MECHANISMS OF ASSOCIATIVE MEMORY CONSOLIDATION DURING SLEEP

by

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Abstract

How are transient memories transformed into lasting ones? While previous research has established the significance of sleep for consolidating memories, the intricate brain mechanisms underlying sleep-dependent memory consolidation are yet to be explored. This thesis investigates the mechanistic role of two cardinal brain oscillations during sleep, sleep spindles and slow oscillations, for consolidating associative memories.

In a first study, a new memory paradigm operationalising multiple aspects of memories, precisely temporal and spatial features, is introduced. The results indicate that the paradigm indeed captures memory aspects that are consolidated during sleep. By combining this paradigm with electrophysiological brain recordings, a second study demonstrates that sleep spindles are most pronounced over learning-related cortical areas. The extent to which spindles track these learning-related cortical areas predicts behavioural measures of memory consolidation. Thereby, the second study provides evidence supporting a mechanistic function of sleep spindles for memory consolidation. That is, sleep spindles specifically occur in encoding relevant cortical areas to facilitate consolidation, presumably by inducing long-lasting changes (*plasticity*) in these areas.

In a third and fourth study, the interplay between the two cardinal sleep oscillations (sleep spindles and slow oscillations) and *memory reactivation* is investigated. Besides inducing plasticity, memory reactivation has been suggested as a potential mechanism underlying sleep-dependent memory consolidation. In the third study, we tested for a synchronisation of sequential memory reactivation by slow oscillations. To this end, we employed a sequential memory paradigm together with novel analysis techniques enabling the tracking of sequential memory reactivation. Results represent first evidence of sequential

memory reactivation in humans and support the hypothesis that reactivation of sequential memories is synchronised by slow oscillations. Applying the same analysis techniques in a fourth study together with an associative memory paradigm, the importance of slow oscillation and sleep spindle coupling for memory reactivation has been tested. Results of study four reveal memory reactivation during slow oscillation-sleep spindle complexes and moreover, that the temporal precision of slow oscillation-sleep spindle coupling predicts memory reactivation strength. Study three and four corroborate a timing function of cardinal sleep oscillations in service of memory consolidation, suggesting the temporal coordination of memory reactivation as a potential mechanistic function of slow oscillations and slow oscillation-sleep spindle complexes.

The final chapter provides a contextualised overview of the work and discusses the interplay between brain oscillations during sleep and the proposed mechanisms, induction of plasticity and memory reactivation. Together, this thesis provides further insights into the mechanisms subserving associative memory consolidation during sleep.

To Mum & Dad

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Publications and Presentations

At the time of thesis submission, the following articles and conference contributions were derived from this doctoral research. For the thesis, published articles have been slightly adjusted to ensure consistency across chapters.

Articles. Published

Petzka, M., Charest, I., Balanos, G. M. & Staresina, B. P. (2021). Does sleep-dependent consolidation favour weak memories? *Cortex*, 134, 65-75. doi.org/10.1016/j.cortex.2020.10.005

Schreiner, T., **Petzka, M.**, Staudigl, T., & Staresina, B. P. (2021). Endogenous memory reactivation during sleep in humans is clocked by slow oscillation-spindle complexes. *Nature Communications*, 12(1), 1-10. doi.org/10.1038/s41467-021-23520-2

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Abbreviations

analysis of variance, ANOVA

confidence interval, CI

electrocorticography, ECoG

electrocorticography, EEG

electromyography, EMG

electrooculography, EOG

functional magnetic resonance imaging, fMRI

Independent component analysis, ICA

liner discriminant analysis, LDA

long term potentiation, LTP

long term depression, LTD

multivariate pattern analysis, MVPA

non-rapid eye movement, NREM

polysomnography, PSG

psychomotor vigilance task, PVT

slow oscillations, SOs

standard deviation, STD

standard error of the mean, SEM

slow wave sleep, SWS

rapid eye movement, REM

reaction time, RT

representation similarity analysis, RSA

targeted memory reactivation, TMR

total sleep time, TST

Chapter 1. General Introduction

Past events and previous experiences lie at the core of humans' personality. They influence actions and thoughts in the present and future, making the ability to remember them as important as experiencing them in the first place. One process enabling us to remember these past events is *memory consolidation* – a process describing the transition of newly made experiences into long-term memories (Müller & Pilzecker, 1900). While memory consolidation intermittently occurs during wakefulness (Wamsley, 2019), it is the period of sleep in which memory consolidation is significantly facilitated (Born & Wilhelm, 2012; Klinzing et al., 2019).

The first evidence for superior memory performance, i.e. less forgetting, following sleep compared to wake was demonstrated at the end of the 19th century (Ebbinghaus, 1885). In a series of studies, Ebbinghaus (1885) recorded his own memory performance for non-sense syllables across different time delays. Based on this, he derived forgetting curves, describing a non-linear decrease in memory performance as a function of time. Interestingly, forgetting was less pronounced when time was spent asleep rather than awake. The finding that forgetting is reduced across a retention interval filled with sleep has then been replicated with larger sample sizes many times (Heine, 1914; reviewed in Rasch & Born, 2013; Jenkins & Dallenbach, 1924).

These early findings have ignited a debate about *why* sleep compared to wake results in less forgetting, putting forth three different accounts. First, the decay theory has argued that forgetting is the consequence of a decline of neurobiological memory traces over time (Thorndike, 1913; reviewed in Rasch & Born, 2013). To then explain the differences in forgetting rates between sleep and wake, it was later argued that the decline happens at a slower rate during sleep. As the overall metabolism is slower during sleep, assumingly all neurobiological processes, including the decay of neurobiological memory

traces, slow down and result in less forgetting over a time interval filled with sleep compared to wakefulness (Ekstrand et al., 1977; Wixted, 2004). Second, the interference theory has explained forgetting as a consequence of learning new information which then retroactively interferes with old information, with negative effects on the ability to retrieve them. While new, interfering information is constantly processed and learned during wakefulness, no processing and learning of new, interfering information occurs during sleep leading to less forgetting (McGeoch, 1932). Both the decay as well as the interference theory have described sleep as a shelter which passively protects and merely postpones deterioration of newly formed memories due to decay or interference (Ekstrand et al., 1977). The third account (memory consolidation), however, has assigned a more active role to sleep. It postulated the occurrence of a physiological process during sleep which transforms newly formed memory traces from a labile into a stable, long lasting state (Born & Wilhelm, 2012; Ekstrand et al., 1977; Müller & Pilzecker, 1900).

Throughout the last century, empirical evidence has substantiated the memory consolidation account (Antony et al., 2019; Belal et al., 2018; Cairney, Guttesen, et al., 2018; Ji & Wilson, 2007; Rasch et al., 2007; Rudoy et al., 2009; Schreiner & Rasch, 2015; Skaggs & McNaughton, 1996; Wilson & McNaughton, 1994; Zhang et al., 2018), which also builds the theoretical framework of this thesis. Although it is widely accepted today *that* physiological processes explain the beneficial effects of sleep for memory, it is still elusive *which* and *how* memories are actually consolidated during sleep. In this thesis both questions are addressed. The first experimental chapter is a behavioural study focusing on the quality of memories being consolidated (*which*). The second, third and fourth experimental chapters describe studies in which brain data were recorded during

both wake and sleep. Together, these three chapters bundle empirical evidence for two main mechanisms underlying sleep-dependent memory consolidation (*how*).

This introduction offers an (non-exhaustive) overview of the current state of research targeting these two questions. Since the main body of the thesis primarily focuses on the mechanisms of sleep-dependent memory consolidation (chapter 3, 4 and 5) and only one study addressed the quality of memories being consolidated (chapter 2), the introduction is weighted accordingly.

1. *Which* memories are consolidated during sleep?

The first studies demonstrated a beneficial effect of sleep on consolidation of declarative memories (Ebbinghaus, 1885; Heine, 1914; Jenkins & Dallenbach, 1924). Declarative memory summarises the ability to consciously remember facts (semantic memory) and events (episodic memory, Tulving, 1972). Declarative memory further enables different facts and events to be associated with and set in relation to each other (Squire, 2004). The fundamental finding that sleep consolidates declarative memories has been conceptually replicated many times with a variety of different declarative memory tasks including wordlist and word pair learning (Abel et al., 2019; Bäuml et al., 2014; Denis, Schapiro, Poskanzer, Bursal, Charon, et al., 2020; Drosopoulos, Schulze, et al., 2007; Ellenbogen, Payne, et al., 2006; Plihal & Born, 1999; Schönauer et al., 2015), object-location/scene associations (Antony & Paller, 2018; Creery et al., 2015; Diekelmann et al., 2011, 2012; Noack et al., 2021; Talamini et al., 2008; van Dongen et al., 2012) and vocabulary

learning (Batterink et al., 2017; Henderson et al., 2012; Schreiner & Rasch, 2015).[1] However, most of these studies applied tasks operationalising either semantic memories (Abel et al., 2019; Bäuml et al., 2014; Henderson et al., 2012; Schreiner & Rasch, 2015) or some aspects of episodic memories, i.e. memory for temporal sequences (Drosopoulos, Windau, et al., 2007; Griessenberger et al., 2012; Wilhelm, Wagner, et al., 2011) or memory for spatial locations (Antony & Paller, 2018; Creery et al., 2015; Diekelmann et al., 2011, 2012; Noack et al., 2021; Talamini et al., 2008; van Dongen et al., 2012). Episodic memory, though, constitutes both the temporal sequence as well as the spatial location of elements (Tulving, 2002). To enable a simultaneous operationalisation of memory for temporal sequences and spatial locations, a new memory paradigm was developed and tested in chapter 2 and 3 (but see Rauchs et al., 2004; Weber, Wang, Born, & Inostroza, 2014 for other memory paradigms operationalising temporal sequences as well as spatial locations). Due to methodological reasons, in chapter 4 and 5, a simpler declarative memory task was used. Here, associations between stimuli had to be encoded. The general benefits of sleep for declarative memories are widely established, yet it is still an open discussion whether all declarative memories equally benefit from sleep or whether sleep preferentially consolidates some declarative memories over others. This discussion has commenced with findings showing that despite memories being stabilised across a period of sleep, some are still forgotten (Bäuml et al., 2014; Denis, Schapiro,

[1] Sleep-dependent consolidation is not limited to declarative memories and has reported for non-declarative, e.g. procedural, memories as well (King et al., 2019; Lutz, Wolf, Hübner, Born, & Rauss, 2018; Plihal & Born, 1999; Schönauer et al., 2015; for a review on sleep-dependent consolidation of motor memories see King, Hoedlmoser, Hirschauer, Dolfen, & Albouy, 2017). Nevertheless, as in all experimental chapters of this thesis declarative memory tasks has been used, the focus lies on sleep-dependent consolidation of declarative memories.

Poskanzer, Bursal, Charon, et al., 2020; Drosopoulos, Schulze, et al., 2007; P. Hu et al., 2006; Lo et al., 2014; Payne et al., 2012; Rauchs et al., 2011; Saletin et al., 2011; Wilhelm, Diekelmann, et al., 2011). According to these findings, not all memories seem to be equally consolidated during sleep and a potential selection process determines the fate of newly encoded memories. Recent studies argue for a preferred selection of weakly encoded memories for sleep-dependent memory consolidation (Denis, Schapiro, Poskanzer, Bursal, Charon, et al., 2020; Diekelmann et al., 2010; Drosopoulos, Schulze, et al., 2007; Schapiro et al., 2017; Sheth et al., 2012). Specifically, when memories are weakly encoded, memory performance after sleep is superior to memory performance after wake. When memories are encoded to a stronger extent, though, memory performance after sleep and wake are often similar. This observation is interpreted as sleep preferentially consolidating weakly encoded memories. The first experimental chapter uncovers an often overlooked confound when comparing sleep-dependent consolidation between weakly and strongly encoded memories, i.e., retrieval difficulty. Here, we first replicate the finding that weakly encoded memories are preferentially consolidated during sleep under normal retrieval conditions. Importantly though, we further demonstrate that, by manipulating the retrieval difficulty, consolidation is not limited to weakly encoded memories and emerges for strongly encoded memories as well. These results challenge the notion that primarily weakly encoded memories are consolidated and give important insights into the quality of memories being consolidated during sleep.

2. *How* are memories consolidated during sleep?

2.1. The sleeping brain oscillates

The sleeping brain oscillates on different time scales. First, across a whole night of sleep, sleep stages indicating different brain states alternate in a cyclic manner. In humans, four different sleep stages can be differentiated based on polysomnography (PSG), the simultaneous recordings of the electrophysiological signal of brain (electroencephalography, EEG), eyes (electrooculography, EOG) and muscles (electromyography, EMG). Sleep stage 1 (N1) constitutes the transition from wake to sleep and comprises 3-8% of total sleep time (TST). In that stage, the amplitude of the EEG signal becomes lower and the frequency slows down from alpha frequencies (8-13 Hz), predominant during wake with eyes closed, to theta frequencies (4-7 Hz). Sleep stage 2 (N2) covers 45-55% of total sleep time and is hallmarked by short oscillatory bursts between 9-15 Hz (sleep spindles) and high amplitude slower oscillations (<4 Hz). When the amplitude of the EEG signal increases and the overall signal becomes slower (<4 Hz), sleep stage 3 (N3) is reached (15-20% of total sleep time). Slow wave activity, the combination between delta waves (oscillations between 1-4 Hz) and slow oscillations (oscillations of <1 Hz), gives sleep stage 3 its alternative name: slow wave sleep (SWS). Even though faster oscillatory bursts (sleep spindles) occur during SWS, slow waves dominate. Sleep stage 1 – 3 can be summarised to non-rapid eye movement (NREM) sleep due to the lack of rapid eye movements (REM). Rapid eye movements are the unique and name-giving characteristic of the 4th sleep stage, REM sleep (20-25% of total sleep time). Besides these typical eye movements, REM sleep is characterised by a low

amplitude and faster EEG which resembles the wake EEG signal (Iber et al., 2007; Lee-Chiong, 2005).

Second, besides cycling through different sleep stages, the sleeping brain oscillates on a shorter time scale between excitation and inhibition of neuronal assemblies. During non-REM sleep, three oscillations are predominant: sleep spindles, slow oscillations (SOs) and hippocampal sharp-wave ripples. Sleep spindles are distinct events oscillating at a frequency of 9-15 Hz in a waxing and waning shape for 0.5-3 seconds. They are subdivided into two different types of spindles: Slow (9-12 Hz) and fast (12-15 Hz) sleep spindles. While the functional purpose of slow spindles is still elusive (Rasch & Born, 2013), fast sleep spindles have been repeatedly associated with memory consolidation (Gais et al., 2002; Holz et al., 2012; Mölle et al., 2011; Schabus et al., 2004). Fast sleep spindles are generated within a thalamo-cortical loop involving the transmission of bursts from the reticular nucleus to thalamo-cortical neurons and their back projection (Steriade et al., 1993). Slow oscillations, on the other side, are distributed alternations between hyperpolarization (down state) and depolarization (up state) of cortical neurons membrane potential. Up and down states fluctuate at a frequency of <1 Hz and lead to wide-ranging cortical silence during the down state and elevated cortical firing during the up state (Klinzing et al., 2019; Volgushev et al., 2006). While sleep spindles and slow oscillations can be recorded with EEG, hippocampal sharp-wave ripples are mainly prevalent using intracranial recordings. Hippocampal sharp-wave ripples consist of two oscillatory patterns: First, ripples which are high oscillatory bursts at ~80Hz and second, slower waves (i.e., sharp waves) below 4 Hz. While sharp-wave ripples comprise both oscillatory patterns, they can also occur in isolation (Buzsáki, 2015).

Sleep spindles, slow oscillations as well as ripples have each been associated with memory consolidation (Gais et al., 2002; Ngo et al., 2013; Schabus et al., 2004; van de Ven et al., 2016), yet theories and empirical findings argue for a synchronisation of the three oscillations to subserve memory consolidation (Diekelmann & Born, 2010; Helfrich et al., 2018; Klinzing et al., 2019; Mikutta et al., 2019; Muehlroth et al., 2019).

2.2. Synaptic & systems consolidation

As described earlier, the memory consolidation account postulates the occurrence of physiological processes which transform labile memory traces into stable representations (Müller & Pilzecker, 1900). These physiological processes take place locally at synapses (synaptic consolidation) as well as more globally across different brain areas (systems consolidation, Dudai, 2004).

Synaptic consolidation describes the activity-dependent remodelling of synapses and dendritic spines within neural circuits representing a memory trace. The activity-dependent remodelling of synapses can be differentiated between long-term potentiation (LTP) and long-term depression (LTD). LTP defines an increase in the synaptic transmission strength as the consequence of increased synaptic activity, whereas LTD reflects a decrease in the synaptic transmission strength due to a lack of synaptic activity (Bear & Malenka, 1994). Empirical evidence demonstrated a direct relationship between sleep spindles and LTP. In vitro, sleep spindle related spike trains have been shown to induce LTP in neocortical pyramidal cells (Rosanova & Ulrich, 2005). Sleep spindles have been furthermore linked to synaptic plasticity as they coincide with an increase in Ca^{2+} activity (Niethard et al., 2018; Seibt et al., 2017). A postsynaptic increase in Ca^{2+} activity triggers LTP (Neveu & Zucker, 1996) and sets early synaptic consolidation

processes in motion (Sejnowski & Destexhe, 2000). While rodent research suggests that sleep spindles, in general, induce synaptic plasticity in cortical neurons, it is still unknown whether the induction of synaptic plasticity particularly in learning-related areas is one mechanism by which sleep spindles reflect memory consolidation. In chapter 3, we tested the prediction that memory consolidation can be explained by sleep spindles being specifically expressed over learning-related cortical areas.

Consolidation not only takes place at the synapse, but also at the systems level. Systems consolidation entails a redistribution of memory representations from one brain system, the hippocampus, which acts as a temporary storage for newly encoded memory traces, to another brain system, the neocortex, where memories are stored for the long-term (Marr, 1971). A new episode experienced during wakefulness is initially encoded in both brain systems. While different areas of the neocortex host different aspects of an episode, for instance, a person in a specific location at a specific time, the hippocampus binds these aspects together into a unique memory trace (McClelland et al., 1995). During sleep, the memory trace is repeatedly reactivated in both systems. *Memory reactivation*, the re-occurrence of a neuronal activity pattern present during encoding, is the pre-requisite of consolidation (Rasch & Born, 2007). Due to repeated memory reactivation, the memory trace is redistributed from hippocampus to neocortex and transformed from a labile into a stable representation (active system consolidation hypothesis, Born & Wilhelm, 2012; Diekelmann & Born, 2010; Klinzing et al., 2019).

The communication between brain areas in general and between the hippocampus and neocortex in particular is enabled by oscillations and their interplay (Buzsáki, 1996). During sleep, neocortical slow oscillations orchestrate the communication between hippocampus and neocortex. The up state of slow oscillations drives the generation of

thalamic spindles which then propagate to the hippocampus to synchronise ripples in their troughs. It has been empirically shown that memory reactivation in the hippocampus is linked to sharp-wave ripples (van de Ven et al., 2016). This can be interpreted as spindles and slow oscillations signalling the hippocampus when to reactivate (Rothschild et al., 2017). Simultaneously, thalamic spindles also propagate to the neocortex synchronising hippocampal and neocortical reactivation and potentially inducing synaptic consolidation in encoding related areas (Klinzing et al., 2019).

Taken together, theories addressing systems consolidation suggest that memory reactivation in hippocampal and cortical sites is a fundamental mechanism of memory consolidation and further, that sleep oscillations (slow oscillations, spindles and ripples) synchronise memory reactivation within and across brain areas. Empirical evidence supporting these theories comes from animal as well as human research.

Animal research provided the first empirical evidence for memory reactivation in the hippocampus and neocortex. In a seminal paper, hippocampal cell activity was recorded during encoding and pre- and post-encoding sleep (Wilson & McNaughton, 1994). Intriguingly, hippocampal cells that were active while encoded a spatial content were re-activated during post-encoding sleep. Since the hippocampal activity pattern was absent during pre-encoding sleep, it was concluded that the activity pattern during encoding modulated the activity pattern during post-encoding sleep. In humans, indirect evidence of memory reactivation was firstly provided by findings demonstrating superior memory performance for memories which were auditorily or olfactorily cued during sleep (Rasch et al., 2007; Rudoy et al., 2009). For example, during encoding, participants associated objects with spatial locations. The presentation of objects at spatial locations was additionally accompanied by a semantically related sound (e.g., a cat was accompanied

by a “meow”) resulting in unique object-location-sound associations. During post-encoding sleep (N2/N3 sleep stages), some of the sounds were played without waking participants up. After sleep, participants were tested on their memory for the location of each object. Results revealed that memory performance for object-location pairs was higher when the associated sounds were presented (cued) during sleep compared to object-location pairs for which the sounds were not presented (non-cued) (Rudoy et al., 2009). Superior memory performance for cued memories can be explained by cued memories being reactivated in response to the sounds and therefore consolidated. The protocol of presenting sounds which were incorporated in previously encoded memories is referred to as targeted memory reactivation (TMR, Rudoy et al., 2009; Schreiner & Rasch, 2015). TMR has been extensively explored and is an established tool to increase memory consolidation (Cairney, Lindsay, Sobczak, Paller, & Gaskell, 2016; Creery et al., 2015; Rasch et al., 2007; Schreiner, Lehmann, & Rasch, 2015; Schreiner & Rasch, 2015; Wang et al., 2019; for a review see Oudiette & Paller, 2013; for a meta-analysis see Hu, Cheng, Chiu, & Paller, 2020).

However, direct evidence for memory reactivation in humans that goes beyond behavioural TMR findings is still scarce (but see Belal et al., 2018; Schönauer et al., 2017; Schreiner, Doeller, Jensen, Rasch, & Staudigl, 2018; Zhang et al., 2018). This can be explained, at least partly, by a longstanding lack of suitable methods to measure memory reactivation in humans. Now, recent developments of novel analysis techniques including multivariate pattern analysis (MVPA) provide the possibility to capture reactivation of memory traces in neuroimaging data (Grootswagers et al., 2017; Norman et al., 2006). Chapter 4 and 5 exploit these new methods to provide evidence for endogenous as well as induced (via TMR) reactivation. Further, in both chapters, reactivation and its interplay

with sleep oscillations is investigated. The analyses in both chapters share the same general approach: MVPA is applied to EEG data obtained during wakefulness and sleep. More precisely, a classification algorithm is trained on EEG wake data measured during a perception task to find a model that best differentiates between category-specific neural patterns (e.g., neural patterns during object vs. scene perception). Importantly, the memory task comprises only one of the categories (e.g., encoding of adjective-object pairs) which is hypothesised to be reactivated during post-encoding sleep. The model that differentiates between category-specific neural patterns (e.g., neural patterns during object vs. scene perception) is then tested on sleep data in such a way that above chance classification reflects neural activity during sleep which resembles one category-specific neural pattern more than the specific pattern of a different category. Evidence for reactivation is assumed if above-chance classification of the stimulus category that was encoded before sleep is observed during sleep.

3. Aims of this thesis

The aims of this thesis are twofold. First, the thesis investigates which memories are consolidated during sleep (chapter 2). According to recent studies, sleep-dependent consolidation processes favour different types of memories to a different extent. More precisely, weaker memories might benefit more from post-learning sleep than stronger memories. In chapter 2 we test the hypothesis that sleep-dependent benefits for weaker over stronger memories might be the consequence of how memories are tested in the experimental task design (i.e., which testing conditions are applied). That is, under standard testing conditions, sleep-dependent consolidation effects for stronger memories

might be obscured by ceiling effects. To test this hypothesis, we developed a new memory paradigm and systematically manipulated memory strength as well as the testing conditions (to push stronger memories away from ceiling). The results of chapter 2 have implications for future testing protocols and further the understanding of which memories are consolidated during sleep.

The second aim of this thesis is to investigate the mechanistic role of two cardinal brain oscillations during sleep, sleep spindles and slow oscillations, for consolidating associative memories. While both sleep oscillations have been associated with memory consolidation, the underlying mechanisms are often theoretically outlined but empirical evidence is scarce. In chapter 3, the same paradigm of chapter 2 in combination with electrophysiological brain recordings is used to investigate the mechanistic function of sleep spindles. While rodent research suggests that sleep spindles, in general, correlate with synaptic plasticity in cortical neurons (Niethard et al., 2021; Rosanova & Ulrich, 2005; Seibt et al., 2017), it is still unclear whether the induction of plasticity particularly in learning-related areas is one mechanism by which sleep spindles reflect memory consolidation. By testing the hypothesis that memory consolidation can be explained by an overlap between encoding and sleep spindle topographies (i.e., reflecting the tracking of learning-related areas by sleep spindles), chapter 3 provides important insights into potential underlying mechanisms of how sleep spindles subserve consolidation.

In chapter 4 and 5, electrophysiological brain recordings are combined with multivariate pattern analysis to test the hypothesis that sleep oscillations orchestrate the reactivation of newly encoded memories during post-encoding sleep. While memory reactivation has been demonstrated before (Belal et al., 2018; Schönauer et al., 2017; Schreiner et al., 2018; Zhang et al., 2018), it is still unknown (i) how memory reactivation of sequential

memories evolves over time and (ii) how memory reactivation interplays with sleep oscillations, specifically with sleep spindles, slow oscillations and sleep spindles that are coupled to slow oscillations. Both points are addressed in chapter 4 and 5, respectively which makes those chapters to an important building block in identifying memory reactivation in humans and in understanding its synchronisation by sleep oscillations.

Chapter 2. Does sleep-dependent consolidation favour weak memories?

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Abstract

Sleep stabilizes newly acquired memories, a process referred to as memory consolidation. According to recent studies, sleep-dependent consolidation processes might be deployed to different extents for different types of memories. In particular, weaker memories might benefit more from post-learning sleep than stronger memories. However, under standard testing conditions, sleep-dependent consolidation effects for stronger memories might be obscured by ceiling effects. To test this possibility, we devised a new memory paradigm (Memory Arena) in which participants learned temporospatial arrangements of objects. Prior to a delay period spent either awake or asleep, training thresholds were controlled to yield weak or strong memory opportunities. After the delay period, retrieval difficulty was controlled via the presence or absence of a retroactive interference task. Under standard testing conditions (no interference), a sleep-dependent consolidation effect was indeed observed for weaker memory opportunities only. Critically though, with increased retrieval demands, sleep-dependent consolidation effects were seen for both weaker and stronger memory opportunities. These results suggest that all memories are consolidated during sleep, but that memories of different strengths require different testing conditions to unveil their benefit from post-learning sleep.

1. Introduction

How do fleeting experiences become long-term memories? Research has established the importance of post-learning sleep for the strengthening of recently acquired memories, a process referred to as memory consolidation (Diekelmann & Born, 2010; Jenkins & Dallenbach, 1924; Müller & Pilzecker, 1900; Rasch & Born, 2013). However, the principles governing sleep-dependent consolidation, i.e., superior memory retention after sleep compared to wake, are still poorly understood. Does post-learning sleep benefit all memories equally, or are particular types of memories prioritized for consolidation processes? Consistent with the latter scenario, evidence has accumulated in recent years for a somewhat selective sleep-dependent consolidation process.

On the one hand, a greater benefit from post-learning sleep has been shown for emotionally salient compared to neutral stimuli (P. Hu et al., 2006), for events with high compared to low future relevance (Wilhelm et al., 2011) and for items intended to be later remembered compared to items intended to be forgotten (Rauchs et al., 2011; Saletin et al., 2011). To the extent that emotional salience, high future relevance and the intention to remember entail deeper processing during encoding (Craik & Lockhart, 1972), these results suggest that sleep-dependent consolidation may prioritise stronger memories. Differential post-sleep memory outcomes might then result from a synaptic downregulation process during sleep through which weaker memories are pruned but stronger memories are preserved (Tononi & Cirelli, 2006).

On the other hand, there is evidence supporting the notion that sleep-dependent consolidation favours weaker memories. For instance, Bäuml, Holterman and Abel (2014) compared sleep-dependent consolidation of items that were restudied with items

that were retrieved during a practice period. As retrieval practice usually results in stronger memories than restudy ('testing effect', Roediger & Butler, 2011), their finding of restudied (i.e., weaker) and not retrieved (i.e., stronger) items showing a sleep-dependent consolidation effect suggests that weaker memories differentially benefit from sleep-dependent consolidation. Two other studies, also indirectly manipulating memory strength, came to the same conclusion. They have shown greater sleep-dependent consolidation effects for word pairs with low compared to high semantic relatedness (Lo et al., 2014; Payne et al., 2012), where low semantic relatedness typically yields weaker memories. Moreover, experimentally facilitating consolidation during sleep via targeted memory reactivation (Rasch, Büchel, Gais, & Born, 2007; Rudoy, Voss, Westerberg, & Paller, 2009; Schreiner & Rasch, 2015) has been demonstrated to be more effective for items less well remembered prior to sleep (i.e., weaker memories) (Cairney et al., 2016; Creery et al., 2015). Finally, one study directly manipulated pre-sleep memory strength, either by having participants learn some stimuli to a lower criterion than others, or by imposing a retroactive interference task immediately after learning. Again, results indicate greater sleep-dependent consolidation benefits for weaker than for stronger memories (Drosopoulos, Schulze, et al., 2007).

How can these different lines of results be reconciled? One possible explanation for the result of weaker memories being preferentially consolidated during sleep is a ceiling effect for stronger memories. That is, elevating the strength of pre-sleep memory traces beyond a certain threshold might conceal the retention benefit typically afforded by sleep. In other words, sleep possibly benefits both weaker and stronger memories, but different testing protocols (mitigating ceiling effects) are needed to uncover these benefits. One effective means to reduce the impact of ceiling effects is to retroactively weaken memory

traces through interference, thereby moving them away from ceiling. For instance, one study had participants learn word pairs to a 100% accuracy criterion (corresponding to rather strong pre-sleep memories) and applied retroactive interference immediately before the final (post-sleep) retrieval session (Ellenbogen, Hulbert, et al., 2006). This procedure indeed revealed a sleep-dependent consolidation effect, despite the initially high learning criterion. Critically though, that study did not vary pre-sleep memory strength, such that it is unclear whether sleep protects both weaker and stronger memories from retroactive interference.

In light of the extant findings, we hypothesised that both weaker and stronger memories might benefit from post-learning sleep, but that an increase in retrieval difficulty is needed to uncover sleep-dependent consolidation of stronger memories. To assess the beneficial effect of sleep-dependent consolidation on weaker and stronger memories as a function of retrieval difficulty within the same paradigm, we systematically manipulated (i) pre-sleep memory strength by varying a training threshold (providing weaker vs. stronger memory opportunities) and (ii) retrieval difficulty by inducing retroactive interference. To this end, we devised a new memory paradigm ('Memory Arena'), designed to capture de-novo learning of temporal and spatial aspects of episodic memory. Specifically, the Memory Arena paradigm has participants learn both the temporal and spatial position of 20 individual object images placed on a circle. Learning is in principle completed when all 20 objects are placed in the correct temporal order to their correct position (100% performance). Importantly though, memory strength can be experimentally controlled by terminating training at different performance levels. Retroactive interference was induced by having participants learn a new temporospatial arrangement of the same objects directly before the final retrieval.

Our first aim was to replicate the greater benefit of sleep-dependent consolidation for weaker relative to stronger memory opportunities (using a standard testing protocol without retroactive interference). Indeed, we found that weaker memory opportunities (training threshold of 1x50% accuracy) showed a sleep-dependent consolidation effect, whereas stronger memory opportunities (training threshold of 2x70% accuracy) did not. We then tested whether increased retrieval demands, i.e., the need to overcome retroactive interference, would yield a sleep-dependent consolidation effect for stronger memory opportunities as well. Intriguingly, this manipulation revealed sleep-dependent consolidation effects for both types of pre-sleep memories (weaker and stronger). These results suggest that post-learning sleep might benefit all memories, but that different testing conditions are differentially sensitive to unveiling consolidation of weaker vs. stronger memories.

2. Results

2.1. General

To capture both the temporal and spatial components of episodic memory we designed a new paradigm, called *Memory Arena* (see Figure 1 for the task design).

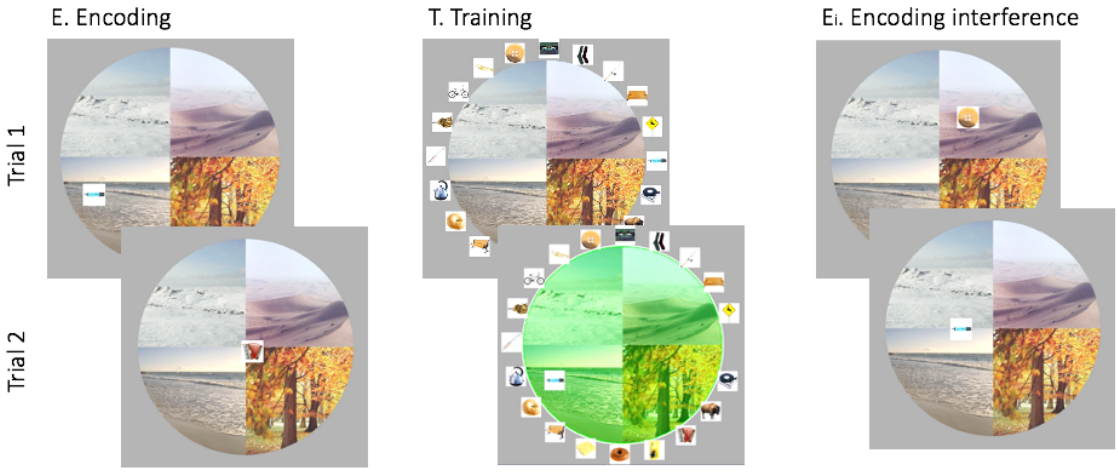

Figure 2.1. Task design. During encoding 20 objects were presented in the *Memory Arena*. After clicking on an object, the current object disappeared and the next object was shown. The training session started with all 20 objects randomly arranged around the arena. Objects had to be dragged and dropped in the correct sequence to the correct spatial position. Feedback was given after each trial and any errors were corrected. Interference was induced by encoding of the same objects but in a different sequence and at different spatial positions. Retrieval (not shown) followed the same procedure as training, but no feedback and correction were provided.

Our study design included the between-subjects factors Delay (sleep vs. wake), Memory Strength (weaker vs. stronger) and Retrieval Difficulty (no interference vs. interference) resulting in 8 conditions with 15 participants each (Figure 2).

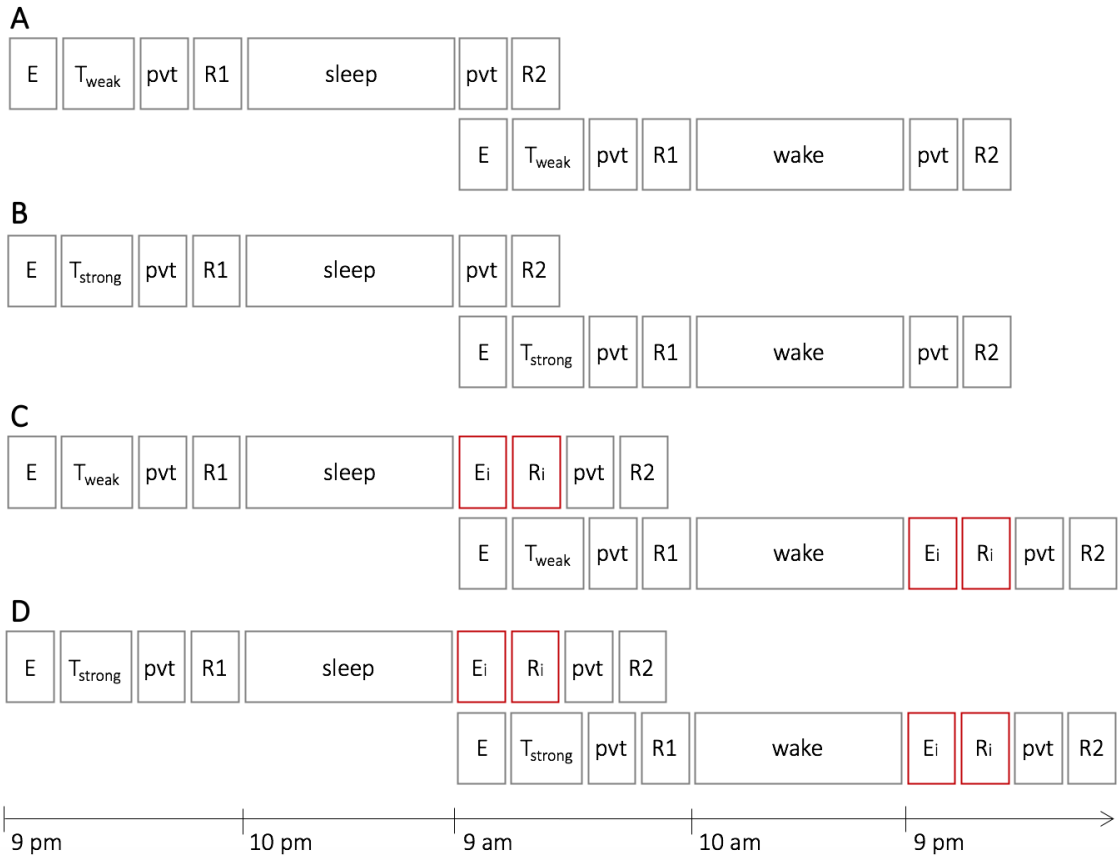

Figure 2.2. Study design. 120 participants were randomly assigned to one of 8 conditions (groups). All *sleep* groups performed the encoding, training and pre-delay retrieval in the evening and the post-delay retrieval 12 hours later in the morning. The *wake* groups performed the encoding, training and pre-delay retrieval in the morning and the post-delay retrieval 12 hours later in the evening. Besides the between-subjects factor Delay (sleep vs. wake), pre-sleep memory strength was manipulated via the training threshold (**A** and **C** for weaker memory opportunities and **B** and **D** for stronger memory opportunities). Additionally, half of the participants were given an interference task before the post-delay retrieval to increase retrieval difficulty (between-subjects factor Retrieval Difficulty, **A** and **B** for no interference, **C** and **D** for interference). E = encoding, T = training, pvt = Psychomotor Vigilance Task, R1 = pre-delay retrieval, R2 = post-delay retrieval, i = interference

First, we assessed whether the encoding strength manipulation (1x50% vs. 2x70% training performance) affected the training duration, quantified both in terms of training rounds required and total time spent to reach criterion, including the encoding part (see Supplemental Information, Table S1 for descriptive data). We conducted a 2x2x2

ANOVA with the between-subjects factors Delay, Memory Strength and Retrieval Difficulty and the training duration or the total number of training rounds as dependent variables. For strong memories (2x70% criterion), participants required significantly longer training time (main effect of Memory Strength: $F(1, 112) = 8.05$, $p = .005$, $\eta_p^2 = 0.07$, $mean_{2x70\%} = 1128.13$ sec, $95\%CI_{2x70\%} = 92.24$ sec, $mean_{1x50\%} = 909.85$ sec, $95\%CI_{1x50\%} = 120.61$ sec) and more training rounds (main effect of Memory Strength: $F(1, 112) = 15.58$, $p < .001$, $\eta_p^2 = 0.12$, $mean_{2x70\%} = 4.88$, $95\%CI_{2x70\%} = 0.49$, $mean_{1x50\%} = 3.22$, $95\%CI_{1x50\%} = 0.67$). The time of the day for training (evening for all sleep groups and morning for all wake groups) neither impacted training duration (main effect of Delay: $F(1, 112) = 0.03$, $p = .869$, $\eta_p^2 < 0.01$, $BF_{01} = 5.08$) nor the number of training rounds needed to reach the criterion (main effect of Delay: $F(1, 112) = 1.40$, $p = .239$, $\eta_p^2 = 0.01$, $BF_{01} = 2.89$). Likewise, there was no significant difference in training duration and number of training rounds between no interference and interference groups (duration: main effect of Retrieval Difficulty: $F(1, 112) = 0.02$, $p = .884$, $\eta_p^2 < 0.01$, $BF_{01} = 5.10$; rounds: main effect of Retrieval Difficulty: $F(1, 112) = 0.01$, $p = .937$, $\eta_p^2 < 0.01$, $BF_{01} = 5.13$).

Next, we identified one performance metric for all subsequent analyses. The *Memory Arena* paradigm yields two separate measures for memory performance, i.e., sequence memory and spatial memory. This allowed us to proceed with the memory measure most sensitive to our critical encoding strength manipulation (1x50% vs. 2x70%, pre-delay Memory Strength). At the same time, we wanted to ensure that pre-delay memory performance did not differ between sleep and wake groups (factor Delay) or between no interference and interference groups (factor Retrieval Difficulty). We thus compared sequence performance as well as spatial error at pre-delay retrieval in two separate 2x2x2

ANOVAs, each including the between-subjects factors Delay, Memory Strength and Retrieval Difficulty. As expected, the 2x70% training threshold led to better pre-delay retrieval performance than the 1x50% training threshold for both measures (main effect of Memory Strength for sequence performance: $F(1, 112) = 73.44$, $p < .001$, $\eta_p^2 = 0.40$; main effect of Memory Strength for spatial error: $F(1, 112) = 46.80$, $p < .001$, $\eta_p^2 = 0.29$). Critically though, the corresponding effect size was markedly higher for sequence ($\eta_p^2 = 0.40$) than for spatial memory performance ($\eta_p^2 = 0.29$). Consequently, we focused our subsequent analyses on sequence performance (but see Supplemental Information, Table S2 and Figure S1-S3, for analyses using spatial memory performance and Table S3 and Figure S4-S6 for analyses using overall memory performance). Importantly, neither Delay (sleep vs. wake) nor Retrieval Difficulty (no interference vs. interference) had a significant effect on sequence or spatial memory performance at pre-delay retrieval (all $F < 1.29$, all $p > .258$, all $BF_{01} > 3.36$), ensuring there were no other baseline (pre-delay) differences between groups.

To account for potential differences in attention between pre- and post-delay retrieval, we compared the number of attention lapses (reaction times > 500ms, Basner & Dinges, 2011) during the psychomotor vigilance task (PVT). Results showed that there was no significant change in the number of lapses in any of the conditions from pre to post-delay retrieval (all $t < 1.59$, all $p > .135$), ruling out fatigue as a confounding factor for our results.

Consolidation, i.e., the change in sequence memory performance from pre to post-delay retrieval, was calculated as a relative change. In the following, sequence consolidation denotes the performance during post-delay retrieval relative to pre-delay retrieval, meaning that values > 100% reflect an increase, values < 100% reflect a decrease and

values = 100% reflect a stabilization of sequence memory performance. Sleep-dependent consolidation is then defined as the differential consolidation effect for the sleep group compared to the corresponding wake group (factor Delay).

As a first analysis we conducted a 2x2x2 ANOVA with sequence consolidation as the dependent variable and Delay (sleep vs. wake), Memory Strength (weaker vs. stronger) and Retrieval Difficulty (no interference vs. interference) as between-subjects factors. Across all groups, post- relative to pre-delay performance was higher in sleep groups than in wake groups (main effect for Delay: $F(1,112) = 32.69$, $p < .001$, $\eta_p^2 = 0.23$) and lower for high retrieval difficulty in comparison to low retrieval difficulty (main effect for Retrieval Difficulty: $F(1,112) = 44.07$, $p < .001$, $\eta_p^2 = 0.28$). Neither the main effect for Memory Strength nor any of the two way interactions reached significance (all $F < 2.06$ all $p > .154$, all $BF_{01} > 2.95$). Critically though, we found a significant three way interaction ($F(1, 112) = 6.21$, $p = .014$, $\eta_p^2 = 0.05$), suggesting that sleep-dependent consolidation effects for weaker and stronger memory opportunities might differ as a function of retrieval difficulty. We thus conducted two sets of subsidiary ANOVAs: First, breaking up the factor Retrieval Difficulty, we conducted separate ANOVAs to test for sleep-dependent consolidation effects for weaker vs. stronger memory opportunities under standard testing conditions (no interference, see section 2.2.) and with an increase in retrieval difficulty (interference, see section 2.3.). Second, breaking up the factor Memory Strength, we conducted separate ANOVAs to assess sleep-dependent consolidation effects as a function of retrieval difficulty for weaker and stronger memory opportunities, respectively (see section 2.4.).

2.2. No interference: Only weaker memories show a sleep-dependent consolidation effect

To test whether weaker memory opportunities show a greater sleep-dependent consolidation effect than stronger memory opportunities under standard testing conditions, a 2x2 ANOVA with the between-subjects factors Delay (sleep vs. wake) and Memory Strength (weaker vs. stronger) was conducted on sequence consolidation for the *no interference* groups. Overall, sequence consolidation was significantly greater in the sleep groups than in the wake groups (main effect of Delay: $F(1,56) = 11.59$, $p = .001$, $\eta_p^2 = 0.17$). Interestingly though, this sleep-dependent consolidation effect was modulated by the initial memory strength (interaction of Delay x Strength: $F(1,56) = 5.78$, $p = .020$, $\eta_p^2 = 0.09$). Post hoc t-tests revealed that sequence consolidation did not significantly differ between the sleep and the wake group for stronger memories ($t(20.40) = 1.11$, $p = .28$, $d = 0.41$, $BF_{01} = 1.82$). However, for weaker memory opportunities, the sleep group showed significantly greater sequence consolidation than the wake group ($t(26.63) = 3.25$, $p = .003$, $d = 1.19$, Figure 3A). These results are consistent with the notion that sleep-dependent consolidation selectively benefits weaker memories.

As mentioned in the introduction, beneficial effects of sleep for weaker and not for stronger memories might result from stronger memories being at ceiling. Indeed, the distribution of pre-delay sequence performance for stronger memory opportunities significantly deviated from a normal distribution (assessed via Shapiro–Wilk tests) for the wake ($W = 0.56$, $p < .001$) and the sleep group ($W = 0.80$, $p = .004$) and was skewed towards high performance values (Figure 3B). Conversely, the distribution of pre-delay sequence performance for weaker memory opportunities did not significantly differ from

a normal distribution (wake group: $W = 0.95, p = .513$, sleep group: $W = 0.92, p = .229$). Under normal testing conditions (in the absence of retroactive interference), post-delay sequence performance for stronger memory opportunities was still at ceiling for both the wake ($W = 0.78, p = .002$) and the sleep group ($W = 0.85, p = .018$), thus likely to obscure any benefit of sleep for the consolidation of stronger memory opportunities (see Supplemental Information, Table S5 for Shapiro-Wilk tests of all distributions and Table S4 for t-tests of pre- vs. post-delay sequence performance).

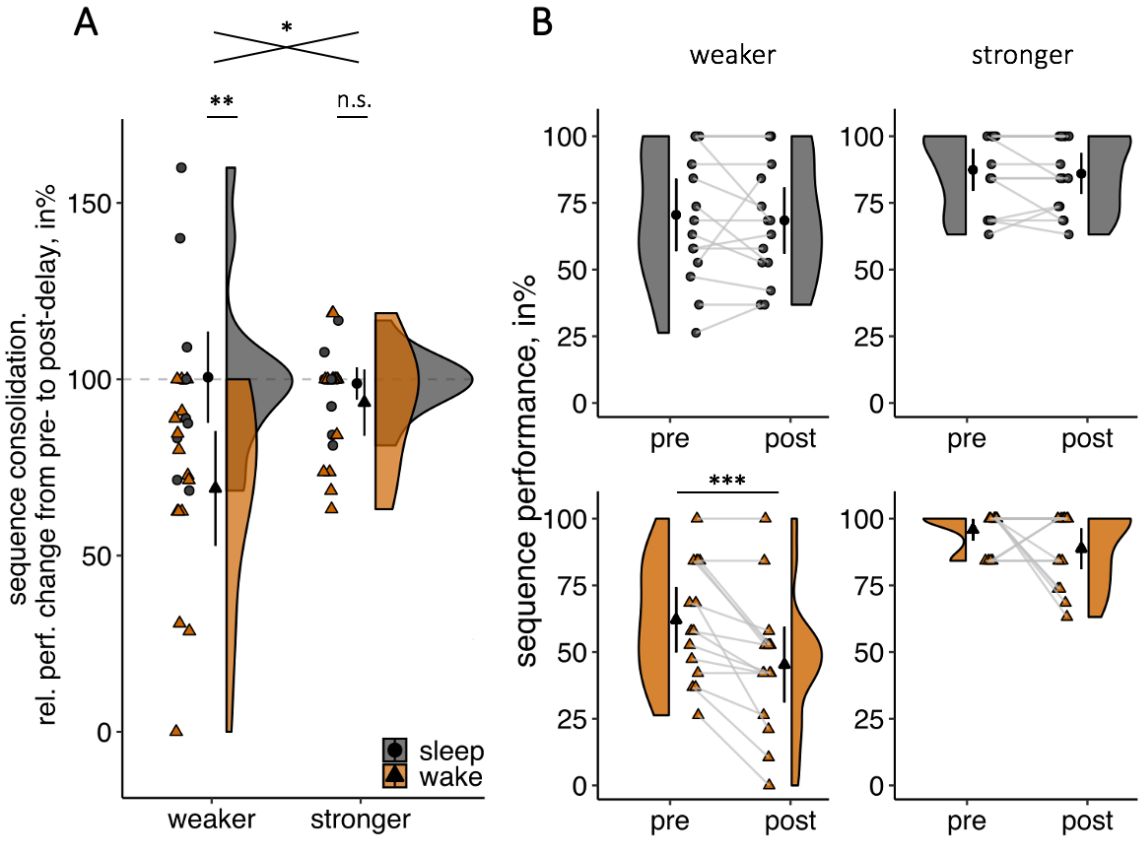

Figure 2.3. Consolidation effects for *no interference* groups. **A.** For weaker memory opportunities, sequence consolidation (relative performance change from pre- to post-delay retrieval) is significantly greater in the sleep group (grey, circle) than in the wake group (orange, triangle), whereas there is no statistical difference between the sleep and wake group for stronger memory opportunities. **B.** For stronger memory opportunities (*right column*), pre- as well as post-delay sequence performance was at ceiling, while pre-

and post-delay sequence performance for weaker memory opportunities (*left column*) were normally distributed. Only sequence performance for weaker memory opportunities in the wake group significantly decreased from pre- to post-delay retrieval. Single participant data (grey filled circles for sleep groups and orange filled triangles for wake groups), density plots and group means with 95% CIs are shown in A and B. * = $p \leq .05$; ** = $p < .01$; *** = $p < .001$; n.s. = not significant, $p > .1$

2.3. Interference: Stronger memories also show a sleep-dependent consolidation effect

As mentioned above, stronger memories may require an increase in retrieval difficulty to mitigate possible ceiling effects and to unveil the beneficial effect of post-learning sleep for consolidation. To test this hypothesis, we again compared sequence consolidation in a 2x2 ANOVA with the between-subjects factors Delay (sleep vs. wake) and Memory Strength (weaker vs. stronger), this time focusing on the high retrieval difficulty (interference) groups.

Results demonstrated that sequence consolidation in the sleep groups was again significantly higher than in the wake groups (main effect of Delay: $F(1,56) = 21.22$, $p < .001$, $\eta_p^2 = 0.28$). Importantly though, both weaker and stronger memory opportunities showed a significant sleep-dependent consolidation effect (no Delay x Strength interaction: $F(1,56) = 0.98$, $p = .327$, $\eta_p^2 = 0.02$, $BF_{01} = 2.80$). For weaker memory opportunities, sequence consolidation was significantly greater in the sleep group than in the wake group ($t(27.61) = 2.27$, $p = .031$, $d = 0.83$), which replicated the pattern observed with low retrieval difficulty (see above). Critically though and in contrast to the low retrieval difficulty conditions, sequence consolidation was also significantly greater in the sleep group than in the wake group for stronger memory opportunities ($t(27.93) =$

4.64, $p < .001$, d = 1.70, Figure 4A). Indeed, the increase in retrieval difficulty effectively eliminated ceiling effects during post-delay retrieval (sleep group: $W = 0.96$, $p = .722$, wake group: $W = 0.98$, $p = .981$, Figure 4B). These findings indicate that both stronger and weaker memories benefited from post-learning sleep, but that stronger memories required additional retrieval demands to show a benefit from post-learning sleep.

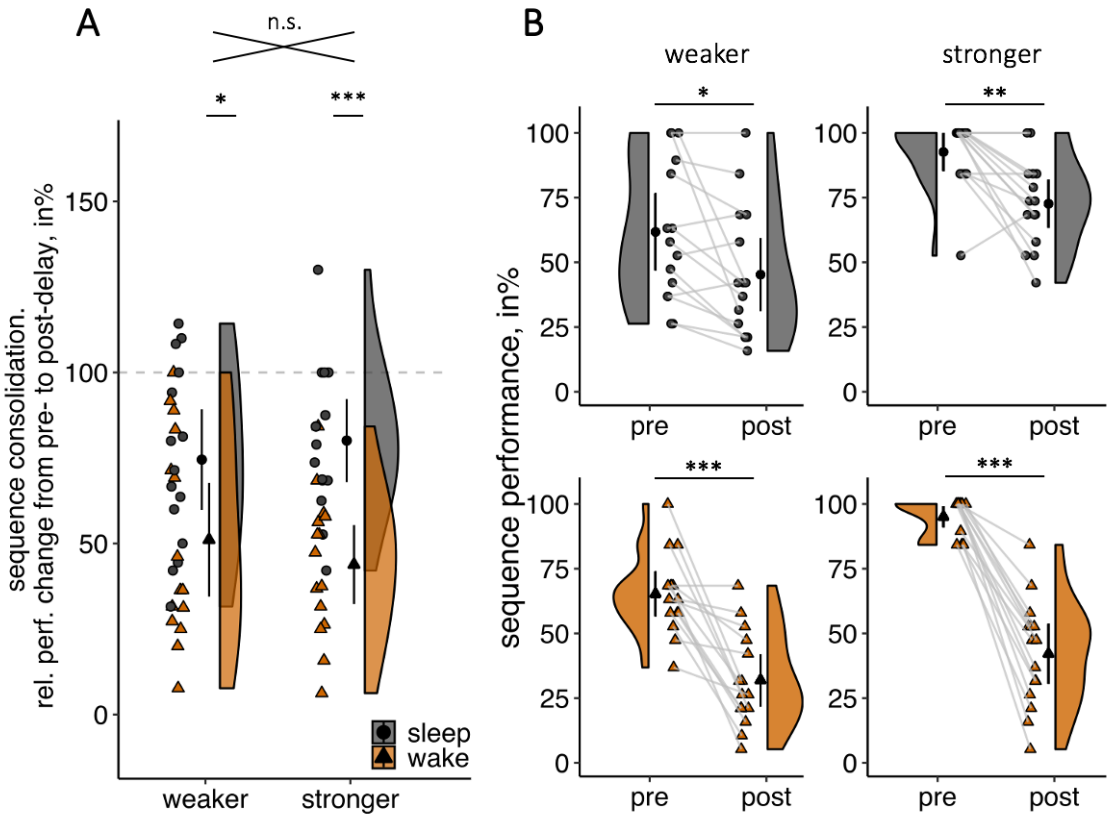

Figure 2.4. Consolidation effects for *interference* groups. **A.** After inducing retroactive interference, sequence consolidation (relative performance change from pre- to post-delay retrieval) in the sleep group (grey, circle) is significantly greater for both weaker and stronger memory opportunities. **B.** For stronger memory opportunities (*right column*), pre-delay sequence performance was at ceiling, while post-delay sequence performance was normally distributed. For weaker memory opportunities (*left column*), pre- as well as post-delay sequence performance were normally distributed. Sequence performance of all memories significantly decreased from pre- to post-delay retrieval. Single participant data (grey filled circles for sleep groups and orange filled triangles for

wake groups), density plots and group means with 95% CIs are shown in A and B. * = p ≤ .05; ** = p < .01; *** = p < .001; n.s. = not significant, p > .1

2.4. Sleep-dependent consolidation of stronger memories is modulated by retrieval difficulty

Our previous analyses showed a sleep-dependent consolidation effect for stronger memories when retrieval difficulty was increased (interference; see section 2.3.), but not under standard testing conditions (no interference; see section 2.2.). To directly test for changes in sleep-dependent consolidation effects from low to high retrieval difficulty separately for weaker and stronger memory opportunities, two 2x2 ANOVAs with the between-subjects factors Delay (sleep vs. wake) and Retrieval Difficulty (no interference vs. interference) were performed. For weaker memory opportunities, the increase in retrieval difficulty did not affect sleep-dependent consolidation effects (no interaction Delay x Retrieval Difficulty: $F(1,56) = 0.33$, $p = .570$, $\eta_p^2 = 0.01$, $BF_{01} = 3.45$). For stronger memory opportunities, we found a significant increase in sleep-dependent consolidation effects from low to high retrieval difficulty (interaction of Delay x Retrieval Difficulty: $F(1,56) = 11.21$, $p = .001$, $\eta_p^2 = 0.17$, Figure 5).

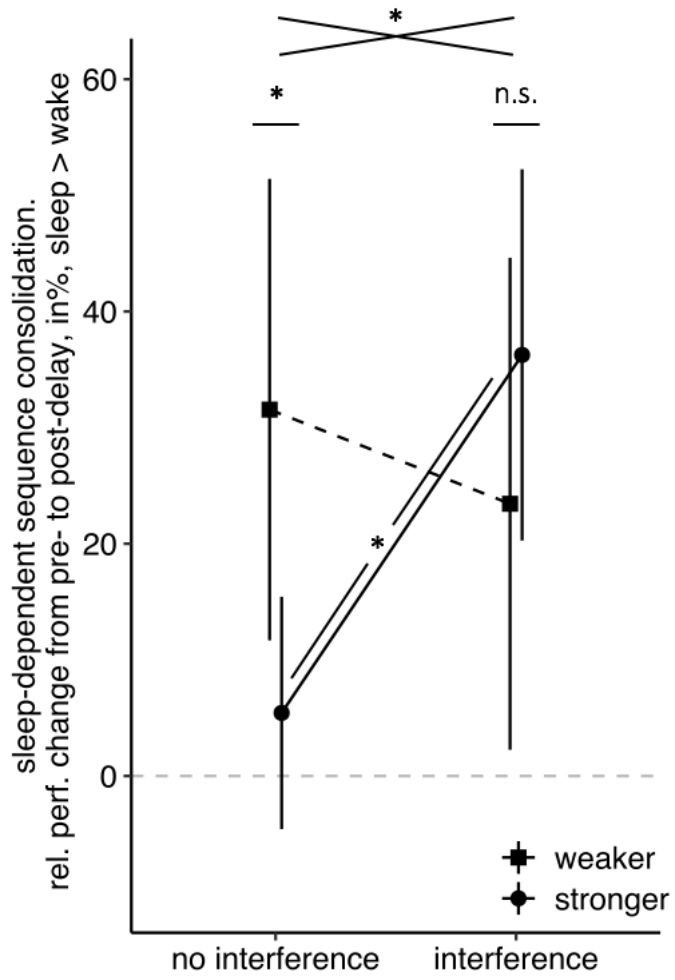

Figure 2.5. Sleep-dependent consolidation effects. With low retrieval difficulty (no interference, *left*), the difference in sequence consolidation (relative performance change from pre- to post-delay retrieval) between sleep and wake is significant for weaker memories only. With an increase in retrieval difficulty (interference, *right*), sleep-dependent consolidation effects are seen for both weaker and stronger memory opportunities. For stronger memory opportunities, the sleep-dependent consolidation effect was significantly greater for high retrieval difficulty (retroactive interference) compared to low retrieval difficulty (no interference). Shown are differences in means between sleep and wake groups and the corresponding 95% CIs. * = $p \leq .05$; n.s. = not significant, $p > .1$

3. Discussion

The aim of the present study was to assess whether sleep-dependent memory consolidation favours weaker over stronger memories. To this end, we devised a novel memory paradigm (*Memory Arena*, Figure 1) and experimentally controlled delay type (sleep or wake), pre-delay memory strength (weaker or stronger) and retrieval difficulty (no interference or interference) (Figure 2). Under standard retrieval conditions (no retroactive interference), our data indeed suggest that weaker memories benefit from sleep while stronger memories seem not to (Figure 3). This finding is in agreement with a growing body of evidence for sleep-dependent consolidation processes favouring weaker memories. Some of these studies used rather indirect manipulations of memory strength, e.g. by comparing retrieval vs. restudy (Bäuml et al., 2014), by varying the difficulty of motor sequences from low element sequences (resulting in stronger procedural memories) to high element sequences (resulting in weaker procedural memories, Kuriyama, Stickgold, & Walker, 2013) or by changing the difficulty of a problem solving task (Sio et al., 2013). Other studies directly manipulated memory strength either by varying the number of presentations (Denis, Schapiro, Poskanzer, Bursal, Charron, et al., 2020; Drosopoulos, Schulze, et al., 2007; Schapiro et al., 2017; Sheth et al., 2012), by inducing retroactive interference immediately after encoding to weaken memories (Drosopoulos, Schulze, et al., 2007; McDevitt et al., 2015) or by comparing participants with high vs. low pre-sleep memory performance (Diekelmann et al., 2010; Djonlagic et al., 2009).

One factor potentially accounting for diminished sleep-dependent consolidation effects for stronger memories is that memory strength is often manipulated by repeated encoding

and retrieval of the study material (Denis, Schapiro, Poskanzer, Bursal, Charron, et al., 2020; Drosopoulos, Schulze, et al., 2007; Schapiro et al., 2017; Sheth et al., 2012). That is, it has been argued that online retrieval emulates a consolidation process similar to that occurring during sleep (Antony et al., 2017). Consequently, stronger memories might already be sufficiently consolidated before sleep, yielding less need for further consolidation during sleep. Convergent evidence for this notion comes from studies examining post-learning sleep spindle activity, with spindles being considered a key mechanistic vehicle of memory consolidation (Fernandez & Lüthi, 2020b; Peyrache & Seibt, 2020). In particular, an increase in spindle power during a post-learning nap has been reported after a high difficulty learning task (producing weaker memories) but not after a low difficulty learning task (producing stronger memories) (Schmidt et al., 2006). Likewise, spindle density has been linked to consolidation specifically of weaker memories (Denis, Mylonas, et al., 2020). Two other nap studies used targeted memory reactivation (TMR) to experimentally bolster consolidation. Interestingly, TMR resulted in better post-sleep memory performance only for weakly encoded memories (Cairney et al., 2016; Creery et al., 2015). Collectively, these findings suggest that sleep-dependent consolidation processes are preferentially deployed for weaker compared to stronger memories.

However, one alternative explanation – at least for the behavioural effects described above – is that beneficial effects of sleep for stronger memories are obscured by ceiling effects. In the present study, we demonstrate that under normal retrieval conditions (without interference), ceiling effects for stronger memories during pre-delay retrieval still persist during post-delay retrieval and would thereby conceal possible sleep-dependent consolidation effects. One way to eliminate ceiling effects during post-delay

retrieval is to induce retroactive interference directly before retrieval. This approach has been taken in a series of studies explicitly testing the protective effect of sleep against retroactive interference. Indeed, despite training participants to 100% pre-delay memory accuracy, the introduction of retroactive interference after the delay and before the final retrieval revealed a beneficial effect of sleep over wake, i.e. a sleep-dependent consolidation effect (Ellenbogen, Hulbert, Jiang, & Stickgold, 2009; Ellenbogen et al., 2006; but see Bailes, Caldwell, Wamsley, & Tucker, 2020; Pöhlchen, Pawlizki, Gais, & Schönauer, 2020). In line with these studies, we used retroactive interference to increase retrieval difficulty and thereby push memory performance from ceiling. Critically, this manipulation revealed sleep-dependent consolidation effects for weaker as well as for stronger memories (Figure 4). One interesting question for future research is whether this 'rescue' of sleep-dependent consolidation effects for strong memories relies on interference manipulations, or whether other means of increasing retrieval demands, e.g., dual task manipulations, produce similar effects.

Our current results thus suggest that post-learning sleep benefits all memories, but that greater levels of initial memory strength call for adjusted testing protocols. It is interesting to note that weaker memories benefitted from sleep irrespective of subsequent retrieval demands, at least with respect to the presence vs. absence of retroactive interference as employed here. That said, an important goal for future research is to establish the lower memory strength boundaries for sleep-dependent consolidation effects to occur. In particular, if initial memory strength is too low, a floor effect would likely hinder any benefit from subsequent sleep.

It deserves mention that besides retrieval difficulty, a number of other factors appear to impact sleep-dependent consolidation. One such factor is the duration of sleep. While some studies used 2h daytime naps as a delay period (Cairney et al., 2016; Creery et al., 2015; Denis, Mylonas, et al., 2020; Schmidt et al., 2006), others followed a whole-night protocol (Bäuml et al., 2014; Diekelmann et al., 2010; Drosopoulos, Schulze, et al., 2007). Importantly, Schapiro et al. (2017) demonstrated that a full night of sleep and a nap show differential selectivity for weaker or stronger memories. In line with other nap studies (Cairney et al., 2016; Creery et al., 2015; Schmidt et al., 2006), they found that a 2h nap selectively benefitted weaker memories. However, the selective benefit for weaker memories diminished after a full night of sleep. A possible interpretation of these results is that weaker memories are reactivated earlier during sleep, i.e., are prioritized as they are more prone to forgetting. A full night of sleep, however, provides sufficient time to reactivate both weaker and stronger memories. While tempting, this interpretation requires additional research systematically controlling nap vs. full night of sleep and weaker vs. stronger memories. Another factor to be considered is the particular definition of weaker and stronger memories. For example, Tucker and Fishbein (2008) used a similar retrieval vs. restudy manipulation as Bäuml et al. (2014) but came to different conclusions. They found a sleep-dependent consolidation effect for items subjected to retrieval practice (thought to result in stronger memories, see introduction), but not for items restudied (thought to result in weaker memories), which is the exact opposite pattern as in Bäuml et al. (2014). However, in their retrieval practice condition, Bäuml et al. (2014) had participants retrieve fewer items more frequently compared to Tucker and Fishbein (2008), likely to result in stronger memories. This illustrates the difficulty of categorically designating a particular memory as weak or strong based on behavioural

assays alone. Real-time brain imaging might be used as a complementary measure to assess post-learning memory strength (Ezzyat et al., 2018).

4. Methods

4.1. Participants

Overall, 128 participants took part in the study. Eight participants were excluded – 6 participants based on Actigraph recordings (5 participants in a sleep group slept less than 5 hours between pre- and post-retrieval and 1 participant in a wake group slept during the day), 1 participant did not finish the experiment and 1 participant was erroneously assigned to the wrong condition. The remaining 120 participants were included in the analyses (age = 20.58 ± 2.08 [mean ± SD], female = 83, n = 15 per group). Target sample size was based on two relevant studies using between-subjects designs. Drosopoulos et al. (2007) used 10 participants per group to demonstrate a greater sleep-dependent consolidation effect for weaker than for stronger memories. Ellenbogen et al. (2006) used 12 participants per group to show that sleep-dependent consolidation effects are impacted by retrieval demands.

Participants had no history of neurological or psychiatric disorders and had a normal sleep-wake cycle as assessed with a sleep diary. For taking part in the study, participants received either monetary reimbursement or study credits. The study was approved by the University of Birmingham Research Ethics and Governance Committee and written informed consent was obtained from participants before the start of the experiment.

4.2. Task design & procedure

To capture both the temporal and spatial components of episodic memory we designed a new paradigm, called *Memory Arena*. It consists of a circle divided into four quarters, each depicting a different scene background (upper left: arctic landscape, upper right: desert, lower right: autumn forest, lower left: sea, Figure 1). On top of these backgrounds, individual objects are presented in different spatial positions. Participants have to learn the temporal (sequential) and spatial position of each object.

Twenty target objects were randomly selected from a pool of 50 common animate and inanimate objects (Konkle, Brady, Alvarez, & Oliva, 2010, coloured and presented on a white 90x90 pixels square). The spatial position of each object was restricted by the outline of the *Memory Arena* and by the position of other objects. Thus, there was no overlap between objects but it was possible that an object covered multiple background scenes.

During the encoding part of the *Memory Arena*, all 20 objects were presented one after another and participants confirmed an object's spatial position by clicking on the object. The current object then disappeared and the next object was presented (Figure 1). Participants were encouraged to associate the objects with each other and with the background scenes into a narrative.

A training session was introduced directly after the encoding part. The training started with all 20 objects randomly arranged around the arena. Participants had to drag and drop the objects in the correct sequence to the correct spatial position. If an error was made regarding the sequence or spatial position, the arena turned red and the error was corrected. If the object was placed at its correct sequential and spatial position, the arena

turned green. The sequence position was scored as correct if object *i* was placed at the i^{th} position. The spatial position of an object was scored as correct if the overlap between its position and the correct position was higher than 25%.

After feedback and potential error correction, the object remained at its correct spatial position and the next object had to be placed in the arena. When all 20 objects were placed, participants received feedback about their overall performance ((n correct objects / n of total objects), where an object was classified as correct when both the sequence and spatial position were correct).

To manipulate pre-sleep memory strength, participants in different groups finished training after meeting two different levels of performance. Pre-sleep memory strength was defined as 'weaker' for participants with a performance criterion of 50%, reached in one training round (1x50%) and defined as 'stronger' when the performance criterion was set to 70%, reached in two consecutive training rounds (2x70%).

After finishing the training session, participants performed a pre-delay retrieval task. The retrieval started, like the training session, with all 20 objects randomly arranged around the arena and the objects had to be dragged and dropped in the correct sequence to the correct spatial position. Importantly though, no feedback was provided, and errors were not corrected meaning that the objects remained at the spatial position where they were dropped.

As we compared memory performance between two retrieval tasks performed at different times of day (pre vs. post-delay retrieval, AM vs. PM or PM vs. AM), an alternative explanation for a change in memory performance might be a change in attention/level of alertness. We thus employed a psychomotor vigilance task (PVT) directly before both

retrieval tasks. The PVT started with a white fixation cross presented in the middle of the screen. After an average of 6 seconds (with a jitter of ± 4s), the fixation cross was replaced by a counter starting at 0 and counting forwards to 2000 in 20ms steps. During that time, participants had to press the space bar as fast as possible. Feedback about their reaction time was provided after the key press (displayed for 2s).

In session one encoding, training, PVT and pre-delay retrieval were completed. 12 hours later participants returned to the lab for the second session. For half of the participants the second session started with an interference task, designed to increase the post-delay retrieval difficulty. Participants were not informed about the interference task until the beginning of the second session. During the interference task, participants were asked to encode the same objects presented in a different sequence and at different spatial positions (Figure 1). The new spatial position of every object was more than 5 pixels away from its original spatial position (centre to centre distance). Encoding of the interfering temporospatial arrangement was implemented in the same way as the original encoding. Following encoding, participants performed a retrieval session of the interfering arrangement (no training was conducted for the new arrangement). Finally, participants performed a second PVT and the retrieval task for the original arrangement (post-delay retrieval). All participants in the no-interference condition directly started with the PVT and the post-delay retrieval of the original arrangement.

4.3. Study design

We used a 2 (Delay: sleep vs. wake) x 2 (Memory Strength: weaker vs. stronger) x 2 (Retrieval difficulty: no interference vs. interference) between-subjects design and participants were randomly assigned to one of the resulting 8 conditions (Figure 2).

Participants in the sleep conditions performed the first session including encoding, training, PVT and pre-delay retrieval in the evening around 9 pm. After finishing the first session they went home to sleep. 12 hours later, at 9 am, they returned to the lab to perform the second session. Half of the participants additionally conducted the interference at the beginning of the second session while the other half directly started with the PVT and post-delay retrieval. Participants in the wake conditions followed the same protocol shifted by 12 hours, i.e., performing the first session (encoding, training, PVT and pre-delay retrieval) at 9 am and returning to the lab 12 hours later at 9 pm for the second session (interference, PVT and post-delay retrieval or PVT and post-delay retrieval, respectively).

4.4. Data collection & analysis

The *Memory Arena* was implemented with MATLAB 2016a (MathWorks). Behavioural responses were recorded using the mouse. Data were prepared and analysed using MATLAB and statistical analyses were conducted with the statistical software R. For data visualization raincloud tools in R were used (Allen, Poggiali, Whitaker, Marshall, & Kievit, 2019; van Langen, 2020).

To capture memory performance, we considered two variables: sequence performance and spatial error (placement distance). Sequence performance was based on correct transitions within the sequence (3rd object is chosen after the 2nd object) rather than on the absolute sequential position (3rd object is chosen at 3rd position), as the absolute sequential position is not necessarily the most sensitive measurement for memory performance. For example, if the second object in the sequence was erroneously placed first but then the order was correctly remembered for all subsequent objects, scoring the absolute positions would yield a performance score of 0. However, by scoring the transition between objects, all but the last (which now comes after the 19th placement but should have come first) are correct. Hence, the sequence performance was calculated based on the difference between object x_i and object x_{i-1}, where $_i$ is the selected sequence position of object x. If the transition is correct this difference is 1. The sum of the correct transitions was then divided by the total number of possible transitions (n = 19 when $n_{obj} = 20$) and multiplied with 100 to get a percentage score. The spatial error was calculated using the Euclidean distance (in pixels) between the centre of the original position and the centre of the placed position of every object.

To test the effects of our experimental factors (Figure 2), parametric ANOVAs were applied. Welch's t-tests were used as post-hoc comparisons as variances between groups were not always equal. Note that for Welch's t-tests, degrees of freedom are adjusted according to the Welch–Satterthwaite equation. For effect sizes, we report partial eta squared (η_p^2) for ANOVAs and Cohens d for Welch's t-tests. Shapiro-Wilk tests were applied to test for normal distributions of pre- and post-delay performance.

As traditional null-hypothesis testing does not allow for conclusions about the absence of an effect, we also conducted Bayesian analyses for all non-significant effects using the BayesFactor package in R (Morey & Rouder, 2015). According to the BayesFactor package, we used a Cauchy distribution (0, 0.707) as a prior. The Bayes factor BF_{01} ($BF_{01} = 1/BF_{10}$) informs about the likelihood to observe the data if the null hypothesis is true ($P(D \mid H_0) / P(D \mid H_1)$). A Bayes factor ($BF_{01}$) between 1-3 can be considered as anecdotal evidence, 3-10 as moderate, 10-30 as strong, 30-100 as very strong and >100 as extreme evidence for H_0 (Lee & Wagenmakers, 2013).

5. Acknowledgements

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6. Author contributions

Marit Petzka: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Visualization Ian Charest: Conceptualization, Software George Balanos: Resources, Supervision Bernhard P. Staresina: Conceptualization, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition

7. Supplemental Information

Analogous to sequence consolidation, we conducted a 2x2x2 ANOVA for spatial consolidation with Delay (sleep vs. wake), Memory Strength (weaker vs. stronger) and Retrieval Difficulty (no interference vs. interference) as between-subjects factors. Spatial consolidation reflects the relative change in error rate (placement distance) from pre- to post-delay retrieval. Thus, values > 100% show an increase in error rate, < 100% show a decrease and values = 100% show a stabilization of error rate. Overall, post-relative to pre-delay error rate was significantly higher in wake groups than in sleep groups (main effect for Delay: $F(1,112) = 9.22$, $p = .003$, $\eta_p^2 = 0.08$) and higher for high retrieval difficulty compared to low retrieval difficulty (main effect for Retrieval Difficulty: $F(1,112) = 25.71$, $p < .001$, $\eta_p^2 = 0.19$). In contrast to sequence consolidation, we did not find a significant three way interaction ($F(1, 112) = 0.34$, $p = .560$, $\eta_p^2 < 0.01$, $BF_{01} = 4.57$). Nevertheless, to fully characterise sleep-dependent consolidation effects for spatial memory, we conducted the same subsidiary ANOVAs as described in the main text.

First, a 2x2 ANOVA with the between-subjects factors Delay (sleep vs. wake) and Memory Strength (weaker vs. stronger) was used for the *no interference* groups only. Spatial consolidation did not significantly differ between sleep and wake groups (main effect Delay: $F(1,56) = 2.59$, $p = .113$, $\eta_p^2 = 0.04$, $BF_{01} = 1.28$) and there was no modulation by initial memory strength (interaction of Delay x Strength: $F(1,56) = 1.49$, $p = .228$, $\eta_p^2 = 0.03$, $BF_{01} = 2.06$, Figure S1A).

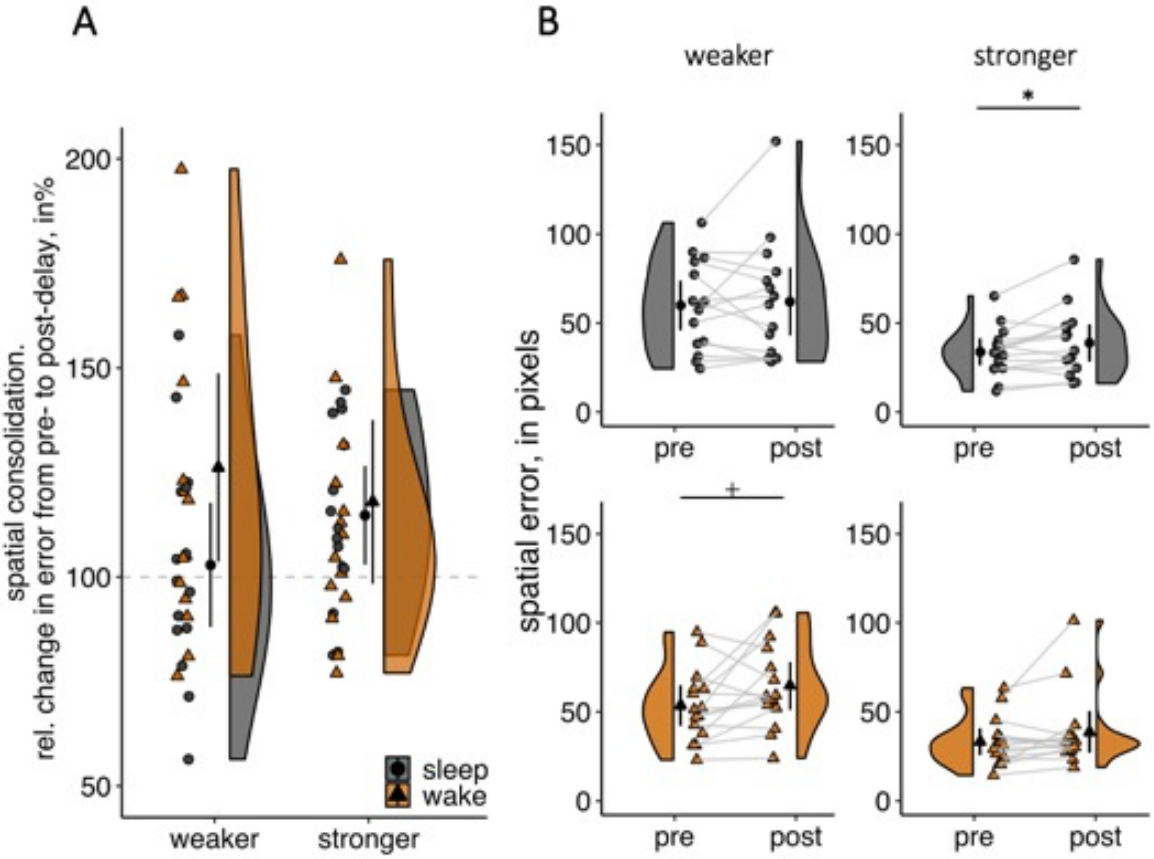

Figure S 2.1. Spatial consolidation effects (error rate) for *no interference* groups. **A.** Neither for weaker nor for stronger memory opportunities did spatial consolidation (relative change in error rate from pre- to post-delay retrieval) significantly differ between the sleep (grey, circle) and the wake group (white, triangle). **B.** Pre- and post-delay error rate for weaker (*left column*) and stronger memory opportunities (*right column*). Single participant data (grey filled circles for sleep groups and orange filled triangles for wake groups), density plots and group means with 95% CIs are shown in A and B. + = .1 ≥ p > .05; * = p ≤ .05

Second, a 2x2 ANOVA with the between-subjects factors Delay (sleep vs. wake) and Memory Strength (weaker vs. stronger) for the *interference* groups was conducted. In line with our notion that an increase in retrieval difficulty unveils sleep-dependent consolidation processes, we found a significant difference between the sleep and the wake groups (main effect Delay: $F(1,56) = 7.68$, $p = .008$, $\eta_p^2 = 0.12$). Similar to sequence

consolidation (see main text, section 3.3.), the sleep-dependent consolidation effect for spatial memory was comparable for weaker and stronger memory opportunities (no Delay x Strength interaction: $F(1,56) = 0.12, p = .727, \eta_p^2 < 0.01, BF_{01} = 3.64$, Figure S2A), albeit only reaching significance for stronger memory opportunities ($t(18.14) = -2.31, p = .033$, $d = 0.84$) and not for weaker memory opportunities ($t(15.33) = -1.64, p = .121, d = 0.60$, $BF_{01} = 1.07$).

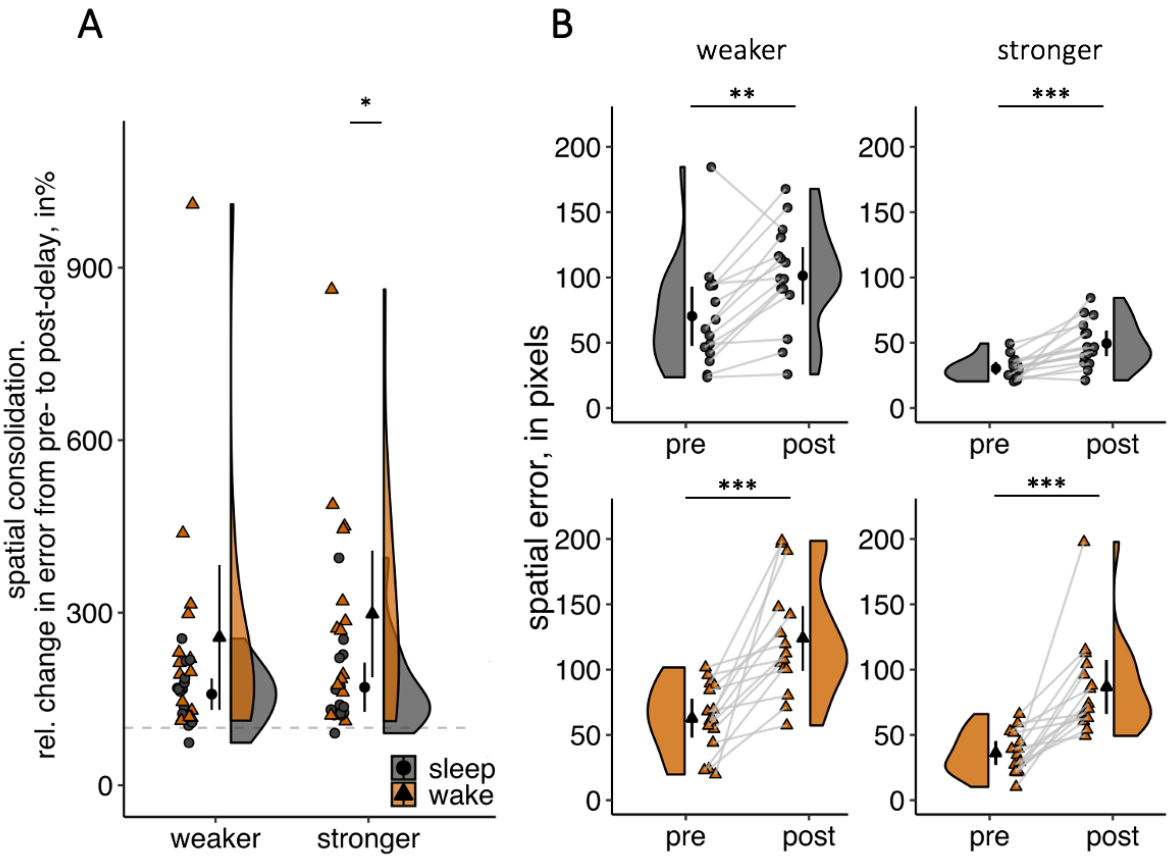

Figure S 2.2. Spatial consolidation effects (error rate) for i*nterference* groups. **A.** Overall, spatial consolidation is significantly worse (greater relative change in error rate from pre- to post-delay retrieval) for the wake group (white, triangle) than for the sleep group (grey, circle). Note that results did not qualitatively change when outliers (N = 3) were excluded. **B.** Pre- and post-delay error rate for weaker (*left column*) and stronger memory opportunities (*right column*). Single participant data (grey filled circles for sleep groups and orange filled triangles for wake groups), density plots and group means with 95% CIs are shown in A and B. * = p ≤ .05; ** = p < .01; *** = p < .001

Lastly, we conducted two 2x2 ANOVAs with the between-subjects factors Delay and Retrieval Difficulty for weaker and stronger memory opportunities, respectively. For stronger memory opportunities the sleep-dependent consolidation effect significantly increased from low to high retrieval difficulty (interaction Delay x Retrieval Difficulty for stronger memories: $F(1,56) = 4.88, p = .031, \eta_p^2 = 0.08$). Note that an increase in sleep-dependent consolidation is reflected by more negative values as the error rate was used. For weaker memory opportunities, there was an overall trend for a sleep-dependent consolidation effect (main effect Delay, $F(1,56) = 3.95, p = .0518, \eta_p^2 = 0.07$), without a significant interaction of Delay x Retrieval Difficulty ($F(1,56) = 1.51, p = .225, \eta_p^2 = 0.03$, $BF_{01} = 2.24$, Figure S3).

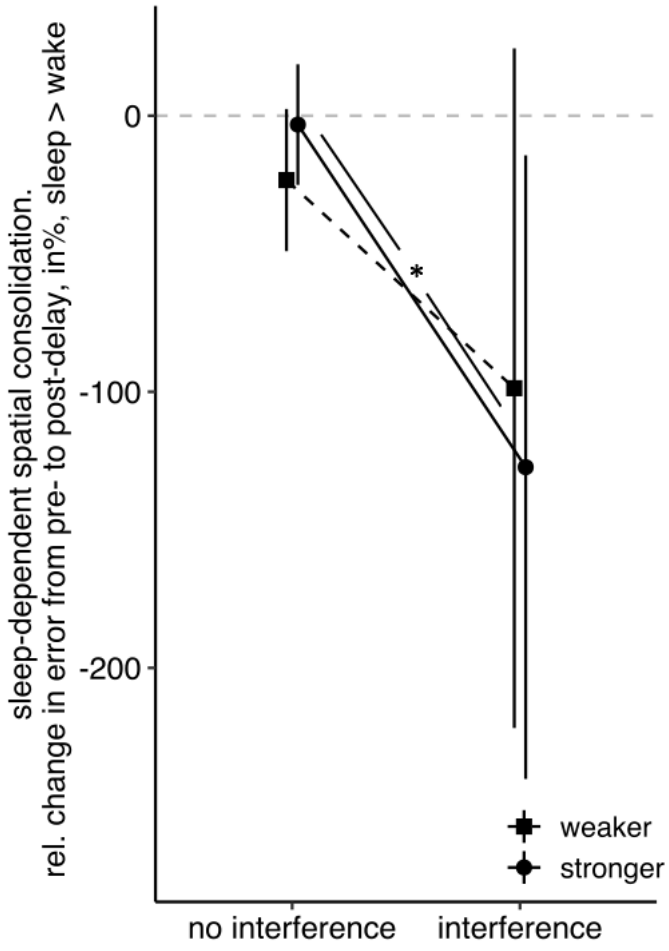

Figure S 2.3. Sleep-dependent consolidation effects. For stronger memory opportunities, the difference in spatial consolidation (relative change in error rate from pre- to post-delay retrieval) between sleep and wake significantly increased with higher retrieval difficulty (interference). Shown are differences in means between sleep and wake groups and the corresponding 95% CIs. * = $p \leq .05$

In sum, while increased retrieval difficulty also unveiled a sleep-dependent consolidation effect for strong spatial memories, effects for weak spatial memories were more subtle than for their sequence memory counterparts. One explanation might be that sequence memory was more sensitive to our initial memory strength manipulation (see main text, section 2.1. and 2.2.). Note also that both variables were operationalized on different scales. While sequence performance for an object is a binary outcome (correct or incorrect position in the sequence), spatial performance (i.e., spatial error) is a continuous variable (Euclidean distance from centre to centre in pixels). Further studies are needed in which temporal and spatial measures are more closely matched to adjudicate whether there are differential beneficial effects of sleep on temporal and spatial aspects of memory.

As our memory strength manipulation was defined based on the overall performance during training, we also used overall performance as dependent variable and conducted the same analyses as with sequence and spatial performance. Overall performance is the combined sequence and spatial performance, with higher values denoting better performance. Therefore, overall performance consolidation (relative change from pre- to post-delay) can be interpreted analogous to sequence consolidation: > 100% means an increase in performance, < 100% means a decrease and values = 100% mean stabilization of performance.

The results of the 2x2x2 ANOVA with Delay (sleep vs. wake), Memory Strength (weaker vs. stronger) and Retrieval Difficulty (no interference vs. interference) as between-subjects factors matched the results for sequence performance. We found two significant main effects for Delay ($F(1,112) = 12.72$, $p < .001$, $\eta_p^2 = 0.10$) and Retrieval Difficulty ($F(1,112) = 52.50$, $p < .001$, $\eta_p^2 = 0.32$). The three way interaction - despite only showing a trend towards significance ($F(1, 112) = 3.09$, $p = .081$, $\eta_p^2 = 0.03$) - still suggests that sleep-dependent consolidation effects for weaker and stronger memory opportunities might differ as a function of retrieval difficulty. Therefore, we conducted the same subsidiary ANOVAs as described in the main text.

For the no interference groups, the 2x2 ANOVA with the between-subjects factors Delay (sleep vs. wake) and Memory Strength (weaker vs. stronger) showed almost the same pattern as in the main text. Overall performance consolidation was significantly greater in sleep compared to wake groups (main effect Delay: $F(1,56) = 5.00$, $p = .029$, $\eta_p^2 = 0.08$) and this difference was modulated by the initial memory strength (interaction of Delay x Strength: $F(1,56) = 5.39$, $p = .024$, $\eta_p^2 = 0.09$). Post hoc t-tests showed no significant difference in overall performance consolidation between sleep and wake group for stronger memory opportunities ($t(28) = -0.08$, $p = .938$, $d = 0.03$, $BF_{01} = 2.90$). However, for weaker memory opportunities, the sleep group showed significantly greater overall performance consolidation than the wake group ($t(27.93) = 2.72$, $p = .011$, $d = 0.99$, Figure S4A).

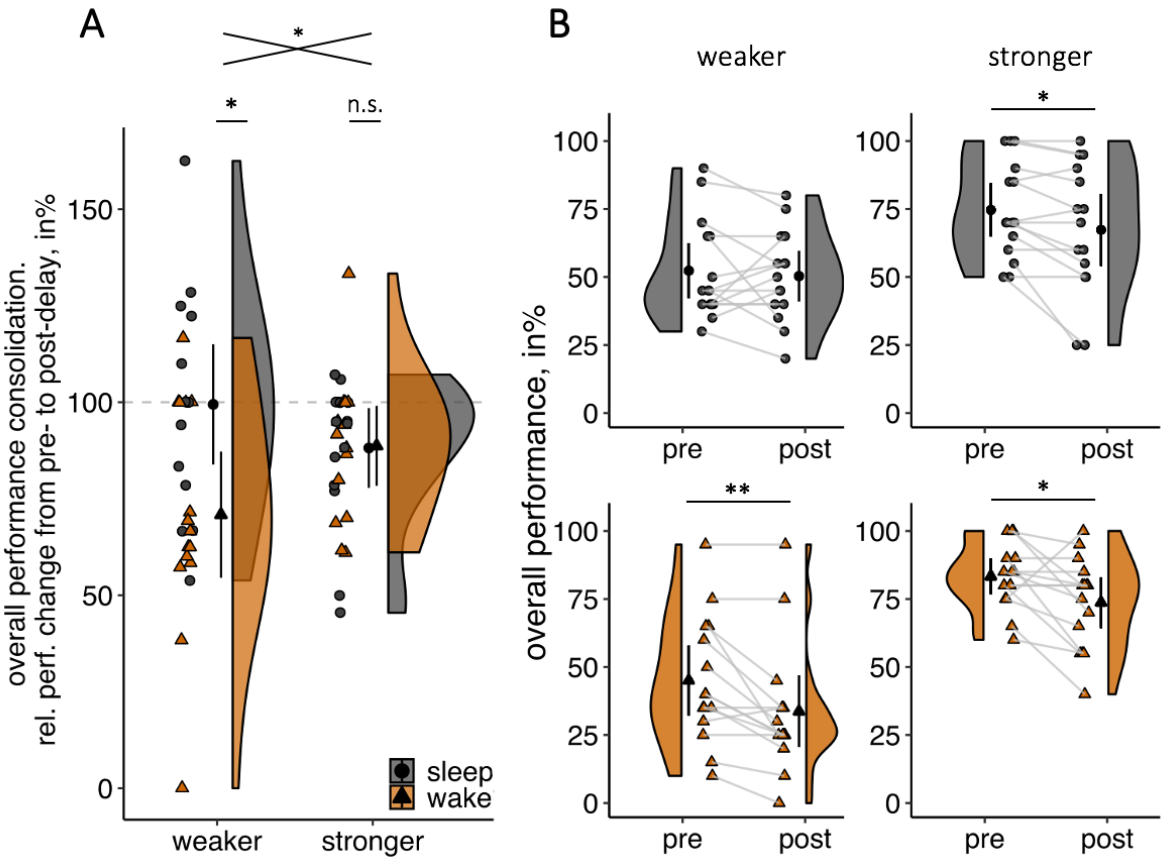

Figure S 2.4. Consolidation effects for *no interference* groups. **A.** For weaker memory opportunities, overall performance consolidation (relative performance change from pre- to post-delay retrieval) is significantly greater in the sleep group (grey, circle) than in the wake group (orange, triangle), whereas there is no statistical difference between the sleep and wake group for stronger memory opportunities. **B.** Pre- and post-delay error rate for weaker (*left column*) and stronger memory opportunities (*right column*). Single participant data (grey filled circles for sleep groups and orange filled triangles for wake groups), density plots and group means with 95% CIs are shown in A and B. * = $p \leq .05$; ** = $p < .01$; n.s. = not significant, $p > .1$

After increasing retrieval difficulty by inducing retroactive interference, we still found a higher overall performance consolidation in the sleep groups than in the wake groups (main effect of Delay: $F(1,56) = 7.73$, $p = .007$, $\eta_p^2 = 0.12$). Importantly, both weaker and stronger memory opportunities showed a significant sleep-dependent consolidation effect

(no Delay x Strength interaction: $F(1,56) = 0.17$, $p = .684$, $\eta_p^2 = 0.003$, $BF_{01} = 3.57$), indicating that both weaker and stronger memories benefited from post-learning sleep (Figure S5A).

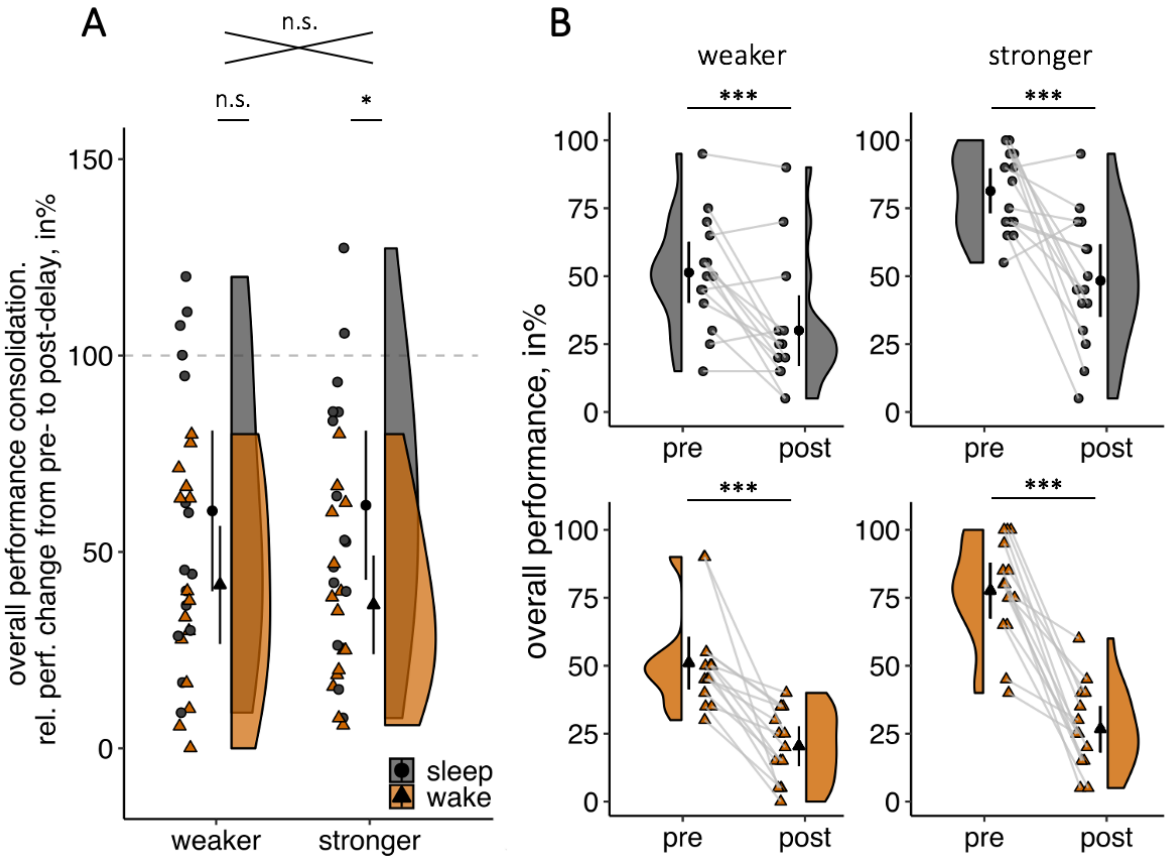

Figure S 2.5. Consolidation effects for *interference* groups. **A.** After inducing retroactive interference, overall performance consolidation (relative performance change from pre- to post-delay retrieval) is significantly greater in the sleep group (grey, circle) than in the wake group (orange, triangle) for stronger memory opportunities. **B.** Pre- and post-delay error rate for weaker (*left column*) and stronger memory opportunities (*right column*). Single participant data (grey filled circles for sleep groups and orange filled triangles for wake groups), density plots and group means with 95% CIs are shown in A and B. * = p ≤ .05; ** = p < .01; n.s. = not significant, p > .1

In a last step, we conducted two 2x2 ANOVAs with the between-subjects factors Delay and Retrieval Difficulty for weaker as well as for stronger memory opportunities. For weaker memory opportunities, the increase in retrieval difficulty had no impact on sleep-dependent consolidation effects (no interaction Delay x Retrieval Difficulty for weaker memories: $F(1,56) = 0.38$, $p = .541$, $\eta_p^2 = 0.01$, $BF_{01} = 3.41$). For stronger memory opportunities, sleep-dependent consolidation effects significantly increased from low to high retrieval difficulty (interaction of Delay x Retrieval Difficulty: $F(1,56) = 4.21$, $p = .045$, $\eta_p^2 = 0.07$, Figure S6).

Taken together, the results using overall performance largely correspond to the results using sequence performance as dependent variable.

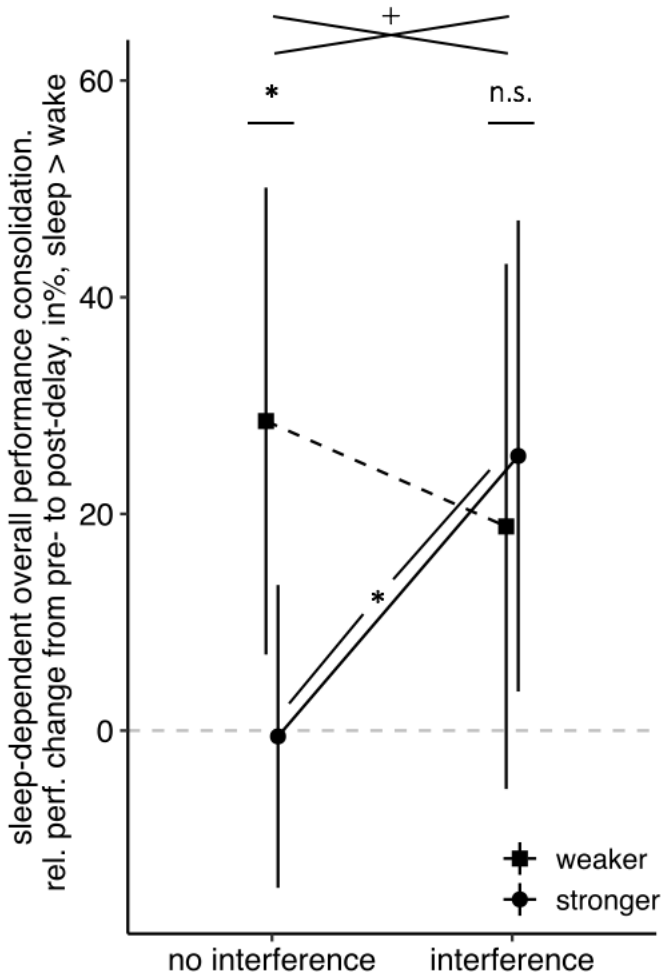

Figure S 2.6. Sleep-dependent consolidation effects, overall performance. With low retrieval difficulty (no interference), the difference in overall performance consolidation (relative performance change from pre- to post-delay retrieval) between sleep and wake is significant for weaker memory opportunities only. With an increase in retrieval difficulty (interference), sleep-dependent consolidation effects are seen for both weaker and stronger memory opportunities. Shown are differences in means between sleep and wake groups and the corresponding 95% CIs. + = .1 ≥ p > .05; * = p ≤ .05; n.s. = not significant, p > .1

Table S 2.1. Descriptive data of duration (in seconds, encoding + training) and number of training rounds needed to reach the criterion. Mean ± 95% CIs

	Weaker				Stronger			
	No interference		**Interference**		**No interference**		**Interference**	
	Sleep	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**
Duration	909.06± 240.27	866.87± 177.41	996.15± 391.47	867.31± 188.68	1065.82± 130.52	1256.70± 208.00	1079.53± 187.81	1110.46± 248.21
Rounds	3.07± 1.65	3.27± 1.30	3.40± 1.85	3.13± 0.98	4.33± 0.58	5.47± 1.22	4.40± 0.95	5.33± 1.27

Table S 2.2. Descriptive data of spatial memory performance (placement distance) pre- and post- delay and the corresponding dependent t-tests. Mean ± 95% CIs, t-values, p-values. Significant effects are highlighted in bold.

	No interference				Interference			
	Weaker		**Stronger**		**Weaker**		**Stronger**	
	Sleep	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**
Pre-delay	59.98± 13.85	53.58± 11.28	33.77± 7.62	33.21± 7.51	70.34± 22.60	62.70± 14.71	30.31± 4.60	36.02± 8.94
Post-delay	62.03± 18.84	64.68± 13.34	38.80± 10.35	38.57± 11.63	101.29± 21.79	123.85± 24.43	49.46± 9.88	86.73± 20.52
t-value	-0.41	-2.01	**-2.34**	-1.59	**-3.80**	**-4.84**	**-4.33**	**-5.19**
(p-value)	(.69)	(.06)	**(.03)**	(.13)	**(<.01)**	**(<.01)**	**(<.01)**	**(<.01)**

Table S 2.3. Descriptive data of overall memory performance pre- and post- delay and the corresponding dependent t-tests. Mean ± 95% CIs, t-values, p-values. Significant effects are highlighted in bold.

	No interference				Interference			
	Weaker		**Stronger**		**Weaker**		**Stronger**	
	Sleep	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**

Pre-delay	52.33± 10.14	45.00± 12.86	74.67± 9.79	83.33± 6.59	51.33± 11.20	51.00± 9.66	81.33± 8.21	77.66± 10.19
Post-delay	50.33± 9.15	33.67± 13.13	67.33± 13.23	73.67± 9.33	30.00± 12.95	20.33± 7.21	48.33± 13.35	26.67± 8.48
t-value	0.57	**3.65**	**2.75**	**2.45**	**4.16**	**5.65**	**4.33**	**8.56**
(p-value)	(.58)	**(<.01)**	**(.02)**	**(.03)**	**(<.01)**	**(<.01)**	**(<.01)**	**(<.01)**

Table S 2.4. Descriptive data of sequence memory performance pre- and post- delay and the corresponding dependent t-tests. Mean ± 95% CIs, t-values, p-values. Significant effects are highlighted in bold.

	No interference				Interference			
	Weaker		**Stronger**		**Weaker**		**Stronger**	
	Sleep	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**	**Sleep**	**wake**
Pre-delay	70.52± 13.53	62.11± 12.18	87.37± 7.85	95.78± 4.00	61.75± 14.88	65.26± 8.66	92.63± 7.29	95.09± 4.04
Post-delay	68.42± 12.41	45.26± 14.14	85.97± 7.61	88.77± 7.54	45.26± 14.06	31.93± 10.06	72.63± 9.30	42.11± 11.55
t-value	0.58	**4.45**	0.81	1.66	**2.94**	**5.51**	**3.99**	**10.79**
(p-value)	(.57)	**(<.01)**	(.43)	(.12)	**(.01)**	**(<.01)**	**(<.01)**	**(<.01)**

Table S 2.5. Test statistic (W) and corresponding p values of the Shapiro-Wilk Test for pre- and post-delay sequence performance. Significant effects are highlighted in bold.

	Weaker				Stronger			
	No interference		**Interference**		**No interference**		**Interference**	
	Sleep	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**
Pre-delay	0.92	0.95	0.90	0.95	**0.80**	**0.56**	**0.62**	**0.63**
	(.23)	(.51)	(.10)	(.58)	**(<.01)**	**(<.01)**	**(<.01)**	**(<.01)**
Post-delay	0.92	0.94	0.91	0.95	**0.85**	**0.78**	0.96	0.98
	(.23)	(.43)	(.12)	(.55)	**(.02)**	**(<.01)**	(.72)	(.98)

Table S 2.6. Test statistic (W) and corresponding p values of the Shapiro-Wilk Test for pre- and post-delay spatial performance. Significant effects are highlighted in bold.

	Weaker				Stronger			
	No interference		**Interference**		**No interference**		**Interference**	
	Sleep	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**	**Sleep**	**Wake**
Pre-delay	0.96	0.95	**0.86**	0.94	0.97	0.92	0.92	0.96
	(.62)	(.59)	**(.03)**	(.36)	(.82)	(.17)	(.22)	(.67)
Post-delay	**0.87**	0.95	0.97	0.93	0.92	**0.68**	0.97	**0.80**
	(.03)	(.51)	(.84)	(.24)	(.18)	**(<.01)**	(.84)	**(<.01)**

Chapter 3. Sleep spindles track cortical learning patterns for memory consolidation

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Abstract

Memory consolidation, the transformation of labile memory traces into stable long-term representations, is facilitated by post-learning sleep. Computational and biophysical models suggest that sleep spindles may play a key mechanistic role for consolidation, igniting structural changes at cortical sites involved in prior learning. Here we tested the resulting prediction that spindles are most pronounced over learning-related cortical areas and that the extent of this learning-spindle overlap predicts behavioural measures of memory consolidation. Using high-density scalp Electroencephalography (EEG) and Polysomnography (PSG) in healthy volunteers, we first identified cortical areas engaged during a temporospatial associative memory task (power decreases in the alpha/beta frequency range, 6-20 Hz). Critically, we found that participant-specific topographies (i.e., spatial distributions) of post-learning sleep spindle amplitude correlated with participant-specific learning topographies. Importantly, the extent to which spindles tracked learning patterns further predicted memory consolidation across participants. Our results provide empirical evidence for a role of post-learning sleep spindles in tracking learning networks, thereby facilitating memory consolidation.

1. Introduction

Sleep after learning bolsters memory retention, a process referred to as sleep-dependent memory consolidation (Diekelmann & Born, 2010; Jenkins & Dallenbach, 1924; Müller & Pilzecker, 1900). In recent years, sleep spindles – transient 12-15 Hz oscillations generated within thalamo-cortical loops - have emerged as a prime mechanistic vehicle to support consolidation (Born & Wilhelm, 2012; Fernandez & Lüthi, 2020b; Klinzing et al., 2019; Lüthi, 2014; Mednick et al., 2013; Peyrache & Seibt, 2020). Previous studies have linked spindles to consolidation in terms of their density (the number of discrete spindle events per minute) (Gais et al., 2002), power (Holz et al., 2012) and activity, a combination of duration and amplitude (Schabus et al., 2004). Despite their ubiquity, however, the specific role spindles play for memory consolidation remains poorly understood.

Ultimately, effective learning requires structural brain changes, beginning at the synaptic level (Bailey & Kandel, 1993; Josselyn et al., 2015). A hallmark computational/biophysical framework (Sejnowski & Destexhe, 2000) suggests that spindles are particularly well-suited to induce changes in synaptic plasticity. Specifically, spindles gate influx of calcium (Ca^{2+}) into pyramidal dendrites, setting early synaptic consolidation processes in motion. Empirical support for this model has been provided by in-vitro application of spindle-like firing patterns (Rosanova & Ulrich, 2005) as well as by showing a direct modulation of Ca^{2+} activity in cortical pyramidal dendrites as a function of spindle power during natural sleep in rodents (Seibt et al., 2017). Moreover, cortical microelectrode array recordings in humans have shown that spindles group co-firing of single units within 25 ms, i.e. within a time window conducive to spike-timing-

dependent plasticity (Dickey et al., 2021). Critically, however, in order for spindles to promote memory consolidation in an adaptive fashion, they need to show some degree of regional specificity. That is, not only would global synaptic consolidation be of limited use in an ever-changing landscape of tasks, but it would also be at direct odds with extant models emphasising the role of sleep in global synaptic downscaling (Tononi & Cirelli, 2006). Instead, adaptive consolidation has to be selective, specifically strengthening local circuits involved in prior learning.

The current study thus set out to assess (i) whether spindles correspond to specific pre-sleep learning patterns and (ii) whether this learning-spindle overlap supports memory consolidation. To this end, we employed a demanding memory task giving rise to rich and idiosyncratic activation patterns during encoding (*Memory Arena*, Figure 1). After learning, participants took a 2-hour nap before their memory retention was tested. This protocol allowed us to examine whether spindles recorded during this nap would correspond to participant-specific learning patterns and whether the extent of this learning-sleep overlap would predict behavioural expressions of consolidation.

2. Results

2.1. Behavioural results

We employed a recently developed memory paradigm called 'Memory Arena' (Petzka et al., 2021) in which participants learn the temporospatial arrangement of objects in a circular enclosure across multiple training rounds (see Figure 1A and Methods for details). Memory for the temporospatial arrangement was assessed in a first retrieval

block (pre-sleep retrieval) which was followed by a 2-hour nap (see Table S1 for descriptive data of sleep stages). Following the nap, participants were instructed to learn a new temporospatial arrangement of the same objects (retroactive interference), after which they were asked to retrieve the original arrangement (post-sleep retrieval, Figure 1C). As the dependent measure, we use sequence memory performance as our previous work suggested this was the measure most sensitive to capture sleep-dependent consolidation. As expected, we found a significant decrease in sequence performance from pre- to post-sleep retrieval of the original sequence ($t(18) = 4.65$, $p < .001$, Figure 1D). For further analyses, memory consolidation is defined as memory retention, i.e., the relative change in sequence performance from pre- to post-sleep retrieval.

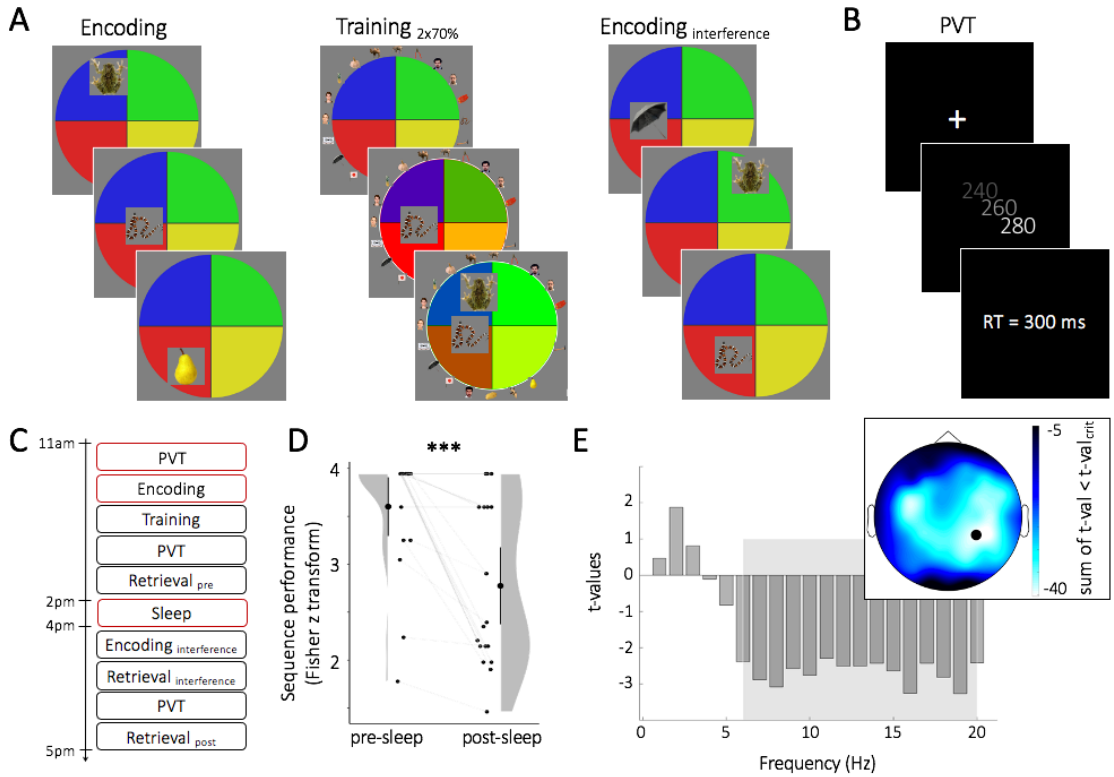

Figure 3.1. Task, experimental design, behavioural results and group EEG encoding pattern. **(A)** *Memory Arena.* During encoding 20 objects were presented in a specific sequence at different spatial positions. Both sequence and spatial position had to be encoded. Training (and retrieval) started with all 20 objects arranged around the arena and participants had to drag and drop the objects in the correct sequence to the correct spatial position. During training, feedback was given after each trial and errors were

corrected. Training was completed after reaching a performance criterion of 70% twice in a row. Retroactive interference was induced by encoding the same objects but in a different sequence and at different spatial positions. **(B)** Every trial of the Psychomotor Vigilance Task (PVT) started with a fixation cross. After a delay of 8-15 sec a counter started (numbers count). Participants had to press a key as fast as possible and received feedback about their reaction time (RT). **(C)** Participants performed the PVT, encoding, training and first retrieval before the 2-hour nap. After the nap, an interference session was employed (1x encoding and retrieval, no training), followed by the second retrieval of the originally learned arena. **(D)** Sequence memory significantly decreased from pre- to post-sleep. Single participant data, density plots and group means with 95% CIs are shown. *** = $p < .001$. **(E)** Comparison of oscillatory power during Memory Arena vs. PVT (thresholded at $p < .05$ cluster corrected), revealing a significant power decrease from 6 – 20 Hz during encoding (grey rectangle), most pronounced over temporo-parietal areas (bars shown for electrode CP4 - black circle on topography plot).

2.2. EEG results: Spindle amplitude tracks encoding patterns

To assess whether sleep spindles track learning sites, we first derived an 'encoding pattern' for each participant. To specifically unravel learning-related activity, we contrasted oscillatory power (1-20 Hz) during encoding with power during a control condition (Psychomotor Vigilance Task, PVT). On the group level, this contrast revealed a significant power decrease in the alpha/beta frequency range (6-20 Hz) during encoding relative to the PVT, particularly over right temporo-parietal areas (Figure 1E). This result is consistent with previous findings linking decreases in alpha/beta power to memory processes (Griffiths et al., 2019, 2021; Hanslmayr et al., 2012; Lega et al., 2017; Noh et al., 2014). The reliable group effect notwithstanding, there was considerable variability in participant-specific effect topographies of the 6-20 Hz power decrease (Figure S1), allowing us to explore whether these participant-specific encoding patterns would bias particular event characteristics during subsequent sleep.

As outlined in the introduction, we hypothesized that the topography (i.e., spatial distribution) of sleep spindles might be modulated by engagement during pre-sleep learning. We thus algorithmically detected sleep spindles during the post-learning nap (see Methods) for every channel and extracted their amplitude as well as duration and density. As a control, we performed the same analyses for algorithmically detected slow oscillations (SOs), which have also been linked to memory consolidation (Heib et al., 2013; Holz et al., 2012; Huber et al., 2004). At the group level, spindles and SOs showed the established prevalence over centro-parietal and frontal areas, respectively (see Figure S2 for amplitude, density and duration topographies of spindles and SOs). Furthermore and in line with previous observations (Helfrich et al., 2018; Mölle et al., 2002; Muehlroth et al., 2019; Schreiner et al., 2021), detected spindle events were on average temporally coupled to the up-state of the SO signal (0.3-1.25 Hz, see Figure S3).

We next turned to the question whether inter-individual differences in the topography of sleep events relate to inter-individual differences in learning topographies. For each participant, the encoding topography (6-20 Hz power relative to the PVT across 58 channels) was correlated (Spearman's rho) with the corresponding topography of 6 different sleep patterns (amplitude, duration and density for spindles and SOs across 58 channels). Note that all sleep measures are positively scaled except the SO amplitude. For simplicity, we unified all scales by taking the absolute value of the SO amplitude. As encoding activity is associated with a decrease in power (negatively scaled values), encoding-sleep overlap would be signified by negative correlations.

Participant-specific correlation values were then evaluated at the group level. First, we conducted a 2 (event type: spindles vs. SOs) x 3 (event characteristic: amplitude, duration, density) repeated measures ANOVA (Figure 2A), assessing whether particular sleep event topographies track idiosyncratic encoding topographies. Results revealed that topographies of overall spindle characteristics correlated with encoding patterns to a greater extent than SO characteristics (main effect of event type $F(1,18) = 13.12$, $p < .001$, Figure 2B). This was the case for event amplitudes ($t(18) = -3.16$, $p = .005$), durations ($t(18) = -2.53$, $p = .021$) as well as densities ($t(18) = -2.14$, $p = .046$). Furthermore, the correlation between all three spindle characteristics with the encoding pattern was significantly smaller than 0 (spindle amplitude: $mean_r = -0.38$, $t(18) = -3.50$, $p = .003$, spindle duration: $mean_r = -0.27$, $t(18) = -3.26$, $p = .004$, spindle density: $mean_r = -0.30$, $t(18) = -3.56$, $p = .002$). There was a trend for the main effect of event characteristic (amplitude topographies correlating strongest with encoding patterns, $F(2,36) = 2.61$, $p = .078$) and no interaction ($F(2,36) = 0.63$, $p = .532$).

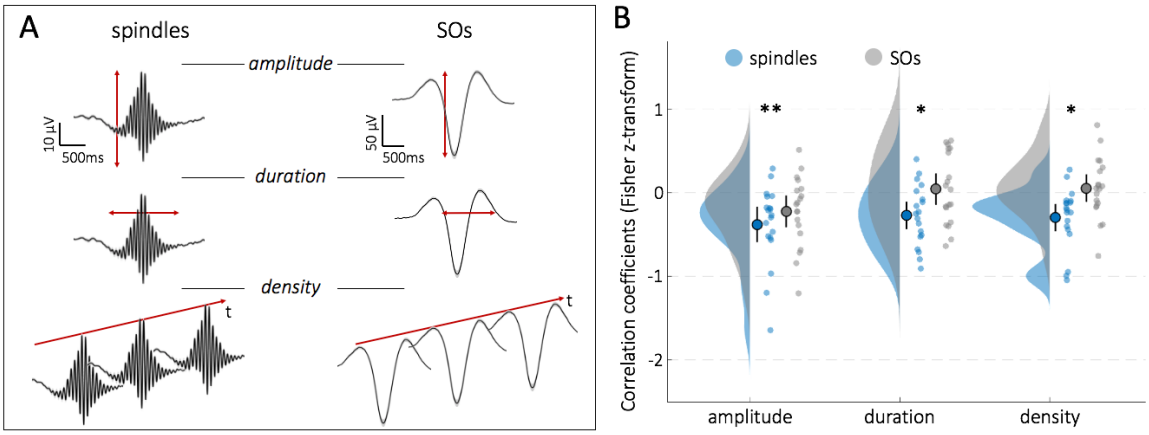

Figure 3.2. Sleep spindles track cortical learning sites. **(A)** Grand average (mean ± 95% CI) of spindles and SOs at electrode position Cz. Amplitude, duration and density were extracted for each participant and channel. **(B)** Across participants, encoding topographies were significantly more strongly correlated with topographies of spindles than SOs (density plots, group means, 95% CIs and single participant data are shown. ** = p < .01. * = p < .05).

To account for the possibility that spindle amplitude, duration and density topographies are correlated amongst each other, we conducted follow-up partial correlations between encoding patterns and spindle characteristics. Interestingly, the link between encoding and spindle amplitude remained significantly different from 0 when partialling out spindle duration or spindle density (for both: $mean_r < -0.23$, $t(18) < -2.11$, $p < .049$). When partialling out spindle amplitude, however, the overlap between spindle duration/density and encoding pattern was not significantly different from 0 (duration: $mean_r = -0.15$, $t(18) = -1.82$, $p = .086$; density: $mean_r = -0.01$, $t(18) = -0.15$, $p = .885$). Together, these results suggest that the spindle-encoding overlap is predominantly driven by spindle amplitude.

To examine whether the overlap with encoding patterns might be restricted to spindles that are coupled to SO up-states, we directly compared the encoding-spindle overlap (amplitude topographies) for spindle events with higher vs. lower coupling (see Methods). We observed no significant difference between the two event types ($t(18) = -0.66$, $p = .521$, see Figure S4).

Finally, we tested whether the overlap of sleep spindle amplitude and encoding activation is linked to behavioural expressions of memory consolidation. To this end, we correlated the encoding-spindle amplitude overlap with the relative change in sequence performance from pre- to post-sleep retrieval (memory retention) across participants. Indeed, a significant negative correlation was observed ($r = -0.58$, $p = .010$), indicating that participants who showed greater retention of sequence memory also had a greater overlap (signified by a more negative value) between encoding and sleep spindle topography (Figure 3A). To ensure that the link with behaviour was driven by participant-specific encoding-spindle overlap, we first shuffled the encoding topographies between participants while retaining participant-specific sleep spindle topography and behavioural

performance. Likewise, we shuffled the sleep spindle topographies between participants while retaining participant-specific encoding topography and behavioural performance (see Figure 3B for visualization). That way we generated a distribution under the null hypothesis that (a) the encoding topography or (b) sleep spindle topography is irrelevant for the observed correlation with behaviour. As shown in Figure 3C, the empirical correlation between encoding-spindle overlap and behaviour significantly exceeded the null distributions in both cases (p = .011 for (a) and p = .018 for (b) based on 1000 permutations).

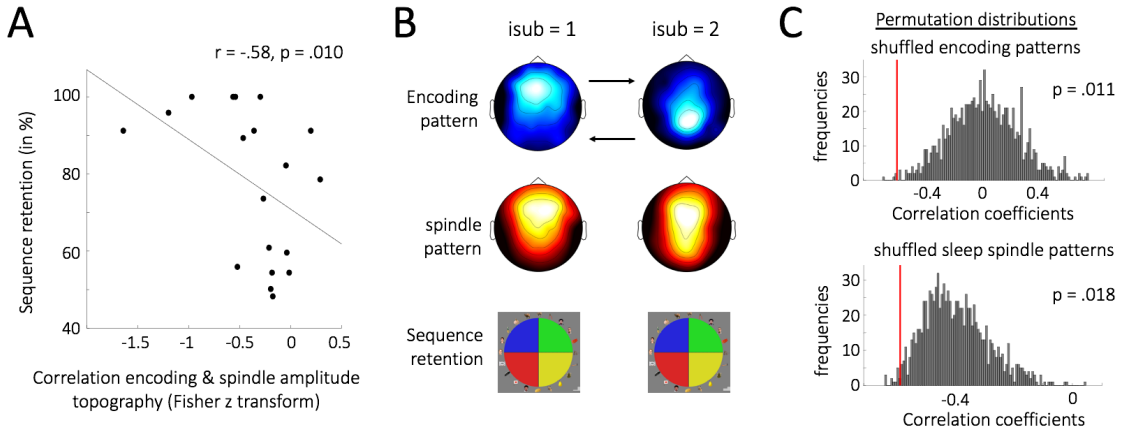

Figure 3.3. Extent of encoding-spindle overlap predicts memory consolidation. **(A)** The correlation between encoding and spindle amplitude topographies is predictive for sequence retention. The more negative the correlation between spindle amplitude and encoding topographies (i.e., the greater the overlap), the greater the levels of sequence retention across sleep. **(B)** Schematic for generating the null distributions in C. Encoding or sleep spindle topographies were shuffled across participants while the respective other topography (spindle amplitude or encoding power) and behavioural performance were retained. **(C)** The observed correlation between encoding-spindle amplitude overlap and sequence retention exceeds both null distributions of encoding or spindle pattern exchangeability.

3. Discussion

Despite accumulating evidence linking sleep spindles to memory consolidation (Born & Wilhelm, 2012; Fernandez & Lüthi, 2020b; Gais et al., 2002; Holz et al., 2012; Klinzing et al., 2016; Lüthi, 2014; Mednick et al., 2013; Schabus et al., 2004), their specific function has remained elusive. Here we tested predictions derived from biophysical/computational models, i.e., that spindles are preferentially expressed over learning-related sites where they might induce early stages of synaptic plasticity (Sejnowski & Destexhe, 2000). Using a recently developed paradigm sensitive to sleep-dependent memory consolidation (Petzka et al., 2021) and geared towards eliciting rich idiosyncratic encoding patterns (Figure 1), we first demonstrate that sleep spindles – in particular the topography of spindle amplitudes – track cortical patterns of memory encoding (Figure 2). This overlap of encoding patterns with spindles was significantly stronger than that with slow oscillations (SOs), ruling out spurious correlations driven by generic signal properties across EEG channels. Importantly, we additionally reveal a functional link between the observed encoding-spindle overlap and memory consolidation, expressed in greater overlap associated with greater levels of memory retention (Figure 3).

Previous work has revealed a number of spindle characteristics that render them well-suited for inducing local plasticity in task-dependent/learning-related brain regions. For instance, intracranial recordings in humans have shown that sleep spindles are local rather than global (Nir et al., 2011; Piantoni et al., 2017) and scalp EEG/MEG has demonstrated high levels of inter-subject variability of spindle topographies (Cox et al., 2017; Klinzing et al., 2016). Moreover, spindle topographies vary as a function of prior learning tasks at

the group level – spindles over left frontal areas are related to consolidation of verbal material (Clemens et al., 2005), whereas spindles over parietal areas are related to consolidation of visuospatial memories (Clemens et al., 2006). However, as brain activity was not measured during learning in these studies, the link between learning activation and post-learning spindle topography remained conjectural. Another study employed simultaneous EEG-fMRI recordings and found a BOLD increase in learning-related ventro-temporal regions time-locked to sleep spindles at electrode position Cz (Bergmann et al., 2012), but whether spindles per se would be preferentially expressed at learning-related sites remained unclear due to limited EEG coverage. Finally, we recently showed that learning content can be decoded in the presence of centrally recorded sleep spindles (Cairney, Guttesen, et al., 2018; Schreiner et al., 2021), but decoding was based on raw EEG data rather than on spindle topographies. In short, despite converging evidence that spindle expression can be local, idiosyncratic and flexible, we here provide first evidence that they track participant-specific learning patterns in service of memory consolidation. These results dovetail with a recent computational study showing that spindles promote independent reactivation of multiple memories at network locations corresponding to awake training (Wei et al., 2018).

It deserves mention that the spatial resolution afforded by scalp EEG is relatively coarse, only capturing macro-scale topographies of brain networks. Intracranial recordings/Electrocorticography (ECoG) would provide much finer resolution, albeit at the expense of comprehensive whole-brain coverage and consistency across participants. That said, a recent study used cortical microelectrode array recordings (Utah Arrays) in four pre-surgical epilepsy patients and demonstrated different spindle- and unit firing dynamics across a 10 x 10 electrode grid covering < 15 mm^2 (Dickey et al., 2021). This

suggests that spindle deployment might be sufficiently fine-tuned in space to selectively strengthen local microcircuits of learning networks.

A key open question is how exactly the deployment of spindles to learning-related cortical sites is governed. One speculative possibility is that circuit-specific encoding activation establishes transient synaptic tags. Spindles are initially broadcast widely and stochastically during sleep but resonate more strongly when coinciding with those synaptically primed circuits. This leads to higher spindle amplitudes and a concomitant increase in Ca^{2+} influx, completing the tag-and-capture cycle suggested to underlie long-term-potentiation (Redondo & Morris, 2011). Increased spindle amplitude, as observed here and in other studies (Bergmann et al., 2012; Cox et al., 2014; Yordanova et al., 2017), would thus reflect elevated local neural co-activation as a vestige of prior task engagement.

Another possibility is that the same thalamic circuits control deployment of attentional resources during wake task performance and spindles during sleep. For instance, a recent study capitalised on the orientation-specific response potentiation (OSRP) in mouse primary visual cortex (V1), which reflects enhanced firing to a visual grating of particular orientation several hours after initial exposure/training. Importantly, neurons in the lateral geniculate nucleus (LGN) of the thalamus already showed orientation-selective tuning during and immediately after training. During post-training sleep, thalamocortical coherence mediated by sleep spindles drove post-sleep orientation-selective tuning in V1 (Durkin et al., 2017). Additional work is needed to elucidate whether similar mechanisms apply to more complex tasks in humans, but accumulating evidence across species has linked thalamic microcircuits to a wide range of cognitive tasks (Halassa & Kastner, 2017). This scenario, in which thalamocortical dynamics during learning bias the path of

spindle deployment during sleep, is reminiscent of models of hippocampal functioning. Specifically, according to the hippocampal indexing theory (Teyler & Discenna, 1986), the hippocampus retains pointers to cortical circuits involved in learning. Upon presentation of a partial cue, hippocampus drives reinstatement (pattern completion) in cortical target sites. Recent work in rodents (Rothschild et al., 2017) and humans (Ngo et al., 2020) points to a cortical-hippocampal-cortical loop around hippocampal ripples, and a tentative scenario might be that the initial cortical response in this loop is mediated by the aforementioned thalamo-cortical spindle projections.

Apart from spindles, memory processing during sleep has been linked to slow oscillations (SOs) and delta (1-4 Hz) rhythms, together referred to as slow wave activity (SWA) (Born & Wilhelm, 2012; Marshall et al., 2006; Rasch & Born, 2013; Tononi & Cirelli, 2006). One seminal study showed that SWA was specifically increased over central cortical areas thought to be involved in prior motor learning (Huber et al., 2004). Likewise, wake immobilisation of a participant's arm led to a decrease in SWA over corresponding motor areas (Huber et al., 2006). Interestingly though, analogous effects were seen in the spindle/sigma band in both studies, raising the possibility that both SOs/SWA and spindles contribute to sleep-dependent consolidation. One pressing question is whether consolidation relies on concomitant or on sequential occurrence of these two sleep events. Speaking to the importance of concomitant SWA-spindles, a recent rodent study showed that Ca^{2+} activity was increased threefold when spindles were coupled to slow oscillations (Niethard et al., 2018). The importance of coupled SO-spindle complexes has been further corroborated by a series of recent findings linking the precision of SO-spindle coupling to memory function in ageing (Helfrich et al., 2018; Muehlroth et al., 2019) and to reinstatement of prior learning experiences during sleep (Schreiner et al., 2021).

However, we did not observe greater overlap of encoding patterns with spindles coupled vs. not coupled to SOs in the current study (Figure S4). Interestingly, a recent EEG/MEG study showed that the topography of spindles was unaffected by the topography of concurrent SOs (Klinzing et al., 2016). This raises the possibility that consolidation of learning patterns relies, at least in part, on the sequential occurrence of spindles and SOs. Indeed, in the original framework (Sejnowski & Destexhe, 2000) as well as in a recent computational model (Wei et al., 2018), it is proposed that SOs, which show enhanced prevalence during later sleep stages, further potentiate strong synapses, incidentally leading to downscaling of weak synapses (Tononi & Cirelli, 2003). In other words, sleep-dependent consolidation might rely on a multi-stage tagging and capture sequence, initiated by wake task performance, potentiated by thalamocortical sleep spindles in conjunction with hippocampal ripples, and completed by SOs. Whole-night recordings would be better-suited to test this notion than the current nap design.

Apart from the nap design, there are additional limitations in the present study. First, conclusions remain correlative rather than causal. A previous study (Lustenberger et al., 2016) used transcranial alternating current stimulation (tACS) to enhance sleep spindles, leading to improved post-sleep motor memory performance. It would be intriguing to test whether 'playing back' participant-specific learning patterns in the form of exogenously induced spindle topographies enhances consolidation in our current paradigm. However, the spatio-temporal precision required for this endeavor (simultaneously inducing different spindle amplitudes at different locations across cortex) would exceed capacities of current human non-invasive brain stimulation (NIBS) tools. Second, the nature of our paradigm precludes a direct link between encoding patterns and behavioural performance during pre-sleep retrieval as well as requires an external baseline (PVT). Specifically,

unlike in one-shot event-related memory paradigms, retrieval performance here is the product of encoding and multiple rounds of training including feedback. Further, the absence of clear events (i.e., trials) entails the absence of a baseline for the EEG analysis. Consequently, an external baseline had to be used. As the PVT resembles the nature of a pre-stimulus baseline in event-related memory paradigms (fixation of a fixation cross in the center of the screen), we utilized it as the external baseline. It is worth considering that the observed encoding pattern was calculated relative the external baseline and cannot be interpreted independently of the activity PVT. Nevertheless, the encoding pattern observed here (alpha/beta power decreases over temporo-parietal areas) is consistent with previous studies of memory formation (Fellner et al., 2013; Griffiths et al., 2016; Hanslmayr et al., 2009; Klimesch et al., 1996).

To conclude, the present study demonstrates that sleep spindles track cortical areas engaged during prior learning and that the extent of learning-spindle overlap predicts levels of memory consolidation. An exciting avenue for future work will be to elucidate the spatiotemporal dynamics between spindles, ripples and slow oscillations across the hippocampus and neocortical sites, both in close temporal proximity and across a whole night of sleep.

4. Methods

4.1. Participants

22 participants were tested. Due to technical issues during data collection, 3 participants had to be excluded resulting in 19 participants for the final sample ($mean_{age}$ = 20.7, $range_{age}$ = 18-31, female = 15).

Pre-screening ensured that participants had no history of neurological or psychiatric disorders and a normal sleep-wake cycle. Participants were instructed to get up one hour earlier than normal and avoid caffeine the day of the experiment. After participating in the study, participants received monetary reimbursement. The study was approved by the University of Birmingham Research Ethics and Governance Committee and written informed consent was obtained from all participants before the start of the study.

4.2. Paradigm and procedure

Memory Arena and PVT were implemented via custom scripts in MATLAB 2016a (MathWorks, Natick, USA). For the PVT, functions of the Psychophysics Toolbox Version 3.0.14 (Brainard, 1997) were used.

Memory Arena

The *Memory Arena* consists of a circle divided into coloured quarters (upper left: blue, upper right: green, lower right: yellow, lower left: red). Within the circle, objects are sequentially presented in different spatial positions. Participants have to learn both the sequence in which the objects were presented as well as the spatial position of each object.

20 target objects (images of 5 faces, 5 natural objects, 5 animals and 5 manmade objects) were randomly selected from a stimulus pool of 40 objects (coloured and presented on a grey 90x90 pixels square) (Kriegeskorte et al., 2008; Mehrer et al., 2021). The spatial position of each object was restricted by the position of other objects. Consequently, there was no overlap between objects, but objects possibly covered more than one colour wedge.

Psychomotor Vigilance Task (PVT)

A white fixation cross was presented in the middle of the screen. After on average 6 seconds (jitter ± 4s), a counter replaced the fixation cross. The counter started at 0 and counted forward in 20 ms steps to 2000. Upon start of the counter, participants had to press the space bar as fast as possible. After the key press, feedback about their reaction time was displayed for 2s (Figure 1B). Overall, the PVT lasted 2 minutes.

Procedure

The experimental session started at 10 am with the application of electroencephalography (EEG), electromyography (EMG) and electrooculography (EOG).

Approximately one hour later at 11 am, participants started with a short practice session (~20 sec) of the PVT which was followed by the actual task. Before they continued with the Memory Arena, participants received written instructions and performed a practice session (with 3 objects) of each Memory Arena part (encoding, training and retrieval). Participants were instructed to associate and combine the objects into a coherent story.

During the encoding part of the Memory Arena, all 20 objects were sequentially presented within the circle. Participants confirm processing of each object by clicking on it. The current object then disappeared, and the next object was presented.

Directly after the encoding part, a training session was conducted. The training session started with all 20 objects arranged around the arena. The objects had to be dragged and dropped in the correct sequence to their correct spatial position. If an error was made regarding the sequence or spatial position, the arena turned red and the error was corrected. Sequence errors were defined as object *i* not being placed at the i^{th} position. Spatial errors were defined as the overlap between correct and chosen position of an object being less than 25%. After potential error corrections, the object remained at its correct spatial position and the next object had to be selected and placed in the arena. When all 20 objects were placed, feedback about the overall performance was presented. The overall performance was defined as the number of correct objects divided by the total number of objects, where an object was classified as correct when sequence as well as spatial position were correct. Participants finished training after reaching 70% overall performance in two consecutive runs.

A second PVT then followed the training session. After the PVT, the pre-sleep retrieval was completed. Like the training, the retrieval started with all 20 objects arranged around the arena which had to be dragged and dropped in the correct sequence to their correct spatial position. Importantly though, errors were not corrected and no feedback was provided.

Participants started the 2-hour nap between 1pm - 2.30pm (see Table S1 for descriptive sleep data). Following the nap, participants continued with an interference task. We were particularly interested in using an experimental design that is suitable to capture sleep-dependent memory consolidation. In a previous study, we used the same task (Memory Arena) and found sleep-dependent consolidation effects with a combination of a 2x70% training threshold (70% overall performance in two consecutive runs) and the induction

of retroactive interference directly after sleep (Petzka et al., 2021). Therefore, in this study, we applied the same methods.

During the interference task, participants had to encode the same 20 objects but in a different sequence and at different spatial positions (Figure 1A). The difference between the old and the new, interfering, spatial position of every object was at least 5 pixels (Euclidean distance). Encoding of the interfering positions was conducted in the same way as the original encoding. Subsequently, participants had to retrieve the interfering sequence and spatial positions (without prior training). Lastly, another PVT and the post-sleep retrieval (of the original sequence and spatial positions) were performed. Until the start of the post-sleep retrieval, participants were unaware of the final test.

4.3. EEG data recording

EEG data were recorded using a Brain Products 64-channel EEG system and were sampled at a rate of 1000 Hz. Electrodes were arranged according to the 10-20 system (including FCz as reference, AFz as ground and left and right mastoids). Two electrodes were placed on the chin to record muscle activity (electromyography, EMG) and two electrodes recorded eye movements (electrooculography, EOG).

4.4. Behavioural analysis

In a previous study using the same paradigm, we found that sequence performance was most sensitive measure to capture sleep-dependent memory consolidation (Petzka et al., 2021). Consequently, all following analyses focus on sequence performance.

To calculate sequence performance, the selected order of all 20 object was correlated with a vector ranging, in ascending order, from 1 to 20. This correlation approach is preferable

to simply counting the correct sequence position of every object, as it reflects both the correct sequence position of every object as well as correct transitions between objects. Correlation values were Fisher z-transformed for further statistical analyses. To test for a significant sequence performance change from pre- to post-sleep retrieval, a paired t-test was computed.

For correlating the change in sequence performance with the EEG data, a sequence retention score was calculated as the relative change from pre- to post- sleep sequence retrieval: 100*(post/pre).

4.5. EEG analysis

EEG analyses were performed using the FieldTrip toolbox (Oostenveld et al., 2011) and custom written scripts in MATLAB.

Encoding pattern

To remove eye movements from the data, an independent component analysis (ICA) was used. Data were down-sampled to 200 Hz, re-referenced to linked mastoids, filtered (high-pass: 1 Hz, low-pass: 100 Hz, band-stop filter: 48-52 Hz), demeaned and segmented in 2 second epochs for a first visual artifact rejection. All phases of the Memory Arena and the PVT were concatenated, coarse artifacts were removed based on outliers regarding amplitude, kurtosis and variance (implemented in *ft_rejectvisual*) and bad channels were rejected. Based on those data, the unmixing matrix was obtained and bad components were identified. The raw data were then preprocessed again, as the first preprocessing was optimized for ICA. The data were down-sampled to 200 Hz, re-referenced to linked mastoids, filtered (high-pass: 0.3 Hz, low-pass: 40 Hz) and

demeaned. The unmixing matrix was applied to the new preprocessed data and bad channels were interpolated.

To derive encoding patterns, the encoding part of the Memory Arena was contrasted against the PVT. Note that the PVT was conducted in temporal proximity to the Memory Arena and requires sustained attention but no memory-related processes. The data recorded during the encoding part of the Memory Arena and the PVT were segmented into 1 second epochs (50% overlap), tapered with a Hanning window and transformed from time to frequency domain using Fast Fourier Transformation. To facilitate reproducibility of results, artifacts were defined based on the 95th percentile uniquely for each frequency bin distribution (across epochs). All 1 second epochs above the 95th percentile were labelled as artifacts and excluded. Note that results did not qualitatively change as a function of the chosen percentile (applying the 90th or 85th percentile as a threshold revealed similar results). Power spectra obtained from encoding and PVT were contrasted (encoding - PVT), yielding absolute power changes during encoding relative to the PVT.

Significant frequency bins were defined based on the group statistics by applying a two-sided cluster-based permutation test with 1000 randomisations (Maris & Oostenveld, 2007). The topography for each participant was then derived by collapsing power values across the significant frequency bins for each channel separately resulting in a 1 x channel (=58) vector.

Event Detection

Sleep spindles and SOs were detected for each participant, based on established detection algorithms (Ngo et al., 2013; Staresina et al., 2015). Like wake data, sleep data were

down-sampled to 200 Hz, re-referenced to linked mastoids and filtered (high-pass: 0.3 Hz, low-pass: 40 Hz). Bad channels were matched between wake and sleep data, excluded and interpolated. Finally, to identify and mark coarse artifacts, data were visually inspected. Channelwise event detection of both sleep spindles and slow oscillations were conducted on data from non-rapid eye movement (NREM) sleep stages 2 and 3. Events were only included if free of artifacts between 1s before and 1s after the event.

To detect fast sleep spindles, data were band-pass filtered between 12-15 Hz (4^{th} order two-pass Butterworth filter). The envelope of the signal was calculated with a moving average of 200ms. An amplitude criterion (mean + 1.25*SD) was applied to the signal. Sleep spindles were detected when the signal exceeded the amplitude criterion for more than 0.5 but less than 3 seconds (duration criterion).

The maximum of the envelope of each detected spindle was used as the amplitude measure. Duration was the time from beginning to end of each event and density was calculated as the number of detected events / total (artifact free) time spent in NREM sleep stage 2 and 3.

To detect slow oscillations, data were band-pass filtered between 0.3-1.25 Hz (4^{th} order two-pass Butterworth filter). Zero crossings were identified, and three criteria (duration criterion, trough to peak criterion and amplitude criterion) had to be fulfilled. The length criterion was met if one positive to negative crossing was followed by a second positive to negative crossing within a time window of 0.8 to 2 seconds. Based on all sufficiently long events, mean and standard deviation were calculated for trough to peak amplitudes as well as for absolute values of trough amplitudes. All events exceeding both means + 1.25*SDs were considered slow oscillations.

The amplitude of SOs was defined as the most negative trough (downstate). To facilitate comparability between sleep measures, the absolute value of SO amplitudes was used. Thus, the downstate became positively scaled to match all other sleep measures. Duration was defined as the time between the first positive to negative and the following positive to negative crossing. Slow oscillation density was calculated by dividing the number of detected events by the total (artifact free) time spent in NREM sleep stages 2 and 3.

Amplitude, duration and density of detected spindles and SOs were extracted per channel and averaged across events (amplitude, duration) resulting in 6 different 1 x channel (=58) vectors.

Coupling of sleep spindles

For each spindle event, the phase of the EEG trace filtered in the SO frequency band was extracted. To this end, the data around each spindle event were filtered from 0.3 – 1.25 Hz. After applying a Hilbert transform, the instantaneous phase angle at the maximum of the envelope of each detected spindle was extracted.

To compare spindles with higher vs. lower coupling, all spindle events per channel were classified based on their phase value. The 50% of spindle events with a phase value closest to 0 degrees were classified as spindles with a higher coupling. The remaining 50% of spindle events were classified as spindles with a lower coupling.

Comparison between encoding and sleep pattern

For each participant, the 1x58 vector obtained from encoding was correlated (Spearman's rho) with every 1x58 vector of the sleep characteristics (amplitude, duration and density for spindles and SOs). Correlations were then Fisher z-transformed for group statistics.

4.6. Statistics

Correlation distributions between encoding and sleep topographies were tested with a 2 (event type: spindles vs. SOs) x 3 (event characteristic: amplitude, duration, density) repeated measures ANOVA. Paired sampled t-tests and one-sample t-tests were used for post-hoc comparisons.

Partial correlations were conducted to test for mediating effects of spindle characteristics (e.g., spindle density) on the correlation between encoding and another spindle characteristic (e.g., spindle amplitude).

To test for an association between sequence retention and the encoding-spindle overlap, the Spearman's rho correlation was conducted. To rule out that the correlation with behaviour is solely driven by either sleep spindles or by encoding power, we applied a permutation approach and shuffled topographies between participants (1000 permutations). To obtain the observed correlation, we derived, for each participant, (i) behavioural performance, (ii) encoding topography and (iii) sleep spindle topography. By shuffling only one topography (encoding or sleep spindles) between participants while retaining the other participant-specific topography and behavioural performance, two null distributions were generated: First, a distribution under the null hypothesis that the participant-specific encoding topography is irrelevant for the correlation with behaviour (shuffling the encoding topographies). Second, a distribution under the null hypothesis that the participant-specific sleep spindle topography is irrelevant for the correlation with behaviour (shuffling the spindle topographies). The observed correlation was then tested against both null distributions.

5. Acknowledgements

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6. Author Contributions

Conceptualisation: M.P., A.C., B.P.S.; Methodology: M.P., I.C., B.P.S.; Investigation: A.C.; Formal Analysis: M.P., B.P.S.; Writing - Original Draft: M.P., B.P.S.; Writing – Review & Editing: M.P., A.C., B.P.S.; Visualisation: M.P., B.P.S.; Supervision: G.M.B., B.P.S.; Funding Acquisition: B.P.S.

7. Supplemental Information

Table S 3.1. Descriptive sleep data in minutes (mean ± SEM). n = 19. TST = total sleep time.

N1	**N2**	**N3**	**REM**	**TST**
18.39	52.53	10.42	16.24	103.58
±2.46	±3.66	±2.56	±2.85	±2.28

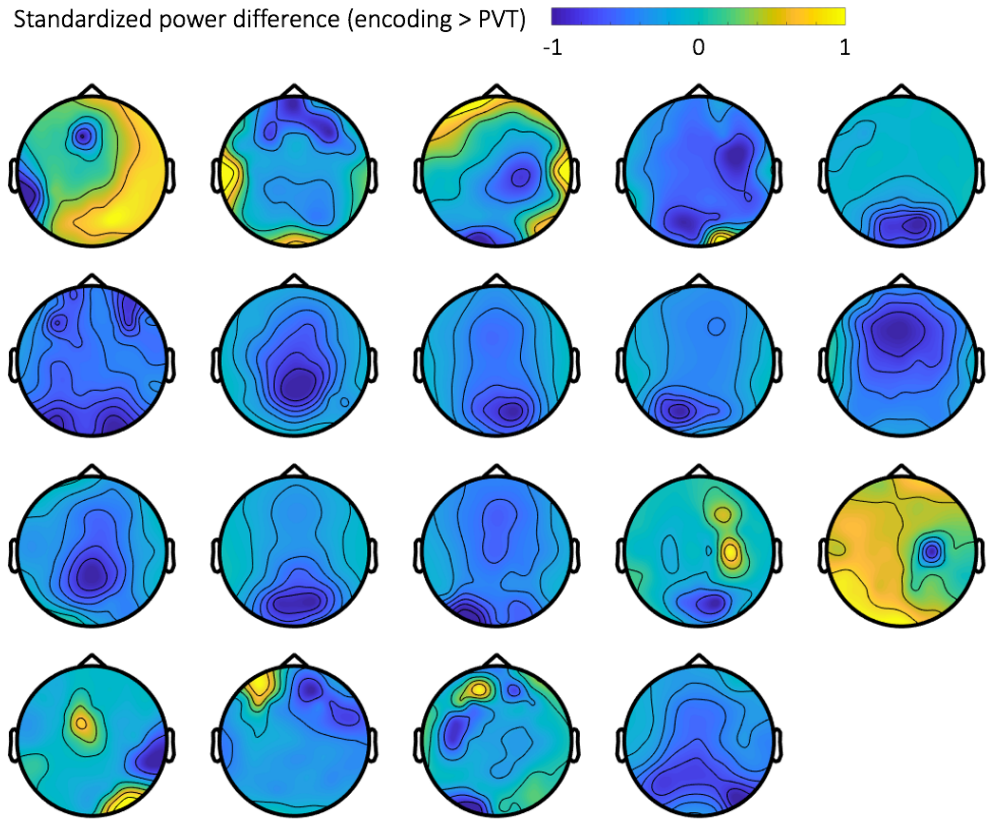

Figure S 3.1. Participant-specific topographies of the 6-20 Hz power changes during encoding relative to the PVT. Power changes were standardized between 1 and -1 for comparability across participants.

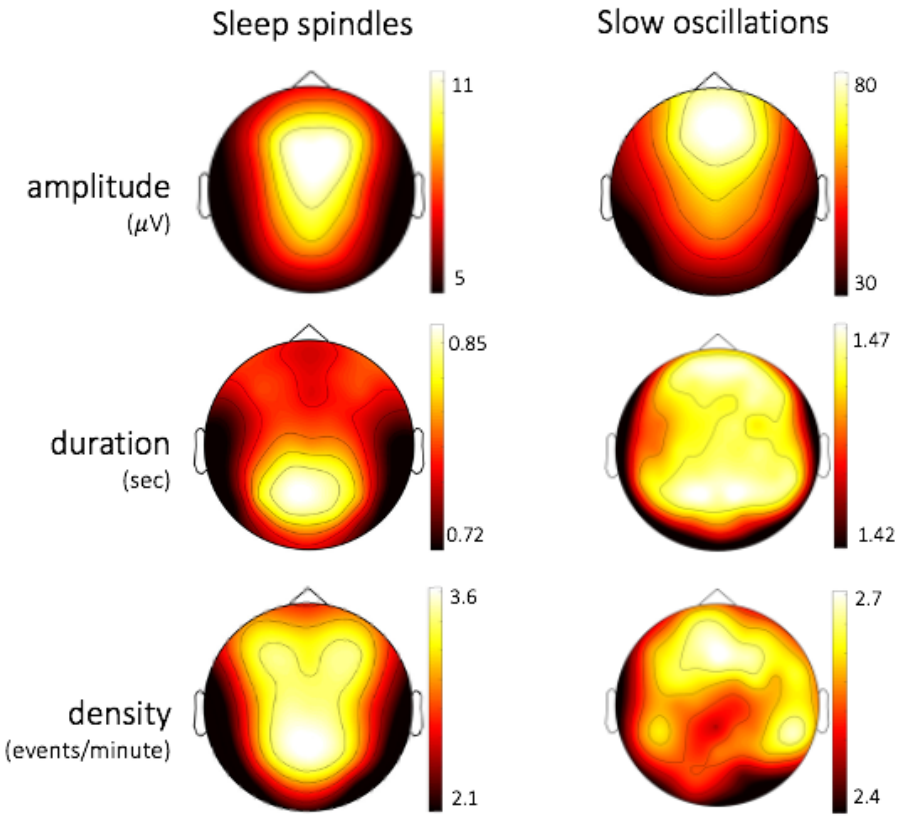

Figure S 3.2. Group-level topographies of amplitude, duration and density of sleep spindles and slow oscillations.

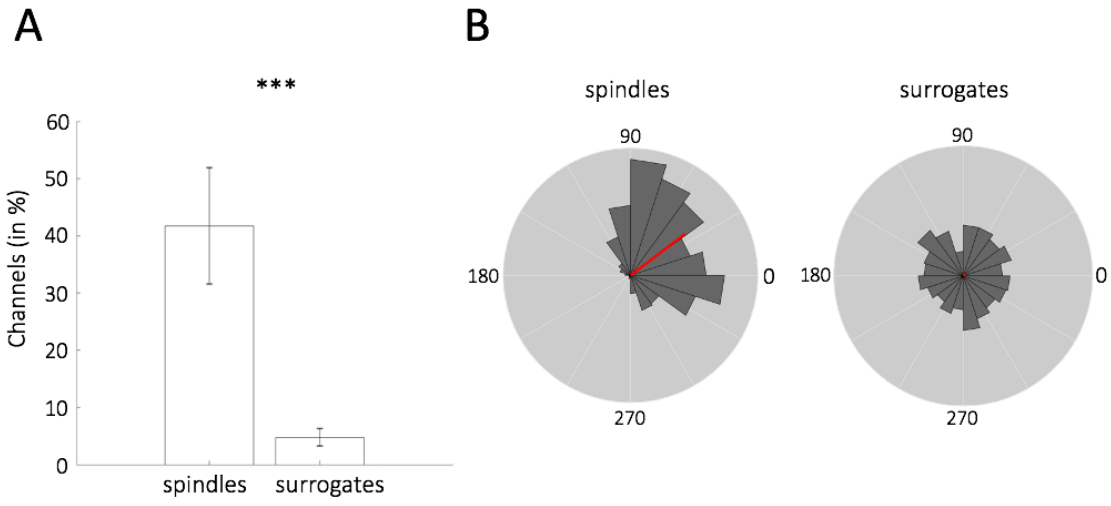

Figure S 3.3. Coupling of spindles and surrogate events to the phase of the signal filtered in the SO frequency range (0.3 -1.25). **(A)** Mean (+/- 95% confidence intervals) percentage of channels on which sleep spindles and surrogate events are significantly

coupled (defined by a significant deviation from a uniform distribution, Rayleigh test: $p < .05$). Spindles are coupled to SOs on significantly more channels than surrogates. Surrogates were matched control events - for each detected spindle, a spindle-free epoch within 15 seconds before or after the actual spindle event was identified (Ngo et al., 2020). The instantaneous phase angle of the SO filtered and Hilbert transformed signal was then extracted at the centre of the spindle-free epoch. **(B)** The corresponding phase (in degrees) of spindle maxima (left) and surrogate centres (right) plotted across all detected events on channels with significant spindle coupling (including all participants, fixed-effects). While spindles significantly cluster at a phase of 37 degrees (Rayleigh test: $z = 158.86$, $p < .001$, resultant vector length = 0.59), surrogates do not deviate from a uniform distribution (Rayleigh test: $z = 0.94$, $p = 0.392$).

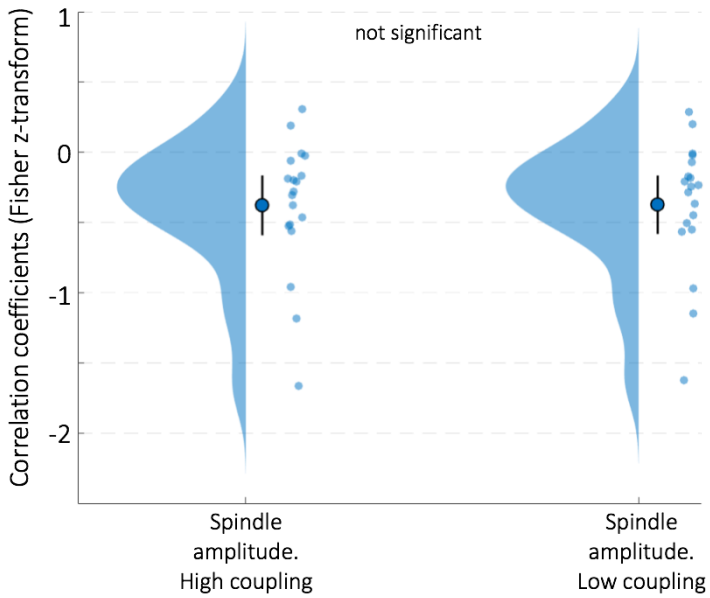

Figure S 3.4. No differential encoding-spindle amplitude overlap for spindles with higher (left) vs. lower (right) coupling to the SO up-state.

Chapter 4. Slow oscillations are the pacemaker for sequential memory reactivation during sleep

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This chapter represents preliminary results.

Abstract

Consolidation of memories relies on their reactivation during post-learning sleep. While most memories incorporate a sequential order of events, the timescale on which sequential events are reactivated during sleep in humans is still elusive. To identify and characterise reactivation of sequential memories, we here employed a sequential memory task and recorded high-density scalp electroencephalography (EEG) during a post-learning nap. During the post-learning nap, we presented sounds associated with the encoded sequences (targeted memory reactivation, TMR) to induce sequential memory reactivation and applied multivariate pattern analysis (MVPA) to then capture reactivation. In response to the sequence-related sounds, we found reactivation of the first but not the second sequence element. Critically, when realigning the data to sound evoked slow oscillations, reactivation of the second sequence element was nested in their up-states. Our results provide first evidence of sequential memory reactivation during sleep in humans and moreover, attribute the timing of sequential memory reactivation to slow oscillations.

1. Introduction

How are memories consolidated during sleep? Memory reactivation, the re-occurrence of a neural pattern representing a memory trace, has been proposed as the mechanism for systems consolidation. Due to repeated reactivation, memory traces are redistributed from hippocampus to neocortex and hence, transformed from labile into stable representations (Born & Wilhelm, 2012; Diekelmann & Born, 2010; Klinzing et al., 2019). Evidence for reactivation during sleep as a mechanism for memory consolidation was provided by recent studies exploiting the development of new methods (e.g. representation similarity analysis, RSA and multivariate pattern analysis, MVPA, Grootswagers, Wardle, & Carlson, 2017; Kriegeskorte, 2008; Norman, Polyn, Detre, & Haxby, 2006) allowing to measure reactivation. That is, by applying RSA or MVPA to pre-sleep wake as well as sleep data, it was demonstrated that memory traces are reactivated during post-learning sleep and further, that the strength of reactivation predicted memory consolidation (Schreiner et al., 2018, 2021; Zhang et al., 2018). However, while these studies focused on paired associations, more naturalistic memories tend to incorporate a cascade of sequentially ordered events. The time scale on which sequentially ordered events are reactivated during sleep has yet to be explored.

In rodents, sequential reactivation (replay) has been proposed to occur in a temporally compressed manner in both hippocampal and cortical areas (Ji & Wilson, 2007; Nádasdy et al., 1999; Skaggs & McNaughton, 1996). In humans, however, empirical evidence for sequential memory reactivation during sleep, to the best of our knowledge, is still lacking. One study demonstrated a re-occurrence of reactivation patterns in a 1 Hz rhythm indicating a timing of multiple reactivation patterns by slow oscillations (Schreiner et al.,

2018). Slow oscillations are hypothesised to time simultaneous memory reactivation in hippocampal and neocortical areas to enable a redistribution of memory traces from hippocampus to neocortex (Rasch & Born, 2013). Whether sequential memory reactivation follows the rhythm of slow oscillations or whether it happens on a much faster time scale is still unknown.

This study aims to assess the time scale of sequential memory reactivation during post-learning sleep in humans. To this end, we employed a sequential memory task in which participants had to encode (object-)face-scene sequences whereas objects were always the cues and faces and scenes were always the targets (faces = first and scenes = second sequence element). To induce sequential reactivation of faces and scenes, sounds that were semantically related to the objects (sound cues) were played during subsequent sleep (targeted memory reactivation, TMR, Rudoy, Voss, Westerberg, & Paller, 2009; Schreiner & Rasch, 2015). Multivariate pattern analysis revealed reactivation of the first (faces) but not the second (scenes) sequence element in response to sound cues. Intriguingly, when realigning the data to slow oscillations evoked by sound cues, reactivation patterns of the second sequence element emerged. Our findings identify slow oscillations as the pacemaker for sequential memory reactivation during sleep.

2. Results

2.1. Targeted reactivation of sequential memories increases memory performance

We employed a sequential memory task in which participants associated an object (presented visually and auditorily) with two sequence elements presented in a fixed sequential order: a face and then a scene (Figure 1A). A fixed order was used as we wanted to increase the chances to detect sequential reactivation during sleep (see discussion). Memory for faces and scenes was assessed via a cued recall directly before sleep (retrieval 1, Figure 1A). Based on retrieval 1 performance, half of the correctly remembered face-scene sequences were assigned to a cued and the other half to a non-cued condition. A face-scene sequence was correctly remembered when both the face and the scene were correct. Sound cues (semantically related with the objects) of face-scene sequences of the cued condition were then played during the following 2-hour nap (targeted memory reactivation, TMR, see Table S1 for descriptive data of sleep stages). Memory for faces and scenes was assessed again directly after the nap (retrieval 2) and the next morning following a full night of sleep at home (retrieval 3, Figure 1B).

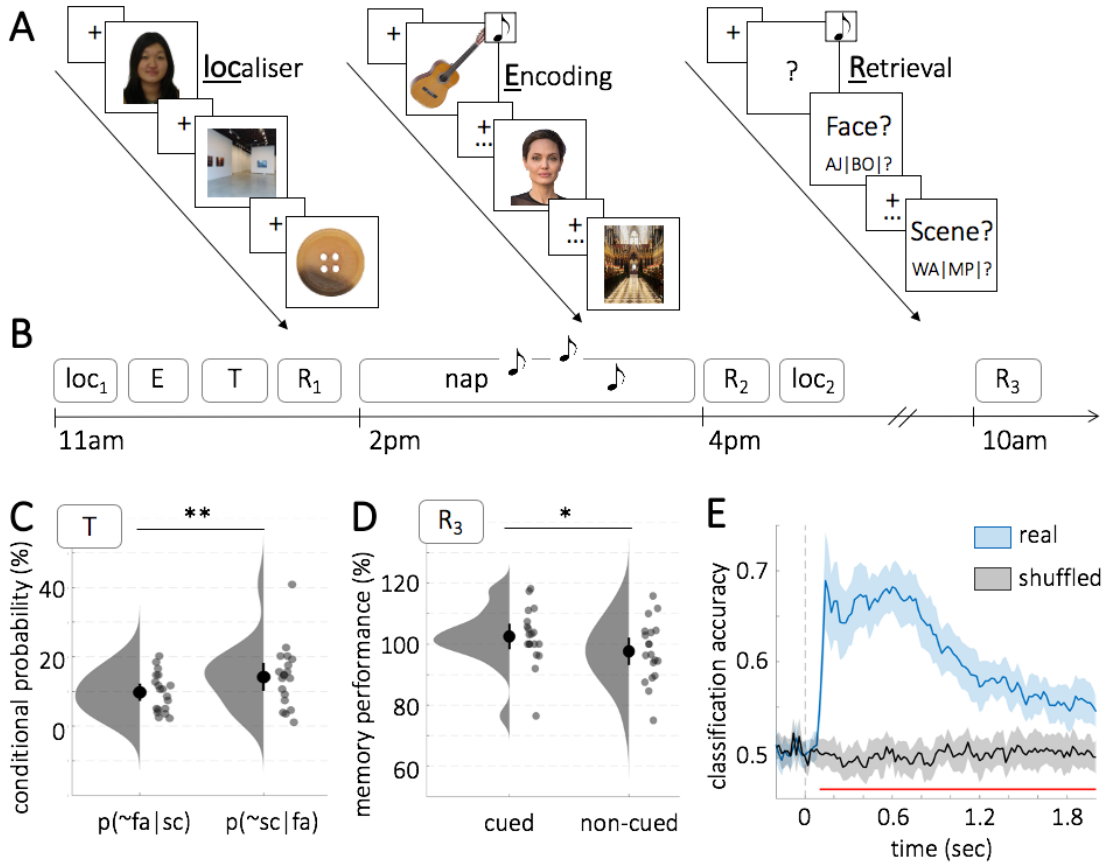

Figure 4.1. Task and experimental design, behavioural results and localiser task. **(A)** During the localiser task, unfamous faces, scenes and objects (not used for the memory part) were presented in a randomised order. During encoding, sequences were presented which always started with an object (presented visually and auditorily) followed by one of two faces (Angelina Jolie, AJ or Barack Obama, BO) followed by one of two scenes (Westminster Abbey, WA or Machu Picchu, MP). A cued recall was used to assess memory performance (retrieval). A question mark was presented together with a sound cue (presenting an object auditorily). After 5 seconds, a legend asking for the correct face was shown which was then followed by a legend asking for the correct scene. **(B)** Participants performed the first localiser (loc1), encoding (E), training (T) and first retrieval (R1) before taking a 2-hour nap. While participants slept, sound cues were presented (targeted memory reactivation, TMR). Following the nap, a second retrieval (R2) and a second localiser (loc2) were conducted. Participants did a third retrieval (R3) the next morning. **(C)** During training (T), the probability to forget a face (fa) given that the scene (sc) was correctly remembered is significantly lower than the probability to forget a scene given that the face is correctly remembered. Density plots, group means with 95% CIs and single participant data are shown. ** = $p < .01$. **(D)** Memory performance of retrieval 3 (R3, proportion of R2 memory) is significantly higher for the cued compared to the non-cued condition. Density plots, group means with 95% CIs and single participant data are shown. * = $p < .05$. **(E)** Stimulus categories (faces vs. scenes)

could be classified based on the EEG localiser data from ~150ms post stimulus onset (dotted line, time = 0). The red line indicates significantly higher classification accuracies for the actual class labels (blue) compared to a distribution where class labels were shuffled (black).

To test whether participants encoded faces and scenes in the correct sequential order, we calculated conditional probabilities for two different outcomes: First, we calculated the probability to forget a face given that the scene is correctly remembered (p(~face|scene)) and second, we calculated the probability that a scene is forgotten given that the face is correctly remembered (p(~scene|face)). We hypothesized that the first outcome (p(~face|scene)) is less likely if memories were encoded in the correct sequential order as forgetting the face interrupts the sequence and therefore, makes it more difficult to remember the scene. Indeed, we found a significantly lower probability for the first (p(~face|scene)) compared to the second (p(~scene|face)) outcome ($t(18) = -2.99$, $p = .008$). Conditional probabilities were calculated based on the training data (retrieval with feedback, see Figure 1B and methods). To obtain representative values for conditional probabilities of these two outcomes, enough trials of either just remembering the face or just remembering the scene are required. During retrieval 1, most trials were either completely correct (face and scene remembered) or incorrect (face and scene forgotten). Therefore, conditional probabilities were calculated based on the training data. Lower conditional probabilities for forgetting faces given that scenes are correctly remembered suggest that faces were, on average, encoded first (Figure 1C).

Next, we turned to the question whether cueing during sleep results in greater memory performance. Memory performance was defined as the number of hits (correctly remembering both the face and the scene) and compared between cued and non-cued

sequences. Memory performance at retrieval 2 (proportion of retrieval 1 hits that were also remembered at retrieval 2) did not differ between cued and non-cued sequences ($t(18) = -0.02$, $p = .987$, see Figure S1). Interestingly and in line with previous findings (Cairney, Guttesen, et al., 2018), memory performance at retrieval 3 (proportion of retrieval 2 hits that were also remembered at retrieval 3) was superior for cued vs. non-cued sequences ($t(18) = 2.69$, $p = .015$, Figure 1D) suggesting that the detection of TMR effects relies, at least partly, on overnight consolidation processes (see discussion).

2.2. Sequence elements (faces and scenes) can be classified during the localiser task

EEG data of the localiser task were used to extract neural patterns representing face vs. scene processing. This was done by training a classifier (linear discriminant analysis, LDA) on the EEG localiser data to differentiate between face and scene trials. Later, this classifier was applied to the EEG sleep data to search for the re-occurrence of category-specific neural patterns in response to the sound cues (reactivation).

During the localiser, faces and scenes, which were not shown during the memory part, were presented in a randomised order and participants had to identify the correct category (face, scene) of the presented image. As expected, participants performed the task with high accuracy (faces: $mean_{acc} = 99.5\%$, $95\%CI_{acc} = 0.48\%$; scenes: $mean_{acc} = 98.8\%$, $95\%CI_{acc} = 1.15\%$). To extract category-specific neural patterns, a multivariate pattern analysis (MVPA) was conducted on the pooled localiser data from -0.2 pre- to 2 sec post-stimulus. A fivefold cross-validation (train and test the classifier on the same task data, see methods) revealed a sustained above-chance classification starting at ~150ms post-stimulus ($p < .001$ at each time point, cluster-corrected, Figure 1E). Therefore, based on

the localiser data, neural patterns representing the processing of face and scene images could be extracted.

2.3. Targeted memory reactivation elicits reactivation of the 1st sequence element (faces)

We hypothesised that the presentation of sound cues (objects) during sleep induce the sequential reactivation of faces and scenes. In other words, we tested whether category-specific neural patterns, obtained from the localiser data, re-emerged in response to sound cues presented during sleep in the same order as they were encoded (faces = first sequence element, scenes = second sequence element). For this, we trained a classifier on each time point of the localiser data (0 to 2 sec) to extract neural patterns representing faces and scenes. We then applied the obtained classifier weights to each time point of the sleep data (-0.2 to 2.5 sec after sound cues, see Figure S2 for the time frequency decomposition and event-related potential). In the resulting localiser time x sleep time matrix, positive values reflect evidence for neural patterns representing face processing and negative values indicate evidence for neural patterns representing scene processing. As shown in Figure 2A (bottom), we indeed found a positive cluster in response to the sound presentation peaking at around 600 ms post-cue (corrected using a cluster-based permutation test, p = .016). While the sound presentation elicited a positive cluster indicating reactivation of face representations, no negative cluster reflecting reactivation of scene representations survived the correction for multiple comparisons (all negative clusters $p > .253$).

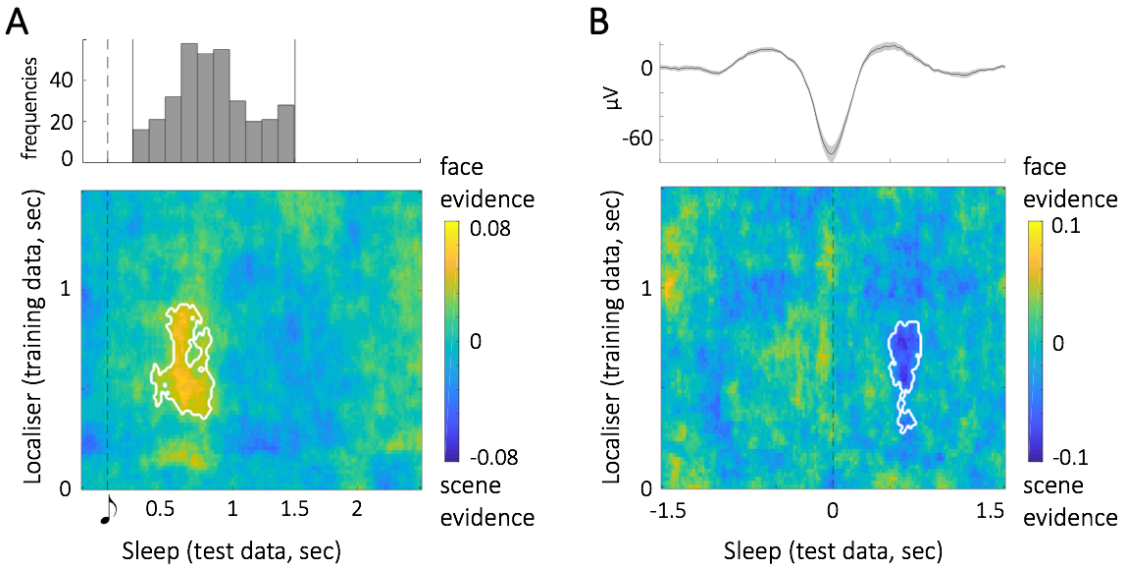

Figure 4.2. Sequential memory reactivation in response to sound cues and evoked slow oscillations. **(A)** Top: The histogram shows how many down-states of evoked slow oscillations (number of events, frequencies) were detected at which time point across all participants (fixed-effects). Bottom: Face representations were classified ~600 ms after sound cues (dotted line = sound onset; white contour lines indicate the significant cluster when comparing the actual data vs. data with shuffled class labels, cluster-corrected, $p < .05$). Top: The histogram shows how many down-states of evoked slow oscillations were detected at which time point within a pre-defined detection time window (solid vertical lines, 0.2-1.5 sec post-cue, dotted line = sound cue onset) across all participants (fixed-effects). Most down-states were detected ~800ms after sound cues but the variation across the whole detection time window was high. **(B)** Top: Grand average of detected slow oscillations evoked by sound cues (mean ± SEM). Bottom: Scene representations were classified ~600 ms after down-states of evoked slow oscillations (dotted line = down-state, white contour lines indicate the significant cluster when comparing the actual data vs. data with shuffled class labels, cluster-corrected, $p < .05$).

However, we assumed that slow oscillations might time the reactivation of sequential memories. Thus, in a next step, we detected slow oscillations evoked by the sound cues and tested for reactivation of category-specific patterns in relation to their down-state.

2.4. Evoked slow oscillations synchronise reactivation of the 2nd sequence element (scenes)

Slow oscillations were detected in a pre-defined time window between 0.2 – 1.5 seconds after sound presentation ('evoked slow oscillations', see Table S2 for the number of detected slow oscillations relative to sound cues). We applied such a narrow detection window to ensure that detected slow oscillations were induced by the sound cues and thus, were related to the cued sequences. Nonetheless, extending the detection window to 2 sec did not qualitatively change the results.

To directly test whether reactivation of scene representations is indeed paced by evoked slow oscillations, we realigned the data according to the down-state of evoked slow oscillations and re-run the previously described classification analysis. In short, we again trained a classifier on each time point of the localiser data, but this time applied the classifier weights to each time point of the sleep data around the downstate of evoked slow oscillations (-1.5 to 1.5 sec with 0 being the down-state of evoked slow oscillations). The resulting localiser time x sleep time matrix revealed a significant negative cluster peaking at around 600 ms after the down-state (corrected using a cluster-based permutation test, $p = .042$), whereas no positive cluster reached significance after correction (all positive clusters $p > .848$). The significant negative cluster indicates reactivation of scene representations which is nested in the up-state of evoked slow oscillations.

Even though slow oscillations were detected in such a narrow time window (0.2 to 1.5 sec), the timing of evoked slow oscillations varied on a trial-by trial basis. More precisely, down-states were, on average, detected after 0.84 sec after sound onset but varied across

the whole detection window (std = 0.32, see Figure 2A top). The findings that no scene reactivation can be found in response to sound cues, but that scene evidence is then timed by evoked slow oscillations can be reconciled by such a variation of evoked slow oscillations. That is, if reactivation of scene representations is paced by slow oscillations and moreover, slow oscillations vary in response to sound cues, reactivation patterns of scene representations would be concealed in averaging the sound-locked data and this is what we see in Figure 2A.

Together, our results suggest that slow oscillations evoked by sound cues time reactivation of sequential memories.

3. Discussion

In our study, TMR combined with MVPA were used to assess reactivation of sequential memories during post-learning sleep. After confirming that participants encoded sequences in a sequential order (Figure 1C), we found superior memory performance for cued compared to non-cued sequences (Figure 1D). When applying MVPA to the sound cues presented during sleep, reactivation of the first but not the second sequence element was revealed (Figure 2A). Critically, when realigning the sleep data to sound evoked slow oscillations, reactivation of the second sequence element emerged during the up-state of slow oscillations (Figure 2B).

It has been assumed that reactivation of memories is induced by presenting associated sound cues during sleep (TMR). This assumption is based on behavioural as well as physiological findings. Behavioural findings demonstrating superior memory

performance for cued compared to non-cued items, as we do in our study as well, suggest memory reactivation in response to sound cues. Accordingly, superior memory performance for cued items can be explained, in theory, by cued items being constantly reactivated and therefore consolidated (Rudoy et al., 2009; Schreiner & Rasch, 2015; for a review see Oudiette & Paller, 2013 ; for a meta-analysis see Hu, Cheng, Chiu, & Paller, 2020).

Besides behavioural findings, recent studies also demonstrate physiological changes in response to sound cues. In response to sound cues (previously associated with learning content) evoked slow oscillations show a higher amplitude and spindle power compared to sound cues without any previously learned association (Laventure et al., 2018; Schreiner et al., 2015; Schreiner & Rasch, 2015). Moreover, learning content previously associated with the sound cues could be decoded when a classifier was trained and tested on sleep data locked to sound cues (Cairney, Guttesen, et al., 2018; Wang et al., 2019). Based on these physiological findings, we were interested in directly testing for reactivation in response to sound cues. As reactivation of memories entails the re-emergence of neural patterns during post-learning sleep which were activated during wakefulness, we trained a classifier on wake data to extracted category-specific neural patterns. When testing the classifier on sleep data, we found direct evidence for memory reactivation in response to sound cues after ~600ms. This finding aligns nicely with another study demonstrating evidence for memory reactivation in response to sound cues after ~400ms (Schreiner et al., 2018). Interestingly, Schreiner and colleagues (2018) found a re-occurrence of memory reactivation ~1 sec after the first reactivation pattern, indicating a timing by slow oscillatory activity. Corresponding to their interpretation, we found in our study that the reactivation pattern of the second sequence element was nested

in the up-state of evoked slow oscillations, arguing for a timing of reactivation by slow oscillations.

It is worth considering that evidence for face reactivation was not observed when realigning the data to evoked SOs (Figure 2B). This suggests that reactivation of the first element in a sequence (i.e., the face) was directly induced by the sound cue, whereas reactivation of the second item in a sequence (i.e., the scene) was then timed by slow oscillations after the sound cue. A recent rodent study proposed that information processing in response to sound cues during sleep follows a cortical-hippocampal-cortical loop (Rothschild et al., 2017). More precisely, the presentation of sound cues during sleep biased auditory cortical activity which then predicted hippocampal reactivation during sharp-wave ripples. Hippocampal activity, in return, drove reactivation in the auditory cortex and hence, closed the information processing loop. Applying this loop to our results, one could speculate that sound cues reactivate cortical areas representing the first sequence element which then drives hippocampal reactivation of the second sequence element during sharp-wave ripples. Hippocampal together with cortical reactivation is synchronised by evoked slow oscillations and nested in their up-states. Thus, variations in the temporal coordination of the cortico-hippocampal-cortical loop might have obscured the decoding of the second sequence item when locking the data to the sound cues and the decoding of the first sequence item when realigning the data to the evoked slow oscillations. However, due to the limited spatial resolution of scalp EEG, the involvement of hippocampal activity and sharp wave-ripples for reactivating sequential memories in our study remains elusive. Future research may unify methods suitable to record and detect hippocampal sharp-wave ripples with sequential memory paradigms to gain insights into the interplay between hippocampal and cortical reactivation. Similarly,

we cannot draw any conclusions about the meaning of sleep spindles in our paradigm. It has been shown that TMR cues elicit evoked slow oscillations which are accompanied by an increase in spindle power during their up-states (Cairney, Guttesen, et al., 2018; Göldi et al., 2019; Oyarzún et al., 2017; Schreiner & Rasch, 2015). A precise nesting of sleep spindles in slow oscillations up-states is predictive for memory consolidation (Helfrich et al., 2018; Muehlroth et al., 2019) and memory reactivation (Schreiner et al., 2021). Consequently, the reactivation of scene evidence which is reported in our study might be also modulated by a coupling of slow oscillations and spindles rather than slow oscillations alone. Owing to a low number of spindles evoked by the sound cues, we cannot address this question here, and it remains open for future research.

A low number of evoked spindles, however, may explain that a full night of sleep in this study was required to unfold the benefits of cueing on memory consolidation (Figure 1D). Sleep spindles induce synaptic plasticity (Niethard et al., 2018; Rosanova & Ulrich, 2005; Seibt et al., 2017; Sejnowski & Destexhe, 2000) and hence, lead to long-lasting changes presumably in cortical networks representing memory traces. A low number of spindles evoked by the sound cues may not be sufficient to induce synaptic plasticity to such an extent that it is expressed in behavioural findings directly after the nap. It might be enough though to preserve the cued memories so that they can be further consolidated during a following night. Whether and how sleep spindles can preserve memories for a later consolidation is yet unknown and requires further investigation.

Apart from a low number of evoked spindles, there are additional limitations in the present study. To increase the chances to detect sequential reactivation during post-learning sleep, we presented the sequence elements in a fixed order (the face was always the first and the scene was always the second sequence element). Randomising the

sequence order within or across participants might result in a mental restructure of the sequence order by participants to make the task easier. That is, learning a face followed by a scene is potentially easier than learning a scene followed by a face as the former aligns with our sentence structure and grammar (a person does something somewhere). As mentally restructuring the sequence order obscure the decoding of the actual sequence order, a fixed sequence (face-scene) was presented.

To rule out that the decoding results (increased classifier accuracy for the face category which is followed by increased classifier accuracy for the scene category) accidentally emerge whenever a sound is presented (independently of whether the sound was previously associated with a face-scene sequence), we incorporated control sounds in our task design. Control sounds seem not to elicit such an order in classifier accuracies (increased face and then scene classifier accuracies, results are not shown).

It is worth noting that the first sequence element (face) is remembered more frequently than the second sequence element (scene). As previous studies argue that weakly memory traces tend to be more consolidated, i.e., more reactivated, than stronger one (Denis, Mylonas, et al., 2020; Denis, Schapiro, Poskanzer, Bursal, Charon, et al., 2020; but see Petzka et al., 2021, chapter 2), an imbalance in memory performance between both sequence elements might be reflected in a difference between classifier accuracies of both sequence elements. However, as we are specifically interested in the order rather than in the magnitude of classifier accuracies, potential differences can be neglected.

In sum, our results demonstrate reactivation of sequential memories in response to sound cues which are moreover synchronised by slow oscillations. Our results not only provide mechanistic evidence for behavioural TMR findings but also shed light on the role of slow oscillations for sequential memory reactivation.

4. Methods

The presented data in this paper are a subset of a larger data set. This larger data set also include a wake group (between-subjects). The data of the wake group were collected in parallel to the presented data of the sleep group. However, as the results of the paper exclusively focuses on the sleep group, information about the wake group is not reported.

4.1. Participants

37 participants were tested. 18 participants had to be excluded due to the following reasons: less than one round of cueing (n = 9), technical issues (n = 4), did not reach the training threshold (n = 3), cancelled due to other issues (participant had a headache n = 1, fire alarm went off during nap n = 1). 19 participants were included in the final sample ($mean_{age}$ = 20.3, $range_{age}$ = 19-23, female = 12). For the final analysis (Figure 2B), another participant had to be excluded as the number of evoked slow oscillations was too low (n evoked slow oscillations = 3). The sample size aligns with previous human sleep and memory studies (Helfrich et al., 2018; Ngo et al., 2015).

Pre-screening ensured that participants had no history of neurological or psychiatric disorders and a normal sleep-wake cycle throughout the experiment. On the day of the experiment, participants were instructed to get up one hour earlier than normal and avoid caffeine. Moreover, they had to abstain from alcohol the night before. Participants received a monetary reimbursement after participating in the study. Written informed consent was obtained from all participants before the start of the study. The study was approved by the University of Birmingham Research Ethics and Governance Committee.

4.2. Paradigm and procedure

All tasks were implemented via custom written scripts in MATLAB 2016a (MathWorks, Munich, Germany) using functions of the PsychoPhysics Toolbox Version 3.0.14 (Brainard, 1997). The description of the tasks follows the order in which they were conducted in the experimental sessions.

Localiser

150 objects, unfamiliar faces and scenes were presented in a randomised order. The localiser was conducted to later classify brain activity according to these categories. Each trial started with a fixation cross presented for 2 ± 0.1 sec in the centre of the screen. One of 150 stimuli (either an object, face or scene) was then shown for 2 sec. After 2 sec, a legend appeared below the stimulus prompting participants to indicate with a key press which category the stimulus belongs to.

The localiser was conducted twice, at the beginning and end of the first experimental session. While the structure of the task was identical, different objects, faces and scenes were used.

Familiarisation

During the familiarisation, 90 objects accompanied by a semantically related sound were presented twice, e.g., a cat together with a "meow". The same 90 objects acted as cues for the 90 sequences that had to be associated during the following encoding. The purpose was to strengthen the connection between the object and sound. Each trial started with a centred fixation cross presented for 2 ± 0.1 sec. Afterwards, an object was presented for 2 sec. The semantically related sound was played twice for 500ms, at the beginning (0 – 500ms) and end (1500 – 2000 ms) of the object presentation. After 2 sec, a legend

appeared below the object inviting participants to listen to the sound again or to continue with the next trial. Participants were free to listen to the sounds as often as they wanted to.

Encoding

Participants had to encode 90 sequences comprising an object as a cue and a face and a scene as a target. Each trial started with a centred fixation cross presented for 3 ± 0.1 sec. A trial unique object was then presented for 2 sec which was accompanied by a semantically related sound (0.5 sec). For example, the image of a guitar was accompanied by the sound of a playing guitar. These were the same objects presented during the familiarisation. To signify the continuity of the sequence, a centred fixation cross together with three dots was shown for 2 ± 0.1 sec, followed by the first target of the sequence, the face. The face was either Angelina Jolie or Barack Obama and was on the screen for 2 sec. After the face, the fixation cross together with three dots were again presented for 2 ± 0.1 sec, followed by the second target of the sequence, the scene. The scene was either Machu Picchu or the inside of the Westminster Abbey. Angelina Jolie/Barack Obama and Machu Picchu/Westminster Abbey respectively were used as targets as they were familiar to all participants and consequently, facilitate the feasibility of the cued recall.

During encoding, participants had to create a short story connecting the object with the face and scene. Importantly, they had to stick to the order in which the sequence was presented. After the scene was shown on the screen for 2 sec, a legend additionally occurred below the scene requesting participants to indicate via button press whether they were successful in creating a mental story. On button press the next trial started.

Training

A training session was introduced to ensure an appropriate number of hits for a later allocation to a cued vs. not-cued condition (see section Nap & Targeted memory reactivation). Each training trial started with a centred fixation cross for 3 ± 0.1 sec, followed by the visual and auditorily presentation of the object for 0.5 sec. A question mark then appeared on the screen indicating the retrieval of the face and scene. After 4.5 sec, a legend was shown and participants had to indicate first, the face and second, the scene the object was presented with. They always had the choice to respond with "don't know". Whenever participants did a mistake or chose the "don't know" response for at least one of the two targets, the whole sequence was presented again.

Participants finished the training when they correctly remembered 75 out of the 90 encoded sequences. Memory performance was assessed in respect to the training threshold when all 90 sequences were tested. If the training threshold was not reached, not remembered sequences were tested again. After each trial, memory performance was assessed again and the training finished as soon as the training threshold was reached.

Psychomotor Vigilance Task (PVT)

To assess the vigilance of participants, a PVT was used. A centred fixation cross was presented for 6 ± 4 sec. Whenever a counter replaced the fixation cross, participants had to press the space bar as fast as possible to stop the counter. Following the key press, feedback about the reaction time was provided. Overall, the PVT lasted 2 minutes.

Retrieval

Each retrieval trial started with a centred fixation cross presented for 2 ± 0.1 sec. After the fixation cross, the object related sound was presented for 0.5 sec, followed by a

question mark (4.5 sec) which prompted participants to retrieve the associated face and scene. Then, a legend appeared and participants had to indicate first, which face and second, which scene the object was presented with. In both cases, participants could also choose a "don't know" or "don't recognise the sound" option. A trial was counted as a hit when both face as well as scene were correct.

Nap & Targeted memory reactivation

Between retrieval and nap, participants had a light lunch (sandwich). At ~2 p.m. they went to the laboratory bedroom for 120 min. They had the opportunity to sleep while their brain, muscle and eye activity was recorded with polysomnography (PSG).

During late sleep stage 2 and early sleep stage 3, the presentation of object sounds was initiated (targeted memory reactivation, TMR). Based on memory performance at retrieval 1, correctly remembered sequences were divided into a cued and non-cued condition. This way, baseline memory performance was equal between cued and non-cued sequences. For example, if 30 sequences were correctly remembered (face as well as scene had to be correct), 15 sequences were assigned to the cued and the other 15 were assigned to the non-cued condition. Whenever an odd number of sequences was correctly remembered, the remaining sequence was randomly assigned to one of the two conditions. In addition to the object sounds, the same amount of control sounds was presented. Referring to our example, in addition to the 15 object sounds from the cued condition, 15 control sounds were presented. Control sounds were generated by shuffling the power spectrum of the real sounds in the frequency domain and thus, resembled noise. Object and control sounds were presented (~30dB) in a randomised order with an inter-stimulus interval of 5 sec. After one round of cueing, the presentation order was shuffled and the cueing continued.

Procedure

The experiment consisted of two experimental sessions. The first session started at 11 am with the application of electroencephalography (EEG), electrooculography (EOG) and electromyography (EMG). Approximately one hour later, participants were given written instructions and the opportunity to practice each task. Following the practice, the actual tasks including localiser, familiarisation, encoding, training, PVT and retrieval 1 were conducted.

Participants then went to bed at ~ 2 pm to take a 2-hour nap (see Table S1 for descriptive sleep data). 30 min after waking up, participants continued with a second PVT, retrieval 2 and the second localiser. The following day at 11 am, participants returned to the lab for the second experimental session comprising a third retrieval (without EEG).

4.3. EEG data recording

EEG data were recorded using a Brain Products 64-channel EEG system and sampled at 1000 Hz. Electrodes were arranged according to the 10-20 system (including FCz as reference, AFz as ground and left and right mastoids). To record muscle activity (electromyography, EMG) and eye movements (electrooculography, EOG), two electrodes were placed on the chin and two electrodes around the eye.

4.4. Behavioural analysis

Conditional probabilities for two different outcomes were calculated based on the training data: First, we calculated the probability of forgetting a face given that the scene is correctly remembered ($p(\sim face|scene) = p(\sim face \cap scene) / p(scene)$) and second, we calculated the probability of forgetting a scene given that the face is correctly remembered

(p(~scene|face) = p(~scene∩face) / p(face)). To obtain representative values for conditional probabilities of these two outcomes, enough trials of either just remembering the face or just remembering the scene are required. During retrieval 1, most trials were either completely correct (face and scene remembered) or incorrect (face and scene forgotten). Therefore, conditional probabilities were calculated based on the training data.

Memory performance at retrieval 2 was calculated as the proportion of hits at retrieval 1 which were correctly remembered at retrieval 2. Memory performance at retrieval 3 was calculated as the proportion of hits at retrieval 2 that were correctly remembered at retrieval 3. A trial counted as a hit trial when face and scene were correctly remembered.

For all behavioural analyses paired-sample t-tests were used to test for statistical differences between conditions.

4.5. EEG analysis

Preprocessing

Preprocessing of EEG data was performed using the FieldTrip toolbox (Oostenveld et al., 2011). To remove eye movements from the wake data, an independent component analysis (ICA) was applied. Data were down-sampled to 200 Hz, filtered (high-pass: 1 Hz, low-pass: 100 Hz, band-stop: 48-52 Hz), demeaned and inspected for coarse artifacts. Bad channels were discharged before applying the ICA to identify bad components and obtain the unmixing matrix. The raw data were then preprocessed again because the first preprocessing was optimised for applying the ICA. Data were down-sampled to 500 Hz, filtered (low-pass: 200 Hz, band-stop: 48-52, 98-102, 148-152 Hz) and demeaned. The previously defined bad channels were excluded and the unmixing matrix was applied to

the new data. Bad components were removed, bad channels were interpolated, and data were re-referenced to common average.

According to the wake data, sleep data were down-sampled to 500 Hz, filtered (low-pass: 200 Hz, band-stop: 48-52, 98-102, 148-152 Hz) and demeaned. Bad channels were interpolated, and data were re-referenced to common average.

Multivariate analysis

Multivariate pattern analysis (MVPA) was conducted using the MVPA-light toolbox (Treder, 2020) running in MATLAB. As a classifier a linear discriminant analysis (LDA) was used (Lemm et al., 2011).

Before applying the LDA to the localiser data, data were segmented ([-0.2 – 2 sec post-stimulus]), smoothed in time with a running average time window of 50ms and baseline corrected (-0.2 to 0 sec). Then, a z-transformation across trials was applied to each time point. On each time point of the z-transformed data, a classifier was trained with all 58 channels serving as features and face and scene trials serving as the two classes. To avoid overfitting, data (trials) were divided into a training and test set using fivefold cross-validation (Lemm et al., 2011). Cross-validation was repeated five times and averaged, since the assignment of trials into training and test set was random. To operationalise the ability of the classifier to differentiate between face and scene classes, the accuracy metric was used. Accuracy can be interpreted in such a way that 0.5 reflects random and 1.0 reflects perfect performance. For example, 0.5 accuracy means that 50% of all tested face trials are assigned to the class face and the other 50% of tested face trials are assigned to the class scene. Consequently, the classifier cannot distinguish between face and scene trials. Accuracy of 1, on the other side, means that all tested face trials are correctly

assigned to the class face reflecting a perfect differentiation between trials. For statistical comparisons, decoding performance was calculated again but this time with shuffled class labels (faces and scenes).

To investigate evidence for face and scene representations during sleep, the temporal generalization method was used (King & Dehaene, 2014). Before, localiser and sleep data were segmented, smoothed with a running average time window of 200ms and a z-transformation on each time point across trials was applied. The classifier was now trained on each time point of the localiser data and tested on each time point of the sleep data resulting in a time x time matrix. As localiser and sleep data were independent data sets, no cross-validation was required. As a metric for classification, we used accuracy ([0-1]) and subtracted the chance performance (0.5). However, since both classes, faces and scenes, were components of all testing trials, we labelled all testing trials as face trials. Consequently, the accuracy measurement has to be interpreted differently. That is, above chance classification (positive values, [0.01-0.5]) can be interpreted as face evidence, whereas below chance classification (negative values, [-0.5- -0.01]) indicates scene evidence. For statistical comparisons, the same analysis was conducted 10 times with shuffled class labels (faces and scenes) of the localiser data. The 10 resulting time x time matrices were averaged and provide values under the null hypothesis.

Event Detection

To detect evoked slow oscillations for each participant, established detection algorithms were applied (Ngo et al., 2013; Staresina et al., 2015). Data classified as N2 or N3 sleep were band-pass filtered between 0.3-1.25 Hz (4^{th} order two-pass Butterworth filter) and zero crossings were detected. For slow oscillations, three criteria had to be fulfilled: A length criterion, a peak to trough criterion and an amplitude criterion. The length criterion

was met, if a positive to negative crossing was followed by a second positive to negative crossing within 0.8 to 2 sec. Based on all sufficiently long events, mean and standard deviation (std) were calculated for trough to peak amplitudes as well as for absolute values of trough amplitudes. The peak to trough criterion and the amplitude criterion were fulfilled, if events exceed the mean + 1.25*std. Then, evoked slow oscillations were identified on electrode position Fz whenever a sound cue was presented 0.2 to 1.5 seconds before the trough (down-state). Sleep data were realigned to the down-state of evoked slow oscillations (time = 0).

Statistics

Paired sampled t-tests were used to test for behavioural differences between conditions (Figure 1 C, D).

To correct for multiple comparisons, FieldTrip's cluster-based permutation test (Maris & Oostenveld, 2007) was applied (1000 randomisations) to compare classifier accuracies between real and shuffled labels across time (Figure 1 E). Furthermore, a permutation test was conducted to compare time x time classification matrices (real vs. shuffled labels, Figure 2). All cluster-based permutation tests were two-sided.

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6. Author Contributions

Conceptualisation: M.P., B.P.S.; Methodology: M.P., B.P.S.; Investigation: M.P.; Formal Analysis: M.P., B.P.S.; Writing- Original Draft: M.P., B.P.S.; Writing – Review & Editing: M.P., S.C., G.M.B., B.P.S.; Funding Acquisition: B.P.S.

7. Supplemental Information

Table S 4.1. Descriptive sleep data in minutes. n = 19. (mean ± SEM)

N1	N2	N3	REM	TST
15.64	46.97	12.89	18.69	102.58
±2.29	±2.98	±2.36	±2.89	±4.69

Table S 4.2. Amount of object (N object cues) and control cues (N control cues) that were presented during sleep and the number of detected slow oscillations evoked by object sounds (N evoked slow oscillations, object cues) and evoked by control sounds (N evoked slow oscillations, control cues).

N object cues	N evoked slow oscillations (object cues)	N control cues	N evoked slow oscillations (control cues)
113.17	18.56	112.72	14.22
±11.95	±1.79	±11.99	±1.15

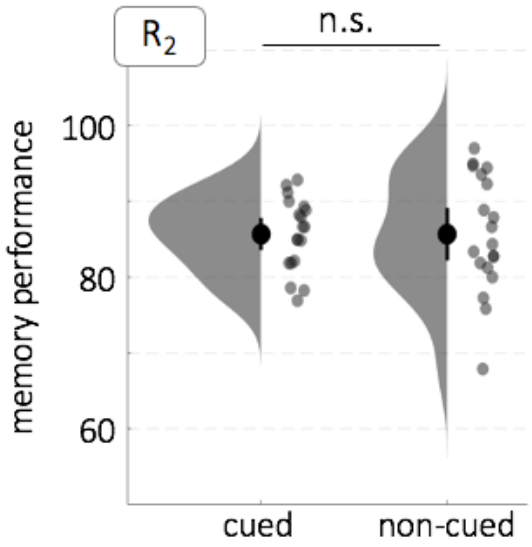

Figure S 4.1. Memory performance of retrieval 2 did not significantly differ between cued and non-cued conditions. Density plots, group means with 95% CIs and single participant data are shown. n.s. = not significant.

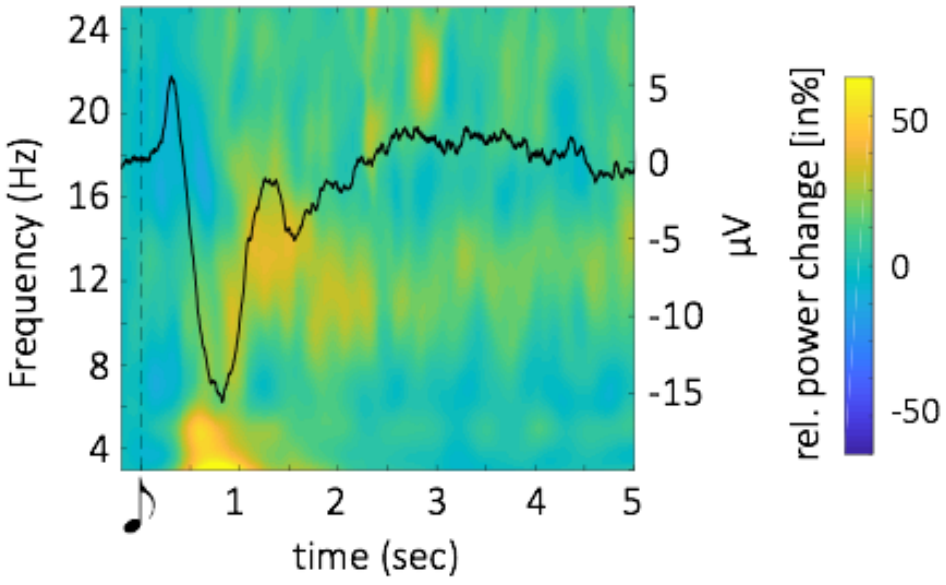

Figure S 4.2. Time-frequency decomposition and event-related potential to sound cues. Time frequency decomposition and event-related potentials were extracted based on re-referenced data to linked mastoids and analyses were performed using FieldTrip. For event-related potentials, data were segmented, baseline corrected (-0.2 to 0 sec), detrended and averaged across all object cue trials within participants and then across

participants. Time-frequency analysis was conducted using Morlet wavelets with an increase in cycles (starting with 5 cycles) for a frequency range of 3-30 Hz in 1 Hz steps. Power was then calculated on 50ms long epochs. To get rid of potential artifacts, we rejected the most extreme 1% of the trial distribution per time x frequency bin. After averaging across the remaining trials, the relative power change from a baseline (-0.3 to -0.1) was calculated and contrasted between sound cues and control cues (sound > control).

Chapter 5. Endogenous memory reactivation during sleep in humans is clocked by slow oscillation-spindle complexes

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Abstract

Sleep is thought to support memory consolidation via reactivation of prior experiences, with particular electrophysiological sleep signatures (slow oscillations (SOs) and sleep spindles) gating the information flow between relevant brain areas. However, empirical evidence for a role of endogenous memory reactivation (i.e., without experimentally delivered memory cues) for consolidation in humans is lacking. Here, we devised a paradigm in which participants acquired associative memories before taking a nap. Multivariate decoding was then used to capture endogenous memory reactivation during non-rapid eye movement (NREM) sleep in surface EEG recordings. Our results reveal reactivation of learning material during SO-spindle complexes, with the precision of SO-spindle coupling predicting reactivation strength. Critically, reactivation strength (i.e. classifier evidence in favour of the previously studied stimulus category) in turn predicts the level of consolidation across participants. These results elucidate the memory function of sleep in humans and emphasize the importance of SOs and spindles in clocking endogenous consolidation processes.

1. Introduction

How do we strengthen memories while we sleep? The prime vehicle of systems consolidation is thought to be the reactivation of information encoded during prior wakefulness (Diekelmann & Born, 2010; Paller et al., 2021; Rasch & Born, 2013; Walker & Stickgold, 2004). Through reactivation, memory representations are relayed between the hippocampus and neocortical long-term stores, transforming initially labile representations into long-lasting memories during sleep (Buzsáki, 1996; Marr, 1971). The communication between the hippocampus and neocortical networks is thought to be facilitated by an intricate interplay of the cardinal NREM sleep-related oscillations, namely cortical slow oscillations (SOs), thalamo-cortical sleep spindles, and hippocampal sharp-wave ripples (Latchoumane et al., 2017; Maingret et al., 2016; Oyanedel et al., 2020; Sirota et al., 2003; Skelin et al., 2019; Staresina et al., 2015). SOs reflect fluctuations of the membrane potential and orchestrate transitions from neuronal silence (hyperpolarization, i.e., downstate) to neuronal excitation (depolarization, i.e., upstate, Amzica & Steriade, 2002; Steriade et al., 1993). Importantly, they initiate time windows of excitability and inhibition not only in cortical but also in subcortical areas (Fernandez & Lüthi, 2020a; Isomura et al., 2006; Timofeev, 2011). They trigger the emergence of sleep spindles in the thalamus (Mak-Mccully et al., 2017), which nest in the excitable up-states of the SOs. Spindles have been shown to gate Ca^{2+} influx into dendrites, thereby facilitating synaptic plasticity (Rosanova & Ulrich, 2005; Seibt et al., 2017). Importantly, recent evidence from two-photon imaging in mice suggests that Ca^{2+} influx is strongly amplified when spindles coincide with SO up-states (Niethard et al., 2018). Lastly, hippocampal ripples are transient network oscillations and have been closely linked to reactivation/replay of learning experiences (Buzsáki, 2015; Joo & Frank, 2018). They

have been shown to occur in the excitable troughs of the spindle, suggesting that spindles might facilitate information transfer from the hippocampus to neocortical target sites (Helfrich et al., 2019; Ngo et al., 2020). The efficacy of systems consolidation through memory reactivation might thus hinge on concurrent SO-spindle coupling, ensuring optimal conditions to ignite structural changes in cortical target sites (Clemens et al., 2007; Jiang, Gonzalez-Martinez, & Halgren, 2019; Oyanedel et al., 2020; Staresina et al., 2015).

Indeed, recent work in humans has revealed a key role of SO-spindle coupling during NREM sleep for behavioural expressions of consolidation. For instance, the precision of SO-spindle coupling, i.e., the exact timing of spindle maxima with respect to the SO upstate, has been shown to correlate with retention of declarative learning material (Mikutta et al., 2019; Zhang et al., 2018). Moreover, levels of SO-spindle coupling track the rise and decline of memory performance across development (Hahn et al., 2020; Helfrich et al., 2018; Muehlroth et al., 2019). What is unknown, however, is whether there is a link between SO-spindle coupling and physiological expressions of consolidation, i.e., memory reactivation. A recent rodent study revealed that precise SO-spindle coupling is key for maintaining the reactivation of neural ensembles (Kim et al., 2019), but whether and how this relates to episodic memory consolidation in humans is unclear.

In humans, the study of memory reactivation during sleep has mainly relied on targeted memory reactivation (TMR) protocols (Oudiette & Paller, 2013; Schreiner & Staudigl, 2020). This experimental technique follows the rationale that reminder cues are presented during sleep to exogenously trigger memory reactivation. Intriguingly, presenting auditory reminder cues during NREM sleep reliably induces SO-spindle complexes (Cairney, Guttesen, et al., 2018; Oyarzún et al., 2017; Schreiner et al., 2015). However,

to what extent TMR-induced processes reflect natural/endogenous consolidation processes remains unknown.

Building on the work summarized above, we propose that SO-spindle complexes might clock endogenous memory reactivation in service of consolidation during human sleep. To test this notion, we devised an experimental paradigm in which participants acquired associative memories before taking a nap. Multivariate decoding was then used to assess endogenous memory reactivation during NREM sleep. In this work, we show that memory reactivation is specifically bound to the presence of SO-spindle complexes, with the precision of their coupling correlating with reactivation strength. Reactivation strength in turn predicts the extent of consolidation across participants. These findings elucidate the memory function of sleep in humans and illustrate the importance of SO-spindle coupling for clocking endogenous consolidation processes.

2. Results

Twenty participants (age: 20.75 ± 0.35; 17 female) took part in two experimental sessions. In both sessions they performed an episodic learning task, with memory performance being assessed before and after taking a 120 min nap (Figure 1A). Depending on the experimental session, participants learned to associate verbs with images of objects or scenes during the pre-sleep learning phase. These stimulus categories were chosen as they recruit distinctive brain networks (e.g., lateral occipital complex for objects, parahippocampal place area for scenes (Epstein & Kanwisher, 1998; Malach et al., 1995), thus facilitating the analytical readout of endogenous, experience-dependent memory reactivation during sleep. Specifically, learning-related memory reactivation

during sleep would manifest as enhanced representational evidence for the stimulus category learned before sleep (i.e., greater evidence for object representations after word-object encoding and greater evidence for scene representations after word-scene encoding, respectively).

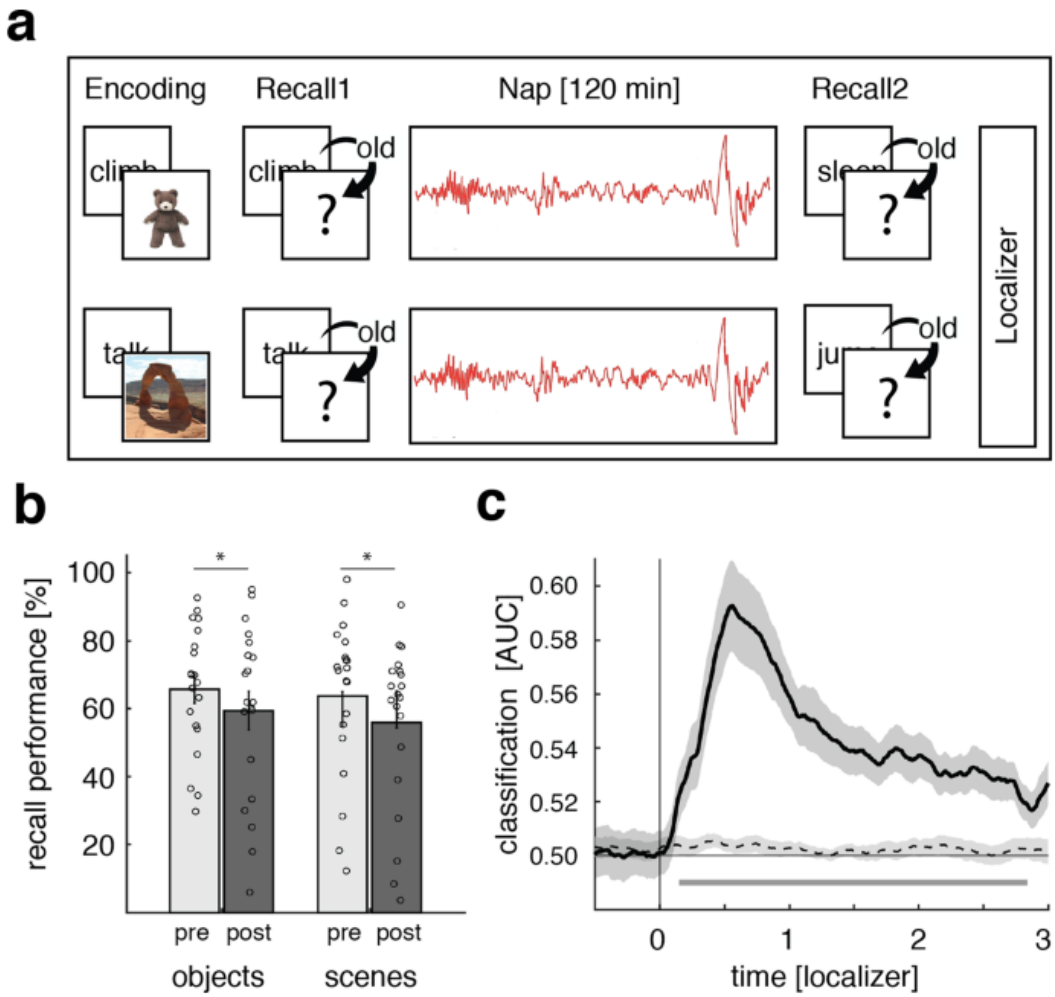

Figure 5.1. Experimental procedure, behavioural results, and localizer task.**(a)** During encoding, participants were presented with 120 verb-object or verb-scene combinations (depending on experimental session). Memory performance was tested before and after a 120 min nap period. At the end of each session, participants performed a localizer task in which they processed a new set of object and scene images. **(b)** Behavioural results for both experimental sessions pre- (light gray) and post-sleep (dark gray). Bar graphs show mean (±SEM) percentage of recalled image exemplars out of correctly recognized verbs. Dots indicate individual memory performance of participants ($N = 20$). Stars denote significant differences as derived from a repeated measures ANOVA

($p = 0.001$). **c** Stimulus categories (objects vs. scenes) could be reliably decoded (above chance) from the localizer EEG data, starting around 150 ms post stimulus onset (the black solid line indicates decoding performance (±SEM)). The horizontal dashed line indicates surrogate decoding performance, which was estimated by shuffling the training labels 250 times. The vertical solid line indicates stimulus onset (time = 0). The lower horizontal gray line shows the temporal extent of significant decoding results as derived from a dependent-samples *t*-test (two-sided, $p = 0.002$, cluster corrected across time).

Memory performance was tested both before and after the sleep period in a stepwise manner. First, participants made word-recognition judgments (old or new). Then, for recognized words only, recall of the associated image exemplar (object or scene, depending on experimental session) was assessed. The resulting recall performance was then normalized by the amount of correctly recognized items (i.e., "hits"). To avoid any impact of pre-sleep testing on our behavioural consolidation measures (Antony et al., 2017; Roediger & Karpicke, 2006), only half of the learned material was tested before sleep, while the remaining half was tested after sleep. Finally, at the end of the experimental sessions participants performed an independent "localizer task", where a new set of object and scene images was presented (including both stimulus categories, irrespective of experimental session). This localizer served to train a linear classifier to distinguish object- vs. scene-related electroencephalographic (EEG) patterns.

2.1. Behavioural results and category classification during the localizer task

First, we calculated d-prime (d′, Macmillan & Creelman, 2005) as a general measure of recognition memory performance (for a detailed overview of memory measures as well as sleep characteristics see Tables S 1 and S 2). Both pre- and post-sleep d' levels confirmed that participants could reliably discriminate between old and new items (i.e., $d' > 0$; pre-sleep objects: $d' = 2.11 \pm 0.14$, scenes: $d' = 2.02 \pm 0.22$; post-sleep objects:

$d' = 1.76 \pm 0.19$, scenes: $d' = 1.69 \pm 0.23$). Out of hits, participants recalled the correct image for $64.31 \pm 3.23\%$ before sleep (objects: $64.90 \pm 3.99\%$, scenes: $63.72 \pm 5.20\%$) and for $57.61 \pm 3.91\%$ after sleep (objects: $59.39 \pm 5.71\%$, scenes: $55.82 \pm 5.47\%$).

To test for potential differences in memory performance between test times and stimulus categories, we conducted ANOVAs for recognition memory (d') and cued recall, including the factors category (object vs. scene) and test-time (pre- vs. post-sleep). Results indicated that memory performance (both recognition and recall) declined over the course of sleep (main factor test-time: recognition memory: $F_{1,19} = 10.91$; $p = 0.004$; cued recall: $F_{1,19} = 15.53$; $p = 0.001$). Importantly though, no difference in memory performance between categories was observable (main effect category: recognition memory: $F_{1,19} = 0.21$; $p = 0.65$; cued recall: $F_{1,19} = 0.38$; $p = 0.54$) and no interaction between test-time and learning category (recognition memory: $F_{1,19} = 0.003$; $p = 0.95$; associative memory: $F_{1,19} = 0.69$; $p = 0.41$), ensuring that task difficulty was highly comparable between image categories (also see Table S1).

The localizer task at the end of each session was employed to derive the neural signatures of object vs. scene processing, which were then used to track category-specific memory reactivation during NREM sleep (see below). Participants were presented with novel sets of object and scene images and performed a continuous recognition task on these images. Specifically, each image was presented twice (mean distance between successive presentations = 8.06, range = 2–33) and participants were instructed to indicate whether a given item was “new” (first presentation) or “old” (second presentation). As expected, participants showed high accuracy levels on this task (objects: 97.02 ± 0.61 correct

decisions; scenes: 92.57 ± 4.44 correct decisions), with performance again matched between image categories ($t_{(19)} = 1.05, p = 0.31$).

To extract the category-specific (i.e., object and scene) patterns of neuronal activity, we pooled the localizer data across experimental sessions and performed multivariate classification (linear discriminant analysis; LDA) on these data (Figure 1c). Using fivefold cross-validation (see Methods), above-chance classification accuracy emerged around 150 ms following image onset, was sustained until 2800 ms and peaked at 600 ms ($p = 0.002$, corrected for multiple comparisons across time). Hence, the localizer data allowed us to isolate brain patterns associated with the processing of object and scene images, which we then used to guide analysis of category-specific reactivation during sleep (for results concerning the stability of the decoding approach see Figure S 1).

2.2. Endogenous memory reactivation during NREM sleep is clocked by SO-spindle complexes

As mentioned above, theoretical models and recent empirical findings point to particular role of SO-spindle coupling for memory consolidation. We thus tested the resulting prediction that the joint presence of SOs and sleep spindles (henceforth referred to as "SO-spindle complexes") would drive endogenous memory reactivation during human sleep. SOs and sleep spindles were detected in the EEG data using established algorithms (Ngo et al., 2013; Staresina et al., 2015). To isolate SO-spindle complexes, we identified events where SO down-states were followed by sleep spindles within a time window of 1.5 s (for a time–frequency representation of the SO-spindle complexes see Figure 2a; for a peri-event SO-spindle histogram, see Figure S 2). To determine whether learning-related (i.e., category-specific) neuronal activity would be differentially reactivated

during SO-spindle complexes, we first trained a classifier on the concatenated localizer data from both experimental sessions [–0.5 to 3 s]. Importantly, the localizer tasks of both sessions included object and scene images, to ensure that multivariate measures of potential reactivation not merely reflect session-specific EEG properties. The resulting training weights were then applied on both sessions' sleep data, centred around the downstate of SO-spindle complexes (for related results where the data were locked to different spindle features see Figure S 3). Classifier testing labels reflected the stimulus category used in the preceding encoding session (object or scene), such that above-chance classification signifies endogenous activation patterns more strongly resembling the just-learned stimulus category than the alternative stimulus category.

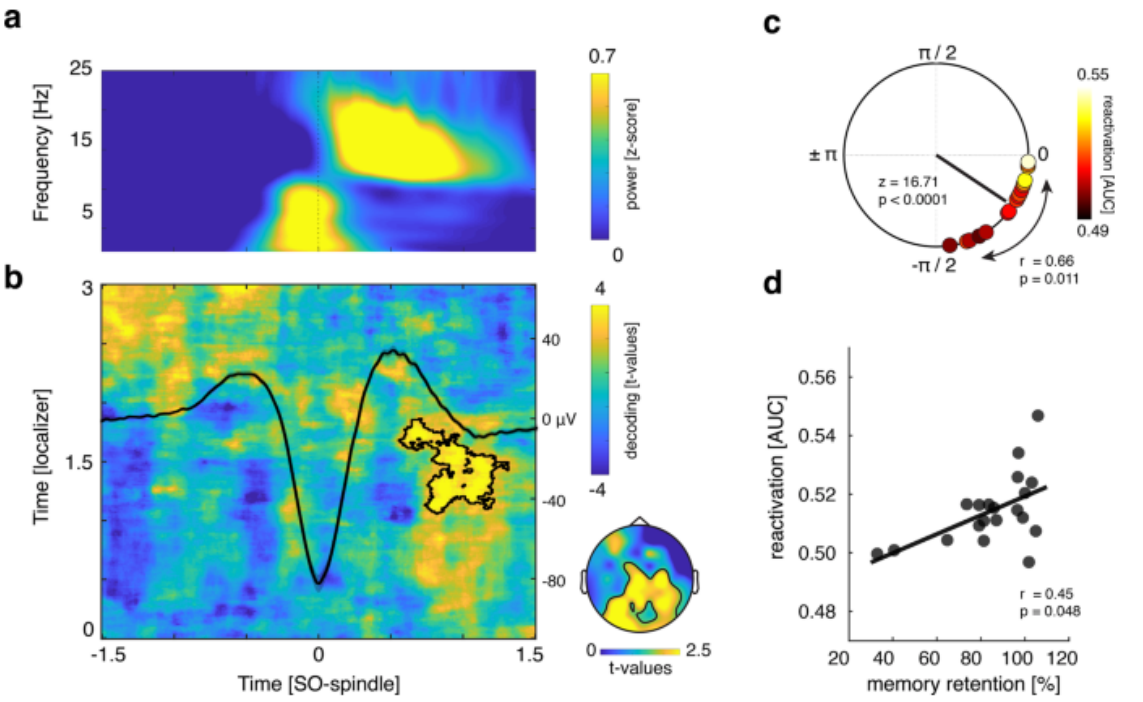

Figure 5.2. SO-spindle locked memory reactivation. **(a)** Time–frequency representation of all SO-spindle segments (z-scored across time; only positive values are displayed, with yellow indicating power increases). **(b)** Learning-related brain patterns (objects vs. scenes) were decodable during SO-spindle complexes (contour lines indicate the extent of the significant cluster, $p = 0.016$ corrected; colour range (blue to yellow)

represents *t* values against surrogate decoding performance, which was estimated by shuffling the training labels 250 times). The averaged EEG trace (all instances in which SO down-states were followed by sleep spindles within 1.5 s at channel Cz in microvolt [μV]) illustrates the relationship of the observed reactivation signal with ongoing oscillatory activity. The topographical insert illustrates the results of a "searchlight decoding procedure", indicating that bilateral parietal and occipital areas exhibited stimulus-category related effects (please note that statistical tests were done for illustrative purposes only). (**c**) Phases of the SO-spindle modulation derived from channel Cz, illustrating the clustering of spindle power toward the SO upstate (upstate corresponding to 0 and downstate to ± π, with –π/2 reflecting the down- to upstate transition; Rayleigh test: $p < 0.0001$; $z = 16.71$). The black line illustrates the mean coupling direction and vector length ($-36.78° \pm 5.48°$, mean vector length = 0.91). Circular-linear correlation analysis between the individual mean SO-spindle coupling phase (circles) and the mean reactivation strength (area under the curve [AUC] scores; colour coded, with white indicating high classification performance and black low classification performance) revealed a positive association ($r = 0.66$; $p = 0.011$). (**d**) Reactivation strength correlated positively with behavioural levels of associative memory consolidation (Spearman's Rank Correlation, $r = 0.45$, $p = 0.048$).

As shown in Figure 2b, results revealed a cluster of significant above-chance classification from 800 to 1200 ms relative to the SO downstate ($p = 0.016$, corrected for multiple comparisons across time, localizer time-window [1000 to 1800 ms]), emerging between maximum and offset of coupled sleep spindles (for the corresponding accuracy map see Figure S 4; for participant specific classification values see Table S 3). No negative cluster survived correction for multiple comparisons (cluster with smallest $p > 0.6$).

But does endogenous memory reactivation indeed require the joint presence of SOs and spindles? To address this question, we performed the same decoding procedure, but locking the data to solitary SO or spindle events (thus, SOs without spindles and vice versa). For both types of events, when testing accuracy levels against chance at any

localizer time × sleep time point, no significant cluster of above-chance classification emerged (in both cases cluster with the smallest $p > 0.2$, see Figure S 5; similarly, testing the classifier on Slow spindle—SO-locked data did not yield any significant cluster of above-chance classification (cluster with smallest $p = 0.67$; see Figure S 6)).

2.3. Precision of SO-spindle coupling correlates with reactivation strength

If SO-spindle coupling is indeed instrumental for consolidation, its precision should impact the extent of endogenous memory reactivation. To quantify the preferred phase of SO-spindle modulation, we determined in every participant the SO phases corresponding to the spindle peak amplitudes (electrode Cz). In 16/20 participants we found significant nonuniform distributions ($p < 0.05$; Rayleigh test, mean vector length: 0.34 ± 0.03). In line with previous findings, we found a significant nonuniform distribution across participants (Rayleigh $z = 16.71, p < 0.0001$), with spindles peaking near the SO upstate (corresponding to 0°; mean coupling direction: –36.78° ± 5.48°; see Figure 2c).

To further test whether the precision of SO-spindle coupling would be relevant for the reactivation of memories we computed a circular-linear correlation between each participant's preferred SO-spindle phase (averaged across sessions) and their mean reactivation strength (averaged across the significant cluster shown in Figure 2b). The individual SO-spindle modulation phase was significantly correlated with decoding accuracy ($r = 0.66$; $p = 0.011$). The distribution indicated that the closer the spindles were nested towards the SO upstate, the higher the fidelity of the associated reactivation signal (see Figure 2c, for a scatter plot see Figure S 7; for additional analyses estimating the impact of trait-like characteristics in this context, see Supplementary Notes).

To ensure that the results described above were not driven by differential wake classification characteristics, we conducted a partial circular-linear correlation with the mean decoding levels from the localizer tasks (averaged across the significant cluster shown in Figure 1c) as a covariate. Again, we observed a positive relationship between the individual SO-spindle modulation phase and decoding accuracy ($r = 0.65$; $p = 0.012$).

2.4. Reactivation strength predicts consolidation of associative memories

If SO-spindle triggered reactivation reflects memory-related processes, one would expect a functional link with behavioural expressions of consolidation. To address this question, we correlated, across participants, levels of post-sleep memory retention and reactivation strength. Specifically, a "retention index" (proportion of post-sleep recalled images (out of hits) in relation to pre–sleep memory performance; see Methods section for details) was collapsed across sessions and correlated with decoding accuracies averaged across the significant cluster reported above. As shown in Figure 2d, we observed a significant positive relationship between the two variables (Spearman rho $= 0.45$, $p = 0.048$). Of note, no association between decoding accuracy and recognition memory performance was detectable ($r = 0.02$, $p = 0.93$), indicating that reactivation strength was specifically linked to the consolidation of hippocampal-dependent associative memories (Davachi, 2006). However, the correlation between reactivation and consolidation of associative memory was not significantly greater than that with recognition memory ($z = 1.35$; $p = 0.17$). Lastly, we again controlled this analysis for localizer decoding levels using a partial correlation, which substantiated the results (Spearman rho $= 0.45$, $p = 0.049$).

3. Discussion

Our results demonstrate that consolidation relies on endogenous memory reactivation clocked by SO-spindle complexes. In particular, we found that during the presence of SO-spindle complexes, activation patterns were biased towards the previously encoded learning material (Figure 2a, b). Moreover, the precision of SO-spindle coupling predicted the fidelity of memory reactivation (Figure 2c). Finally, reactivation strength predicted the amount of consolidation across participants, highlighting its functional significance for behavior (Figure 2d).

NREM sleep oscillations (SOs, spindles, and ripples) have long been implicated in the memory function of sleep, and recent work has emphasized the importance of their temporal synchronization (Klinzing et al., 2019). Specifically, the precise timing of SOs, spindles, and ripples is thought to enable the relay of hippocampus-dependent memories to cortical networks (Rasch & Born, 2013). Indeed, recent work in rodents has shown that their co-occurrence is necessary for effective consolidation as assessed via fear conditioning (Latchoumane et al., 2017) or an object-in-place recognition task (Maingret et al., 2016). However, how these tasks relate to expressions of episodic memory in humans is not entirely clear. Human iEEG work with epilepsy patients has corroborated the triple-interaction of these sleep oscillations (Helfrich et al., 2019; X. X. Jiang et al., 2019; Staresina et al., 2015), but none of these studies has assessed memory reactivation or the effects on behaviour. Investigation of healthy participants via scalp EEG has shown that brain patterns across sleep differ as a function of prior learning tasks (Schönauer et al., 2017), but these activation patterns were not directly related to wake activity or to discrete SOs/spindles. Another study employed simultaneous EEG-fMRI and found

univariate signal increases in learning-related areas during spindles (Bergmann et al., 2012), but it remained open whether such reactivation bears relevance for memory consolidation. Finally, the advent of TMR protocols (Rasch et al., 2007; Rudoy et al., 2009) has shown evidence for both SO-spindle complexes and information processing in response to external reminders (Bar et al., 2020; Cairney, Guttesen, et al., 2018; Göldi et al., 2019; Oyarzún et al., 2017; Schechtman et al., 2021; Schreiner et al., 2018; Wang et al., 2019), but it is unclear whether and how such exogenous memory reactivation relates to endogenous reactivation in service of memory consolidation. In sum, different lines of research across species point to a key role in coupled sleep oscillations, but the dynamics of endogenous reactivation in humans and its relevance for memory consolidation has remained unclear.

In the current study, we tackled this question by employing two learning sessions per participant, each using different and analytically discriminable learning stimuli (object and scene images, Figure 1a). To ensure that multivariate measures of reactivation not merely reflect session-specific EEG properties, we included an object/scene localizer task in each session and trained a linear classifier on the combined data. This allowed us to track the re-emergence of learning categories during the nap periods. It deserves mention that decoding levels were modest in general and not every participant reached above-chance classification (18/20, see Figure 2d and Table S 3). Several reasons might limit the effect size when decoding memory reprocessing during sleep. First, the signal of interest (i.e., sleep electrophysiology) is inherently noisy. Guided by theoretical considerations we limited the search-space for memory reactivation to the presence of SO-spindle complexes. Still, it is unlikely that each single SO-spindle complex is associated with memory reactivation. Including the presence of ripples as a criterion may

increase sensitivity, but even SO-spindle-ripple complexes are unlikely to yield robust memory reactivation in every instance (Swanson et al., 2020). Second, our data show that SO down-states represent viable reference points for time-locking the analysis of memory reactivation. However, there is considerable variability in signal characteristics across SOs and spindles (e.g., event durations or peak times), and such across-event variability diminishes classification power which relies on spatiotemporal activation patterns common across events. That said, decoding levels observed here are in line with previous TMR studies examining sleep-related memory reactivation with multivariate classification (Belal et al., 2018; Cairney, Guttesen, et al., 2018; Wang et al., 2019). Importantly, we found that higher decoding performance correlates with the behavioural expression of memory consolidation across participants, further corroborating the functional significance of reactivation.

Another key feature of our paradigm was the assessment of both item- and associative memory performance. Interestingly, the strength of memory reactivation during sleep predicted consolidation levels for associative memory only. This finding could indicate that reactivation particularly benefits hippocampus-dependent memories (Davachi, 2006). However, it might also reflect the fact that reactivation pertained to the categorical features of the learning material, which was also the aspect relevant for associative- and not item memory. Moreover, while performance levels were carefully matched between object and scene tasks (Figure 1b), performance was lower for associative memory than for item recognition. Thus, differential effects of reactivation for associative- vs. item memory could also suggest differential benefits of sleep for weaker vs. stronger memories (Cairney et al., 2016; Creery et al., 2015; Drosopoulos, Schulze, et al., 2007; Schapiro et al., 2017; but see Petzka et al., 2021).

Owing to the limited spatial resolution of scalp EEG (especially for transient high-frequency oscillations), our current data remain agnostic with regard to hippocampal ripples. That said, a recent iEEG study has shown that both hippocampal ripples and hippocampal–cortical interactions are most eminent when preceded by a cortical SO-spindle complex (Helfrich et al., 2019). To the extent that reactivation observed here is linked to hippocampal engagement, the timing of our effects (Figure 2a, b) is consistent with accumulating evidence that the hippocampal–cortical dialog is in fact initiated by cortex (Helfrich et al., 2019; Navarrete et al., 2020; Ngo et al., 2020; Rothschild, 2019; Rothschild et al., 2017). One tentative interpretation of our results might thus be that cortical SO-spindle complexes trigger hippocampal memory reactivation while ensuring that the cortical target area is optimally tuned for synaptic plasticity and memory reprocessing (Niethard et al., 2018; Rosanova & Ulrich, 2005; Sejnowski & Destexhe, 2000). Indeed, recent rodent work has shown that optogenetic induction of SO-locked spindles enhances SOs-spindle-ripple coupling and the consolidation of hippocampus-dependent memories (Latchoumane et al., 2017). Our finding that reactivation peaks towards the end of spindles (Figure 2b) is consistent with the idea that mnemonic reprocessing and integration into neocortical networks continue after sleep spindles, i.e., during periods of spindle "refractoriness" (Antony et al., 2018). Likewise, intracranial recordings in humans have shown that hippocampal–cortical connectivity ("mutual information") mediated by hippocampal ripples occurred ~500–1500 ms after the SO downstate (Helfrich et al., 2019), again matching the time window in which we observed memory reactivation. Together, one tentative scenario might be that memory processing is most beneficial after SO-spindle complexes, i.e., at time points of elevated cortical plasticity.

Analytically, our approach relied on (i) matching behavioural performance between sessions, (ii) pooling sleep data across both sessions, and (iii) deriving evidence for the reactivation of learning material across all aggregated SO-spindle complexes. These design features leave some interesting questions open for future work. First, to what extent might trait-like participant characteristics drive both reactivation and memory processes? Using our sleep questionnaires, we were able to rule out subjective sleep quality and circadian rhythm as confounds (see Supplementary Notes), but there may be other trait-like factors impacting reactivation and consolidation. An alternative design would be to conduct a longitudinal study in which within-participant levels of learning and consolidation are experimentally manipulated across multiple sessions (e.g., by varying encoding depth or task difficulty). Second, while aggregating all SO-spindle events is essential for the classification approach, it leaves open whether reactivation occurs during each SO-spindle event. An alternative approach might be to use intracranial recordings to identify single neurons that are tuned to stimuli used in a specific learning session and then track engagement of these neurons during individual SO-spindle complexes. Such more fine-grained methods might provide additional insights into reactivation-related characteristics (e.g., accuracy and frequency of reactivation processes). In conclusion, our results indicate that endogenous memory reactivation in service of sleep-dependent consolidation is clocked by the fine-tuned coupling of SOs and spindles. Future work employing simultaneous recordings from the hippocampus will further elucidate the intricate dynamics underlying the hippocampal–cortical dialog of systems consolidation.

4. Methods

4.1. Participants

Twenty healthy, right-handed participants (mean age: 20.75 ± 0.35; 17 female) with normal or corrected-to-normal vision took part in the experiment. An additional five participants had to be excluded due to insufficient sleep (less than 30 min sleep during one of the sessions). The sample size was determined in accordance with previous human sleep and memory studies (e.g., Helfrich et al., 2018; Ngo et al., 2015). Pre-study screening questionnaires (including the Pittsburgh Sleep Quality Index, PSQI, Buysse, Reynolds, Monk, Berman, & Kupfer, 1989), the morningness–eveningness questionnaire (Horne & Ostberg, 1976), and a self-developed questionnaire querying general health status and the use of stimulants) indicated that participants did not take any medication at the time of the experimental session and did not suffer from any neurological or psychiatric disorders. All participants reported good overall sleep quality. Furthermore, they had not been on a night shift for at least 8 weeks before the experiment. All participants were instructed to wake up by 7 a.m. and avoid alcohol the evening before and caffeine on the day of the experimental sessions. They confirmed at the beginning of each experimental session their adherence to the requirements. The study was approved by the University of Birmingham Research Ethics Committee and written informed consent was obtained from participants.

4.2. Stimuli and procedures

Overview

The experiment consisted of two experimental sessions (object and scene condition), separated by at least 1 week (mean = 8.5 ± 0.85 days). The order of the two sessions was counterbalanced across participants. On experimental days participants arrived at the sleep laboratory at 11 a.m. The experimental session started with the set-up for polysomnographic recordings during which electrodes for electroencephalographic (EEG), electromyographic (EMG), and electrocardiographic (ECG) recordings were applied. Before the experimental sessions, participants were habituated to the environment by spending an adaptation nap in the sleep laboratory.

At around 12 a.m. the experiment started with a modified version of the psychomotor vigilance task ("PVT", Dinges & Powell, 1985), followed by the memory task (for details see Memory Task below). The sleep period began at ~1 p.m. and participants were given 120 min to nap (mean total sleep time: 101.63 ± 2.23 min; for sleep characteristics see Table S 2). Afterwards, the vigilance of all participants was assessed using the PVT and memory performance was tested again. At the end of each session a localizer task was conducted (see Localizer Task for details).

Stimuli

A set of in total 360 verbs and 240 images (half objects and half scenes) served as experimental stimuli during both sessions. Objects were images of animals, food, clothing, tools, or household items presented on a plain white background (e.g., a hammer). Scenes were images of nameable landscapes or places (e.g., a coffee shop). All images were taken from (Konkle et al., 2010).

Experimental tasks

For the recording of behavioural responses and the presentation of all experimental tasks, Psychophysics Toolbox Version 3 (Brainard, 1997) and MATLAB 2018b (MathWorks, Natick, USA) were used. Participants completed a practice run (five trials) of each experimental task in advance to ensure they fully understood the instructions. Responses were made via keyboard presses on a dedicated PC. Across all experimental phases, presentation order of stimuli was randomized across participants.

Psychomotor vigilance task

The vigilance of the participants was assessed using a modified version of the "PVT" (Dinges & Powell, 1985) before the encoding phase and right after the sleep period. Participants were presented with a centred fixation cross on the computer screen. Every 2–10 s the fixation cross was replaced by a counter counting up from 0 to 2 s in steps of 20 ms. Participants were instructed to stop the counter as fast as possible by pressing the space bar. After each trial participants were provided with feedback about their reaction time. The task was administered for 5 min. For PVT related results see Figure S 8.

Familiarization

The experiment began with an image familiarization phase. The purpose of this part was (i) to facilitate learning of the verb-image pairs in the main encoding session and (ii) to provide the proper image names for subsequent cued recall. Each trial started with a fixation cross, presented for 1.5 ± 0.1 s. Subsequently, participants saw one of 130 images showing objects or scenes (depending on the experimental session). About 120 of these images were part of the subsequent learning material and were accompanied by a caption naming the exemplar. Ten additional images, which were not further used during the

experiment, were accompanied by an erroneous description. Each stimulus combination was presented for 2.5 s on the computer screen. The participants' task was to press a button whenever they encountered a wrong image-word combination.

Encoding

Participants learned pairwise associations between 120 verbs and images. The images comprised either objects or scenes (depending on experimental session).

Each trial started with a fixation cross, presented for 1.5 ± 0.1 s. Afterwards, a verb (e.g., "jump") was presented for 1 s on the computer screen and immediately followed by the to-be-associated image for 4 s. Participants were instructed to form a vivid mental image or story linking the verb and the object/scene. After the presentation of the image (4 s), they had to indicate whether the image they had formed was realistic or bizarre. In addition, participants were informed that their memory performance for verb- image pairs would be tested later. The learning block was run twice with varying trial order to reach satisfactory levels of pre-sleep memory performance (as determined in a pilot study).

Pre-sleep memory test

In order to prevent any testing effect on our behavioural measures of memory consolidation (Antony et al., 2017; Roediger & Karpicke, 2006), only half of the learned verb-image combinations was tested during the pre-sleep memory test. Thus, the pre-sleep memory test included 60 randomly chosen verbs intermixed with 30 new verbs, which were not seen by the participants before ("foils"). Each trial started with a fixation cross, presented for 1.5 ± 0.1 s. After the fixation cross, a verb was presented on the computer screen. After 3 s, participants had to indicate whether the verb was "old" (i.e., part of the learning material) or "new"' (i.e., it was not seen during learning) within the

next 10 s. In case of "new" responses, participants immediately moved on to the next trial. In case of "old" responses, participants were required to type a description of the image they had in mind or to type "do not know" in case they could not recall the target image. Trials were coded as correct if (i) the participant typed the same caption as shown during the familiarization phase or (ii) the description unambiguously matched the content of the image

Sleep period

The nap period began at ~1 p.m. Participants had the opportunity to sleep in a laboratory bedroom for 120 min, while their brain activity was monitored using polysomnography).

Post-sleep memory test

Twenty minutes after waking up, participants performed another memory test on the remaining 60 study items. This followed the same procedures as the pre-sleep memory test with the exception that new foil verbs were used.

Localizer task

During the localizer task participants were presented with a new set of images comprising objects and scenes (90 objects and 90 scenes, irrespective of session). Each trial started with a fixation cross, presented for 1.5 ± 0.1 s. Subsequently, a randomly chosen image (object or scene) was presented on the computer screen for a minimum of 2.5 and a maximum of 10 s. Each image was presented twice during the task and participants were instructed to indicate whether it was shown for the first ("new") or second ("old") time (mean distance between successive presentations = 8.06, range = 2–33).

By administering the localizer task at the very end of each session, we assured that participants engaged exclusively with a given stimulus category before sleep (objects or

scenes, respectively). The rationale of this approach was to keep the category-specific representations during learning as pure as possible, in an effort to bias their reactivation during the subsequent sleep period. However, presenting both stimulus categories during the localizer task ensured that category-specific classifier evidence during sleep would not merely reflect general differences between sessions (e.g., electrode impedances, electrode positions, etc.).

4.3. EEG

A Brain Products 64 channel EEG system was used to record electroencephalography (EEG) throughout the experiment. Impedances were kept below 10 kΩ. EEG signals were referenced online to electrode FCz and sampled at a rate of 1000 Hz. Furthermore, EMG and the ECG was recorded for polysomnography. Sleep architecture was determined offline according to standard criteria by two independent raters (Iber et al., 2007).

4.4. Data analysis

Behavioural preprocessing

To assess recognition memory performance, we calculated the sensitivity index d′ [i.e., z(Hits)—z(False Alarms)] according to signal detection theory. Proportions of 0 and 1 were replaced by $1/2N$ and $1–1/2N$, respectively, with N representing the number of trials in each proportion (i.e., $N = 60$, see ref. Macmillan & Creelman, 2005).

For associative memory performance we calculated the proportion of correctly recalled images relative to the number of recognized words (i.e., (recalled images/hits) ∗∗ 100). To correlate levels of memory retention and reactivation strength we derived a "retention index". We computed the proportion of post-sleep recalled images (out of hits) in relation

to pre-sleep memory performance (i.e., (recalled out of hits post-sleep/recalled out of hits pre-sleep) ∗ 100) and collapsed these measures across sessions.

EEG data analysis

EEG data were preprocessed using the FieldTrip toolbox for EEG/MEG analysis (Oostenveld et al., 2011). All data were downsampled to 200 Hz. Subsequently, the localizer and sleep data were segmented into epochs. The temporal range of the epochs was [–1 to 3] s around stimulus onset for localizer trials. As in other studies concentrating on the coordination of SOs and spindles (Demanuele et al., 2017; Hahn et al., 2020; Helfrich et al., 2018; Muehlroth et al., 2019; Staresina et al., 2015) we specifically focused on electrode Cz due to the spatial distribution of both oscillations. Both oscillations show strong presence over central areas, rendering Cz an optimal target zone for investigating concomitant activity of SOs and (fast) spindles. Hence, for the sleep data, slow oscillation—spindle epochs [–2.5 to +2.5 s] time-locked to SO down-states were extracted from channel Cz (for details see Event detection).

Noisy EEG channels were identified by visual inspection, discarded, and interpolated, using a weighted average of the neighbouring channels. The localizer data were additionally subjected to an independent component analysis (Jung et al., 1998) and ICA components associated with eye blinks and eye movements were identified and rejected.

Event detection and SO-spindle coupling

SOs and sleep spindles were identified for each participant, based on established detection algorithms (Ngo et al., 2013; Staresina et al., 2015). Following standard procedures, all sleep data were re-referenced against linked mastoids for sleep scoring and event detection (Cox & Fell, 2020; Iber et al., 2007; Silber et al., 2007); please note that the

classification results reported in Figure 2b remained unchanged when using a CAR scheme. SOs were detected as follows: Data were filtered between 0.3–1.25 Hz (two-pass FIR bandpass filter, order = three cycles of the low frequency cut-off). Only movement-free data (as determined during sleep scoring) from NREM sleep stages 2 and 3 were taken into account. All zero-crossings were determined in the filtered signal at channel Cz, and event duration was determined for SO candidates (that is, down-states followed by up-states) as time between two successive positive- to-negative zero-crossings. Events that met the SO duration criteria (minimum of 0.8 and maximum of 2 s, 0.5–1.25 Hz) entered the analysis. 5-s-long segments (±2.5 s centred on the downstate) were extracted from the unfiltered raw signal.

For spindle detection, data were filtered between 12–18 Hz (De Gennaro & Ferrara, 2003; Ngo et al., 2020; two-pass FIR bandpass filter, order = three cycles of the low frequency cut-off), and again only artifact-free data from NREM sleep stages 2 and 3 were used for event detection. The root mean square (RMS) signal was calculated for the filtered signal at channel Cz using a moving average of 200 ms, and a spindle amplitude criterion was defined as the 75% percentile of RMS values. Whenever the signal exceeded this threshold for more than 0.5 s but less than 3 s (duration criteria), a spindle event was detected. Epochs time-locked to the minimum spindle trough (−2.5 to +2.5 s) were extracted from the unfiltered raw signal for all events. To isolate SO-spindle complexes, we determined for all SOs whether a spindle was detected following the SO (SO downstate + 1.5 s). Finally, SO-spindle events were extracted (−2.5 to +2.5 s with regards to the SO downstate) from the raw signal at channel Cz.

For the analysis of SO-spindle coupling (Helfrich et al., 2019; Staresina et al., 2015), we filtered the SO-spindle data in the SO range (0.3–1.25 Hz, two-pass Butterworth bandpass filter), applied a Hilbert transform and extracted the instantaneous phase angle. Next, we filtered the same data segments in the spindle range (12–18 Hz two-pass Butterworth bandpass filter), Hilbert transformed the signal and extracted the instantaneous amplitude. Only data points within ±1.5 s were considered to avoid filter-related edge artifacts. Then we detected the maximal sleep spindle amplitude in channel Cz and isolated the corresponding SO phase angle. The preferred phase of SO-spindle coupling was then obtained from averaging all individual events' preferred phases of each participant, and the resulting distribution across participants was tested against uniformity (Rayleigh test, CircStat toolbox; Berens, 2009).

Multivariate analysis

Multivariate classification of single-trial EEG data was performed using MVPA-Light, a MATLAB-based toolbox for multivariate pattern analysis (Treder, 2020). For all multivariate analyses, a LDA was used as a classifier (Treder, 2020). Prior to classification, all data were re-referenced using a common average reference (CAR).

For classification within the localizer task, the localizer data were *z*-scored across all trials for each time point separately. Next, data from both sessions were collapsed and subjected to a principal component analysis (PCA), which transforms the data into linearly uncorrelated components, ordered by the amount of variance explained by each component (Jackson, 1991). PCA was applied to reduce dimensionality and limit over-fitting (Jiang & Guo, 2007) and the first 30 principal components were retained for further analysis (Grootswagers et al., 2017; Pinheiro-Chagas et al., 2019; Sankaran et al., 2018).

To quantify whether object and scene representations can be differentiated in the localizer, the classifier was trained and tested to discriminate between object and scene trials. Data were smoothed using a running average window of 150 ms. The EEG channels served as features and a different classifier was trained and tested on every time point. As metric, we used Area Under the ROC Curve (AUC), which indexes the mean accuracy with which a randomly chosen pair of Class A and Class B trials could be assigned to their correct classes (0.5 = random performance; 1.0 = perfect performance). To avoid overfitting, data were split into training and test sets using fivefold cross-validation (Lemm et al., 2011). Since cross-validation results are stochastic due to the random assignment of trials into folds, the analysis was repeated five times and results were averaged. For statistical evaluation, surrogate decoding performance was calculated by shuffling the training labels 250 times. Resulting surrogate performance values were then averaged, providing baseline values for each participant under the null hypothesis of label exchangeability.

To investigate differential evidence for object vs. scene representations as a function of prior learning during SO-spindle complexes (Figure 2b), we used the temporal generalization method (King & Dehaene, 2014). Prior to decoding, a baseline correction was applied based on the whole trial ([–0.5 to 3 s] for localizer segments; [–1.5 to 1.5 s] for SO-spindle segments). Next, localizer and sleep data were *z*-scored across trials and collapsed across sessions. PCA was applied to the pooled wake-sleep data and the first 30 principal components were retained. Localizer and sleep data were smoothed using a running average window of 150 ms. A classifier was then trained for every time point in the localizer data (Figure 2b, vertical axis) and applied on every time point during SO-spindle complexes (horizontal axis). No cross-validation was required since localizer and

sleep datasets were independent. As metric, we again used AUC (see above). For statistical evaluation, surrogate decoding performance was calculated by shuffling the training labels (stemming from the localizer task) 250 times. Again, the resulting performance values were averaged, providing baseline values for each participant under the null hypothesis of label exchangeability.

To resolve the topography of diagnostic features, we conducted a "searchlight decoding procedure". In brief, PCA components were projected back to sensor space and the classification procedure was repeated across moving kernels of small electrode clusters, with neighbouring electrodes being selected as features [feature number range: 5 to 9]. Classifiers were trained for every time point in the localizer data and applied on every time point during SO-spindle complexes. Finally, classification values were collapsed across our time windows of interest [localizer time: 1000 to 2000 ms; SO-spindle time: 800 to 1200 ms] and tested against chance level (corrected for multiple comparisons across space). A broad cluster of above-chance classification comprising bilateral parietal and occipital areas emerged ($p_{cluster} = 0.004$).

Time–frequency analysis

Time–frequency analysis of the SO-spindle segments was performed using FieldTrip. Frequency decomposition of the data, using Fourier analysis based on sliding time windows (moving forward in 50 ms increments). The window length was set to five cycles of a given frequency (frequency range: 1–30 Hz in 1 Hz steps). The windowed data segments were multiplied with a Hanning taper before Fourier analysis. Afterwards, power values were *z*-scored across time [–4 to 4 s]. The longer time segments were

chosen to allow for resolving low frequency activity within the time windows of interest [−1.5 to 1.5 s] and avoid edge artifacts.

4.5. Statistics

Behavioural retrieval data were subjected to a 2 (Category: Object/Scene) × 2 (Test-Time: Pre-sleep/Post-sleep) repeated measures ANOVA. To test for potential differences in memory accuracy between sessions in the localizer task, a paired sampled *t*-test was computed. The statistical significance thresholds for all behavioural analyses were set at $p < .05$. Spearman correlation was used to assess the relationship between memory retention and reactivation strength. To control for mean decoding levels from the localizer tasks (averaged across the significant cluster), a partial Spearman correlation was used. SPSS (IBM Corp., Version 26) and Matlab was used for behavioural data analyses.

FieldTrip's cluster permutation test (Maris & Oostenveld, 2007) was used to deal with the multiple comparisons problem for all classification analyses. A dependent-samples *t*-test was used at the sample level to identify clusters of contiguous time points across participants and values were thresholded at $p = 0.05$. Maxsum (sum of all *t* values in cluster) served as cluster statistic and Monte Carlo simulations were used to calculate the cluster *p* value (alpha = 0.05, two-tailed) under the permutation distribution. Analyses were performed at the group level. The input data were either classification values across time (Figure 1c) or time x time classification values (Figure 2b). In all cases a two-sided cluster permutation test with 1000 randomizations was used to contrast classification accuracy against chance performance.

Non-uniformity of the preferred phase with regard to SO-spindle coupling was assessed using the Rayleigh test (CircStat toolbox). The nonlinear relationship between SO-spindle coupling and reactivation strength was determined with a circular-linear correlation as implemented in the CircStat toolbox. A partial circular-linear correlation modified from the CircStat toolbox was used to control for the mean decoding levels from the localizer task. In all cases the statistical significance thresholds were set at $p < 0.05$.

5. Acknowledgements

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6. Author Contributions

T.S., M.P., and B.P.S. conceived the study and designed the experiment. T.S. conducted the experiment. T.S., M.P., T.St., and B.P.S. analysed the data and wrote the paper.

7. Supplemental Information

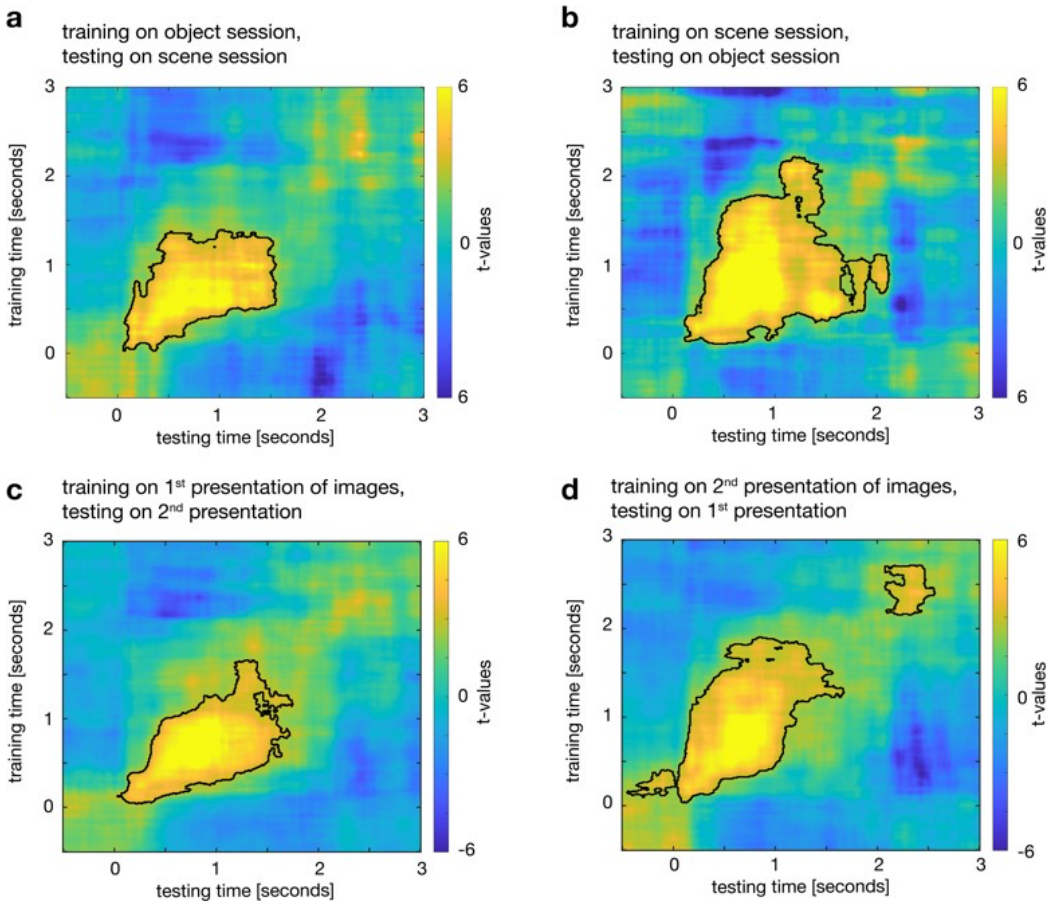

Figure S 5.1. Stability of EEG object vs. scene decoding during the localizer tasks. **(a + b)** To estimate the across-session stability of our decoding approach, we trained a classifier on the localizer data of object sessions and tested it on the localizer data of the scene sessions. **(a)** We found robust above- chance classification when training the classifier on the localizer of the object session and applying the training weights to the corresponding data from the scene session (two-sided dependent-samples t-test; p = 0.002, cluster corrected across time). **(b)** The same result pattern emerged when training on the scene session data and applying the training weights to the object session data (two-sided dependent-samples t-test; p = 0.002, cluster corrected across time). **(c + d)** During the localizer task, each image was presented twice. To estimate the across-presentation stability of our decoding approach, we trained a classifier on the first image presentation tested it on the second presentation. **(c)** We observed a significant cluster of above-chance classification when training the classifier on the first presentation and applying it to the second presentation (two-sided dependent-samples t-test; p = 0.0018, cluster corrected across time). **(d)** A highly comparable result pattern emerged when training on the second presentation and applying it to the first presentation (two-sided dependent-samples t-test; p = 0.002; p =0.019, cluster corrected across time).

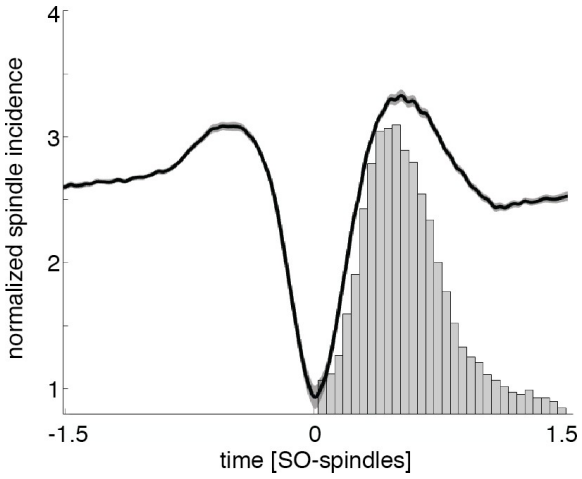

Figure S 5.2. Perievent histogram of sleep spindles (amplitude maxima) following SO down- states (time = zero; normalized by number of spindles). Note that due to our definition of SO-spindle complexes (with sleep spindles following SOs), no spindles appear prior to SO down-states (time-point zero).

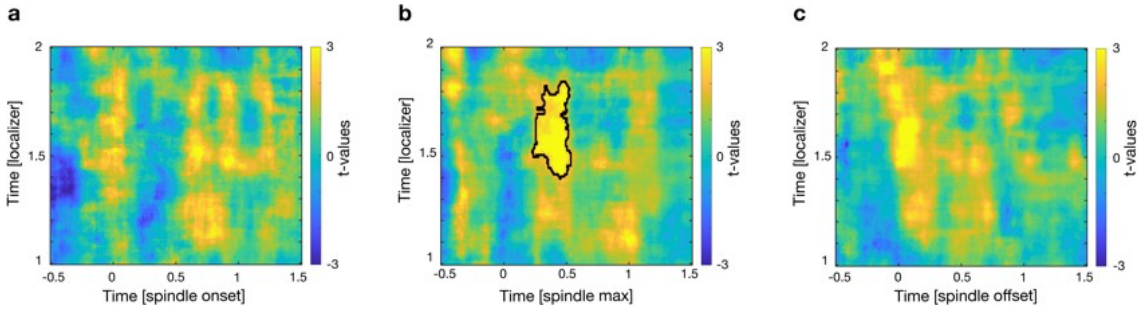

Figure S 5.3. Classification locked to different spindle features. To test for the impact of different features of SO-spindle complexes on the synchronisation of reactivation events, the classification was computed on SO-spindle data locked to the onset, maximum and offset of spindles. Informed by the main analysis, the utilized time-window of both the localizer task and SO-spindle complexes were adjusted accordingly [localizer time: 1000-2000ms; SO-spindle time: -500 to 1500ms relative to respective feature (spindle onset, maximum amplitude and offset)]. Testing accuracy levels against chance at any localizer time x sleep time point for data locked to spindle on-and offsets **(a + c)** did not lead to any significant above chance classification (two-sided dependent-samples t-test; cluster with the smallest p-value for spindle onset: $p = 0.25$; spindle offset: $p = 0.051$, cluster corrected across time). However, testing the classifier on SO-spindle data locked to the spindle maximum **(b)** peaks yielded a positive cluster of significant above chance classification (two-sided dependent-samples t-test; $p = 0.019$, SO-spindle time [250 to 500ms], localizer time-window [1400 to 1800ms], cluster corrected across time).

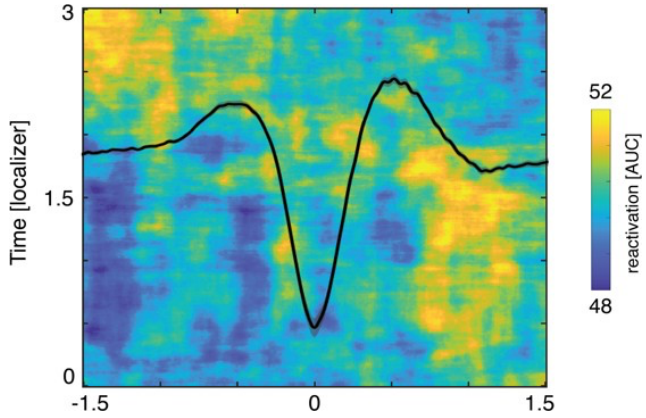

Figure S 5.4. Accuracy map for the classification during SO-spindle complexes. Corresponding accuracy map for the main decoding result reported in Fig. 2b. Color range (blue to yellow) represents decoding performance (Area Under the Curve).

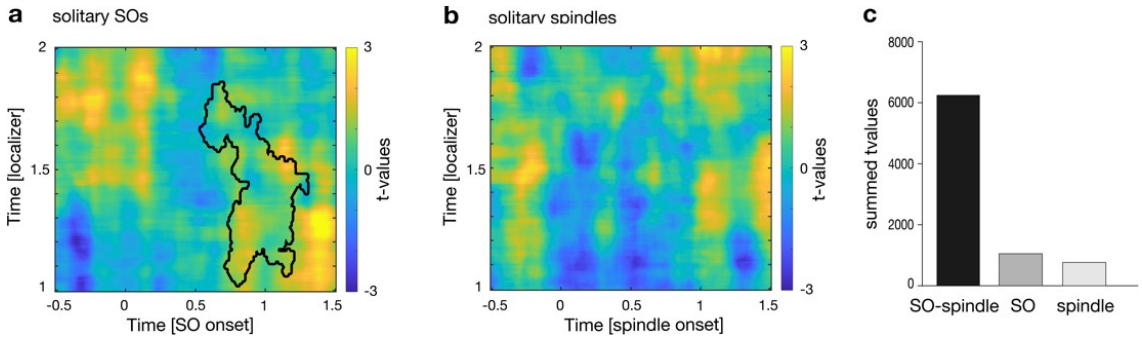

Figure S 5.5. Memory reactivation during solitary SO and spindle events. **(a + b).** To test whether endogenous memory reactivation indeed requires the joint presence of SOs and spindles, we performed the decoding procedure on solitary SO or spindle events (thus, SOs without spindles and vice versa). Time-windows of both the localizer task and SO-spindle complexes were restricted according to the main results [localizer time: 1000-2000ms; SO-spindle time: -500 to 1500ms relative to respective event (SO down-state, spindle maximum)]. For both types of events, when testing accuracy levels against chance at any localizer time x sleep time point, no significant cluster of above chance classification emerged (two- sided dependent-samples t-test: in both cases cluster with the smallest $p > 0.2$; cluster corrected across time). The black contour lines in (a) illustrate the extent of the significant cluster derived from the main analysis (classification during the presence of SO-spindle complexes). **(c)** Summed t-values of the significant classification cluster (as derived from the main analysis, corresponding to the black contour lines in Figure 2b and Figure S 5a) for SO-spindle complexes, solitary SOs and solitary spindles.

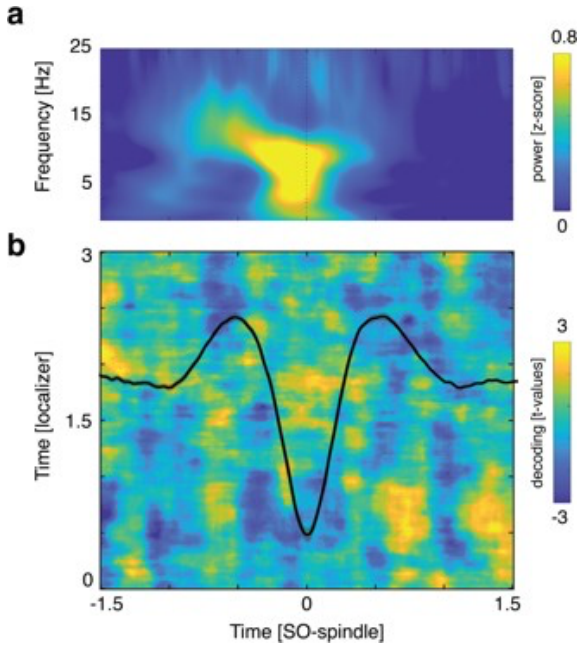

Figure S 5.6. SO-slow spindle locked memory reactivation. **(a)** Time frequency representation of all slow spindle-SO segments (z-scored across time; only positive values are displayed). **(b)** When testing accuracy levels against chance at any localizer time x sleep time point, no significant cluster of above- chance classification emerged (two-sided dependent-samples t-test; cluster with smallest p value: 0.67, cluster corrected across time). The black line illustrates the averaged EEG trace of all slow spindle-SO segments (electrode Fz).

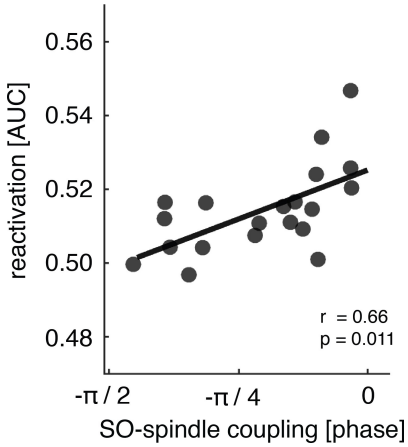

Figure S 5.7. Precision of SO-spindle coupling correlates with reactivation strength. Circular- linear correlation analysis between the individual mean SO-spindle coupling phase (circles) and the mean reactivation strength revealed a positive association ($r = 0.66$; $p = 0.011$).

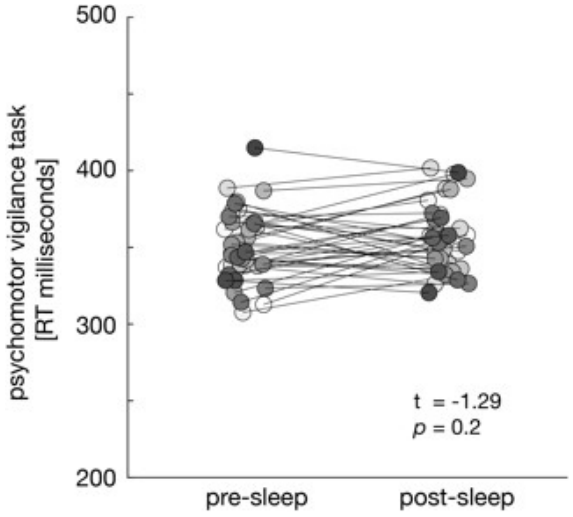

Figure S 5.8. PVT results. Before encoding and after the sleep period participants' vigilance state was assessed using a modified version of the psychomotor vigilance task (PVT). During the pre- encoding task the mean reaction time was 350.6 ± 3.6 ms, while the average response time during the post-sleep PVT was 355.2 ± 3.6 ms. Reaction times did not differ between testing times (pre-encoding vs. post-sleep; t = -1.29, p = 0.20).

Table S 5.1. Overview of memory performance. Associative memory % refers to the percentage of correctly recalled images (relative to the total number of stimuli), while associative memory [out of hits] refers to the percentage of recalled image exemplars out of correctly recognized verbs. Statistical differences between conditions (objects vs. scenes) were assessed using dependent samples t-tests (two-sided).

	Objects	Scenes	*t*	*P*
Recognition [Hits] %				
pre-sleep	72.16 ± 4.16	70.91 ± 4.26	0.47	0.64
post-sleep	63.16 ± 4.19	63.58 ± 4.76	-0.79	0.86
post relative to pre	87.41 ± 2.82	87.97 ± 2.88	-0.16	0.85
Recognition [Correct Rejections] %				
pre-sleep	90.00 ± 2.14	85.33 ± 4.48	1.13	0.27
post-sleep	88.00 ± 2.89	83.33 ± 5.22	0.98	0.33
post relative to pre	97.49 ± 1.75	97.33 ± 3.60	0.04	0.96
Recognition [d']				
pre-sleep	2.11 ± 0.14	2.02 ± 0.22	0.47	0.64
post-sleep	1.76 ± 0.19	1.69 ± 0.23	0.37	0.71
Associative Memory %				
pre-sleep	49.16 ± 4.87	46.75 ± 3.08	1.13	0.41
post-sleep	40.08 ± 4.94	36.50 ± 4.46	1.11	0.28
post relative to pre	76.52 ± 5.27	72.61 ± 4.51	0.71	0.48

Associative Memory [out of hits] %				
pre-sleep	64.90 ± 3.99	63.72 ± 5.20	0.33	0.73
post-sleep	59.39 ± 5.71	55.82 ± 5.47	-1.03	0.31
post relative to pre	86.95 ± 5.52	82.55 ± 4.84	0.50	0.55

Table S 5.2. Sleep characteristics. Data are means ± s.e.m. N1, N2: NREM sleep stages N1 & N2, SWS: slow-wave sleep, REM: rapid eye movement sleep, WASO: wake after sleep onset. Statistical differences between conditions (objects vs. scenes) were assessed using dependent samples t-tests (two-sided).

Sleep stage [%]	Objects	Scenes	*t*	*P*
N1	2.9 ± 1.6	13.5 ± 2.1	-0.6	0.53
N2	39.5 ± 2.6	48.1 ± 3.1	-1.7	0.09
SWS	22.9 ± 3.3	19.4 ± 2.6	-1.2	0.25
REM	21.9 ± 3.4	16.7 ± 2.8	1.4	0.15
WASO	2.3 ± 0.9	1.4 ± 0.7	0.7	0.49
Total Sleep Time [min]	102.6 ± 3.4	100.6 ± 2.9	0.6	0.53
# spindles	184.8 ± 12.9	188.9 ± 17.9	-0.1	0.85
Spindle density	2.9 ± 0.1	2.82 ± 0.1	0.3	0.74
Spindle duration	0.81 ± 0.01	0.78 ± 0.01	1.3	0.19
Spindle frequency	14.02 ± 0.1	14.06 ± 0.1	-0.2	0.81
# SOs	445.5 ± 31.2	474.1 ± 43.8	-0.5	0.59
SO density	6.9 ± 0.25	7.1 ± 0.32	-0.4	0.68
SO duration	1.38 ± 0.01	1.38 ± 0.01	0.5	0.61
#SO – spindle comp. range	49.4 ± 3.6 [25-88]	50.7 ± 4.9 [12-89]	-0.3	0.79

Table S 5.3. Participant-specific decoding performance averaged across the significant cluster of localizer – SO-spindle classification.

P1	0.501
P2	0.497
P3	0.517
P4	0.547
P5	0.510
P6	0.504
P7	0.507
P8	0.534
P9	0.515
P10	0.504
P11	0.508
P12	0.499
P13	0.520
P14	0.511
P15	0.515
P16	0.526
P17	0.512
P18	0.511
P19	0.524
P20	0.516

7.1. Supplemental Notes

Assessing the impact of trait-like characteristics on the interplay of memory reactivation with the preferred SO-spindle phase and the behavioural expressions of consolidation.

Our results suggest that memory reactivation is linked to the preferred SO-spindle phase as well as to behavioural expressions of consolidation. However, SO-spindle coupling and consolidation might also be governed by other, trait-like participant characteristics. We thus examined - using hierarchical regressions - whether subjective sleep quality (as

determined the Pittsburgh Sleep Quality Index (PSQI)) or circadian rhythm (derived from the Morningness-Eveningness Questionnaire (MEQ)) would account for memory performance or the preferred phase of SO-spindle coupling above and beyond reactivation strength during sleep.

In Step 1, reactivation strength explained 29.5% of the variance in memory performance ($R^2 = 0.295$, $F_{1,18} = 7.51$, $P = 0.013$). In Steps 2 and 3, neither subjective sleep quality (PSQI global score collapsed across the two sessions, range = 2-5 across participants), nor circadian rhythm (MEQ score collapsed across the two sessions, range = 31-66 across participants) explained significant amounts of additional variance (Step 2: $\Delta R^2 = 0.001$, $\Delta F_{2,16} = 0.014$, $P = 0.91$; Step 3: $\Delta R^2 < 0.01$, $\Delta F_{3,13} < 0.01$, $P = 0.98$). Similarly, using the preferred phase of SO-spindle coupling as the dependent variable, in Step 1 reactivation strength explained 37.2% of the variance ($R^2 = 0.372$, $F_{1,18} = 10.65$, $P = 0.004$). In Step 2 and 3, neither subjective sleep quality nor circadian rhythm explained significant amounts of additional variance (Step 2: $\Delta R^2 = 0.07$, $\Delta F_{2,16} = 0.18$, $P = 0.67$; Step 3: $\Delta R^2 = 0.01$, $\Delta F_{3,13} = 0{,}30$ $P = 0.59$). Please note that the distribution of the preferred phase values (all clustering between $-\pi / 2$ and 0 degrees) enabled us to add these circular data to the linear regression analysis. Nevertheless, to further test for potential associations between subjective sleep quality, circadian rhythm and the preferred phase of SO-spindle coupling, we administered additional circular- linear correlations. Neither sleep quality ($rho = 0.04$, $p = 0.98$) nor circadian rhythm ($rho = 0.24$, $p = 0.47$) correlated significantly with the preferred phase.

Together, although other trait-like characteristics not captured in the present analysis might have an influence, these results rule out that the link between memory reactivation and consolidation or the phase of SO-spindle coupling is driven by participants' subjective sleep quality or circadian rhythm.

Chapter 6. General Discussion

In this chapter, the main findings of all experimental chapters will be summarised, relate to each other and limitations will be highlighted. It will be discussed to which extent answers to the two questions raised in chapter 1 (i.e., *Which* and *how* are memories consolidated during sleep?) are advanced by the main findings of this thesis. Consistent and inconsistent findings within the thesis will then be addressed and embedded in the existing literature. Future research directions and ideas emerging from findings of this thesis and related findings in the field will be outlined.

1. Main findings

1.1. Which memories are consolidated during sleep? – Weaker as well as stronger memories are consolidated during sleep

It is well established that sleep benefits declarative memory consolidation (Diekelmann & Born, 2010; Jenkins & Dallenbach, 1924; Müller & Pilzecker, 1900; Rasch & Born, 2013). Yet, it is still unknown whether all declarative memories benefit equally from sleep or whether a selection process determines a preferred consolidation of some memories over others.

Recent findings suggest a preferred consolidation of weakly over strongly encoded memories during post-encoding sleep (Cairney et al., 2016; Creery et al., 2015; Denis, Schapiro, Poskanzer, Bursal, Charon, et al., 2020; Denis, Mylonas, et al., 2021; Drosopoulos, Schulze, et al., 2007; Lo et al., 2014; Payne et al., 2012; Schapiro et al., 2017, 2018). However, performance for weakly and strongly encoded memories is often tested under the same retrieval conditions which, in some instances, result in ceiling

effects for stronger memories and hence, complicate the detection of sleep-dependent consolidation effects.

In chapter 2, we tested the hypothesis that both weakly and strongly encoded memories may benefit from post-learning sleep, but that an adjustment of retrieval conditions is required. We hypothesised that, for strongly encoded memories, retrieval difficulty has to be increased to mitigate ceiling effects as ceiling effects may obscure potential sleep-dependent consolidation effects. To this end, we developed a new memory paradigm (*Memory Arena*, Chapter 2, Figure 1 and Chapter 3, Figure 1) and manipulated in a between-subjects design (a) the retention interval which was either experienced asleep or awake, (b) memory strength that was either weak or strong (applying a training threshold of 1x50% for weakly encoded memories vs. 2x70% for strongly encoded memories) and (c) retrieval difficulty that was either normal or difficult (standard retrieval condition vs. inducing retroactive interference to increase retrieval difficulty). Under standard retrieval conditions (without retroactive interference), we replicated the finding that weakly compared to strongly encoded memories are preferentially consolidated during sleep. Intriguingly, and in line with our hypothesis, an increase in retrieval difficulty (by inducing retroactive interference) revealed sleep-dependent consolidation effects for weakly as well as for strongly encoded memories. It is worth noting that the results of chapter 3 further substantiate this conclusion. In both chapters, the *Memory Arena* paradigm was used to capture memory consolidation. This offered the possibility to apply the most suitable thresholds (for memory strength and retrieval difficulty) to measure sleep-dependent consolidation in chapter 3. Thus, in chapter 3, the same thresholds for memory strength and retrieval difficulty were applied as for the sleep group with high memory strength and high retrieval difficulty in chapter 2: Memories were strongly

encoded by applying a training threshold of 2x70% and following sleep, retrieval difficulty was increased by inducing retroactive interference. In chapter 3, we then showed that consolidation of memories can be predicted by an overlap between encoding and spindle topographies, even though the memories were strongly encoded. Therefore, in chapter 3, we demonstrate complementary results substantiating and furthering our conclusions from chapter 2 such that strongly encoded memories are consolidated during post-encoding sleep and that sleep spindles are a vehicle to provide their consolidation.

Diverging from our conclusions, a recent study argues for sleep spindles specifically predicting memory consolidation of weakly encoded memories (Denis, Mylonas, et al., 2021). While Denis et al. (2021) did not take encoding topographies into account, it is still worth considering that memory strength in this study, like in others (Creery et al., 2015; Denis, Schapiro, Poskanzer, Bursal, Charron, et al., 2020; Schapiro et al., 2017), was manipulated within rather than across participants. Precisely, memory strength was manipulated within participants and across word pairs in such a way that for each participant, some word pairs were presented more frequently during encoding than others. Results revealed that weakly encoded word pairs are preferentially consolidated during sleep (Creery et al., 2015; Denis, Schapiro, Poskanzer, Bursal, Charron, et al., 2020; Denis, Mylonas, et al., 2021; Schapiro et al., 2017).

However, while these studies did not vary retrieval demands in dependence of weakly and strongly encoded word pairs, we did not manipulate memory strength on the individual item level. One might speculate that, independently of our memory strength manipulation, some items were more strongly encoded than others. For example, within the stronger memory condition (training threshold of 2x70%) memory strength might still vary on the individual item level. Consequently, based on our findings, we cannot argue

for the absence of a selection process within each memory strength condition. If a selection process according to memory strength is assumed, however, our results imply that a possible selection is based, at least partly, on the memory strength of an item relative to the memory strength of other items rather than on an absolute memory strength threshold. This is because we found a sleep-dependent consolidation effect also in the strongly encoded condition.

To further characterise such a selection process, a future study has to incorporate (a) a memory strength manipulation on an individual item level (relative memory strength as Denis et al., 2021, did), (b) a memory strength manipulation of multiple items (between-subjects factor to manipulate the absolute memory strength threshold as we did in chapter 2) and (c) a variation of retrieval difficulty depending on an item's memory strength (e.g., by manipulating the target-lure similarity in an item recognition task). This study can test three different predictions of how a selection process might look like: First, the selection process can be based on an absolute memory strength criterion that is independent of the number of items that are competing for consolidation (e.g., items with memory strength $> x_{thres}$ are not consolidated at all, independently of the memory strength of other competing items). Second, the selection process can be based on a relative memory strength criterion that is independent of the absolute memory strength (e.g., $item_1$ is always consolidated if memory strength of $item_1$ < memory strength of $item_2$; x_{thres} in this case does not matter). Third, the selection process can rely on both, on an absolute as well as relative memory strength criterion.

Together, a follow-up study described here would address the existence as well as the characteristic of a potential selection process for sleep-dependent memory consolidation and provide important information about which memories are consolidated during sleep.

1.2. How are memories consolidated during sleep? Sleep spindles and slow oscillations orchestrate memory reactivation

Sleep-dependent memory consolidation happens on a synaptic as well as on a systems level. While synaptic consolidation includes the remodelling of synapses and dendritic spines representing a memory trace, systems consolidation incorporates changes in memory representations across different brain areas (Dudai, 2004; Klinzing et al., 2019). The two mechanisms underlying consolidation which are discussed in this thesis (induction of plasticity and reactivation) might be attributed, at least partly, to the synaptic and systems level, respectively. The induction of plasticity as a mechanism subserving consolidation is often addressed by synaptic consolidation approaches, whereas memory reactivation is often defined as a mechanism by systems consolidation approaches.

However, in chapter 3 we deviated from a strict categorisation into a synaptic and systems level by deriving a hypothesis from findings mainly addressing synaptic consolidation and testing this hypothesis on the systems level. Biophysical models and rodent research suggest that sleep spindles are a prime candidate to induce changes in synaptic plasticity in cortical neurons (Dickey et al., 2021; Niethard et al., 2018; Rosanova & Ulrich, 2005; Seibt et al., 2017; Sejnowski & Destexhe, 2000). Consequently, memory consolidation might rely on sleep spindles inducing synaptic plasticity specifically in cortical areas representing encoded memory traces. To test the hypothesis that memory consolidation can be explained by an overlap between encoding and spindle topographies, the memory paradigm of chapter 2 was applied in combination with EEG. We first demonstrated that the amplitude of sleep spindles correlated with encoding patterns and further, that the overlap between encoding and spindle amplitude pattern was associated with memory consolidation. Chapter 3 suggests that the induction of synaptic plasticity in encoding

related areas might be a mechanism of how sleep spindles subserve memory consolidation. While this is a tempting interpretation, it is worth noting that the evidence presented is chapter 3 is indirect (due to calcium activity as a proxy of synaptic plasticity not being measured), correlative and based on a small sample size (n = 19). Moreover, the *Memory Arena Task,* despite being a useful task for capturing temporal and spatial memory components, it has its downsides. That is, the lack of clearly bounded events makes it challenging to analyse the EEG signal due to missing event onsets and a pre-event baseline. To counteract this limitation, in chapter 3 an external baseline was used that closely resembles a pre-event baseline in an event-related memory paradigm. Nevertheless, replicating the finding from chapter 3 with an event-related memory paradigm would provide important substantiation of the presented results.

Future research is yet required to replicate the findings and also provide direct evidence (on the synaptic level) for our interpretation, e.g., by simultaneously measuring calcium and spindle activity during post-learning sleep in rodents and comparing calcium and spindle activity between cortical areas engaged and not engaged in pre-sleep learning.

In chapter 4 and 5, evidence for another mechanism, i.e., memory reactivation, is provided. Theories state that memory consolidation is acquired by a simultaneous reactivation of memory representations in hippocampal and cortical areas (Rasch & Born, 2007). Consequently, memory representations are redistributed from hippocampal to cortical areas and hence, transformed from labile into stable representations (Marr, 1971). The simultaneous reactivation across brain areas (i.e., hippocampus and neocortex) is thought to be synchronised by sleep oscillations, including slow oscillations, sleep spindles and hippocampal sharp-wave ripples (Buzsáki, 1996; Diekelmann & Born, 2010; Paller et al., 2021; Walker & Stickgold, 2004). In humans, empirical evidence for

reactivation of information encoded during wakefulness is rare (but see Belal et al., 2018; Schönauer et al., 2017; Schreiner, Doeller, Jensen, Rasch, & Staudigl, 2018; Zhang, Fell, & Axmacher, 2018), leaving the interplay between memory reactivation and sleep oscillations mostly unaddressed. Exploiting new developed methods including MVPA (Grootswagers et al., 2017; Norman et al., 2006), we provide evidence for a temporal coupling of reactivation and sleep oscillations (slow oscillations and sleep spindles). In chapter 4, we investigated the timescale on which sequential memories are reactivated by applying MVPA and tested for a potential synchronisation by slow oscillations. To induce memory reactivation in this study, we applied a TMR protocol. In chapter 5, we used MVPA again to then investigate the interplay between endogenous memory reactivation and slow oscillation and sleep spindles. In chapter 4 and 5 slow oscillations up-state were identified as the critical time window of memory reactivation. However, while in chapter 4, memory reactivation (of the second sequence element) was nested in the slow oscillations up-state, in chapter 5, memory reactivation was nested in the slow oscillations up-state when spindles co-occurred. Solitary slow oscillations, without an accompanied sleep spindle, did not synchronise reactivation of previously encoded memories. Directly comparing both studies and drawing conclusions about slow oscillation-sleep spindle coupling in respect to sequential memory reactivation is difficult. Analysing slow oscillation-sleep spindle coupling in chapter 4 was limited due to a low number of spindles evoked by sound cues. A full night study design potentially results in a higher number of spindles evoked by sound cues and is more suitable to address the importance of slow oscillation-sleep spindle coupling for sequential memory reactivation.

Owing to the limited spatial resolution of scalp EEG, we cannot draw reliable conclusions about hippocampal activity and hippocampal ripples. fMRI and/or iEEG are more

suitable to capture hippocampal activity/ripples and should be leveraged to elaborate the interplay between the cortex and hippocampus as well as the interplay between slow oscillations, sleep spindles and ripples during sleep-dependent memory consolidation.

2. (In)consistency across studies

2.1. Sequence memories benefit from post-encoding sleep

Naturalistic, episodic memories incorporate both spatial locations and also the temporal sequence of events. Yet, declarative memory tasks often measure memory for spatial locations (Kunz et al., 2021; Rudoy et al., 2009; Shrager et al., 2007; Talamini et al., 2008; Wang et al., 2019) or for temporal sequences of events (DuBrow & Davachi, 2013, 2016; Ezzyat & Davachi, 2014; Faber & Gennari, 2015; Michelmann et al., 2019; Tubridy & Davachi, 2011) rather than a combination of both (but see Herweg et al., 2020; Rauchs et al., 2004; Weber, Wang, Born, & Inostroza, 2014). Moreover, research addressing consolidation of declarative memories during sleep has mainly focused on memory for spatial locations, e.g., associations between objects and spatial locations, and tends to neglect memory for temporal sequences or even temporal durations of events. Specifically, memory for spatial locations has been repeatedly shown to be consolidated during sleep (Cairney, Lindsay, et al., 2018; Noack et al., 2021; Talamini et al., 2008; van Dongen et al., 2012), has been associated with sleep spindles (Antony et al., 2018; Creery et al., 2015; Wang et al., 2019) and can be strengthened by TMR (Cairney et al., 2016; Oudiette et al., 2013; Rudoy et al., 2009). Consolidation of memory for temporal sequences, on the other side, has mainly been investigated as a component of procedural memories. For example, the finger tapping task or the serial reaction time task require

participants to (explicitly or implicitly) encode a motor sequence by continuously pressing the same progression of keys (Povel & Collard, 1982; Robertson, 2007). Both tasks have been demonstrated to be sleep-dependent (Brown & Robertson, 2007; B. R. King et al., 2017; Walker et al., 2003).

So far, only a few studies explored to which extent temporal sequences as a component of declarative memories are consolidated during sleep (Drosopoulos, Windau, et al., 2007; Griessenberger et al., 2012). Here, participants had to encode the sequential order of images or nouns. Sleep directly following encoding led to superior memory performance for the sequential order compared to wake (Drosopoulos, Windau, et al., 2007; Griessenberger et al., 2012). Building on these findings, we demonstrate in chapter 2 that temporal sequences of objects were consolidated during post-learning sleep and further, in chapter 3, that memory consolidation of temporal sequences was associated with an overlap between encoding and spindle topographies. Our results, together with the previous findings, suggest that the temporal sequence of events is consolidated during sleep.

The memory paradigm that we developed offers the opportunity, besides measuring memory performance for temporal sequences, to also compare sequence and spatial memory consolidation or to investigate possible interactions between them. As recent literature suggests that both temporal and spatial aspects of memories are represented in the hippocampal signal (Herweg et al., 2020) and hippocampus-dependent memories are consolidated during sleep (Diekelmann & Born, 2010), one could speculate that both temporal and spatial aspects of memories are equally well consolidated during sleep.

In chapter 2 and 3, however, we did not follow up on this research idea as our primary interest was to identify one measure which was most sensitive to our memory strength manipulation (for a comparison between weakly and strongly encoded memories), which appeared to be sequence memory.

2.2. Sleep actively consolidates memories

As outlined in the general introduction, within the last century, three different accounts for explaining superior memory performance after sleep compared to wake were put forward (decay, interference, consolidation account). Both the decay and interference account have described sleep as a passive protector of memories against decay or interference which merely postpones the deterioration of newly formed memories (Ekstrand et al., 1977). The memory consolidation account, on the other side, has attributed a more active role to sleep in such a way that physiological processes during sleep actively transform newly formed memory traces into stable representations (Born & Wilhelm, 2012; Ekstrand et al., 1977; Müller & Pilzecker, 1900). Empirical evidence supporting the consolidation account was provided by elegantly designed behavioural studies (Gais et al., 2006; Schönauer et al., 2014; Talamini et al., 2008) as well as by studies recording brain activity to directly measure these physiological processes during sleep (Ji & Wilson, 2007; Niethard et al., 2018; Seibt et al., 2017; Wilson & McNaughton, 1994). In line with the latter, chapter 3, 4 and 5 recorded brain activity during post-encoding sleep to further characterise physiological processes underlying sleep-dependent memory consolidation, i.e., the induction of plasticity by sleep spindles (which was tested indirectly) and memory reactivation.

However, the memory consolidation account as the only explanation of superior memory performance after sleep has been recently challenged, as memory consolidation and their underlying physiological processes have been shown during wake as well. For example, memory consolidation not just depends on sleep following learning but also varies as a function of retrieval conditions. Actively retrieving memories result in superior memory performance and thus, in greater memory consolidation, compared to passively restudying them. Furthermore, actively retrieving memories protects them against interference (Potts & Shanks, 2012) similar as sleep does (Ellenbogen, Hulbert, et al., 2006) and a direct comparison between memory performance after a period of sleep, a period of wake and a period of wake with retrieval practice revealed a similar large benefit of sleep and retrieval practice on memory consolidation (Denis, DiPietro, et al., 2021). Based on these findings, it has been argued that active retrieval emulates a consolidation process similar to that occurring during sleep (Antony et al., 2017).

Besides behavioural findings and theories arguing for sleep-like consolidation processes during retrieval, physiological processes underlying memory consolidation (induction of plasticity and memory reactivation) are not exclusive to sleep and have also been observed during wake. According to the active systems consolidation hypothesis, memory representations in hippocampus and neocortex are simultaneously reactivated during sleep and thus, are redistributed from the hippocampus, a temporary storage of memories, to the neocortex, where memories are long-lastingly stored (Born & Wilhelm, 2012; Diekelmann & Born, 2010). Repeated memory reactivation results in structural changes in cortical areas and stabilises the memory trace in the long-term (Klinzing et al., 2019). Yet, memory reactivation and its relevance for consolidation has been repeatedly demonstrated during wake rest and retrieval. More concrete, the re-exposure of encoding-

related cues which assumingly triggers reactivation (targeted memory reactivation, TMR) also has a stabilizing effect on memory performance when presented during wake (Tambini et al., 2017) and resembles TMR effects during sleep (Oudiette & Paller, 2013; Rudoy et al., 2009; Schreiner & Rasch, 2015). In the same vein, applying RSA or MVPA to brain data revealed memory reactivation of neural patterns activated during encoding in subsequent wake rest periods which was further predictive for memory consolidation (Schapiro et al., 2018; Schlichtinga & Prestona, 2014). Similarly, neural patterns activated during retrieval resembles neural patterns activated during sleep (Schreiner & Staudigl, 2020) which further highlights the similarities between memory reactivation during retrieval and sleep. Intriguingly, a recent study presented a newly developed method to operationalise plastic changes in cortical areas in humans and revealed an induction of structural plasticity in cortical areas representing a memory trace already 1 hour after learning which was free of any sleep (Brodt et al., 2018). Together, these findings strongly suggest active memory consolidation during wake.

If active memory consolidation occurs during both sleep and wakefulness, then how can the superior memory performance after sleep compared to wakefulness be explained? Memory consolidation starts already during wakefulness and can be triggered by rehearsal or retrieval (Himmer et al., 2019). Nevertheless, subsequent sleep is required to ensure a further stabilisation of memories. Consequently, it is reasonable to assume that either the quality of physiological processes underlying memory consolidation or the quantity in which they occur differ between wake and sleep states.

It is yet still unknown to which extent physiological processes underlying sleep and wake-dependent consolidation are different or alike, whether they are hard-wired and always follow the same pattern or whether they flexibly change across the wake-sleep cycle.

Further, the amount of induced plasticity or the number of reactivation events has never been compared between sleep and wakefulness leaving it open for future research to investigate whether the quantity in which physiological processes occur differ between sleep and wakefulness.

2.3. Sleep spindles & slow oscillations: sometimes coupled and sometimes not

Sleep-dependent memory consolidation relies on the communication between the hippocampus and neocortex that synchronises memory reactivation in both areas and thus, enables a redistribution and stabilisation of memory traces (Diekelmann & Born, 2010; Rasch & Born, 2013). During sleep, the communication between the hippocampus and neocortex is thought to be facilitated by an interplay of slow oscillations, sleep spindles and hippocampal sharp-wave ripples (Helfrich et al., 2019; Klinzing et al., 2019; Staresina et al., 2015). Slow oscillations are changes between neuronal excitation and inhibition in cortical as well as subcortical areas (Isomura et al., 2006; Timofeev, 2011). They initiate the generation of thalamic spindles that propagate to the neocortex where they are nested in the up-state of slow oscillations and to the hippocampus where they synchronise hippocampal ripples in their troughs (Ngo et al., 2020; Staresina et al., 2015). Thus, the simultaneous occurrence of all three cardinal sleep oscillations enables synchronised memory reactivation in hippocampal and cortical sites (Helfrich et al., 2019; Ngo et al., 2020) and subserve memory consolidation (Hahn et al., 2020; Helfrich et al., 2018; Muehlroth et al., 2019).

In chapter 3 and 5, coupling between slow oscillations and sleep spindles was investigated and the results are somewhat inconsistent. In chapter 3, our main finding revealed that sleep spindles track encoding patterns in favour of memory consolidation, assumingly by

an induction of plasticity in these encoding-related areas. While recent evidence (Hahn et al., 2020; Helfrich et al., 2018; Muehlroth et al., 2019) and our results of chapter 5 highlight the importance of slow oscillation-sleep spindle coupling for memory consolidation and reactivation, in chapter 3 we found that sleep spindles track encoding patterns independently of whether they are coupled or not.

Interestingly, a recent study demonstrated that spindle topographies were unaffected by concurrent SO topographies (Klinzing et al., 2016) and theoretical and computational models (Sejnowski & Destexhe, 2000; Wei et al., 2018) propose a sequential consolidation process with sleep spindles and slow oscillations acting at different stages in the process. Together, this evidence raises the possibility that (synaptic) consolidation of encoding patterns relies, at least partly, on sleep spindles independently of slow oscillations.

It is reasonable to assume that sleep spindles fulfil different mechanistic functions which are potentially influenced by their co-occurrence with slow oscillations and hippocampal sharp-wave ripples. First, sleep spindles offer an optional time window for the induction of synaptic plasticity (Peyrache & Seibt, 2020). To ensure an efficient induction of synaptic plasticity in neuronal networks, no new input should be simultaneously processed as it results in interference and disrupts the consolidation process. Interestingly, presenting sound cues during sleep spindles results in a diminished brain response (Dang-Vu et al., 2011) and high gamma power is accompanied by a higher arousal threshold to sound cues (Lecci et al., 2017), suggesting that the brain is less responsive to external cues during sleep spindles. In the same vein, theoretical approaches argue for less information processing during oscillations such as alpha oscillations (Hanslmayr et al., 2012, 2016) or sleep spindles (Helfrich et al., 2021). Oscillations reflect highly

synchronised neuronal activity across brain regions, but information processing requires variance in neuronal activity to code information. Variance in neuronal activity is present during desynchronised brain states which then enables the (re-) processing of old and new information (Hanslmayr et al., 2012, 2016; Helfrich et al., 2021). Sleep spindles as a synchronised brain state offer perfect conditions for the induction of plasticity due to reduced information processing of new information. To which extent a coupling of sleep spindles with slow oscillations or hippocampal sharp-wave ripples, however, is relevant for the induction of plasticity is still elusive. While a recent rodent study demonstrated that Ca^{2+} activity is increased threefold when spindles were coupled to slow oscillations (Niethard et al., 2018), other studies linked sleep spindles in general to plastic changes (Rosanova & Ulrich, 2005; Seibt et al., 2017). Sleep spindles are prime vehicles for plastic changes. It is yet to be explored how the coupling of sleep spindles to other cardinal sleep oscillations influences the amount of induced plasticity.

Besides offering a time window for the induction of plasticity, sleep spindles are also involved in triggering and synchronising memory reactivation across brain areas (Helfrich et al., 2019; Ngo et al., 2020). Plastic changes can occur during solitary sleep spindles (Rosanova & Ulrich, 2005; Seibt et al., 2017). Yet, memory reactivation requires the co-occurrence of sleep spindles with slow oscillations and sharp-wave ripples (Latchoumane et al., 2017; Maingret et al., 2016). According to the previously described theoretical approaches (Hanslmayr et al., 2012, 2016; Helfrich et al., 2021) which argue for increased information processing during desynchronised brain activity, memory reactivation should ideally occur before or after sleep spindles. Indeed, recent evidence suggest an increased information processing after sleep spindles by demonstrating that the presentation of a sound cue directly following a sleep spindle (< 1sec) disrupts the

information process and leads to more forgetting (Antony et al., 2018). Similarly, the benefits of presenting sound cues during sleep (and the induction of slow oscillations and sleep spindles) was blocked when a second sound was presented shortly after (Schreiner et al., 2015). In line with this findings, in chapter 5 we found that memory reactivation, in the grand average, peaked shortly after coupled sleep spindles pointing to an increase in information processing after, not during sleep spindles (but see Cairney, Guttesen, El Marj, & Staresina, 2018).

Together, these two mechanistic functions that can be ascribed to sleep spindles explain the discrepancies between results of chapter 3 and 5. One could speculate that solitary as well as coupled sleep spindles induce plasticity in encoding-related areas (chapter 3) but that sleep spindles coupled to slow oscillations are required to trigger memory reactivation (chapter 5).

A key open question for future research is how memory reactivation and the induction of plasticity interact with each other. Is memory reactivation directly followed by plastic changes? If not, how long can it take for plastic changes to be induced without disrupting the consolidation of the reactivated memory trace? Is there a sequential consolidation process during which memory reactivation always happens first and the induction of plasticity always follows? To date, direct relations between memory reactivation and plastic changes are rare due to different methods required to measure both. For example, while plastic changes have been measured using two-photon calcium imaging in rodents (Niethard et al., 2018), memory reactivation has been investigated using recently developed methods like MVPA and RSA (Cairney, Guttesen, et al., 2018; Schreiner et al., 2018; Zhang et al., 2018). Building on a very recently developed method enabling to measure plastic changes in humans (Brodt et al., 2018), the simultaneous investigation of

memory reactivation and plastic changes throughout a period of sleep might soon be possible.

3. Concluding remarks

During the last century, the significance of sleep for memory consolidation has been established. While the intricate brain mechanisms underlying sleep-dependent memory consolidation remained theoretical for a long time, the recent development of new methods has enabled their empirical investigation. By exploiting these methods together with the development of a new memory paradigm, this thesis further the understanding of mechanisms underlying sleep-dependent memory consolidation and relate them to cardinal brain oscillations during sleep, i.e., slow oscillations and sleep spindles.

Sleep spindles have been previously identified as a vehicle to induce plasticity. In the first part of the thesis (chapter 2 and 3), it is demonstrated that memory consolidation can be explained by sleep spindles tracking cortical patterns active during learning ascribing a mechanistic function to sleep spindles for memory consolidation. That is, sleep spindles specifically occur in encoding relevant cortical areas to facilitate consolidation, assumingly by inducing long-lasting changes (plasticity) in these areas.

In the second part of the thesis (chapter 4 and 5), slow oscillations and sleep spindles have been linked to another mechanism underlying sleep-dependent memory consolidation, memory reactivation. In both chapters, slow oscillations up-states were identified as the critical time window for memory reactivation, whereas the last chapter additionally

demonstrate that the precise coupling between slow oscillations and sleep spindles predicts memory reactivation strength.

Together, this thesis provides important insights into the mechanisms subserving associative memory consolidation during sleep, raises new interesting research questions and hopefully stimulates future work to further investigate both mechanisms independently but also in relation to each other.

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