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Investigating the Role of Targeted Memory Reactivation in Sleep Spindle Production

Justin W. Hopper, *The University of Western Ontario*

Supervisor: Grahn, Jessica A., *The University of Western Ontario*
Co-Supervisor: Batterink, Laura J., *The University of Western Ontario*
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Abstract

In ‘targeted memory reactivation’ (TMR) paradigms, information learned during wakefulness is paired with a cue, and reactivated during sleep by presenting that same cue. TMR improves memory. In a prior study (Antony et al., 2012), participants learned two melodies. One melody was cued during a nap, and performance was better than for the uncued melody. The current study reanalyzed these data to characterize sleep spindle density during TMR cue-periods relative to non-cued periods, and whether spindle density correlated with performance. During TMR stimulation, spindle density was significantly higher than during non-stimulation in four time windows. Compared to the non-TMR group, higher spindle density occurred in two windows in the TMR group. Within-subject, spindle density was not correlated with accuracy, while between-subjects, spindle quantity correlated with post-nap accuracy improvements ($r = .507$). Thus, spindle density is altered at specific times by TMR, but TMR-specific density changes may not predict performance.

Keywords

Targeted memory reactivation, sleep spindles, memory consolidation, slow oscillations, hippocampal sharp wave ripples, slow wave sleep, electroencephalography

Summary for Lay Audience

'Targeted memory reactivation' (TMR) is a technique that uses a stimulus like sound or smell associated with prior learning to boost the memory-bolstering processes that happen during sleep. One of these processes is called sleep spindles, which are fast brainwaves that burst in an rhythmic, repetitive manner. In this study, a non-invasive electrophysiological monitoring method (elecroencephalography; EEG) was used to record electrical activity on the scalp that been shown to represent the activity of the surface layer of the brain underneath. Using data collected first reported in a previous, auditory TMR study (Antony et al., 2012) we investigated the occurrence of sleep spindles relative in time to the sounds during the sleep recordings. This was contrasted to silent periods within the same EEG recordings, as well as against a different group of nappers who were presented no sounds during sleep.

In the original study, participants produced a melody with button presses before napping, and after napping, with the melody presented to them during their nap. They found that participants were better at producing the that they had been presented during the nap when compared to a control melody.

We found higher concentrations of sleep spindles in four small time windows within the periods of sound stimulation when comparing to periods of no sound stimulation of the same participants' recordings. When we compared to the group that received no sounds at all, we found that the TMR group had significantly higher concentrations of sleep spindles in two small time windows. This indicated that while TMR alters sleep spindle concentration, this may not alone correspond to better performance in cued tasks.

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Table of Contents

List of Figures

Introduction

Sleep is a naturally recurring state of mind and body, characterized by altered consciousness, relatively inhibited sensory activity, reduced muscle activity and inhibition of nearly all voluntary muscles at various times throughout. Though humans spend about one-third of their time sleeping, much is still unknown about the function of sleep. There is good evidence that sleep promotes the consolidation of long-term memory, which is the process by which newly acquired memories are strengthened and integrated with prior knowledge. In fact, nearly 100 years ago Jenkins and Dallenbach (1924) first reported a slower rate of forgetting during a period of sleep compared to an equivalent period of waking, and decades of more recent research confirm this basic finding. Sleep may be an ideal period for memory consolidation because it is relatively free from competing sensory stimuli to be encoded (Rasch & Born, 2013).

There are several neural features of sleep that may facilitate the process of memory consolidation. The focus of this thesis project is on sleep spindles, which are transient, rhythmic bursts of neural oscillatory activity occurring between 11 and 16 Hz. Spindles are produced as a result of activity in the thalamus, the thalamic reticular nucleus and thalamocortical relay neurons (Llinás & Steriade, 2006), and their length may also be affected by neocortical feedback (Bonjean et al., 2011). Spindles are thought to play an important role in memory consolidation processes. Tasks requiring learning often result in increases in spindle measures (Gais et al., 2004; Schmidt et al., 2006). In addition, spindle activity correlates with memory retention after sleep (Clemens et al,. 2013; Van der Helm et al., 2011) and predicts performance in a directed-forgetting paradigm (Saletin et al., 2011). Spindles are capable of potentiating cortical synapses,

and thus, their role in learning and memory-related processes may be supported by their plasticity-enhancing properties (Rosanova and Ulrich, 2005). The goal of the current study is to further understand the role of sleep spindles in memory consolidation, using an auditory learning paradigm, by investigating whether learning-related cues enhance spindle activity, and if so, examining if this increase corresponds to memory performance.

Neural Mechanisms of Memory Consolidation and Sleep

Sleep is divided into four stages, which are characterized by unique neural signatures. Stages N1, N2, and N3 are known as "non-REM sleep", and the fourth stage is rapid-eye movement (REM) sleep. Stage N1 mostly consists of encephalographic (EEG) theta waves (4–8 Hz). Stage N2 is a deeper stage of sleep consisting of theta activity (4–8 Hz), K complexes, which are solitary EEG spikes usually greater than 100 μV, and sleep spindles. Stage N3, also known as slow-wave sleep (SWS), consists of the deepest stage of sleep and is characterized by delta waves (high amplitude brainwaves of 0.5-4 Hz). During the final stage, REM sleep, brain activity consists of primarily theta activity. Also, during REM, there is a great deal of cyclical, coordinated ocular activity (Okawa et al., 2017). Certain stages of sleep appear to be more important for memory consolidation than others. In particular, SWS appears to be important for memory consolidation, in part because its neurophysiology appears to be suited to that purpose (Diekelmann, Wilhelm, & Born, 2009).

Two Stage Model Overview

A modern framework for understanding memory consolidation is the two-stage model of memory (Marr, 1971; McClelland, McNaughton, & O'Reilly, 1995). It

proposes two separate memory stores: one store allows learning at a fast rate and holds the information only temporarily; the other learns at a slower rate and acts as the long-term store. New events are encoded in simultaneously in both stores. In successive periods of consolidation, the recently encoded memory traces are re-activated in the short-term store, which promotes synchronized re-activation in the long-term, slow-learning store. Over time, memory representations in the long-term store are reinforced and memories become redistributed, relying to a greater extent on the long-term store. With repeated re-activation of new memories, in combination with related older memories, the short-term store reinforces the long-term store to gradually integrate the new memories into the existing network of long-term memories (Lange, Dimitrov, & Born, 2010).

Active Systems Model (Diekelmann & Born 2010)

One model of understanding consolidation of memory during sleep is the Active Systems model. This model suggests, using the two-stage framework of memory, that memories are encoded into a temporary store (hippocampus), and during SWS are reactivated to be redistributed to the long-term store (neocortex). This reactivation process occurs through the nesting of hippocampal sharp wave ripples, sleep spindles, and slow oscillations (SOs) – high amplitude, low frequency slow waves of approximately 0.8 Hz – within each other, as described in greater detail below. Events experienced during waking are originally encoded in parallel in both the neocortex and the hippocampus, as well as nearby medial temporal lobe structures. During SWS, newly developed memory traces are repeatedly reactivated and become progressively redistributed, strengthening synaptic connections within the neocortex, and solidifying

memory representations. SOs are generated primarily in neocortical networks, though they synchronize neuronal activity not only in the neocortex, but also in various other brain regions relevant to memory consolidation. SOs provide a global temporal frame whereby the depolarizing up phases repetitively drive the reactivation of memories in hippocampal circuits in parallel with thalamo-cortical spindles (Diekelmann & Born 2007, 2010; Born & Wilhelm, 2012).

A number of lines of evidence support the general idea that these sleep-related neural signals play an important role in memory consolidation. Various metrics of SWS correlate with memory improvement after sleep, including a longer duration of SWS, as well as higher SWS wave amplitude and density (Diekelmann et al., 2009; Ellenbogen, Hulbert, Stickgold, Dinges, & Thompson-Schill, 2006; Gais & Born, 2004). Slow waves have higher amplitudes and lower frequencies after a period of learning relative to a period where no intentional learning occurs (Heib et al., 2013; Kim, Pardilla-delgado, & Alger, 2017; Mölle, Eschenko, Gais, Sara, & Born, 2009; Mölle, Marshall, Gais, & Born, 2004). Moreover, electrically inducing SOs starting in stage N2, boosts overall slow-wave amplitude as well as memory performance post-sleep (Massimini et al., 2007; Tononi, Riedner, Hulse, Ferrarelli, & Sarasso, 2010). These findings provide insight into the potential role of SOs in coordinating neural reorganization of memories during sleep.

Also of importance for memory consolidation as understood by the Active Systems model are sleep spindles (Cairney, Marj, & Staresina, 2018; Cox, Cox, Hofman, & Talamini, 2012; Wei, Krishnan, Komarov, & Bazhenov, 2018). These bursts occur during sleep stages N2 and N3 and have been shown to play an important role in the consolidation of memory. Intracranial EEG recordings show that the spindles occur in

travelling rotational waves across the cortex (Muller et al., 2016). This repetitive spatial characteristic of spindle occurrence provides some insight into their proposed role in synchronizing activity from different cortical areas to allow for the integration of inter-regional activity into complete memories (Sanda et al., 2019; Skelin et al., 2021).

Sleep spindles often co-occur with slow oscillations across the cortex during stage N3 sleep (Eschenko, Ramadan, Mölle, Born, & Sara, 2008). Spindles have been found to occur specifically nested within the upstate (or excitable phase) of each slow oscillation (Sanda et al., 2019). More precise phase-locked co-occurrence between spindles and slow oscillations corresponds to greater performance in declarative memory tasks after sleep (Kim et al., 2017). The Active Systems model proposes that the performance increase arising from spindle/slow oscillation co-occurrence is because of a strong correlation between the presence of sleep spindles and hippocampal sharp wave ripples, which are high frequency oscillations (~140 Hz+) within the hippocampus, difficult to detect via conventional EEG because of the structural depth of the hippocampus and the high frequency of the oscillations (Buzsáki, 2015; Clemens et al., 2013; Coon et al., 2019, Beijamini, Valentin, Jäger, Born, & Diekelmann, 2021; Born & Wilhelm, 2012; Bringmann, 2018). Rodent studies indicate that disrupting hippocampal sharp wave ripples disrupts memory consolidation (Girardeau et al., 2009; Ego-Stengel & Wilson, 2010). In another rodent study, researchers manipulated hippocampo-cortical temporal coordination during sleep after training on a spatial memory task designed to trigger encoding. They bolstered coordination between hippocampal sharp wave-ripples, delta waves, and spindles across the cortex using rhythmic electrical stimulation, which resulted in a higher prefrontal response to the task and higher recall performance the next

day, whereas controls performed at chance. These results indicate that hippocampo-cortical interplay during sleep plays a causal role in memory consolidation during sleep, through a mechanism involving specific, coordinated patterns of sharp wave-ripples, SOs and spindles (Maingret, Girardeau, Todorova, Goutierre, & Zugaro, 2016).

Multiple lines of evidence also provide support for the role of each neural signature (SOs, sleep spindles, and hippocampal sharp waves) in memory consolidation during sleep. In an EEG paradigm, spindle activity during NREM sleep has been found to differ based on the previous learning condition. Spindles were found to increase after learning, and correlate with performance on an accuracy test of learned information. Further, parietal sleep spindles were found to accompany task-specific reactivation seen in functional magnetic resonance imaging (fMRI; Schönauer et al., 2017). Inducing memory reactivation in NREM sleep evokes a brief increase in spindle activity, during which the content of reactivated memories can be reinforced (Cairney et al., 2018). Manually disrupting spindles by presenting a competing auditory stimulus during reactivation removes the retention benefits associated with sleep (Schreiner, Lehmann, & Rasch, 2015). Therefore, spindles may facilitate the reestablishment and consolidation of new memories.

Studies in rodents also provide some of the strongest, most direct evidence that memory consolidation involves reactivation of specific, recently acquired memories during sleep (Wilson & McNaughton, 1994). In one rodent study, hippocampal neurons associated with spatial processing fired in the same order during sleep as during prior learning, potentially indicating a memory “replay”, contributing to memory consolidation (Pavlides & Winson, 1989). In human fMRI, hippocampal areas that were activated

during route learning in a virtual environment were similarly activated during subsequent SWS (Peigneux et al., 2004), consistent with the idea of memory reactivation as an underlying neural mechanism supporting memory consolidation. A similar result has also been found with intracranial EEG in participants engaged in episodic free recall of previously viewed photographs, where an increase in the rate of hippocampal ripples occurred 1-2 seconds before recall events. During recall events, visual neural areas showed hippocampal sharp wave-coupled activation patterns associated with recalled content (Norman et al., 2019). These findings indicate that sharp wave ripples may play a role in triggering spontaneous recall and coordinating the reestablishment of cortical representations during memory retrieval in humans.

Sleep Spindles and Memory Consolidation

Most natural episodic memories are multi-modal, therefore encoded across many neural areas. Spindles mostly occur locally, detected via EEG in small regions across the scalp (Rasch & Born, 2013). Thus, sleep spindles may support memory consolidation by facilitating the reprocessing of separate memory components in local networks of neurons (Bergmann, Mölle, Diedrichs, Born, & Siebner, 2012). In support of this theory, spindle-coupled memory reactivations are topographically constrained to the same cortical areas activated during the learning process (Cox, Hofman, de Boer, & Talamini, 2014), and cued memory reactivations evoke spindles across learning-centric neural areas.

Spindle refractoriness—the minimum period of time between spindle occurrences—may play a central role in information processing by limiting interference and protecting the process of memory consolidation from extraneous information. In humans, spindles have refractory periods of 3–6 s (Antony et al., 2018), which may

provide a natural restriction to the process of memory reactivation (Ngo et al., 2015). Documented memory improvements of cueing reactivations in NREM sleep disappear when cues are presented directly after spindle offset (Antony et al., 2018).

Spindle refractoriness may also optimize periodic or oscillatory communications between neural areas during NREM sleep. In accordance with the Active Systems model (Rasch & Born, 2013), the coupling of cortical slow oscillations and sleep spindles mark clusters of hippocampal-sharp wave ripple events, which represent small memory units. This unified oscillatory pattern may facilitate communication between cortical and subcortical memory networks (Latchoumane, Ngo, Born, & Shin, 2017). This oscillatory pattern may be based on the natural repeating refractory period of sleep spindles. Sharp-wave ripples may occur separately from sleep spindles and vice-versa; however, declarative memory benefits from sleep are more closely linked to spindle-ripple co-occurrence than to either sharp-wave ripples or spindles independently (Xia et al., 2017). The broader oscillatory pattern of sleep spindle occurrence throughout the cortex may facilitate the interaction between local spindle-ripple clusters contributing to the reinstatement of local, specific memory units, and cortical slow oscillations, which may contribute to broader representations across the cortex (Antony et al. 2014).

In another study, intracranial recordings from the human medial temporal lobe and prefrontal cortex in epilepsy patients were combined with EEG recordings to test if coupled SO-spindle activity mediated hippocampal-neocortical information transfer during a full night of sleep. They found bidirectional interactions between the prefrontal cortex and hippocampus suggesting NREM (non-REM) sleep as a potential optimal time for information transfer to the neocortex (Helfrich et al., 2019). The same research group

found, through EEG, structural MRI, and sleep-dependent memory assessment, precise time locking between slow oscillations and sleep spindles, the quality of which predicted memory retention after sleeping (Helfrich et al, 2018). Individually, these findings show strong support for the role of each neural signature in memory consolidation during sleep (SOs, sleep spindles, and hippocampal sharp waves). It is likely that, in accordance with the Active Systems model, SOs, sleep spindles, and hippocampal sharp wave ripples operate in concert during sleep to facilitate communication between areas of the neocortex and the hippocampus, enabling newer memories to be integrated into existing schema.

As underscored by these studies, the reactivation of new memories is a fundamental component of memory consolidation. During natural sleep, memory reactivation occurs spontaneously, and these specific neural signatures (hippocampal sharp waves, spindles, and slow oscillations) are thought to be the neural mechanisms that represent memory reactivation. There are features of memories that may render their reactivation more likely. These features are sometimes referred to as “usefulness”, i.e., emotionally salient, reward-based, and more recent memories are more likely to be replayed than other types of memories (Oudiette & Paller, 2013). Memory reactivation, while naturally occurring, may also be triggered externally, by sensory cues provided to a sleeper during sleep. This process of externally triggering reactivation is called targeted memory reactivation (TMR).

Targeted Memory Reactivation

When sleepers are exposed to stimulus-associated cues from an initial learning period, memory benefits in subsequent wake have been observed, a paradigm referred to

as TMR. In TMR paradigms, to-be-learned information is paired with sensory cues during learning, and then those cues are used to reactivate memories during sleep. Both olfactory and auditory cues have been shown to produce memory benefits (Bar et al., 2020; Cellini & Capuozzo, 2018; Shimizu et al., 2018). In a seminal TMR study, new memories were cued in humans during sleep by presenting an odor that had been introduced during a prior learning phase. Re-exposure to the odor during SWS improved the preservation of hippocampus-dependent declarative memories, but not hippocampus-independent procedural memories. In addition, fMRI showed increased hippocampal activity in response to re-exposure to the odor during SWS, providing neural evidence of hippocampal memory reactivation (Rasch et al., 2007).

In a follow-up auditory TMR study, participants learned locations of objects on a screen while hearing specific sound cues for each picture (Rudoy, Voss, Westerberg, & Paller, 2009). During a subsequent nap, half of the sounds were presented covertly during SWS, with the other half serving as uncued control sounds. After the nap, participants more accurately recalled picture locations for trials that were cued during SWS than for uncued trials (Creery, Oudiette, Antony, & Paller, 2015). These findings suggest that cues trigger memory reactivation of newly learned items during sleep, strengthening the associated memory representations. The cueing-induced improvement in subsequent memory tasks compared to uncued baseline performance is thought to be dependent on mechanisms that occur during SWS (Cairney, Durrant, Hulleman, & Lewis, 2014).

In addition to memories for spatial locations, TMR has also been shown to influence fear extinction (Hauner, Howard, Zelano, & Gottfried, 2013), word recall

(Fuentemilla et al., 2013), language learning (Batterink & Paller, 2017; Schreiner & Rasch, 2017), skill learning (Antony, Gobel, Hare, Reber, & Paller, 2012; Lahav, Saltzman, & Schlaug, 2007), and even social stereotypes (Hu et al. 2015). Intact medial temporal regions appear necessary for effective TMR, at least for declarative memory tasks. In a spatial-word task, TMR improved word recall for controls and epilepsy participants with unilateral damage, but not bilateral damage. The degree of recall improvement post-TMR correlated with the amount of medial temporal damage across participants (Fuentemilla et al., 2013). Effective TMR also requires sufficiently strong associations between the cues and learned information and may additionally depend on whether simultaneous memory processing is competing with TMR-cued processing (van Dongen, Thielen, Takashima, Barth, & Fernández, 2012). TMR benefits are not observed if memory is tested after insufficient encoding or too much memory decay before sleep, or if memory performance is initially at ceiling (Cairney et al., 2016). In addition, sleep-based consolidation depends on the importance of remembering the target information (Oudiette, Antony, Creery, & Paller, 2013; Wilhelm et al., 2011), indicating that motivation may also influence consolidation. Basic sensory processing of cues at the sleep phase is also necessary. For example, one fMRI TMR study found no reliable TMR memory benefit overall (Van Dongen et al., 2012), possibly because the scanner noise masked the auditory cues.

Work has been conducted aiming to decode the content of retrieved memories in EEG during sleep. In one experiment, participants learned to associate spatial locations of visual objects, along with a semantically related sound with hand movements. During a following nap, half of the paired sounds were cued during SWS. Cued spatial locations

and hand movement discrimination accuracy was above chance and predicted successive memory. These lateralized signals increased with sleep spindle amplitude following cues, indicating a strong relation between retrieval and spindles. This study also shows that neural activity related to individual memories can be decoded from EEG sleep recordings, indicating offline retrieval (Wang et al., 2019).

The Present Study

Sleep spindles are a feature of NREM sleep, and spindle activity may be enhanced by TMR (Cairney et al. 2018). It is not entirely clear which measures of neural activity in humans index specific instances of memory reactivation (Creery et al., 2015). To further establish TMR as an effective tool for manipulating consolidation and to understand the underlying mechanisms, TMR-induced spindles occurring during SWS, which have been taken as a marker for TMR effectiveness, must be differentiated from spindles that occur naturally within the sleep cycle.

Most auditory TMR paradigms use short, discrete cues, separated by relatively long periods of silence. Continuous stimulus streams are less common, for several reasons including ease of administration and analysis, simpler cue-pairing during the learning phase, and greater number of cues presented over the course of a sleep period. Temporally longer cued pairs allow for analyses that are not possible with traditional designs. Before longer cues can be used, it is necessary to understand what the time course of spindle activity elicited by TMR cues is, and how it differs from spindle activity that occurs spontaneously. However, there are benefits to using a continuous auditory stimulus for TMR, including the facilitation of time-based analyses, in which

researchers can monitor the time course of the neural signal in response to a particular stimulus as it is presented.

Additionally, discrete stimuli, because of their brevity, are often less naturalistic than continuous stimuli have the potential to be. Most naturalistic sounds that humans derive meaning from are not as short as those administered in TMR studies, a practice restricted by the nature of the paired-stimulus design. Emotionally salient stimuli, often studied with TMR, are often especially long, relative to most discrete, experimental auditory stimuli (Cairney et al., 2014). The purpose of the current study was to compare spindle occurrences evoked by TMR relative to endogenously during non-TMR periods, and to further establish whether TMR cues (and particularly continuous TMR cues) enhance spindle activity, providing additional evidence on whether TMR effects operate by the hypothesized mechanism of memory reactivation. TMR-related spindles are expected to predict memory consolidation of cued events, whereas non-TMR-related spindles may correlate with overall memory consolidation for all trial types – it is possible that these could occur spontaneously regardless of trial type (cued or uncued). To investigate this, sleep spindles occurrence was tracked, and temporal dynamics across continuous trials were compared between trial types. To further the understanding of the mechanisms behind TMR, more research is required into what specific learned material can be externally reactivated and which specific spindle components are associated with reactivation during sleep.

The current study analyzed existing data first reported by Antony et al. (2012). In that study, participants learned to produce two different melodic sequences in time with moving visual symbols. One of the sequences was then presented during an afternoon

nap, and results showed that memory for the sequence was enhanced relative to the non-presented sequence. Neural signatures related to memory processing during sleep indicated that auditory stimulation that does not disrupt sleep can influence memory consolidation.

Here we set out to determine the relationship between spindle density during stimulation and subsequent memory performance when continuous cues were used for TMR. Specifically, we assessed whether spindle density would be higher during continuous cued TMR relative to a similar period of non-stimulation, and whether individual differences in spindle density during TMR would relate to melody performance gains. We hypothesized that during TMR stimulation, the density of sleep spindles would be higher than during periods of non-stimulation. We also hypothesized that, across individuals, higher spindle density would predict gains in cued sequence accuracy, as assessed through behavioural pre- and post- measures. If higher spindle density predicts greater accuracy, this would provide evidence for the co-ordinating role of sleep spindles facilitating memory consolidation, as suggested by previous theories and studies (Clemens et al., 2013; Muehlroth et al., 2019; Staresina, Bergmann, Bonnefond, & Der, 2016).

Methods

Procedure of Previous Study

In a 2012 study (Antony et al., 2012), after preparation for electrophysiological recordings, participants (n=16) learned to play two simple melodies. All melodies were 12 items long, following a fixed order. Each melody had a different melodic contour, rhythm, and pitch. The participants first listened passively to each sequence repeated five times each.

Next, in a following training phase, participants completed a total of 4 training blocks. In each block, melodies were repeated five times each. During training, a screen 168 cm away from where the participant was seated showed circles that ascended in four columns toward four stationary circular target markers (Figure 1). Participants were told that their performance accuracy for the two sequences would be assessed. Participants then attempted to press a computer keyboard button corresponding to each target during circle-target overlap. In blocks 1 and 2, button presses evoked a musical tone only if a response occurred during the interval from 200 ms before to 150 ms after complete circle-target overlap.

After block 2, less advance warning was given before circle-target overlap. The size of the mask (providing advance warning information of incoming notes) was individualized so that poorer performers received more advance notice than more accurate performers. The time from appearance of a circle until when it reached the target location was initially 2056 ms and became either 370, 498 or 625 ms after block 2.

In block 4, a novel melody was introduced so that specific learning of the trained melodies could be distinguished from baseline improvement resulting from general

learning of the basic mechanisms of the task (pressing buttons to target timings). Each sequence was repeated five times. Block 4 constituted the pre-nap test.

After block 4, participants were asked to nap. No mention of sound presentation was made, other than to inform participants that quiet white noise would be presented throughout the nap. The experimenter monitored sleep online to tell when the participants had entered SWS, though comprehensive sleep staging was completed offline according to American Sleep Association standards. Unbeknownst to participants, one of the learned melodies was played repeatedly upon detection of slow-wave sleep. Each sequence lasted 6.3 seconds, and between each sequence there was a silent interval that was also approximately 6.3 seconds (mean = 6.52 seconds; range = 4.23 to 7.11 seconds). White noise was administered in the background at ~35 dB, and when the sequences were administered, the volume of the white noise was decreased slightly so that the overall sound intensity remained stable, maintained at approximately 35 db. Sequences were repeated an average of 21 times per participant. The sleep period lasted 90 min, and post-nap testing began 10 min later. The post-nap test block (Block 5) was administered in the same way as the pre-nap test block (Block 4), except that a different novel sequence was used (Figure 2).

For both pre-nap and post-nap test blocks (Blocks 4 and 5), behavioural accuracy for each note was computed according to whether the correct key was pressed within -200 to 150 ms relative to target onset. Pre-to-post change in accuracy scores (percent correct responses) were computed by subtracting each individual's mean score for the pre-nap test block from their score in the post-nap block. Positive values indicate an improvement from pre-nap to post-nap.

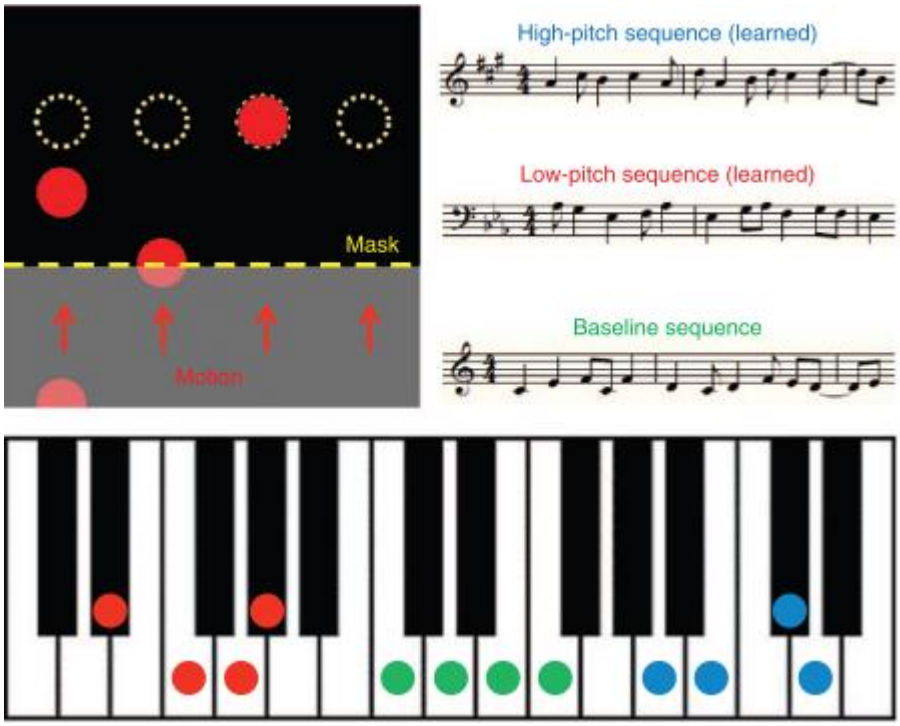
A second group of 16 subjects (mean age = 19.8 years, mean musical experience = 5.7 years) followed the same protocol as the sleep sounds group, but did not receive any auditory cueing during a 90-min sleep period.

EEG Data Acquisition and Sleep Staging (Original Study)

Tin electrodes in an elastic cap were placed at 21 standard scalp locations, left and right mastoids, adjacent to the eyes, and on the chin. Electrophysiological data were sampled at 1,000 Hz with a band-pass of 0.1–100 Hz, down-sampled to 250 Hz, and re-referenced to average mastoids. Recordings were manually sleep scored offline according to the American Sleep Association guidelines.

Figure 1.

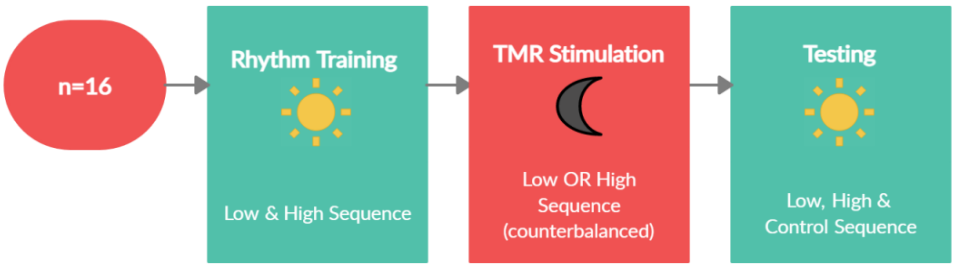
Melody Sequence Task



Participants learned to play melodies on a computer keyboard with four fingers of the left hand while watching circles that indicated which key to press when. Circles ascended at 10.8 cm s^{-1} toward four stationary targets (dashed yellow outlines). After initial learning trials, the amount of advance information was reduced using an opaque mask (shown here as transparent). Two melodies were repeatedly practiced (red and blue). Baseline melodies (green) were played during testing periods before and after the nap. Either the high melody (eight subjects) or the low melody (eight subjects) was presented covertly (cued) during sleep (from Antony et al., 2012).

Figure 2.

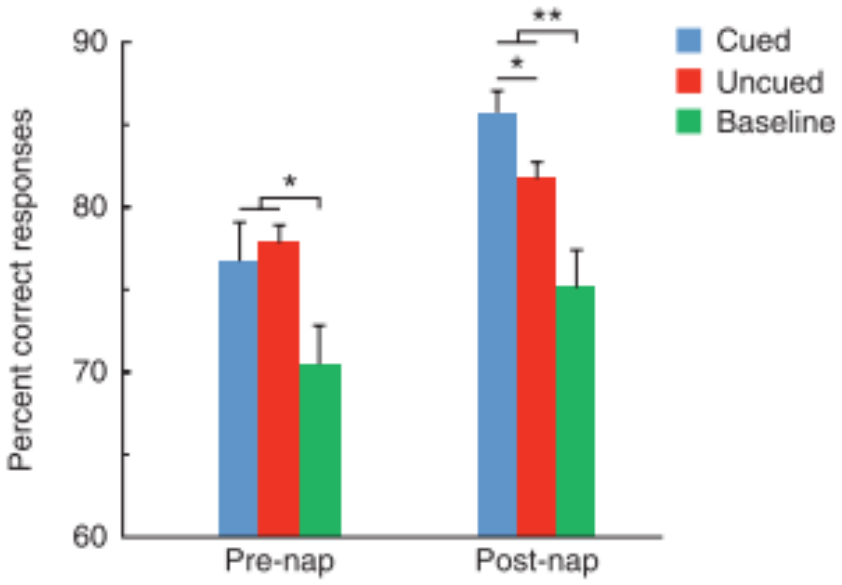
Procedure



Participants were first trained on both sequences, after which one of the sequences was cued during a nap, and after nap participants were tested on both sequences, as well as a control (baseline) sequence (n =16). This procedure was replicated without TMR cueing during nap for the sham group (n = 16).

Figure 3.

Behavioural Results (Original Study)



Post-nap accuracy scores (percent correct responses) for the melody performance task were significantly higher for the cued condition than the uncued, both of which were significantly greater than the baseline (control) accuracy scores (from Antony et al., 2012).

Current Data Analysis

For each participant in the TMR group (*n*=16), epochs of 8.75 seconds were extracted timelocked to the onset of the first tone in a sequence, (hereafter referred to as "stimulation" epochs; mean number of epochs per participant = 21, SD = 3.3, range = 17 to 26). Each note in a sequence was played for approximately 300 ms, such that each stimulation epoch consisted of 6.3 sec of auditory stimulation followed by 2.45 sec of silence. From the remaining EEG data for each participant, corresponding "control" epochs with the same duration of 8.75 seconds silence (periods of no auditory stimulation) of stage N2 and stage N3 sleep were manually identified and extracted (mean number of epochs = 19, SD = 5.2, range = 14 to 32). The proportion of epochs corresponding to N2 versus N3 sleep was not statistically different between the stimulation and control epochs (Chi Square = 0.31, p = .58; mean number of stimulation N2 epochs = 5.12; mean number of stimulation N3 epochs = 16.51; mean number of control N2 epochs = 3.82; mean number of control N3 epochs = 15.24).

For participants in the control group (n = 16), who did not receive auditory cueing during sleep, "sham" epochs were extracted, corresponding to stage N2 and N3 sleep (mean number of epochs = 78, SD = 23, range = 43 to 113), using the sleep staging indicated by Antony et al. (2012) to maintain reliability and consistency between studies. The time when sleep cues would have been presented (had the participant been assigned to the TMR group) was estimated by using a yoking procedure. For each participant in the TMR group, I computed the lag time from the onset of SWS to the first sequence presentation time and applied this lag to a yoked participant in the control group. The higher number of overall epochs in the sham group when compared to the TMR group is

due to a recording restriction, as the spaces between cued sequences were not long enough to extract control epochs from. As the sham group received no sounds stimulation, there was no such restriction for this group, this allowed us to extract more control epochs from the sham recordings.

Next, sleep spindles were detected during NREM sleep based on amplitude-duration thresholding using the following steps: (1) filtering the EEG with a 12–15 Hz bandpass filter, (2) calculating the root mean square (RMS) of each 100 msec interval of the filtered signal, (3) counting the number of times the RMS power crossed a constant detection threshold of 10 μV for 0.5–3 seconds, marking any time point with a spindle at any channel as 1 (spindle present = true) or 0 (spindle present = false) (Gais & Born, 2004; Warby et al., 2014). The Boolean output of this amplitude-duration thresholding step was then verified using a second step, which involves measuring the signal-to-noise ratio (SNR) of power in the bandpass (9–18 Hz, 8th-order Butterworth filter) against the bandstop (9–18 Hz) signal (Muller et al., 2016). The SNR metric is calculated on 500 ms sliding windows in each channel. When the SNR metric reaches 0 dB, the signal and noise power are equal. A static SNR threshold of 5 dB was used to verify spindle occurrence. This method provides a conservative technique for identifying epochs in multichannel data that is relatively unaffected by amplitude.

Boolean spindle detection for both stimulation and control epochs were concatenated and averaged across timepoints and across epochs at the individual level, and then averaged across participants. This produced an average proportion epoch, representing the average Boolean spindle detection across participants at each time point (hypothetical range = 0 to 1, with 0 indicating no spindles in any channels at that time

point for any participant and 1 indicating a spindle in at least one channel at that time point for every participant). These data were then smoothed using MATLAB's *smoothdata* function (local moving average) using a 50 time-point window (corresponding to 200 ms of EEG data).

TMR Group: Within-Subject Statistical Analysis

For participants in the TMR group, for each time point, I tested whether there was a significant difference between spindle proportion of the stimulation epochs and control epochs, using a running two-tailed paired-sample t-test between the stimulation proportional average epoch (all TMR epochs for a given participant's recording) and their non-stimulation proportional average epoch, corrected for multiple comparisons using False Discovery Rate (FDR). Continuous periods of ten or more time points in which the difference between epochs showed an FDR-corrected p-value < .05 were considered to be statistically significant sections (Vanden Bosch der Nederlanden et al., 2016).

TMR Group: Correlational Analysis

A single behavioural measure of cueing-related change in performance was assessed by first calculating the difference between pre- and post-accuracy scores separately for cued and uncued trials, and then calculating the difference (cued vs. uncued) of these difference scores (i.e., [cued post-accuracy – cued pre-accuracy] – [uncued post-accuracy – uncued pre-accuracy]; hereafter referred to as "***behavioural cueing index***"). Positive values on this measure indicate a greater boost in performance for the cued trials than uncued after the nap period (i.e., a cueing benefit), whereas negative values indicate a greater boost in performance for uncued trials relative to cued trials. To investigate the potential relation between spindle density and behavioural

performance, mean spindle count difference per epoch ([mean spindle count in cued epochs] – [mean spindle count in uncued epochs]), for each individual was correlated with the behavioural cueing index at the individual level by computing Pearson's product-moment correlations.

TMR Group Versus Sham Group: Between-Subjects Analysis

To test for differences in spindle count between groups, as in the within-subject analysis, statistical significance was determined using two-tailed independent-samples t-tests across all time points between the stimulation grand average epoch and the sham grand average epoch, which we corrected for multiple comparisons using False Discovery Rate (FDR). Again, continuous periods of ten or more time points in which the difference between epochs showed a p-value < .05 were regarded as statistically significant sections (Vanden Bosch der Nederlanden et al., 2016).

Sham Group: Correlational Analysis

To investigate the potential relation between spindle density and behavioural performance, mean spindle quantity was correlated with overall accuracy change (pre- to post- nap) scores by computing a Pearson's product-moment correlation.

Results

Results of Previous Study

As reported by Antony et al. (2012), pre- to post-nap change in accuracy for the cued sequence was greater than performance for the uncued sequence (cued, 7.9 ± 2.4%; uncued, 2.6 ± 1.5%; p = .02; Antony et al., 2012). Further, accuracy differences correlated with the percentage of time spent in SWS, as well as spindle activity during SWS at the F4 electrode, which is located over premotor cortex. The authors therefore suggested that this spindle correlation may indicate memory integration in the premotor cortex.

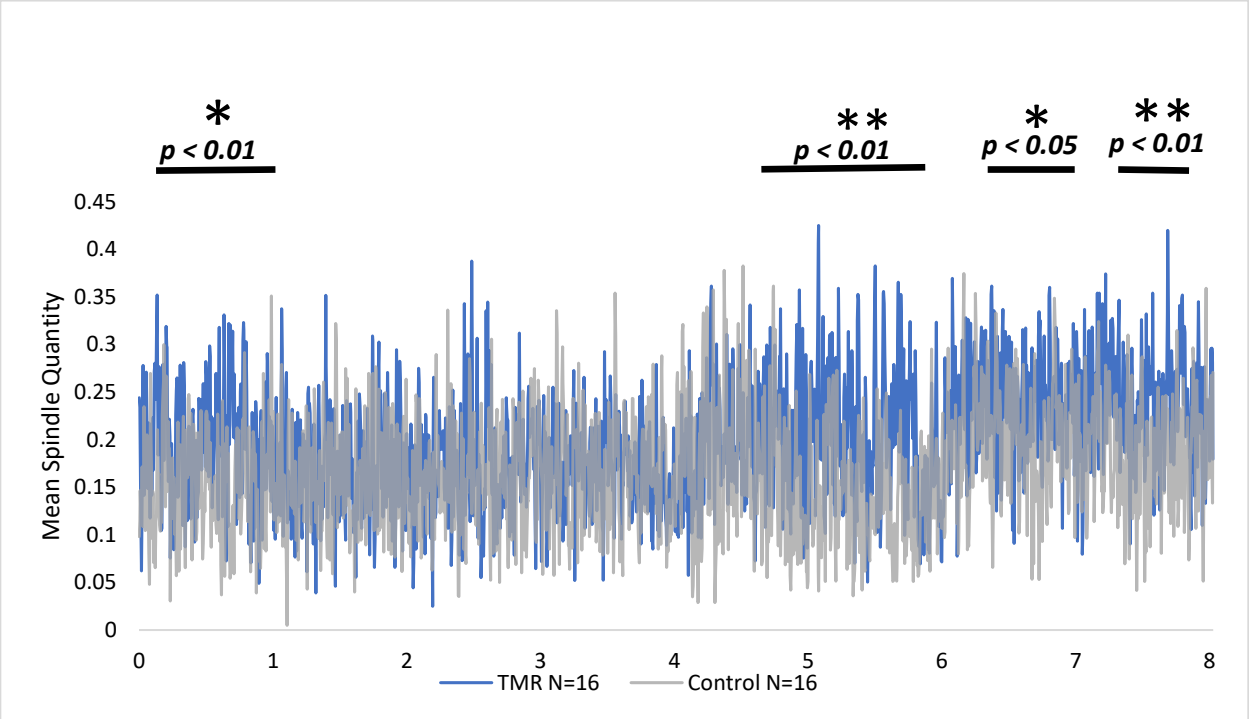
Current Study

TMR Group: Within-Subject Results

A significantly greater number of spindles were found in the TMR epochs compared to the control epochs from 0.17 to 0.96 seconds after onset of the first stimulus note, as well as in three later time intervals (4.62 to 5.71, 6.28 to 6.97, and 7.19 to 7.71 seconds relative to stimulation epoch onset; Figure 4). In contrast, there were no time windows that showed the reverse effect, in which the control epochs showed a significantly greater number of spindles than the TMR condition, using the 10 continuous time point criterion (Vanden Bosch der Nederlanden et al., 2016). However, when averaged across the entire epoch, overall spindle density between the epochs did not significantly differ (mean number of spindles for cued condition = 0.731, SD = 0.352; mean number for uncued = 0.705, SD = 0.461; $t = 1.124$, $p = .17$).

Figure 4.

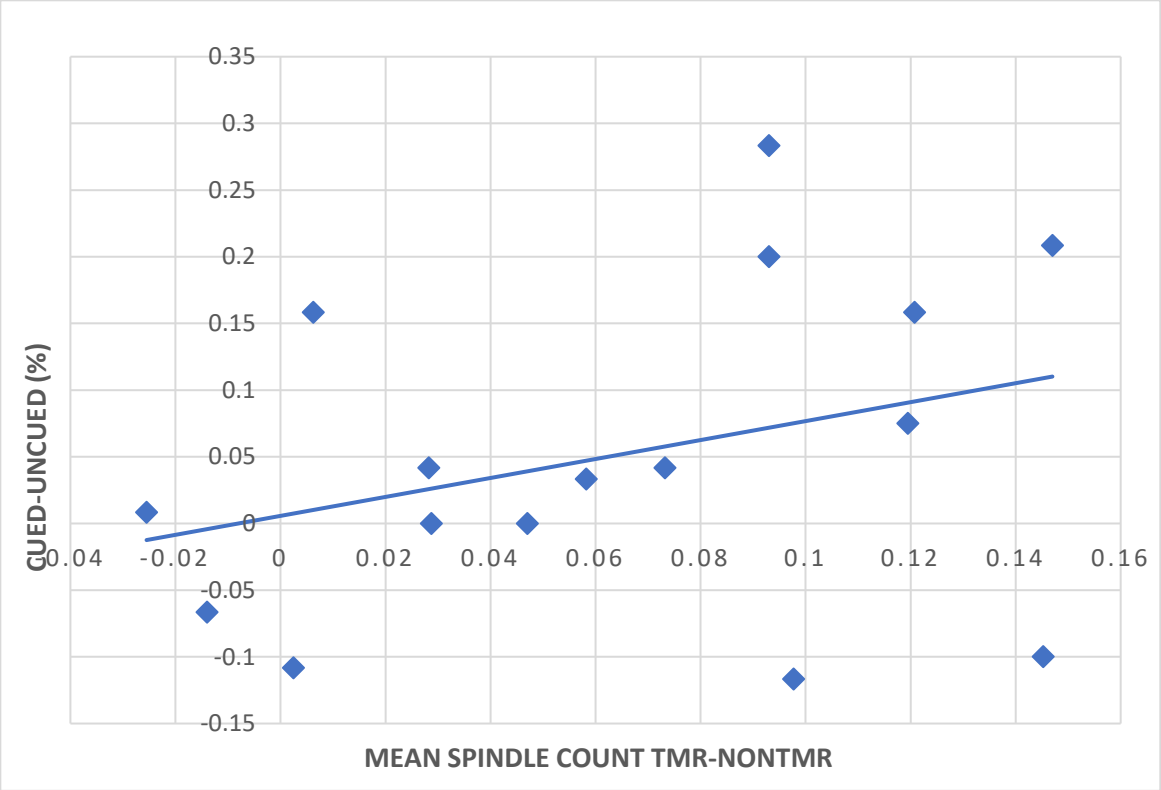
Mean Spindle Proportion - TMR vs. Control (Within Subject)



In contrast to our hypothesis, the behavioural cueing index did not significantly correlate with spindle quantity differences between TMR and control epochs, across the entire epoch (r = .196, *p* = .465; Figure 5).

Figure 5.

Spindle Count vs Cued-Uncued Accuracy - TMR Condition

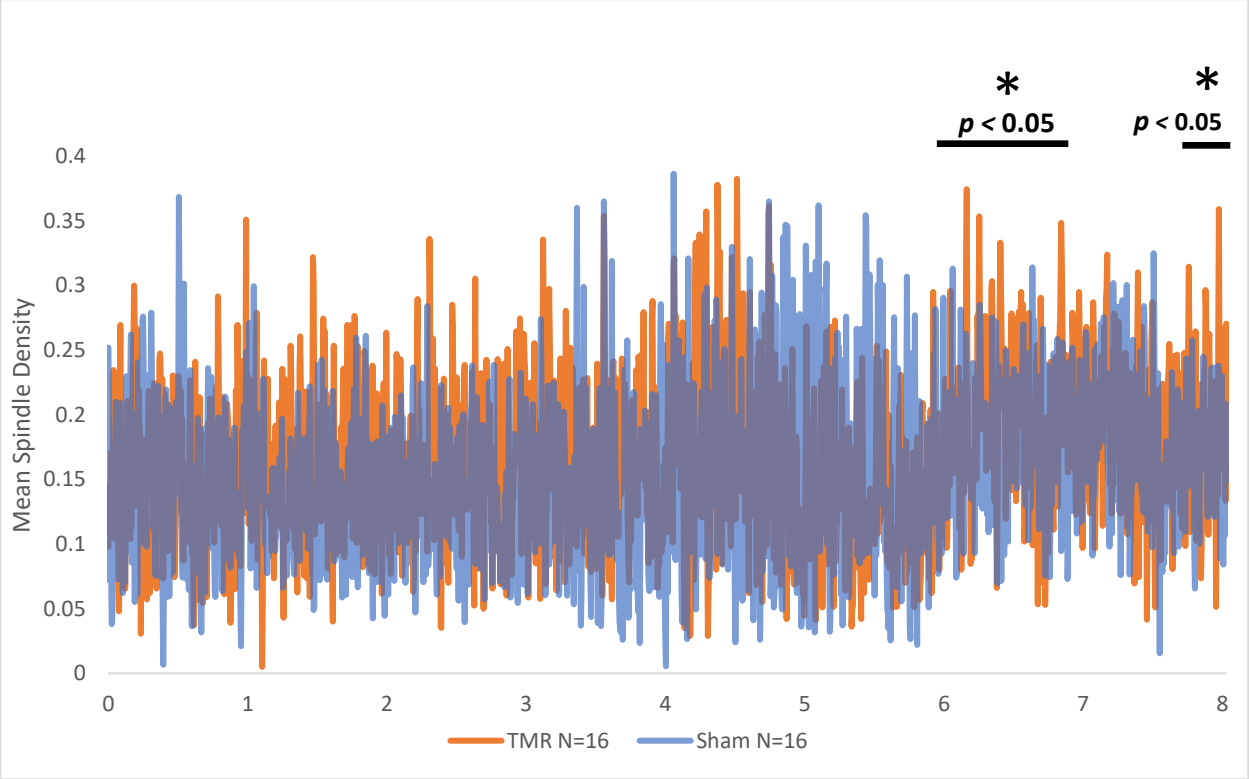


TMR Group Versus Sham Group: Between-Subjects Results

A significantly greater number of spindles were found in the TMR group compared to the sham group from 5.94 to 6.86 seconds, and 7.73 to 8.75 seconds directly after stimulus onset (Figure 6). There were no windows that showed the reverse effect, in which the sham group showed a significantly greater number of spindles than the TMR group, using the 10 continuous time point criterion (Vanden Bosch der Nederlanden et al., 2016). Averaged across the entire epochs, overall spindle density between the epochs did not significantly differ (mean number of spindles for TMR group = 0.731, SD = 0.352; mean number for sham group = 0.78, SD = 0.547; $t = 1.273$, $p = .113$).

Figure 6.

Mean Spindle Proportion - TMR Group vs. Sham Group (Between Subjects)



Sham Group: Correlational Results

In the sham group, mean spindle quantity significantly correlated with pre-to-post nap change in accuracy (post-nap minus pre-nap baseline; r = .507, *p* = .045, Figure 5), consistent with previous findings (Diekelmann et al., 2009; Ellenbogen, Hulbert, Stickgold, Dinges, & Thompson-Schill, 2006; Gais & Born, 2004; Muehlroth et al., 2019). In contrast, in the TMR group, mean spindle quantity did not significantly correlate with pre- to post- nap change in accuracy performance (final performance minus baseline; *r* = .136, *p* = .617). However, a Fisher Z-test revealed no significant difference between correlations (*z* = 1.075, *p* = .141).

Figure 7.

Spindle Count vs Overall Performance Accuracy - Sham Group

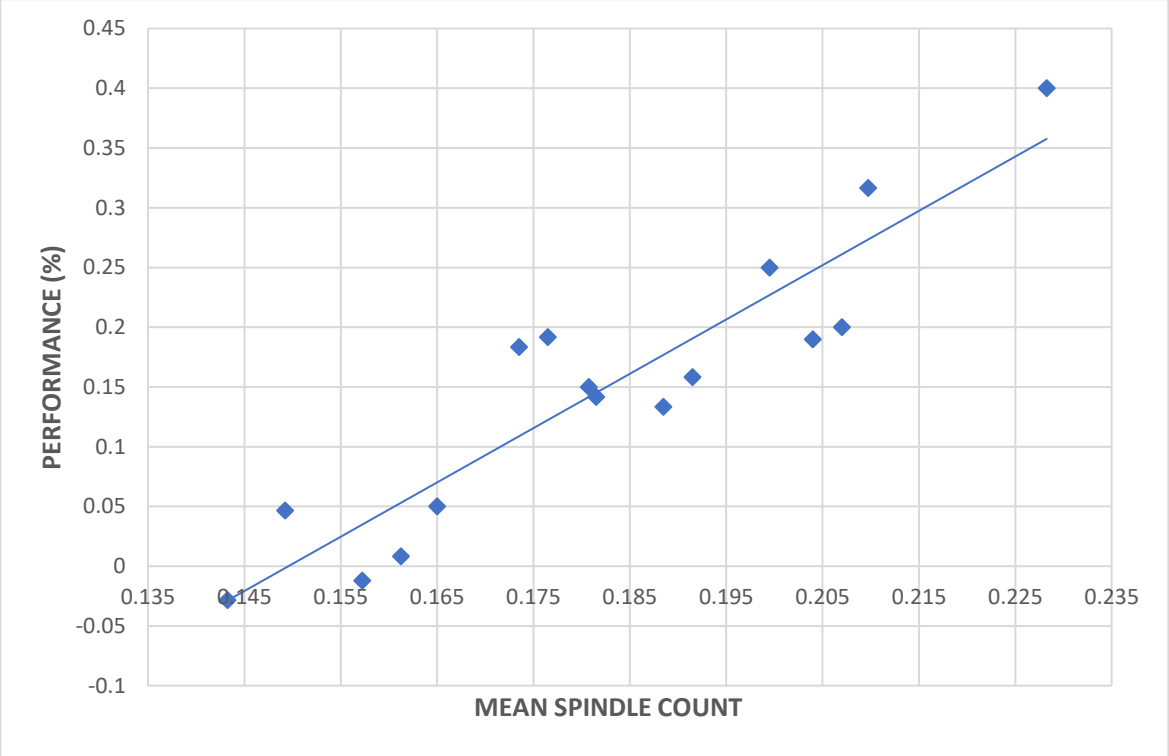
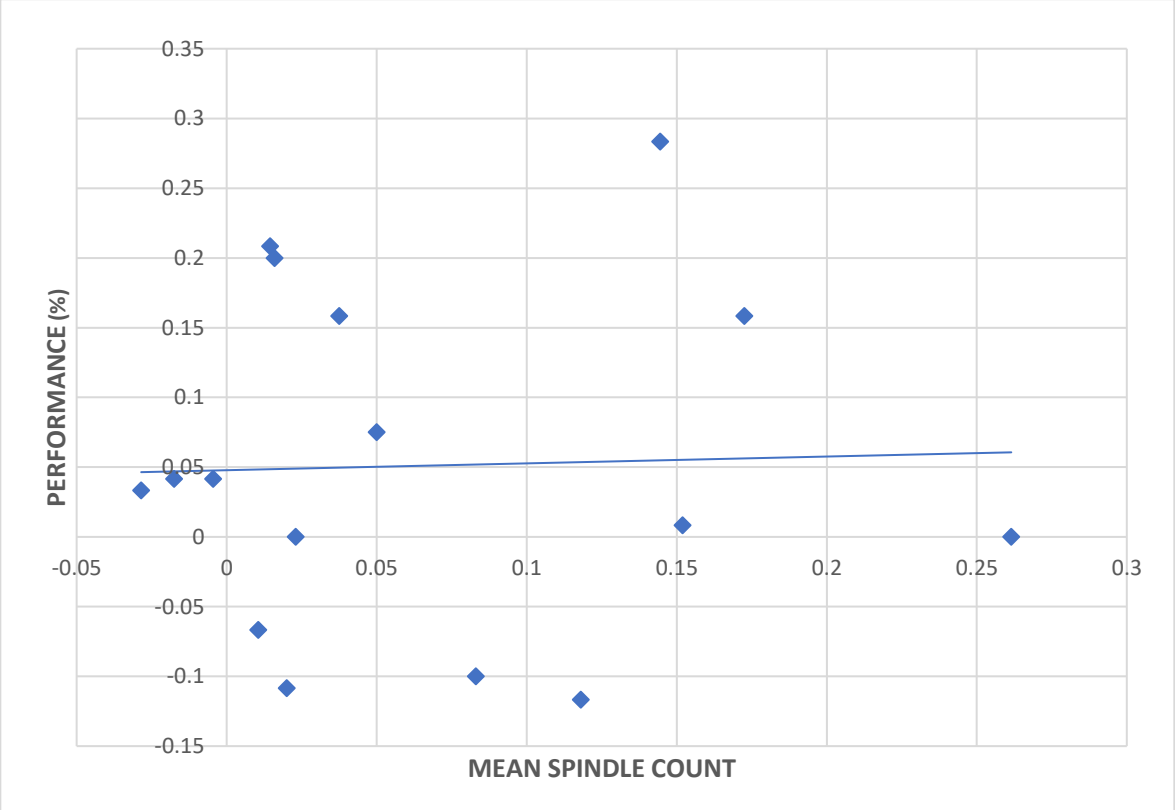


Figure 8.

Spindle Count vs Overall Performance Accuracy - TMR Condition



Discussion

The purpose of the current study was to identify spindle occurrence because of TMR and endogenous spindle occurrence during non-TMR periods, and to further establish whether TMR cues (and particularly continuous TMR cues) enhance spindle activity, thus investigating how TMR effects operate by the hypothesized mechanism of inducing memory reactivation. To achieve this aim, we examined whether TMR cues are more likely to elicit spindles than periods without cueing and investigated the relationship between spindle count during continuous TMR stimulation and memory performance. We also aimed to characterize the temporal dynamics of any elicited spindles relative to a continuous memory-related sequence.

Our hypothesis was that the mean sleep spindle count would be higher during periods of TMR stimulation than periods of non-stimulation. The hypothesis was partially supported. Within participants, although overall spindle count was not statistically higher than the control epochs, there were several time periods within the TMR epochs where spindle counts significantly exceeded those in the control epochs. There was no time when the control condition spindle count exceeded the TMR condition spindle count. While this did not correspond specifically to our hypothesis, it is indicative of a potentially quite complex dynamic of spindle occurrence relative to TMR cues.

Previous TMR studies using odor and discrete-sound cues found a TMR-related increase in spindle density, with increases predicting subsequent memory performance (Creery et al., 2015; Oyarzún et al., 2017). This also been demonstrated in other continuous auditory TMR paradigms (Shimizu et al., 2018, Gao et al., 2020). In one

paradigm, participants listened to classical music while taking a virtual lecture on microeconomics. During a subsequent nap, roughly half of the participants were presented with the music that they had heard during the lecture. The control participants were presented with white noise of the same duration. The study showed significantly better next-day performance for those participants that had received TMR cueing, along with significantly greater frontal theta activity (filtering with sleep spindle parameters) during the nap in contrast to the control group (Gao et al., 2020). The continuous nature of the stimuli as well as the overall long duration when compared to more traditional discrete auditory TMR paradigms indicates that the process of TMR cueing can elicit the same neural patterns without a strict restriction for short cues. There have been yet no studies in continuous paradigms showing time-based analyses relative to cue onset, as have been shown in the current study.

We also hypothesized that, across individuals, higher mean spindle count for TMR epochs would predict improvements in cued sequence accuracy, as assessed through behavioural pre- and post- measures. This hypothesis was not supported by the data; there was no significant correlation between cued sequence accuracy and spindle count in the TMR condition ($r = 0.196$, $p = 0.465$). In contrast, the sham group showed a significant positive correlation between spindle count and memory performance. This result is consistent with previous studies that have found a positive relationship between spindle density and subsequent memory (Diekelmann et al., 2009; Ellenbogen, Hulbert, Stickgold, Dinges, & Thompson-Schill, 2006; Gais & Born, 2004; Muehlroth et al., 2019).

This significant correlation between spindle count and overall performance was not present in the TMR group, nor did *overall* spindle density show any significant differences between TMR and control epochs (within-subject) or TMR and sham groups (between-subjects). This was not expected, considering that prior work suggests that TMR should increase spindle activity (Clemens et al., 2013; Staresina, Bergmann, Bonnefond, & Der, 2016). However, differences were only revealed when using a more fine-grained approach to parse spindle occurrence. There are a few possible explanations for the lack of significant difference in overall spindle count. Considering the length of the window together with the finding that spindle count in the TMR epochs is higher than the control epochs at several timepoints, it is possible that the increases in spindle count at certain times resulted in decreases in other time windows, leaving the overall net spindle density unchanged. As mentioned earlier, sleep spindles range from 0.5-1.5 seconds in duration on average and have a refractory period of 3-6 seconds (Cox, Hofman, & Talamini, 2012; Antony et al., 2018). Therefore, it is likely that only one spindle may be expressed within the 8.75 second window. Auditory stimulation may have influenced any potential oscillatory pattern of spindle production, but a longer post-stimulus window would be helpful to determine this. One major limitation of this study is that, because the data was collected for the purposes of different analyses, the period of time between stimulus windows was not long enough to capture two spindle occurrences without interference from the next TMR stimulus onset.

Future work is required to determine the nature of the changes in spindle activity after continuous auditory TMR stimulation. To evaluate endogenous persistence of TMR-induced changes or to identify any larger scale oscillatory patterns in spindle

occurrence, longer periods of time between stimulation windows would provide important information. Similarly, longer periods of stimulation (~15-16 seconds) would allow for at least two spindles to propagate, considering the longest estimates for both spindle duration and refractoriness. Finally, in the current study, the control periods were quite limited in time, as control periods could only be drawn from between TMR periods during SWS (which is a small subset of the nap recording period). A within-subjects design in which participants experience a control nap and a TMR nap would allow for more and better-quality control data, with longer control data windows that would not be influenced by any carryover effects of TMR stimulation. One area for future work is to determine what is driving the small, significant periods of greater sleep spindles in the TMR condition when compared to both the control epochs within-subject and the sham groups between-subjects. Is it systematic and replicable with similar paradigms?

This study provides evidence for the value of a more sophisticated metric than simply spindle count when investigating continuous cues for TMR in the auditory domain. The presence of a strong correlation between spindle count and memory accuracy for the sham group but not for the TMR condition suggests the occurrence of something more complex than a uniform increase in spindle count across the TMR window. The periods of time that were marked as significant, most of which are later in the TMR window, also suggest that there is a longer delay between TMR stimulation and spindle occurrence than observed in response to shorter duration TMR cues. Most studies at present evaluating continuous TMR cues have not examined the temporal dynamics of spindle count in a similar way to this study, and more investigation on spindle occurrence relative to cue onset in recent studies would provide insight on any potential consistency

in this construct across studies. Prior work has indicated the potential co-ordinating role of sleep spindles in synchronizing neural activity during sleep, in turn facilitating memory reintegration (Clemens et al., 2013; Muehlroth et al., 2019; Staresina, Bergmann, Bonnefond, & Der, 2016). The proximity of stimuli to one another may end up disrupting oscillatory patterns in the same way that the introduction of a secondary auditory stimulus has been shown to (Schreiner, Lehmann, & Rasch, 2015).

The Active Systems Model of consolidation proposes that slow oscillations drive the occurrence of hippocampal memory replay, together with sharp-wave ripple events in the hippocampus, and sleep spindles throughout SWS. As a result, sharp-wave ripples and reactivated memory information are thought to become nested into oscillatory troughs of the spindle, which is collectively fed back to the neocortex in the form of a spindle-ripple event, thereby facilitating the transfer of hippocampal-dependent memories to neocortical sites for long-term storage (Rasch & Born, 2013). The findings of this study, specifically, the within-subject finding that a significantly greater number of spindles were found in the TMR epochs than the control epochs in several periods may be evidence for an enhanced consolidation process, in which TMR introduces hippocampal replays, prompting an enhanced sequence of spindle-ripple events. Consequently, because of TMR, newly assimilated memories may swiftly become autonomous of the hippocampus during SWS, and more efficiently become stable within neocortical memory systems. While not conclusive, this study provides evidence for a systematic difference between spindles that occur spontaneously and those that occur relative to TMR stimulation, in that spindles that occur relative to TMR cueing show short, specific, later-going increases in concentration, when compared within-subject and between

groups. The mechanistic difference is not entirely clear, but the evidence points to differences in spindle production as one of the potential causes of enhanced memory performance.

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Curriculum Vitae

Name:	Justin Hopper
Post-secondary Education and Degrees:	Bishop's University Lennoxville, Quebec, Canada 2011-2016 H. B.A. George Brown College Toronto, Ontario, Canada 2017-2018 Counselling Cert. The University of Western Ontario London, Ontario, Canada 2018-Present M.Sc.
Honours and Awards:	B.E.S.T. Grant Undergraduate Scholarship 2015 Stuart A. Cobbett Scholarship Undergraduate Scholarship 2012-2014
Related Work Experience	Teaching Assistant The University of Western Ontario 2018-Present Teaching Assistant Bishop's University 2014-2016

Presentations:

Hopper, J., Batterink L., & Grahn, J. A. (2020, May). Does musical stimulation during slow-wave sleep potentiate slow oscillations and associated declarative memory performance?

Neuromusic, Aarhus, Denmark – Cancelled due to Covid-19.

Hopper, J., Batterink L., & Grahn, J. A. (2019, June). Does musical stimulation during slow-wave sleep potentiate slow oscillations and associated declarative memory performance?

Rhythm Production and Perception Workshop (RPPW), Traverse City, Michigan.

Hopper, J., Drumheller A., & Mendrek, A. (2016, May). The effect of musical auditory stimulation on sleep latency and quality.

Eastern Townships Social Sciences Convention, Magog, Quebec.

Hopper, J., Drumheller A., & Mendrek, A. (2015, November). Does auditory intervention during sleep affect sleep architecture?

CPAC Conference, Ottawa.