cells. After a sound, the spontaneous replay of trajectories observed in the hippocampus was biased towards the trajectory associated with that sound. Notably, this study found that replay events after sounds appeared to be very similar to naturally occurring replay events.

TMR effects have now been repeatedly replicated by multiple labs. A recent meta-analysis (Hu et al., 2020) found that TMR during sleep was capable of improving declarative memory (as measured using tasks like recalling words), procedural memory (measured using tasks that require participants to learn and execute a skill). TMR effects were detectable when TMR was performed in either stage 2 or stage 3 of nonREM sleep, but not in REM sleep. In almost all cases, the effect of TMR on consolidation is inferred the same way as in the first two experiments: participants perform learning, followed by a period of sleep, and then a memory test. In within-subjects experiments only a subset of learned items are reactivated in sleep; other experiments use a between-subjects design where subjects receive either TMR or sham TMR. Both methods have revealed a consistent effect of TMR on memory performance after sleep. The findings that TMR can elicit memory reactivation, and also that TMR improves

performance for specific memories, have greatly strengthened the theory that reactivation in sleep contributes to memory consolidation.

1.3 Targeted Memory Reactivation as a Memory Enhancement Therapy

The ability of TMR to modulate memory consolidation has also raised the possibility that it could be used outside of the lab to facilitate learning (Oudiette & Paller, 2013). The current literature suggest that TMR could potentially be useful wide range of situations including enhancing learning of academic material (Gao et al., 2020) and new skills (Johnson et al., 2021). TMR could also potentially be useful in clinical practice. Many therapies, such as neurorehabilitation after a stroke, or psychotherapy for mental illness require the patient to learn new skills, a type of learning which is known to be facilitated by TMR. More speculatively, TMR might be able to reduce the subjective memory issues, which are often reported by older adults and people with mental illnesses (Reid & MacLullich, 2006).

Using TMR outside of a research environment requires two major developments. First, researchers must demonstrate that the benefits of TMR can extend outside of specific laboratory memory tasks. In Chapter 2 I addressed this question using a face-memory task intended to more closely mirror real-world learning. This research led to the discovery of a novel effect where small arousals during sleep can reverse the effects of TMR, which I explore more in Chapter 3.

In this thesis, chapter 2 is a published research study and chapter 3 is a manuscript currently undergoing review. Chapter 4 has not yet been submitted for publication Nathan Whitmore is

the first author of all three manuscripts, contributions of other authors have been described in the author contributions sections. The text of the submitted and published articles has been edited for consistent formatting and to remove a redundant section from the introduction of chapter 3.

Chapter 2--Targeted Memory Reactivation of Face-Name Learning Depends on Ample and Undisturbed Slow-Wave Sleep

2.1 Abstract

Face memory, including the ability to recall a person's name, is of major importance in social contexts. Like many other memory functions, it may rely on sleep. We investigated whether targeted memory reactivation during sleep could improve associative and perceptual aspects of face memory. Participants studied 80 face-name pairs, and then a subset of spoken names with associated background music was presented unobtrusively during a daytime nap. This manipulation preferentially improved name recall and face recognition for those reactivated face-name pairs, as modulated by two factors related to sleep quality; memory benefits were positively correlated with the duration of stage N3 sleep (slow-wave sleep) and negatively correlated with measures of sleep disruption. We conclude that (a) reactivation of specific face-name memories during sleep can strengthen these associations and the constituent memories, and that (b) the effectiveness of this reactivation depends on uninterrupted N3 sleep.

2.2 Introduction

We often rely on face recognition and name recall — such as when we notice friends from a distance and call to them by their names. Most people are extraordinarily adept at recognizing faces of individuals, even those they've met just once. Yet, there are also times when we fail to recognize someone—and it can be embarrassing when we forget a name that we should have remembered.

What determines which memories continue to be enduringly available and which are forgotten? Given that the human brain is remarkably active during sleep, researchers have asserted that neural events during sleep may function to stabilize and strengthen recently acquired memories(Marr, 1971; Paller, 1997; Paller et al., 2020; Winson, 1974). The delineation of these neural events and their specific ramifications for memory has become increasingly central to memory research and the science of learning.

A prevalent view is that memories can benefit due to spontaneous replay during sleep(Born & Wilhelm, 2012; Pavlides & Winson, 1989; Sejnowski & Destexhe, 2000; Sirota et al., 2003). In recent years, Targeted Memory Reactivation (TMR) has emerged as a useful tool for investigating this process(Oudiette & Paller, 2013). In the TMR procedure, information that people learn is associated with a sound or smell during learning. Researchers then present the same sensory cue while people sleep, without waking them. After sleep, people remember information associated with the cue stimulus better than other information that was equally well-learned, a frequently reported finding confirmed in a recent meta-analysis (Hu et al., 2020). Moreover, the notion that TMR benefits memory through reactivation is supported by neuronal evidence of hippocampal place cell replay engaged following the presentation of learning-related sounds during sleep(Bendor & Wilson, 2012).

In addition to functioning as a powerful research tool, TMR offers the potential to enhance memory with a simple, non-invasive intervention during sleep, which may be useful in many scenarios. Because learning face-name associations is an important and widely relevant form of memory, we asked whether TMR could enhance this type of learning.

We developed a procedure whereby participants learned about people ostensibly in either a Japanese History class or a Latin-American History class. Learning was accompanied by a background music track, either traditional Japanese music or traditional Latin-American music, respectively. Each classroom had 40 pupils. To learn the names of these pupils, participants viewed each face adjacent to the corresponding written name while also hearing the spoken name. Recall training, feedback, and visualization practice served to solidify this learning. Next, we assessed both face recognition and name recall. Then, during a period of sleep, some of the spoken names and associated background music were softly presented. After awakening, participants were exposed to additional faces and names, potentially interfering with the original learning. Finally, we assessed memory again. Figure 2.1 shows the experimental procedure schematically, and additional details are provided in *Methods*.

This experimental design thus makes it possible to determine the extent to which face memory can be selectively improved by reactivation during sleep. Such results, along with analyses of relationships between memory change and characteristics of slow-wave sleep, could have implications for understanding the physiological mechanisms that generally enable memory reactivation during sleep to be effective.

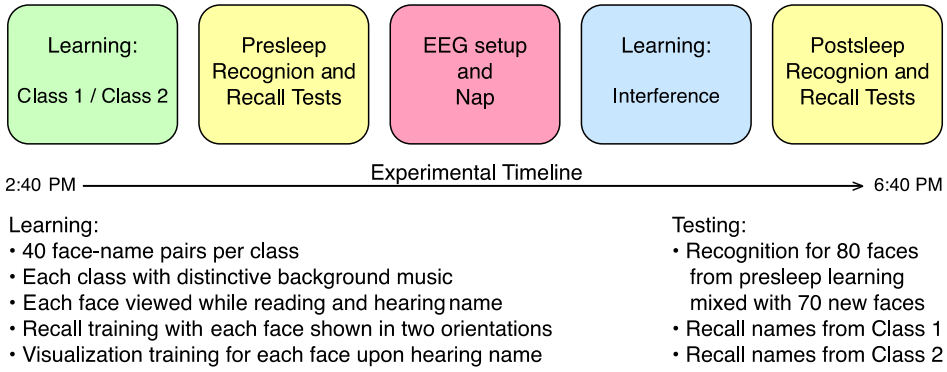

Figure 2.1 *Experimental procedure overview.*

2.3 Results

The influence of TMR on memory varied with duration of stage N3 sleep

Memory testing demonstrated effective learning of faces and associated names. On the name recall test, participants correctly recalled a mean of 74.04 (SD=4.83) names before sleep and 75.00 (SD=4.03) names after sleep (in each case from a total of 80 face-name associations). When requested, hints were provided in the form of up to three starting letters. Across all 80 pairs in the test, participants requested a mean of 0.77 (SD=0.69) hint letters per pair before sleep and 0.80 (SD=0.75) after sleep. On the presleep recognition test, participants successfully recognized 97% (SD=3%) of the identical old faces and 92% (SD=7%) of the old faces that were rotated from their original view. For new faces, 74% (SD=24%) were endorsed correctly as new faces. Postsleep recognition scores were similar (97%, 91%, and 85% correct for old, rotated, and new, SDs=4%, 9%, 15%, respectively).

To examine the effect of TMR, we first computed the change in the number of names successfully recalled across sleep. This value, Δrecall, did not differ significantly between the cued class, designated *class C*, and the uncued class, designated *class U* [mean Δrecall 0.75 and 0.21, SDs=2.03, 1.70 respectively; $t(23)=0.93$, $p=0.36$].

In initial planned comparisons, we analyzed the cueing effect on *Δrecall* (defined as the difference between class-C Δrecall and class-U Δrecall) in relation to several sleep measures: total sleep duration, N2 duration, N3 duration, and REM duration. As shown in Figure 2.2A, the size of the cuing effect on Δrecall was correlated with the duration of stage N3 sleep [$\chi^2(1, N=24)=5.18$, p=0.02]. This cueing effect was not significantly correlated with the duration of other sleep stages. Taking into account the total sleep duration during the nap, a follow-up exploratory analysis revealed an even stronger association between the cueing effect and relative duration of N3 sleep [$\chi^2(1, N=24)=14.52$, p<0.001, FDR $p=0.001$].

We found similar effects with face recognition. To measure recognition accuracy, we collapsed performance across old and rotated faces and computed d' statistics for class C and class U before and after sleep. Overall, recognition accuracy increased after sleep [mean increase=0.43, SD=0.47, $t(22)=4.37$, $p<0.001$], but Δrecognition (change in d') did not differ between class C and class U [mean Δrecognition 0.37 and 0.49, SD=0.50.0.54 respectively; $t(22)=1.25$, $p=0.22$]. Nevertheless, the cuing effect on Δrecognition was associated with N3 duration (Figure 2.2B), paralleling the results for name recall [$\chi^2(1, N=23)=5.56$, $p=0.02$].

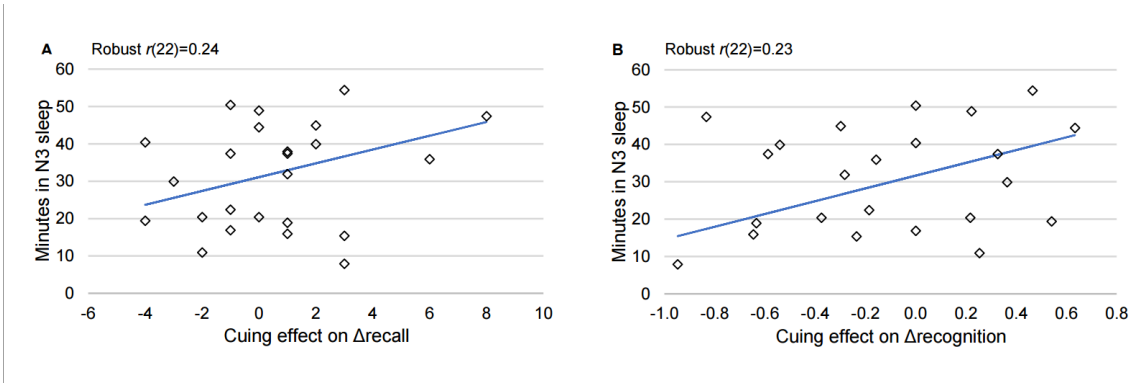

Figure 2.2 N3 duration was correlated with the cuing effect on Δrecall (A), and with the cuing effect on Δrecognition (B).

The influence of TMR on recall depended on undisturbed sleep

A previous report found that participants who self-reported sleep disruption during TMR did not benefit from memory cues(Göldi & Rasch, 2019).To objectively assess sleep disruption related to cue delivery during sleep, we developed the Sleep Disruption Index to quantify arousals in the EEG after spoken name cues. As an arousal is conventionally defined by an abrupt shift in the spectral content of the EEG(Iber, C et al., 2007), we summed the absolute change in EEG power at Cz (increase or decrease) across a broad frequency band from 0.38-20.35 Hz during the 5 seconds after spoken-name cue onset relative to the 5 prior seconds. The Sleep Disruption Index thus focuses on periods surrounding name cue presentation. Additionally, we computed another measure that encompasses the whole nap period, the Sleep Fragmentation Index, which quantifies sleep-stage transitions per hour(Haba-Rubio et al., 2004). We then used a correlational analysis to examine whether the memory benefit of TMR was associated with these indices of sleep disruption.

As shown in Figure 2.3A, cue-related sleep disruption, as assessed by the Sleep Disruption

Index, was negatively correlated with the cuing effect on Δrecall [χ^2 (1, N=24)=3.98, p=0.046]. There was a trend for a similar effect with the Sleep Fragmentation Index [χ^2 (1, N=24)=2.57, p=0.109]. We did not observe correlations between either sleep index and recognition performance.

Because the Sleep Disruption Index encompasses a broad frequency range, we conducted follow-up analyses to identify whether certain frequency bands were associated with the cuing effect on Δrecall. We divided the 0.38-20.35 Hz spectrum into six frequency bands, each 3.33-Hz wide, and then we correlated cue-evoked power in each band with the cuing effect on Δrecall. Cue-evoked power in the highest beta band (17.03-20.35 Hz) was inversely correlated with the cuing effect; a greater cuing effect was seen with less beta power after a cue [χ^2 (1, N=24)=10.1, p=0.0015, Figure 2.3B]. We also found marginal negative correlations between the cuing effect on Δrecall and power in the lower alpha band [7.04-10.37 Hz, χ^2 (1, N=24)=3.83, p=0.05] as well as power in a lower beta band [13.7- 17.03 Hz, χ^2 (1, N=24)=3.43, p=0.06]. As abrupt increases in alpha and beta are associated with arousal from sleep(Iber, C et al., 2007), these data suggest that TMR depends on uninterrupted sleep.

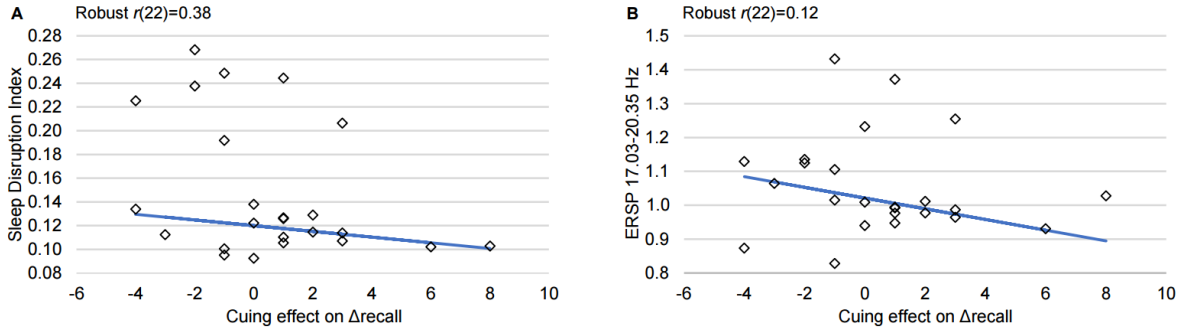

Figure 2.3 (A) Sleep disruption after a cue was inversely correlated with the cuing effect on Δrecall. (B) Greater post-cue EEG power in the high beta band (17.03-20.35 Hz) was associated with a smaller cuing effect

Measures of N3 duration, cuing, and sleep disruption are highly intercorrelated

N3 duration was highly correlated with the Sleep Disruption Index [$r(22)=-0.6$, $p=0.002$], Sleep Fragmentation Index [$r(22)=-0.65$, $p=0.001$], number of name cues presented during sleep [$r(22)=0.59$, $p=0.002$], and total number of sleep-stage transitions [$r(22)=-0.44$, $p=0.03$]. To test whether these variables independently explained variance in the cueing effect on Δrecall, we performed multiple regression. The overall regression model predicted the cuing effect on Δrecall [$r(17)=0.55$, $p=0.01$]. Several variables had significant coefficients: Sleep Fragmentation Index [$\chi^2(1, N=24)=6.39$, $p=0.01$], Sleep Disruption Index [$\chi^2(1, N=24)=6.23$, $p=0.01$], and number of name cues [$\chi^2(1, N=24)=5.23$, $p=0.02$]. Therefore, these measures represent correlated but separable facets of overall sleep quality.

Additional exploratory analyses

We also performed correlations between cuing effects and several other sleep and participant variables, as described in Supplemental Tables 2.1 and 2.2. Notably, in addition to the correlations with time in N3, the cuing effect on Δrecall was positively associated with participant age and percent of N3 sleep during the nap. The cuing effect on Δrecall was negatively associated with total sleep-stage transitions during the nap, which is a component of the Sleep Fragmentation Index. In addition to the association with N3 time, cuing effect on Δrecognition was negatively associated with measures of stage 2 sleep (percent stage 2 sleep and sigma power) as well as with participant age. We also correlated N3 time and sleep disruption with other memory measures (Supplemental Table 2.3). The finding that older participants showed a larger cuing effect may have reflected higher sleep quality in these individuals; these older participants (the oldest of which was 31 years old) had a lower Sleep Fragmentation Index [$\chi^2(1, N=24)=4.08$, $p=0.04$] and a lower Sleep Disruption Index [$\chi^2(1, N=24)=4.69$, $p=0.03$], and

they received a larger number of spoken cues [χ^2 (1, N=24)=4.79, p=0.03].

We used a decision-tree partition analysis (JMP 15) to ask whether we could classify participants as TMR responders or non-responders based on number of spoken cues, N3 duration, or sleep disruption index. Sleep Disruption Index was the single strongest predictor of the Δrecall cuing effect. Participants with a Sleep Disruption Index < 0.13 (n=15) had a positive Δrecall cuing effect [mean=1.53 names, SD=2.75, t(14) = 2.16, p=0.049], indicating superior recall for the cued class. Participants with a Sleep Disruption Index > 0.13 did not have a significant Δrecall cuing effect. For the cuing effect on Δrecognition, number of spoken cues was the single strongest predictor; participants with >157 spoken cues had a nonsignificant trend for a positive cuing effect [mean=0.37, SD=0.27, t(3) = 2.73, p=0.072], whereas those with fewer spoken cues had a negative cuing effect [mean=-0.22,SD=0.42, t(18) = 2.29, p=0.03].

2.4 Discussion

In this experiment we observed that presenting cues during sleep influenced two important memory abilities: recognizing a face that was recently viewed and recalling a person's name when seeing their face. To the extent that slow-wave sleep was ample and undisturbed, cues reactivated recent memories during sleep and thereby improved both the ability to recognize faces and the ability to recall the associated names. We conclude that memory reactivation during sleep can improve memory for people's faces and names. Notably, benefits of memory reactivation were greater in those individuals with longer periods of slow-wave sleep and without signs of sleep disruption during their afternoon nap in the laboratory.

Delivering auditory or olfactory cues during slow-wave sleep with the goal of targeted memory reactivation has previously been shown to improve many types of memory(Hu et al., 2020). The conclusion that memory reactivation during sleep supports memory function has received

support from these studies conducted in many countries, during both afternoon and nocturnal sleep, and in both laboratory and home environments. In this research area, however, learning of face-name associations has not previously been examined. Our results add to this literature by showing that these additional types of memory, name recall and face recognition, are subject to memory reactivation during sleep. The experiment also provided novel evidence that might be generally applicable with respect to any type of learning. That is, objective measures of N3 duration and sleep disruption after cues moderated the degree to which memory reactivation during sleep produced name-recall improvement.

Compatible findings have been reported from studies of TMR in word-learning paradigms using self-reports of sleep disruption (Göldi & Rasch, 2019) and using measures of REM duration (Batterink et al., 2017). We propose that variation in quality and quantity of sleep represent important factors sometimes overlooked in the broader TMR literature.

Such variation may explain some instances in which TMR does not benefit memory. Meta-analysis has shown that the extant use of TMR methods produced a significant, small-to-moderate effect size overall, but the effect size was highly variable even across similar studies(Hu et al., 2020). Differences in sleep quality and disruption may explain some of this variability; these parameters are not usually reported. Moreover, our results suggest that optimizing sleep quality during TMR may increase the overall size of TMR benefits on memory or other aspects of cognition.

Our results also raise a further question: what is the relationship between momentary arousal, N3 duration, and TMR effects? (Göldi & Rasch, 2019) proposed that sleep disruption caused by auditory stimulation may introduce interference into newly reactivated memories. Conversely, arousals may have no direct effect on memory, but simply reduce N3 duration, which in turn

reduces TMR benefits. A third possibility is that short N3 duration and high arousability both reflect a latent factor of sleep quality that mediates TMR effects, as suggested by the inter-correlations between measures of arousal and N3.

Whereas N3 duration was associated with cuing effects in both the recognition and recall tests, we observed several differences between the tests; for instance, sleep disruption was associated with cuing effects in the recall test but not in the recognition test. The correlations between participant variables such as sleep parameters and the cuing effect on memory may be shaped by several factors that differ between the tests, including ceiling effects, total amount of forgetting, and unintended spreading of reactivation from the cued class to the uncued class. There are multiple ways to quantify memory performance (e.g., as shown in Supplemental Table 2.1), and measures vary in sensitivity and other psychometric properties. Modified protocols, for instance with a between-subjects design or memory testing at a longer delay, may shed light on the role of these factors. When memory is tested immediately after sleep with TMR, evidence that reactivation was helpful might be most clearly evident for memories that would otherwise fall just short of being strong enough to be remembered at that time point.

In our procedure, sleep cues included both specific spoken names from the learning phase as well as the background track for learning, which was a unique music genre. We therefore cannot determine whether TMR benefits were due to the spoken names, the music, or both. In prior experiments, TMR benefits have been observed using spoken words (Cairney et al., 2018; Schreiner & Rasch, 2015) as well as short music tracks (Antony et al., 2012; Sanders et al., 2019).

Other limitations of this study could also be addressed in future research. In particular, it was not possible to measure how sleep disruption after cues affected the fate of individual memory

items. Future studies that take into account item-specific sleep disruption measures may shed more light on mechanisms of memory change. Finally, because we recorded only one sleep session, it is unclear whether differences in sleep quality represent stable individual differences. Future studies could employ additional manipulations that address such questions.

Overall, the present results show that provoking memory reactivation during sleep can influence learning of face-name associations, with consequences for whether people can recall the correct name after sleep. Furthermore, the magnitude of this effect was found to depend on the length of N3 sleep and on the absence of signs of arousal after spoken cues. We propose that N3 duration and cue-evoked arousal are important factors shaping how memory reactivation during sleep influences subsequent memory performance. These factors thus have relevance for future research aimed at making progress in understanding the neural mechanisms whereby learning is influenced by subsequent sleep even when there is no sensory input during sleep. Considerations of N3 disruption might be especially relevant when memory modification is desirable, including in clinical contexts(Paller, 2017). Measures of sleep disturbance should thus be examined closely in future studies seeking to determine if applications of targeted memory reactivation can produce clinical or other benefits.

2.5 Methods

Participants and Experimental Design

Participants (*N*=24) were 8 males and 16 females 18-31 years old (mean=23.38, SD=4.44) recruited from the Northwestern University population and surrounding community. Figure 2.1 shows an overview of the procedure, which was approved by the Northwestern university institutional review board. The design provided within-subject comparisons of memory performance as a function of whether cues were presented during sleep. To increase the

chance of napping, participants were asked to go to bed 1 hour later or wake up 1 hour earlier than normal, and to avoid nicotine or caffeine on the day of the study. After participants arrived in the lab and gave written informed consent, the following phases transpired: learning, pre-nap test, bioelectric recording setup, nap, and post-nap test. After completing these steps, they were paid for their participation.

Procedure

Learning Phase. Participants completed a face-name learning task intended to simulate learning the names of pupils in two classes, with 40 face-name pairs per class. Each class was associated with a distinct instrumental music track, traditional Latin-American music or traditional Japanese music. Participants completed all learning tasks for Class 1 followed by all learning tasks for Class 2.

Face-name learning started with initial exposure plus interspersed name recall. Participants viewed sets of five face-name pairs, each shown one at a time while the spoken name was played over speakers. In addition to the name, which included a first name and a surname, a one-sentence fact about the person (such as “I love cats and fall weather”) was also viewed. Each to-be-learned person was presented twice during initial exposure with the same biographical information, with the face shown once in a quarter-right and once in a quarter-left orientation, to encourage learning of facial structure. Prompts for name recall appeared after every set of five pairs; each of the five faces appeared and participants attempted to type the corresponding first name. Following each recall attempt, feedback was provided (the face paired with the correct name simultaneously shown and spoken).

Following initial exposure with interspersed name recall for all 40 pupils in the class, participants began recall training. In this task, the same faces (both orientations) were shown, one at a time,

as prompts for recall of the corresponding first name. When a correct name was recalled and entered via the keyboard, the facial image was dropped from the training list. The remaining facial images were shown repeatedly, in random order. Visual and auditory feedback was again provided on each trial. Training was complete when the participant gave the correct first name for each person in the class on two occasions (i.e., after seeing both facial orientations).

Participants were then asked to visualize each person in the class when the corresponding name was spoken. This task was intended to promote face visualization in response to names, even with names presented during sleep. Each visualization attempt was followed by a prompt to identify the visualized person from two same-sex alternatives, using a foil face randomly chosen from the same class as the visualized face. The two faces were presented 3.5 s after name onset. Feedback was given after the participant made each choice (the face paired with the spoken and written name).

Following training for Class 1, all training steps were repeated for Class 2, with the order of the Japanese History class and the Latin-American History class counterbalancing across subjects. Participants completed all learning steps for both classes in approximately 45 minutes.

Pre-sleep and post-sleep tests. The first test given was a self-paced recognition test for faces. Participants viewed 230 faces sequentially and were asked to decide if each person was in Class 1, Class 2, or was a new person not seen before. They were asked to perform this categorization regardless of whether the face was rotated from the view seen earlier. Each test included the 80 previously learned faces identical to the images seen earlier (*old*), 70 new faces (*new*), and each of the 80 previously learned faces from a different angle (*rotated*). Old faces were presented at quarter-right orientation, whereas rotated faces were profile views, facing right in the pre-sleep test and left in the post-sleep test. New faces were presented with equal

numbers at quarter-right orientation and the rotated orientation for that test (profile left or profile right). New faces in both recognition tests were faces not shown previously. Faces were presented in random order. Due to a technical issue, recognition data were not available for one participant.

Name recall followed recognition testing. Participants were asked to type a person's first name given a picture of the corresponding face. Faces appeared in either a quarter-right or quarter-left orientation one at a time in random order. Participants could receive hints by pressing the tab button to receive a letter, up to the first three letters of the person's first name. No feedback was provided during this test. Each participant performed a cued recall test for Class 1 and then for Class 2.

The same recognition and recall tests were also given starting approximately 10 min after the end of the sleep phase, immediately following an interference task. In the interference task, participants were asked to memorize 20 new face/name pairs in a simplified procedure with no recall step or biographical details. This manipulation was intended to increase difficulty and simulate the type of interference that occurs in real-world face-name learning.

Physiological recording. We recorded electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG) signals during the nap using a BioSemi Active2 system with 32 scalp channels and 4 electrodes on the face. Data were acquired with a sampling rate of 512 Hz, filtered between 0.1 and 100 Hz during recording, and re-referenced to the right mastoid. EOG was recorded with electrodes lateral to the left and right outer canthus and underneath the right eye. EMG was recorded from the chin. Setup and recording began immediately before the start of the sleep period and electrodes were removed after sleep and before the interference task.

Nap and TMR. After EEG setup, participants slept on a futon in the same chamber where they completed the learning and testing tasks, with background white noise at a low level (43-44 dB). Participants slept for a mean of 59 min (range: 32 to 92 min). TMR began after participants had been asleep for a mean of 7.3 minutes (range: 1.6 to 21 minutes). TMR was manually initiated when the experimenter visually detected signs of stage N3, which has characteristic EEG slow waves. TMR was paused when sleep transitioned to any other sleep stage or to wake and resumed when participants re-entered N3. Offline, raters blind to when cuing occurred determined sleep stages according to standard rules for adults(Iber, C et al., 2007).

The specific TMR cues presented were selected as follows. The class randomly assigned to be cued was Class 1 for half of the subjects and Class 2 for the other half. There was thus a cued class and an uncued class (*Class C* and *Class U*, respectively). During TMR, the background music presented while learning class C was played continuously at low volume and half of the spoken names from Class C were presented at 10-s intervals. The specific names played during sleep were chosen to match presleep recall accuracy and number of hints required between cued and uncued names in class C. Intensity of the spoken names was controlled manually to deliver cues at the highest volume possible without causing arousal (45-50 dB peak). This design was chosen to reactivate both the general context of the cued class and specific items in this class, as both strategies may promote memory benefits (Antony et al., 2012; Cairney et al., 2018; Schreiner & Rasch, 2015).

Behavioral data analysis

To test whether cuing differentially influenced memory, we compared the change in memory performance across sleep for class C to that for class U. We defined the *cuing effect* by taking the difference between the two change scores as follows: (class C postsleep – class C

presleep) – (class U postsleep – class U presleep). Statistical significance was assessed using a two-tailed *t* test. Due to a technical error, we were not able to test whether TMR effects differed for the two conditions within class C (cuing with spoken name+background music and cuing with only background music).

To measure memory performance in the name recall test, we counted the number of faces for which the participant correctly entered the first name (with or without hints) in each class. To measure recognition performance, we computed a d' statistic with log-linear correction(Hautus, 1995) for each class based on the hit rate for that class and the false alarm rate pooled across both classes. Recognition statistics were computed treating both rotated and old faces as “old.”

Because participants designated each face as either “class 1”, “class 2”, or “new” during the recognition test, we considered either a “class 1” or “class 2” response as an “old” response, independent of whether the participant attributed the old face to the correct class.

Arousal analysis

Arousal during sleep is conventionally defined as an abrupt shift in the spectrum of the EEG (Iber, C et al., 2007).Therefore, we quantified arousal after TMR cues by measuring the absolute difference of the power spectra in two windows: [-5 0] seconds relative to cue onset and [0 5] seconds relative to cue onset. Arousal was calculated as the mean of abs(1-(postcue power/ precue power)) across linear-spaced 0.256-Hz-wide frequency bins from 0.38 to 20.35 Hz. Power was calculated using a short-time FFT (newtimef, EEGLAB 14.1.1b, 2-s window)(Delorme & Makeig, 2004). These parameters were chosen to cover the range of frequencies in which arousal-related activity appears, while providing sufficient frequency and time resolution to compute the sleep disruption index. As a secondary analysis to identify the frequencies correlated with TMR effects, we divided this frequency range into six 3.33-Hz-wide

bins and correlated the event-related spectral-power change for each bin (postcue power / precue power) with the cuing effect.

Correlation analysis

Because we observed outliers and heteroskedasticity in many of our correlates (especially higher variance in sleep measures when the cuing effect was low), we computed correlations between the cuing effect and sleep variables using robust linear regression. These regressions were performed with a Cauchy error distribution as implemented in JMP 15. Robust regressions were calculated with cuing effect as the independent variable and sleep measures as the dependent variable. Significance was determined using a Wald test to determine whether including the cuing effect significantly improved prediction of the dependent variable. Robust *r* values correspond to the variance in the unweighted data explained by the Cauchy regression line. We also computed correlations using ordinary least squares, which gave similar results (Supplemental Tables 2.3 and 2.4). Intercorrelations between sleep measures were computed using ordinary least squares as these correlations were not heteroskedastic. P values were adjusted for multiple comparisons using the false discovery rate(Benjamini et al., 2009).

2.6 Acknowledgements

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2.7 Author Contributions

This is a manuscript current published in a peer reviewed journal (Whitmore et al., 2022). N.W.W. and K.A.P. designed the experiment. N.W.W. and A.M.B. collected, analyzed, and interpreted data. K.A.P. supervised the project. N.W.W. and K.A.P. wrote the manuscript, with input and feedback from all authors.

2.8 Supplemental information

Supplemental Table 2.1: Participant and sleep metrics and their correlation with cuing effects as measured by Δrecall. Stage transitions and sleep fragmentation index were calculated as described in Haba-Rubio et al. (2004). SO (0.5-1 Hz), delta (1-4 Hz), and sigma (12-16 Hz) power were calculated between the first and last sleep epoch of recordings using short-time FFT (spectopo, EEGLAB 14.1.1b, 2-s window) after removing time periods containing artifacts. Bold text indicates significant correlations. Percent sleep times were calculated as the proportion of total sleep time spent in a specific phase. Sleep disruption index was calculated as described in the methods. We also calculated correlations using ordinary linear regression (OLS) which found a similar but less robust pattern due to outliers and heteroskedasticity in the data.

Measure	Mean	SD	Cauchy χ^2	Cauchy *p*	Cauchy *p* (FDR)	Cauchy r	OLS r	OLS *p*
Total sleep minutes	59.04	15.34	2.2	0.14	0.25	-0.44	-0.08	0.71
Stage 2 minutes	19.17	13.12	1.13	0.29	0.45	-0.61	-0.13	0.54
Stage 3 minutes	31.33	13.82	5.18	**0.02**	0.07	**1.15**	0.24	0.26
REM minutes	1.48	4.68	0	0.99	1	-0.66	-0.4	0.05
Age (years)	23.38	4.44	31.45	**<0.001**	<0.001	0.68	**0.44**	**0.03**
Number of name cues	118.63	41.19	5.67	**0.02**	0.07	3.97	0.27	0.2
Minutes between tests	113.42	14.82	2.06	0.15	0.26	-54.02	-0.17	0.42
Minutes of sleep after last cue	35.66	14.49	0.92	0.34	0.48	-73.01	-0.24	0.26
Total stage transitions	23.63	11.20	8.05	**0.005**	0.03	-1.46	*-0.37*	*0.07*
Sleep fragmentation index	24.81	13.17	2.57	0.11	0.26	-1.63	*-0.35*	*0.09*
Slow oscillation power (μV^2)	515.14	358.31	2.07	0.15	0.26	15.94	0.13	0.56
Delta power (μV^2)	117.88	89.28	0.24	0.62	0.7	0.97	0.03	0.89
Sigma power (μV^2)	2.43	1.32	0.62	0.43	0.52	-0.1	-0.22	0.31
Percent stage 3 sleep	53.76	22.04	14.51	**<0.001**	0.001	0.02	0.3	0.16
Percent stage 2 sleep	31.76	18.23	0.82	0.37	0.48	-0.01	-0.13	0.55
Percent REM sleep	2.02	6.47	0	1	1	-0.01	-0.42	**0.04**
Sleep disruption index	14.81	5.75	3.98	**0.046**	0.13	-0.01	*-0.38*	0.07

Supplemental Table 2.2: Correlation between participant/sleep metrics and Δrecog. Statistics were computed as described in the previous table.

Measure	Cauchy χ^2	Cauchy *p*	Cauchy *p* (FDR)	OLS r	OLS *p*
Total sleep minutes	0.84	0.36	0.85	0.17	0.4
Stage 2 minutes	0.01	0.93	1	-0.2	0.37
Stage 3 minutes	5.56	**0.02**	0.11	0.22	0.29
REM minutes	0	1	1	-0.39	*0.06*
Age (years)	15.13	**<0.001**	**<0.001**	-0.48	**0.02**
Number of name cues	0.12	0.73	1	0.2	0.36
Minutes between tests	0.25	0.62	1	0.22	0.3
Minutes of sleep after last cue	0.7	0.4	0.85	-0.04	0.85
Total stage transitions	2.04	0.15	0.51	0.23	0.29
Sleep fragmentation index	0.07	0.79	1	0.19	0.38
Slow oscillation power (μV^2)	0	0.95	1	0.09	0.67
Delta power (μV^2)	0.87	0.35	0.85	0.15	0.48
Sigma power (μV^2)	10.59	**0.001**	**0.01**	-0.15	0.50
Percent stage 3 sleep	0.05	0.82	1	0.14	0.53
Percent stage 2 sleep	5.02	**0.03**	0.13	-0.33	0.12
Percent REM sleep	0	1	1	0.38	0.07
Sleep disruption index	0.31	0.58	1	-0.20	0.36

Supplementary Table 2.3: Memory measures**.** Whereas name recall and face recognition scores were not significantly influenced by TMR, the cuing effect was positively correlated with minutes in N3 sleep (for both measures) and negatively correlated with the sleep disruption index (for name recall). P values were computed using robust regression with a Cauchy distribution as described in the methods and r values were calculated from ordinary least squares regression. TMR had a larger influence in participants with more N3 sleep and less sleep disruption.

Mean ± SEM	Pre-sleep		Post-sleep		Cuing effect	Cuing x N3 duration	Cuing x sleep disruption
	Uncued	Cued	Uncued	Cued			
Names recalled	37.08 ±2.50	36.96 ±3.20	37.29 ±1.55	37.71 ±2.94	0.54 ±2.84	*r* = 0.24 *p* = 0.02	*r* = -0.38 *p* = 0.046
Names recalled with no hints	23.54 ±2.27	23.46 ±2.32	23.75 ±2.35	22.96 ±2.24	-0.71 ±0.81	*r* = -0.19 *p* = 0.75	*r* = 0.02 *p* = 0.75
Mean hints per name recalled	0.85 ±0.17	0.84 ±0.16	0.87 ±0.18	0.87 ±0.17	-0.01 ±0.07	*r* = -0.03 *p* = 0.43	*r* = -0.09 *p* = 0.87
Recognition d' for faces	2.56 ±0.19	2.58 ±0.18	3.05 ±0.22	2.95 ±0.22	-0.12 ±0.09	*r* = 0.22 *p* = 0.02	*r* = -0.2 *p* = 0.58

Supplemental Table 2.4: Differences in sleep and participant measures between the 12 participants with the highest N3 duration and the 12 participants with the lowest N3 duration (median split). Measures are computed as described in Supplementary Table 2.2.

Measure	High N3	Low N3	*p*
Total sleep minutes	62.75	55.33	0.24
Stage 2 minutes	12.46	25.88	**0.01**
Stage 3 minutes	43.38	19.29	**0.00**
REM minutes	1.23	1.83	0.77
Age (years)	24.75	22.18	0.18
Number of cues	137.50	99.75	**0.02**
Minutes from presleep test to postsleep test	115.17	111.67	0.57
Minutes of sleep after last cue	33.77	37.54	0.54
Total stage transitions	18.42	28.83	**0.02**
Sleep fragmentation index	17.49	32.13	**0.00**
Slow oscillation power (μV^2)	721.54	308.74	**0.00**
Delta power (μV^2)	165.61	70.14	**0.01**
Sigma power (μV^2)	2.56	2.31	0.64
Percent stage 3 sleep	0.71	0.36	**0.00**
Percent stage 2 sleep	0.18	0.45	**0.00**
Percent REM sleep	0.02	0.03	0.70
Sleep disruption index	0.12	0.18	**0.01**

Chapter 3--Sleep Disruption Selectively Weakens Reactivated Memories

3.1 Abstract

A widely accepted view in memory research is that recently stored information can be reactivated during sleep, leading to memory strengthening. Two recent studies have shown this effect can be reversed in participants with highly disrupted sleep. To test whether weakening of reactivated memories can result directly from sleep disruption, in this experiment we varied the intensity of memory reactivation cues, such that some produced sleep arousals. Prior to sleep, participants (local community members) learned the locations of 75 objects, each accompanied by a sound naturally related to that object. Location recall was tested before and after sleep, and a subset of the sounds were presented during sleep. Reactivation with arousal weakened memories, unlike the improvement typically found. We conclude that reactivated memories can be selectively weakened during sleep, and that memory reactivation may strengthen or weaken memories depending on additional factors such as concurrent sleep disruption.

3.2 Introduction

The TMR procedure relies on the premise that sensory stimulation can be delivered during sleep without producing awakening or arousal from sleep. However, sleep may indeed be disrupted under some circumstances. Göldi and Rasch (2019) described a TMR procedure in participants' homes, unsupervised by laboratory personnel, and they found that when

participants reported that reactivation cues disturbed their sleep, reactivated items were remembered less well than items not reactivated. However, there were no measures of sleep physiology to assess the sleep disruption. Subsequently, in a laboratory study, Whitmore and colleagues (2022) found that participants with shallow sleep and large numbers of arousals evident in EEG recordings did not show the normal benefits of TMR, and in some cases showed weakening of reactivated memories.

What might explain these effects? We hypothesized that reactivation combined with sleep disruption introduces errors into memory traces. This model rests on two claims. The first claim is that memory engrams can be modified when memories are reactivated during sleep, thereby allowing for strengthening and consolidation. The second claim is that if sleep does not remain undisturbed during reactivation, but is instead disrupted, consolidation can go awry, inducing errors in the reactivated memory trace.

An initial prediction of this hypothesis is that memory reactivation accompanied by sleep disruption should produce forgetting, unlike reactivating a memory without sleep disruption. To test this prediction, we developed an experiment in which we reactivated memories during sleep with a varying amount of sleep disruption.

3.3 Methods

Participants (*N*=24) were a convenience sample of 11 males and 13 females 18-30 years old (mean=21.6, SD=3.79) recruited from the Northwestern University population and surrounding community. Inclusion criteria included being between 18-35 years old, self-reporting being able to sleep in the afternoon in the lab, and currently not having a sleep disorder. Our target sample

size was selected to be comparable to that used in other sleep reactivation experiments in and outside our lab. To increase the likelihood of falling asleep, participants were asked to get 1 hour less sleep than normal the night before and to avoid nicotine and caffeine on the day of the study. After participants arrived in the lab and gave written informed consent, the following six phases transpired: initial learning, bioelectric recording setup, pre-sleep memory test, sleep, post-sleep memory test, and a test to assess which sounds (if any) were heard during the nap. Figure 3.1 shows an overview of the procedure, which was approved by the Northwestern University Institutional Review Board.

Procedure

Participants learned the locations of 75 pictures of common objects presented individually on a Perlin noise background (Figure 3.1). The learning consisted of two parts. In the first part, participants were shown each object in its correct location and then immediately asked to move the object from the center of the screen to the correct location. Participants were shown the correct location after placement and received visual feedback (either “correct” or “incorrect” with the correct location of the object shown). Correct responses were those placed less than 3 cm from the correct location. The sound associated with each object was played when it first appeared on the screen and when participants made a correct response.

After performing this procedure for all 75 objects, participants began the second part, which required learning to criterion. Objects were presented in the center of the screen and participants were asked to move them to the correct location. As in the first part, sounds were presented when the object first appeared and when the participant made a correct response. Objects were presented in a random order, constrained so that the same object could not be shown twice in a row unless it was the only object remaining. After placing the object,

participants received feedback in the same manner as during the first part. If the participant placed the object center within 3 cm of the correct location, the criterion was considered achieved and the object was not shown again; otherwise, the object was included in the rotation. Learning ended when the participant correctly placed all the objects.

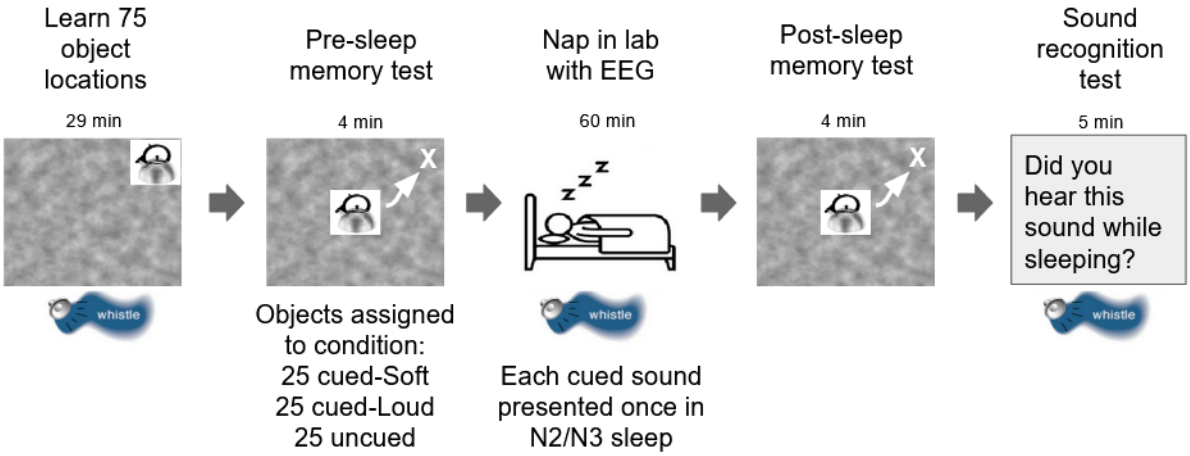

Figure 3.1*:* Illustration of experimental procedure, with mean duration for each phase. Participants first learned locations of 75 objects paired with sounds on a Perlin-noise background. Bioelectric recording set-up was next (not shown, mean time 29 min). Participants then took a memory test where they moved objects (illustrated here by white arrow and X) from the center to the correct location. Following memory testing, participants slept in the lab while object sounds were presented in N2 and N3 sleep. Following sleep, participants performed a second memory test, identical to the first. Finally, we played each of the 75 sounds and participants indicated whether or not they had heard each sound during sleep.

Bioelectric recording

Following learning, we attached bioelectric recording electrodes for EEG, EMG, and EOG (electroencephalography, electromyography, and electro-oculography). Data were recorded using a Neuroscan Synamps2 system with 26 scalp channels plus horizontal and vertical EOG, and chin EMG. Data were recorded at 1000 Hz with a high-pass filter at 0.1 Hz and a low-pass

filter at 100 Hz.

Pre-sleep test

After bioelectric recording setup, participants completed a pre-sleep memory test. In this test, objects were presented in random order and the participant attempted to place each object at its correct location. No feedback or sounds were presented during the pre-sleep test and each object was tested once.

After the test was complete, objects were divided into three sets comprising 25 to be cued with loud sounds during sleep, 25 to be cued with soft sounds, and 25 not cued. Objects were assigned to sets so as to match pre-sleep memory performance across sets. In this procedure, the objects were first ranked by accuracy and sequentially assigned to sets, so that each set received an equal mix of high-, medium-, and low-accuracy objects.

Sleep period

Participants slept on a futon in the same chamber where they completed the behavioral tasks. When participants reached stage N2 sleep (determined by the experimenter's real-time sleep staging), their initial arousal threshold was determined by presenting a probe sound (bike bell) not related to the memory task. If the sound did not elicit an arousal, the intensity was raised and the sound presented again, repeating this procedure until an arousal occurred. The intensity that prompted an arousal was used as the initial intensity for the sounds in the loud set.

After finding the arousal threshold, we waited for the participant to return to stable N2 sleep and then began presenting the cue sounds. Sounds were presented in random order, with loud and soft sounds intermixed. Loud sounds were presented at approximately 43 dBa; with intensity continually adjusted to reliably produce brief arousals but avoid prolonged awakenings, defined

as more than a minute of wake or N1 following the cue. Quiet sounds were presented at low intensity (approx. 28 dBa) and adjusted to avoid arousal. The mean intensity of quiet sounds was 33% of the initial arousal threshold [SEM=4.3%] and 7% of the mean intensity of loud sounds [SEM=0.6%]. Decibel values were determined by testing using Decibel X on a Redmi Note 9 placed at the location of the participant's head.

Sounds were presented with at least a 10-s interstimulus interval. If a sound triggered an arousal, cueing was paused until the participant returned to stable N2 or N3 sleep. Each sound was presented only once, allowing us to correlate each object's spatial memory fate with the sleep physiology surrounding sound presentation. After all sounds were presented, the participant was allowed to sleep for 5 min before they were awakened.

Immediately after the participant awoke, we informed them that we had played sounds during the nap and asked them if they remembered hearing any sounds. This was the first time that participants were explicitly told that sounds would be presented during the nap.

Post-sleep test

Participants performed the post-sleep memory test, which was identical to the pre-sleep test, approximately 5 min after awakening.

Sound recognition test

Following the post-sleep memory test, we presented the 75 object-associated sounds one at a time. Participants were asked to indicate whether they heard each sound during their sleep, with three possible responses: Definitely yes, possibly, and definitely no.

Sound intensity analysis

We defined the forgetting ratio (FR) across the sleep period as post-sleep error (in cm) / pre-sleep error (in cm). We computed a 3-level repeated-measures ANOVA to compare FR for cued-loud objects, cued-soft objects, and uncued objects. We also computed a two-tailed repeated-measures *t* test to compare FR for all cued objects to uncued objects.

EEG data processing

EEG data were analyzed in EEGLAB 2020.0 (Delorme & Makeig, 2004). Prior to analysis, we visually inspected the data and replaced EEG channels with poor signal quality using interpolation. No other data pre-processing or cleaning was performed. Data from one participant were excluded from arousal and EEG spectrum analyses due to a technical failure that prevented stimulus times from being recorded.

Arousal analysis

To measure the effects of EEG arousal on memory, we performed offline manual scoring to classify each sound cue as arousal-provoking or non-arousal-provoking. During this classification, the rater was blinded to the type of cue (loud versus soft). The rater examined a segment of time from 10 s before to 10 s after each cue, and scored the cue as arousal-provoking if an arousal meeting the AASM criteria (Iber et al., 2007) occurred in the 10 s after the cue. We computed FR for three conditions: cued objects that produced an arousal, cued objects that did not produce an arousal, and uncued objects. We then tested whether FR differed for objects cued with arousal, objects cued with no arousal, and uncued objects in a 3-level repeated-measures ANOVA.

Effects of cue perception on memory fate

To assess the impact of consciously perceiving sounds during sleep on memory fate, we compared FR for objects where participants reported hearing the associated sound to FR for objects where participants did not report hearing the sound. In this analysis, sounds were considered heard if the participant reported definitely or possibly hearing them during the sleep period. We tested whether FR differed for cued-heard and cued-unheard objects using a two-tailed repeated-measures *t* test.

EEG predictors of memory fate

To identify EEG features associated with enhancement/weakening of memory, we correlated FR for each cued object with the power spectrum from the 10 s before the cue. Spectra were computed from electrode Cz using the spectopo function in EEGLAB v2020.0. Each spectrum consisted of 21 2.5-Hz-wide frequency bins, spanning 0-52.5 Hz. Correlations were performed using a mixed model in JMP 15, with participant as a random factor and spectral power in each bin as a fixed factor.

3.4 Results

Spatial recall accuracy was matched across conditions before sleep, and declined after sleep, especially for cued objects

At the pre-sleep test, participants recalled locations with a mean error of 6.8 cm [SEM=0.52]. Recall accuracy did not differ between cued-loud, cued-soft, and uncued objects [$F(2,46)=1.22$, $p=0.3$].

Recall error increased at the post-sleep test to a mean of 7.47 cm [SEM=0.53], which was significantly worse than pre-sleep [$t(23)=2.89$, $p=0.008$]. As shown in Figure 3.2, recall was less accurate after sleep in all three conditions and there was a larger increase in error (FR) for all

cued objects combined compared to uncued objects [$t(23) = 2.39$, $p = 0.025$]. While this effect was significant in our planned comparison of all cued objects versus uncued objects, no significant differences were found in the omnibus ANOVA comparing cued-loud, cued-soft, and uncued sets [$F(2,46)=1.64$, $p=0.21$].

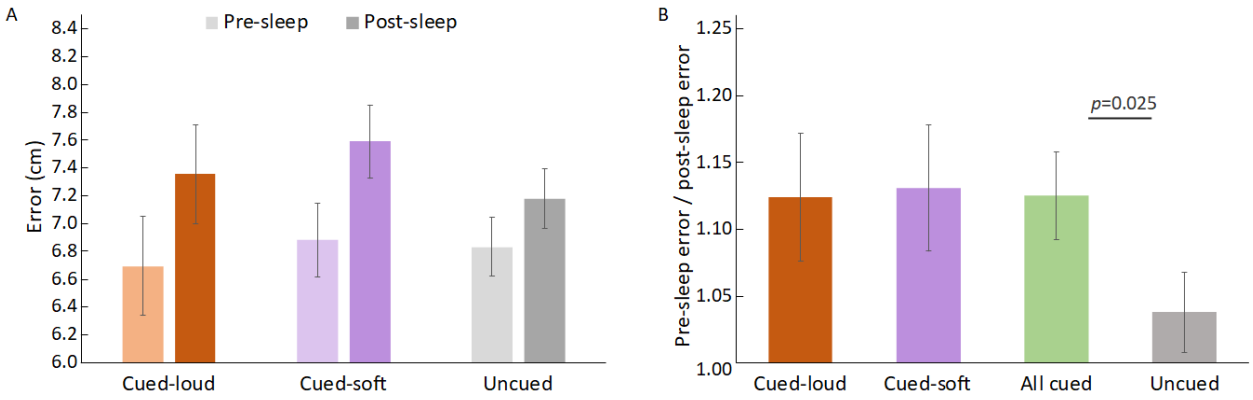

Figure 3.2: (A) Mean spatial error in centimeters for all conditions on pre- and post-sleep tests. Error bars represent the SEM for the post-sleep error minus pre-sleep error within subjects. (B) Forgetting Ratio (fold change in spatial error) for all conditions.

Forgetting was increased when cues generated an arousal

To understand the effects of arousal during sleep on reactivation, we categorized cued objects according to whether arousal was apparent in post-stimulus EEG recordings. Arousals were common for both types of sound. Arousals occurred following 43% of the soft sounds and 59% of the loud sounds.

As shown in Figure 3.3, forgetting was greater for objects that were cued with arousal than for uncued objects. On the pre-sleep test, recall did not differ by condition [$F(2,44)=2.05$, $p=0.14$] whereas differences were apparent after sleep. An ANOVA comparing FR for uncued objects, objects cued with arousal, and objects cued without arousal revealed a significant effect

[F(2,44)=3.51, *p*=0.04]. Post-hoc testing using two-tailed t tests of all pairs with Bonferroni correction (alpha per test=0.017) showed a significant difference between uncued objects cued with arousal and uncued objects [*t*(22)=3.28, *p*=0.003].

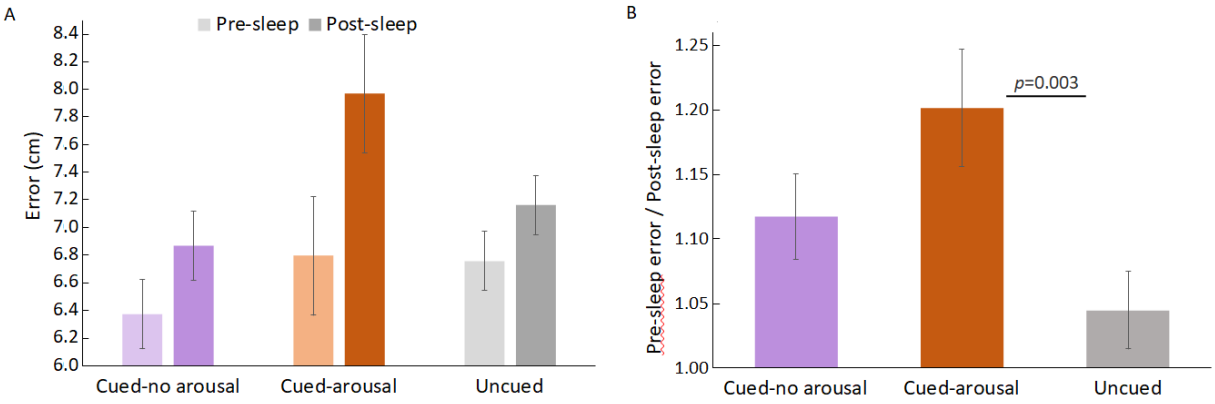

Figure 3.3: (A) Mean error for uncued objects and objects cued with/without arousal. Error bars represent the SEM for post-sleep error minus pre-sleep error within subjects. (B) Forgetting ratio for these three conditions.

Pre-cue alpha power predicts the effect of cueing on memory

To identify EEG features that predicted the effects of cueing, we tested whether EEG spectral power at Cz in the 10 s prior to a cue predicted the memory fate of the associated object. We divided the spectrum into 21 2.5-Hz wide bins, spanning 0-52.5 Hz. Increased power in the high alpha bin (10-12.5 Hz) before the cue was associated with more forgetting of the cued object [*t*(642.5)=2.37, *p*=0.018]. However, following FDR multiple-comparisons correction this correlation was nonsignificant (*p*=0.38).

Subjective recall of sleep cues did not influence memory performance

Because participants sometimes report hearing cue sounds during sleep, we examined possible

relationships between subjective reports of hearing a sound during sleep and changes in the corresponding object-location memories. At the end of the experiment, participants were presented with each cue sound and asked if they remembered hearing it while sleeping. On average, participants reported definitely hearing 11% of the sounds played during sleep [SEM = 3%] and possibly hearing 28% of the sounds [SEM = 4%]. We combined these two categories for this analysis. There was no significant difference in FR [$t(23)=0.88, p=0.39$] between cued objects with sounds heard during sleep [mean FR=1.25, SEM=0.17], and cued objects with sounds not heard during sleep [mean FR=1.09, SEM=0.04].

3.5 Discussion

In this experiment, we observed that reactivating memories in the context of disrupted sleep produced a selective forgetting effect, where spatial recall accuracy after sleep was decreased for the reactivated objects. These results support our hypothesis that sleep disruption can reverse the typical accuracy-enhancing effect of TMR.

Surprisingly, we did not observe a difference in memory outcomes for objects cued with soft sounds and objects cued with loud sounds. One potential explanation is that many soft sounds also triggered an arousal; the presence or absence of an arousal may be more relevant to memory than the absolute sound volume. Supporting this hypothesis, objects cued with arousal had a significantly higher forgetting ratio than uncued objects and slightly (but not significantly) higher forgetting ratio than objects cued without arousal. Soft sounds may be more likely to trigger arousals when sleep is repeatedly interrupted, as it was in this experiment.

Another surprising observation was that we did not observe a memory-enhancing effect of TMR; objects reactivated without arousal were comparable to uncued objects, with a slight but not significant increase in error. The lack of benefit for objects without arousal might indicate that

the effects of sleep disruption can “spread” and affect not only memories reactivated with arousal but also related memories or memories reactivated around the same time.

Our results also suggest that sleep state prior to a cue can influence the fate of a memory. When cues were presented during periods of sleep with relatively high alpha power, the corresponding object locations were recalled less accurately. Given that alpha power during sleep is thought to reflect sleep depth and arousability (McKinney et al., 2011), a reasonable interpretation is that cues presented in periods of light sleep are more likely to trigger arousal and weakening.

Finally, we found that participant reports of hearing cues did not significantly predict memory fate. This result contrasts with those of Göldi and Rasch (2019), where only participants who reported sleep disturbed by cues showed worsening of memory induced by TMR. This difference across experiments may reflect differences in the questions used to assess sleep. Göldi and Rasch asked participants whether the sounds woke them, whereas we asked if participants remembered hearing each sound. Asking about waking (as opposed to memory for hearing the sound) may therefore be a better proxy of TMR-induced arousal.

Our results parallel the effects of disrupting memory reconsolidation. While retrieval during wake typically strengthens memory (Roediger & Butler, 2011), Misanin and colleagues (1968) showed that a retrieval cue followed by electroconvulsive stimulation produced forgetting of the reactivated information in rats. Similarly, retrieval while protein synthesis is inhibited produces forgetting (Nader et al., 2000). Because both of these interventions produce amnesia by disrupting early consolidation (Haubrich & Nader, 2018), these results imply that consolidation processes are required after retrieval of a memory to prevent forgetting. If consolidation after retrieval is disrupted, the memory becomes weakened. Paralleling this idea, TMR with uninterrupted sleep may allow a full consolidation process to occur, thereby strengthening

memory, whereas interrupting sleep may prevent stabilization from occurring. Cueing followed by sleep disruption may thus produce a destabilizing effect, leading to memories that tend to be less accurate upon awakening.

Our results add to the literature on memory processing during sleep by showing that it is possible for memory reactivation to lead not only to strengthening, but also to weakening. Weakening may be particularly perpetuated when sleep is disrupted. This finding raises several interesting possibilities. The ability to produce both strengthening and weakening may prove useful in understanding the discrete processes that operate during memory reactivation in sleep. The results also suggest potential practical applications, such as using TMR to weaken memories of traumatic events, which could be explored in future research.

3.6 Author Contributions

This is a manuscript current undergoing review (Whitmore & Paller, 2022). N.W.W. and K.A.P. designed the experiment. N.W.W. collected, analyzed, and interpreted data. K.A.P. supervised the project. N.W.W. and K.A.P. wrote the manuscript.

3.7 Acknowledgements

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Chapter 4—Designing and Testing of a System for TMR Outside the Sleep Lab

4.1 Introduction

Experiments with TMR have shown that it is both a useful tool for investigating questions in memory and potentially for enhancing learning outside of the sleep lab. For example, TMR can improve retention of information learned in class (Gao et al., 2020) and a TMR intervention was shown to improve motor function in stroke survivors (Johnson et al., 2021).

A major barrier to expanding TMR research is that the technique requires experimenters to present cues manually while monitoring sleep using polysomnography. While this process is effective, it requires a specialized sleep facility and extensive training of operators and requires participants to commute and sleep outside their homes.

These requirements impose large limitations on the experiments that can be done using TMR. For example, very few studies have examined the effects of multiple TMR sessions, owing largely to the logistical difficulties involved. These requirements also make it impractical to study or use TMR as a translational therapy, for example in rehabilitation.

Therefore, it is highly desirable to find ways to perform TMR in participants own homes, ideally using an automated system that does not require direct control by an operator. This chapter describes a system we developed and tested to perform TMR at home.

Previous research on TMR outside of the sleep lab

Previous research on home-TMR can be divided into two categories: unsupervised approaches, which present TMR cues during sleep irrespective of sleep stage, and supervised approaches, which attempt to present TMR cues in a specific sleep stage.

Unsupervised home TMR has shown mixed results in replicating the effect of in-lab TMR. (Neumann et al., 2020) found that TMR with an olfactory cue delivered at home could improve vocabulary learning in children. In this study, children were exposed to rose odor while studying, and the memory was reactivated during sleep by placing rose-scented incense sticks near the pillow. Children who received this reactivation performed better on vocabulary tests than those who did not, and the effect was sleep-specific, with no benefit for children who received the rose odor only while awake. Similarly, Ritter et al. (2012) found that participants produced more creative solutions to a problem when information about the problem was reactivated during sleep using odor. In this experiment, reactivation was performed using oil diffusers that participants plugged in before sleeping at home. Participants were first briefed on a problem they had to solve and sent home to sleep. Participants who received the same scent that was presented during the briefing produced more creative solutions than those who received no scent or an unrelated scent. This indicated that the procedure reactivated memories and facilitated problem solving.

Other unsupervised TMR experiments have not shown effects of TMR. Göldi & Rasch (2019) attempted to replicate previous findings that TMR could improve vocabulary learning using home-TMR. In this study, participants started an audio file before going to sleep, and words from the vocabulary task were presented at low volume 30 minutes after participants started playing the file. Contrary to previous findings in the lab, performance was not higher for reactivated words. Further analysis of this data showed an effect of self-reported sleep

disruption—participants who reported their sleep was undisturbed showed a typical TMR effect, but participants who reported that the sounds disrupted their sleep performed worse for reactivated items, resulting in a null effect overall. A similar, unpublished study in our lab also found no benefit of home TMR for vocabulary learning.

These results suggest that unsupervised home TMR may be less effective than TMR performed in the lab due to the inability to target cueing to optimal sleep states. For this reason, several research groups have focused on "supervised" home TMR, in which a sleep sensor (typically EEG) is used to identify optimal sleep states for cueing.

In several experiments, researchers performed TMR using a modified version of the Zeo headband, customized for TMR applications by the company SheepDog Sciences. In this system, data is sent from a wearable EEG headband to a laptop, which performs automated sleep staging and plays TMR cues in sleep stage N3. TMR with this system has been shown to increase feelings of ownerhip in a rubber hand experiment ((Honma et al., 2016), and improve creative problem solving (Sanders et al., 2019) . However, an experiment using this device with a spatial memory task (Weber & Paller, n.d.) did not fully replicate the well-documented effect of TMR on spatial memory; while there was no overall effect of TMR on memory, a subset of participants with good learning performance showed a significant effect 72 hours (but not 24 hours) after TMR.

Limitations of current technologies and data

Based on previous research and our own experience with devices, we identified a number of limitations of existing technologies:

- Unsupervised TMR is simple and can be performed with only a phone and no additional hardware. However, because it cannot selectively target deep sleep it is likely to produce

sleep disruption which interferes with the memory benefits of TMR. Furthermore, unsupervised systems can incidentally present stimuli in wake, and cannot monitor the physiological responses to TMR cues. Unsupervised TMR using smell is promising but not suitable for many experiments and settings.

- A major limitation of existing supervised TMR systems is that they rely heavily on proprietary software and hardware, which poses challenges for science. For example, the Zeo device frequently used for home TMR studies is no longer produced and is essentially impossible to obtain. Furthermore, these systems often rely on black-box sleep staging models which do not offer the ability to tune detection parameters or inspect the raw data.
- EEG-based systems can suffer from poor signal quality when used in the home environment, which impedes their ability to deliver cues in the correct sleep stages. Furthermore, obtaining good signal quality requires significant effort from participants such as applying conductive gel to the skin for some systems. These systems are also quite expensive (around $500-$4000 for typical sleep systems) which limits their utility for running very large-scale studies.

In addition to technical limitations, a general limitation is that little research has demonstrated that home-TMR and lab TMR have the same effects on memory. The best-known TMR effect is a reduction in error for declarative memories which are reactivated during sleep (Hu et al., 2020)but only one study (Neumann et al., 2020) has clearly shown this effect with home-TMR, and it differed from typical lab protocols in several ways (use of children, limited blinding of conditions). Therefore, more research is needed to directly compare the effects of lab-based and home TMR.

4.2 Design of a novel home-TMR system

To address the technical limitations we identified, we developed a novel system for supervised home-TMR. Our system consists of a Fitbit smartwatch which transmits physiological data (heart rate and motion) to a smartphone, which uses a machine-learning algorithm to identify periods of slow-wave sleep and present cues in those periods.

This approach is conceptually similar to that used by sleep-stating algorithms developed by Fitbit (Beattie et al., 2017) as well as other research groups (Faust et al., 2019), which use motion and heart rate data to stage sleep, achieving significant agreement with human raters examining polysomnogram recordings (de Zambotti et al., 2018). Although we use a Fitbit device to collect data, we do not use the Fitbit sleep staging algorithms, the Fitbit simply provides raw data to an algorithm we developed ourselves. We chose to use a custom algorithm for several reasons: the Fitbit algorithm cannot stage sleep in real time, it does not allow experimenters to choose the specificity/sensitivity tradeoff, and the Fitbit algorithm is not publicly available.

Our system offers several desirable features for home TMR:

- The Fitbit is easy for participants to wear and use and does not require skin preparation.
- All raw data is stored on the phone and streamed to experimenters, allowing experimenters to measure sleep physiology and verify that the device is functioning correctly.
- The cueing parameters such as stringency of N3 detection and sounds to play can be configured remotely by the experimenter, or the app can be integrated with a behavioral task app which selects sounds to play

- The system uses open-source software and relatively low-cost hardware, allowing a lab to feasibly run very high volumes of participants.

Technical description of the system

A custom application running on the Fitbit acquires data (consisting of heart rate in BPM, acceleration on X Y and Z axes, and rotation on these axes) once per second. This data is transmitted via Bluetooth to a paired phone.

The first step of processing the data consists of feature extraction, as shown in Figure 4.1. Briefly, the phone computes a time-frequency representation of the last 240 seconds of accelerometer, gyro, and heart rate data. The result of this is a time-frequency matrix of the data, which measures the amount of variability as a function of both time (in seconds before the current time) and frequency. This transformation is similar to that used in other sleep-staging algorithms (Beattie et al., 2017), and is useful because it allows for characterization of different sleep phenomena (i.e. high-frequency vs low-frequency heart rate variability).

Following feature extraction, the time frequency features, along with current values from all sensors and total motion integrated over the last 240 seconds are input to a neural network trained to predict the probability of N3 sleep (Figure 4.1). This network produces a value, p(N3) corresponding to the probability of N3 sleep.

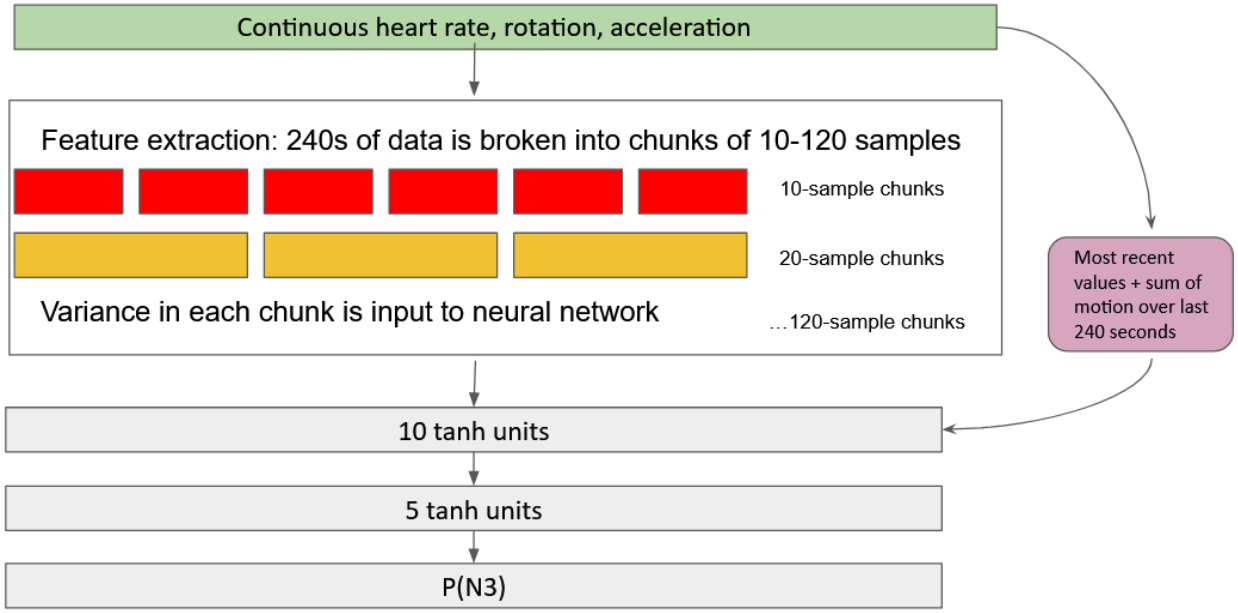

Figure 4.1 Schematic of the feature extraction system and neural network

The phone begins playing sound cues when the average value of p(N3) reaches a threshold set by the experimenter, and cues continue until either the maximum number of cues or maximum running time are reached, or an arousal is detected. When arousal is detected, cueing is paused, and the volume of the sounds is lowered. Full details of the control logic are shown in Figure 4.2.

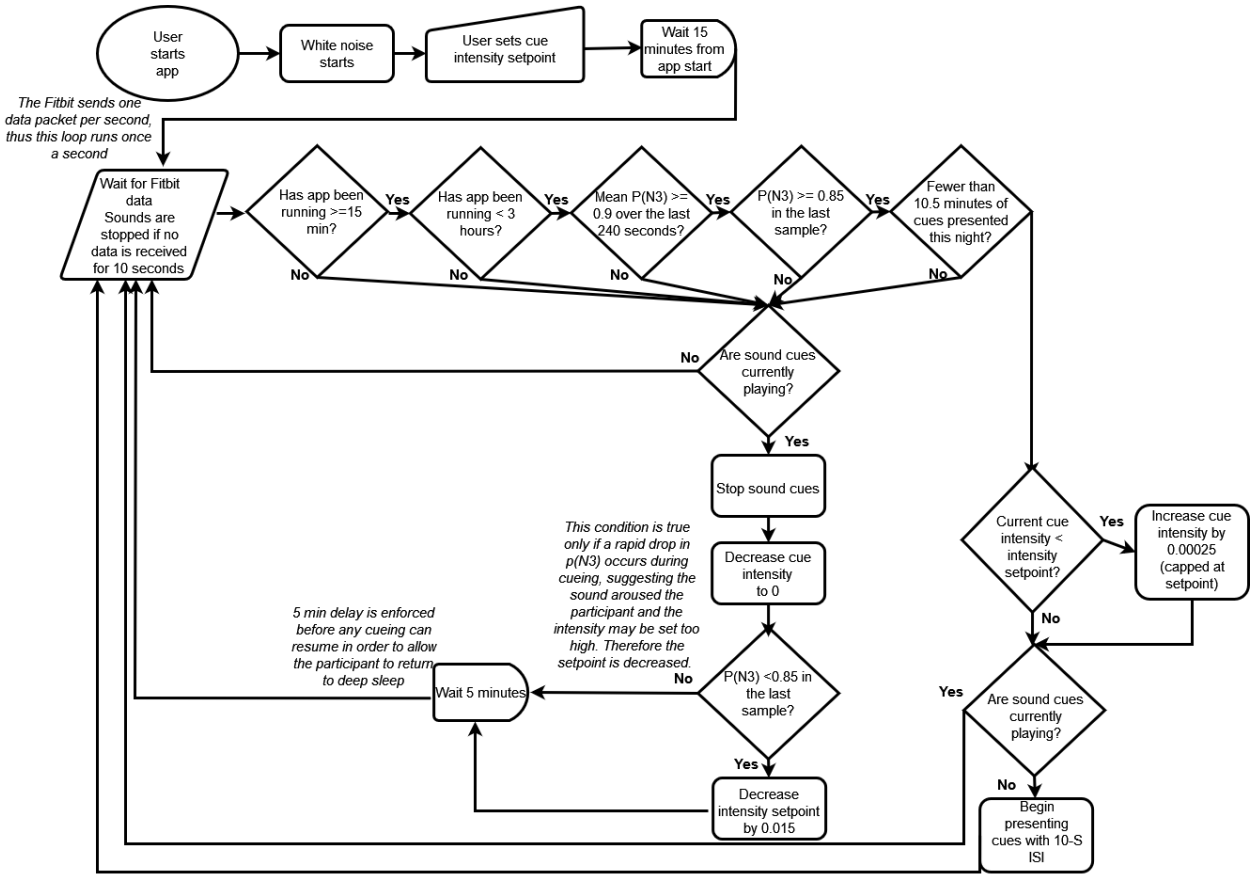

Figure 4.2 Flow chart illustrating how sounds are controlled in the home TMR system

Neural network training and testing

We trained the neural network on a dataset of 24 participants for whom we acquired both Fitbit data and polysomnographic sleep scores. 12 participants were young adults who slept in the lab overnight for an unrelated study and 12 were middle-aged adults who slept at home. For the young adults, sleep stage was determined by manual scoring; for middle-aged adults sleep stage was determined using the automatic scoring with a Dreem 2 headband, which participants wore while sleeping at home.

Prior to training, we computed features for the Fitbit data as described above. To speed training, we subsampled the data by a factor of 5, to yield one sample every 5 seconds. Preliminary

testing showed that this did not meaningfully affect classifier accuracy, likely because successive samples contained mostly redundant information. In total, 178,948 observations were included in the dataset.

We then trained a perceptron neural network with two hidden layers to predict whether each second would be scored as N3 based on the Fitbit features. Training was performed using the Neural module of JMP 15.2 using the “squared” regularization penalty. To evaluate the network’s overall performance in classifying N3 sleep, we also trained a separate version of the model with one-third of the subjects (50,425 observations) held out from training as a validation set. The model achieved an AUC of 0.77 in classifying sleep as N3 or non-N3, indicating significantly above-chance performance.

We also evaluated a number of alternative classifier schemes, including linear discriminant analysis and a convolutional neural network. Of these, the two-layer perceptron combined with our feature extraction algorithm performed the best.

Phone-based memory tasks

We created an app-based implementation of a standard spatial memory task (described in Figure 4.3) for learning and testing object locations on the phone. In this task, a grid covered the phone’s entire screen, and participants learned the correct locations of objects on the grid. We tested memory by asking participants to move objects from the center of the grid to their correct location. The app recorded accuracy and response times during each phase.

After participants completed their bedtime memory task, the app selected 25 objects to be cued during sleep using a matching algorithm to minimize the difference in bedtime memory performance between the groups of cued and uncued objects. Because it is impossible to exactly match performance and some differences remained, the app also counterbalanced

participants so that the “slightly better” group of objects was cued in half of participants and the “slightly worse” group was cued in the other half.

4.3 Experiment 1-Testing the memory effects of home TMR

After initial design and testing of the home-TMR system, we performed an experiment to test whether home-TMR with this system would improve spatial memory for reactivated objects, as has been shown for in-lab TMR.

In this experiment (shown in Figure 4.3), we provided participants with a Fitbit and smartphone which was used for TMR over the 5-day experiment. Participants learned correct locations for 50 objects on a grid; half of these objects were reactivated during sleep. Participants received TMR for three consecutive nights; the same group of objects was reactivated on each night. Memory was measured using a memory test on the phone following each night of TMR. We predicted that participants would place the objects reactivated during sleep closer to their correct locations, replicating the typical effect of TMR on spatial memory.

Participants also wore a Dreem 2 headband while sleeping, which allowed us to correlate EEG and sleep physiology features with the behavioral results.

Participants

We collected data from 120 adults who we recruited using flyers placed on campus. The study protocol was approved by the Northwestern University IRB. Participants were paid for their time.

Prior to analysis we filtered participants though a set of inclusion criteria. These criteria were:

- Performed training, bedtime memory test, and at least one morning memory test
- Had at least 25 cue presentations
- No more than four stimuli were presented when the Fitbit read a heart rate of zero (indicative of a poor heart rate signal)
- Objects were correctly allocated to cued and uncued conditions (on occasion a bug in the allocation algorithm caused this to fail)

This process yielded 61 participants for analysis. Participants were 28% male and ranged from 18-25 years old (mean=20.6 years).

Task

The experiment consisted of four days, three of which involved TMR and memory testing and one of which was an adaptation night. During the initial visit, participants received the Fitbit, Dreem 2 headband, and an Android phone with the study software.

Day 1 Participants visited the lab to pick up the equipment. Participants did not complete any memory tasks on this day but did use the home-TMR system and Dreem headset at night to allow for acclimation to the equipment and auditory cues.

At night, participants put on the Fitbit and Dreem device and started the home-TMR app on the

phone. The phone played continuous white noise, and participants were instructed to use a slider in the app to set the white noise volume to a comfortable level. The intensity of the white noise was also used as the initial setpoint for sounds played during the night.

During this night, the app played a placeholder sound (electronic ding noise, not related to the memory task) using the same algorithm used to control TMR dues. This was intended to allow participants to adapt to the sounds and reduce sleep disruption from unfamiliar sounds in the second night.

Day 2 On day 2 participants completed a spatial learning task using the phone provided by the lab. The learning task consisted of 5 blocks of 10 objects. Within each block, participants were first shown the correct location of each object. The objects then appeared in the center of the screen in random order, and participants were asked to move them to the correct location. After moving the object, participants received feedback. If objects were placed within 120 pixels (approximately 2 cm) of the correct location, the placement was considered correct, and the object was dropped from the rotation. A block ended when the participant placed all objects correctly. The phone played the sound associated with each object when it first appeared on the screen, and when the participant placed it correctly.

Following the completion of all blocks, participants completed the bedtime memory task. In this task, all 50 objects are presented sequentially in the center of the screen, and the participant is instructed to move each object to its correct location. After the participant completed the bedtime memory test, the app selected 25 objects to be cued at night.

This night, participants again put on the Fitbit and Dreem and started the home TMR app and calibrated the volume before going to sleep. During this night, and all subsequent nights,

sounds linked to the cued objects were presented during sleep windows determined by the home TMR system.

Day 3-4 Participants completed a memory test in the morning (identical to the memory test on day 2) asking them to place objects on the correct locations. They then used the home TMR system and Dreem 2 at night as on previous nights.

Day 5 Participants completed a final memory test in the morning and returned the equipment. When returning the equipment, we asked participants whether they remembered hearing any of the sounds from the memory task while they were sleeping.

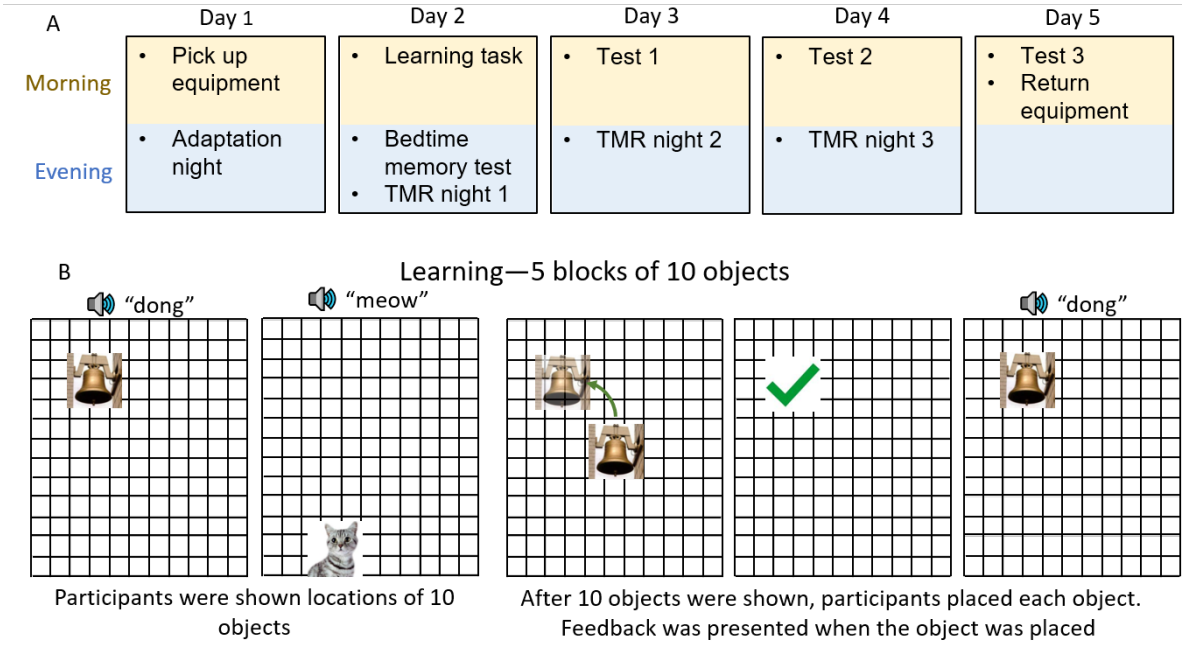

Figure 4.3 A. Sequence of events in the study. B. Procedures for the learning phase, including presentation of objects (left) and trials of location recall with the drop-out method (right). The sound of each object was played whenever the object appeared on the screen in its target location. The memory test used the same procedure for location recall except that participants

were not given feedback or shown the correct location of the objects, and the sounds were not played.

Automated TMR

Sounds were played at 10-second intervals when N3 sleep was detected (average P(N3) over a 240-second window at least 0.9, and the most recent P(N3) was at least 0.85). Cue start/stop and cue volume was controlled by the algorithm shown in Figure 4.2.

We configured the system to present sounds for a maximum of 10.5 minutes per night, which corresponded to 2.5 presentations of each sound cue. The system was configured to only present sounds in a time window from 15 minutes after the system was turned on to 3 hours after the system was turned on. Both of these constraints were imposed to minimize the chance of disrupting sleep with the sounds, and are consistent with the protocols used in previous lab-TMR studies (Rudoy et al., 2009).

Memory performance measurement

We measured memory error as the ratio of mean spatial error at a morning memory test to mean spatial error at the bedtime memory test, e.g., mean test1 error/mean bedtime test error. We computed this statistic separately for the cued and uncued groups of objects.

TMR effect measurement

For each test, we computed the TMR effect by computing (error for cued objects-error for uncued objects). A negative value indicates a benefit of TMR for memory—for example if a participant has a TMR effect of -0.1, it means that the error for the cued objects increased by 10% less than the error for uncued objects. We computed whether TMR effects differed

significantly from zero using the Wilcoxon signed-rank test, which is a non-parametric one-sample test.

We conducted two broad analyses of the TMR effect: one to assess the effect of TMR at the final memory test, and one to describe how TMR effects varied over the three memory tests. In the primary analysis, we examined memory performance at the last test. While participants nominally took three memory tests, 8 participants failed to perform the morning memory test on one or more mornings. Therefore, our primary memory analysis examined memory performance at the time of last test, and we treated number of memory tests and number of nights cued as predictors in our model.

In the secondary analysis, we considered 53 participants who completed all three memory tests. We evaluated the effect of TMR at each test to observe how it changed over time.

Controlling for the effects of initial memory performance

We observed that TMR effects were correlated with the pre-sleep difference in memory performance between the cued and uncued groups of objects. (Figure 4.4). This appeared to reflect a regression-to-the-mean effect: when initial memory performance was worse for the cued set, the cued set improved more at the last test (Figure 4.4). Because this effect adds variability which could obscure other correlations, we first controlled for this effect before correlating the TMR effect with other variables. In this procedure, we used linear regression to predict the TMR effect based on only the difference in initial memory performance between the cued and uncued groups of objects. We then subtracted this predicted effect from the TMR effect we observed. We computed the correlation separately for each test, to account for the possibility that the influence of this effect can depend on the time since the initial memory test.

We used the corrected TMR effect for analyses where we correlated the TMR effect with other variables, but we did not correct the mean values for the cued and uncued object. While differences in initial memory contributed to variance in the TMR effect they did not systematically bias the mean error for the cued and uncued objects. This is because the phone based spatial task performed counterbalancing so that half of the participants had better performance for the cued objects, and half of the participants had better performance for the uncued objects. This resulted in no significant difference in mean initial memory score using a Wilcoxon signed-rank test [mean=0.76 pixels, z(60)=0.8,p=0.42].

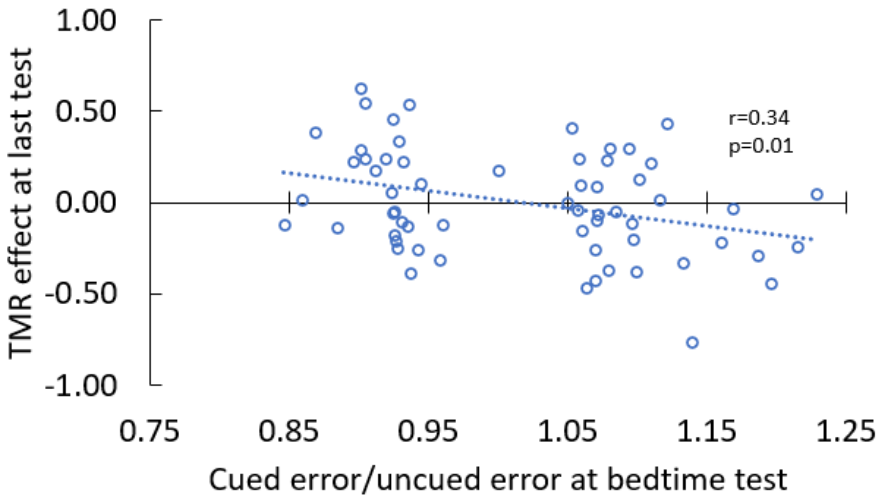

Figure 4.4 An example of the linear regression used to control for variation in memory performance in the bedtime task. The bimodal distribution on the X axis reflects how participants were counterbalanced to equalize pre-sleep memory performance between groups.

Testing predictors of the TMR effect

To examine variables that predicted the effects of TMR, we correlated TMR effect at last test with each variable of interest using linear regression.

Dreem 2 sleep staging

While sleeping, participants wore a Dreem 2 headset, which performs automatic sleep staging and has attained high agreement with expert human raters (Arnal et al., 2020). We used this data to compute the time participants spent in each sleep stage, as well as the percentage of cues delivered in each sleep stage. Because some participants did not have sufficient high-quality Dreem data for staging (as assessed by the Dreem algorithm) only a subset of participants were included in these analyses.

4.4 Experiment 1 results

TMR cues delivered by the system were targeted to N3 sleep

For 45 participants who had EEG data with sufficient quality to permit sleep staging during cueing, we compared the percent of cues delivered in each sleep stage to the percent of overall time spent in that sleep stage. This analysis served as an independent test that the algorithm targeted N3 sleep in a group of participants separated from the original test and validation set.

Results (Figure 4.5) revealed that the system successfully targeted N3. Compared to the total time in each stage, the time when cues were played was more likely to be N3 [$t(44)=3.56$, $p<0.001$] and less likely to be classified as N2 [$t(44)=2.56$, $p=0.03$] or REM [$t(44)=2.61$, $p=0.01$]. Although N2 was underrepresented in the cued sleep, a substantial number of cues were presented in N2 due to the higher base rate of N2 sleep. We did not observe differences between the total sleep and cued sleep in wake or N1, which may be because these stages were rarely observed in the training set, providing little opportunity for the model to learn how to identify them.

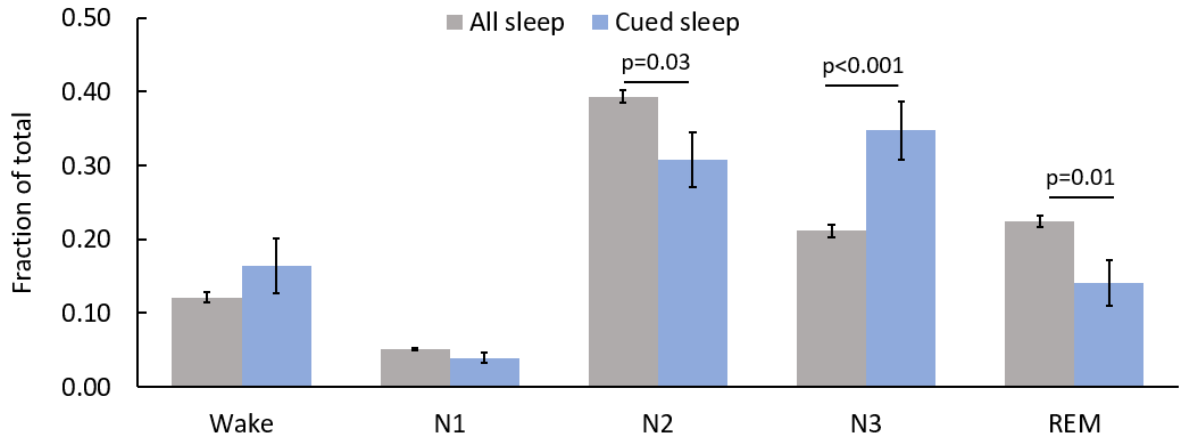

Figure 4.5 Sleep stages in which cues were delivered (blue) vs overall distribution of sleep stages (gray). Sleep stage was determined by automatic scoring of sleep by the Dreem 2 in 45 participants who had usable EEG data while cues were delivered.

Participants did not generally notice TMR cues

In the total sample (including participants with unusable data), 16/120 participants (13%) reported hearing sounds from the memory task, and no participants reported that the sounds disrupted their sleep or awoke them. Among the participants included in analysis, 7/61 (11%) reported hearing sounds.

Participants efficiently learned and retained object locations

Participants required a mean of 1.61 [SEM=0.09] repetitions per object in the learning task to reach the learning criterion. In the bedtime task performed after learning, participants mean error remained below the criteria (Figure 4.6), indicating that the learning procedure created at least an effective short-term memory. Participants usually began the bedtime test shortly after completing the learning (mean delay 11 minutes, SEM=5 minutes).

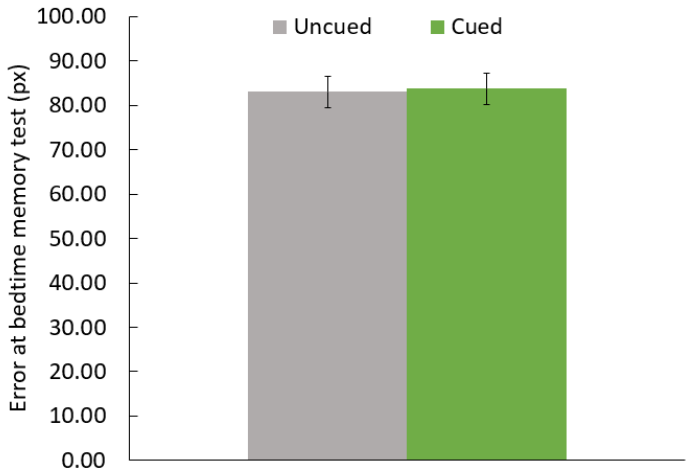

Figure 4.6 Error at the bedtime test remained below the learning criterion (120 pixels). Error did not differ between cued and uncued groups of objects [Wilcoxon signed-rank test; mean difference=0.76 pixels, z(60)=0.8,p=0.42]

Participants gradually forget object locations, but do not show an overall effect of TMR

The mean spatial error increased from 83 pixels at bedtime test to 105 pixels at last test, indicating significant forgetting [$t(60)=9.48$, $p<0.001$]. No significant TMR effect was found at the last test or at any of the individual time points. (Figure 4.7)

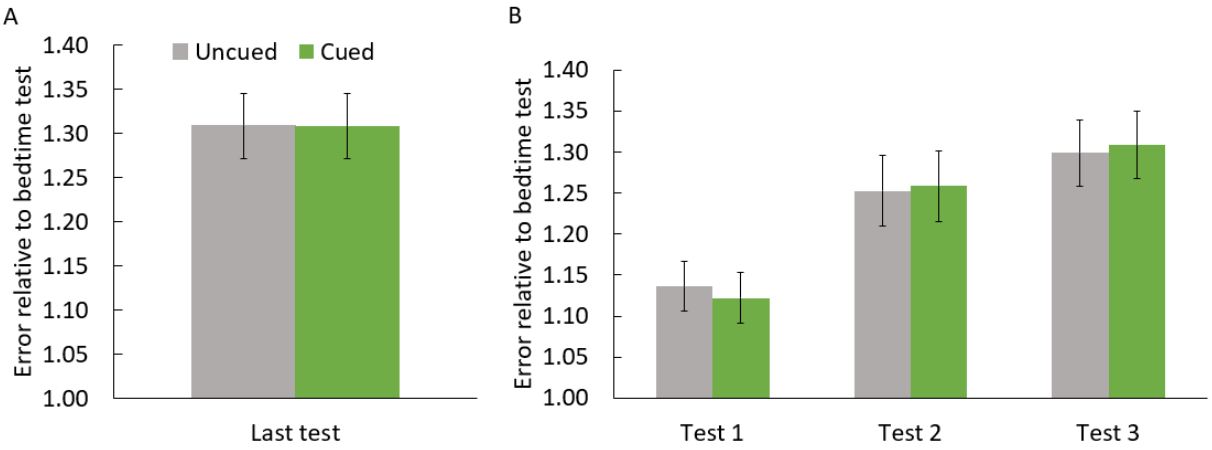

Figure 4.7 Mean spatial error increased by about 30% for both cued and uncued objects at the last test (compared to the bedtime test immediately after learning. No significant difference in error was found between the cued and uncued objects. B. In participants who completed all 3 morning tests (n=41) error continued to increase throughout the experiment, reflecting forgetting. There was no significant difference between cued and uncued objects at any time point. Error bars reflect the SEM for the within-subjects analysis of cued error-uncued error.

TMR effect was associated with cue intensity and sleep stage targeting

Previous research has suggested that individuals differ in the extent to which they respond to TMR, due to factors such as level of sleep disruption (Whitmore et al., 2022). Therefore, we hypothesized that our subjects might consist of a mix of “responders” and “non-responders”. Therefore, we sought to identify parameters that influenced TMR response. We found that memory benefit from TMR was associated with a low cue amplitude and marginally associated with the percentage of cues delivered in sleep stage N3 (Figure 4.8, Table 4.1)

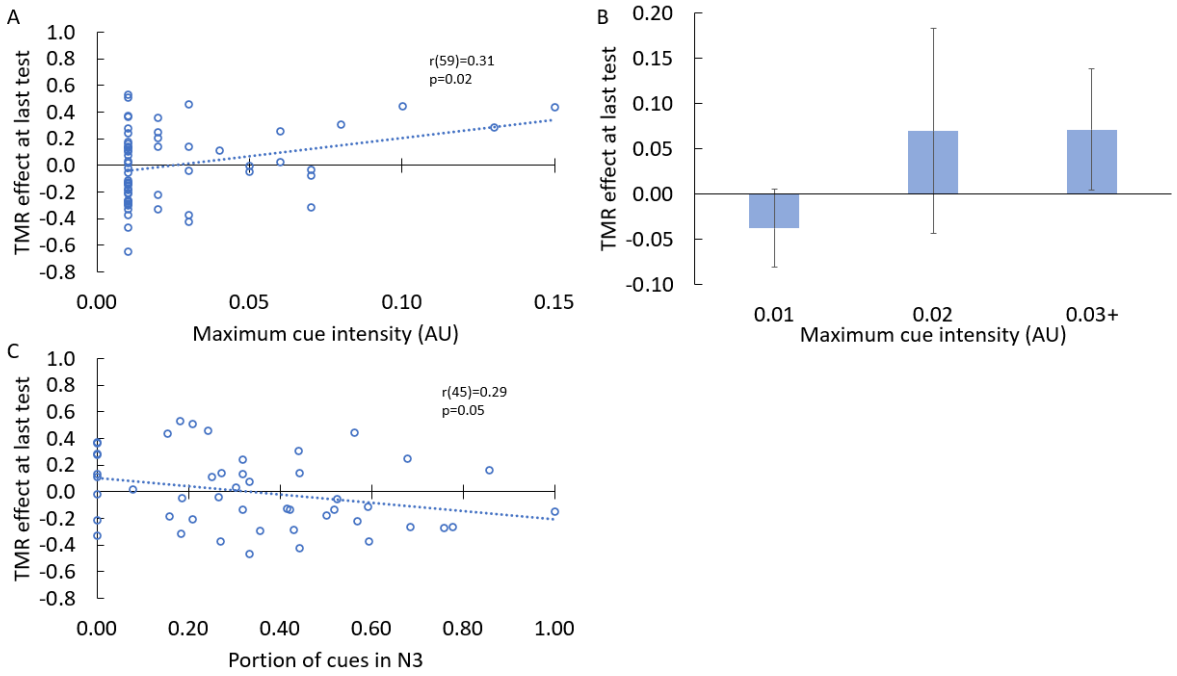

Figure 4.8 A. Correlations between the TMR effect and maximum cue intensity. B. Mean TMR effect as a function of maximum cue intensity. C. Correlation between TMR effect and proportion of cues in N3. TMR effect shown here is corrected for initial differences in memory.

Measure	Mean (SEM)	*P*	r	Rationale
Total number of cues	145.13 (8.52)	0.36	-0.12	Increased number of cues may produce more reactivation and stronger effect
Arousals per cue	0.07 (0.01)	0.81	- 0.03	Measure of sleep disruption during cueing, disruption can reduce TMR effects
Maximum cue volume	0.03 (0.00)	0.02	0.31	Volume is set by the user before sleep; excessively loud or soft cues might be ineffective
Number of sounds on adaptation night	48.23 (3.66)	0.26	-0.15	Receiving cues on the adaptation night might reduce sleep disruption on the first TMR night
Portion of sound cues delivered in N3	0.34 (0.04)	0.05	-0.29	Cueing in stages other than N3 might reduce the effects of TMR (Chapter 2)
Number of sound cues delivered in N3	16 (2.71)	0.07	-0.26	Cues might be especially effective in N3 sleep (Chapter 2)
Portion of sound cues delivered in N2+N3	0.66 (0.04)	0.15	-0.21	Cues may work equally well in N2 and N3, but worse in other sleep stages.
Portion of sound cues in wake/N1	0.19 (0.03)	0.53	0.09	Cues in wake/N1 may be especially likely to be noticed and disrupt sleep.
Portion of sound cues in REM	0.13 (0.03)	0.18	0.2	Reactivation in REM may produce unique effects not seen in other sleep stages (Hutchison et al., 2021)
Portion of total sleep time in N3	0.24 (0.01)	0.92	0.01	Proxy for overall depth of sleep, which was shown to affect TMR in the prior study (chapter 1)
Portion of participants reporting hearing sounds	0.11 (0.04)	0.88	-0.02	In (Göldi & Rasch, 2019), participants who reported hearing cues had poorer memory
Mean error at initial test (pixels)	83.39 (3.51)	1.00	0.00	TMR effects may depend on the strength of initial learning (Creery et al., 2015)
Morning memory tests performed	2.87 (0.05)	0.61	0.07	If TMR effects evolve over time, participants who completed all tests might show a different effect than those completing only some tests
Number of nights cued	2.18 (0.11)	0.62	-0.06	Repeated cueing on multiple nights may increase the total reactivation and provide a stronger TMR effect
Participant age in years	20.56 (0.23)	0.92	-0.01	Our previous study (chapter 2) found TMR effects were associated with age

Table 4.1 Correlations between the TMR effect (corrected for initial differences in memory) and sleep/participant variables. Correlation is a linear regression. Sign of the r value indicates the direction of the correlation; a negative r indicates higher values of the independent variable are associated with more benefits of TMR for memory.

Participants cued with optimal and non-optimal parameters diverge over time

Based on this analysis, we explored the trajectory of the TMR effect in participants cued with "optimal parameters" (defined as receiving at least 25 sound cues on the adaptation night and using a maximum volume of less than 0.02) versus those with non-optimal parameters. We opted to select participants using these parameters because these are factors that can be directly controlled by the experimenter to reduce sleep disruption. As seen in Figure 4.9, while we did not observe a statistically significant difference between optimal and non-optimally cued participants, we did observe a trend where the TMR effect diverged for the two groups over time.

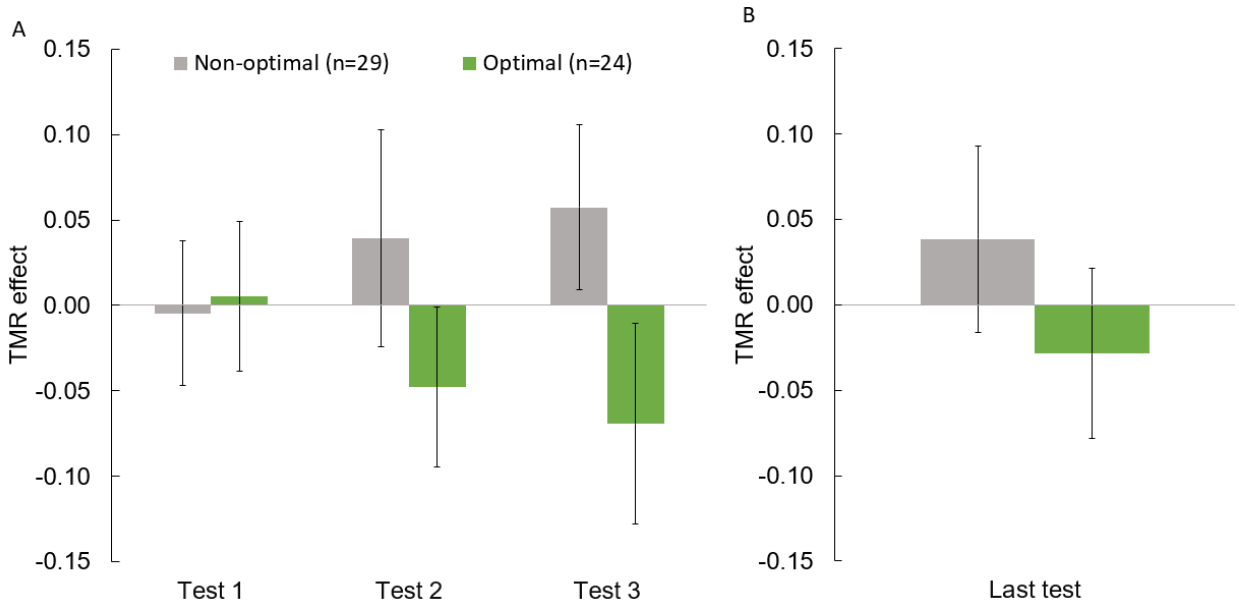

Figure 4.9 For participants cued with optimal parameters, we found a trend towards increasing benefit over time, while TMR produced an increasing detriment over time when participants were cued with non-optimal parameters. Results are shown for participants with tests on all three days (A) and at last test (B). TMR effect shown here is corrected for initial differences in memory.

4.5 Interim discussion

Based on these results, we hypothesized that we did not observe an overall TMR effect due to sleep disruption which occurred in some participants. Participants who received cueing likely to cause sleep disruption (intensity >=0.02 or few cues on adaptation night) showed a reversed TMR effect, while participants who received more optimal cues showed a typical TMR effect. This effect is consistent with the effect of presenting loud TMR cues that we described in chapter 2.

Our finding that N3 sleep duration is associated with TMR benefits replicates that seen in chapter 1, where an increased amount of time in N3 sleep predicted more benefit from TMR. This may reflect a number of different processes (1) Participants may be less arousable in N3 than in other sleep stages, (2) arousals may reduce the amounts of time in N3, or (3) TMR cues may produce greater benefits in N3 regardless of arousal. While the study described in Chapter 1 found that cueing benefit was related to the total amount of N3 in the nap, this study found that TMR benefit was related only to the percent of cues presented in N3, and not to the total amount of N3. A potential explanation is that while cues were delivered for nearly in entire portion of N3 in experiment 1, in this experiment the cued time was only a small fraction of the

total N3 time. Thus, both results are consistent with the idea that TMR has larger effects in participants with high amounts of N3 during cueing.

4.6 Experiment 2—can the parameters of home TMR be optimized?

Because our initial experiment suggested that the home TMR system could improve memory contingent on low cue volume, we conducted a follow-up study to replicate this effect. This experiment was identical to the original experiment, except for the following modifications:

- Participants were not able to set the initial volume higher than 0.02
- A new algorithm required participants to receive at least 25 adaptation cues before they could begin the memory test. If participants did not receive 25 cues on the first adaptation night, they performed the adaptation procedure again
- Participants could receive up to 30 minutes of cueing per night, vs 10.5 minutes in the previous experiment.
- We improved the algorithm used to allocate objects to cued and uncued groups to perform better matching; for this reason we did not use statistical controls for pre-sleep memory performance in this experiment.

Participants

We collected data from 40 participants, of these 24 passed inclusion criteria and were included in analysis. The goal N (24 participants) was planned before the start of the experiment., Participants were recruited and paid using the same methods as the prior experiment.

4.7 Experiment 2 results

TMR with an optimized protocol improves spatial memory at last test

As shown in Figure 4.10, the optimized TMR protocol used in experiment 2 significantly improved memory for the cued objects relative to the uncued objects at last tests [Wilcoxon signed-rank test; z(23)=-2.69, p=0.007].

In the secondary analysis examining only participants who took all three memory tests (n=18) a significant difference between cued and uncued objects emerged at the second memory test and persisted in the third test [Wilcoxon signed-rank test; z(17)=-1.63, -2.24,-2.29, p=0.103,0.025,0.022 for test 1, 2 and 3 respectively].

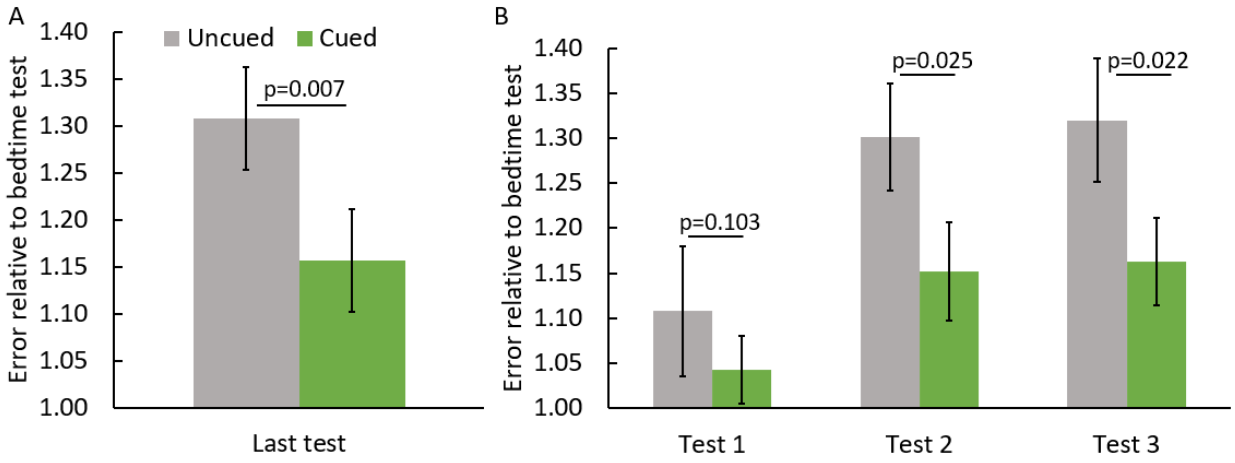

Figure 4.10 A. TMR effects at the final memory test. B. TMR effects at each time point, for the participants who performed all three morning memory tests.

4.8 Discussion

In this series of experiments, we designed and tested a novel wearable system for presenting auditory cues during sleep. Our results confirm that the system can target deep sleep and produce memory benefits that mirror those of targeted memory reactivation performed in the sleep lab. These results demonstrate that home sleep interventions with a smartwatch-based system are feasible and suggest important considerations for cue intensity and control algorithms.

An important finding in our experiment was that participants remained unaware that TMR cues were presented in almost all (89%) of cases, and no participants reported that the cues disrupted their sleep. In the optimized cueing experiment, only 2/24 participants reported hearing the cues. This is a significant improvement over unsupervised home TMR, where participants frequently report hearing the cues and cues disturbing their sleep (Göldi & Rasch, 2019). Presenting cues without participants noticing is important for usability and to avoid accidentally unblinding participants in experiments where they are assigned to conditions. This result also confirms that the system can target states where participants are soundly asleep (as opposed to light sleep).

The ability to target deep sleep was also reflected by the Dreem 2's automatic sleep staging of the times in which cues were delivered. Cues were delivered disproportionately in N3 sleep, with most of the cues not in N3 were delivered in N2. In lab-TMR experiments aimed at enhancing memory, cues are typically presented in either N3 or a combination of N2 and N3, as memory-related sleep features like spindles and slow waves occur in both of these stages. (Dijk et al., 1993). These results show that the system can target deep nonREM and present cues

without waking participants, both important advances for conducting sleep intervention studies at home.

We also demonstrate that TMR at home has the typical effect observed in the lab, where TMR with quiet sounds can improve performance in a spatial memory task. We also found that TMR with very loud sounds (above 5% of the phone's maximum output) consistently reverses the TMR effect, consistent with findings in our lab (Chapter 2). These two findings suggest that home-TMR can be meaningfully used to investigate memory processes and may be useful in clinical and translational contexts.

While most lab-TMR experiments involve only a single night, using home-TMR afforded us the ability to study the effects of multiple sessions of TMR, interspersed with memory tests each morning. Our data suggest that this is a fundamentally valid way to study multiple sessions for TMR; for example, forgetting followed a typical forgetting curve despite the practice effects from morning memory tests. TMR effects appeared to become larger over time; in experiment 2 we observed no significant TMR effect at test 1 but a significant effect at tests 2 and 3; a similar trend occurred for optimally-cued participants in experiment 1. Notably, overall error was lower in test 1 compared to later tests; the size of the TMR effect may be proportional to the overall amount of forgetting which has occurred.

Our results also highlight the critical importance of auditory cue intensity and sleep disruption in home TMR. Initially we suspected that allowing users to calibrate the volume of the white noise and cues would produce the best effects; however locking intensity to a consistent low level in experiment 2 produced better TMR effects than allowing users to calibrate the intensity in experiment 1. Therefore, our current recommendation is that home-TMR experiments should

use cues which are just barely audible in a quiet room, though optimal means of calibrating intensity is an important topic for future research.

Finally, these results suggest numerous avenues for future research. The EEG and wearable data acquired during this experiment may allow us to develop more accurate algorithms for targeting N3 and other sleep stages. Future analyses may also use this data to study how sleep parameters and experimental-design factors like the number and timing of cues influence relate to TMR response, in a large sample of participants. The ability to run TMR experiments at large scale using home devices may prove useful for answering fundamental questions about TMR and memory processing in sleep in the future.

4.9 Author Contributions

This chapter has not yet been submitted as a manuscript. Nathan Whitmore and Ken Paller designed the experiment. Nathan Whitmore collected, analyzed, and interpreted data and wrote software. Torin Kovach and Jasmine Harris wrote software. Ken Paller supervised the project. Nathan Whitmore wrote the chapter with input from Ken Paller.

5.0 Acknowledgments

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Chapter 5—Conclusions and Future Directions

5.1 Summary

In the previous three chapters, we demonstrated three findings important for using TMR to improve memory

- First, we demonstrated a novel finding that TMR can either strengthen or weaken memories, depending on whether the TMR cues produce sleep disruption. We demonstrate this effect across multiple studies and measures in all three chapters.
- Secondly, we designed a novel system to perform TMR at home, and demonstrated that it can deliver auditory cues targeting specific sleep phases without awakening participants
- Finally, we demonstrated that using TMR at home can enhance memory in the same manner observed in laboratory studies, contingent on avoiding sleep disruption.

Our first finding, that the effects of TMR can be reversed by sleep disruption, is important for several reasons. First, it demonstrates that avoiding sleep disruption is critical in using TMR to improve memory. Second, it demonstrates a novel phenomenon that may shed light on the mechanisms of memory consolidation. Third, it suggests a possible mechanism by which sleep disorders such as apnea could impair memory. Finally, it raises the possibility that sleep disruption could be used therapeutically, for example to reduce the impact of distressing memories.

Our findings on sleep disruption also open numerous avenues for future research. For

example, does sleep disruption also weaken memories that were acquired some time ago, and thus have already been subject to some consolidation? We are currently running a study to tackle this question, in which we disrupt sleep while reactivating memories acquired a week previously. These results will hopefully shed additional light on whether sleep disruption parallels the effects seen in wake reconsolidation studies. If so, memories that have already undergone some consolidation should still be affected by sleep disruption. Alternatively, memories may be vulnerable to sleep disruption only immediately after encoding, arguing against a reconsolidation-like effect. The results could also bear on the feasibility of using sleep disruption to weaken unwanted memories outside the laboratory, such as when an individual has experienced some traumatic event. In general, the ability to produce both weakening and strengthening of memories opens up new possibilities for dissecting TMR mechanisms, as discussed in the next section.

Our work on TMR outside of the sleep lab also opens up opportunities for future research. In particular, our novel system for home TMR may offer the ability to run new types of TMR experiments, such as testing the effects of TMR over extended periods of time, and in the context of classroom education or clinical therapy. Another exciting possibility enabled by this technology is high-throughput TMR studies involving hundreds or thousands of participants. Such studies could shine light on questions that have not been adequately addressed by traditional lab-TMR studies, such as whether the effects of TMR change with age (Wilhelm et al., 2020).

Furthermore, the ability to present stimuli linked to specific sleep states as demonstrated here may have applications beyond improving memory. For example, in collaboration with colleagues in the lab group, I developed a modified version of the model described here that targets REM

to induce lucid dreaming. Preliminary results suggest this system can increase the rate of lucid dreams by three-fold (Whitmore, Konkoly, Mazurek, Mallett, & Paller 2022, unpublished findings). Targeted auditory cueing during sleep may prove to be useful for a wide range of applications including dream incubation (Haar Horowitz et al., 2020), enhancing creativity (Sanders et al., 2019), and improving sleep quality (Besedovsky et al., 2017)

5.2 The effects of sleep disruption suggest TMR is a multi-stage process

While it is widely accepted that reactivation during sleep can strengthen specific memories, a major open question remains: what does it mean (at a cognitive and neural level) for a memory to be reactivated?

Our data on sleep disruption can shed some light on this question. The effects of sleep disruption suggest that memory reactivation involves at least two distinct phases: a “selection” phase where a specific memory is targeted for modification, and a “strengthening” phase where the memory undergoes a transformation which enhances recall. The two-phase model is inferred from the observation that sleep disruption weakens reactivated memories more than those not reactivated. In disrupted sleep, selection proceeds normally (implied by selective effects on reactivated memories) but the memory is not strengthened.

5.3 What mechanisms might underlie the sleep disruption effect?

Why might strengthening fail when sleep is disrupted? One hypothesis is that strengthening depends on a dialog between multiple cortical and subcortical areas for some time after a TMR

cue, mediated by phenomena like sleep spindles and slow oscillations (Ngo et al., 2020) . Arousal in sleep causes a marked shift in oscillations (Iber et al., 2007) and rapidly abolishes spindles and slow waves, which may disrupt this process.

Several lines of evidence support this hypothesis. Reactivated information is decodable from EEG for several seconds after a TMR cue (Wang et al., 2019), indicating that information is being processed in the time window where arousals usually occur. Antony et al., (2018) found that TMR cues benefitted memory more when a sleep spindle occurred after the TMR cue; since sleep disruption disrupts spindles it may prevent this process.

Disrupting brain networks after a cue could produce forgetting of reactivated memories though several mechanisms. Phase-locking across frequencies and brain regions appears to mediate the benefits of sleep for memory (Cordi et al., 2018; Hahn et al., 2022; Muehlroth et al., 2019); the random perturbation induced by an arousal may destroy this phase locking and corrupt the contents of a memory replay. Similarly, during an arousal neurons may begin to respond more to sensory input, resulting in a “confused” state representing both features of the memory and features of the current environment. Finally, oscillations like spindles and hippocampal theta (Creery et al., 2022) may function to induce plasticity (Huerta & Lisman, 1995) and stabilize reactivated memories; interrupting this process may result in forgetting similar to that seen in classical reconsolidation experiments (Tronson & Taylor, 2007).

Currently, many questions remain regarding how sleep disruption weakens memory, as well as the mechanisms of TMR in general. In the future, TMR with sleep disruption may provide a valuable tool for understanding memory reactivation; for example varying the timing of sleep disruption relative to reactivation may help delineate the time course of memory consolidation

processes. Future experiments may also explore the types of errors induced by sleep disruption, which may provide clues to how it affects the cognitive processing of memory. Finally, future experiments could explore the effects of disrupting specific sleep processes like spindles to better understand the mechanisms of TMR

5.4 What can TMR be used for?

Increasing our ability to use TMR in new contexts raises an additional question: what kinds of memory and learning tasks are likely to benefit from TMR?

A key challenge in answering this question is that the mechanisms by which TMR improves memory remains unclear. While the classical hypothesis of TMR proposes that TMR works by accelerating transfer of memory data from the hippocampus to cortex; this transfer process has never been directly observed (for instance, by testing whether TMR before a hippocampal lesion reduces retrograde amnesia in animals). Rather, transfer is inferred because TMR induces replay processes similar to those thought to induce systems consolidation. Some memory researchers have questioned whether transfer of memories from hippocampus to cortex occurs at all, and instead argue that the hippocampus and cortex are responsible for fundamentally different types of memory (Yonelinas et al., 2019).

Currently, it is prudent to say that while acceleration of systems consolidation is a plausible explanation for the effects of TMR (and naturally occurring memory reactivation), it is not the only plausible explanation. For example, reactivation may produce retrieval-induced forgetting which reduces interference in memories (Murayama et al., 2014) , and promote pruning of unneeded synapses (González-Rueda et al., 2018). These processes are not mutually exclusive, and reactivation may in fact improve memory through several independent mechanisms.

Given this, our best guide to the possibilities achievable with TMR is behavioral data, which generally show that TMR is effective for a wide range of declarative learning and skill learning tasks, with no effect for conditioning (Hu et al., 2020). This observation may help extend TMR to many contexts—for example, a TMR intervention for emotional health could focus on using TMR to improve cognitive reappraisal skills.

5.5 Conclusion

Overall, the results of these studies demonstrate that (1) sleep disruption is a critical factor influencing the effects of TMR and that (2) when sleep disruption is controlled, TMR can be a useful tool for improving learning, even outside of a sleep laboratory environment. The results described here provide insight into the mechanisms of TMR, and our home-TMR system offers a practical and effective way of performing TMR experiments and therapies in any location where people sleep.

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