The Role of Post-Learning Reactivation in Memory Consolidation

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List of Abbreviations

TMR	**Targeted Memory Reactivation**
REM	**Rapid-Eye Movement**
NREM	**Non-Rapid-Eye Movement**
SWS	**Slow-Wave Sleep**
SWA	**Slow-Wave Activity**
SHY	**Synaptic Homeostasis Hypothesis**
iOtA	**Information Overlap to Abstract**
MSL	**Motor Sequence Learning**
SRTT	**Serial Reaction Time Task**
M1	**Primary Motor Cortex**
SMA	**Supplementary Motor Area**
MTL	**Medial Temporal Lobe**
mPFC	**Medial Prefrontal Cortex**
dlPFC	**Dorso-Lateral Prefrontal Cortex**
vlPFC	**Ventro- Lateral Prefrontal Cortex**
ERP	**Event-Related Potential**
fMRI	**Functional Magnetic Resonance Imaging**
EEG	**Electroencephalography**
PSG	**Polysomnography**
MEG	**Magnetoencephalography**
tDCS	**Transcranial Direct-Current Stimulation**
BOLD	**Blood-Oxygen Level Dependent**
MVPA	**Multi-Voxel Pattern Analysis**
EPI	**Echo-Planar Imaging**
HRF	**Hemodynamic Response Function**

Abstract

The role of post-learning reactivation in memory consolidation

James Cousins, The University of Manchester
For the degree of Doctor of Philosophy (PhD) *26th September 2014*

Memories are gradually consolidated after learning, and subsequent offline periods containing sleep are suggested to support the stabilisation, enhancement, reorganisation and integration of representations within long-term memory networks. The spontaneous reactivation of specific memory traces during sleep is proposed as a key mechanism underlying sleep-dependent consolidation, but the neurophysiological underpinnings of this 'memory replay' remain unclear. The research described in this thesis utilised a method of manipulating memory reactivation during sleep (targeted memory reactivation), in combination with behavioural experimentation, polysomnography (PSG), electroencephalography (EEG), and functional magnetic resonance imaging (fMRI), to refine current understanding of the neural processes underlying sleep-dependent memory consolidation.

In Chapter 2 we developed a motor sequence learning paradigm that combined visuo-motor performance with sound stimuli, which enabled the targeted memory reactivation (TMR) of specific motor memories during sleep in subsequent chapters via the replay of the associated sounds during sleep. Chapter 3 used this task to cue the reactivation of a learned motor sequence during slow-wave sleep (SWS), which enhanced motor skill for the cued sequence relative to an uncued sequence, and also made the sequence of motor movements more available for conscious recall. Furthermore, these effects were associated with key neural features of sleep (slow oscillations and spindles). These findings indicate that reactivation not only enhances procedural memories, but plays a part in the reorganisation of representations that leads to the emergence of explicit knowledge. A great deal of research has shown that the neural systems supporting procedural memories evolve over time, particularly within cortico-striatal and cortico-cerebellar networks. Chapter 4 used fMRI to show that reactivation is instrumental to this neural plasticity by comparing brain activity at retrieval of a sequence that was cued during SWS with a sequence that was not. The cued sequence showed increased activation in bilateral caudate nucleus and left hippocampus, mediated by time spent in slow-wave sleep, while functional connectivity was also altered by TMR between caudate and hippocampus. These findings indicate that the behavioural enhancements associated with TMR of procedural learning are related to overnight plasticity in motor memory networks. Lastly, Chapter 5 expanded on the reorganisation of memories investigated in Chapter 3, asking whether reactivation mediates the generalisation of representations that can sometimes create false memories. Learned lists of semantically associated words were reactivated during NREM sleep, but revealed no evidence that TMR effected false memory formation. However TMR was found to reduce the recognition of studied items, which may indicate that certain TMR procedures can interfere with consolidation rather than enhance it.

Collectively these results provide new insights to the role played by reactivation in memory consolidation. We have provided evidence for both the enhancement and reorganisation of procedural memories during sleep, and indicate that such effects are supported by alterations to underlying neural plasticity. We also show the importance of slow-wave sleep and associated neural features in this consolidation process.

Declaration

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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This thesis is dedicated to Peter Tonkin

The author

James Cousins completed an undergraduate degree in Experimental Psychology at the University of Bristol, graduating with First Class Honours. He then worked as a research assistant at the University of Wollongong for a brief period before returning to the UK to study. He subsequently gained a Distinction for his Masters in Cognitive Neuroscience at the University of York, before embarking on his PhD at the University of Manchester.

Rationale for submitting the thesis in an alternative format

The work in this thesis forms the basis of a pilot study and three articles that were prepared for submission to scientific journals and are at various stages of publication. These articles form chapters 3-5 and have each been written to include a review of the literature that is relevant to the data presented. Chapter 1 will provide a broad introduction to all of the research undertaken in this thesis and outline the key research questions and aims.

The author was the primary investigator for all work presented in this thesis, performing the majority of experiment conceptualisation, design, data collection, analysis, interpretation and writing. Contributions from co-authors included sleep scoring (where scoring by two experimenters is a requirement), supervisor guidance for project design, analysis and interpretation, and assistance with collecting data (Chapter 5 – experiment 2).

Chapter 1

Introduction

Preface

This thesis concerns some of the unanswered questions surrounding memory reactivation during sleep, and how such reactivation could promote sleep-dependent memory consolidation.

Why do we sleep? This profound alteration in consciousness and brain activity appears to be a universal trait of the animal kingdom, and yet it remains one of the most intriguing unsolved questions in biology. Technological advances in neuroimaging techniques over the past 20 years have heralded considerable advances in our understanding of human brain function, which has illuminated the previously hidden world of sleep, as well as highlighting the remarkable plasticity of the brain throughout adult life. There is now compelling evidence for a strong association between this plasticity and sleep, whereby sleep does not simply protect memories from the interfering processing of wake experience, but it actively promotes alterations to memories that stabilise, enhance, abstract commonalities and integrate memories within existing networks.

Memory systems are widely considered to not simply retain a literal record of the past for later retrieval, but they are constructive in nature (Bartlett, 1932). Encoded information is transformed in relation to existing knowledge in order to maximise the utility of learning to direct future behaviour (Conte & Ficca, 2013). The reorganising properties of sleep appear to be a critical element in assisting that constructive process.

Many factors during encoding and prior to sleep determine which memories are selected to undergo consolidation. Furthermore, a range of neurophysiological processes that occur during sleep are suggested to contribute to memory consolidation. One neural feature in particular has been singled out as a vital component of this consolidation process: the reactivation or 'replay' of memories during sleep. Specific neuronal firing sequences associated with wake behaviour are reinstated during subsequent sleep in rodents (Wilson & McNaughton, 1994), suggesting a form of memory replay, and disrupting processes associated with these replays impairs post-sleep memory (Girardeau, Benchenane, Wiener, Buzsáki, & Zugaro, 2009). Similarly in humans, brain regions and patterns of activity associated with previous learning experiences appear to

be recapitulated during sleep (Peigneux et al., 2003), and this activity correlates with consolidation measures at retrieval (Deuker et al., 2013; Peigneux et al., 2004).

To establish a causal role for reactivation and its associated neural features in sleep-dependent memory consolidation, some recent techniques have been used to directly manipulate sleep, including trans-cranial magnetic stimulation (tDCS) (Marshall, Helgadóttir, Mölle, & Born, 2006), closed-loop auditory stimulation (Ngo, Martinetz, Born, & Mölle, 2013), and the targeted reactivation of specific memory traces. This latter method of targeted memory reactivation (TMR) involves the combination of learning materials with sensory cues (olfactory or auditory), then re-presenting those cues during sleep to bias sleep-dependent consolidation in favour of the cued memory (Rasch, Büchel, Gais, & Born, 2007). TMR provides a novel method to investigate the role for reactivation in many forms of memory consolidation, particularly when combined with neuroimaging techniques such as electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). By utilising this method in a series of experiments, the goal of this thesis is to explore some of the unanswered questions surrounding memory reactivation and precisely how it promotes sleep-dependent memory consolidation. The thesis is organised along 5 broad themes.

Firstly, a recent focus of memory models and research is sleeps role in reorganising and transforming memories, which includes the abstraction of commonalities between learned items (e.g., Ellenbogen, Hu, Payne, Titone, & Walker, 2007), the emergence of explicit knowledge for implicitly encoded information (e.g., Fischer, Drosopoulos, Tsen, & Born, 2006) and the generalisation of episodic memories that may lead to false memory formation (e.g., Payne et al., 2009). Prominent sleep and memory models propose a mechanistic role for reactivation in this reorganisation of memory representations (Diekelmann & Born, 2010), but a direct relationship has not yet been observed experimentally. TMR provides a unique opportunity to establish a causal role for reactivation in this process, and this was the focus of Chapters 3 and 5.

Second, at the outset of this thesis it had not been resolved as to whether procedural memories could be manipulated with TMR (Rasch et al., 2007). As a result, the extent to which reactivation underscored sleep-dependent consolidation of procedural memories was unclear. However, two recent studies have shown that specific auditory cues presented during sleep can bias consolidation of a motor sequence skill (Antony,

Gobel, O'Hare, Reber, & Paller, 2012; Schönauer, Geisler, & Gais, 2014). Questions remains as to how this reactivation relates to important sleep features such as slow oscillations and sleep spindles. Chapters 3 and 4 aimed to further our understanding of the conjunction between these neural features of sleep and memory reactivation.

Third, the underlying plastic changes associated with reactivation have received little attention. Procedural learning is known to involve dynamic changes within motor memory networks over time, particularly in terms of changes within the basal ganglia (Doyon & Benali, 2005) and connectivity between striatum and hippocampus (Albouy, King, Maquet, & Doyon, 2013). Most theoretical accounts of memory consolidation conceptualise memory reactivation as a driving force behind this plasticity (e.g., Diekelmann & Born, 2010), but this has yet to be tested directly with TMR, and this was precisely the goal of Chapter 4.

Fourth, TMR as a technique is still very much in its infancy, and there are a number of remaining questions regarding how the technique works, the specific relationship between learning material and cues that is optimal to bias consolidation, and the circumstances under which it enhances or interferes with consolidation. By using TMR in different learning contexts we aimed to further understanding of the complexities underscoring this procedure (Chapters 3-5).

Lastly, TMR affords a unique opportunity to determine precisely the time that reactivation occurs, and therefore characterise the underlying brain activity with modern neuroimaging analysis techniques. An important next step is to link the neural activity of memory encoding during wakefulness with reactivation of that memory during sleep in humans, and determine how this brain activity relates to behavioural outcomes of consolidation. This consideration was a central concern in the development of paradigms within this thesis, and forms part of a wider project to utilise a similar paradigm in concert with pattern analysis techniques (e.g., Fuentemilla, Penny, Cashdollar, Bunzeck, & Düzel, 2010) to explore these questions. This paradigm development is outlined in detail within Chapter 2, but experiments using pattern analysis do not form part of this thesis. The final discussion chapter attempts to integrate our findings within current theoretical models of sleep-dependent memory

consolidation, and suggests ways we can further our understanding of reactivation as an underlying mechanism.

The following introduction will describe the underlying brain activity of typical sleep. It then moves on to summarise the benefits to different forms of memory afforded by sleep and how they relate to underlying neurophysiology. We will then focus on the spontaneous reactivation of memories during sleep, how it relates to behavioural outcomes and the current understanding garnered from a variety of neuroscientific approaches. Lastly, the insights offered by studies utilising TMR will be considered, before outlining the unique opportunity this technique provides to really chip away at that very difficult question, why do we sleep?

The neurophysiology of sleep

Sleep is not a homogenous state, but is composed of separate physiological stages defined by their distinct electroencephalogram (EEG) oscillatory patterns (Figure 1.1). They are broadly divided into rapid eye movement sleep (REM) and non-REM sleep (NREM), which is further subdivided into stages 1-4, with stages 3 and 4 now combined and classified as slow-wave sleep (SWS) (Iber et al., 2007). REM sleep and NREM sleep periods occur in ultradian cycles throughout the night, lasting approximately 90 minutes. Typically, sleep occurring early in the night is dominated by SWS, while later in the night REM sleep is more prevalent.

The earliest sleep stage 1 is characterised by a transition between the wake-like alpha rhythm (8-12Hz) and theta (4-7Hz), contains sharp-waves called vertex spikes (Fuller, Gooley, & Saper, 2006) and is associated with the visual imagery of hypnogogic dreams. This stage is typically short lived and followed by stage 2, which is defined by the presence of thalamo-cortical spindles and k-complexes on a background of theta activity. A k-complex is a cortically generated negative sharp wave (<0.5secs), followed by an extended positive component. They can be induced by auditory stimulation, indicating a potential role in maintaining sleep (Cash et al., 2009). They have not typically been associated with memory processing, although it was recently suggested they might be instrumental in the global cortico-hippocampal dialogue thought to underscore declarative memory consolidation (Genzel, Kroes, Dresler, & Battaglia, 2014). Sleep spindles are rapid bursts of relatively high frequency activity (12-15Hz).

Processing of auditory stimuli during spindles is severely reduced, suggesting they also function to maintain sleep (Dang-Vu, McKinney, Buxton, Solet, & Ellenbogen, 2010). The thalamic gating of external sensory processing during spindles (Schabus et al., 2012) may facilitate internal memory consolidation processes (Dang-Vu, 2012) and indeed a number of studies have linked spindles to memory consolidation (e.g., Barakat et al., 2011). Spindles are further delineated into slow spindles (12-13.5Hz) that are associated with activity in superior frontal gyrus, and fast spindles (13.5-15Hz) that are linked to sensorimotor regions, medial frontal cortex and hippocampus (Schabus et al., 2007).

While spindles are most prevalent during stage 2 sleep, they also occur during the latter NREM stage of SWS. This is defined by very low frequency slow oscillations (<1Hz) and delta activity (1-4Hz), representing a global alternation between depolarised "up-states" of heightened neuronal activity and hyperpolarised "down-states" of relative quiescence (Amzica & Steriade, 1998). The slow oscillation is cortically generated and involves waves propagating across the cortex predominantly from prefrontal regions (Massimini, Huber, Ferrarelli, Hill, & Tononi, 2004). This slow-wave activity (SWA) is proposed to maintain homeostasis in memory networks through synaptic downscaling (Tononi & Cirelli, 2003, 2014). However it is also suggested to play a role in long-term potentiation (LTP) of memories, by synchronising the cortico-hippocampal communication that underscores long-term transfer of declarative memories from hippocampus to neocortex (Diekelmann & Born, 2010) (see Box 1). Hippocampal sharp wave ripples (SWR's) are transient high frequency oscillations (<100ms) that are a common feature of SWS, although they also occur during wakefulness (Carr, Jadhav, & Frank, 2011). SWR's originate in the hippocampus, they synchronise with spindle activity and neocortical slow oscillations (Clemens et al., 2007), and they are suggested to promote synaptic plasticity (Buzsaki, 1986). They are also tightly linked to the reactivation of neuronal populations associated with prior learning (Wilson & McNaughton, 1994), discussed in more detail later.

REM sleep is characterised by rapid-eye movements and muscle atonia, and was initially defined as "paradoxical sleep" on account of EEG activity so closely resembling the high frequency activity associated with wakefulness (30-80Hz). REM sleep is associated with vivid dream imagery (Crick & Mitchison, 1983), mediated by heightened activity across a number of brain regions including visual cortex, thalamus

and amygdala (Maquet, 1996). There are also profound neurochemical changes behind these different brain states, such as cholinergic activity that is associated with learning processes (Hasselmo, 1999; Hobson & Pace-Schott, 2002) although discussion of these are beyond the scope of this thesis (for review see Rasch & Born, 2013).

Lastly, it is important to note similarities and differences between human and rodent sleep architecture (Figure 1.1), since evidence from neuroscientific approaches in both species are crucial to understanding the mechanistic role of reactivation in memory consolidation. Despite some differences in circadian rhythms and the time scale of transitions between stages, rodents show remarkable similarities in terms of the presence of key neural features such as SWR's, spindles and slow-waves, making them a suitable experimental model from which to infer properties of human sleep.

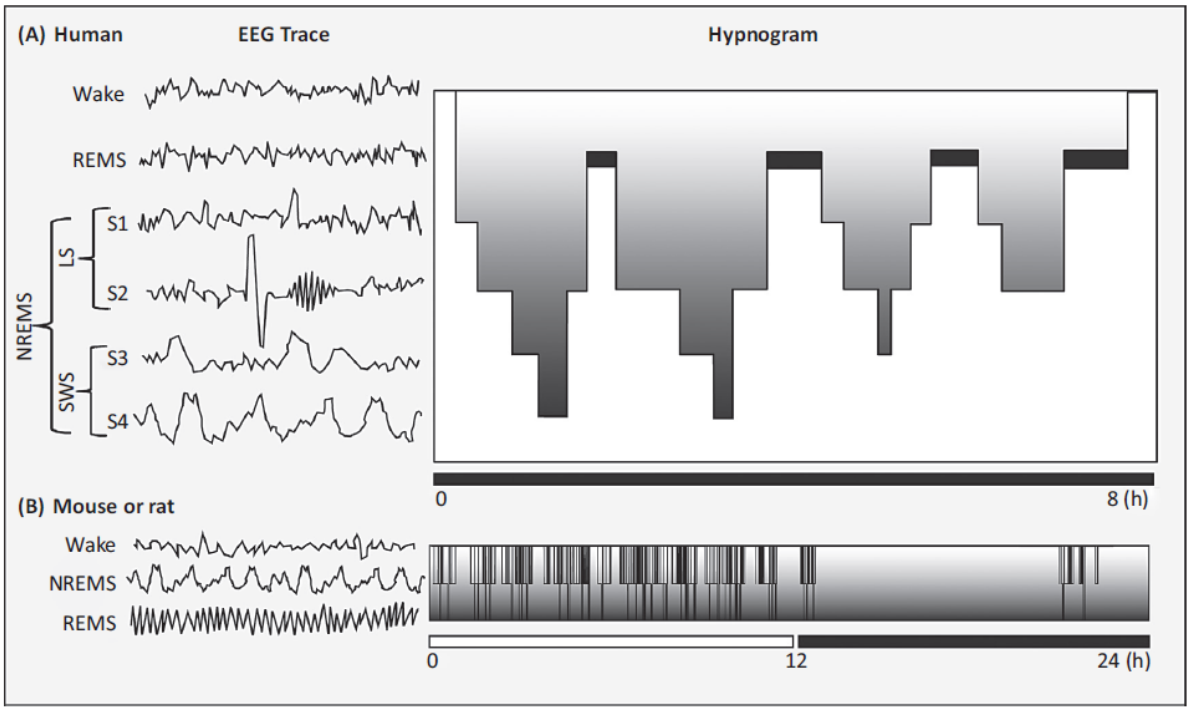

Figure 1.1: Sleep Architecture. (A) Human sleep is broadly divided into rapid-eye movement (REM) sleep and non-REM sleep (Stages 1-4), and alternates between these in roughly 90minute cycles. The deeper NREM sleep stages (SWS) dominate earlier in the night, while the latter half of the night contains comparatively more REM sleep. (B) The same REM/NREM sleep distinction is present in the neurophysiology of rodent sleep, but NREM is not further subdivided and is often referred to as SWS in the literature. Also cycles are shorter and occur predominantly during the daytime. Diagram reference: Genzel et al. (2013).

Box 1: A brief history of memory models and reactivation

There is a surprising level of agreement between memory models with regard to the proposed role for post-learning reactivation in memory consolidation, particularly for the evolution of declarative memories. Two-stage models have dominated theoretical accounts of declarative memory, proposing a fast learning transitory memory store that encodes new information alongside slow learning long-term memory stores where memories are gradually reorganised and re-enforced (Marr, 1970). The two systems are necessary to avoid catastrophic interference between incoming information and stored memories. This has been computationally modelled in **Complimentary Learning Systems** (McClelland, McNaughton, & O'Reilly, 1995), where the fast learning store trains the slow learning store progressively through reactivation of memories. The fast learning store is generally conceptualised as the hippocampus, and the neocortex as the long-term store (Frankland & Bontempi, 2005). Thus, over time memories become less hippocampal-dependent. **Multiple Trace Theory** (Nadel & Moscovitch, 1997), suggests that reactivation of memories creates new traces within the hippocampus, therefore the hippocampus retains information to facilitate retrieval from long-term stores. **The Schema Model** (Morris, 2006) posits that systems consolidation of newly encoded information is guided by consolidation of hippocampal traces and also by pre-existing neocortical schema that rapidly incorporate new information, and this potentially involves sleep reactivation (Wang & Morris, 2010).

Additional models provide a focus on the specific sleep processes involved. The **Hippocampo-Neocortical Dialogue Model** (Buzsaki, 1996) proposed the sleep-wake cycle underscores this process, where wake encoding in cortical networks is transferred to the transient hippocampal store, and this flow of information is reversed in NREM sleep to allow transfer from the hippocampus to the neocortical long-term store (Hasselmo, 1999). The **Dual Process Hypothesis** (Plihal & Born, 1997) suggested REM to be instrumental for procedural memory consolidation, while SWS is important for declarative memories, although this distinction has not been well supported by subsequent experimental evidence. **The Sequential Hypothesis** (Giuditta et al., 1995) posits interactions between both stages. This accounts for the cyclical nature of SWS/REM and suggests REM sleep strengthens and stabilises a process begun during preceding SWS periods. The related **Active Systems Consolidation** model (Diekelmann & Born, 2010) underlines the importance of SWS and neuronal replay, but

also suggests a role for REM sleep in synaptic consolidation (Figure 1.2). Systems consolidation principles have also been applied to procedural memory to account for offline performance stability and enhancement (Krakauer & Shadmehr, 2006; Walker, 2005), although the specific regions supporting this are debated.

These models broadly agree that a process of memory reactivation during sleep underpins memory consolidation. **The Synaptic Homeostasis Hypothesis (SHY)** (Tononi & Cirelli, 2003, 2014) instead suggests that slow oscillations globally downscale synaptic connections in order to maintain homeostasis within memory networks, and this downscaling of weak connections reduces noise within preserved memory traces and stabilises them. Reactivation is proposed to "protect" specific memories from downscaling, rather than providing long-term potentiation (LTP) as suggested by other models.

There has been a recent proliferation of models that specifically describe qualitative changes to memories during sleep through processes of schema formation and integration, combining elements of the above models: **Sleep-dependent Memory Triage** (Stickgold & Walker, 2013) proposes an important role for REM sleep in this process, while the **Information Overlap to Extract (iOtA)** model (Lewis & Durrant, 2011) combines the overlapping reactivation of memory traces and synaptic downscaling during SWS to account for schema development. **Recurrency and Episodic Memory Results in Generalisation (REMERGE)** (Kumaran & McClelland, 2012) has computationally modelled how hippocampal reactivation could lead to generalisation during sleep, while others posit interactions between SWS and REM sleep (Landmann et al., 2014) perhaps supported by the reactivation of novel combinations of memories (Spencer, 2013).

This brief outline of contemporary sleep and memory models highlights that memory reactivation is widely considered to be a mechanism that supports long-term memory processing in some form, although the underlying neurophysiology supporting consolidation are still debated.

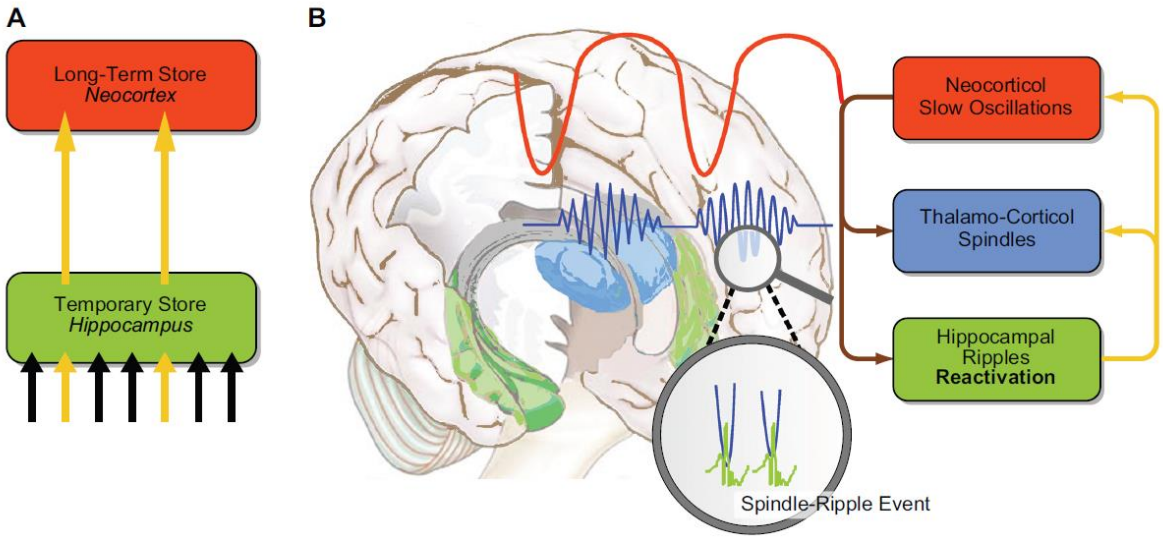

Figure 1.2: Active Systems Consolidation. (A) Schematic of the two-stage model. The temporary store (the hippocampus for declarative memory) encodes new memories during wakefulness that are then repeatedly reactivated during SWS, and this gradually redistributes them to cortical long-term stores. (B) Systems consolidation is achieved during SWS via cortico-hippocampal communication under top-down control of neocortical slow oscillations (red). Hippocampal memory traces are repeatedly reactivated during up-phases of the slow oscillation in concert with hippocampal sharp wave-ripples (green) and thalamo-cortical spindles (blue), forming spindle-ripple events. Not shown, ensuing REM sleep periods provide synaptic consolidation processes that stabilise memory representations that were transferred during SWS. Diagram reference: Rasch and Born (2013).

Sleep and memory consolidation

The modification of memories during learning and the offline consolidation of memories are instances of brain plasticity, that is, the brains capacity to adapt structurally and functionally over time to accommodate new information. It is perhaps obvious that memories must be reactivated in some form to then undergo plasticity processes, and there is a wealth of research outlining how conscious and unconscious reactivation of memories during wakefulness can transform and reconsolidate memories

(Bridge & Paller, 2012; Karim Nader & Hardt, 2009; Nader, Schafe, & Le Doux, 2000). However, this thesis focusses on the covert reactivation of memories during sleep, and the way in which that shapes the long-term development of memory as a flexible system.

Within minutes of a memory being encoded, the representation transforms at the cellular level (synaptic consolidation), and over longer periods these new memory representations are redistributed to other neuronal circuits throughout the brain for long-term storage (systems consolidation) (Frankland & Bontempi, 2005), which may take days, months and even years (Takashima et al., 2006). Not all offline memory processing is sleep-dependent, but the marked changes in neural activity and underlying neurochemistry associated with sleep do allow processes to occur that cannot during wakefulness (Spencer, 2013). These optimal conditions for consolidation may account for the profound change in consciousness that defines sleep (Diekelmann & Born, 2010).

Memory is subdivided into declarative (explicit) memory that is available for conscious recall, encompassing memories for events (episodic) and facts (semantic) (Cohen & Squire, 1980) that are generally hippocampal-dependent (Squire, 1992). Non-declarative memory (implicit) is non-conscious and regarded as hippocampal-independent. It includes procedural skills for perceptual and motor tasks, conditioning, and priming effects. However many procedural tasks involve both implicit and explicit elements that interact and shift across the life-time of a memory (Doyon & Benali, 2005), and the hippocampus supports some forms of procedural learning (e.g., Albouy et al., 2008). These dichotomies can be useful in the experimental context, but it is important to keep in mind that everyday learning is not partitioned between these subtle distinctions (Conte & Ficca, 2013).

The supposition that memory reactivation during sleep fuels memory consolidation is born from a wide range of converging sources that indicate an active process during sleep, rather than passive protection of memories from interference (Vertes & Siegel, 2005). Notwithstanding limitations to some approaches with regards to confounding variables, such as the influence of circadian rhythms (Gerstner & Yin, 2010), evidence has been provided from sleep deprivation studies (Maquet, Schwartz, Passingham, & Frith, 2003), correlations between behavioural outcomes and neural substrates (Barakat

et al., 2011), modifying sleep through auditory or trans-cranial direct current stimulation (tDCS) (Marshall et al., 2006; Ngo et al., 2013), recording neuronal firing patterns in rodents (Wilson & McNaughton, 1994), measuring sleep-dependent cellular mechanisms in animal models (Bushey, Tononi, & Cirelli, 2011), pharmaceutical suppression of sleep neurophysiology (Vogel et al., 1990), disturbed sleep patterns in neuropsychological patients (Autret et al., 2001), comparison of wake and sleep consolidation periods with nocturnal sleep and napping (Durrant, Taylor, Cairney, & Lewis, 2011) and targeted memory reactivation (Rasch et al., 2007). Discussion will now outline sleeps contribution to declarative, procedural, and qualitative change to memories as explored via these methods.

Declarative memory

The comparison of sleep and wake consolidation periods tend to show 'less forgetting' of declarative memories associated with sleep rather than an enhancement per se. For example, word-pair learning involves encoding semantically un-related words (e.g., SHEEP-CUP), and this is enhanced relative to wake consolidation, particularly after an early period of SWS (Plihal & Born, 1997). Similar results have been obtained for picture recognition (Hu, Stylos-Allan, & Walker, 2006) and spatial tasks (Tucker & Fishbein, 2008).

Correlational evidence between consolidation effects and specific features also suggest that sleep actively processes memories (e.g., Backhaus & Junghanns, 2006). For instance, spindle density predicts post-sleep word-pair retrieval (Schabus et al., 2004). Moreover, a causal role for slow oscillations in memory consolidation was established by artificially enhancing slow oscillations via tDCS stimulation (0.75Hz), which increased word-pair learning (Marshall et al., 2006). Such evidence led to the proposal of Active Systems Consolidation (Diekelmann & Born, 2010), where hippocampal replay of memories underscores their transfer to long-term neocortical stores (see Box 1). Studies utilising fMRI support this systems consolidation, finding decreased hippocampal involvement over time (Takashima et al., 2006), reduced hippocampal connectivity (Takashima et al., 2009), and increased responses in several cortical regions (Sterpenich, et al., 2009). However, it is possible that reduced hippocampal activity over time merely reflects weaker memory traces, therefore care should be taken

to interpret neuroimaging research that compares neural correlates of memory at different time points.

Procedural memory

Procedural memory encompasses both motor and perceptual tasks. Learning of some perceptual tasks benefits from sleep, such as visual texture discrimination (Karni, Tanne, Rubenstein, Askenasy, & Sagi, 1994; Gais, Plihal, Wagner, & Born, 2000; Mednick, Nakayama, & Stickgold, 2003). Motor tasks that demonstrate sleep effects include mirror tracing (Plihal & Born, 1997), pursuit rotor task (Smith & MacNeill, 1994), and motor sequence learning (MSL) tasks such as occulomotor sequence learning (Albouy et al., 2006), finger tapping (Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002) and the serial reaction time task (SRTT) (Nissen & Bullemer, 1987).

Walker et al. (2002) found a 20% increase in finger tapping speed after a night of sleep, relative to an equivalent period of wake. These improvements are specific to the learned sequence (Fischer, Hallschmid, Elsner, & Born, 2002), while more difficult bimanual tasks gain the greatest sleep benefit (Kuriyama, Stickgold, & Walker, 2004). Learning a different sequence prior to sleep blocks improvements (Walker, Brakefield, Hobson, & Stickgold, 2003), indicating a sequence specific consolidation mechanism during sleep. Also, circadian factors alone cannot account for these effects (Fischer et al., 2002; Walker et al., 2002). The immediate post-sleep performance boost associated with MSL was proposed to show ‘delayed learning’ (Walker, 2005), but controlling for circadian effects, learning effects, and response inhibition (slowed responses after accumulation of fatigue, interference or attentional factors) was found to eliminate this immediate gain (Brawn, Fenn, Nusbaum, & Margoliash, 2010; Rickard, Cai, Rieth, Jones, & Ard, 2008). Despite this, converging evidence shows sleep to stabilise motor sequence representations to facilitate more rapid re-learning and protect skills from interference (Debas et al., 2010; Korman et al., 2007; Walker et al., 2003).

The serial reaction time task (SRTT) is a form of MSL that demonstrates robust performance improvements after sleep (Brown & Robertson, 2007; Cohen, Pascual-Leone, Press, & Robertson, 2005; Robertson, Pascual-Leone, & Press, 2004a; Robertson, 2007; Song & Cohen, 2014; Spencer, Sunm, & Ivry, 2006). Explicitly learned sequences consistently undergo sleep consolidation, but also implicit

probabilistic sequences if they contain hippocampal-dependent contextual associations (Spencer et al., 2006), supporting the crucial role of the hippocampus in sleep-dependent consolidation (Diekelmann & Born, 2010). The SRTT is utilised for experiments in this thesis and so will be discussed in more detail in later chapters.

Correlational evidence provides support for specific sleep features actively processing procedural memories, with stage 2 and REM sleep providing the most consistent involvement. For example, finger tapping has been linked to stage 2 sleep duration (Walker et al., 2002), spindles (Fogel & Smith, 2006; Nishida & Walker, 2007), and REM sleep duration (Fischer et al., 2002). SWS has also been correlated with the consolidation of some skills (Huber, Ghilardi, Massimini, & Tononi, 2004; Tamaki et al., 2013), perhaps due to declarative components of some 'implicit' procedural tasks (Rasch & Born, 2013). Indeed the whole range of sleep processes are linked to procedural memory consolidation (Gais et al., 2000; Plihal & Born, 1997; Smith, Conway, & Rose, 1998), which may reflect the complex contribution of implicit and explicit factors to procedural learning tasks.

Lastly, the neural plasticity associated with procedural learning across sleep has been explored with neuroimaging methods using a range of procedural tasks. In terms of MSL, comparison of sleep and wake retention intervals show increased activation in regions including primary motor cortex (M1), cerebellum and hippocampus, changes suggested to promote faster and more accurate motor performance (Walker, Stickgold, Alsop, Gaab, & Schlaug, 2005), although decreases in similar regions have also been observed (Fischer, Nitschke, Melchert, Erdmann, & Born, 2005). Sleep was also found to increase functional connectivity between striatum and hippocampus in relation to MSL (Albouy, et al., 2013) and spindles are also related to alterations in brain activity within motor memory networks after MSL (Barakat et al., 2013; Fogel et al., 2014). Reactivation is suggested to be involved in this motor plasticity, but it has yet to be established directly with TMR.

Reorganising memories during sleep

The discussed sleep-dependent improvements to declarative recall and procedural skill could potentially be accounted for by synaptic consolidation processes, whereby connections within memory representations are optimised without necessarily being reorganised within the brain (Rasch & Born, 2013). The strongest support for systems

consolidation during sleep is provided by instances of qualitative alterations to memories. This encompasses processes of schema formation and integration, with the abstraction of implicit rules and relationships between stimuli, and the integration of new memories within existing networks, which optimises learned information to facilitate future behaviour (Conte & Ficca, 2013). Two-stage models propose reactivation to drive this long-term reorganisation of memories (Diekelmann & Born, 2010), but this has yet to be firmly established experimentally.

A remarkable demonstration of this reorganisation is provided by a study where participants simply listened to a stream of tones that unbeknownst to them followed a probabilistically determined sequential structure (Durrant et al., 2011). A subsequent night of sleep or a nap improved their ability to recognise novel sequences that shared the same structure, indicating a role for sleep in abstraction of the underlying statistical probabilities. Improved performance correlated with SWS duration, and was supported by changes in functional connectivity between striatum and parahippocampal regions (Durrant, Cairney, & Lewis, 2013).

This abstraction can also lead to shifts between implicit and explicit memory, as demonstrated with the number reduction task (NRT), where participants gain sudden insight to a hidden rule after sleep (Wagner, Gais, Haider, Verleger, & Born, 2004). This effect relies on early SWS-rich periods (Yardanova et al., 2008; Yordanova, Kolev, Wagner, & Verleger, 2010) and is associated with increased SWS alpha power (Yordanova, Kolev, Wagner, Born, & Verleger 2012). A more gradual emergence of awareness for underlying rules has been observed with the IOWA gambling task (Pace-Schott, Nave, Morgan, & Spencer, 2012), and abstraction of relationships among novel elements has been shown with a transitive inference task (Ellenbogen et al., 2007). Implicitly learned SRTT sequences have also been shown to become gradually more available for explicit recall after sleep (Drosopoulos, Harrer, & Born, 2011; Fischer et al., 2006). This effect is strongest in children, perhaps on account of their greater amount of SWS, and is associated with enhanced hippocampal activity at retrieval (Wilhelm et al., 2013). It is unresolved whether reactivation of sequence representations supports this transition between implicit and explicit memory.

This abstraction of commonalities can be viewed in terms of schema formation (i.e., forming a framework of knowledge). Once a schema has been formed, it can be used to

generalise to new information and assist learning (Eichenbaum, 2004). Sleep has also been shown to facilitate this schema formation and generalisation, whereby newly learned words could be generalised to other similar words after a consolidation period containing sleep (Fenn, Nusbaum, & Margoliash, 2003). Similarly children can generalise a newly learned artificial grammar to new sentences after a nap (Gómez, Bootzin, & Nadel, 2006; Hupbach, Gomez, Bootzin, & Nadel, 2009). In the procedural domain, sleep also supports the generalisation of performance of SRTT sequence performance from the learning hand to the non-learning hand, which suggests sleep facilitates the emergence of an effector independent representation (Cohen et al., 2005; Witt, Margraf, Bieber, Born, & Deuschl, 2010).

Schema integration has been explored by utilising the lexical competition effect, where the integration of newly learned words can be measured by the amount they inhibit RT's for the recognition of familiar words that are lexical neighbours. Sleep was shown to underscore this assimilation of new words into the mental lexicon (Dumay & Gaskell, 2007) and has been associated with spindle activity (Tamminen, Payne, Stickgold, Wamsley, & Gaskell, 2010). Similarly with semantic memory, sleep has been shown to assist the integration of new words based on their meaning, and again this was associated with spindles (Tamminen, Lambon Ralph, & Lewis, 2013).

These processes of abstracting commonalities (gist) may also lead to the formation of false memories, which has been studied with the Deese-Roediger McDermott (DRM) paradigm. Participants learn lists of words that are missing a semantically related associate, and retrieval of this un-studied associate during retest is taken as a measure of false recall. Sleep increases false recall of un-studied words when tested with free recall (Diekelmann, Born, & Wagner, 2010; McKeon, Pace-Schott, & Spencer, 2012; Payne et al., 2009), indicating sleep may be involved in the generalisation of episodic memories and the extraction of a "gist" representation that leads to false memories. However, recognition testing shows a decrease in false memories after sleep (Fenn, Gallo, Margoliash, Roediger, & Nusbaum, 2009; Lo, Sim, & Chee, 2014), therefore further research should establish why these tests differ and whether reactivation plays a part in this process.

The reorganisation of memories during sleep has also been measured by its effects on behaviour such as problem solving and creativity (Cai, Mednick, Harrison, Kanady, &

Mednick, 2009; Sio, Monaghan, & Ormerod, 2013). The Remote Associates Task (RAT) challenges participants to generate the common associate of 3 unrelated words, and this ability is improved after a nap containing REM sleep (Cai et al., 2009). The contributions of REM and SWS to such effects is much debated, with some models indicating SWS to be instrumental (e.g., Lewis & Durrant, 2011), while others prefer REM sleep (Stickgold & Walker, 2013), or a combination of the two (Landmann et al., 2014; Spencer, 2013). These models do agree that reactivation supports these processes, but its causal role in the reorganisation of memory representations is not yet known. There has also been very little work to measure the neural plasticity that results from these forms of qualitative alterations to memories, with only one study showing enhanced hippocampal activity after the emergence of explicit knowledge for an implicit SRTT (Wilhelm et al., 2013). It is unresolved whether reactivation induces this plasticity.

Selectivity of sleep-dependent memory consolidation

Sleep does not process all recently encoded memories equally. Instead a range of factors during or shortly after encoding determine which memories are "tagged" for consolidation (Stickgold & Walker, 2013). Emotional memories are preferentially preserved by sleep (Hu, Stylos-Allan & Walker, 2006), and this is associated with amygdala activity at encoding (Payne & Kensinger, 2011). Knowledge of future relevance (Wilhelm et al., 2011) or reward (Abe et al., 2010; Fischer & Born, 2009) also tags memories for sleep-dependent consolidation, with striatal activity at encoding influencing the latter (Knutson & Adcock, 2005). In procedural consolidation, explicit awareness of SRTT sequence regularity effects subsequent consolidation (Drosopoulos et al., 2011; Song, 2009) and hippocampal activity at encoding determines the extent of subsequent MSL consolidation (Albouy, et al., 2013).

This selectivity is perhaps best illustrated by directed forgetting paradigms, whereby advising participants to not remember certain stimuli prevents them from being tagged and consolidated across sleep (Saletin, Goldstein, & Walker, 2011). Items 'to be forgotten' most probably decay across sleep rather than being actively and specifically obliterated during sleep, although one study did indicate the latter by showing that fast spindles correlate with the reduction in recall of 'to be forgotten' items (Saletin et al., 2011). To summarise, there are a number of selective mechanisms tagging different

forms of memory. Important questions remain as to the precise neural substrates of these tagging mechanisms operating before and during sleep, as well as the way in which they interact with reactivation to facilitate consolidation.

Sleep and memory reactivation

Spontaneous memory reactivation

The most compelling evidence for the occurrence of memory reactivation comes from the observation that neuronal firing sequences that are related to behaviour in rodents are spontaneously reinstated during subsequent rest periods and sleep (Pavlides & Winson, 1989). Wilson and McNaughton (1994) recorded from hippocampal place cells that encode spatial location (O'Keefe and Dostrovsky, 1971) and identified pairs of place cells that tended to fire one after the other as the rat traversed a linear track. During subsequent SWS, these pairs of cells had an increased tendency to fire together, suggesting reactivation of memory for traversing the track (Figure 1.3).

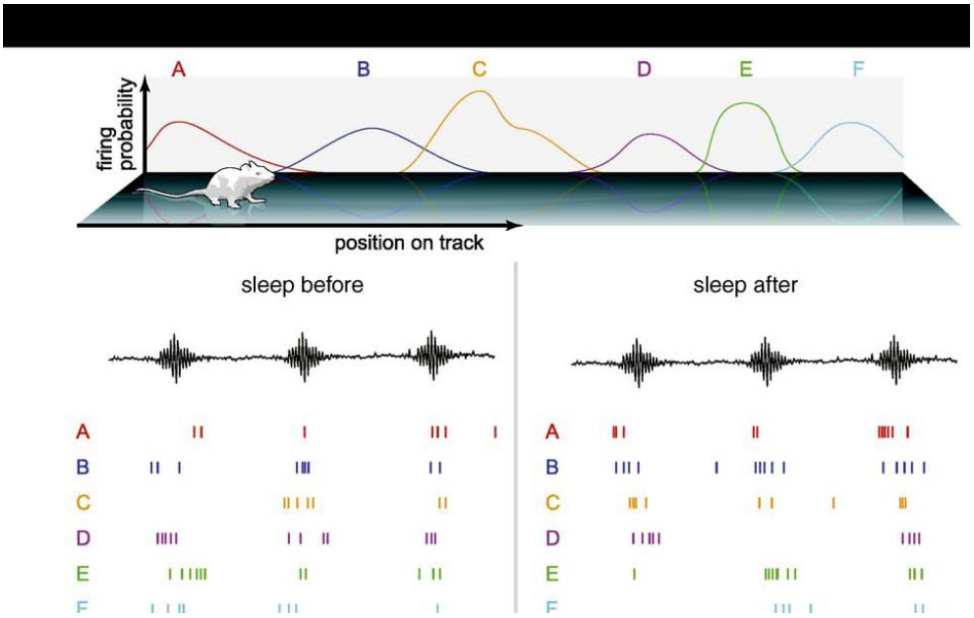

Figure 1.3: Reactivation of neuronal firing sequences in rodents. As the rodent moves along a linear track the firing probability of place cells A-F peak at different points on the track, creating a specific temporal sequence of firing (above). This sequence is then reactivated at a faster rate during SWR's in subsequent sleep (below right), but is not correlated with firing of the same place cells before sleep (below left). Diagram reference: O'Neill, Pleydell-Bouverie, Dupret, & Csicsvari, (2010).

Further research utilising more complex tracks and open exploration (Csicsvari, O'Neill, Allen, & Senior, 2007) have revealed a great deal about the nature of this 'replay'. During SWS, reactivations usually occur within SWR's (Kudrimoti, Barnes, & McNaughton, 1999) and occur in the same temporal order as encoding at 6-20 times the speed of their rate during wake behaviour (Lee & Wilson, 2002; Nádasdy, Hirase, Czurkó, Csicsvari, & Buzsáki, 1999). Predominantly replays have been studied in the hippocampus, but they have also been identified in the parietal lobe (Qin, McNaughton, Skaggs, & Barnes, 1997), medial prefrontal cortex (mPFC) (Benchenane et al., 2010; Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009), thalamus and putamen (Ribeiro et al., 2004), motor and somatosensory areas in monkeys (Hoffman & McNaughton, 2002), ventral striatum (Lansink et al., 2008; Pennartz et al., 2004), and visual cortex (Ji & Wilson, 2007). The latter study found hippocampal reactivations to lead those in V1, which is consistent with the idea of information transfer from the

hippocampus to cortical regions during SWS (Diekelmann & Born, 2010). In addition, reactivations in mPFC (Peyrache et al., 1999) and ventral striatum (Lansink et al., 2008) are modulated by reward, while hippocampal reactivation more frequently replays decision points in a maze (Peyrache et al., 2009), indicating replay of information that is behaviourally relevant to the animal. This replay has also been studied extensively in birds (Dave & Margoliash, 2000; Shank & Margoliash, 2009). Together, these studies show that not only spatial location information is reactivated during sleep, but many complex aspects of waking behaviour within interacting neural networks throughout the brain.

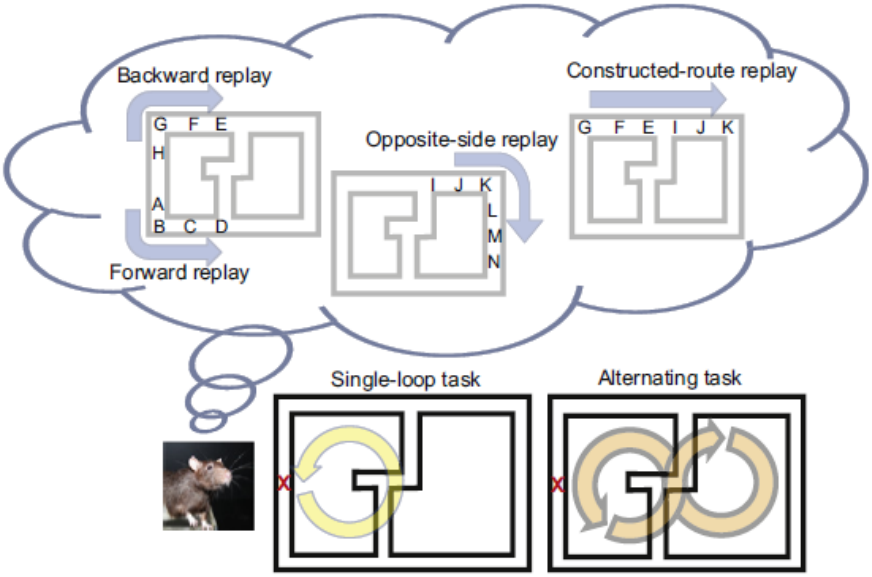

Figure 1.4: Reactivation during wakefulness (Gupta, van der Meer, Touretzky, & Redish, 2010). The rat is rewarded at the red X for performing either a single-loop task (Left only or Right only) or alternating loop task. Switching between these tasks allowed examination of recency and frequency of replays during pauses in the maze. Forward and backward replays were observed (top left), as well as non-local replays (top middle) and replay of novel trajectories (top right). Diagram reference: Derdikman & Moser (2010).

Memory reactivation is also common during SWR's that occur during wakefulness, and it remains unclear if they share similar functions to sleep reactivation (for review see Carr et al., 2011). Generally speaking wake reactivations are more diverse, in that they occur forward of the rats current location (preplay) and backward (Johnson & Redish, 2007), as well as distant from the animal (Karlsson & Frank, 2009). Additionally, the reactivation of a novel trajectory was observed in one study (Gupta et al., 2010) although only in a single rodent (Figure 1.4). It has been proposed that replay of novel sequences, rather than recapitulation of the learning experience, might underlie qualitative memory alterations during sleep in humans (Spencer, 2013), but this novel replay has not yet been observed during sleep.

Conversely, there is limited evidence for the occurrence of memory reactivation during REM sleep (Poe, Nitz, McNaughton, & Barnes, 2000), with only one study finding reactivation of neuronal firing sequences at a less compressed time scale (0.5-2.5 times the speed of waking behaviour) (Louie & Wilson, 2001). REM sleep periods are relatively short and infrequent in rodents, which may account for the limited supporting evidence, but clearly further work is needed to confirm the presence of memory reactivation during REM sleep.

The spontaneous reactivation of memories observed in rodents is difficult to link to consolidation on account of animals being too well trained to be able to measure improvements, although one study did show reactivation to predict subsequent spatial memory performance (Dupret, O'Neill, Pleydell-Bouverie, & Csicsvari, 2010). Also, indirect evidence comes from studies that selectively disrupt the hippocampal SWR's that accompany the majority of reactivation events (Buzsaki, 1986), causing severe disruption to consolidation of spatial learning (Girardeau et al., 2009; Girardeau & Zugaro, 2011). SWR's are known to occur within spindle troughs (Siapas & Wilson, 1998), while spindles are themselves synchronised with cortical slow oscillations (Clemens et al., 2007), further indicating that SWR's are important for the hippocampal-cortical dialogue thought to underlie systems consolidation (Diekelmann & Born, 2010). Interestingly, hippocampal ripples have also been identified in humans by recording from electrodes implanted in epileptic patients, during naps and quiet wakefulness, and this activity was associated with subsequent recall (Axmacher, Elger, & Fell, 2008).

A handful of studies have identified spontaneous reactivation during sleep in humans with positron-emission tomography (PET) and fMRI, albeit with less temporal and spatial resolution than the single cell recording methods used in rodents. Hippocampal activity associated with route learning was found to be reactivated during subsequent SWS, and the level of activation correlated with post-sleep performance (Peigneux et al., 2004). Work from this lab also showed reactivation of procedural learning in REM sleep for an implicit SRTT in premotor cortex (PMC), cuneus (Maquet et al., 2000) and striatum (Peigneux et al., 2003), as well as enhanced functional connectivity between PMC and posterior parietal cortices (Laureys et al., 2001). This reactivation might optimise motor networks that enable performance improvements the following day. These findings may also indicate a dissociation between SWS and REM sleep for declarative and non-declarative memory reactivation. However, Yotsumoto et al. (2009) showed reactivation of a non-hippocampal task during NREM sleep. They trained specific parts of the visual field using a texture discrimination task, and showed that areas of primary visual cortex (V1) that represented those parts of the visual field were reactivated during subsequent NREM sleep, while improvement in task performance was correlated with V1 reactivation.

The discussed PET and fMRI methods use a univariate approach that averages activity over long periods of sleep, to identify regions that are more active after different learning conditions. Recently a multivariate approach to fMRI, multi-voxel pattern analysis (MVPA), has identified patterns of activity for specific memories being reactivated at a single time-point, during rest periods and sleep (Deuker et al., 2013). This is discussed in more detail in the final sub-section (The neural signature of reactivation). Lastly, a novel source of evidence for reactivation of sequences during sleep in humans was provided by a study of sleepwalking patients, who were found to re-enact a sequence of learned motor movements during SWS (Oudiette et al., 2011), presumably as a result of reinstated activation in motor regions.

Studies of spontaneous memory reactivation provide invaluable insight to the neurophysiological basis of sleep-dependent memory consolidation. The disadvantage of these methods is that their relationship with consolidation can only be inferred from correlations between activity and post-sleep performance measures. TMR compliments

these findings by providing a means to explore the causal relationship between reactivation and memory consolidation.

Targeted memory reactivation

This method involves pairing learning material with a contextual odour or with sounds that are specifically associated with individual stimuli, and then re-presenting those cues during sleep to manipulate consolidation of the associated memories. Some early attempts were made at cueing memories during sleep with the use of a ticking clock for example (Smith & Weedon, 1990), but this study in particular suffered from a very small sample size, while similar studies suffered from poor control over sleep measurement, and might have also been ignored because of the stigma associated with 'sleep learning' (for review see Oudiette & Paller, 2013).

TMR of declarative memories: More recently, a seminal study by Rasch and colleagues (2007) presented an odour (contextual stimulus) while participants learned a declarative object location task or procedural finger tapping task. Subsequent presentation of the odour during SWS enhanced memory for the spatial location task only (i.e., less forgetting of cued memories), while REM or wake odour exposure had no influence upon consolidation. In addition, fMRI revealed activation in the left hippocampus during odour presentation, suggesting hippocampal-dependent reactivation of memory for the task. TMR during SWS was also found to accelerate spontaneous consolidation of this task (Diekelmann, Biggel, Rasch, & Born, 2012) and protect reactivated memories from interference, while TMR during REM sleep had no effect (Cordi, Diekelmann, Born, & Rasch, 2014), and wake TMR made memories labile and vulnerable to interference (Diekelmann, Büchel, Born, & Rasch, 2011). However others have shown TMR during wakefulness can enhance memories (Oudiette, Antony, Creery, & Paller, 2013).

TMR with auditory stimuli is potentially more disruptive to sleep, but can be used to cue specific memories rather than the context of a whole task. This was first demonstrated with a similar spatial memory task to Rasch and colleagues (2007), pairing pictures in spatial locations with semantically associated sounds and cueing half of the sounds during NREM sleep of a subsequent nap. Location recall was significantly enhanced for cued items, while the amplitude of event-related potentials was higher in response to cues for items that were recalled more accurately after sleep (Rudoy, Voss,

Westerberg, & Paller, 2009). This task has also been used to show that TMR recovers memories associated with low reward during sleep and wakefulness (Oudiette et al., 2013), which suggests that TMR overrides the selective mechanism that tags memories for consolidation. TMR of word-pairs with semantically unrelated sounds was also shown to enhance subsequent recall, but not in patients with medial temporal lobe damage (MTL) (Fuentemilla et al., 2013), further supporting the important role of these structures in sleep-dependent memory consolidation. In addition, vocabulary learning of a new language was recently enhanced with TMR, via presentation of verbal cues for newly learned words during NREM sleep (Schreiner et al., 2014). Interestingly, this study showed a boost to cued memories, rather than less forgetting. This use of verbal cues also demonstrates that the brain can process relatively complex external stimuli during sleep, a finding that was recently supported by the presence of ERP's in response to verbal cues that indicate semantic processing during sleep (Kouider, Andrillon, Barbosa, Goupil, & Bekinschtein, 2014). This preserved processing during sleep is also demonstrated by the retained ability to learn conditioned responses during sleep (Arzi et al., 2012).

TMR of emotional memories: An additional form of memory consolidation that has been explored with TMR is emotional memory. A recent study showed that sound cues presented during SWS enhanced emotional memories in humans (Cairney, Durrant, Hulleman, & Lewis, 2014), an effect that was dependent on the amount of SWS obtained. Consistent with this, sound cues have also enhanced conditioned fear memories in mice (Rolls et al., 2013). Another study paired electrically induced odours with fear stimuli and these "odours" enhanced fear memories when presented during NREM sleep, while TMR of a different odour interfered with consolidation (Barnes & Wilson, 2014). Conversely, a study in humans showed that presentation of a contextual odour during SWS actually extinguished the associated fear memory rather than enhanced it (Hauner, Howard, Zelano, & Gottfried, 2013). Subtle variations in methodology of these TMR studies likely account for the enhancing or extinguishing effects (Oudiette, Antony, & Paller, 2014), and they highlight that further work is needed to establish how TMR manipulates memories under different conditions. Also REM sleep has been strongly linked to emotional memory consolidation (Walker & van der Helm, 2009), although a recent study found that TMR with sounds during REM

sleep influenced emotional and neutral memories to the same degree (Sterpenich et al., 2014).

TMR of procedural memories: Procedural memories have also been manipulated with TMR in two studies. The first of these found that replay of a melody associated with a learned visuo-motor sequence during SWS improved performance accuracy of that sequence relative to an uncued sequence, and the amount of improvement was correlated with regional spindles (Antony et al., 2012). A similar result has since been shown utilising the SRTT (Schönauer et al., 2014). Earlier work in birds showed sensorimotor replay in response to playback of birdsong during sleep (Dave & Margoliash, 2000), therefore a similar replay in response to TMR could underscore consolidation effects in humans. Learning of these MSL tasks would most likely have engaged the hippocampus (Schendan, Searl, Melrose, & Stern, 2003), lending further support for the hippocampus as a crucial structure for reactivation (Diekelmann & Born, 2010). Together these studies show that cues must have a tight temporal association with motor learning in order for them to effectively reactivate procedural memories, because contextual odour stimuli were not effective cues for a similar MSL task (Rasch et al., 2007). Interestingly, contextual environmental sounds were ineffective cues for a declarative word pair learning task (Donohue & Spencer, 2011). A number of questions remain as to the necessary relationship between cues and stimuli for TMR to be effective.

TMR and memory reorganisation: Very little research has been conducted to investigate the influence of TMR on qualitative alterations to memories. TMR with contextual odours presented throughout sleep improved creative solutions to a problem experienced prior to sleep (Ritter, Strick, Bos, van Baaren, & Dijksterhuis, 2012), but the odour was also present during pre and post-sleep wakefulness, making the specific contribution of sleep processing unclear. Some models implicate REM sleep in this memory reorganisation (e.g., Stickgold & Walker, 2013), and a recent study partially supports that notion. Sterpenich et al. (2014) found that TMR during REM sleep of newly encoded faces with associated sounds increased false recognition of new faces at retrieval, suggesting generalisation of the reactivated memories. Further demonstration of TMR influencing reorganisation of memories during sleep would provide crucial evidence for active systems consolidation (Diekelmann & Born, 2010) because: (1) these transformed memories provide the strongest evidence that sleep actively

reorganises memories (systems consolidation), and (2) reactivation is suggested to be the key mechanism underlying that reorganisation.

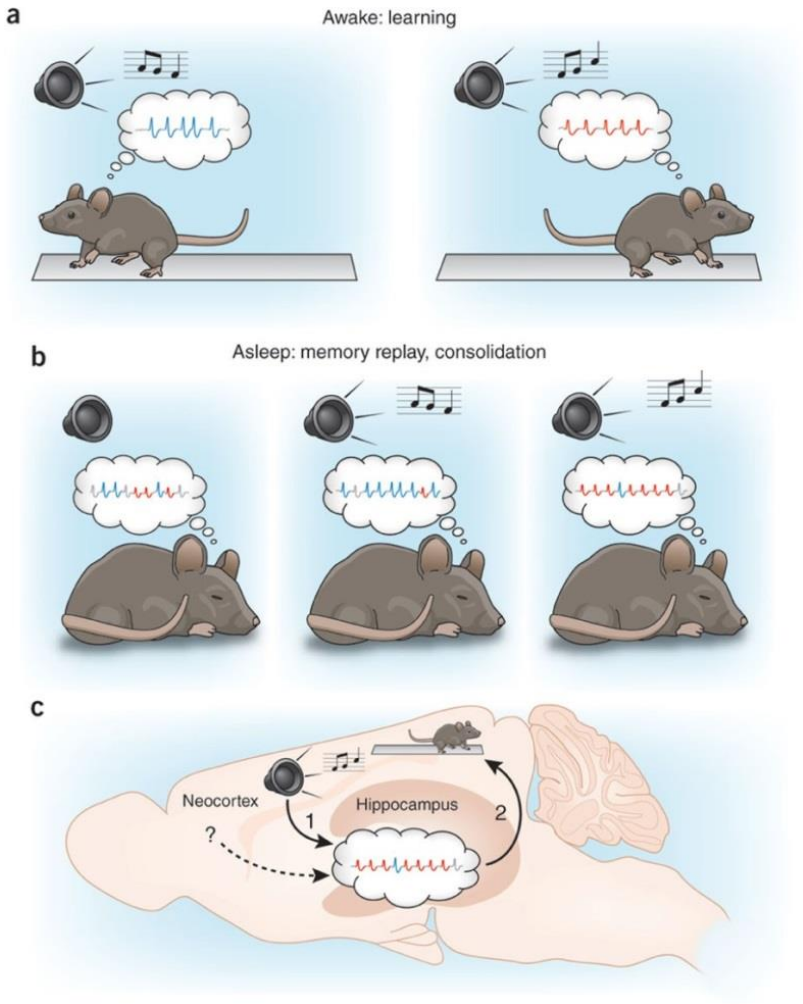

Figure 1.5: The influence of TMR on neuronal firing sequence reactivation in rodents (Bendor & Wilson, 2012). (**a**) The rat was trained to run to the left in response to one sound, and right in response to a different sound. Thus the neuronal populations that fire are different for these two positions (blue for left, red for right) and their associated sound cues. (**b**) During subsequent sleep, spontaneous reactivation of both populations occurred (left). Replaying sounds biased which neuronal population fired (centre and right) (**c**) Sounds are initially processed in neocortical auditory areas, which then presumably triggers reactivation of hippocampal neuronal activity associated with that sound. Diagram: Kelemen & Born (2012).

The neural substrates of TMR: Crucially, Bendor and Wilson (2012) provided an important link between the spontaneous reactivation of neuronal populations in rodents, and the consolidation effects for specific cued memories observed in human TMR studies, by showing that cues bias hippocampal replay events (Figure 1.5). This confirms the assumption that TMR studies in human subjects also influence neuronal reactivation.

Three fMRI studies have explored the neural substrates of TMR in humans, illuminating the brain activity that occurs during cueing and the resulting plasticity measured at retrieval. Hippocampal activity was first observed in response to cues for a spatial memory task (Rasch et al., 2007), and this was extended in a study using Rudoy and colleagues (2009) object-location task (van Dongen et al., 2012). They found enhanced parahippocampal activity in response to auditory cues, although the behavioural effect was not apparent, perhaps on account of the many issues associated with participants sleeping in the noisy MRI scanner environment. Post sleep memory accuracy was associated with enhanced functional connectivity at retest between parahippocampus and medial prefrontal cortex (mPFC), which suggests that reactivation played a part in these connectivity changes. The third study involved memorising face stimuli, and TMR of these stimuli during REM sleep was associated with plastic changes in regions underlying multi-sensory integration, indicating that TMR was instrumental in solidifying associations between stimuli and their associated cues. There are currently no studies identifying plastic changes to procedural memories after TMR during sleep, changes that are associated with normal sleep (Albouy et al., 2008, 2013; Fischer et al., 2005; Walker et al., 2005).

Furthermore, reactivated memories appear to interact with neural features of slow-wave activity (SWA) and spindles. A pair of EEG studies have shown that TMR with an odour increased SWA (Rihm, Diekelmann, Born, & Rasch, 2014) and spindle density in task related brain regions (Cox, Hofman, de Boer, & Talamini, 2014), but these studies did not show the associated performance enhancement, therefore the relationship between TMR and these plasticity processes remains poorly understood.

A small number of studies have used pattern analysis neuroimaging techniques (machine learning) to identify specific memories in humans, particularly with fMRI multi-voxel pattern analysis (MVPA) (for review see Haynes & Rees, 2006). Here, the

full spatial pattern of blood-oxygen-level dependent (BOLD) activity relating to a viewed stimulus is measured and classified during repeated presentations in a training session, providing a template of neural activity associated with that stimulus (class). In the subsequent testing session, it is possible to predict which stimulus is being viewed or retrieved by the participant by probabilistically assigning the cued activity to one of the previously learned classes, effectively matching the BOLD response to a template in order to decode the mental representation (O'Craven & Kanwisher, 2000). These 'classifiers' have been used to determine which episodic memories participants are recalling based purely on hippocampal activity patterns (Chadwick, Hassabis, & Maguire, 2011). Another study trained a MVPA classifier to distinguish between different objects and scene imagery, and found it could predict hypnogogic dream content (Horikawa, Tamaki, Miyawaki, & Kamitani, 2013). MVPA has also identified spontaneously reactivated memories during sleep (Deuker et al., 2013), and the extent of this reactivation correlated with memory consolidation measures after sleep.

While the findings of MVPA research are encouraging, development of an EEG classifier to identify reactivation would be a far more practical research tool, overcoming the expense, noise, movement artefacts and lack of ecological validity associated with sleeping in an MRI scanner. Classifiers utilising magneto-encephalography (MEG) and EEG follow the same principles as fMRI, but use a more diverse range of information available at the sensors to create classifiers, including temporal, frequency, sensor and source based information. For instance, an MEG classifier was used to identify reactivation of indoor or outdoor natural scenes during a 5second period when participants attempted to maintain the image in working memory (Fuentemilla et al., 2010), therefore similar methodology could be used to identify reactivated memories during sleep.

Summary

Targeted memory reactivation offers a unique opportunity to identify similar types of reactivation in EEG during sleep, because it allows us to determine when reactivations are going to occur. As part of a technically challenging wider project, paradigms developed within this thesis included elements that would assist with creation of an EEG classifier. Potentially this could then be applied to un-disturbed sleep, allowing identification of spontaneous reactivations and comparison to cued reactivations. TMR

also presents opportunities to answer remaining questions regarding sleep-dependent memory consolidation, and the specifics of how the technique itself works needs clarification. For instance, under most circumstances TMR biases consolidation of cued memories (e.g., Rudoy et al., 2009), but it has also been shown to reduce fear memories (Hauner et al., 2013), therefore the conditions under which it enhances, interferes, or extinguishes memories is unresolved. Its relationship to sleep features (e.g., spindles) remains unclear, and the types of plasticity induced after TMR of procedural memories have yet to be explored with fMRI. Lastly, the role for reactivation in qualitatively altering memories has yet to be experimentally manipulated with TMR.

Chapter 2

Establishing offline consolidation of an audio-visual procedural memory task (a pilot study)

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Abstract

Procedural learning develops rapidly with continued practice, and more recently offline periods have been implicated in the stabilisation, enhancement and reorganisation of skill representations in long-term memory, particularly during sleep. The spontaneous reactivation of specific memories during sleep is proposed to be the mechanism underlying sleep-dependent consolidation, but many of the neurobiological underpinnings of this 'replay' remain unclear. We added auditory cues to a procedural memory paradigm (serial reaction time task), with the aim of developing a task that could be cued during sleep by the replay of those auditory cues. We first performed two experiments to establish that the adapted serial reaction time task (SRTT) benefits from offline consolidation. Experiment 1 used a SRTT containing 6 audio-visual cues, and contrasted reaction-time and accuracy improvement after a 20minute retention interval (immediate group, N=14) with improvement in a group who experienced a 24 hour retention interval containing sleep (24h group, N=14). The longer retention interval was predicted to provide enhanced speed/accuracy at retest, supported by sleep-dependent consolidation processes, but no group differences were found. Experiment 2 utilised a simplified version of the SRTT with only 4 audio-visual cues. Sequence performance was significantly enhanced for the 24h group (N=20) compared to the immediate group (N=20). We conclude that offline consolidation processes improved performance in the 24h group, consistent with previous findings of sleeps benefit for procedural learning, although the specificity of this effect to sleep cannot be established due to the presence of both sleep and wakefulness during the 24h group retention interval. Importantly, we were successful in our aim to develop a paradigm that may be used to cue a procedural memory during sleep, allowing us to explore the causal role for reactivation in procedural memory consolidation in future studies.

Introduction

Selecting a paradigm to address the aims of this thesis required three key considerations: (1) the task must benefit from sleep-dependent memory consolidation. (2) It must contain features allowing it to be cued during sleep via olfactory or auditory

cues. (3) It must provide distinct patterns of electroencephalographic (EEG) activity that could be classified with pattern analysis techniques.

The SRTT can be adapted to fit the above criteria. (1) It consistently benefits from sleep-dependent consolidation (Robertson et al., 2004a), and key brain regions supporting performance have been shown to spontaneously reactivate during REM sleep (Maquet et al., 2000; Peigneux et al., 2003). (2) The task features 4-6 visual stimuli, therefore auditory tones can be associated with each of these stimuli and subsequently used as cues during sleep. Indeed, this was successfully achieved in a recent study (Schönauer et al., 2014). (3) The bimanual button presses and visual stimuli appearing in different hemifields of visual space potentially provide features associated with motor and visual neural responses that could be classified. For example, a stimulus that appears on the left side of the screen and requires a left handed button press will be associated with neural activity in right visual and motor cortices, the opposite of stimuli appearing on the right, therefore an EEG classifier might be able to use these differences to predict which target has appeared. This same classifier could then potentially be applied to sleep, to predict which stimulus has been cued with TMR.

The SRTT consists of 4-6 visual cues which appear at set screen locations after a fixed interval. Participants must respond with the corresponding button press as quickly and accurately as possible. The cues follow a sequence (e.g., 2-3-1-4-2-3-4-1-3-4-2-1) that participants begin to anticipate, with or without awareness of the sequence, leading to a gradual reduction in reaction times (RT's). Probabilistic sequences have been used to ensure participants are only implicitly aware of the sequence (e.g., Fischer et al., 2006), and also to explore the level of complexity of sequence learning that can be achieved. It has been shown that second, third and fourth order adjacent and non-adjacent sequential dependencies can be learned (Remillard, 2008).

Explicit sequence learning of the SRTT is generally considered to be instances where participants are instructed of the presence of a sequence, or they spontaneously become aware of a sequence during learning. It should be noted however that learning in these instances likely evolves through contributions from both implicit and explicit learning systems. The reduction in RT's during training reflects both the learning of transitions in the sequence, and also learning the visuo-motor mapping between each visual cue and the associated motor response (Robertson, 2007). Typically these two forms of

learning are measured by subtracting RT's for trials containing the sequence from random trials where there is no sequence, providing a measure of sequence specific skill.

Fischer et al. (2006) trained participants on an implicit SRTT with 6 target positions, then tested their ability to implicitly perform the task, and also explicitly generate the sequence from memory. Both periods of sleep and wake led to consolidation of the implicit task, but only after a period of sleep could participants explicitly predict the sequence above chance, suggesting a role of sleep in making implicit knowledge available for explicit recall. Interestingly, those participants who developed explicit sequence knowledge no longer showed an improvement in implicit sequence skill, indicating an interaction between procedural and declarative systems.

Further research shows that explicit SRTT sequences benefit from sleep-dependent consolidation, while implicit SRTT sequences consolidate regardless of sleep or wake consolidation periods (Robertson, Pascual-Leone, & Press, 2004). Also, consolidation of the SRTT can be blocked by a declarative learning task over wake, but not over sleep, again suggesting interactive neural systems for declarative and procedural memory consolidation (Brown & Robertson, 2007). Cohen and colleagues (2005) adapted the SRTT to separate consolidation of the goal of performing the sequence and the movement itself, by manipulating which hand participants used in the task. They found the goal of performing the sequence to be enhanced by sleep, but consolidation of the movement itself was enhanced during wake, indicating separate and distinct mechanisms for consolidation that are influenced differently by sleep and wake. Furthermore, sleep was recently shown to enhance the ordinal representation of a SRTT sequence (e.g., '2' is the second ordinal position in 1-2-4), while transitions between items only improve with practice (e.g., '2' follows '1' in 1-2-4) (Song & Cohen, 2014). In sum, the implicit SRTT provides a useful tool for exploring the effects of awareness and interacting mechanisms of memory consolidation. The explicit SRTT demonstrates the most robust improvement in procedural skill after sleep therefore constitutes a useful paradigm for the aims of the current study.

Experiment 1

Introduction

Our aim was to establish that an adapted version of the SRTT benefits from spontaneous offline consolidation. Performance improvement of an 'Immediate' (IM) group, who were given only a 20 minute retention interval prior to retest, was compared to a group with a 24 hour period (24h) containing sleep in which to consolidate the task before the retest. Participants learned a 12-item SRTT with 6 different cue positions (Figure 2.1) and were tested before and after the retention interval.

We predicted the longer retention interval of the 24h group would provide enhanced speed/accuracy for sequence trials at retest relative to the IM group, supported by sleep-dependent consolidation processes. We expected random trial improvement would not differ between the two groups, indicating that the longer retention period benefits sequence specific learning and not learning of the visuo-motor mapping. In addition, previous research has shown that an implicitly encoded sequence becomes available for explicit recall after sleep (Fischer et al., 2006; Wilhelm et al., 2013), therefore participants also performed an explicit generative task before and after the retention period to test whether their level of explicit sequence knowledge was influenced by a longer consolidation period. We predicted greater improvement in explicit sequence knowledge for the 24h group compared to the IM group.

Materials & Methods

Participants

Twenty-eight participants (Mean age = 23 years, 17 female) from the University of Manchester took part in return for course credits. Participants were screened for any history of neurological or psychiatric diseases or sleep disorders. Participants were asked to abstain from caffeine and alcohol 24 hours prior to testing, and during retention periods. The study was approved by the research ethics committee within the School of Psychological Sciences, University of Manchester. All participants signed the consent form to indicate they understood requirements prior to the experiment. They were

advised their identity and data would remain anonymous and they could withdraw from the study at any time.

Serial reaction time task

The computer monitor was approximately 80cm in front of the participant. The display present throughout contained 6 white boxes labelled A to F on a black background, each box 5x5cm and 3cm apart. This was identical to Fischer et al. (2006), except the boxes in the current study were arranged vertically with A to C on the left of the screen, and D to F to the right hand side of the screen with a central fixation point (Figure 2.1) This composition was used for the following reasons: (1) Clear separation of stimuli between left and right visual fields would serve as an important feature for an EEG classifier. For example, a classifier could learn that right visual cortex activity tends to predict a visual stimulus is being viewed in the left hemifield (Cue A, B or C), therefore reactivation of the same region during TMR would indicate reactivation of a memory representation for either A, B or C. (2) Previous finger tapping studies have found that increasingly difficult finger transitions are more sensitive to sleep-dependent consolidation (Kuriyama et al., 2004). There was no one-to-one mapping between key positions and cue locations in our stimuli, which we expected to increase the difficulty of finger transitions between cues and make the task more sensitive to sleep-dependent consolidation.

Participants were instructed that they would perform a reaction time task, and cues would follow a 12-item repeating sequence which they should try to learn. They were asked to place their fingers on the correct keys. For each trial a solid circular cue appeared in the centre of a box, remaining until the participant pressed the correct key. Presentation and recording of responses was performed using Cogent v1.29 in Matlab 2007b (The MathWorks Inc., Natick, MA, 2000). Reaction time (RT) was defined as the time between stimulus presentation and making a correct button press, including the time making any incorrect presses. Cues disappeared immediately after a correct press, followed by a 300ms inter-trial interval before the next cue. A blue cue indicated the trial was random, green indicated the trial followed the fixed sequence. A pure tone was played at the same time as cue onset for a duration of 200ms, the frequency of which was consistent with the cue across trials (A = 98Hz (Gmaj), B = 175Hz (Fmaj), C = 294Hz (Dmaj), D = 440Hz (Amaj), E = 659Hz (Emaj), F = 988Hz (Bmaj)). All tones

were major musical notes and created to sound as distinct from one another as possible using Audacity 1.2.6.

The sequence trials followed the 12-item sequence B-A-E-F-C-B-D-E-A-C-F-D. This sequence followed a pattern of two left hand keys, followed by two right hand keys and so on. This was another strategy to aid classifier development, one reason being that the switch between left and right hemisphere neural activity is slowed and therefore potentially easier to detect. Each of the 6 positions appears twice in the sequence.

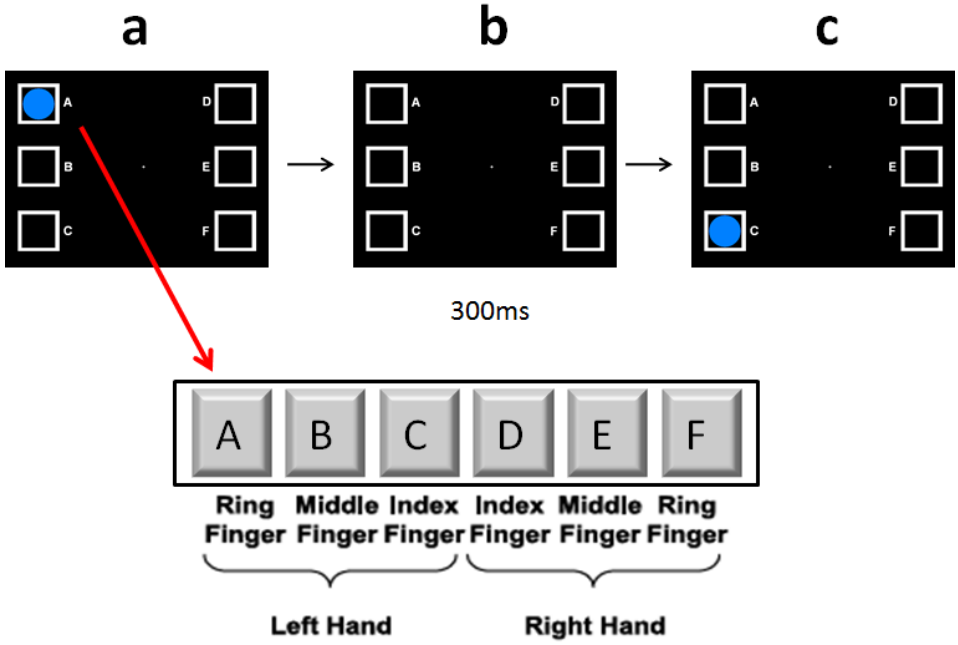

Figure 2.1: SRTT stimulus presentation for experiment 1. (a) The cue appears (blue indicates the current trials are random, while green would indicate trials will follow the repeating sequence). The participant is required to push the corresponding key as fast as possible. If the wrong key is pressed, the cue remains on screen until a correct press is made, at which point the cue disappears. (b) There is a 300ms intertribal interval. (c) The next cue appears.

Random trials were designed to match sequence trials in every respect except for the presence of the 12-item sequence, allowing any differences between the two to be assigned to learning of the sequence. They followed four constraints: (1) the same cue could not appear on consecutive trials, (2) each cue was presented twice within a string of 12-items, (3) the pattern of two left, 2 right keys was emulated, and (4) no strings of 4 or more items matched the sequence. Random blocks were different from one another but identical for each participant. Participants performed two training blocks containing 200 random and 480 sequence trials in total. They also performed a test block before (SRTT Pre) and after (SRTT Post) the retention period, containing 180 sequence trials sandwiched between 50 random trials at the beginning and end of the block (Figure 2.2).

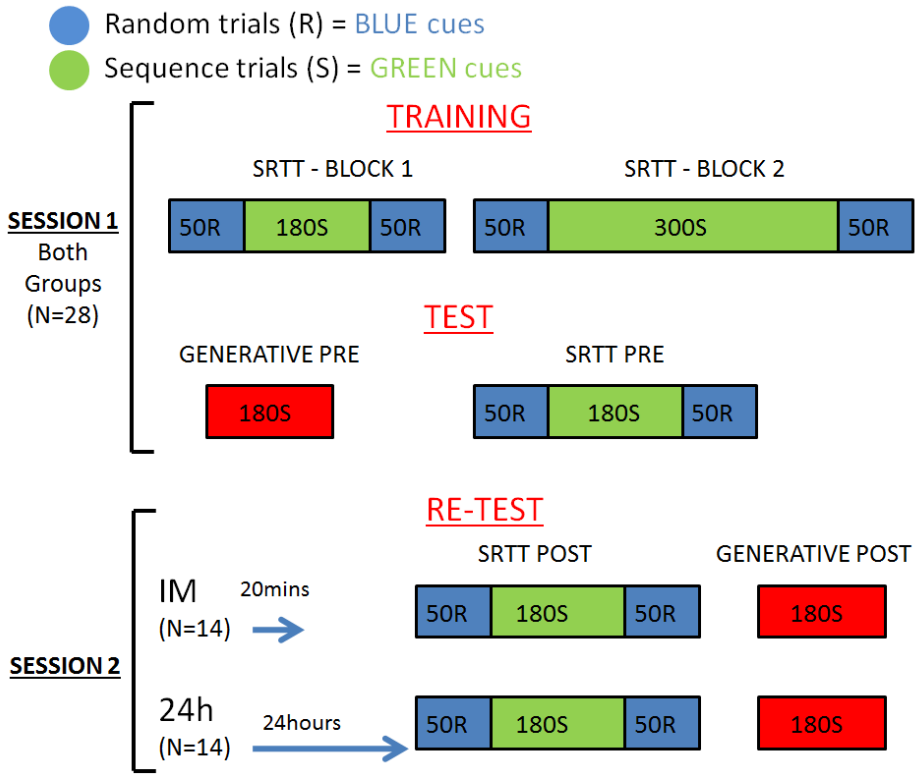

Figure 2.2: Schematic of experiment 1 procedures. In the first session, both groups performed the initial SRTT training blocks (Blocks 1 and 2) followed by test blocks for the generative task (Generative Pre) and SRTT (SRTT Pre). Blocks consisted of a number of sequence trials (S) sandwiched between 50 random trials (R) on either side. Cue colour indicated the switch between random (blue) and sequence (green). Session 2 took place 20 minutes later for the IM group, or 24 hours later for the 24h group. This session involved post retention tests of the SRTT (SRTT Post) and generative task (Generative Post).

Generative task

Participants were asked to push buttons for the sequence from memory. Each time participants pushed a key the green cue would appear in the correct position on screen accompanied by the tone, regardless of whether the correct key was pushed. The task consisted of a single block of 180 trials which followed the same sequence as the SRTT, performed both before (Generative Pre) and after (Generative Post) the retention interval. Improvement was calculated as the difference between Session 1 and Session 2 test performance.

Design & procedure

Participants were randomly assigned to one of two groups. An independent samples t-test confirmed no significant age difference between the immediate group (IM) (N = 14, Mean = 21.9 years, SD = 2.85) and 24hour group (24h) (N = 14, Mean = 24 years, SD = 5.4), $t(26) = 1.31$, $p=0.2$. Testing took place between the hours of 9am and 4pm. Those assigned to the IM group performed the second test session 20mins after the first session. The 24h group performed the second session exactly 24 hours after the first session (Figure 2.2).

In Session 1, participants were seated with headphones in a quiet room. They performed two training blocks of the SRTT, and were then asked to generate the sequence themselves as accurately as possible from memory in the generative task (Generative Pre), before performing a test block of the SRTT (SRTT Pre). After the retention period, they performed a second SRTT test (SRTT Post), followed by the second generative task (Generative Post). The order of these was identical for all participants.

Statistical analysis

The mean of the last 50 random and 50 sequence trials of each block of the SRTT were analysed for both RT and accuracy (percentage of errors). RT's longer than 1000ms were rejected, indicating lack of proper engagement with the task. A 3-way mixed ANOVA was used with group (24h vs. IM) as the between subjects variable, while block (pre vs. post-retention) and trial (sequence vs. random) were within subjects variables. For the generative task, the percentage of correctly predicted trials was calculated across the entire block and taken as a measure of explicit sequence knowledge. A 2-way mixed ANOVA was used with group (24h vs. IM) and block (pre vs. post retention). Paired sample and independent group t-tests were used for post-hoc and planned comparisons. All statistical tests were 2-tailed, significance level $p<0.05$.

Results

SRTT

Mean RT across all trials and conditions are presented in Figure 2.3. Data were analysed in a 3-way Mixed ANOVA with factors group (24h vs. IM), block (pre vs. post-

retention) and trial (sequence vs. random), which revealed a significant main effect of block, $F(1, 26) = 68.37$, $p<.001$, as both group RT's improved between pre and post retention period tests. There was also a significant main effect of trial, $F(1, 26) = 42.35$, $p<.001$, demonstrating that RT's were significantly lower for sequence trials than for random trials, indicating sequence learning.

However, there was no significant trial*group interaction, $F(1, 26) = 0.03$, $p=0.86$, therefore the improvement for both sequence and random trials was equivalent in both groups. There was no main effect of group, $F(1, 26) = 1.6$, $p =0.22$, and no group*block interaction, $F(1, 26) = 2.28$, $p=0.14$, indicating the type of retention period made no difference to the level of improvement between pre and post retention period tests.

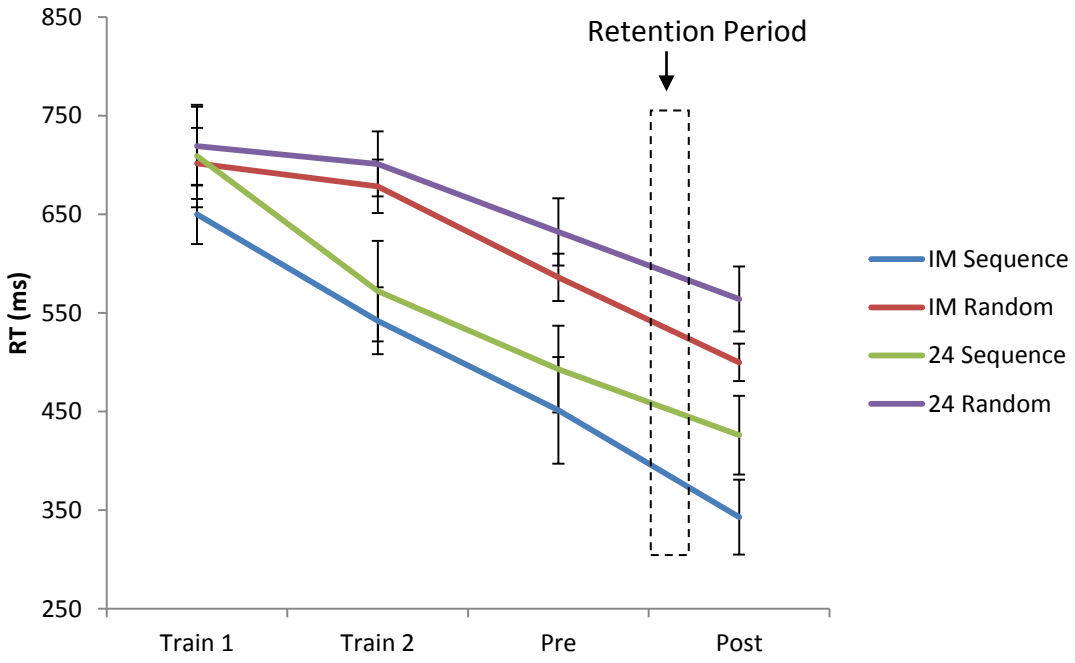

Figure 2.3: Mean RT's for SRTT performance across all blocks of experiment 1. Statistical analysis focussed on the pre and post-retention period tests. The gradual reduction in RT's across all blocks demonstrates a training effect, but there were no significant differences between groups. Data are presented as Mean ± S.E.M.

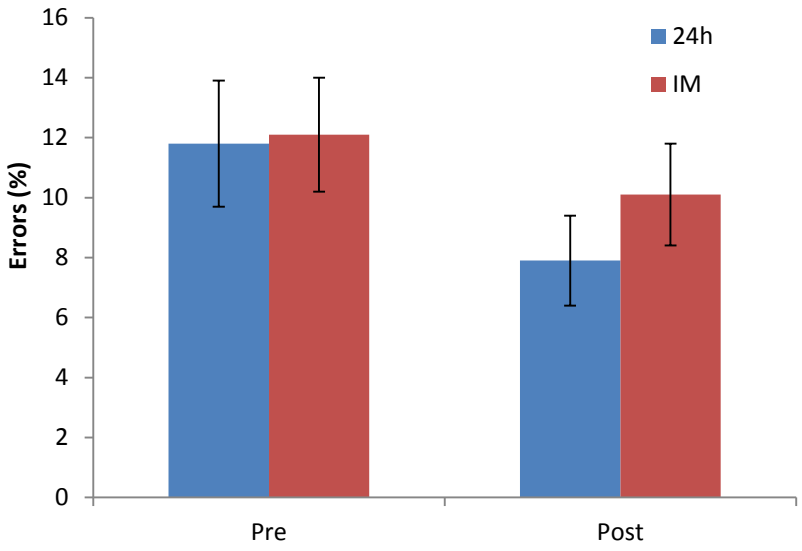

Figure 2.4: Mean errors for SRTT performance during pre and post retention interval tests (experiment 1). The percentage of incorrect responses made by both retention groups (IM vs. 24h), collapsed across sequence and random trials. There were no significant differences between groups. Data are presented as Mean ± S.E.M.

Accuracy was low compared to other SRTT studies, perhaps reflecting a higher level of difficulty (Figure 2.4). A main effect of block showed errors were significantly lower after the retention period, $F(1, 26) = 7.66$, $p<0.01$. A main effect of trial showed errors to be higher for random trials than for sequence trials, $F(1, 26) = 17.23$, $p<0.001$. Accuracy for the 24h group improved slightly more than the IM group, but not significantly so because there was no main effect of group, $F(1, 26) =0.21$, $p=0.64$, and no block*group interaction, $F(1, 26) = 0.52$, $p=0.48$. Therefore the type of retention period had no effect on accuracy improvement.

Generative task

A two-way mixed ANOVA with group (24h vs. IM) and block (pre vs. post-retention interval) showed both groups significantly improved in their ability to generate the sequence explicitly after the retention period, $F(1, 26) = 20.05$, $p<.0.001$. However there was no main effect of group, $F(1, 26) =0.73$, $p=0.4$, and no interaction, $F(1, 26) =0.16$, $p=0.69$, showing that the type of retention period had no effect on the emergence of explicit sequence knowledge.

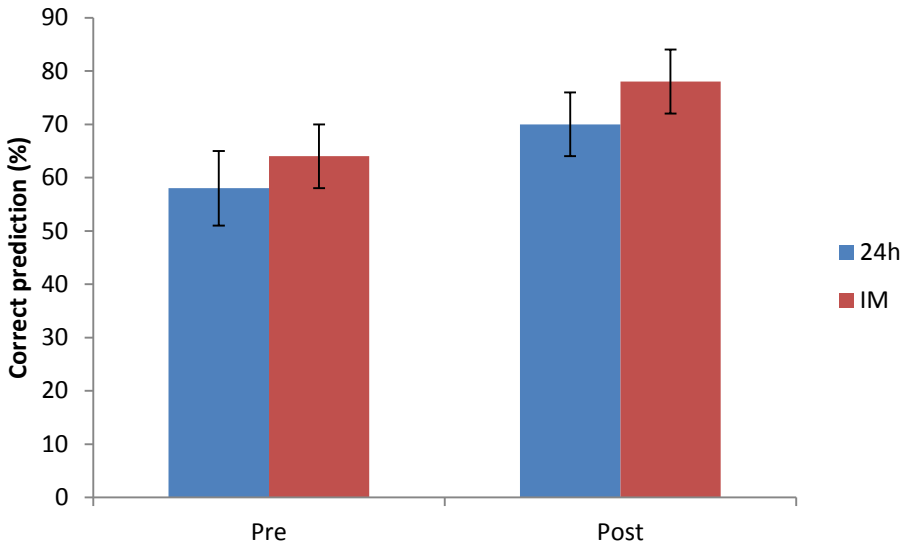

Figure 2.5: Mean explicit sequence knowledge for experiment 1. The percentage of correctly predicted trials made by both retention groups (IM vs. 24h) for pre and post retention tests, showing no significant group differences. Increase in predictive ability demonstrates a training effect. Data are presented as Mean ± S.E.M.

Discussion

This adapted version of the SRTT did not show the predicted sequence skill improvement associated with an extended offline consolidation period. Similarly, explicit sequence knowledge improvement did not differ between groups. Both groups improved on the procedural and explicit measures, reflecting a training effect on account of the additional training experienced during the retest. In light of previous literature showing sleep-dependent consolidation of the SRTT (e.g., Cohen et al., 2005), we conclude that methodological flaws most likely account for our null results. These are now discussed with reference to how they were rectified in experiment 2.

Stimuli composition: The non-direct visuo-motor mapping between keys and cue positions may have made learning the task too difficult, reflected in error rates exceeding those observed in previous SRTT studies. For example, error rates in the seminal SRTT study were only 2% for the majority of sequence blocks (Nissen & Bullemer, 1987), compared with our rates in excess of 10%. In addition, mean RT's were 100-150ms slower across blocks than a similar study (Spencer et al., 2006), and

large individual differences were observed in participant's ability to perform the task. Together this indicates a high level of difficulty. Our stimuli were designed to assist a potential EEG classifier, but this has interfered with sleep-dependent consolidation. To rectify this, experiment 2 utilised 4 cues arranged horizontally, similar to the main body of SRTT literature.

Training: The added difficulty of a non-direct visuo-motor mapping most likely requires additional training, therefore any small observable improvement associated with consolidation would be masked by the larger improvements associated with training. Motor skill learning entails a fast acquisition phase which is illustrated by incrementally less improvement being made across experimental blocks (i.e., the slope of a graph of block performance becomes less steep), followed by smaller increments across consolidation periods or additional practice (Doyon & Benali, 2005). Figure 2.3 clearly shows that large improvements are still being made across the whole experiment (i.e., the slope of the graph does not change), suggesting participants were still within the fast learning stage and so this training effect would conceal any smaller consolidation effects. Experiment 2 adjusted the stimuli composition as discussed, while following the training process and overall design of a closely matched SRTT study (Spencer et al., 2006).

Generative task interference: To minimise interference of this explicit memory test on SRTT test blocks, it was performed between training and pre-retention SRTT test, and after the post-retention SRTT. However it may have still interfered in several ways. Firstly, it may have increased between-subject variance, on account of differing strategies used by participants when performing the generative task. That is, some participants performed the generative task rapidly in the same way as SRTT performance, therefore it served as additional SRTT training, while others performed it very slowly. This highlights a related problem with this task, that it is not a good measure of explicit knowledge since participants can utilise explicit motor skill to perform it. Also, the expectations of performing the generative task can impact strategy while performing the SRTT. In this way, some participants take more care explicitly learning the 12-item sequence during performance of the SRTT, perhaps to the detriment of RT's, while others rely entirely on an implicit strategy. In light of these issues, this task was omitted from experiment 2.

Instructions: Explicit versions of the SRTT engage sleep-dependent consolidation more often than implicit versions (Song, 2009), therefore we instructed participants to explicitly learn the sequence. However, debriefing indicated that this instruction split the strategies of participants, where some made an effort to memorise the sequence throughout, while others focussed on reacting as fast as possible under the assumption they would acquire the sequence gradually. This may have contributed to between subject variance, masking consolidation effects. Experiment 2 did not ask participants to learn the sequence.

Sequence/random trials: Random trials were constrained to match the structure of sequence trials, in particular matching the pattern of two left finger presses followed by two right, which means the random trials formed a probabilistic sequence. For example, a left press of 'A' would mean the next trial has a 50% chance of being 'B' or 50% chance of 'C'. Learning this probabilistic sequence may partially account for random improvement that closely matched sequence improvement. This is a minor point as it had no bearing on the null finding in sequence trials. Random trials of experiment 2 were less structured to ensure they represented learning of the motor mapping only.

Retention period: The twenty minute retention period for the IM group may not have allowed participants enough time to be distracted from the experiment, which may have encouraged conscious rehearsal of the task. A short retention period is necessary to allow aspects of the task to decay from working memory, and also to allow recovery from fatigue. SRTT performance has been shown to improve through consolidation after 4 hours, but not after 1 hour (Press, Casement, Pascual-Leone, & Robertson, 2005), therefore 1 hour was considered a suitable delay for the IM group in Experiment 2.

In summary, the non-direct visuo-motor mapping between cues and keys appears to have created high error rates, between-subject variance and inflated RT's, masking any sleep benefit associated with the SRTT. The paradigm was modified too far in the direction of being useful to an EEG classifier; therefore experiment 2 utilised a simplified SRTT. The additional issues outlined above were also addressed in experiment 2.

Experiment 2

Introduction

This experiment utilised a simpler version of the SRTT with only 4 cue locations and direct visuo-motor mapping between screen positions and key positions. An additional change was made in order to create neural activity that would be more easily classified in future experiments recording EEG, whereby cues were no longer solid circles but a mixture of non-symmetrical objects and faces.

The organisation of neural systems supporting face perception remains a popular and contentious issue in cognitive neuroscience, with some proposing a specialised module in the Fusiform Face Area (FFA) (Kanwisher, 2000) while others posit a more distributed processing model (Haxby et al., 2001). The current study aimed to take advantage of the considerable evidence for faces and objects creating distinct neural representations measurable by EEG and fMRI.

The FFA lies on the lateral aspect of the fusiform gyrus of the right hemisphere (Kanwisher, McDermott, & Chun, 1997). The FFA produces a larger blood-oxygen level dependent (BOLD) response to faces than objects, even when lower level features differ widely such as line drawings of faces (Spiridon & Kanwisher, 2002) or even faces of cats (Tong, Nakayama, Moscovitch, Weinrib, & Kanwisher, 2000). Using a multi-voxel pattern analysis (MVPA) approach, Haxby et al. (2001) identified a distributed population code in ventral temporal cortex that responds significantly to faces, including the 3 key face areas and their surrounding cortices.

Face perception has also been studied extensively with EEG. Face stimuli consistently produce a larger negative event-related potential (ERP) component at occipito-temporal electrodes between 140ms and 200ms (N170 component), when compared with non-face stimuli (Calder, Rhodes, Johnson & Haxby, 2011), which is thought to reflect activation of face selective areas. An additional component often found to be face sensitive is the vertex positive potential (VPP), which usually accompanies the N170 (Botzel & Grusser, 1989; Jeffreys & Tukmachi, 1992). There is also evidence for gamma oscillations (>30Hz) being induced over these same electrodes during face perception (Lachaux et al., 2005), showing that non-phase locked activity may also be

utilised to classify what is being viewed or reactivated. These components might provide important features to train an EEG classifier.

An important question is whether these same components will be present when participants are asleep and not actually viewing the stimuli, but reactivating a memory of the visual stimuli. Reactivation of perceptual learning has been shown in V1 during NREM sleep (Yotsumoto et al., 2009), while MVPA has shown the reinstatement of stimulus-specific neural activity during resting periods and sleep (Deuker et al., 2013). Furthermore, hypnogogic dream content has been decoded using MVPA (Horikawa et al., 2013). Also, it was recently shown that ERP's associated with semantic processing of verbal stimuli are still present after participants have fallen asleep (Kouider et al., 2014). Thus, similar EEG components for viewing faces and objects might be apparent during reactivation of those representations during sleep.

The SRTT in this experiment will involve an object and a face appearing consistently in the same location on the left (Positions 1 and 2), and the same on the right (Positions 3 and 4). This could assist an EEG classifier, because activity measured in left occipito-temporal cortex would indicate a stimulus is being viewed or reactivated in the right visual field, position 3 or 4. By looking at the shape of this ERP it may be possible to discern if a face (3) or an object (4) is being viewed or reactivated.

We predicted sequence RT for the 24 hour group would improve significantly more than the immediate group after the retention period, but random performance would not differ.

Materials & Methods

Participants

Forty participants (Mean age=25 years, 20 female) from the University of Manchester took part with the same screening/ethics protocol as Experiment 1.

Serial reaction time task

Visual cues appeared above four white lines (6cm) arranged horizontally, corresponding to keys labelled 1-4 (Figure 2.6). The gap between position 2 and 3 was larger so that 1-

2 will appear predominantly in the left visual field and 3-4 in the right. Keys 1 and 2 required a key press with the middle and index finger of the left hand, cue 3 and 4 the middle and index finger of the right hand. The cues were non-symmetrical objects or faces. The same face or object appeared for each position (1= face A, 2= lamp, 3= face B, 4= tap). Face images were taken from the Psychological Image Collection at Stirling (PICS; http://www.pics.psych.stir.ac.uk), object images from a variety of web-based sources. Visual stimuli were matched for lower level perceptual features as much as possible. Background luminance was constant and mean luminance for each image was similar. Retinal eccentricity was also roughly equal. Each visual cue was accompanied by a tone of a specific frequency (A = 175Hz (Fmaj), B = 294Hz (Dmaj), C = 440Hz (Amaj), D = 659Hz (Emaj)).

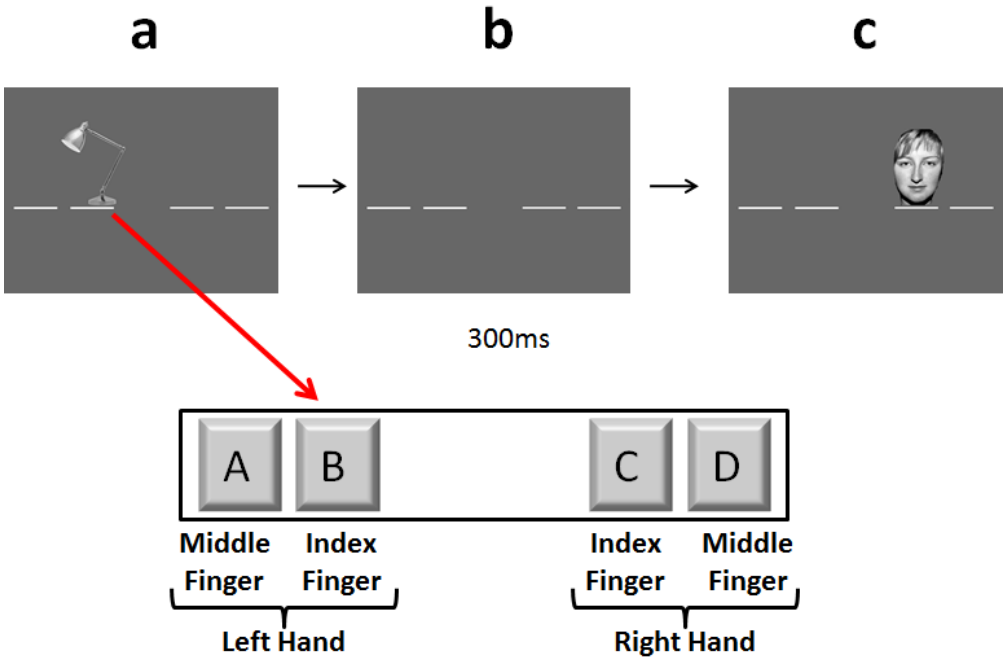

Figure 2.6: SRTT stimulus presentation for experiment 2. (a) The stimulus appeared until a correct key press was made. (b) This was followed by a 300ms inter-trial interval. (c) The next stimulus appeared.

Sequence trials followed the 12-item sequence 2-3-1-4-3-2-4-1-3-4-2-1, where each position occurred once for every quadruplet. Random trials followed the constraints: (1) the same cue did not appear on consecutive trials, (2) each position occurred equally frequently across each block of 72 trials, and (3) strings of 4 or more items matching the sequence were limited. Random blocks were different from one another but identical for each participant.

Participants were instructed to press the keys corresponding to the cues as quickly as possible while keeping errors to a minimum. They were told the nature of the cues (objects/faces) was irrelevant to the task. They were advised that most blocks would follow a sequence but the order would be random for some blocks, and each subsequent trial would not begin until they made the correct key press. Each block contained 72 trials. The first and second sessions contained 11 blocks in total and were identical. The first seven blocks follow the sequence while the final 4 blocks alternated between sequence and random (Figure 2.7) .Feedback advising fastest/average RT and errors was provided between each block during a fixed 30second break.

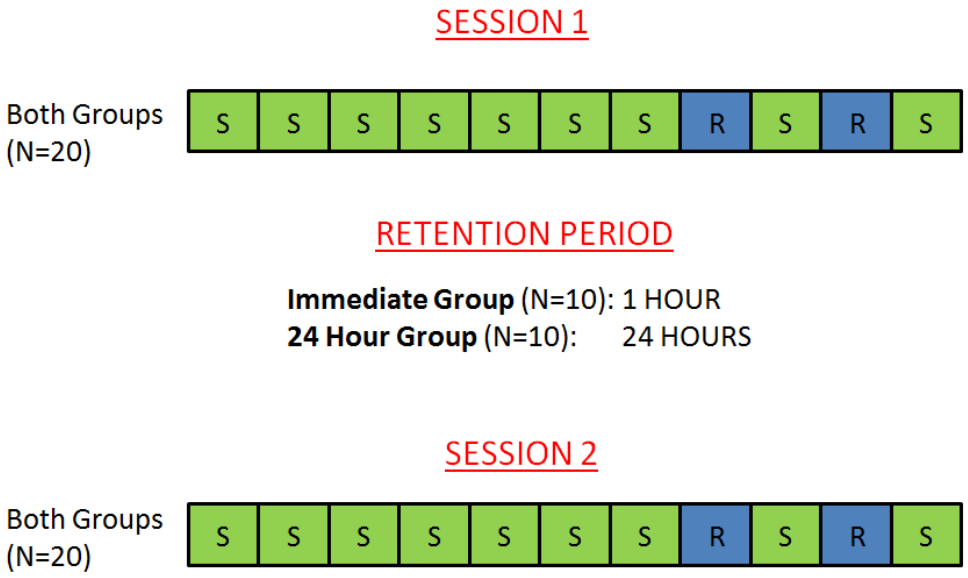

Figure 2.7: Schematic of experiment 2 procedures. In session 1, both groups performed the SRTT containing 11 blocks of 72 trials, 9 of which were sequence (S) and 2 random (R). Session 2 took place one hour later for the IM group, or 24 hours later for the 24h group. This session was identical to the first session. The last 4 blocks of each session were compared to provide a measure of consolidation.

Design & procedure: Participants were randomly assigned to two groups (24h vs. IM), with no significant age difference between 24h (N = 20, Mean age= 22.9 years, SD = 2.9, 12 female) and IM groups (N = 20, Mean age= 23.4 years, SD = 2.8, 10 female), $t(20)=0.59$, $p=0.58$. Testing took place between 3-7pm, with the IM group performing session 2 one hour after session 1, and the 24h group performing the second session exactly 24hours after the first. Participants were seated and provided with headphones, then performed the learning session. Participants returned to the lab after the retention interval and performed the retest session.

Statistical analysis: The variable block (pre vs. post) was collapsed into a single improvement measure, by subtracting mean RT for session 2 from session 1 for both sequence and random blocks separately. A 2-way mixed ANOVA was used with group (24h vs. IM) and trial (sequence vs. random). Paired-samples and independent-groups t-tests were used for post-hoc and planned comparisons. All statistical tests were 2-tailed, significance level $p<0.05$.

Results

A mixed ANOVA with the factors of group (24h vs. IM) and trial (sequence vs. random) revealed a main effect of trial, $F(1, 38) = 13.26$, $p<0.001$, demonstrating a greater performance improvement for sequence trials than random trials collapsed across groups. There was also a main effect of group, $F(1, 38) = 6.65$, $p<0.05$, showing the 24h group improved significantly more than the IM group collapsed across all trials.

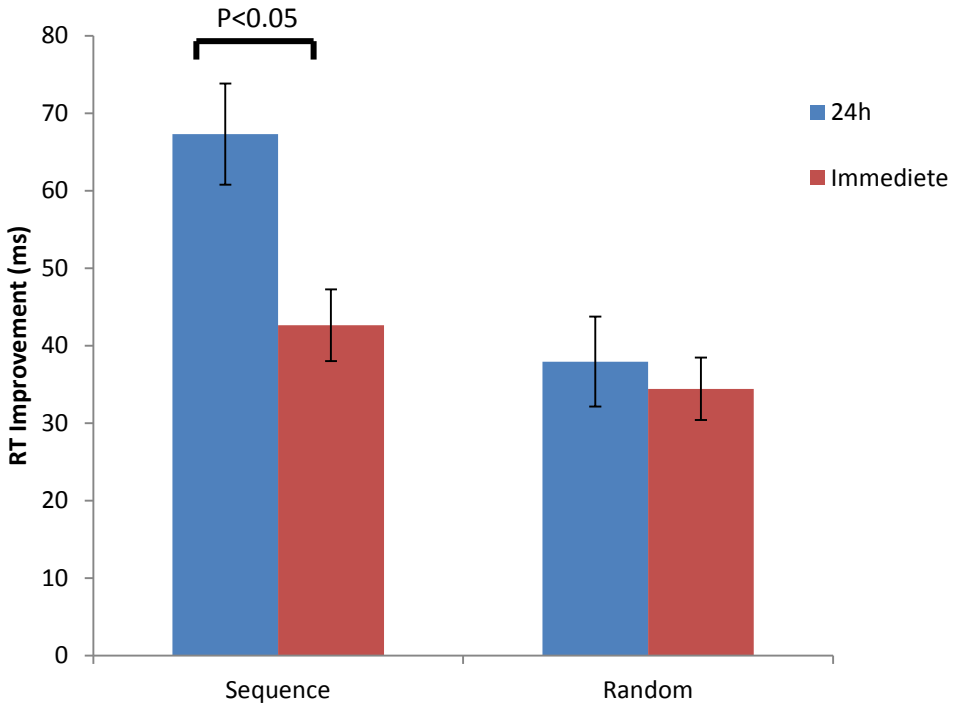

Figure 2.8: Mean RT improvement for SRTT performance across sessions of experiment 2. Both groups improved after the retention period. The 24h retention period led to a significantly larger improvement on sequence trials, but had no effect on random trials, indicating enhanced consolidation of specifically the sequence. Data are presented as Mean ± S.E.M.

We also found a significant interaction between trial and group, $F(1, 38) = 4.15$, $p<0.05$, driven by a significant difference between the two groups for sequence trials, $t(38)= 3.09$, $p<0.01$, but not random trials, $t(38)= 0.52$, $p=0.61$ (Figure 2.8). This demonstrates sequence specific consolidation after a longer retention period. In addition, within-subjects contrasts showed the 24h group improved significantly more for sequence trials compared with random trials, $t(19)= 3.82$, $p<0.001$. This was not the case for the IM group, $t(19)= 1.2$, $p=0.25$. If we calculate sequence-specific skill (random minus sequence skill), the 24h group skill improved by 26.1ms.

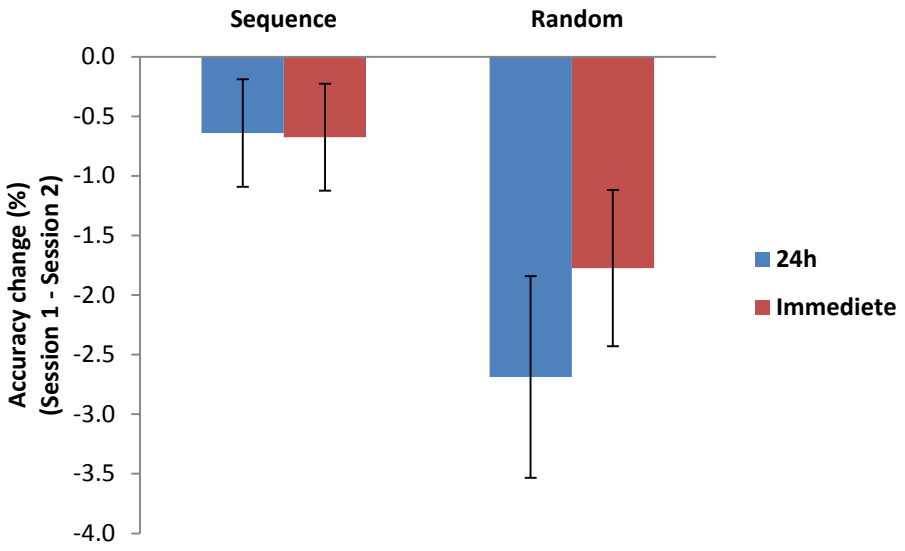

Figure 2.9: Mean decline in accuracy for SRTT performance across sessions of experiment 2. The change in accuracy within both groups was very low, and no significant group differences were found. Data are presented as Mean ± S.E.M.

The change in accuracy across sessions was calculated in the same way as RT data. Error rates were typically low for sequence trials (Mean=3.4±3.0%) and random trials (Mean=6.7±4.2%), and all deteriorated slightly after the retention period. A mixed ANOVA with group (24h vs. IM) and trial (sequence vs. random) showed a main effect of trial, $F(1, 38) = 9.92$, $p<0.01$, indicating a greater number of errors for random trials as expected, but no main effect of group, $F(1, 38) = 0.27$, $p=0.61$, or group*trial interaction, $F(1, 38) = 0.7$, $p=0.41$.

General Discussion

As predicted, the 24h retention period led to a significantly greater performance improvement for sequence trials, but had no effect on random trials, suggesting the longer retention period containing sleep enhanced offline consolidation of the sequence representation. This is consistent with Robertson et al. (2004), who found a 35ms increase in sequence-specific skill for participants who had a slept rather than stay awake during a 12h retention period, compared with 26.1ms in the current study. Other studies have found enhanced performance of between 17-21ms (Fischer et al., 2006;

Brown & Robertson, 2007; Cohen et al., 2005), therefore our finding appears to be a reliable one. It is not possible to discern from our data whether the effect was sleep or time dependent, but the research outlined above implies sleep likely played a prominent role.

To conclude, our adapted version of the SRTT has replicated the well-established sleep/time consolidation effect associated with the task, making it suitable for a subsequent sleep study involving cued reactivation via replay of the SRTT tone sequence during sleep.

Chapter 3

Cued memory reactivation during slow-wave sleep promotes explicit knowledge of a motor sequence*

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The results were also presented at the Alpine Brain Imaging Meeting (ABIM; Champery, 9th January 2014), the Cognitive Neuroscience Society Annual Meeting (CNS; Boston, 8th April 2014) and the International Workshop on Learning and Memory Consolidation (San-Sebastian, 11th July 2014).

Abstract

Memories are gradually consolidated after initial encoding, and this can sometimes lead to a transition from implicit to explicit knowledge. The exact physiological processes underlying this reorganization remain unclear. Here, we used a serial reaction time task (SRTT) to determine whether targeted memory reactivation (TMR) of specific memory traces during slow-wave sleep promotes the emergence of explicit knowledge. Human participants learned two 12-item sequences of button presses (A and B). These differed in both cue order and in the auditory tones associated with each of the four fingers (one sequence had four higher pitched tones). Subsequent overnight sleep was monitored, and the tones associated with one learned sequence were replayed during slow-wave sleep. Upon waking, participants demonstrated greater explicit knowledge (p=0.005) and more improved procedural skill (p=0.04) for the cued sequence relative to the uncued sequence. Furthermore, fast spindles (13.5-15Hz) at task-related motor regions predicted overnight enhancement in procedural skill (r=0.71, p=0.01). Auditory cues had no effect on post-sleep memory performance in a control-group who received TMR prior to sleep. These findings suggest that targeted memory reactivation during sleep can alter memory representations and promote the emergence of explicit knowledge, supporting the notion that reactivation during sleep is a key mechanism in this process.

Introduction

Sleep benefits many forms of memory consolidation. It aids the assimilation of memories into existing knowledge networks (Tamminen et al., 2010, 2013), facilitates inferential thinking (Ellenbogen et al., 2007), and assists in the emergence of explicit knowledge for underlying statistical regularities (Drosopoulos et al., 2011; Fischer et al., 2006; Wagner et al., 2004; Wilhelm et al., 2013; Yardanova et al., 2008).

The spontaneous reactivation of recently learned memories during sleep has been proposed as a mechanism which may underpin the re-organization of memory traces

(Born et al., 2006). In rodents, neuronal firing sequences that were expressed during encoding are reinstated in subsequent periods of sleep (Wilson & McNaughton, 1994), while human neuroimaging studies show reactivation of learning related brain regions during post-encoding sleep (Maquet et al., 2000; Peigneux et al., 2003), and this predicts subsequent post-sleep performance improvement (Peigneux et al., 2004; Yotsumoto et al., 2009).

Recent studies have intentionally elicited memory reactivation by covertly presenting cues that were paired with new memories at learning during subsequent sleep. Such targeted memory reactivation (TMR) biases consolidation of both declarative (Cairney et al., 2014; Fuentemilla et al., 2013; Rasch et al., 2007; Rudoy et al., 2009) and procedural (Antony et al., 2012; Schönauer et al., 2014) memory in humans when implemented during slow-wave sleep (SWS), but not when implemented during wakefulness. TMR is thought to influence the neural replay of recently formed memories, and has been shown to bias specific neuronal firing sequences in rodents (Bendor & Wilson, 2012). Together these findings suggest that cues provide a tool for manipulating naturally occurring memory consolidation processes.

While TMR during sleep can enhance specific memories, its impact on other transformations of memory that occur during normal sleep, such as the emergence of explicit awareness of an implicitly learned sequence (e.g., Fischer et al., 2006), remains to be explored. Here, we build on the finding that TMR influences procedural skill consolidation across sleep (Antony et al., 2012; Schönauer et al., 2014), by using a serial reaction time task (SRTT) to examine how TMR effects the overnight emergence of explicit knowledge. Participants learned two four-finger button pressing sequences, in which each finger was associated with a specific auditory tone, and four separate tones (higher or lower in pitch) were used for each sequence. To cue memory replay, the tones associated with one sequence were replayed to participants during subsequent SWS (experimental-group) or prior to sleep (control-group). Upon waking the following morning, we measured explicit knowledge of sequence order and reaction times when performing each sequence. We predicted enhanced performance on both measures for the cued sequence, indicating a role for memory replay in both forms of consolidation. The electrophysiological correlates of TMR, and how they relate to consolidation, are largely unknown. We explored this by conducting a thorough analysis

of spindles (12-15Hz) and slow oscillations (~1Hz) during replay of cues and surrounding periods of SWS.

Materials & Methods

Participants: Thirty-eight healthy right-handed volunteers were screened for history of neurological, psychiatric diseases, sleep or motor disorders and asked to abstain from caffeine and alcohol 24 hours prior to testing. Participants were randomly assigned to experimental and control groups. Six participants were excluded from all analyses for corrupted sleep data (N=1), RT performance at learning >2SD from group means (N=2) and >2SD disparity between group mean RT for the two sequences at learning (N=3). Thirty-two participants remained in experimental (N=16, mean age=24.8 years, 8 females, 8 males) and control (N=16, mean age=23.2 years, 8 females, 8 males) groups.

Design and procedure: Participants were fitted for polysomnographic (PSG) recording between 7-8pm, then performed an adapted SRTT (Nissen & Bullemer, 1987) containing interleaved blocks of two 12-item sequences, A (1-2-1-4-2-3-4-1-3-2-4-3) and B (2-4-3-2-3-1-4-2-3-1-4-1). Sequences were matched for learning difficulty, contained 3 repetitions of each item, and did not share strings of 5 or more items.

Each sequence was accompanied by either high or low pitched pure tones, counterbalanced across participants. Tones were musical notes grouped closely within the 4^{th} (low) (C/D/E/F) and 5^{th} octave (high) (A/B/C#/D).

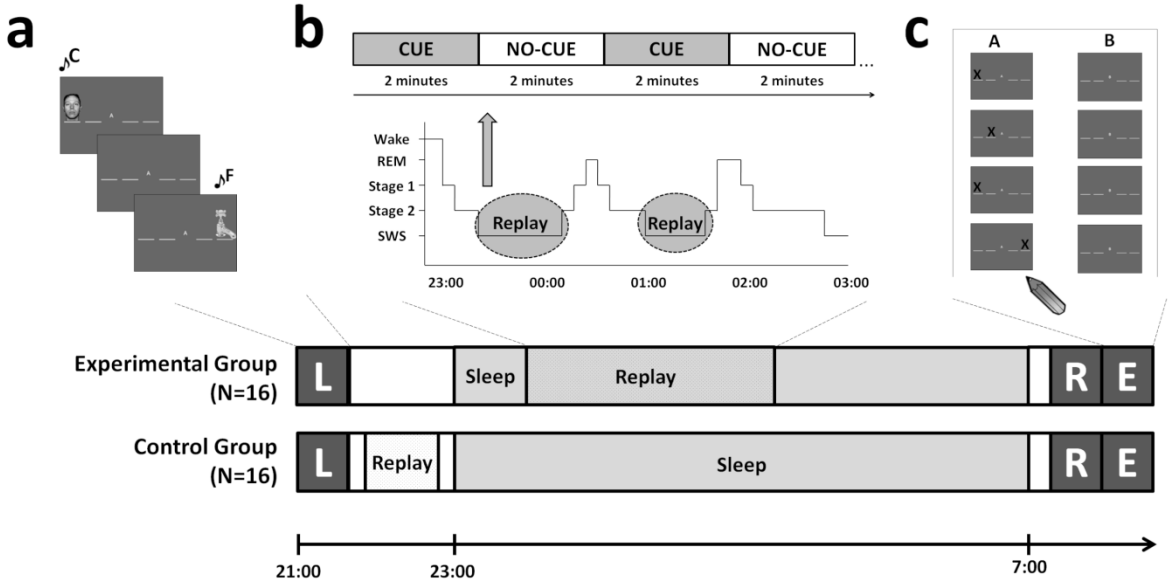

Figure 3.1: Experimental procedures. (a) Serial Reaction Time Task (SRTT) learning (L): Visual stimulus appeared contingent with a unique tone. Correct key response was followed by a 300ms interval before next stimulus. Interleaved blocks of Sequence A containing low tones (4^{th} Octave: C, D, E and F), and B containing high tones (5^{th} Octave: A, B, C# and D), and random blocks containing high and low tones were performed. (b) One sequence was played to the experimental-group during SWS, with 12 repetitions (CUE) followed by silence (NO-CUE). (c) In the morning, participants were re-tested on the SRTT (R) before their explicit sequence knowledge was assessed (E), by marking sequence order on paper.

For each trial, a visual cue appeared with a tone in one of four spatial locations, corresponding to keys of the same configuration, and pressed with individual fingers of the left hand as quickly as possible while minimising errors. 'A' or 'B' appeared centrally on the screen indicating the sequence. Participants were not asked to explicitly learn the sequences. Visual cues were objects or faces, appearing in the same position for both sequences (1=face1, 2=lamp, 3=face2, 4=water tap). Participants were told that the nature of the cues (objects/faces) was irrelevant. Stimuli disappeared only after a correct response and were followed by a 300ms inter-trial interval (Figure 3.1a).

Blocks containing 3 repetitions of a sequence were performed in pairs separated by a 2s fixation cross. Each pair was followed by a 30s break with reaction time (RT) and error-rate feedback. Blocks were interleaved pseudo-randomly, with no runs of more than 2-blocks of the same sequence. Sequence A and B were counterbalanced across cued and uncued conditions, so half the participants were cued with sequence A. Tones (high/low pitch) were counterbalanced across sequences. Participants performed 20 blocks each of cued (SEQ_C) and uncued sequences (SEQ_U). Four random blocks containing no repeating sequence followed, with 'R' displayed centrally. Half of these blocks contained tones from SEQ_C (RAND_C) and half used tones from SEQ_U (RAND_U).

The control-group listened to cues while awake, 20 minutes after training. SEQ_C was played on PC speakers (48dB) imbedded in brown noise with tones 650ms apart, similar to mean pre-sleep performance. Replay blocks (CUE) lasted 2 minutes and contained 12 sequences, followed by 2 minutes of silence (NO-CUE). To prevent rehearsal, which may influence skill and memory, participants performed a number comparison task during replay. A pair of 3-digit numbers appeared on the screen, joined by a similar target number 3000ms later. Participants pressed keys with the index finger of each hand to indicate which number was nearer the target. They had 3000ms to respond followed by a 500ms inter-trial interval.

All participants were permitted to read in bed prior to 11pm lights out. PC speakers played brown noise all night. In the experimental group, tones of SEQ_C were played during the first three extended periods of SWS (Figure 3.1b), using the same replay protocol as in the control group. Cues were stopped immediately upon arousal or leaving SWS.

Participants were awoken 7-8am, and allowed 20 minutes to overcome sleep inertia. At re-test, 12 blocks of each sequence (SEQ_C and SEQ_U) preceded 12 repetitions of each random block (RAND_C and RAND_U). 'REST' was displayed centrally during the 30s breaks. Order of learning (i.e., whether interleaved blocks began with sequence A or B), replay and re-test was counterbalanced across participants.

Free recall was used as the measure of explicit knowledge. Participants marked sequence order on printed out screen-shots arranged vertically on a page, for each sequence, with sequence order counterbalanced (Figure 3.1c). All participants except

one (N=31) completed the explicit test. Alertness was measured using the Stanford Sleepiness Scale (Hoddes et al., 1973).

Behavioural analysis: Pre-sleep performance comprised mean of the last 4 blocks of SEQ_C and SEQ_U, and 2 blocks of RAND_C and RAND_U. Sequence was subtracted from random RT to separate learning of the sequence from sensori-motor mapping, providing a measure of 'sequence-specific-skill'. Post-sleep performance used a mean of the last four blocks of SEQ_C and SEQ_U, and the first four blocks of RAND_C and RAND_U, minimising differences in training between SEQ and RAND because they were performed side-by-side. Post-sleep performance was subtracted from pre-sleep performance to determine improvement. Trials containing incorrect button presses, prior to the correct press, were included. Response latencies greater than 1000ms were excluded. For explicit recall, individual items were only correct if in the correct sequence position and within a segment containing >2 consecutive correct items (Willingham & Goedert-Eschmann, 1999), minimising the influence of guessing. To determine if explicit recall was above chance, recall of both sequences was re-scored against 10 randomly generated sequences for each participant. The mean of these 10 random sequence scores was taken as 'chance', and the mean of these chance scores across all participants was taken as the average number of items that would be guessed by chance.

Mixed ANOVA and paired sample t-tests were used for planned comparisons of cued and uncued sequence RT and recall, except where Shapiro-Wilk tests indicated a non-normal distribution, then Friedman's ANOVA and Wilcoxen signed-rank tests were used. Associations between behavioural measures and EEG features were tested with Pearson's correlations, or Spearmans Rho for non-normal distributions. All statistical tests were 2-tailed, significance level $p<0.05$. All means presented in the text ± standard deviation.

EEG recording and analysis: Scalp electrodes were attached according to the 10-20 system at fourteen standard locations, F3, F4, C3, C4, C5, C6, CP3, CP4, CP5, CP6, P7, P8, O1, and O2, referenced to the combined mean of left and right mastoid. Left and upper electromyogram, left and right electrooculagram, and a forehead ground electrode were also attached. Impedance <5Ω was verified at each electrode, and digital sampling rate was 200Hz. Data were scored by two trained sleep researchers according to The

AASM Manual (American Academy of Sleep Medicine, Westchester, IL). The second scorer was blind to CUE/NO-CUE periods. Correlations with behavioural measures focussed on groups of electrodes: 2 'Frontal' (F3 and F4), 2 'Parietal'(P7 and P8) and 8 'Central' (C3, C4, C5, C6, CP3, CP4, CP5 and CP6) electrodes. These groupings were excluded from analysis if any electrode had to be removed due to noise.

Complete 2-minute epochs of CUE/NO-CUE periods were extracted for every channel and concatenated separately, providing a CUE and NO-CUE time series for each participant. Adjacent CUE/NO-CUE periods were rejected if they contained visually identified artefacts (e.g., movement), followed by band pass filtering for slow (12-13.5Hz) and fast (13.5-15Hz) spindles separately, using a linear finite impulse response filter in EEGLab v.9.0, via MATLAB 2010 (The MathWorks Inc., Natick, MA, 2000). An automated detection algorithm (Ferrarelli et al., 2007) determined the number of spindle events at each electrode. Spindle density was calculated as total spindles divided by length of the CUE/NO-CUE period time series. To explore regional spindle effects, we subtracted spindle density in left (non-learning) from right (learning) hemisphere. Outcome measures were the difference in spindle density between left and right electrodes (Lateralised Spindles) for the three electrode groups in CUE/NO-CUE periods. Power spectral density was analysed over CUE/NO-CUE periods using Welch's method, with power averaged over each time series. This utilized a 4s Hamming window length with 50% overlap, focussing on slow oscillations (0.3-1Hz). Outcome measures were the combined mean power within the three electrode groups in CUE/NO-CUE periods. To correct for multiple comparisons, correlations between behavioural measures and separate EEG features were false discovery rate (FDR) corrected (Benjamini & Hochberg, 1995), a method that accounts for the expected proportion of falsely rejected hypotheses. Thus, each EEG feature (e.g., fast spindles) was corrected based on a total of 6 comparisons, given that we measured 3 groups of electrodes (frontal, central and parietal) over 2 periods of interest (CUE and NO-CUE).

Results

Reaction times were significantly faster for sequence trials compared to random trials prior to sleep in both experimental and control-groups ($p<0.001$), confirming learning of both cued and uncued sequences. Importantly, RT's for cued and uncued sequences, and randomly sequenced trials, did not differ prior to sleep ($p>0.3$) (Figure 3.2 and Table 3.1).

To examine the effects of cueing on explicit recall, we performed a mixed Friedman's ANOVA (since explicit recall was not normally distributed) with factors group (experimental/control) and replay (cued/uncued). This showed no main effect of group, $F(1,29)=0.19$, $p=0.7$, a marginal main effect of replay, $F(1,29)=4.13$, $p=0.05$, and a significant interaction, $F(1,29)=5.61$, $p=0.025$. Post-hoc Wilcoxen signed-rank tests in the experimental-group showed significantly better explicit recall of the cued ($M=4.9\pm3.5$) than uncued sequence ($M=1.7\pm2.2$) ($p=0.005$) (Figure 2c). Cued and uncued sequence-recall did not differ in the control-group ($p=0.6$), indicating that the marginal main effect of replay was driven by the experimental-group. Furthermore, the cued sequence was recalled significantly better than chance in the experimental-group ($p<0.001$), while the uncued sequence was not ($p=0.16$). In the control-group, uncued-sequence-recall was above chance ($M=3.1\pm3.1$) ($p=0.01$) while cued-sequence-recall was not ($M=2.6\pm3.9$) ($p=0.22$), but these did not differ significantly ($p=0.55$). Overall, we show that TMR during sleep, but not wakefulness, increases explicit knowledge of the cued sequence.

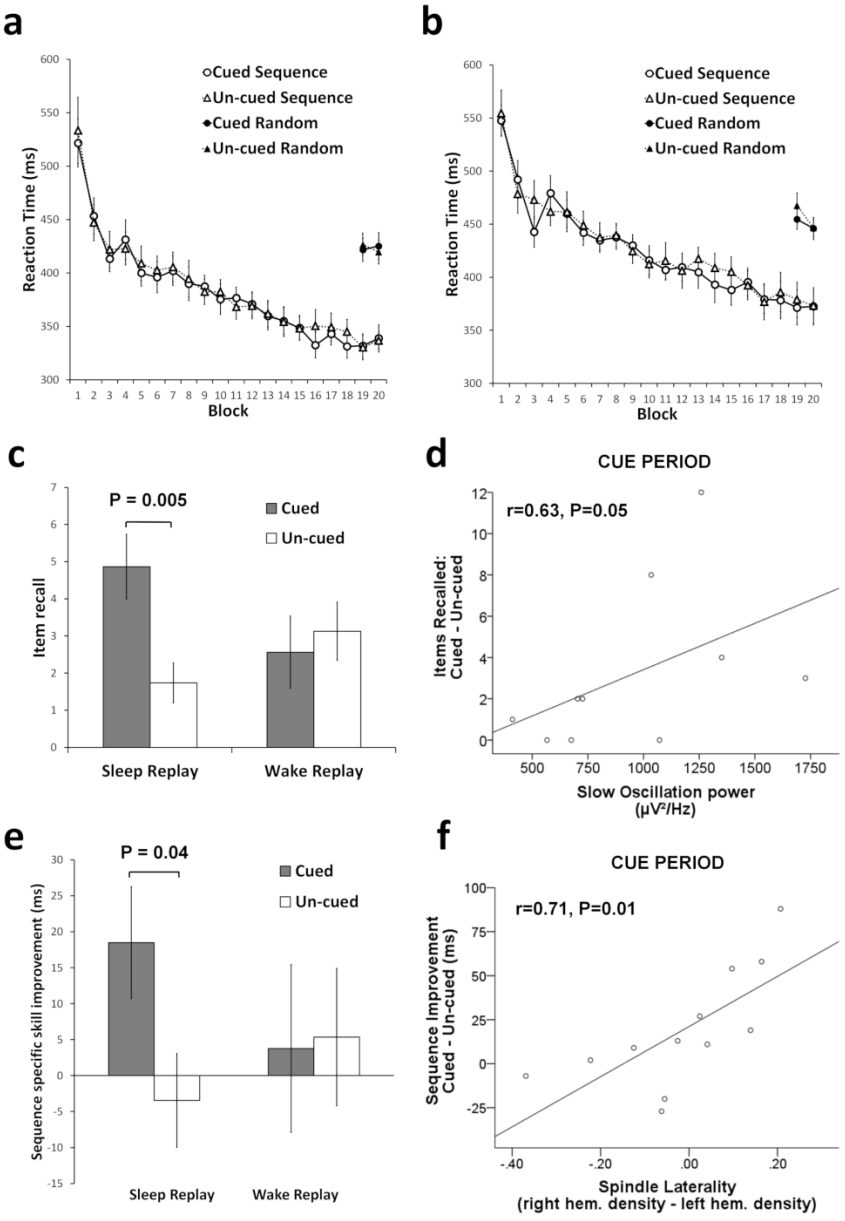

Figure 3.2: The cueing effect and neural correlates. (a) Pre-sleep SRTT performance across all blocks of learning in the experimental-group and (b) control-group. (c) Cues led to significantly more correctly recalled sequence items for the experimental-group, but not control-group. (d) Correlation between slow oscillation power in central electrodes and the explicit-cueing-effect during CUE (N=10) in the experimental-group. (e) SRTT sequence-specific-skill improvement was significantly better for the cued than uncued sequence in the experimental-group only. (f) Spindle Laterality at central electrodes predicted the SRTT cueing effect in the experimental-group during CUE (N=12), and NO-CUE. Data are presented as Mean ± S.E.M. Correlations are presented with some participants removed due to EEG artefacts.

To explore the relationship between the impact of TMR on explicit knowledge and EEG measures during (CUE) and after TMR (NO-CUE), we subtracted explicit uncued-sequence-recall from cued-sequence-recall for each participant, creating the 'explicit-cueing-effect'. Correlations between the explicit-cueing-effect and slow oscillation power were marginally significant at central ($r=0.63$, $p=0.05$) electrodes (Figure 3.2d), and approached significance at frontal ($r=0.53$, $p=0.077$) but not parietal ($r=0.4$, $p=0.29$) electrodes during CUE periods. NO-CUE periods showed a much weaker relationship for frontal ($r=0.31$, $p=0.32$), central ($r=0.42$, $p=0.23$) and parietal sites($r=0.32$, $p=0.41$). There was no significant difference in mean slow oscillation power between CUE and NO-CUE periods in any electrodes ($p>0.3$). Thus, although cues did not appear to trigger a slow oscillation power increase, the marginal central correlation suggests that slow oscillation power during cue presentation may be linked to the impact of cues upon explicit recall. By contrast, neither fast nor slow lateralised spindles (non-learning minus learning hemisphere spindle density) predicted the explicit-cueing-effect in frontal, central or parietal electrodes ($p>0.18$).

To determine the effect of TMR on procedural skill, we examined overnight enhancement of sequence-specific-skill (random RT minus sequence RT) using a mixed ANOVA with factors group (experimental/control) and replay (cued/uncued). This showed no main effect of group, $F(1,30)=0.77$, $p=0.8$, or replay, $F(1,30)=2.03$, $p=0.2$, and no interaction, $F(1,30)=2.7$, $p=0.1$. Experimental-group sequence-specific-skill increased overnight ($M=18.5\pm31.1$) for the cued sequence and decreased for the uncued sequence ($M=-3.4\pm26.0$) (Figure 3.2e). Planned comparisons showed that this difference was significant, $t(15)=2.32$, $p=0.04$. Control-group sequence-specific-skill improvement did not differ between cued and uncued sequences, $t(15)=-0.15$, $p=0.89$. Thus, TMR during sleep influenced consolidation of sequence-specific-skill, but we can make no firm conclusion about the differential effects of TMR in sleep and wake.

Table 3.1: All SRTT and explicit recall data from experimental and control-groups.

		Experimental-group		Control-group	
		Cued	**Un-cued**	**Cued**	**Un-cued**
SRTT Test Blocks					
Mean RT ± S.E.M (ms)					
Learning	**Sequence**	335.6 ± 9.3	341.2 ± 9.3	375.4 ± 15.3	381.7 ± 16.1
	Random	423.6 ± 11.8	424.7 ± 10.0	450.3 ± 9.0	457.0 ± 9.5
	Difference	88.0 ± 9.8	83.6 ± 8.1	74.9 ± 12.0	75.3 ± 10.9
Retest	**Sequence**	301.1 ± 11.4	323.8 ± 10.9	348.8 ± 15.9	353.8 ± 17.2
	Random	407.6 ± 10.7	403.9 ± 11.5	427.5 ± 9.3	434.5 ± 10.0
	Difference	106.5 ± 11.5	80.1 ± 11.5	78.7 ± 14.8	80.7 ± 14.8
Improvement	**Sequence**	34.4 ± 6.5	17.4 ± 5.6	26.6 ± 4.7	27.9 ± 5.6
	Random	16.0 ± 6.4	20.8 ± 6.2	22.8 ± 12.0	22.5 ± 8.5
	Sequence				
	Skill	18.5 ± 7.8	-3.4 ± 6.5	3.8 ± 11.7	5.4 ± 9.5
Explicit Knowledge Test					
Mean recall ± S.E.M (items)					
Recall		4.9 ± 0.9	1.7 ± 0.6	2.6 ± 1.0	3.1 ± 0.8

To determine how the cued sequence RT advantage relates to EEG features during sleep, we first calculated a 'procedural-cueing-effect' by subtracting uncued from cued sequence RT improvement. Lateralised fast spindles at central electrodes predicted the procedural-cueing-effect during both CUE ($r=0.71$, $p=0.01$) (Figure 3.2f) and NO-CUE ($r=0.69$, $p=0.01$) (FDR corrected). This was not true at frontal electrodes during CUE ($r=-0.08$, $p=0.8$) and NO-CUE ($r=0.01$, $p=0.96$), or parietal electrodes during CUE ($r=0.55$, $p=0.1$) and NO-CUE ($r=0.49$, $p=0.2$). To determine whether cues increased fast spindles, we compared CUE and NO-CUE periods for mean spindle density (rather than laterality), finding no significant differences in frontal ($p=0.6$), central ($p=0.8$), or parietal ($p=0.6$) sites. Thus, cues did not trigger a net increase in fast spindles, but fast spindles over task-related areas did predict cued sequence consolidation. Analysis of slow spindles and slow oscillation power found no significant correlations with the procedural-cueing-effect ($p>0.2$).

To test for a relationship between the impact of cueing on implicit and explicit measures, we correlated two explicit measures (cued-sequence-recall and explicit-cueing-effect) against four procedural performance measures: cued-sequence-improvement (RT improvement without subtracting from random), cued-sequence-specific-improvement (RT improvement after subtracting from random), procedural-cueing-effect and sequence-specific-cueing-effect (RT improvement after subtracting from random, cued minus uncued). No significant correlations were found ($p>0.4$).

Error rates were typically low (3.8-10.8% trials). Comprehensive statistical tests revealed no effect of TMR upon error rates ($p>0.2$), therefore our RT findings represent a pure gain in cued sequence performance, rather than a shift in speed-accuracy trade-off.

Standard sleep scoring confirmed CUE/NO-CUE periods fell entirely within SWS for 12 of 16 participants. Considering all 30s epochs, 96% of CUE periods and 97% of NO-CUE periods were in SWS, while the others were in Stage 2 and were excluded from further EEG analyses. Sleep onset time, total sleep duration, and duration of all sleep stages did not differ between groups ($p>0.3$) (Table 3.2). Replay began 126 ± 55minutes post-SRTT in the experimental-group, compared to 20minutes post-SRTT in the control-group. The number of sequences replayed differed within experimental ($M=131\pm41.2$) and control ($M=129\pm36.9$) groups, but not between groups ($p=0.9$).

Combining CUE/NO-CUE periods gave 43.17±13minutes mean replay. To establish whether sounds disrupted sleep, we compared arousal events when sounds were playing (CUE) and not playing (NO-CUE). This showed no evidence for more events during CUE: arousals (p=0.3), movements (p=0.4), or awakenings (p=0.7). Mean occipital alpha power, which can be an indicator of arousal, did not differ between CUE/NO-CUE periods (p=0.7). No experimental-group participants reported hearing tones. Alertness at encoding and retrieval did not differ between groups, or between sessions within groups (p>0.28).

Table 3.2: Total time spent in sleep stages.

	Experimental-group (N=16) (min ± S.E.M)	**Control Group (N=16)** (min ± S.E.M)
Stage 1	26.5 ± 3.2	28.4 ± 5.4
Stage 2	226.6 ± 9.7	215.0 ± 15.9
Slow-wave sleep	111.25 ± 4.2	111.1 ± 11.4
Rapid eye movement sleep	81.3 ± 5.5	91.1 ± 9.0
Total Sleep Time	445.7 ± 12.1	445.6 ± 22.6

Discussion

This study presents the first evidence that TMR during sleep facilitates the emergence of explicit knowledge. Explicit recall of a sequence cued during sleep was significantly greater than recall of an uncued sequence. Response speed was also influenced by TMR during sleep, with significantly more overnight reaction time improvement for the cued than uncued sequence. This bias was predicted by fast spindles at motor regions in the learning hemisphere.

Explicit awareness of implicitly learned SRTT sequences can emerge spontaneously after nocturnal sleep (e.g., Fischer et al., 2006). The gradual transition between implicit and explicit knowledge facilitates adaptation to a changing environment, and our data suggest that TMR can bias this otherwise spontaneous process. This result suggests that memory reactivation may underpin the emergence of explicit memory during offline consolidation, and lends strong support to the active systems consolidation model (Diekelmann & Born, 2010), which proposes that memories are actively reorganised through reactivation during slow-wave sleep.

The hippocampus has a central role in memory consolidation during sleep (Diekelmann & Born, 2010), and we observed that TMR influences consolidation of the hippocampal-dependent SRTT (Schenden et al., 2003), while non-hippocampal tasks remain to be cued successfully during sleep. Explicit recall of an implicitly learned sequence after sleep has been linked to post-sleep enhancement in hippocampal activity (Wilhelm et al., 2013). Further work will determine whether the behavioural changes we observed after TMR are also associated with this type of long-term plasticity.

Slow-wave activity (SWA) is strongly linked to declarative memory consolidation, and the marginally significant positive relationship we observed between the explicit-cueing-effect and slow oscillation power during cueing tentatively supports this association. This builds on our previous finding that SWS modulates TMR impact in declarative memory (Cairney et al., 2014), and predicts abstraction (Durrant et al., 2011). Importantly, Marshall et al. (2006) demonstrated that slow oscillations causally impact consolidation, while Wilhelm et al. (2013) found that post-training SWA predicts the emergence of explicit knowledge for an implicitly learned SRTT. We do however advise caution when interpreting this relationship, since our correlation was at

a different electrode site to that reported by Wilhelm et al. (2013) and did not survive correction for multiple comparisons.

In procedural consolidation, TMR during sleep influenced sequence-specific skill but not stimulus-response mapping. These findings are consistent with prior observations of spontaneous consolidation of SRTT sequences during sleep, in which nocturnal sleep preferentially consolidated the sequence rather than the mapping component (Robertson et al., 2004; Spencer et al., 2006), and build on this work by suggesting that TMR specifically biases procedural consolidation in favour of the cued sequence. This idea links to the finding that TMR of half a SRTT sequence enhanced performance accuracy of only that portion of the sequence (Schönauer et al., 2014). In addition, our study is the first to show that TMR influences response speed in a procedural skill, rather than accuracy (Antony et al., 2012; Schönauer et al., 2014). Note, however, that the non-significant interaction means we cannot draw conclusions about the differential effects of cueing in sleep and wake.

The predictive relationship we observed between task-specific fast spindles and enhanced reaction times builds on previous findings regarding the role of spindles in procedural memory consolidation. Spindle density at task-related motor regions was shown to predict post-sleep improvement on finger-tapping (Nishida & Walker, 2007), and fast spindles may be preferentially involved in consolidation, as they increase after motor learning (Barakat et al., 2011). Our correlations with fast (13.5-15Hz), but not slow (12-13.5Hz) spindles further supports a functional distinction between the two. Together these findings support a role for regionally specific spindles in procedural consolidation, with TMR biasing consolidation in conjunction with spindles.

Interestingly, behavioural performance measures for explicit and procedural memory consolidation did not correlate, despite the similar pattern exhibited by group means for these measures. This could indicate that procedural and declarative memory systems supporting these two measures are influenced independently by TMR, resulting in differential cueing effects within each participant. However, explicit sequence knowledge is often tightly linked to RT's in the SRTT (Spencer et al., 2006), and despite the absence of a significant relationship between our implicit and explicit measures, it remains possible that improved procedural performance after TMR

facilitated explicit learning during SRTT retest. Further work should disentangle this relationship.

Antony et al. (2012) found no effect of TMR on explicit knowledge, and spindle correlations slightly anterior to those we observed at central electrodes. This may stem from methodological differences between the studies, including differences in nap sleep architecture relative to overnight sleep, a larger number of TMR cues in our study, and the fact that our sound cues were contingent upon visual stimuli rather than motor responses. The different spindle correlations may simply reflect issues of determining precise neural sources with a relatively small number of electrodes. Of note, the SRTT is well-established as a paradigm for exploration of interactions between implicit and explicit learning (Drosopoulos et al., 2011; Fischer et al., 2006; Wilhelm et al., 2013), while the task used by Antony et al. (2012) has not been used for this purpose. Importantly, both studies demonstrate that there is no clear association between the effects of TMR on implicit and explicit memory.

Our behavioural data tentatively support a finite consolidation resource during sleep. Since TMR was ineffective when applied prior to sleep, the control-group demonstrates the sequence consolidation occurring after normal un-stimulated sleep. Interestingly, both procedural and explicit performance measures for the control-group fell midway between the same measures for cued and uncued sequences in the experimental-group (Figure 3.2). This pattern was also observed by Antony and colleagues, who speculated that it implies TMR produces a consolidation bias rather than a pure gain. Similarly, if spindles and slow oscillations reflect the electrophysiological correlates of reactivation, then the inability of cues to increase them also suggests a finite consolidation resource.

Bendor and Wilson (2012) observed that sound cues did not increase the amount of reactivation of neuronal ensembles in the hippocampi of rodents, but could nevertheless bias the content of subsequent replay events up to 10s after cueing. Similarly, our comparison of CUE and NO-CUE periods found no evidence that cues increase slow oscillation power or fast spindles, which are considered to be neural correlates of sleep-dependent memory consolidation (Diekelmann & Born, 2010). We observed correlations between lateralised spindles and procedural memory consolidation both during replay (CUE) and also during 2-minute periods of silence after replay (NO-CUE). This could indicate trait like individual differences, whereby people with

naturally right lateralised spindles benefit more from TMR of our right lateralised task. Additionally, cues in our study may have triggered a short lived increase in slow-wave activity (Rihm et al., 2014), or a continued increase throughout NO-CUE periods and subsequent SWS (Cairney et al., 2014). Our cueing procedure was not designed to discern between these different accounts, therefore additional work is needed to explore these possibilities.

In relation to the cueing procedure itself, a remaining question regards whether TMR cues must exactly match the cues associated with learning for TMR to influence consolidation. In our study, TMR cues did not follow the exact temporal rhythm experienced during learning, since SRTT performance includes an inconsistent gap between stimuli that depends upon response speed, while tones were spaced evenly during replay. The success of this procedure shows that cues do not need precisely the same timing as the learning experience to reactivate memories.

A limitation to our study is that, to avoid interference with SRTT performance, explicit knowledge was not tested prior to sleep. Therefore it remains possible that TMR during sleep may not have promoted the emergence of explicit knowledge, but instead protected existing explicit knowledge against decay. Additionally, the difference in delay of TMR between groups could potentially account for why cueing was unsuccessful in the control-group, as initial consolidation processes may need to be completed before TMR is effective. Notably, however in Antony et al. (2012) TMR during wake did not influence consolidation even when this delay was matched across sleep and wake groups.

This study did not examine other sleep stages, therefore the specificity of reactivation to SWS remains unclear. Reactivation of brain regions involved in SRTT learning has been identified in rapid-eye movement (REM) (Maquet et al., 2000; Peigneux et al., 2003), while others propose lighter sleep stages may be important (Genzel et al., 2013). Further work is needed to determine the role of these stages in reactivation.

The gradual emergence of explicit awareness for statistical regularities forms a critical part of learning and directing appropriate behaviour, and reflects a form of reorganization of memory traces in the brain. This occurs preferentially during sleep and provides evidence for an active consolidation process. We show that experimentally manipulating reactivation of a procedural memory biases explicit knowledge,

suggesting a causal role for reactivation during sleep in this type of consolidation. The complex neuronal processes of reactivation remain to be discovered, but our data suggest distinct roles for slow oscillations and fast spindles for explicit knowledge and procedural memory respectively.

Chapter 4

Targeted memory reactivation of motor learning during slow-wave sleep modifies plasticity in motor networks*

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***This chapter is based on a manuscript that is being prepared for submission for peer review.**

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Abstract

There is strong evidence for a specific role of sleep in memory consolidation, as demonstrated by improved performance after sleep and enhanced neural plasticity underlying that performance. Targeted memory reactivation (TMR) allows the manipulation of sleep-dependent consolidation of specific memory traces, but the underlying neural basis of these altered memories remains unclear. We show with functional magnetic resonance imaging (fMRI) a change in the representation of a motor memory after TMR during slow-wave sleep (SWS). Participants learned two motor sequences (serial reaction time task) associated with different auditory tones (high or low pitch). During subsequent overnight slow-wave sleep, we performed TMR of one sequence by replaying the associated tones. Participants were retested on both sequences the following day during fMRI. As predicted, participants showed faster reaction-times for the cued sequence after TMR. When exploring brain activity associated with performance of the cued, relative to the uncued sequence, regions of increased activation were expressed in bilateral caudate nucleus, left hippocampus, and left thalamus, mediated by the amount of slow-wave sleep obtained. In addition, functional connectivity was found to increase between the caudate nucleus and hippocampus after TMR. This demonstrates that the offline performance gains associated with memory consolidation after TMR are supported by plasticity in key motor networks.

Introduction

The learning of motor skills forms a central part of all daily activities. Motor skill learning and consolidation transforms initially fragile memories that are susceptible to interference into enduring long-term representations that support fast, accurate and automatized performance. Repeated practice leads to ‘fast’ performance gains in early stages and ‘slow’ gains over the course of weeks and months, supported by an intermediate phase of offline consolidation where stabilisation and gains in performance are observed (Doyon & Benali, 2005).

Motor skill learning has been extensively studied in order to better understand the underlying neural substrates and behavioural determinants (Doyon et al., 2009), with a

strong focus on motor sequence learning (MSL) paradigms. These include finger tapping (e.g., Walker et al., 2002), the serial reaction-time task (SRTT) (Nissen & Bullemer, 1987) and occulomotor sequence learning (Albouy et al., 2008). Learning and consolidation of these abilities is measured by reduced reaction-times and improved accuracy. Early stages of MSL have consistently shown increases in cortical and subcortical motor regions, including cerebellar cortices, striatum, primary motor cortex (M1), pre-supplementary motor area (Pre-SMA), and supplementary motor area (SMA) (Floyer-Lea & Matthews, 2005; Penhune & Doyon, 2005). The hippocampus is also associated with occulomotor sequence learning (Albouy et al., 2006, 2008) and SRTT learning regardless of conscious awareness (Schenden et al., 2003), perhaps on account of the medial temporal lobes (MTL) role in representing sequences of events (Hsieh, Gruber, Jenkins, & Ranganath, 2014; Ross, Brown, & Stern, 2009) and contextual associations (Spencer et al., 2006). Activity in these regions changes dynamically over time, including plasticity after offline periods containing sleep (Albouy et al., 2013; Barakat et al., 2013; Fischer et al., 2005; Walker et al., 2005).

Sleep has been consistently linked to offline memory consolidation effects for both declarative (e.g., Plihal & Born, 1997) and procedural memories (Fischer et al., 2002; Korman et al., 2007; Walker et al., 2002). Sleep-dependent consolidation of procedural memories leads to stabilisation from interference (Korman et al., 2007; Robertson, 2009; Walker et al., 2003), delayed gains in performance (Robertson et al., 2004a; Walker et al., 2002), and the emergence of explicit knowledge for implicitly learned sequences (Drosopoulos et al., 2011; Fischer et al., 2006; Wilhelm et al., 2013). To uncover the neural substrates of procedural memory consolidation, fMRI and PET have been used to compare brain activity after sleep retention intervals with equivalent periods of sleep deprivation (Maquet et al., 2003) or daytime wakefulness (e.g., Walker et al., 2005). For example, unimanual performance of a short 5-item explicit sequence was supported by increased activation in contralateral M1, cerebellum, and hippocampus after a consolidation period containing sleep, plasticity the authors suggest would support enhanced performance (Walker et al., 2005). A key contributor to consolidation of MSL is the striatum (Debas et al., 2010; Walker et al., 2005), and interactions between striatum and hippocampus have also been highlighted, with findings of enhanced activation within and functional connectivity between the two regions after sleep (Albouy et al., 2008, 2013).

The active systems consolidation model posits that spontaneous reactivation of newly learned hippocampal-dependent memories during SWS underlies enhanced memory at retrieval and the associated systems-level plasticity (Diekelmann & Born, 2010). Ample evidence for this proposed 'replay' has been identified, with the re-instatement of learning-related neuronal firing patterns in rodents (Wilson & McNaughton, 1994), and spontaneous reactivation of memory-related brain structures during sleep in humans (Maquet et al., 2000, Peigneux et al., 2003, 2004; Yotsumoto et al., 2009). Of particular significance, the amount of reactivation occurring during sleep predicts subsequent memory at retest (Peigneux et al., 2004; Yotsumoto et al., 2009), which suggests neural reactivation plays a part in sleep-dependent memory consolidation. Additional neural events associated with sleep, as measured with electroencephalography (EEG), are linked to these mnemonic effects and neural plasticity, particularly slow oscillations (~1Hz) and short phasic events called thalamo-cortical sleep spindles (12-15Hz). Artificial enhancement of slow-oscillations improves post-sleep memory performance (Marshall et al., 2006; Ngo et al., 2013), while the density, duration and amplitude of spindles increases after motor learning (Fogel & Smith, 2011), and these measures predict procedural performance improvements after sleep (Barakat et al., 2011; Barakat et al., 2013; Nishida & Walker, 2007). In addition, spindles have been associated with increased post-sleep striatal activation (Barakat et al., 2013), and age related decreases in motor memory consolidation and cortico-striatal activity after sleep (Fogel et al., 2014). Together, these studies highlight the neural substrates of reactivation and their potential relationship with plasticity.

The technique of targeted memory reactivation (TMR) was developed to establish a causal role for reactivation in sleep-dependent memory consolidation. Learning related sounds or odours are presented during sleep, selectively reactivating memories and biasing consolidation of declarative (Rudoy et al., 2009; Schreiner et al., 2014) and procedural tasks (Antony et al., 2012; Schönauer et al., 2014). The neural substrates of TMR have been explored for declarative memories, revealing enhanced activity in hippocampus (Rasch et al., 2007) and parahippocampal gyri (van Dongen et al., 2012) during cue presentation. The neural plasticity resulting from TMR in the latter study showed enhanced functional connectivity at retest between parahippocampus and medial prefrontal cortex, although the association between this plasticity and

behavioural outcomes was not firmly established. The plasticity associated with TMR of procedural memories has yet to be investigated.

Together, the evidence discussed shows : (1) Motor sequence learning and sleep-dependent consolidation is supported by dynamic changes in activity within cortico-striatal, cortico-cerebellar and hippocampal networks (Albouy et al., 2008; Debas et al., 2010; Fischer et al., 2005; Walker et al., 2005). (2) Learning-related spontaneous reactivation has been observed in these regions during sleep in rodents (Peyrache et al., 2009; Wilson & McNaughton, 1994) and humans (e.g., Peigneux et al., 2003). (3) Presentation of learning-related cues during SWS also activates these regions (Rasch et al., 2007; van Dongen et al., 2012) and leads to enhanced consolidation of the cued material (Rasch et al., 2007). (4) Plasticity has been observed after TMR, but only for declarative memories and it was unclear how this relates to behaviour (van Dongen et al., 2012). (5) Sleep spindles are linked to the neural plasticity associated with motor learning (Barakat et al., 2013; Fogel et al., 2014).

A question remains as to the role for reactivation in this plasticity, specifically whether the motor skill improvements observed after TMR are supported by underlying plasticity in motor networks, and if so which brain regions are involved. Here we test this by cueing a SRTT during SWS and measuring subsequent differences in functional activity and connectivity during retest the following day using fMRI. Participants learned two 12-item sequences that were associated with high or low pitch tones. During nocturnal sleep, memory for one sequence was reactivated (cued) by replaying the associated tones, while the other sequence was not (uncued). Brain activity while performing cued and uncued sequences at retest was compared in order to ascertain the plasticity associated with TMR. The assumption underlying TMR studies is that cues are manipulating a naturally occurring process that either biases (Antony et al., 2012) or artificially boosts consolidation (Schreiner et al., 2014) in favour of a cued memory. Thus we predicted: (1) TMR would have a behavioural effect evidenced by faster RT's for the cued sequence. (2) TMR would result in a cueing-dependent increase in cerebral activation within structures that are important for sequence consolidation (cortico-striatal, cortico-cerebellar and hippocampal networks), alongside a cueing-dependent increase in functional connectivity between striatum and hippocampus. Additionally, SWS duration is associated with consolidation (e.g., Durrant et al., 2013) and TMR

effects (Cairney et al., 2014; Diekelmann et al., 2011), therefore we predicted plasticity in these regions would be modulated by SWS.

Materials & Methods

Participants

Twenty-five (16 males) healthy participants aged 18-35 years (mean age = 23.8 years, SD ± 4.2) volunteered. Three were excluded because of ceiling performance at learning, falling asleep during the fMRI scanning session, and disrupted SWS as a result of cueing. Data from the remaining twenty-two (14 males) participants were analysed, aged 18-35 years (mean age = 23.5 years, SD ± 4.3). Pre-study questionnaires determined that participants had no history of neurological, psychiatric diseases, sleep or motor disorders and kept a normal sleeping pattern in the week prior to the experiment. Participants were free of any form of medication, except for females using the contraceptive pill. They were asked to abstain from caffeine and alcohol 24 hours prior to testing and between test sessions, and to avoid napping on the experimental day. All participants were right-handed, confirmed by a score of 80% or more on the Edinburgh Handedness scale (Oldfield, 1971). Written consent was acquired in accordance with the University of Manchester and the University of Liverpool ethics committees. Prior to the scanning session, a qualified radiographer from the University of Liverpool screened participants to assess their suitability for MRI.

Experimental task and design

The learning session and replay protocol are described in detail in Chapter 3. Briefly, there was a learning session and a retest session that took place in the MRI scanner (Figure 4.1). Participants arrived at 7-8pm for the first session and were fitted for polysomnography (PSG). They then performed an adapted SRTT (Nissen & Bullemer, 1987) containing psuedorandomly interleaved blocks of two 12-item sequences, A (1-2-1-4-2-3-4-1-3-2-4-3) and B (2-4-3-2-3-1-4-2-3-1-4-1), with no runs of more than 2-blocks of the same sequence. Blocks containing 3 repetitions of the sequence were separated by a consistent 15 second gap where feedback was presented, rather than a mixture of 30 second gaps and 2 second fixations between sequences as in Chapter 3.

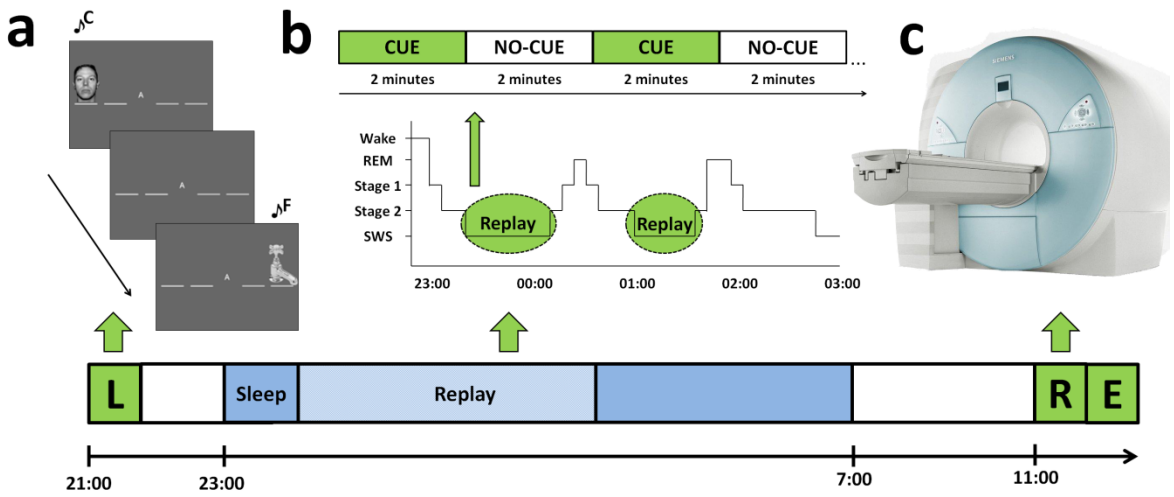

Figure 4.1: Schematic of experiment design. (a) Learning (L) of the SRTT task, consisting of interleaved blocks of the cued and uncued sequence, and also random blocks. (b) The cued sequence is replayed during periods of SWS, in groups of 12 sequences (CUE) and equivalent periods of silence (NO-CUE). (c) Retest (R) of the SRTT takes place the following morning in the MRI scanner, followed shortly afterwards by the explicit memory test outside of the scanner.

This was to ensure the learning session matched the MRI retest session, which incorporated 15 second rest periods to allow the blood-oxygen level dependent (BOLD) response to return to baseline. Sequences were accompanied by either high or low pitched pure tones. Participants performed 20 blocks of each sequence, followed by 4 random blocks containing no repeating sequence, ('R' displayed centrally), containing high (2-blocks) and low pitched tones (2-blocks)

Trials contained an auditory tone and visual cue in one of four spatial locations, corresponding to a four-button box with all fingers of the left hand. 'A' or 'B' appeared centrally on the screen to indicate the sequence. Participants were asked to respond as quickly and accurately as possible, and were not asked to explicitly learn the sequences. Visual cues were objects or faces appearing in the same position for both sequences.

Participants were told the nature of cues (objects/faces) was irrelevant. Stimuli remained on screen until a correct response was made, followed by a 300ms inter-trial interval.

Participants were invited to sleep overnight in the Neuroscience and Psychology of Sleep (NaPS) Laboratory at the University of Manchester, where they were monitored with PSG. Lights out was at 11pm. During periods of SWS, one sequence's tones were replayed (48dB) in the same order as learning and imbedded in brown noise at a speed similar to mean pre-sleep performance, in blocks of 2 minutes replay (CUE), followed by 2 minutes silence (NO-CUE). Sequence A and B were counterbalanced across cued and uncued conditions, and tones (high/low pitch) were counterbalanced across sequences. Cues were stopped for signs in the EEG of arousal or leaving SWS.

Participants were awoken between 7-8am. The retest session took place between 11am-12pm during fMRI, consisting of 24 sequence blocks (12 cued and 12 uncued), followed by 24 random blocks (12 containing cued tones and 12 uncued tones). 'REST' was displayed centrally during 15s breaks. Lastly, free recall was measured outside the scanner with participants marking sequence order on paper. The Stanford Sleepiness Scale assessed alertness prior to learning and retest sessions (Hoddes et al., 1973).

Equipment

All experimental scripts were executed using MATLAB 6.5 (The MathWorks Inc., Natick, MA, 2000) and Cogent 2000 (Functional Imaging Laboratory, Institute for Cognitive Neuroscience, University College, London). Sounds were presented via a pair of Sony noise cancelling headphones during the learning session, via PC speakers during sleep replay, and via an MR compatible headphone system (MR Confon) during retest (fMRI). A serial 4-button box attached to a Domino multicontroller from Micromint recorded participant responses, with a time resolution of approx. 1ms.

fMRI data acquisition

Functional MRI data were acquired using an 8 channel head coil with a Siemens 3T Allegra MR scanner. The BOLD signal was recorded with T2*-weighted fMRI images obtained via a gradient echo-planar imaging (EPI) sequence. We acquired 50 oblique transaxial slices at 25degree tilt, in an ascending sequence, voxel size 3 x 3 x 2.8mm, interslice gap of 40%, a matrix size of 64 x 64, flip angle of 80degrees, repetition time (TR) of 2960ms, and echo time (TE) of 30ms. A structural T1 –weighted image was

also acquired, using a 3D IR/GR sequence with a matrix size of 224 x 256 x 176, cubic voxels with isotropic resolution of $1mm^3$, TR of 2040ms, TE of 5.57ms, inversion time of 1100ms, and flip angle of 8degrees.

Behavioural analysis

The last 4 blocks of SEQ_C/SEQ_U, and 2 blocks of RAND_C/RAND_U comprised pre-sleep performance. Sequence RT was subtracted from random RT to provide a measure of sequence learning that was separate from sensori-motor mapping, although sequence and random were also treated separately in some analyses.

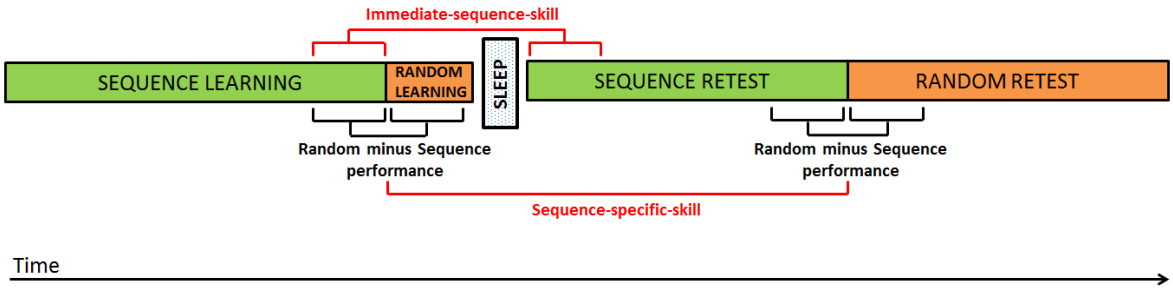

Figure 4.2: Schematic of dependent measures. Participants perform interleaved blocks of the cued and uncued sequences within 'Sequence Learning' and 'Sequence Retest', and random cued and uncued trials within 'Random Learning' and 'Random Retest'. Random performance provides a measure of participants learning of the mapping between buttons and stimuli, with no sequence component. Sequence performance provides a measure of both the mapping and sequence learning. Thus, subtracting performance on sequence blocks from performance on random blocks provides a measure of sequence skill alone. This was performed for the final sequence/random blocks of the pre-sleep learning session, and equivalent sequence/random blocks performed side-by-side at retest, to provide a measure of improvement or 'sequence-specific-skill'. Analyses were also performed on these sequence and random blocks considered separately (Figure 4.3b). To ascertain any immediate performance improvement, sequence blocks at the end of pre-sleep learning and the beginning of post-sleep retest were also compared for a measure of 'Immediate-sequence-skill'.

Two dependent measures were calculated by comparing pre-sleep to post-sleep performance (Figure 4.2): (1) Sequence-specific-skill' (Random RT minus sequence RT) used a mean of the last four blocks of SEQ_C/SEQ_U, and the first four blocks of RAND_C/RAND_U performed immediately after, subtracted from the same pre-sleep comparison to determine improvement. This is consistent with the main analysis used in Chapter 3. (2) 'Immediate-sequence-improvement' referred to the first 4 blocks of SEQ_C/SEQ_U at retest subtracted from pre-sleep sequence performance. This measure identifies whether any cueing effects are present immediately upon re-test in the current experiment. Together these measures account for the way TMR-induced consolidation assists performance of sequences across the retest period. Reaction times >1000ms were excluded, while trials with multiple button presses prior to the correct press were included. For explicit recall, individual items within a segment containing >2 consecutive correct items and in the correct sequence position were marked as correct.

Mixed ANOVA and paired sample t-tests were used for planned comparisons of cued and uncued sequence RT and recall. Associations between behavioural measures and EEG features were tested with Pearson's correlations. Where Shapiro-Wilk tests indicated a non-normal distribution, Wilcoxen signed-rank tests or Spearman's Rho correlations were used. All statistical tests were 2-tailed, significance level $p<0.05$. All means presented in the text ± standard deviation.

EEG recording and analysis

Electrodes were attached at standard locations, F3, F4, C3, C4, C5, C6, CP3, CP4, CP5, CP6, P7, P8, O1, and O2, referenced to the combined mean of left and right mastoid, according to the 10-20 system. Also attached were left and right electrooculagram, left and upper electromyogram and forehead ground electrode. Impedance below 5Ω was verified, and the digital sampling rate was 200Hz. Data were scored according to The AASM Manual (American Academy of Sleep Medicine, Westchester, IL) by two experimenters, the second of which was blind to CUE/NO-CUE periods.

Electrodes were grouped for analysis into 2 'Frontal' (F3 and F4), 2 'Parietal'(P7 and P8) and 8 'Central' (C3, C4, C5, C6, CP3, CP4, CP5 and CP6). Loss of one electrode due to noise resulted in exclusion of that group from further analyses. Epochs of CUE/NO-CUE periods (2mins) were extracted for every channel, and adjacent CUE/NO-CUE periods were rejected if either contained visually identified artefacts

such as movement. Epochs were then concatenated for each participant, creating a separate time series for CUE/NO-CUE periods. Band pass filtering was carried out for slow (12-13.5Hz) and fast (13.5-15Hz) spindles separately with a linear finite impulse response filter in EEGLab v.9.0, via MATLAB 2010. Spindles were automatically detected at each electrode with an algorithm (Ferrarelli et al., 2007). Spindle density was total spindles divided by the length of the CUE/NO-CUE period time series. Previous research has identified localised spindle increases in the hemisphere that predominantly encoded the task, such as right motor regions for a left handed motor task (Nishida & Walker, 2007), and also localised increases during TMR (Cox et al., 2014). To explore these regional spindle effects, spindle density in left (non-learning) hemisphere electrodes was subtracted from right (learning) hemisphere electrodes, providing a 'Lateralised Spindles' measure for the three electrode groups in CUE/NO-CUE periods. Welch's method was utilised for power spectral density analyses, with power averaged over each time series for CUE/NO-CUE. Frequency bands of interest were slow oscillation (0.3-1Hz), slow spindle (12-13.5Hz) and fast spindle (13.5-15Hz). Mean slow oscillation power within the three separate electrode groups during CUE/NO-CUE periods was correlated with behavioural measures. Spindle power laterality was calculated for fast and slow spindles by subtracting learning from non-learning hemisphere power, and this was then correlated with behavioural measures.

fMRI analysis

Functional imaging data were analysed using Statistical Parametric Mapping 8 software (SPM8; Wellcome Department of Cognitive Neurology, London, UK). The first 2 volumes of each functional EPI run were removed to allow for T1 equilibration. Two participants were excluded from analysis for excessive movement >3.5mm. Functional images were re-aligned to correct for motion artefacts using iterative rigid body realignment, minimizing the residual sum of squares between all scans and the first scan. Functional images were then spatially normalised to the Montreal Neurological Institute brain (MNI space), resampled to voxel size 2x2x2mm. Lastly, a spherical Gaussian smoothing kernel (full-width half maximum = 8mm) was applied to each participant's normalised data.

Statistical analysis of MRI data at the single subject level used the general linear model (GLM) (Friston et al., 1994). Blocks of cued and uncued sequences were modelled as

boxcar functions, and button presses for individual trials were also modelled as single events with 0 duration. These were temporally convolved with the hemodynamic response function (HRF). The design matrix also included 6 non-convolved head motion regressors, and lastly baseline activation was modelled with a constant regressor. A first-order autoregressive model with added white noise was used to model serial correlations, estimated with a restricted maximum likelihood algorithm. A high pass filter was utilised by using a cut off period of 128 seconds, removing low frequency noise.

Contrast parameter images were generated for each participant with balanced linear t-contrasts, including one-sample t-tests for the cued>uncued contrast. These contrast images were subsequently entered into a series of second level random effects analyses. To determine the interaction between activity differences for cued/uncued sequences and sleep parameters, we performed 3 separate regression analyses utilising 3 parametric regressors (time spent in stage 2, SWS and REM sleep): The cued>uncued contrast images were entered to a second-level design matrix containing a constant regressor (cued>uncued) and one of the 3 parametric regressors separately.

All analyses were whole brain corrected at $p<0.05$ for family-wise error resulting from multiple comparisons via a Monte Carlo simulation (Slotnick, Moo, Segal, & Hart, 2003). This modelled the entire imaging volume assuming a type I error of $p<0.05$ at a voxel-wise uncorrected threshold of $p<0.005$. The algorithm performed across 1,000 iterations recommended a cluster extent threshold of 51 contiguous voxels to provide a whole-brain corrected probability of $p<0.05$. Clusters entirely in white matter were not reported.

Functional connectivity

We examined the functional connectivity between regions using psychophysiological interactions (PPI). Five separate PPI's were conducted. Each spherical seed region (radius 6 mm) was based on peak coordinates of the group response to the cued>uncued contrast with SWS-time as a second-level covariate. For each participant, the time course of activity for a sphere with a radius of 6mm around the peak coordinate of the seed region was extracted and deconvolved, forming the physiological factor. We were interested in how connectivity with each seed varied after TMR during sleep, therefore our psychological factor was the contrast (cued>uncued). Thus, for each participant our

PPI design matrix included 3 regressors: the physiological factor, the psychological factor, and the interaction (physiological vs. psychological), in addition to the button press regressor convolved with the HRF, and the 6 non-convolved motion regressors. Contrast images for the PPI regressor were then generated using a one-sample t-test. These images formed a second-level random effects analysis. The results represented regions whose functional connectivity was sensitive to whether the sequence had been cued during sleep or not. PPI data was thresholded in the same manner as localised data, i.e., 51 contiguous voxels of $p<0.005$ were considered significant at $p<0.05$ based on our Monte-Carlo simulation. The coordinates used for the PPI analyses are listed below: Left caudate nucleus -20 16 14; right caudate nucleus 20 26 -4 and 18 4 20; left hippocampus -34 -12 -20; left thalamus -2 -12 12.

Results

Behavioural analysis

Reaction times: Firstly we confirmed that sequence learning occurred prior to sleep, by showing that reaction times were significantly faster for sequence trials compared to random trials for both cued, $t(21)=9.2$, $p<0.001$, and uncued, $t(21)=9.22$, $p<0.001$, sequences. Crucially, we also demonstrated that prior to sleep there was no significant difference between RT's for cued and uncued sequences, $t(21)=1.05$, $p=0.31$, or random, $t(21)=0.22$, $p=0.83$, therefore post-sleep differences are attributable to TMR of the cued sequence.

Our sequence-specific-skill measure subtracts RT for sequence blocks at the end of retest, from RT's for random blocks that follow immediately after (Figure 4.2), and compares this to pre-sleep performance. This is consistent with the main analysis performed in Chapter 3 which showed a significant cueing effect. Unexpectedly, sequence-specific-skill for the cued and uncued sequences did not significantly differ, $t(21)=-0.45$, $p=0.66$.

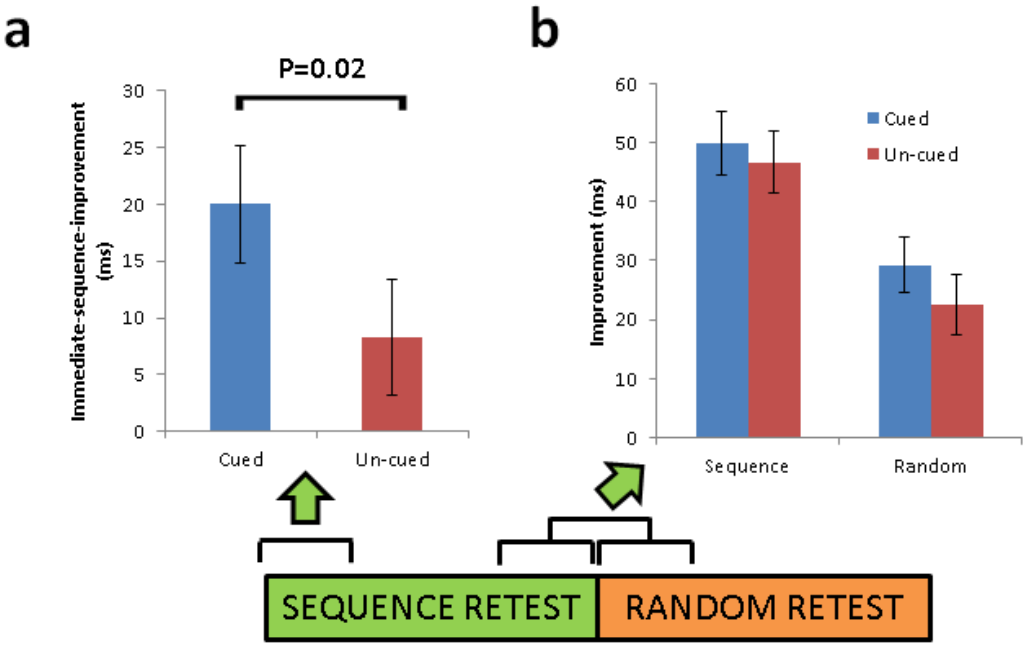

Figure 4.3: Mean reaction time improvement for the SRTT after TMR. (a) Immediate-sequence-improvement comparing the pre-sleep sequence performance to initial blocks of sequence retest, showing a significant cueing effect. (b) Sequence-specific-skill failed to show a cueing effect, therefore sequence and random performance were separated to identify specifically which trials differed, revealing similar improvement for cued and uncued for both measures. Error bars represent standard error mean (S.E.M.).

This measure is influenced by cued/uncued differences in performance improvement for both sequence and random trials, therefore to explore this further we performed analyses that considered sequence-improvement and random-improvement separately. This utilised a repeated measures ANOVA (of late sequence and early random blocks) with factors of sequence (sequence/random) and replay (cued/uncued). This showed a main effect of sequence, $F(1,21)=14.73$, $p=0.001$, but no main effect of replay, $F(1,21)=1.7$, $p=0.21$, and surprisingly no interaction, $F(1,21)=0.21$, $p=0.65$. (Figure 4.3b). Planned comparisons showed the small advantage for the cued sequence was not significant, $t(21)=0.58$, $p=0.57$, while the same was true of the random, $t(21)=1.25$, $p=0.22$.

To account for TMR effects upon sequence performance across the entire retest period, we explored immediate improvement in RT's by subtracting initial blocks of sequence retest from pre-sleep performance, providing a measure of immediate-sequence-improvement. Here we found improvement for the cued sequence was significantly greater than the uncued, $t(21)=2.46$, $p=0.02$ (Figure 4.3a). Chapter 3 also identified a trend for better performance of the cued sequence in the immediate sequence blocks of retest ($p=0.1$) (un-reported in that manuscript). In light of our previous findings and other studies (Robertson et al., 2004; Cohen et al., 2005; Spencer et al., 2006, Schönauer et al., 2014, Song & Cohen, 2014) we can be relatively confident this early improvement reflects an improvement in sequence learning rather than visuo-motor mapping.

The test of explicit knowledge that took place outside of the scanner did not show a group cueing effect. Shapiro-Wilk tests indicated a non-normal distribution, therefore a related-samples Wilcoxen signed-rank test was used, which showed no significant difference between cued and uncued sequence recall ($p=0.68$).

Error rates: Performance of the SRTT can be seen as a trade-off between accuracy and speed, therefore we also explored changes in error rates after our cueing manipulation to ascertain whether the RT improvement we observed reflects a pure gain in speed, or a shift in the trade-off between speed and accuracy. These analyses were identical to the above RT analyses except that the dependent variable was the percentage of errors made per block, and one participant was excluded due to corrupted error data, leaving N=21.

Error rates were very low across the experiment (4.7-7.8% trials). There was a trend for more errors to be made for the cued sequence relative to the uncued sequence prior to sleep, $t(20)=1.86$, $p=0.08$, although this was a difference of only 2% between cued ($M=7.8 \pm 4.7$) and uncued errors ($M=5.8 \pm 3.3$), which represents 0.6 of a trial within a block (32 trials), or only 2.56 trials within the 4 blocks (128 trials) used to calculate that measure. Random trials showed no difference between cued and uncued prior to sleep, $t(20)=0.9$, $p=0.38$.

To determine the effect of cueing on post-sleep error rates, we then examined overnight error changes for sequence-specific-skill (random error rate minus sequence error rate, comparing the final retest blocks and subsequent random blocks), which showed no significant difference in improvement between cued ($M=1.8 \pm 6.1$) and uncued ($M=0.2$

± 1.4), t(20)=1.09, p=0.29. We also performed analyses for sequence-improvement and random-improvement separately within these same blocks. This utilised a repeated measures ANOVA (of late sequence and early random blocks) with factors of sequence (sequence/random) and replay (cued/uncued). This showed no main effect of sequence, F(1,20)=0.69, p=0.42, or replay, F(1,20)=1.19, p=0.29, and no interaction, F(1,20)=1.2, p=0.29.

Lastly we examined immediate-sequence-improvement, and this showed significantly greater error rate improvement for the cued relative to the uncued sequence, t(20)=2.46, p=0.02. Importantly, this shows that the significant RT enhancement for the cued sequence across these blocks represents a gain in speed, rather than being the result of a shift in the speed-accuracy trade-off, because errors were actually less for the cued (M=3.8 ± 2.5) than the uncued sequence (M=4.7 ± 3.3) at these early blocks. This result also appears to show that TMR improved accuracy for the cued sequence, as well as RT. However we advise caution on this interpretation, since the observed differences are very small (i.e., less than one trial per block), and pre-sleep errors were marginally higher for the cued sequence, therefore the cued sequence had a greater potential for post-sleep improvement. Lastly, alertness measures (Stanford Sleepiness Scale) did not differ between encoding and retrieval, t(21)=1.03, p=0.32.

Replay & sleep parameters: Considering all EEG data as 30s epochs, 97% of CUE periods and 95% of NO-CUE periods were in SWS. All others were stage 2 and excluded from further EEG analyses. Sleep onset time, total sleep duration, and duration of all sleep stages are displayed in Table 4.1. The number of sequences replayed varied across participants (Mean=156 ± 49.1). Combining CUE/NO-CUE periods gave 5 2± 16.4 minutes mean replay time. Mean alpha power at occipital electrodes can indicate arousal, and we found this did not differ between CUE/NO-CUE periods, t(20)=0.97, p=0.35, suggesting sounds did not disrupt sleep. Lastly, no participants reported hearing tones during the night.

Table 4.1: Total time spent in sleep stages.

	Duration (min ± S.E.M)
Stage 1	32.7 ± 5.6
Stage 2	217.9 ± 9.8
Slow-wave sleep	102.6 ± 7.2
Rapid eye movement sleep	84.2 ± 5.8
Total Sleep Time	437.9 ± 13.7

EEG analysis

Procedural-cueing effect: To establish the link between sleep EEG features and the advantage for the cued sequence at early blocks of retest, we calculated a procedural-cueing-effect for each participant by subtracting immediate-sequence-improvement for the cued from the uncued sequence. We then correlated this measure with slow and fast spindle power laterality during CUE and NO-CUE periods. Based on prior literature (e.g., Nishida & Walker, 2007) and results from Chapter 3, we expected correlations to be mostly over central motor regions, and more apparent for fast than slow spindles. Broadly speaking this is what we found. The procedural-cueing-effect was significantly predicted by fast spindle power laterality at central electrodes during CUE ($r=0.50$, $p=0.03$) (Figure 4.4a) and NO-CUE periods ($r=0.55$, $p=0.02$) (Figure 4.4b). The same correlation with frontal electrodes was marginally significant during NO-CUE ($r=0.44$, $p=0.05$), but not CUE ($r=0.23$, $p=0.32$). Parietal correlations were not significant for CUE ($r=0.15$, $p=0.53$) or NO-CUE ($r=0.27$, $p=0.26$). Therefore those participants with a stronger bias for fast spindle power at learning hemisphere motor regions tended to show a greater performance improvement for the cued sequence.

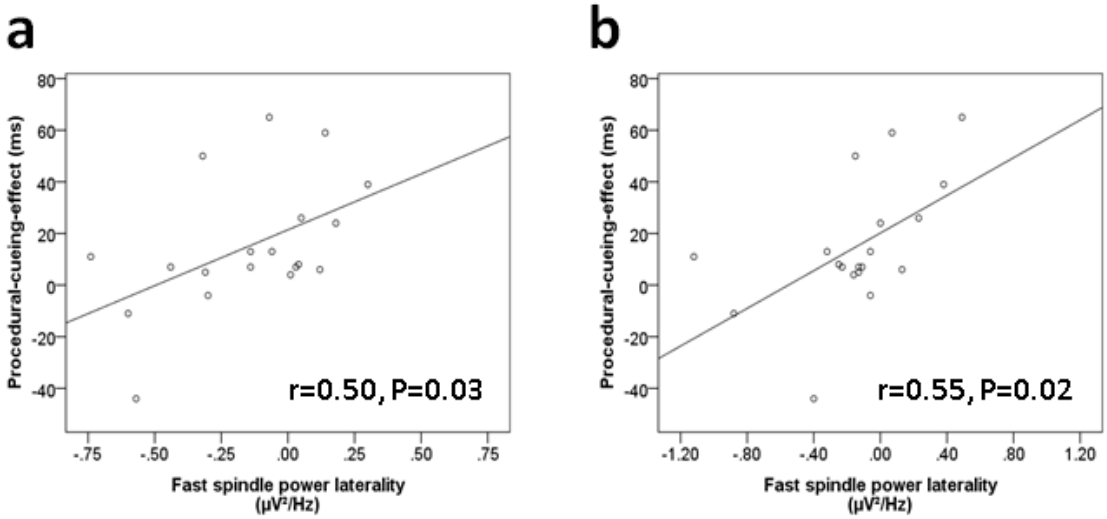

Figure 4.4: Correlations between the procedural-cueing-effect and fast spindles. (a) Correlation between the procedural-cueing effect and fast spindle power laterality at central electrodes during the CUE period (N=19) and (b) during the NO-CUE period (N=19). Correlations are presented with some participants removed (N=3) due to EEG artefacts.

Analysis of slow spindles also showed a significant correlation with the procedural-cueing-effect during NO-CUE at central electrodes (r=0.51, p=0.03), but the CUE period relationship was not significant (r=0.22, p=0.36). In contrast frontal electrodes showed a negative correlation with this measure during CUE that was close to significance (r=-0.42, p=0.07), while the NO-CUE period correlation was virtually absent (r=0.004, p=0.99). Lastly parietal electrodes were not significantly correlated with this measure for CUE (r=0.04, p=0.85) or NO-CUE periods (r=0.001, p=1). Thus, slow spindles also related to procedural cueing effects, although the relationship was less consistent.

It should be noted that Chapter 3 identified correlations between spindle density and the procedural-cueing effect, utilising a spindle counting algorithm rather than the overall power measure used above. This algorithm failed to reveal any significant relationships between spindle laterality (right hemisphere density minus left hemisphere density) and the procedural-cueing-effect at any electrode site in the current study (p>0.05).

Lastly, consistent with Chapter 3 we found no significant correlations between slow oscillation power and the procedural-cueing effect at any electrode site ($p>0.18$). Similarly the duration of sleep stages did not correlate with this behavioural measure ($p>0.1$).

Explicit-cueing-effect: This study found no cueing effect for explicit sequence knowledge at the group level, but there were individual differences in explicit knowledge for both sequences, therefore we correlated the 'explicit-cueing-effect' for each participant with EEG features. This analysis found slow oscillation power at parietal electrodes during NO-CUE predicted the explicit-cueing-effect ($r=0.6$, $p=0.006$) (Figure 4.5a), and there was a similar trend during the CUE period ($r=0.42$, $p=0.08$). This relationship was not apparent at frontal electrode sites for CUE ($r=-0.05$, $p=0.85$) or NO-CUE ($r=0.16$, $p=0.48$), and central sites for CUE ($r=0.17$, $p=0.5$) and NO-CUE ($r=0.19$, $p=0.45$). Thus, despite the lack of a group effect, individual differences in the explicit-cueing-effect were once again associated with slow oscillation power, which supports the marginal correlation between these measures found in Chapter 3, this time in a larger sample.

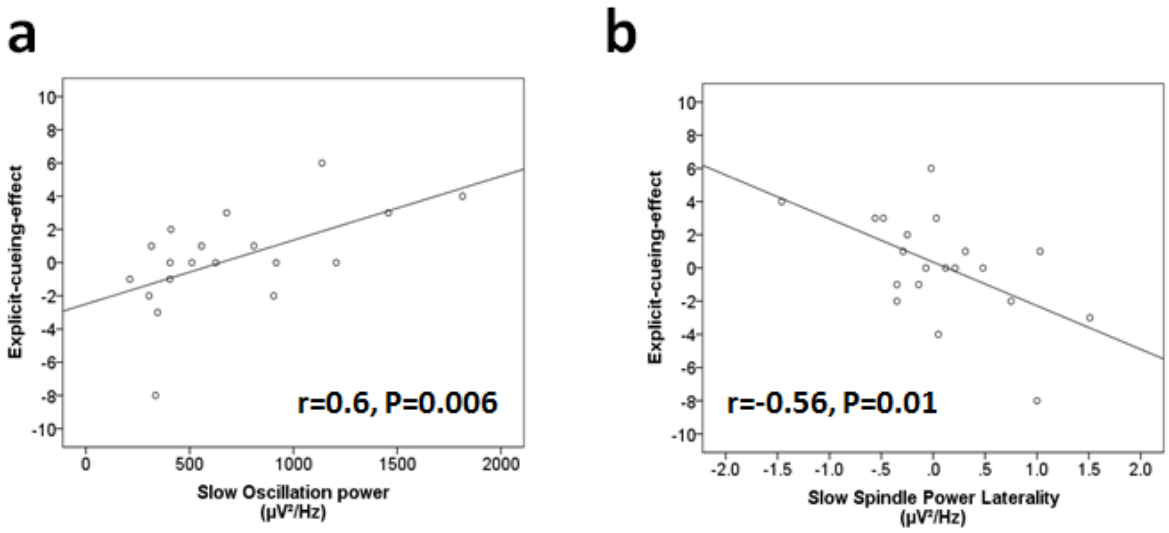

Figure 4.5: Correlations between the explicit-cueing-effect and EEG features. (a) The explicit-cueing-effect was predicted by slow oscillation power at parietal electrodes during the NO-CUE period (N=19). (b) Slow spindle power laterality was negatively correlated with this measure at frontal regions (N=20). Some participants were removed due to EEG artefacts.

For completeness we also correlated the explicit-cueing-effect with slow and fast spindles power laterality. Interestingly, slow spindle power laterality was negatively correlated with this measure at frontal sites during NO-CUE ($r=-0.56$, $p=0.01$) (Figure 4.5b), but not during CUE ($r=-0.21$, $p=0.37$). Central sites were not significantly correlated for CUE ($r=-0.2$, $p=0.42$), or NO-CUE ($r=-0.29$, $p=0.22$), and the same was true of parietal sites for CUE ($r=0.02$, $p=0.92$), and NO-CUE ($r=0.15$, $p=0.51$). Fast spindle power laterality did not significantly correlate with this measure at any electrode ($p>0.1$), and neither did duration in any sleep stage ($p>0.2$).

Functional imaging analysis

To examine the neural responses associated with a procedural memory that had undergone TMR during sleep, we contrasted BOLD activity during performance of the cued sequence with activity during performance of the uncued sequence at retest ($p<0.05$, whole brain corrected). SWS modulates behavioural consolidation effects associated with sleep (e.g., Durrant et al., 2013) and TMR (Cairney et al., 2014; Diekelmann et al., 2011), therefore we added 'time in slow-wave sleep' (SWS-time) as a covariate to the cued>uncued contrast, to explore the interaction between slow-wave sleep and TMR. This identified significant clusters of increased activity for the cued sequence in the predicted ROI's of hippocampal and cortico-striatal networks (Figure 4.6), specifically in the left hippocampus, left medial dorsal nucleus of the thalamus, left caudate nucleus, and two separate clusters in the right caudate nucleus (see Table 2 for complete list of responses). The reverse contrast identified no significant decreases in activity across the whole brain. Thus, a longer duration of SWS was associated with increased activity in task related brain regions for the cued sequence, which suggests TMR triggers plastic changes that rely on on-going SWS to be fully realised.

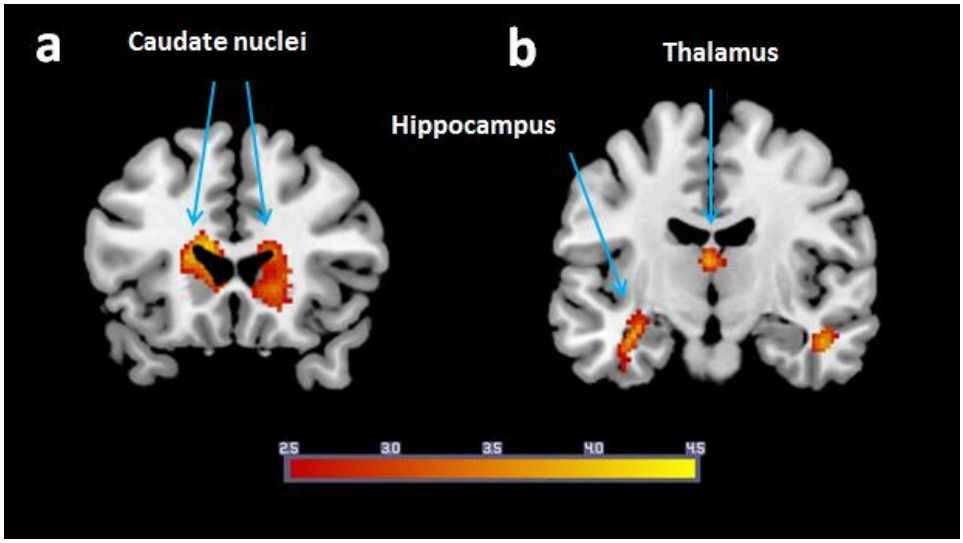

Figure 4.6: Slow-wave sleep modulated increases in activity after targeted-memory reactivation. (a) Increased activity after cueing in bilateral caudate (18, 4, 20, and -22, 12, 16), (b) left thalamus (-2, -12, 12) and left hippocampus (-34, -12, -20). $p<0.05$ whole brain corrected and displayed as coronal projections superimposed on a standard MNI brain. Colour bar indicates t-values.

To further explore the relationship between plastic changes after TMR and how they relate to features of sleep, we conducted further regression analyses with time spent in REM (REM-time) and stage 2 (Stage2-time) as second-level covariates, both of which have been previously linked to motor sequence memory reactivation and consolidation (Maquet et al., 2000; Peigneux et al., 2003; Walker et al., 2002). Firstly, the REM-time analysis identified significant clusters in a number of regions, including bilateral cerebellum, left PMC and left SMA (Figure 4.7) (Table 2 for all regions). Once again, there were no decreases in activity at any region associated with REM-time. Interestingly, these regions are linked to consolidation of MSL tasks (e.g., Walker et al., 2005), yet they do not overlap with regions identified by the SWS-time analysis, perhaps reflecting consolidation of different aspects of the task during these sleep stages. By contrast, the Stage2-time analysis did not reveal any significant increases or decreases in activity.

Table 4.2: Coordinates of local maxima for brain regions showing greater activity for cued relative to the uncued sequence (N=20), modulated by SWS and REM-sleep.

Region	MNI x, y, z (mm)	No. of voxels	Peak T	Peak Z	Peak P(unc)
Cued>Uncued *SWS duration (mins)					
Right caudate	18, 4, 20	103	5.75	4.28	<0.001
Left caudate	-22, 12, 16	474	5.21	4.02	<0.001
Left hippocampus	-34, -12, -20	54	4.0	3.34	<0.001
Right middle temporal gyrus	48, -10, -22	52	3.83	3.23	<0.001
Left thalamus	-2, -12, 12	63	3.83	3.23	<0.001
Right caudate	20, 26, -4	240	3.67	3.13	<0.001
Cued>Uncued *REM sleep duration (mins)					
Left parietal lobe	-22, -50, 36	208	7.01	4.81	<0.001
Left occipital/fusiform gyrus	-40, -72, -10	771	5.57	4.19	<0.001
Left cerebellum	-16, -64, -32	1211	5.45	4.14	<0.001
Left orbitofrontal cortex	-34, 48, -14	119	5.23	4.03	<0.001
Right superior parietal lobe	24, -54, 34	1011	5.21	4.01	<0.001
Right fusiform gyrus	46, -50, -22	237	4.63	3.71	<0.001
Left insula cortex	-28, 24, -6	61	4.43	3.59	<0.001
Right middle temporal gyrus	60, -52, 4	244	4.23	3.48	<0.001
Right cerebellum	30, -66, -44	739	4.22	3.47	<0.001
Right precentral gyrus	60, 6, 10	78	4.03	3.36	<0.001
Right lingual gyrus	4, -78, -6	75	3.87	3.26	<0.001
Right insula	36, 12, 10	142	3.84	3.24	<0.001
Right supramarginal gyrus	48, -36, 34	104	3.82	3.23	<0.001
Left premotor cortex	-48, 10, 46	59	3.74	3.18	<0.001
Left insula	-40, 12, 6	66	3.72	3.16	<0.001
Left supplementary motor area	-20, 8, 62	73	3.69	3.14	<0.001
Right middle frontal gyrus	42, 42, 22	72	3.64	3.11	<0.001
Right middle occipital	42, -78, 26	104	3.64	3.11	<0.001
Left middle frontal gyrus	-40, 38, 32	59	3.6	3.08	0.001
Right occipital lobe	40, -82, -8	60	3.4	2.95	0.002
Left lingual gyrus	-10, -84, -10	88	3.23	2.83	0.002

The main effect of targeted memory reactivation across the whole brain, modulated by slow-wave sleep duration and rapid-eye movement sleep duration, voxel threshold of p=0.05 (whole brain corrected) and extent threshold of k>50 voxels. All active voxels are positive for the cued>uncued comparison.

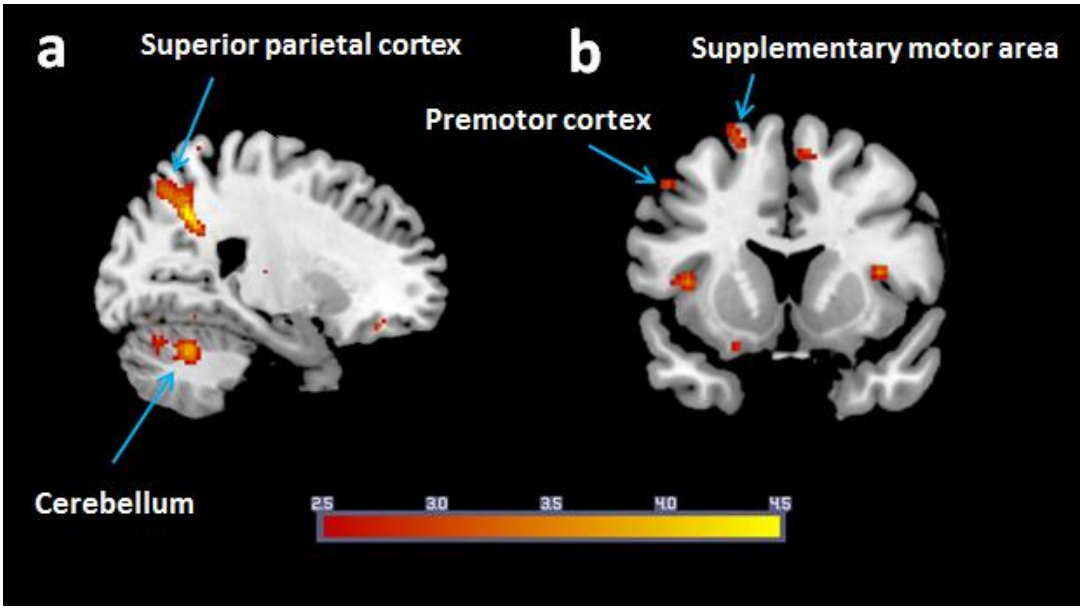

Figure 4.7: Rapid-eye movement sleep modulated increases in activity after targeted-memory reactivation. (a) Enhanced activity in right cerebellum (30, -66, -44) and superior parietal cortex (24, -54, 34), (b) left PMC (-48, 10, 46) and left SMA (-20, 8, 62). $p<0.05$ whole brain corrected. Contrasts displayed as sagittal and coronal projections superimposed on a standard MNI brain. Colour bar indicates t-values.

Lastly, the simple cued>uncued contrast revealed no significant increases across consolidation, but a decrease in activity for the cued sequence in a cluster spanning left caudate and anterior cingulate gyrus, and a second left occipital/cuneus cluster. However, this contrast is less sensitive to effects associated with sleep stages across the night.

Functional connectivity analysis

Further to identifying localised activation differences after TMR during slow-wave sleep, we sought to examine the functional connectivity of task-related regions that showed sensitivity to TMR. This was achieved with five separate PPI analyses seeded in left hippocampus, left thalamus, left caudate nucleus and two separate seeds in the right caudate nucleus, all based on peak coordinates identified in our cued>uncued SWS-time covariate analysis (Figure 4.8). Each analysis explored how connectivity

differed between the seed region and the whole brain for cued and uncued sequences ($p<0.05$, whole brain corrected) (Table 3 for all regions).

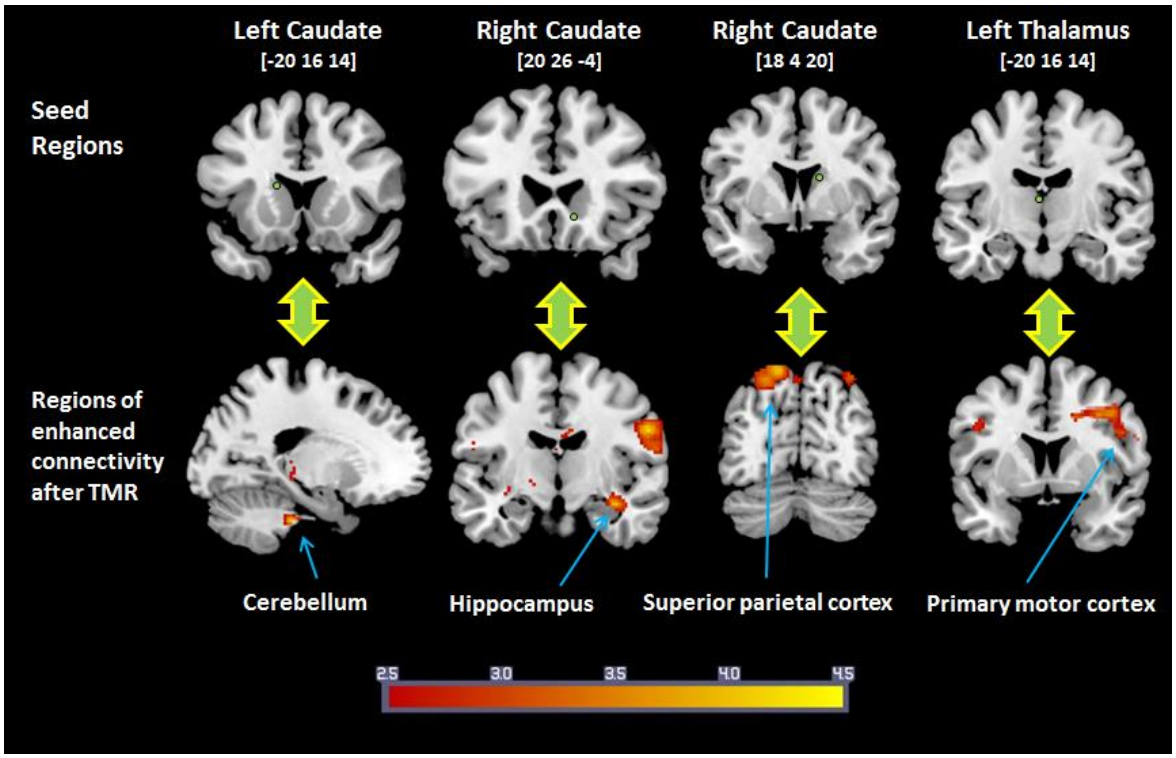

Figure 4.8: Regions of increased functional connectivity after TMR. A psychophysiological interaction (PPI) analysis revealed enhanced connectivity for the cued sequence between a left caudate seed region and left cerebellum (-22, -30, -36). Right caudate seeds were more connected to the right hippocampus (34, -12, -16), and superior parietal cortex (-14, -74, 60). The left thalamus was more strongly connected to the right primary motor cortex (32, 2, 38). Contrasts displayed as sagittal and coronal projections superimposed on a standard MNI brain. Colour bar indicates t-values.

Table 4.3: Coordinates of local maxima for brain regions showing greater functional connectivity (PPI) for cued relative to the uncued sequence (N=20).

Region	MNI x, y, z (mm)	No. of voxels	Peak T	Peak Z	Peak P(unc)
Cued>Uncued					
Seed: Left caudate nucleus	-20, 16, 14				
Left anterior cerebellum/pons	-22, -30, -36	53	4.3	3.55	<0.001
Left fusiform gyrus	-48, -58, -22	59	3.96	3.34	<0.001
Right thalamus	6, -22, 0	117	3.68	3.16	<0.001
Seed: Right caudate nucleus	20, 26, -4				
Right somatosensory cortex	56, -10, 30	231	4.28	3.54	<0.001
Right hippocampus	34, -12, -16	93	4.17	3.47	<0.001
Right superior temporal gyrus	56, -50, 20	206	4.12	3.44	<0.001
Right inferior temporal gyrus	56, -42, -12	156	4.0	3.36	<0.001
Left superior temporal gyrus	-54, -52, 20	87	3.92	3.32	<0.001
Seed: Right caudate nucleus	18, 4, 20				
Left superior parietal lobe	-14, -74, 60	256	4.41	3.61	<0.001
Left fusiform gyrus	-42, -48, -18	68	4.2	3.49	<0.001
Seed: Left hippocampus	-34, -12, -20				
No regions	-	-	-	-	-
Seed: Left thalamus	-2, -12, 12				
Right primary motor cortex	32, 2, 38	106	3.87	3.28	<0.001
Right inferior frontal gyrus	42, 14, 16	59	3.4	2.97	0.002

PPI analysis of connectivity between 5 seed regions and the rest of the brain, voxel threshold of p=0.05 (whole brain corrected) and extent threshold of k>50 voxels. All active voxels are positive for the cued>uncued comparison.

The left caudate nucleus seed (-20 16 14) showed enhanced connectivity for the cued relative to the uncued sequence with left anterior cerebellum and right thalamus. The first right caudate nucleus seed (20 26 -4) also showed enhanced connectivity, with a cluster spanning parts of right hippocampus, amygdala and putamen (Figure 4.8). The second right caudate nucleus seed (18 4 20) showed enhanced connectivity with superior parietal cortex, and the left thalamus seed (-2 -12 12) showed enhanced connectivity with right M1. Lastly, the left hippocampal seed (-34 -12 -20) failed to show any significant enhancements in connectivity. Of note, functional connectivity

was not significantly enhanced for the uncued sequence, relative to the cued sequence, between any of our seed regions and the rest of the brain. Thus, the behavioural enhancements observed after TMR appear to be related to increased connectivity, as well as increased activation, within regions associated with MSL consolidation.

Discussion

We have identified for the first time that TMR of a procedural memory leads to plasticity in motor networks supporting performance. As predicted, we found significantly faster reaction times for a procedural sequence that was cued during SWS relative to an uncued sequence, in accordance with previous work (Chapter 3). This consolidation was associated with enhanced activation in cortico-striatal and hippocampal networks, as modulated by SWS. Furthermore, TMR was also linked to increased functional connectivity between striatum and hippocampus. Lastly, rapid-eye-movement (REM) sleep was found to modulate plasticity after TMR in additional cortico-cerebellar cortices.

A number of neuroimaging studies exploring the neural substrates of sleep-dependent procedural memory consolidation have shown increased post-sleep activity within cortico-striatal networks (for review see Albouy et al., 2013). Specifically within the striatum, dynamic changes across all stages of learning are thought to support motor sequence memories (Doyon et al., 2009) and increased striatal function has been observed when comparing MSL after sleep and wake retention intervals (Albouy et al., 2008; Debas et al., 2010; Walker et al., 2005). We extend these findings by showing that cued reactivation of specific memories during sleep can bias this plasticity in cortico-striatal networks, leading to improved performance that is supported by enhanced functional brain activity in bilateral caudate nuclei, modulated by SWS duration.

The finding of increased activity for the cued sequence in the left hippocampus is also supportive of our original hypothesis. This region was predicted to play a role for several reasons: (1) It is involved in the acquisition of both implicit and explicit SRTT sequences (Schenden et al., 2003), and appears to be necessary to engage sleep-dependent consolidation of the SRTT (Spencer et al., 2006). (2) This region

spontaneously reactivates during sleep after learning of hippocampal-dependent tasks, demonstrated through specific neuronal firing sequences measured in rodents (Wilson & McNaughton, 1994), and regional blood flow changes observed in humans (Peigneux et al., 2004). (3) Hippocampal activity has also been observed during TMR (Rasch et al., 2007), in addition to nearby parahippocampal activity (van Dongen et al., 2012). (4) Post-sleep the hippocampus shows enhanced activity following MSL learning (Albouy et al., 2013; Walker et al., 2005; Wilhelm et al., 2013). For these reasons the hippocampus is central to models of systems-consolidation via the reactivation of specific memory traces during SWS (Diekelmann & Born, 2010). Our finding that TMR of a procedural memory boosts activity in this region suggests that reactivation plays a fundamental role in post-sleep changes in hippocampal function, perhaps as a result of systems level reorganisation of the memory representation (Diekelmann & Born, 2010; Krakauer & Shadmehr, 2006).

Furthermore, TMR during sleep modulated striato-hippocampal connectivity, so that connectivity was enhanced for the cued relative to the uncued sequence. Thus, our data provide further evidence that TMR during SWS can modify connectivity patterns within networks underlying performance (van Dongen et al., 2012), and for the first time we show these connectivity changes with a procedural memory. This result was predicted based on prior MSL research by Albouy and co-workers (2008, 2013), showing that ventral striatum and hippocampal activity during learning predicts subsequent overnight performance gains, and their interaction transforms from competitive to cooperative overnight. They propose a model of sleep-dependent interactions between hippocampus, striatum and prefrontal cortex in motor sequence memory consolidation. Together, these studies suggest that the functional interaction between hippocampus and striatum underscores consolidation of motor sequence learning, and our data support the idea that reactivation during SWS is the mechanism that underlies this process. We suggests that these changes in striatum and hippocampus reflect stabilisation and perhaps reorganisation of the sequence memory representation, which then allows faster motor output when performing the sequence at retest.

The PPI analysis also revealed the left caudate to be more connected to cerebellum and thalamus after TMR, while the left thalamus had increased connectivity to a region of M1 relatively close to the hand representation. The left thalamus also showed enhanced activity for the cued sequence in the SWS-covariate analysis, and specifically the

medial dorsal nucleus which has been linked to memory functions (Aggleton & Brown, 1999; Zola-Morgan & Squire, 1985). Together these findings indicate that reactivation influences functional interactions across a wide range of cortico-striatal and cortico-cerebellar motor systems.

The discussed increases in activity after TMR were all modulated by SWS duration. The amount of SWS obtained is an important factor in the effectiveness of TMR on behavioural measures (Cairney et al., 2014), and a longer SWS period provides the same offline gains as a shorter period containing TMR (Diekelmann et al., 2012). Due to this strong influence of SWS, we included SWS duration as a covariate, as it was not possible to control how much SWS participants obtained. This analysis showed that slow-wave sleep is critical for plastic changes that support enhanced performance after TMR. This agrees with findings that SWS plays a role in some forms of procedural memory consolidation (Huber et al., 2004; Tamaki et al., 2013), and the emergence of explicit knowledge from implicit SRTT learning (Wilhelm et al., 2013). Thus, cues in the current study biased reactivation, and the amount of slow-wave sleep determined the extent of neural reorganisation that subsequently occurred in relation to the cued sequence.

The importance of SWS was also illustrated by our correlations between behavioural measures and neural features of SWS, and these agree with the same analyses from Chapter 3. Slow oscillation power once again predicted the emergence of explicit sequence knowledge after sleep, although at parietal rather than central electrodes (Chapter 3). These regional discrepancies may be due to the non-local nature of slow oscillations travelling across the cortex (Massimini et al., 2004), and previous research correlating consolidation effects with slow-wave activity have also identified inconsistent regions (Wilhelm et al., 2013).

Additional neural features of slow and fast spindle power laterality predicted procedural improvement, most consistently over central electrode sites in close proximity to M1. This is consistent with Chapter 3 and previous studies (Nishida & Walker, 2007), and is in accordance with the proposed role of spindles in neural plasticity associated with systems consolidation (Barakat et al., 2013; Diekelmann & Born, 2010). Interestingly, Fogel et al. (2014) recently showed that impaired motor sequence consolidation in older participants was related to both a decrease in spindles and a concomitant decrease of

activity in the cortico-striatal network after sleep. Our data show a similar relationship in healthy subjects, where spindles were associated with enhanced consolidation after TMR, and TMR was also associated with increased striatal activity after sleep. Correlations were apparent during replay (CUE) and subsequent silent periods (NO-CUE), which may suggest consolidation is ongoing during the silent NO-CUE periods that follow TMR. However our design is not optimised to determine if this is the case, and future research utilising control sounds may clarify this. Fast spindles are associated with activity in hippocampal and sensorimotor regions (Schabus et al., 2007), that is, regions where we observed TMR related plasticity. Thus our correlation between fast spindles and the procedural-cueing effect indirectly suggests fast spindles were instrumental to that plasticity, a speculation that is supported by prior research (Barakat et al., 2013; Fogel et al., 2014). Further research utilising fMRI during TMR is required to more firmly establish if this is the case, and investigate how the neural substrates of spindles and reactivation interact. Additionally, slow spindles were also correlated with the procedural-cueing-effect at central electrodes, while they were negatively correlated with the explicit-cueing-effect at frontal regions. This may indicate a complex role for slow spindles in consolidation, which are predominantly linked with frontal activity (for review see Fogel & Smith, 2011). However a great deal of further work is needed to establish these distinctions between fast and slow spindles and how they relate to memory reactivation.

An unexpected finding was the modulation of TMR related plasticity in a number of regions by time spent in REM sleep. This showed increased activity in cortico-cerebellar networks, specifically within bilateral cerebellum, left PMC and SMA, regions that were predicted to show increases in relation to SWS. PMC and SMA operate within a loop with striatal regions and thalamic nuclei during motor performance (Miyichi et al., 2006), and interact dynamically during learning to create a sequence memory (Penhune & Doyon, 2005). Together with the cerebellum these areas could directly allow faster motor output of the cued sequence. Increases were previously observed in cerebellum when comparing wake and sleep consolidation periods (Walker et al., 2005), where it was suggested the increased motor output after consolidation might demand more cerebellar involvement for error monitoring (Ohyama Nores, Murphy, & Mauk, 2003). Importantly, prior studies compared sleep and wake periods to establish the contribution of sleep to plasticity (Albouy et al., 2013; Debas et al., 2010;

Fischer et al., 2005; Walker et al., 2005) and did not utilise sleep stages as covariates. These should be crucial components to further work in order to uncover the contribution of specific features of sleep underlying motor plasticity.

Interestingly, these regions associated with REM sleep are separate from the 'SWS-dependent' MSL regions we observed. This might suggest that SWS supports consolidation of a sequence representation (caudate and hippocampus), while REM sleep facilitates consolidation within motor circuits that support faster implementation of that sequence memory (cerebellum, PMC and SMA). Also of note, our REM sleep analysis showed several regions of difference not included in our original hypothesis. Most notably a region of posterior parietal cortex that was previously found to spontaneously reactivate (Maquet et al., 2000) and have enhanced connectivity to premotor cortex (PMC) during REM sleep (Laureys et al., 2001) after SRTT learning. We also found enhanced connectivity between this region and the right caudate after TMR. This area is associated with utilising visual inputs to guide movement (Pisella et al., 2000; Rizzolatti et al., 1998), therefore plasticity in this region could support the enhanced performance we observed for the cued sequence.

The implication of these 'REM-dependent' cueing effects is that the plasticity triggered by TMR during SWS not only continued throughout SWS, but also effected changes that relied on subsequent REM sleep. Our findings are consistent with the suggestion that REM sleep occurring later in the night completes consolidation initiated during early SWS periods (Giuditta et al., 1995), perhaps enabling synaptic consolidation to stabilise memories after they were reorganised during SWS (Diekelmann & Born, 2010). A number of studies have implicated REM sleep in procedural learning (Fischer et al., 2002; Smith & Conway, 1998), leading to the suggestion of a distinction between procedural learning and REM sleep, declarative and SWS (Smith, 1995, 2001). However, subsequent findings relating SWS to procedural learning (e.g., Tamaki et al., 2013) and intact memory consolidation after pharmacological suppression of REM sleep (e.g., Rasch et al., 2009), suggest a more complex picture. We extend this by showing plasticity after reactivation of specific procedural memories is associated with REM sleep in some brain regions, and SWS in others.

Given that our fMRI data only relate to plastic changes observed during post-sleep retrieval testing, an intriguing question remains as to what activity occurs in response to

cues during sleep that underlies plasticity. Our cues were presented in SWS, where hippocampal replay events have been most consistently observed in rodents (e.g., Nádasdy et al., 1999), as well as replay in ventral striatum (Lansink et al., 2008) and other regions (Ji & Wilson, 2007; Peyrache et al., 2009; Qin et al., 1997). Similarly, MTL regions reactivate in response to TMR during SWS in humans (Rasch et al., 2007; van Dongen et al., 2012). For procedural memory, spontaneous reactivation has been observed in task-related visual areas during NREM sleep (Yotsumoto et al., 2009). Many of the regions identified across both of our SWS and REM sleep covariate analyses were shown to reactivate spontaneously during REM sleep after SRTT learning, including the caudate, PMC and parietal cortices (Maquet et al., 2000; Peigneux et al., 2003). We speculate that our cues presented during SWS also led to similar reactivation in hippocampus, striatum, and perhaps other motor memory regions, which led to the plastic changes observed after sleep, perhaps during both SWS and REM sleep.

The question as to whether the observed plasticity represents synaptic or systems consolidation is a difficult one to answer with our data alone. Given the relatively short time-scale between encoding and retrieval, and the lack of a pre-sleep measurement of brain activity with which to compare post-sleep activity, it is possible that the plasticity we observed in relation to the cued sequence is the result of synaptic consolidation within regions, rather than systems level consolidation reorganising the memory across brain regions. However, a wealth of previous work shows dynamic alterations to procedural memories across many brain regions over time (Doyon & Benali, 2005), and across sleep (Albouy et al., 2013), therefore we speculate that our findings do show preferential reorganisation of the cued sequence memory across sleep.

There are some limitations to the current work. Firstly, one could argue that activation changes for the cued sequence reflects enhanced performance, or 'production', rather than showing the direct influence of sleep-dependent consolidation on memory related regions. Indeed, increased finger movement frequency is associated with increased cortical activation in M1, cerebellum and PMC (Blinkenberg et al., 1996; Lutz, Koeneke, Wüstenberg, & Jäncke, 2004). We cannot entirely rule out this possibility. However, button presses were modelled as a covariate of no interest to account for this, and the very small differences in performance observed between cued and uncued sequences are unlikely to account for our findings. Paced versions of procedural tasks

can eliminate this confound (Karni et al., 1998; Walker et al., 2005), however this would eliminate the behavioural consolidation effect we wanted to explore, while changing the nature of SRTT performance. Also, a number of other studies have carefully controlled these confounds and still demonstrate increases in the same regions, including bilateral cerebellar cortices, bilateral caudate, and PMC (Steele & Penhune, 2010).

Second, SWS-time and REM-time were not correlated with our behavioural cueing effect, raising the question of whether it is appropriate to include them as fMRI covariates. The reorganisation of neural network activity underlying performance after sleep is not always necessarily accompanied by observable changes in behaviour (Peigneux et al., 2006; Rauchs et al., 2008) therefore it is still valid to explore TMR related plasticity in the absence of correlations with behaviour. Also, these covariates were included based entirely on previous literature showing their role in memory consolidation (e.g., Cairney et al., 2014). On a related point, our main effect comparing cued and uncued sequence performance without covariates highlighted reduced activity in the left caudate, which is at odds with the expected increase after spontaneous sleep-dependent consolidation (Albouy et al., 2008; Debas et al., 2010; Fischer et al., 2005). We propose the SWS-modulated contrast is a more sensitive measure, as it takes into consideration the potential for SWS-dependent consolidation across the entire night, potential that can be biased with TMR.

Lastly, we did not replicate the explicit cueing effect at the group level (Chapter 3), and we speculate this is as a result of methodological differences between the two studies, such as the time of the retest session, the scanner environment and block composition. For example, longer breaks between sequences (15seconds vs. 2seconds) may have encouraged different learning strategies that effected consolidation. This may also account for why consolidation effects were observed at different points of retest in each experiment. Generally speaking, sleep consolidation effects are very sensitive to many aspects of learning and retest, and any one of these could have led to these differences.

To conclude, we show that TMR of a procedural memory is associated with plasticity in striatum and hippocampus, and this plasticity may explain the behavioural effects associated with TMR. We also show that behavioural cueing effects are linked to slow

oscillations and spindles, and they may play a crucial role of inducing neural plasticity after TMR. We provide tantalising hints that REM-sleep that occurs after TMR is important to engage certain neural changes that support post-sleep performance of procedural memories. Lastly, this study explored a single night after initial acquisition of a procedural memory, and an important next step is investigate reactivations role over longer time scales as procedural memories become automatized.

Chapter 5

The effect of targeted memory reactivation during sleep on the formation of false memories

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Abstract

Targeted memory reactivation (TMR) is a recently developed technique to investigate the consolidation of specific memories, and has provided novel evidence for memory reactivation as a primary mechanism for stabilising, strengthening and reorganising memories during sleep. There is still some debate as to the role played by sleep in the generalisation of memory representations that can lead to false memory formation, and it has not been shown whether reactivation underpins this process. To explore this, we used the Deese-Roediger-McDermott (DRM) paradigm, where participants learn lists of semantically associated words (e.g., "house", "door", "frame") that are missing a strong associate or critical lure (e.g., "window"). Musical notes (experiment 1) or verbal cues (experiment 2) were added to the lists at encoding. During subsequent non rapid-eye movement (NREM) sleep, half the list words were cued by replaying the associated sound cues. Neither experiment found any evidence that TMR influenced false recall or recognition of critical lures. This suggests reactivation is not involved in the generalisation of such memory representations during sleep. However, TMR did influence veridical memory for the location of correctly recognised cued words, despite having no effect on free recall or recognition of the words themselves (experiment 1). Conversely, the verbal cues used in experiment 2 impaired veridical memory consolidation, with significantly poorer recognition of cued relative to uncued words. We conclude that the type of cue being used (sounds or words) influences memory consolidation in different ways, either by stimulating consolidation of sensory details, or by interfering with sleep-dependent memory consolidation.

Introduction

The vivid recollection of events that never occurred is an unnerving experience shared by most of us at some point in our lives. But rather than reflecting a broken system, false memories illuminate the constructive nature of memory (Bartlett, 1936). These errors demonstrate key facets of a system that draws upon past experience and knowledge in order to direct the planning and execution of behaviour occurring in the

immediate present, based on myriad factors operating at the encoding, consolidation and retrieval of memory representations (Straube, 2012).

The Deese-Roediger-McDermott (DRM) paradigm is a well-established method for exploring the formation of false memories, specifically the way in which semantic associations can influence retrieval of episodic memories (Roediger & McDermott, 1995). Participants encode lists of related words (e.g., "mad", "fear", "hate") that are missing a semantically associated critical lure (e.g., "anger"). These critical lures, or false memories, are consistently recalled during both recognition testing and free recall (Gallo, 2010). Importantly, susceptibility to false memories in the DRM task is linked to the tendency to create false autobiographical memories outside of the laboratory (Clancy, McNally, Schacter, Lenzenweger, & Pitman, 2002). Many factors at encoding and retrieval contribute to this effect (for review see Straube, 2012), and more recently offline consolidation periods have been suggested as a contributing factor, particularly the reorganising properties of sleep.

Sleep is a neurophysiological state that is crucial to memory formation, and may also play a role in false memory formation. The importance of sleep in the consolidation of declarative memories is well established, and the reactivation of memory representations during sleep is proposed to facilitate their reorganisation and integration within pre-existing networks (Diekelmann & Born, 2010; Lewis & Durrant, 2011). Supporting this, a retention interval containing sleep is beneficial for memory, as evidenced by less forgetting (Plihal & Born, 1997), resistance to interference (Ellenbogen et al., 2006), integration of memories within existing long-term networks (Tamminen et al., 2010), abstraction of rules (Gomez et al., 2006), transitive inference (Ellenbogen et al., 2007), insight into hidden solutions (Wagner et al., 2004), and the emergence of explicit knowledge for implicitly encoded information (Fischer et al., 2006).

Based on these reorganising properties of sleep, it has been proposed that reactivation during sleep might be involved in the generalisation of memories, or the extraction of 'gist'. For example, the Information Overlap to Extract model (iOtA) (Durrant & Lewis, 2011) proposes that overlapping reactivation of memory traces alongside synaptic downscaling (Tononi & Cirelli, 2003, 2014) serves to potentiate and abstract underlying commonalities shared by memories. Conceivably these same processes could promote

false memories. In the DRM paradigm for example, sleep may extract the gist of list words, enabling generalisation of episodic memories to related words and concepts, the result of which being greater false recall of associated critical lures. Supporting this notion, false memories are more persistent than veridical memories over long time periods, perhaps suggesting active maintenance of the gist representation (McDermott, 1996). Furthermore, DRM task performance relies on activity in the medial-temporal lobe (MTL) (Schacter et al., 1996), a region that is heavily implicated in sleep-dependent memory consolidation (Diekelmann & Born, 2010). Conversely, many studies already discussed show that sleep facilitates more accurate recall, therefore we might expect individuals to more accurately discriminate between false and veridical memories after sleep.

A handful of studies have used the DRM task to explore the role sleep plays in false memory formation, providing differing results depending on the type of memory test used at retrieval. Studies utilising free recall require the self-generation of list words after a retention interval. These reveal increased false memories alongside a concomitant increase in veridical recall after sleep relative to an equivalent period of wake (Diekelmann et al., 2010; McKeon et al., 2012; Payne et al., 2009). For example, Payne et al. (2009) showed that veridical recall deteriorated less during sleep compared to wake, while false memories were actually enhanced by sleep. Interestingly, in contrast to previous studies highlighting slow-wave sleep (SWS) as beneficial for declarative memory consolidation (e.g., Marshall et al., 2006), they consistently found the amount of SWS to be negatively correlated with veridical recall. These effects can be interpreted in terms of the reorganising properties of sleep, which facilitate gist abstraction during sleep and lead to false recall (Diekelmann et al, 2010). However, there are several theoretical accounts for DRM paradigm effects (for review see Gallo, 2010) which may also account for such findings. For instance, spreading activation in semantic memory networks at encoding could lead to critical lures being tagged for sleep-dependent consolidation (Roediger, Watson, McDermott, & Gallo, 2001) or gist representations formed during encoding (Brainerd & Reyna, 2005) could also undergo consolidation during sleep.

In contrast, studies using recognition testing have shown sleep to have no effect on the formation of false memories (Diekelmann, Landolt, Lahl, Born, & Wagner, 2008) or to reduce them (Fenn et al., 2009; Lo et al., 2014) relative to a wake period, and to

enhance them relative to a period of sleep deprivation (Darsaud et al., 2011; Diekelmann et al., 2008). Recognition testing involves the presentation of list words, critical lures and un-studied distractor words, and participants must indicate if they have vivid recall of the word (remember), the word is familiar (know) or the word is new. These responses can be viewed as reflecting a continuum of subjective experience, where 'know' responses reflect a vague sense of familiarity, and 'remember' responses demonstrate an illusory recollection that typically includes sensory details and is associated with hippocampal activity (Cabeza, Rao, Wagner, Mayer, & Schacter, 2001). The increase in false memories after sleep deprivation has been related to the effects of fatigue on retrieval mechanisms (Diekelmann et al., 2008). Fenn et al. (2009) found reduced false remembering of critical lures after sleep consistently across 3 experiments, and Lo et al. (2013) recently replicated a similar pattern in older subjects, where the reduction was greatest in those participants with the longest SWS duration. This reduction is suggested to reflect sleeps role in strengthening contextual and sensory details of studied words, thus allowing effective discrimination between lures and studied words at retrieval (Fenn et al., 2009).

In summary, free recall studies indicate that sleep facilitates the formation of false memories, alongside the well-established beneficial effect of sleep on veridical recall. On the other hand, recognition studies highlight the role played by sleep in enhancing contextual and sensory details, which aids the correct rejection of lure words that don't conjure the same distinctive memory representations when cued in a recognition test. The two opposing findings are difficult to reconcile with one another. One might argue the enhanced representation that reduces recognition of false memories would also aid discrimination between studied words and critical lures during the self-cued retrieval of free recall. However, the processes of retrieval in free recall and recognition are very different, and the way in which participants retrieve words within lists during free recall (i.e., they tend to write down words in groups of other related words, with each word presumably cueing the next one in memory) might stimulate gist representations to a greater extent than recognition testing.

The disparate findings for free recall and recognition have yet to be tested within the same study, and the specific role of sleep in false memory formation is still hotly debated, as are the roles for associated sleep features such as slow-wave activity (SWA) and reactivation. Given that reactivation is the proposed mechanism that underlies

sleep-dependent consolidation, we examined the role of reactivation during sleep in the formation of false memories. TMR allows specific declarative or procedural memories to be cued during sleep using odours or sounds that were paired with memories during encoding. This cued reactivation of memories leads to less forgetting (Cairney et al., 2014; Fuentemilla et al., 2013; Rasch et al., 2007; Rudoy et al., 2009), improved recall (Schreiner et al., 2014), stabilisation from interference (Diekelmann et al., 2011), enhanced creativity (Ritter et al., 2012), improved performance at procedural tasks (Antony et al., 2012; Schönauer et al., 2014), the emergence of explicit knowledge (Chapter 3), and more recently the generalisation of memory for faces (Sterpenich et al., 2014). Cues are most often presented during non-rapid-eye movement (NREM) sleep, and SWS in particular has been associated with the effectiveness of TMR on behavioural outcomes (Cairney et al., 2014) and neural plasticity associated with consolidation (Chapter 4).

To the best of our knowledge, TMR has not been used to specifically explore semantic memory effects during sleep, or the formation of false memories. We investigated the proposed role of reactivation during sleep on false memory formation in two experiments using the DRM paradigm, which we adapted for TMR. Participants learned lists of visually presented and semantically related words, that were associated with individual tones from musical instruments (experiment 1) or with verbal cues (experiment 2), before sleeping overnight in the lab. During NREM sleep, half the lists were cued with the associated sounds. Participants performed free recall and recognition tests upon waking, to clarify contradictions in the literature between findings for these two tests. We predicted that veridical and false recall would be enhanced for cued lists relative to uncued lists when tested with free recall. Based on prior literature, we predicted TMR would have no effect on veridical recognition, but would reduce false recognition.

Experiment 1

Materials & Methods

Participants

Sixteen native English speaking, right-handed participants (mean age ± standard deviation [SD] = 20.3 ± 1.3years; 4 males), were recruited. One participant was rejected for insufficient sleep (<3hrs), leaving fifteen (Mean age = 20.3 ± 1.3years; 4 males). Participants were screened for history of neurological or psychiatric diseases, or sleep disorders. The study was approved by the Research Ethics Committee within The School of Psychological Sciences, University of Manchester. Participants gave written consent to indicate they understood experimental requirements, that their identity and data would remain anonymous and they could withdraw from the study at any time. Participants were asked to abstain from caffeine and alcohol for 24 hours prior to testing. Participants confirmed they had kept a normal sleeping pattern in the week running up to the experiment.

Stimuli

We adapted the DRM paradigm (Roediger & McDermott, 1995) to include sound cues. Participants learned 16 lists (8 cued, 8 uncued) of 10 visually presented words (160 words) (e.g., "house", "door", "frame"…) that were semantically related to an unstudied critical lure (e.g., "window"). Lists were selected from Roediger et al. (2001) and each word was presented in order of their relatedness to the critical lure, with the least related word presented last (Appendix A). Each list was presented with a sound that would repeat with each word. Sounds were musical instruments playing a fixed note (e.g., Bass guitar A in 2^{nd} octave, Violin D in 5^{th} octave) selected for their distinctiveness from one another and matched for subjective intensity. Sound stimuli were chosen with a potential wake control group in mind, who would be cued with sound stimuli while awake rather than during sleep (this wake control group was not tested in this study because sleep cueing failed to show the predicted results). Thus, these stimuli were deemed preferable to verbal cues, since verbal cueing would very closely resemble additional rounds of encoding for a wake control group, rather than covert reactivation as intended.

Lists were numbered 1-16 based on their likelihood of inducing false recall (Roediger et al., 2001). Sounds were randomly assigned to lists for pairs of participants, and could only be assigned to each list once across the experiment. Within these pairs of participants, even or odd numbered lists were used as sleep cues for each, ensuring the likelihood of inducing false recall was counterbalanced for cued/uncued lists within participants, and also that all sounds were replayed equally. Each word in a list appeared in one of the four corners of the screen. This was to assist encoding of such a large number of stimuli, and to increase the likelihood of sleep cues successfully cueing associated words, since previous TMR studies have utilised hippocampal-dependent visuo-spatial tasks (e.g., Fuentemilla et al., 2012). We felt that adding a spatial element increased the likelihood of engaging similar effects. List location was randomised and counterbalanced across participants, with each location appearing an equal number of times for cued and uncued lists within participants.

Procedure

Learning session: Participants arrived in the lab at 8pm and were fitted for polysomnography (PSG). The learning session began 9-9.30pm. For each trial, a word appeared in one of four corners of the screen for 500ms, accompanied by a sound lasting between 1000-1500ms (Figure 5.1). Participants were instructed to remember each word, its screen location and the associated sound because they would be tested later. Words appeared at a rate of one every 2000ms. Each list was separated by a 10second fixation to reduce interference between lists.

All lists were displayed once, lasting approximately 10 minutes. Participants then had a short self-paced break before beginning a second round of presentation. The second round helped build sound-word associations, which was essential for TMR to be effective, and also assisted with encoding the increased number of words relative to other sleep studies using the DRM paradigm (e.g., Payne et al., 2009). More lists were included to help gain sufficient statistical power to observe cueing effects with the relatively low number of critical lures (8 cued and 8 uncued). Order of lists was randomised within each of these two rounds, and no list was presented as the first or last 3 lists within both rounds, minimising primacy/recency effects (Ebbinghaus, 1913).

Participants also performed a number-comparison distractor task in a separate room, 10 minutes after list encoding. The potential wake control group mentioned previously would have received TMR during this task, to assess the impact of cueing during wakefulness on false memory formation. A pair of 3-digit numbers appeared on the screen, joined 3000ms later by a target number that was numerically between the two original numbers. Participants indicated which number was closest to the target number with a key press, using the index finger of each hand. Trials were separated by a 500ms fixation, and participants were given 3000ms to respond. This task was performed for 40mins. Participants were in bed for lights out at 11pm.

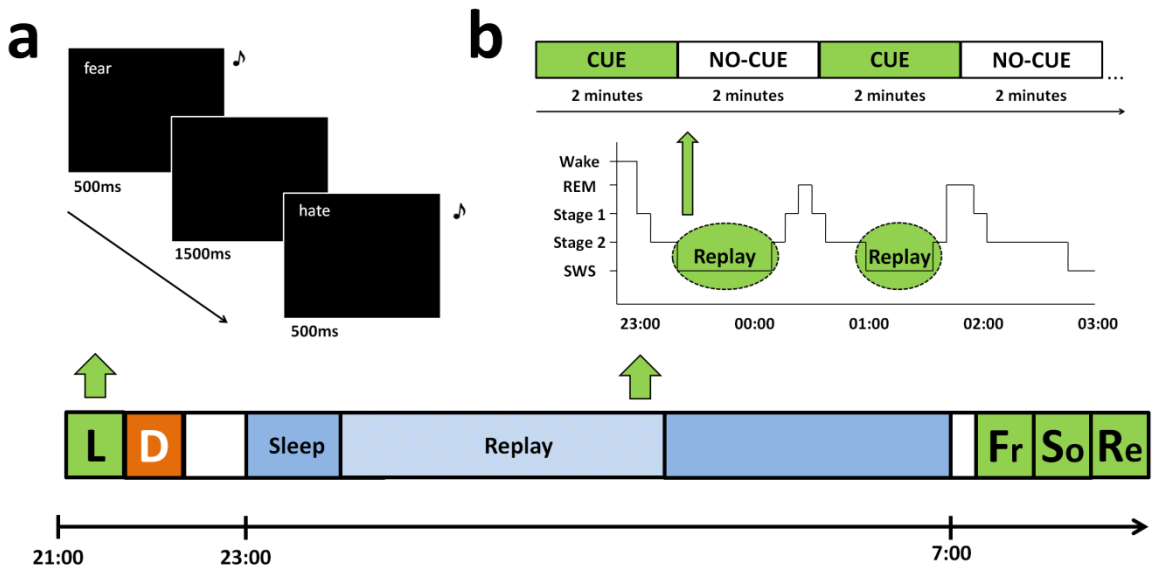

Figure 5.1: Schematic of experiment 1 procedures. (a) During the learning session (L) participants learned all list words with their associated locations and sounds in two rounds of presentation. This was followed by a number-comparison distractor task (D). (b) During periods of SWS, sounds for cued lists were replayed at the same rate as learning, in 2min blocks (CUE) followed by 2mins of silence (NO-CUE). Upon waking, participants performed a free recall test (Fr), sound-association test (So), and recognition test (Re).

Sleep replay: During SWS, sounds were replayed at an identical pace to learning, with a sound every 2s (total of 20s) and a 10s gap between lists (Figure 5.1). Sounds for half the cued lists (4 lists) were replayed in a block lasting 2mins (CUE period), followed by 2mins of silence (NO-CUE period). The next CUE period contained the remaining 4 cued lists. Order of replay was randomised within these two halves, and lists appearing within each half were randomised across participants. Replay was stopped for any sign of arousal or disruption of sleep stage (e.g., moving from SWS to stage 2).

Free recall test: Participants were awoken at 7am and given 20 minutes to overcome sleep inertia. They were then asked to write on paper as many words as they could remember from the learning session, and to write them in the location they appeared.

Sound-association test: This test explored any change in the association between sound cues and words/locations after TMR. Each sound was played in the same way as learning, that is, repeated 10 times at a rate of one sound every 2s. Participants were instructed to note down as many words as they could recall being associated with that sound. They were also instructed to note down the sound-location association and to guess if they were unsure. Participants had the duration of the tones to do this (20s), and a further 20s before the next list was cued. Order of sound presentation was randomised for each participant.

Recognition test: Participants were visually presented with all critical lures (16 words), three words from each studied list (words number 1, 5 and 10) (48 words), and two un-studied words from 16 additional DRM lists (32 words). This meant there were 48 studied and 48 unstudied words, 96 in total. Each word appeared centrally on screen, and participants were asked to make a self-paced Remember/Know/New judgement (Rajaram, 1993; Roediger & McDermott, 1995). They were instructed to use 'remember' if they had a vivid recollection of the word, 'know' if they could not remember anything specific but they knew the word was presented during the learning session, and 'new' if the word was not studied during learning. Remember or know responses were followed by a forced-choice button press to indicate the associated screen location (top left/top right/bottom left/bottom right). Ideally the order of retrieval tests would be counterbalanced across participants. However, the order of tests was

fixed for all participants, since the sound-associations test and recognition test would have a potentially profound influence on free recall, while the reverse impact of free recall is relatively small. The Stanford Sleepiness Scale was used to measure alertness prior to each learning and retest session (Hoddes et al, 1973).

Statistical analysis

Paired sample t-tests were used to compare cued/uncued words in all analyses with significance of $p<0.05$. Wilcoxen signed-rank tests were used wherever Shapiro-Wilk tests indicated data were not normal. Similarly, one-sample t-tests or one-sample Wilcoxen signed-rank tests were used to compare conditions to chance performance.

Free recall: Proportion was calculated as recall divided by the number of possible words that could be recalled. Since the number of possible recalled words differs vastly between false (e.g., only 8 possible items for cued), and veridical recall (e.g., 80 possible words for cued), it was not appropriate to compare false and veridical proportions in a single analysis. For example, recall of 16 studied words represents a proportion of 0.2, while recalling only 2 non-studied critical lures represents a larger proportion of 0.25. For this reason, separate t-tests or non-parametric tests were used to compare cued and uncued words for false and veridical recall/location. In terms of location, we calculated proportion for each condition by dividing the number of words recalled in the correct location by the total number of words recalled in that condition. If a participant did not recall any words from a particular condition (e.g., they recalled no cued critical lures) then they were removed from subsequent location analyses for that condition (this only occurred in 4 instances across all conditions). For critical lures, we deemed the location to be correct if the word was placed in the location of its associated list, although technically the critical lures were never actually presented in that location themselves.

Sound-association test: Recalled words were correct only if written down in association with the correct sound, and the proportion of recalled words was analysed in exactly the same way as free recall. In terms of location, participants could potentially recall 8 sound-location associations correctly for cued and uncued, therefore the number of correctly recalled locations was divided by 8 to give the proportion of correct locations recalled for cued and uncued lists.

Recognition test: Performance was analysed for remember (R), know (K), and combined (R + K) separately. For the statistical tests, to control for base-rate false alarm rates we calculated the sensitivity index (*d'*) [Normalised (hits / (hits + misses)) – Normalised (false alarms / (false alarms + correct rejections))]. Both false and veridical recognition were corrected in relation to the false alarm rate for all new words. Location proportions were calculated in the same way as for free recall.

Polysomnography & sleep scoring

Electroencephalography (EEG) electrodes were attached according to the international 10-20 system at 11 locations: Frontal (F3, Fz, F4), central (C3, Cz, C4), parietal (P3, Pz, & P4) and occipital (O1 & O2), referenced to linked mastoids (M1 & M2). For sleep-scoring, electrodes were referenced to the contralateral mastoid. We also attached left and right electrooculogram, left and upper electromyogram, and a ground electrode. Impedence <5kOhms was verified at each electrode and signals were digitally sampled at 200Hz. Online sleep scoring was performed by a trained experimenter according to the AASM criteria (American Academy of Sleep Medicine, Westchester, IL), using RemLogic© 1.1 software, in order to determine the appropriate stage to replay sounds. Sleep across the whole night was not formally scored, but the experimenter verified online that all participants acquired >6hrs sleep.

Results

Free recall: The proportions for recall in all tests and conditions are displayed in Table 5.1. Free recall data were not normally distributed therefore non-parametric tests were used (Wilcoxen signed-rank test). TMR did not influence false recall, since there was no significant difference in recall between cued and uncued critical lures (p=0.72) (Figure 5.2). Unexpectedly, veridical recall was not enhanced by cueing (p=0.8). In addition, location recall was high for both false and veridical recall, but did not differ between cued and uncued for false (p=0.4) or veridical recall (p=0.44).

The false recall of non-studied items that were not critical lures was also examined. This included intrusions that were related to cued lists (cued-intrusions), related to uncued lists (uncued-intrusions), and intrusions that were not related to any lists (unrelated-intrusions). Unrelated intrusions were typically low (mean number of words ± standard

deviation [SD] = 0.86 ± 2.6). More intrusions were related to cued (1.67 ± 2.0) and uncued (1.53 ± 1.7) lists, but these did not differ significantly from each other (p=0.79).

Sound-association test: When recall was cued with sounds, it revealed a trend for significantly better recall of cued relative to uncued critical lures (p=0.06), but not studied words (p=0.6) (Figure 5.3). This suggests that cueing during sleep led to a stronger association between the sound cue and the generalised list representation (gist), rather than the association between specific words, leading to more false recall of critical lures. However, it should be noted that performance on this test across participants was very poor for both critical lures (mean proportion ± standard deviation [SD] = 0.06±0.02) and studied words (0.02±0.02), with more than half the participants recalling none of the sound associations (N=8). Lastly, we found no significant difference in sound-location associations for cued and uncued lists (p=0.8), and these associations were no better than chance for cued (p=0.8) or uncued (p=0.5).

Table 5.1: Mean and standard error of the proportion of all recall and recognition responses, and signal detection measures (d').

			Cued	Uncued	t	P
Free Recall						
	Critical lures		0.30 ± 0.04	0.28 ± 0.07	N.P.	0.72
	Studied words		0.20 ± 0.03	0.19 ± 0.04	N.P.	0.80
Sound-associations						
	Critical lures		0.07 ± 0.02	0.05 ± 0.02	N.P.	0.06
	Studied words		0.02 ± 0.01	0.03 ± 0.02	N.P.	0.60
Recognition						
	Critical lures	Remember	0.46 ± 0.06	0.48 ± 0.05	N.P.	0.69
		Know	0.28 ± 0.06	0.22 ± 0.05	N.P.	0.44
		Remember + Know	0.74 ± 0.05	0.69 ± 0.08	N.P.	0.46
	Studied words	Remember	0.34 ± 0.05	0.39 ± 0.05	N.P.	0.65
		Know	0.27 ± 0.05	0.25 ± 0.04	0.70	0.50
		Remember + Know	0.60 ± 0.06	0.64 ± 0.05	0.22	0.83
d'						
	Critical lures	Remember	1.69 ± 0.17	1.74 ± 0.15	0.38	0.71
		Know	0.84 ± 0.26	0.66 ± 0.16	N.P.	0.41
		Remember + Know	1.92 ± 0.19	1.79 ± 0.20	0.80	0.43
	Studied words	Remember	1.55 ± 0.17	1.57 ± 0.15	0.15	0.88
		Know	0.74 ± 0.19	0.63 ± 0.16	N.P.	0.28
		Remember + Know	1.69 ± 0.19	1.66 ± 0.22	0.20	0.85
Recognition (false positives)			**Distractors**			
	List words	Remember	0.04 ± 0.02			
		Know	0.09 ± 0.02			
		Remember + Know	0.13 ± 0.03			

N.P. = non-parametric tests were used. *significant at $p<0.05$

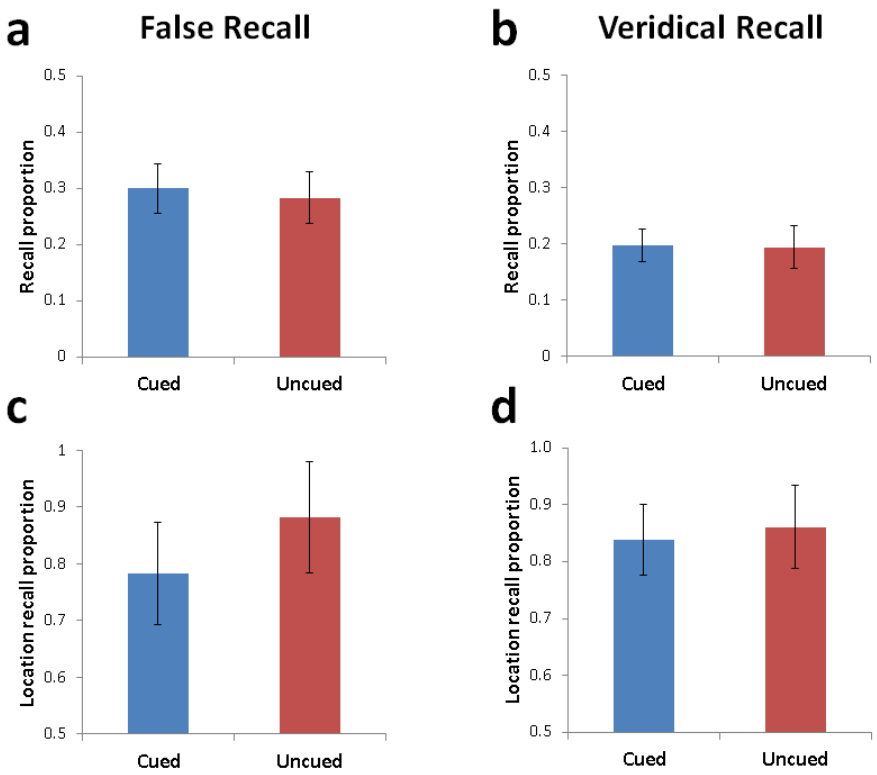

Figure 5.2: Mean free recall proportions for word and location recall in experiment 1. There were no significant differences between cued and uncued lists. Graphs (a) and (b) show the proportion of recalled words for false and veridical recall respectively, while graphs (c) and (d) show the proportion of recalled words that were placed in the correct location, again for false and veridical recall respectively. Error bars represent S.E.M.

Recognition: Performance was analysed separately for remember (R), know (K), and combined recall (R + K), using d' to correct for false positives. There was no significant difference between cued and uncued remember responses for false recognition of critical lures, $t(14)=0.38$, $p=0.71$, or veridical recognition, $t(14)=0.15$, $p=0.88$. Similarly, know responses did not differ between cued and uncued false ($p=0.41$), or veridical recognition ($p=0.28$). Lastly, the same was true for combined recognition of false, $t(14)=0.8$, $p=0.43$, and veridical memory, $t(14)=0.2$, $p=0.85$. The proportion of false positives was very low for remember (0.04 ± 0.02), and know responses (0.9 ± 0.02), suggesting participants used a strict retrieval strategy.

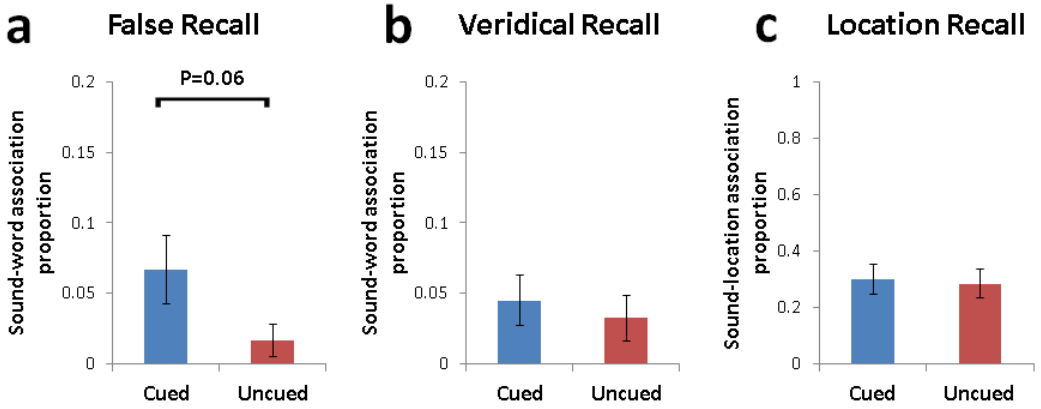

Figure 5.3: Mean proportion of word and location recall for the sound-association test in experiment 1. (a) There was a non-significant trend for an enhanced association between sounds and cued critical lures, relative to uncued lures. (b) Veridical recall did not show any effect of TMR. (c) Location recall was at chance performance for both cued and uncued. Error bars represent S.E.M.

For remember responses, location recall did not significantly differ between cued and uncued lists for false memory (p=0.64), but location of cued words was significantly enhanced for veridical memory (p=0.04) (Figure 5.4). Location of know responses did not differ for cued and uncued words for false (p=0.17) or veridical recognition (p=0.35), and the same was true for combined false, t(14)=0.72, p=0.48, and veridical recognition, t(14)=0.85, p=0.41. This suggests that cueing enhanced the perceptual feature of word location, but only for words that were vividly remembered. Lastly, participant alertness (Stanford Sleepiness Scale) did not differ across sessions, t(14)=-1.28, p=0.22. It should be noted that reported t-tests show relatively small differences and would not survive correction for multiple comparisons.

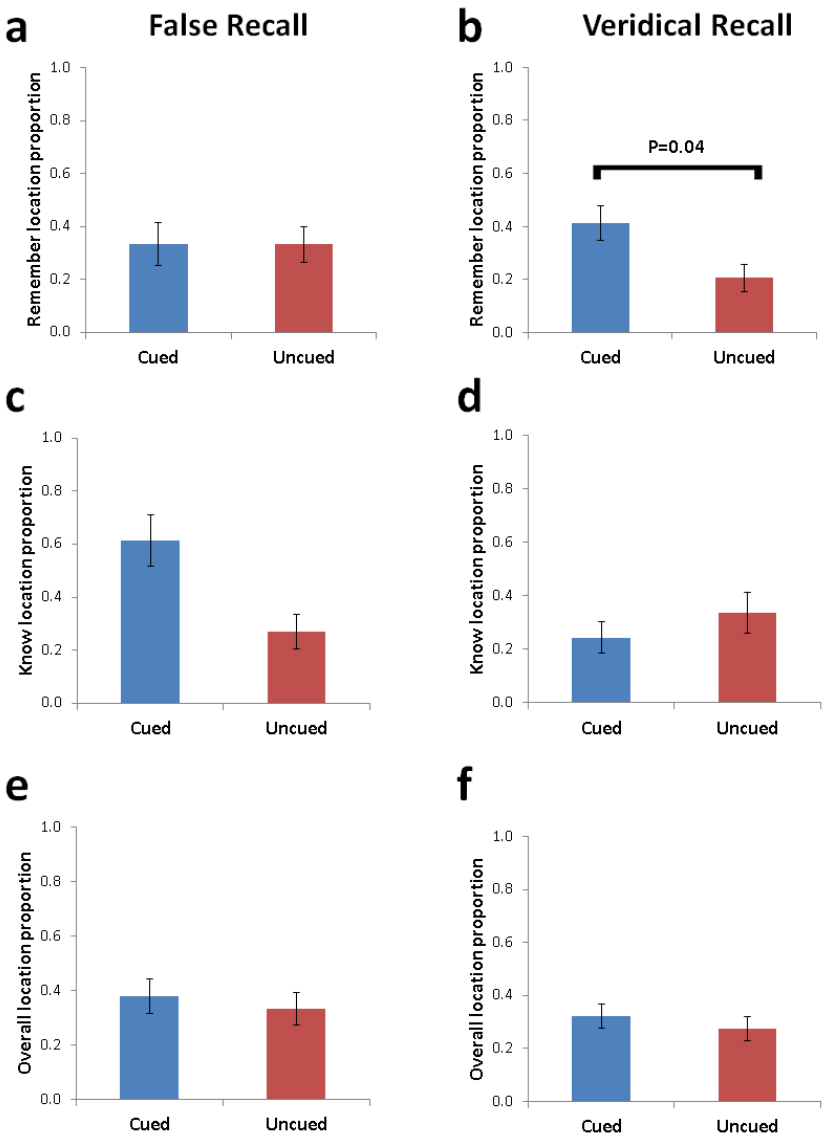

Figure 5.4: Mean location recall for the recognition test in experiment 1. (a) TMR did not influence location recall for remembered critical lures (i.e., a critical lure was deemed in the correct location if it was remembered in the location of its associated list). (b) However TMR did enhance location recall for correctly remembered studied words. (c) In terms of 'know' responses, TMR had no effect on location recall for critical lures or (d) studied words. (e) Location recall for overall recognition performance also showed no effect of TMR for critical lures or (f) studied words. Error bars represent S.E.M.

Debriefing: To ascertain strategy used by participants at encoding, they were asked if they noticed anything about the list words, and if they recalled using an encoding strategy. All participants noticed that list words were related to each other, and a number of them used these associations to help them group words during encoding (N=10), but none knew the lists were designed to induce false memories. The majority found the sound associations were too difficult to learn (N=12), but location associations helped (N=12). Only 5 participants reported hearing sounds during the night, while only 2 of these could accurately recall which sound they heard. Thus, the majority of cueing occurred during sleep. However, a wake control group and detailed analysis of sleep architecture are required in future work to be absolutely confident that this is the case and the observed effects are sleep specific.

Table 5.2: Mean and standard error of the proportion of location responses for both free recall and recognition tests.

			Cued	Uncued	t	P
Free Recall						
	Critical lures		0.78 ± 0.09	0.88 ± 0.10	N.P.	0.40
	Studied words		0.84 ± 0.06	0.86 ± 0.07	N.P.	0.44
Sound-associations	**Sound-location**		0.30 ± 0.05	0.28 ± 0.05	N.P.	0.80
Recognition						
	Critical lures	Remember	0.33 ± 0.06	0.33 ± 0.06	N.P.	0.64
		Know	0.61 ± 0.10	0.27 ± 0.06	N.P.	0.17
		Remember + Know	0.38 ± 0.06	0.33 ± 0.06	0.72	0.48
	Studied words	Remember	0.41 ± 0.07	0.20 ± 0.05	N.P.	0.04*
		Know	0.24 ± 0.06	0.34 ± 0.08	N.P.	0.35
		Remember + Know	0.32 ± 0.05	0.27 ± 0.05	0.85	0.41

N.P. = non-parametric tests were used. *significant at p<0.05

Discussion

We did not find the expected increase in false and veridical recall after TMR during sleep, and there were also no significant differences between cued and uncued words for recognition memory. However, TMR did lead to significantly enhanced recall of word location for remembered words on the recognition test, and a trend for enhanced sound-critical lure association on the sound-association test. Thus, cues did impact upon consolidation of some perceptual details of words, if not for memory of the words themselves.

Focussing on recognition memory, TMR enhanced the perceptual feature of word location, but only for items that were remembered. This is consistent with previous TMR studies finding enhanced spatial associations after cueing (Rasch et al., 2007; Rudoy et al., 2009), and lends further support for a role of memory reactivation during sleep in the consolidation of spatial memories. This is partly consistent with findings of two other sleep studies using the DRM paradigm, where a reduction in false memories after sleep was suggested to reflect sleeps role in enhancing contextual and sensory details (Fenn et al., 2009; Lo et al., 2014). However, we failed to find the same reduction in false memory, perhaps on account of our TMR procedure, discussed in more detail below.

The sound-association test revealed that participants were more likely to recall the gist word associated with a sound after TMR, but not the studied list words. In many ways this enhanced gist representation is what we predicted, except the effect is only apparent when the gist is cued with the associated sound at retest. Debriefing indicated that most participants found it difficult to associate sounds to words during learning, and reported little conscious awareness of associations. This indicates an implicit strengthening of associations between sounds and gist words after TMR. This result appears consistent with the iOtA model (Lewis & Durrant, 2011), where the overlapping cued reactivation of studied words led to abstraction of the gist associated with that sound. However, the null effect of cueing on veridical recall was not predicted, and it may indicate that sounds were not able to cue individual words in order to then abstract the gist during sleep, but rather the sounds cued a gist representation that had been formed previously during encoding (Brainerd & Reyna, 2005). This interpretation seems probable when you consider the association between sounds and individual words during encoding,

whereby there was not a unique sound associated with each word, but rather a unique sound associated with each list, and therefore the gist representation. In sum, this result suggests a gist representation can be reactivated, but it does not necessarily relate to the proposed extraction of gist via the reactivation of associated memory traces (Lewis & Durrant, 2011). It should also be noted that performance across participants was very poor, with many subjects recalling none of the sound-word associations (N=8), therefore further work is needed to establish if this is a robust finding.

Lastly, free recall showed no significant effects of TMR on false or veridical memory. This was unexpected, since sleep has increased both types of memory in previous DRM task studies (Diekelmann et al., 2010; Payne et al., 2009; Spencer et al., 2012), and declarative memory recall is consistently enhanced after TMR (e.g., Fuentemilla et al., 2013).

How do we account for this null finding? It is possible that musical note cues were not appropriate to reactivate individual words. Our only significant result showed that cues were able to strengthen associations between words and their location during recognition testing. The same association was not present for free recall. This may indicate an implicit word-location association that was sensitive to the forced choice location question that followed correct recognition of a word. By contrast, the arguably more explicit memory measures of free recall and recognition for words themselves were not influenced by TMR with musical notes. This may be due to sounds not being distinct enough from one another, and there being too many for participants to learn. The trend for enhanced associations between sounds and critical lures but not studied words supports this conclusion, because it suggests that cues were only able to reactivate the gist representation rather than individual words. We sought to correct this in experiment 2, by replacing the musical instrument cues with verbal cues.

Experiment 2

Introduction

This experiment aimed to explore the role for memory reactivation in false memory formation, this time with a DRM paradigm that included verbal cues rather than musical notes. Verbal cues were recently shown to be effective TMR cues, improving vocabulary for words cued during sleep (Schreiner et al., 2014). Once again we predicted that veridical and false recall would be enhanced for cued lists relative to uncued lists when tested with free recall, while recognition testing would show no cueing effect for veridical recognition and would reduce false recognition.

Materials & Methods

Participants

Sixteen right-handed participants (mean age ± standard deviation [SD] = 23.9 ± 4.2years; 9 males) who were native English speakers were recruited. The same screening and ethics protocol was utilised as experiment 1.

Stimuli

These were similar to Experiment 1 except for some key differences:

(1) The sound cues associated with visually presented words were verbal presentation of the word spoken in a male voice.

(2) Words were presented centrally on screen, coincident with a visual cue appearing in participants' peripheral vision in one of the four corners of the screen (500ms), rather than the word itself appearing in the location. This was achieved by providing a chin rest and displaying visual cues at 12º visual angle from fixation (minimum 7º from the beginning or end of the word) (Figure 5.5a). The justification for this alteration was to allow the comparison of neural features associated with cueing items in different hemifields. A recent study showed that TMR of a declarative memory task lateralised to one hemifield led to a spindle increase at electrodes close to contralateral visual regions during TMR (Cox et al., 2014). Thus, we could potentially perform the same

comparison of lists that were presented with associated visual cues in each hemifield, although this analysis was not performed in the current study.

(3) We replayed 8 un-learned (control) lists, alongside 8 cued lists. This allows comparison of EEG features associated with learned (cued) words and non-learned (control) words, although again this analysis was not conducted in experiment 2.

(4) The number of lists learned by participants remained the same, but we selected them from 32 DRM lists rather than 16, in order to allow tight control of the likelihood of producing false recall for cued/uncued lists, control lists, and un-studied new word lists that were used for the recognition test. For each participant, 16 lists were learned (160 words), with 8 being replayed during sleep (80 words). An additional 8 lists were replayed as control (80 words), and words from another 8 lists were selected for the recognition test as new words. The likelihood of each list (cued, uncued, control, or new) creating a false memory was counterbalanced within participants, while the lists that were cued/uncued/control/new was also counterbalanced between participants. Thus, recognition testing presented participants with the 16 critical lures from cued and uncued lists, as well as 3 learned words (words 1, 5 and 10) from cued/uncued lists (48 words). They were also presented with 8 critical lures from control lists, 3 words from each control list (24 words), 8 critical lures from new lists, and 3 words from each new list (24 words). It was necessary to have new words that were critical lures from un-learned lists, in order to compare with critical words from learned and control lists, since critical lures are typically higher frequency words than list words (Fenn et al., 2009).

(5) There was no sound-association test, since there is already a direct association between each visually presented word and the associated verbal cue.

Procedure

Learning session: This was identical to the learning session of experiment 1. Briefly, participants learned every list in one round, and then a second round. They then performed a distractor task before sleeping overnight in the lab (Figure 5.5).

Sleep replay: This occurred in groups of three 30 second blocks: (1) Replay of a learned (cued) list (R). (2) Replay of a control list (C). (3) Silent NO-CUE period (N). The order of these was randomised (e.g., RCN-NRC-RNC…). It took 12minutes for one

round of replay (i.e., all 8 cued lists and 8 control lists). This would allow comparison of sleep features between the three periods, to explore which features are associated with memory effects (cued vs. control) or simply sound stimulation (cued/control vs. NO-CUE).

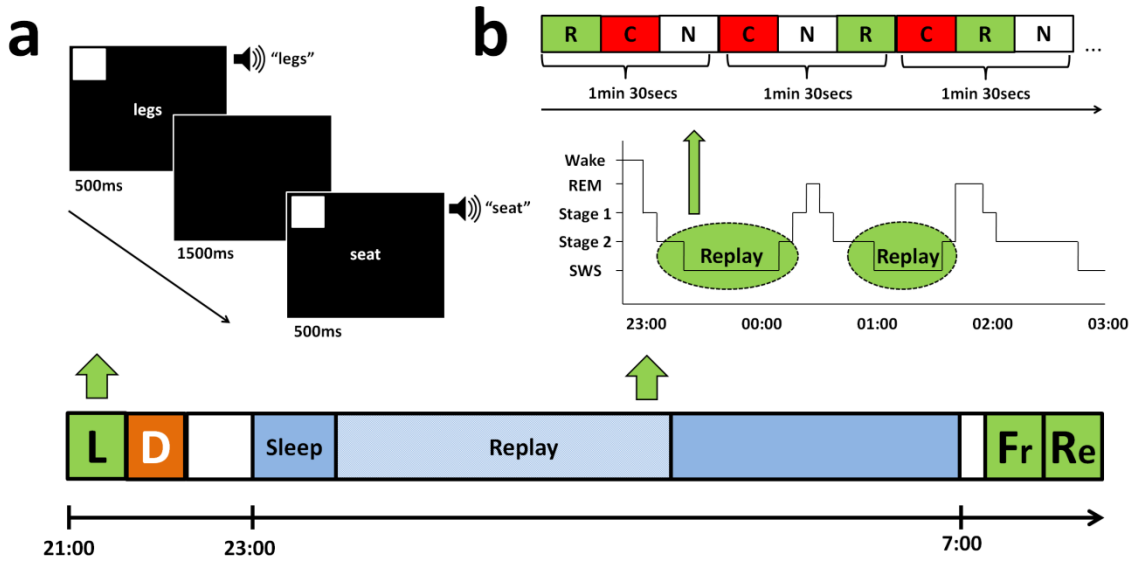

Figure 5.5: Schematic of experiment 2 procedures. (a) The learning session (L) involved presentation of all list words with associated locations and spoken word cues, in two rounds of presentation, followed by a number-comparison distractor task (D). (b) During periods of NREM sleep, replay proceeded in 30sec blocks containing 1 list of cued words (R), 1 list of control words (C), or a silent no-cue period (N). Upon waking, participants performed a free recall test (Fr) and recognition test (Re).

Statistical analysis

Free recall analyses were identical to experiment 1. Calculation of d' for the recognition analysis differed slightly in experiment 2, whereby false alarm rates were calculated separately for veridical and false recall, rather than using a single measure based on responses to all new words: veridical recognition hits were considered in relation to incorrectly recognised un-studied list words (veridical-false-alarms), while false recognition took into account the number of incorrectly recognised critical lures from un-studied lists (lure-false-alarms).

Results

Free recall: We found no difference between cued and uncued for false ($p=0.33$) or veridical recall ($p=0.23$) (Table 5.3). When considering location, there was no difference between cued and uncued for false words ($p=0.28$), but a trend for better recall of uncued locations for studied words ($p=0.07$) (Figure 5.6).

Only two control words were recalled by two separate participants, while none of the critical lures for control words were recalled, demonstrating little learning of items cued only during sleep.

Intrusions that were not related to any lists (unrelated-intrusions) were again very low (0.56 ± 1.32 words) while intrusions that were related to cued lists (cued-intrusions) (1.25 ± 1.66 words), or uncued lists (uncued-intrusions) (1.63 ± 1.5 words) were similar to experiment 1 and did not differ significantly from each other ($p=0.24$).

Table 5.3: Mean and standard error for all participants responses to free recall and recognition testing in experiment 2, including d' indices.

			Cued	Uncued	t	P
Free Recall						
	Critical lures		0.21 ± 0.04	0.26 ± 0.06	N.P.	0.33
	Studied words		0.14 ± 0.02	0.16 ± 0.03	N.P.	0.23
Recognition						
	Critical lures	Remember	0.37 ± 0.06	0.45 ± 0.07	N.P.	0.13
		Know	0.28 ± 0.06	0.26 ± 0.05	N.P.	0.54
		Remember + Know	0.65 ± 0.05	0.70 ± 0.06	1.16	0.26
	Studied words	Remember	0.34 ± 0.05	0.39 ± 0.05	2.33	0.03*
		Know	0.27 ± 0.05	0.25 ± 0.04	N.P.	0.46
		Remember + Know	0.60 ± 0.06	0.64 ± 0.05	0.92	0.37
d'						
	Critical lures	Remember	1.00 ± 0.21	1.21 ± 0.22	1.40	0.18
		Know	0.38 ± 0.19	0.32 ± 0.22	0.32	0.75
		Remember + Know	1.34 ± 0.23	1.50 ± 0.24	1.19	0.25
	Studied words	Remember	1.32 ± 0.17	1.53 ± 0.16	2.58	0.02*
		Know	0.59 ± 0.18	0.54 ± 0.17	0.47	0.64
		Remember + Know	1.50 ± 0.21	1.61 ± 0.20	0.94	0.36

			Controls	Distractors	t	P
Recognition (false positives)						
	Critical lures	Remember	0.06 ± 0.02	0.05 ± 0.03	N.P.	0.41
		Know	0.22 ± 0.07	0.16 ± 0.06	N.P.	0.13
		Remember + Know	0.28 ± 0.06	0.20 ± 0.06	N.P.	0.10
	List words	Remember	0.04 ± 0.02	0.03 ± 0.01	N.P.	0.44
		Know	0.17 ± 0.05	0.14 ± 0.05	N.P.	0.11
		Remember + Know	0.21 ± 0.05	0.16 ± 0.05	N.P.	0.12

N.P. = non-parametric tests were used. *significant at p<0.05

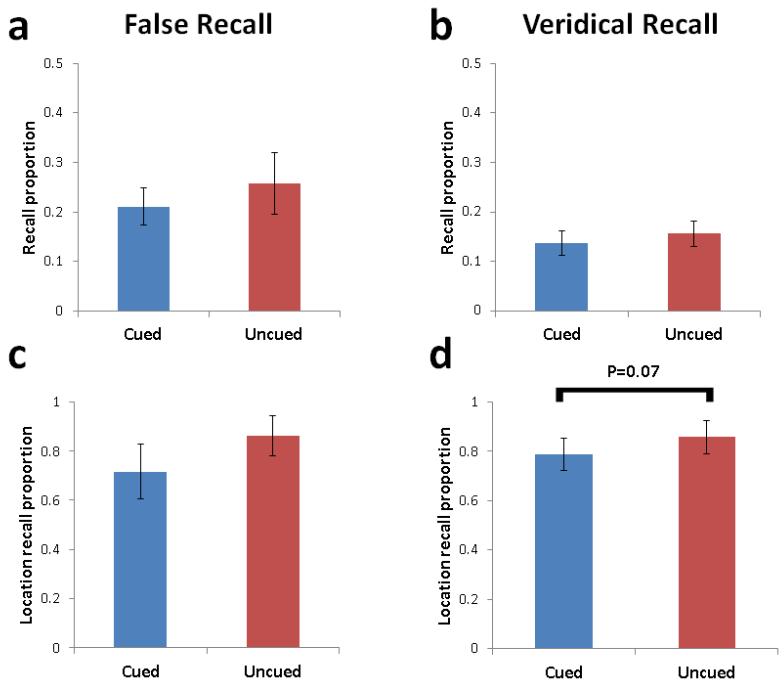

Figure 5.6: Mean free recall proportions for word and location recall in experiment 2. Graphs (a) and (b) again show no effect of TMR on free recall. (c) Location recall of critical lures was also not effected by cueing. (d) However there was a trend for better location recall of uncued studied words. Error bars represent S.E.M.

Recognition: Surprisingly, veridical responses were significantly impaired for cued words relative to uncued words for remember responses (d'), $t(15)=-2.58$, $p=0.02$, (Figure 5.7), while critical lures showed no significant difference between cued and uncued remember responses, $t(15)=-1.4$, $p=0.18$. Know responses did not differ between cued and uncued false, $t(15)=0.32$, $p=0.75$, or veridical recognition, $t(15)=0.47$, $p=0.64$. Lastly, combined recognition of false, $t(15)=-1.19$, $p=0.25$, and veridical memory, $t(15)=-0.94$, $p=0.36$, did not significantly differ. Location memory for recognised words did not differ between cued and uncued words for any condition (Table 2.4): For false recognition, this did not differ for remember ($p=0.45$), know ($p=0.67$), or combined responses, $t(14)=-0.37$, $p=0.72$. For veridical recall there were no significant differences for remember ($p=0.16$), know ($p=1$), or combined responses, $t(14)=0.14$, $p=0.89$.

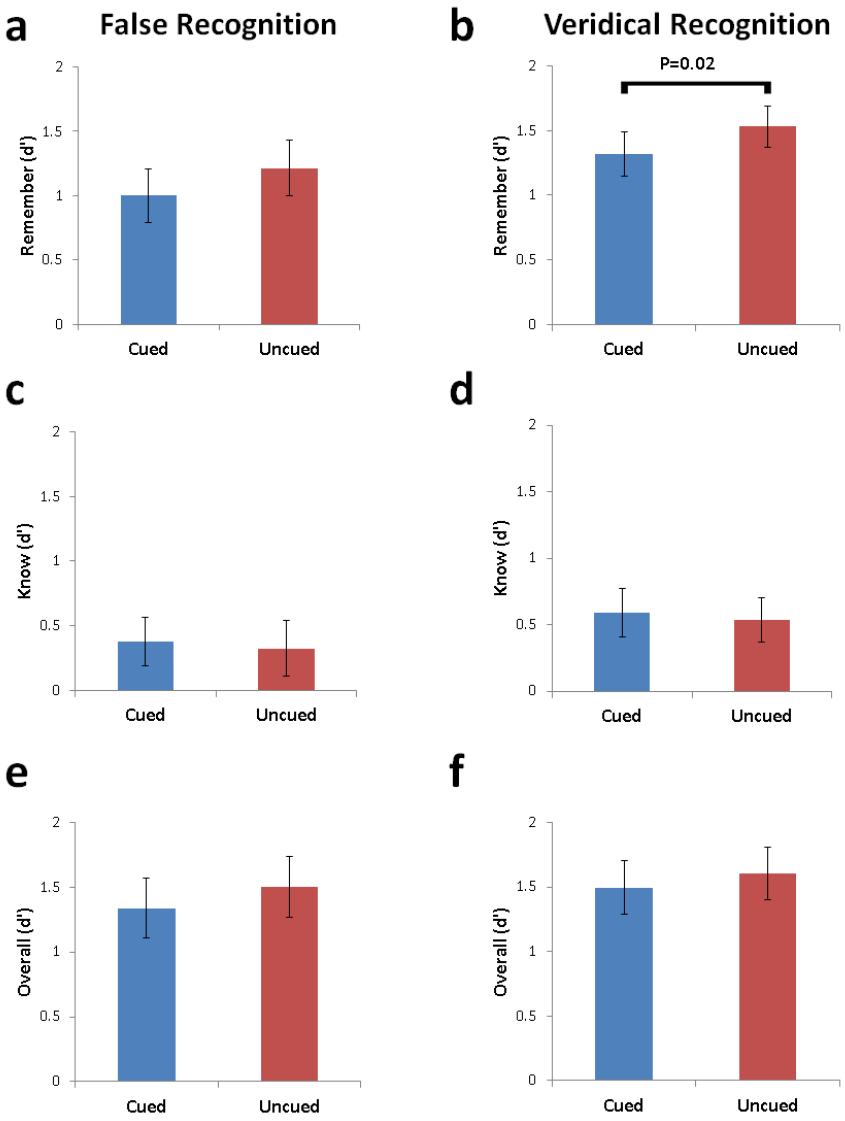

Figure 5.7: Mean recognition memory proportions in experiment 2 (d'). (a) For 'remember' responses, there was no effect of cueing on false memories. (b) However, veridical recall was significantly impaired for cued words relative to uncued words after TMR. There were no significant differences between cued and uncued lists for 'know' (c and d) or overall responses (e and f). Error bars represent S.E.M.

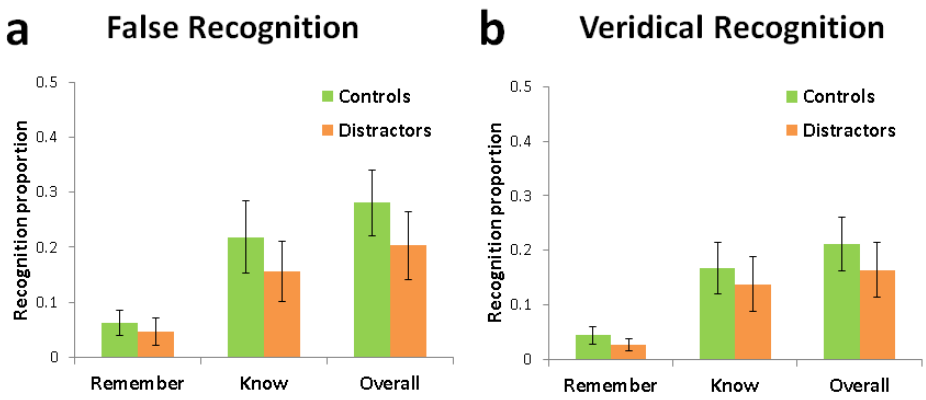

Figure 5.8: Mean recognition memory proportions for control and distractor words in experiment 2. Graphs (a) and (b) indicate low levels of recognition for words played to participants only during sleep (control) and new words presented during the recognition test (distractors), suggesting there was no learning of control words during sleep. Error bars represent S.E.M.

Recognition of control words did not differ significantly from recognition of new non-studied words (distractors) for false remember ($p=0.41$), know ($p=0.13$), and combined ($p=0.1$) responses (Figure 5.8). This was also true of veridical recall for remember ($p=0.44$), know ($p=0.11$) and combined ($p=0.12$) responses, again indicating very little learning of control words during sleep. There were no differences in alertness across sessions, $t(15)=0.66$, $p=0.52$.

Debriefing: All participants noticed the relationship between list words during learning, and many used these associations to help them group words to assist encoding (N=11), but none realised that the task was designed to induce false memory. The majority found location associations helped encoding (N=13). Half the participants reported hearing words during the night (N=8), but only one could accurately recall which word. Interestingly, one participant recalled hearing a critical lure. Again, this shows that most words were replayed during sleep.

Table 5.4: Mean and standard error for location memory in all tests of experiment 2.

			Cued	Uncued	t	P
Free Recall						
	Critical lures		0.72 ± 0.11	0.86 ± 0.08	N.P.	0.28
	Studied words		0.79 ± 0.07	0.86 ± 0.07	N.P.	0.07
Recognition						
	Critical lures	Remember	0.78 ± 0.09	0.70 ± 0.09	N.P.	0.45
		Know	0.48 ± 0.10	0.48 ± 0.11	N.P.	0.67
		Remember + Know	0.64 ± 0.06	0.66 ± 0.07	0.37	0.72
	Studied words	Remember	0.57 ± 0.06	0.56 ± 0.08	N.P.	0.16
		Know	0.64 ± 0.07	0.68 ± 0.07	N.P.	1.00
		Remember + Know	0.56 ± 0.08	0.59 ± 0.14	0.14	0.89

N.P. = non-parametric tests were used. *significant at p<0.05

General Discussion

The aim of these experiments was to reactivate episodic memories for lists of semantically associated words, which was predicted to enhance free recall of studied words and critical lures, and decrease false recognition of critical lures. Contrary to predictions, experiment 2 showed no effect of TMR on false memory formation. Furthermore, recognition was significantly impaired by cueing for veridical recall, while in the free recall test there was a trend for impaired location memory for cued words. The goal of the adjustments made to the TMR protocol for experiment 2 was to ensure the effective cueing of studied words, and the observed differences between cued and uncued retrieval suggests we were successful in that aim, except that TMR impaired consolidation rather than enhanced it.

How do we reconcile these disparate findings? We speculate on two possibilities: (1) TMR was successful in reactivating studied words, and the results indicate that consolidation of semantically associated information has a different relationship to reactivation than consolidation of other types of episodic memory. (2) The procedure of cueing semantically related items in close temporal proximity interfered with

spontaneous consolidation processes, impairing episodic memory consolidation rather than enhancing it. Discussion will focus on each of our findings (i.e., false and veridical free recall, false and veridical recognition) in relation to prior research and models, to determine whether the former explanation can account for our findings across experiments 1 and 2. Given the conflicting prior work on sleep and false memory formation, it is possible to account for some of our findings in relation to prior work, although no single theoretical account can do so completely. We will then dissect the TMR procedures used in each experiment to evaluate the second, more parsimonious explanation for our findings, that cues in experiment 2 interfered with consolidation rather than enhanced it.

False recall and recognition: Our primary aim was to explore the effects of TMR on false memory formation. We expected TMR to enhance false memories for free recall in particular, since previous studies have shown a period of sleep relative to wake leads to greater false recall (Diekelmann et al., 2010; Payne et al., 2009; Spencer et al., 2012). These effects have been interpreted as demonstrating reorganisation of memory representations during sleep which facilitates gist abstraction and generalisation (Deikelmann et al., 2010; Stickgold & Walker, 2013), perhaps relating to overlapping reactivation of memories (Lewis & Durrant, 2011), although potentially other accounts related to encoding processes (Brainerd & Reyna, 2005; Roediger et al., 2001) could be responsible (Gallo, 2010). If we assume that cues in our study reactivated their associated word memories in a manner that is similar to their spontaneous reactivation during normal sleep, then our failure to bias this process with TMR suggests that reactivation is not a mechanism underlying formation of false memories during sleep. Indeed, the circumstances under which generalisation occurs during sleep or wake remain unclear, with some evidence showing it to occur preferentially during wake instead of sleep for word learning (Werchan & Gómez, 2013) and category learning (Hennies, Lewis, Durrant, Cousins, & Lambon-Ralph, 2014). Of note, experiment 1 showed a trend for an enhanced association between critical lures and sound cues after TMR. Thus, cues reactivated the gist representation and strengthened its link to the sound, but they failed to impact upon false recall itself, suggesting reactivation was only involved in strengthening contextual details of the DRM task. Participants also possessed little awareness of these associations, which suggests TMR influenced implicit associations but had no effect on explicit recall measures of word recall and

recognition. This explanation is unlikely in light of previous work that shows TMR to preferentially bias explicit hippocampal-dependent forms of memory rather than more implicit forms of learning (Rasch et al., 2007).

We also found TMR to have no effect on false recognition. False recognition of critical lures has been shown to reduce after a period of sleep relative to wakefulness, perhaps due to sleeps role in enhancing contextual details of episodic memories (Fenn et al., 2009) or weakening links between contextual details and memory for critical lures (Lo et al., 2014). Consistent with this, we did see a slight non-significant reduction in false recognition of critical lures for cued lists (experiment 2), while lack of statistical power to test false memory might explain why it was only a weak trend. However, this account would also predict enhanced veridical memory after TMR, and experiment 2 actually showed the opposite effect.

Veridical recall and recognition: Surprisingly, recognition performance was actually impaired for remember responses after cueing in experiment 2, while the free recall test showed a trend for impaired location recall of cued words. This contradicts a wealth of research showing sleep to improve declarative recall (e.g., Plihal & Born, 1997), and TMR studies that indicate reactivation underlies sleep-dependent consolidation (e.g., Rudoy et al., 2009). Similarly, studies using the DRM paradigm tend to show less forgetting of studied words after sleep for free recall (Diekelmann et al., 2010; Payne et al., 2009; Spencer et al., 2012), although not for recognition memory (Diekelmann et al., 2008; Fenn et al., 2009; Lo et al., 2014). Thus, our data might suggest that reactivation plays an active role in the forgetting of semantically associated episodic memories, perhaps by weakening contextual details that support retrieval during the recognition test. Supporting this, sleep has been shown to decontextualize some declarative memories, reducing the impact of context upon cued recall (Cairney, Durrant, Musgrove, & Lewis, 2011). If we assume that reactivation enhances decontextualisation, then the reduction in contextual information for the words we cued with TMR may have impaired their retrieval. Potentially, this could also account for the different results of experiments 1 and 2. In this scenario, experiment 1 cues did not relate to individual words and so they cued the context (or gist) instead, which enhanced some contextual features (e.g., spatial memory for word location). The cues in experiment 2 however were able to cue individual words, leading those words to be decontextualized and impaired at retrieval. However, such effects have not been

observed previously with the DRM paradigm across normal sleep, which indicates the effect of TMR was unnatural and interfered rather than biased consolidation. Again it should be noted that this effect for veridical recall would not survive strict correction for multiple comparisons, therefore further research should establish if the finding is robust.

Slow-wave sleep: TMR in the current study was carried out during periods of SWS, and alternative support for the notion that TMR in the current study did in fact bias normal consolidation processes is provided by previous SWS associations with the DRM paradigm. Consider that: (1) SWS is causally linked to declarative memory consolidation (Marshall et al., 2006), perhaps due to the spontaneous memory reactivation that occurs during SWS (Wilson & McNaughton, 1994), and yet Payne et al. (2009) found SWS to be negatively correlated with veridical recall of the DRM paradigm. (2) TMR enhances declarative memory consolidation (e.g., Rudoy et al., 2009), presumably by biasing reactivation during SWS in favour of the cued memory, and yet we found that TMR instead had a negative impact on veridical recall. Thus, our data, and indirectly Payne and colleagues' (2009) findings, suggest that SWS reactivation of the DRM paradigm is detrimental to veridical recall. This could indicate generalisation, whereby the 'gist' representation is strengthened to the detriment of veridical representations, and Payne et al. (2009) did show enhanced false memories. However this does not form a complete picture because we did not find an effect of TMR on false memories. In addition, SWS duration was shown to reduce false recognition (Lo et al., 2014), suggesting SWS reduces consolidation of gist rather than enhances it. At the very least, these studies indicate that SWS does not benefit consolidation of semantically associated lists of the DRM paradigm in the same way as other declarative memories. Also the way in which TMR influences these processes is not the same as other declarative memories, although this may relate to our TMR procedure rather than informing us about spontaneous reactivation. Future research should examine sleep architecture in association with TMR of the DRM paradigm to clarify these issues.

Sleep & memory models: Current theories of false memory formation during sleep do not fully account for results in the literature, and similarly our findings do not fit with any one account. The proposed generalisation of memories during sleep would predict reduced veridical memory in line with our findings, but it would also predict increased false memories, and we actually found a non-significant decrease. The iOtA model

(Lewis & Durrant, 2011) suggests the reactivation of overlapping memory traces underpins this process, but our data do not support a role for reactivation in generalisation and gist extraction. Others propose sleep to enhance sensory and contextual details, allowing more effective source monitoring at retrieval (Fenn et al., 2009). This is consistent with our non-significant decrease in false recognition, where the enhanced details allow strategic avoidance of false memories that don't conjure the same detailed percepts at retrieval. However, our observed reduction in veridical recognition contradicts this explanation, especially because the observed reduction was for remember responses, which are strongly associated with the experience of sensory and contextual details. A third speculation might be that reactivation can also facilitate forgetting (Hardt, Nader, & Nadel, 2013; Hauner et al., 2013; Stickgold & Walker, 2013), in which case our cues might have biased a process that actively weakens specific memory traces. A recent TMR study found reactivation of a contextual odour reduced conditioned fear memories rather than enhanced them (Hauner et al., 2013), although it is difficult to directly associate conditioning to declarative learning of the DRM paradigm. Also, directed forgetting paradigms instruct participants not to remember certain items, which lead them to be weakened across sleep, and correlational evidence suggests that fast spindles might actively weaken these memories (Saletin et al., 2011). However, the most probable explanation is that 'to-be-forgotten' items are not tagged to undergo sleep dependent consolidation and so they decay or undergo synaptic downscaling during sleep (Tononi & Cirelli, 2003, 2014), rather than specific memories being reactivated and weakened during sleep. Based on available evidence, this account of TMR influencing a mechanism for forgetting seems unlikely. Lastly, TMR during REM sleep was recently linked with generalisation and false recognition of face stimuli (Sterpenich et al., 2014). This cannot inform as to our current findings with regard to reduced veridical recognition, but it may account for the failure of TMR to influence false memory. Thus, TMR of the DRM during REM sleep in future research may facilitate false memory formation.

In sum, the inability of these frameworks to explain our results, and the discrepancy between our veridical recognition finding and the literature, lends weight to the idea that our cues must have interfered with spontaneous reactivation and consolidation, rather than biased that process in favour of the cued memories. Importantly, a reduction in recognition memory has never been observed after normal sleep with the DRM task,

therefore our finding of reduced recognition memory after cueing violates a key assumption of TMR studies, that they bias consolidation processes already established to occur naturally during sleep. Thus, the interference account appears to be the most feasible one, and understanding exactly how this occurs might allow us to infer some of the properties underlying sleep-dependent memory consolidation.

TMR interfered with consolidation: Close examination of differences between our cueing procedure and other TMR studies may provide clues as to whether some aspect of our cueing procedure interfered with natural consolidation processes.

Firstly, the differing results from experiments 1 and 2 suggest that the type of cue used to reactivate memories will determine what aspect of the task is consolidated, or indeed if that consolidation is interfered with. We found that musical instrument cues benefited some contextual details of the DRM task, while verbal cues impaired veridical recall, despite the fact that cues were intended to reactivate the same task. TMR has been characterised as biasing (e.g., Rudoy et al., 2009), or enhancing (Schreiener et al., 2014) normal consolidation mechanisms, but our data suggest the relationship between cues and consolidation may not always be that simplistic. TMR has only been demonstrated in a handful of studies, the majority of which utilised visuo-spatial tasks (e.g., Rasch et al., 2007), and it remains to be established as to what exactly is cued in every instance.

Second, our study was the first to cue the DRM paradigm, and experiment 2 was the first to cue words that are already well established in participants' mental lexicon, rather than newly learned words. To our knowledge, the only other study to cue with word stimuli utilised newly learned foreign vocabulary (Schreiner et al., 2014), finding improved association memory between these new words and their translation. Perhaps TMR of already well-established words in semantic memory interferes with newly formed episodic memories of those words.

Third, some specific methodological features of our TMR procedures could provide clues as to how TMR interfered with DRM task consolidation. The timing of cue presentation could be crucial for declarative TMR. Verbal cues in experiment 2 were presented every 2 seconds, compared to 5 seconds (Fuentemilla et al., 2012; Oudiette et al., 2013; Rudoy et al., 2009) and 3 seconds for semantic sounds (Cairney et al., 2014), and jittered between 2.8-3.2secs for verbal stimuli (Schreiner et al., 2014). In this scenario, our verbal cues reactivated the associated memory, but before it could be

consolidated the next verbal cue was presented and interfered, which might prevent consolidation of words throughout the list. Reconsolidation theory suggests that reactivated memories during wake become labile (Nader & Hardt, 2009), therefore reactivated memories during NREM sleep might also become momentarily labile, and if further stimulation is received during this brief temporal window it interferes with consolidation. Alternatively, control words were replayed shortly after the cued words in experiment 2, raising the possibility that they interfered with consolidation of cued words. This seems unlikely given that lists were separated by at least 10 seconds, a far longer interval between stimuli than the successful TMR studies already discussed (e.g., Schreiner et al., 2014).

A subset of participants in both experiments reported hearing cues during the night, in which case TMR during wakefulness could have made memories labile and produced interference (Nader & Hardt, 2009). This is also unlikely to have influenced overall retrieval measures, since replay was stopped at any sign of arousal in the EEG, therefore only a very small number of words would have been reactivated in this way. Also TMR studies that tested various wake control groups fail to show any effect on consolidation (e.g., Schreiner et al., 2014). Further analysis of sleep data and a wake control group would be essential to clarify this in future work.

An additional minor point is that cues were presented in SWS during experiment 1, but during NREM sleep in experiment 2. Other TMR studies find significant effects when cueing in NREM (Rudoy et al., 2009; Schönauer et al., 2014), or solely during SWS (e.g., Rasch et al., 2007), therefore it seems improbable that this accounts for different findings across our two experiments.

Lastly, aspects of retrieval testing may have influenced our findings. We used both free recall and recognition tests, with the aim of clarifying contradictory results between the two types of study. It was crucial to test free recall prior to recognition, because the cues presented in the recognition test would heavily influence subsequent free recall, but we acknowledge that free recall may have also impacted upon recognition. However, the fact that no significant cueing effects were found for the free recall test suggests that any impact it did have on recognition would be balanced across cued and uncued conditions.

Conclusion: In trying to account for reduced veridical recognition after TMR, we first suggested that reactivation might play an active role in impairing memory for semantically-related information. However we observed no evidence of a concomitant increase in false memories, and numerous studies show reactivation to strengthen representations rather than weaken them (e.g., Rudoy et al., 2009). The second account we posited forms a stronger case, proposing that TMR somehow impaired spontaneous reactivation and consolidation processes. Unfortunately, the role for sleep in actively consolidating this type of task has yet to be convincingly established, which makes it problematic to resolve these two accounts. TMR as a technique is still under development, and ours is the only study to cue this type of semantically associated information, therefore further research is required to establish the parameters under which cues bias or interfere with different types of memory. At the very least we have established that the type of cue utilised in TMR will affect consolidation in different ways, therefore care should be taken in future work to interpret what has been reactivated via TMR, and how exactly it has influenced sleep-dependent consolidation.

Chapter 6

General Discussion

Introduction

This thesis aimed to investigate the role of post-learning reactivation in memory consolidation. By using targeted memory reactivation (TMR) alongside contemporary neuroimaging techniques during sleep, we have extended current understanding and elucidated some of the neurophysiological correlates of this plastic process.

A key assumption made throughout this thesis is that cues presented during sleep covertly reactivate specific memory representations, although we have not measured reactivation directly. The specificity of consolidation effects to cued memories in humans strongly supports this assumption (e.g., Schonauer et al., 2014). Also sleep cues bias hippocampal memory reactivation in rodents (Bendor & Wilson, 2012), providing direct support for this assumption. The evidence we present has measured the outcome of this reactivation at post-sleep retrieval in the form of behavioural improvements and neural plasticity, and these findings are now discussed in terms of how they expand our knowledge of reactivation as a mechanism of memory consolidation

This discussion will initially summarise the key findings within the thesis, before discussing the wider implications of these findings for the field of sleep and memory consolidation in relation to reactivation in 3 key areas: (1) Qualitative alteration to memories during sleep. (2) Procedural learning and neural plasticity. (3) Sleep and wakefulness. The final section will focus on specific questions regarding the methodology of TMR, before outlining questions that remain in the field and how they may be tackled in future research.

Summary of findings

In Chapter 2 we investigated behavioural outcomes for two procedural learning tasks, a crucial step towards developing a paradigm that could later be used to cue reactivation of procedural memories during sleep. Auditory cues were added to the Serial Reaction Time Task (SRTT) so that they may be used for TMR in later chapters of the thesis. We found that sequence performance was enhanced after a longer retention period containing sleep. We concluded that the task underwent spontaneous consolidation and was therefore a suitable paradigm to explore cued consolidation.

Chapter 3 utilised this paradigm to successfully cue reactivation of specific memory representations via replay of learning-associated sounds during slow wave sleep (SWS). After learning two sequences, we found that TMR of one sequence during SWS was associated with enhanced explicit sequence knowledge relative to an uncued sequence, indicating for the first time a role for reactivation in the emergence of explicit knowledge for a procedural memory. We also found correlations between this effect and the spectral power of slow oscillations during cueing, which indirectly supports the prominent role for slow-wave activity (SWA) in reactivation. Furthermore, sequence-specific procedural skill was enhanced by cueing, and this was associated with localised spindle activity.

Chapter 4 focussed upon a prominent question stemming from the findings of Chapter 3, exploring the underlying neural plasticity supporting behavioural improvement after TMR. A great deal of research has shown that the neural systems supporting procedural memories evolve over time, particularly within cortico-striatal and cortico-cerebellar networks, and we demonstrated for the first time that reactivation is instrumental in this plasticity. After undergoing the same learning and TMR procedure as Chapter 3, we show with fMRI a change in the representation of a motor memory after TMR during SWS, alongside a behavioural improvement. Increased activation was identified in bilateral caudate nucleus, left hippocampus, and left thalamus, mediated by SWS duration. Functional connectivity was also altered by TMR between caudate and hippocampus. Interestingly, REM sleep duration also modulated activation increases in key regions associated with motor learning, including the cerebellum, premotor cortex (PMC) and supplementary motor area (SMA). Thus, the behavioural enhancements after TMR of a procedural task are related to plasticity occurring in regions supporting performance, and there may be some interaction between SWS and REM sleep in that consolidation process.

Lastly, Chapter 5 aimed to build upon the qualitative alterations to memories identified after TMR in Chapter 3, by exploring whether reactivation was instrumental in another qualitative change to representations, the formation of false memories. Lists of semantically associated words were encoded prior to sleep, with each list missing a critical lure that was strongly associated with all words in the list. Half of the lists were cued during sleep with either unrelated sounds or verbal cues. We found no evidence that TMR effected false memory formation, but TMR reduced recognition of studied

items. This shows for the first time that TMR can interfere with natural consolidation of specific memories rather than enhance them as previously shown.

Reactivation and qualitative alteration to memories during sleep

Human memory is an adaptive system that does not maintain a literal record of past experiences or past behaviours, but rather is constructive in nature (Bartlett, 1936) and utilises new information to effectively guide behaviour. One way the system achieves this is through the abstraction of invariant features, and the way in which reactivation underpins these qualitative alterations to memory is a largely unexplored area of research. Moreover, prominent neural oscillations identified with EEG form the basis of how we define sleep, but the interplay between these features and memory reactivation remain poorly understood. The goal of Chapter 3 was to reactivate a specific procedural memory, to assess how reactivation impacts upon the emergence of awareness for that memory's underlying sequential structure, and to explore how that effect is dependent upon underlying neural features (i.e., slow oscillations and sleep spindles). Chapter 5 then investigated another potential consequence of this memory reorganisation in the formation of false memories, utilising the same TMR technique to explore how reactivation mediates that process. This section will examine the wider implications of each of these chapters in turn.

The emergence of explicit knowledge

Chapter 3 showed that reactivation of a procedural memory facilitated the abstraction of the underlying sequence, making it available for explicit recall after sleep. The fact that TMR was applied during SWS lends support to the proposal that reactivation of specific memories during this stage is fundamental to this form of reorganisation (Diekelmann & Born, 2010). Our finding broadly agrees with the role for reactivation posited in many models of sleep-dependent memory consolidation. The Active Systems Consolidation hypothesis suggests that memories are reactivated during sleep to be consolidated and undergo qualitative changes during transferal to the long-term store (Born & Wilhelm, 2012). This has been elaborated upon in further models which broadly characterise processes of schema formation and integration across sleep, notably in Sleep-dependent Memory Triage (Stickgold & Walker, 2013) and the

Information Overlap to Abstract (iOtA) model (Lewis & Durrant, 2011). The iOtA model details the contribution of reactivation to this process during SWS, proposing that the abstraction of underlying commonalities (schema) is achieved via the overlapping reactivation of memory traces alongside synaptic downscaling (Tononi & Cirelli, 2003, 2014). Under this framework, TMR in Chapter 3 biased these processes in favour of the cued memory and facilitated reorganisation of the memory to support explicit sequence knowledge.

The EEG analyses from Chapters 3 and 4 provide further indirect evidence for the importance of slow-wave activity (SWA) in reactivation and the emergence of explicit knowledge. Here we found a relationship between slow oscillation power during TMR and the extent of the cueing effect on explicit sequence knowledge. Active Systems Consolidation indicates that the cortically generated slow oscillations that dominate SWS orchestrate neuronal replay (Diekelmann & Born, 2010). Our finding corroborates and expands understanding of these interrelated mechanisms, by showing that TMR is most influential during high slow oscillation power, presumably because of greater potential for reactivation at this time. Questions remain as to whether TMR directly triggers increased slow oscillation power, which then impacts on the consolidation of reactivated memories, although preliminary evidence using odour cues suggests it does (Rihm et al., 2014). It is also unclear exactly where in the cascade of neural events that our cues exert their influence. Presumably they are initially processed in auditory cortex and thalamic nuclei (Dang-Vu, 2012). However the actual replay event may not occur immediately, as it was shown that cues bias hippocampal replay occurring up to 10seconds after auditory TMR (Bendor & Wilson, 2012).

There are some specific details of our explicit behavioural finding with interesting implications to our understanding of interactions between implicit and explicit memory systems. Sleep can lead to a sudden explicit insight into underlying rules (e.g., Wagner et al., 2004), or rather a gradual emergence of explicit knowledge (Fischer et al., 2006). Comparison of cued and uncued sequence knowledge in Chapter 3 support the latter, where only a small subset of participants showed ‘insight-like’ large differences between the two sequences (N=3), while the rest showed smaller differences (N=7) or no difference (N=5). This suggests that reactivation supports a gradual shift between implicit and explicit knowledge, similar to the emergence of explicit sequence knowledge after undisturbed nocturnal sleep (e.g., Wilhelm et al., 2013).

Also the cueing effects we observed for procedural skill and explicit sequence knowledge were not correlated (Chapters 3 & 4), contrary to previous findings that showed an interaction between the two (Fischer et al., 2006). This may indicate separate consolidation processes, but the very concept of explicit knowledge emerging from procedural learning indicates interacting processes during consolidation. This raises some important issues surrounding the organisation of neural and psychological systems that support implicit and explicit memory.

A pervasive view is that the motor memory system encompasses a collection of implicit skills (Squire, 1984) that are largely independent of the hippocampus, instead relying on cortico-striatal and cortico-cerebellar networks (Kreittzer & Malenka, 2008). Declarative and procedural memory are thought to be processed and retained largely within separate neural circuits (Cohen & Squire, 1980), based on dissociations between the two types of memory in neuropsychological populations (Gabrieli, Corkin, Mickel, & Growdon, 1993; Gabrieli et al., 1997). For example, medial-temporal lobe (MTL) damage is often characterised by severely impaired encoding of new declarative memories (anterograde amnesia), while retaining the ability to acquire a range of skills processed within intact motor regions such as the basal ganglia (Squire, 1992).

However, a more complex picture of partially overlapping systems is beginning to emerge. The gradual emergence of explicit knowledge after TMR (Chapter 3) supports this picture by suggesting that reactivation somehow mediates interactions between these systems. Partial interference has been observed between declarative and procedural memory consolidation (Brown & Robertson, 2007), perhaps due to interactions between striatum and MTL during learning and consolidation (Albouy et al., 2008, 2013; Poldrack et al., 2001). A number of studies also highlight a role for prefrontal cortex in these implicit/explicit interactions (Cohen & Robertson, 2011; Rose, Haider, & Büchel, 2010), and this is a region that is also strongly linked to slow oscillation generation (Massimini et al., 2004), and hippocampal replay (Weirzynski et al., 2009). Adding to this body of work, Chapter 4 found TMR to enhance striato-hippocampal functional connectivity, although a limitation of this study is that the explicit cueing effect was not replicated at the group level. Procedural and declarative consolidation mechanisms must interact to some degree during sleep-dependent consolidation, and this could underscore the observed transfer between implicit and explicit memory, mediated by interactions between MTL, PFC and basal ganglia. The

null correlations we observed between explicit and procedural measures (Chapters 3 & 4) may suggest that TMR influences aspects of the two processes differently, but does not necessarily mean they are entirely independent of one another.

A further challenge to our understanding of how reactivation relates to implicit/explicit interactions in memory consolidation is the way they are measured. The 'explicit' test (Chapters 3 & 4) was designed to separate sequence performance, which relies heavily on implicit movements and familiarity with visual cues, from explicit sequence knowledge. Others have used a generation task (Drosopoulos et al., 2011; Fischer et al., 2006) or pointing at cue positions (Wilhelm et al., 2013), where implicit elements of performance could potentially interfere. Our explicit test performed with pen and paper eliminated many of these implicit factors. However, we acknowledge that this behavioural measurement misses some important features of explicit memory, most notably the subjective experience that separates explicit from implicit memory. It has been argued that any account of consciousness and memory must utilise a range of strategies to account for the four key dimensions of consciousness and memory: cognitive, neural, behavioural and subjective (Paller, Voss, & Westerberg, 2009). Thus, our explicit measure accounts for the behavioural aspect, with some indication of its neural correlates during sleep. To fully account for these dimensions of explicit memory, future studies should acquire introspective accounts of sequence knowledge (subjective). Insight to neural correlates could be gained via exploration of event-related potentials (ERP's) during encoding, consolidation and retrieval, alongside behavioural measures, enabling formation of a more complete picture of the underlying cognitive processes.

The formation of false memories

The models described at the beginning of this section encompass the generalisation of episodic memories proposed to induce false memories during sleep. The principles of implicit rule abstraction can also be applied to the abstraction of commonalities within episodic memories, enabling generalisation of new memories based on existing schema This ability is fundamental to such things as category learning as well as false memories. Evidence for sleep-dependent processing in this form of declarative reorganisation is mixed, although there is some evidence that reactivation facilitates some forms of generalisation (Sterpenich et al., 2014).

The experiments reported in Chapter 5 addressed these issues, perhaps generating more questions than they answered. Using the Deese-Roediger McDermott (DRM) paradigm with TMR during NREM sleep, we found no evidence that reactivation instigates the formation of false memories during sleep. Previous work has created a mixed picture of increases (e.g., Payne et al., 2009) and decreases in false memories across sleep depending on retrieval tests (e.g., Fenn et al., 2009), alongside little change to neural representations of false memories associated with sleep (Darsaud et al., 2012). However, there were indications that our TMR procedure interfered with the established enhancing effect of sleep on declarative memory (e.g., Plihal & Born, 1997). This suggests TMR was not biasing consolidation in the expected way, therefore its influence on false memory is also questionable. Interestingly, a very recent study demonstrated that TMR during REM sleep of newly learned faces increases false alarm rates at retrieval (Sterpenich et al., 2014), suggesting generalisation of the learned face stimuli. REM sleep is proposed by some to be instrumental to this form of generalisation (Stickgold & Walker, 2013), therefore future research should utilise TMR during REM sleep to explore this further.

Summary

The transformation of memories after encoding demonstrates a flexible system that facilitates a range of adaptive behaviours, and our data (Chapters 3) suggest that sleep and memory reactivation during SWS are instrumental in that process. A remaining area of contention regards the specific sleep stages supporting these processes. Sleep-dependent Memory Triage (Stickgold & Walker, 2013) suggests many forms of integration and generalisation are REM sleep dependent, while SWS is the focus of Active Systems Consolidation (Diekelmann & Born, 2010) and the iOtA model (Lewis & Durrant, 2011). Chapter 3 and 4 clearly support the abstraction of explicit sequence knowledge as being SWS-dependent. However, the failure in Chapter 5 to show similar qualitative change to declarative memories after TMR during SWS suggests a more complex picture. The principles of rule extraction, integration and generalisation might share similarities across many types of declarative and procedural memories, but those principles cannot currently be accounted for within a single sleep stage or process.

Reactivation, procedural learning, and neural plasticity

There has been some controversy with regard to the active enhancement of procedural memories during sleep, with the suggestion that controlling certain pre-sleep factors such as fatigue eliminates 'delayed learning' effects. Furthermore, behavioural changes to procedural memories after sleep are associated with plastic changes within motor memory networks in the brain, but it remains unclear whether reactivation facilitates that plasticity. This section will firstly outline the behavioural improvements we observed to procedural skill after TMR (Chapters 3 and 4), before shifting focus to the plasticity induced by TMR (Chapter 4) and how that has bridged our understanding of reactivations' reorganising abilities within motor memory systems.

Behavioural improvement of procedural memory

Walker (2005) proposed a neurocognitive model of procedural memory to account for enhanced motor sequence learning (MSL) after sleep, which posited an enhancement consolidation phase of 'delayed learning'. This active process was questioned after it was shown that controlling for circadian effects, learning effects, and response inhibition (slowed responses after accumulation of fatigue, interference or attentional factors) eliminated such enhancements (Rickard et al., 2008; Brawn et al., 2010). While these sleep effects may not represent immediate enhancement per se, ample evidence suggests representations are stabilised (Debas et al., 2010; Kuriyama et al., 2004; Walker et al., 2003) and transformed to allow more rapid re-learning (e.g., Spencer et al., 2006). Prior work using TMR suggested reactivation to underlie this process (Antony et al., 2012; Schonauer et al., 2014), and findings from chapters 3 and 4 support this. In these studies cued and uncued memories were matched for fatigue, circadian factors and response inhibition and yet TMR was successful in biasing consolidation of the cued memory, further indicating an active consolidation process. With regard to delayed learning, our data do not support the immediate enhancement characterised by Walker (2005), given that even the "immediate" blocks that showed a cueing effect in Chapter 4 represent a measure averaged across 132 trial of re-learning. More recently, sleep has been suggested to enhance the ordinal representation of a SRTT sequence (e.g., '2' is the second ordinal position in 1-2-4), while transitions between items only improve with practice (e.g., '2' follows '1' in 1-2-4) (Cohen &

Song., 2014), therefore TMR combined with similar analyses could establish whether reactivation enhances this ordinal representation.

Neural plasticity of procedural memory

The central finding of Chapter 4 was the enhancement of activity in caudate and hippocampal regions after TMR, coupled with enhanced connectivity between these regions. Importantly, this is consistent with regions showing increased activation after sleep relative to a period of wake (Albouy et al., 2013; Walker, 2005), which suggests TMR biased the plasticity that occurs during normal sleep. Crucially, neural plasticity was accompanied by behavioural improvements, which suggests the plasticity induced by reactivation supports improved performance. A recent model proposes that sleep reorganises the activity and functional interactions between hippocampal, striatal, and prefrontal cortex to facilitate overnight performance enhancements for motor sequence memories (Albouy et al., 2013), and our findings extend this by suggesting reactivation underlies this plasticity.

This TMR related plasticity was also modulated by slow-wave sleep duration, indicating that ongoing SWS is crucial to establish plastic changes in favour of the cued memory. Others have shown that the behavioural effects of TMR rely upon on-going SWS (Cairney et al., 2014), and that a longer period of SWS can provide the same consolidation as a shorter period containing TMR (Diekelmann et al., 2011). We show that the underlying neural plasticity also relies upon on-going SWS. This is consistent with correlations from Chapters 3 and 4, where cueing provided larger behavioural effects if slow oscillation power was greater. These findings agree with Active Systems Consolidation (Diekelmann & Born, 2010), demonstrating links between slow-wave activity, behavioural and neural outcomes.

An unexpected result of Chapter 4 was the modulation of TMR related increases in activity by REM sleep, notably in bilateral cerebellum, left PMC and SMA. This is consistent with the Sequential Hypothesis (Giuditta et al., 1995), which suggests REM sleep later in the night completes consolidation processes initiated in earlier SWS. REM sleep might provide synaptic consolidation of memories cued previously during SWS (Diekelmann & Born, 2010). The Dual Process Hypothesis (Plihal & Born, 1997) proposes SWS to support hippocampal-dependent declarative memories and REM sleep supports hippocampal-independent procedural memories. Under this framework, the

association between TMR related plasticity within key motor memory regions and SWS (caudate and hippocampus) or REM sleep (cerebellum, PMC and SMA) may relate to consolidation of declarative and non-declarative elements of the SRTT respectively. If REM sleep does support hippocampal-independent consolidation, the cerebellum increases we observed are particularly relevant given that this region is considered to support non-declarative functions (Manto et al., 2012). The neurophysiology of REM sleep is certainly receptive to plasticity processes, with the presence of ponto-geniculo-occipital waves that relate to cellular processes involved in plasticity (Datta, 2000), and the upregulation of immediate early gene activity localised to learning related brain regions (Ulloor & Datta, 2005). Interestingly studies utilising PET have shown brain regions involved in implicit SRTT learning are reactivated during REM sleep but not NREM sleep (Laureys et al., 2001; Maquet et al., 2000; Peigneux et al., 2003). Thus, although no TMR occurred during REM sleep in Chapter 4, the observed REM sleep modulated plasticity suggests that TMR in earlier SWS periods may have triggered a process that continued during subsequent REM sleep periods.

In Chapter 4 we observed the final result of plasticity induced by reactivation, so it is interesting to consider the type of neural reactivation during sleep that induced this reorganisation. The neural substrates of declarative spatial memories have been explored with TMR, identifying enhanced hippocampal (Rasch et al., 2007), and parahippocampal activity during cueing (van Dongen et al., 2012), regions that were closely associated with learning. This has not yet been investigated with procedural memory, but logic dictates that we would expect reactivation to occur within the same regions involved in performance of the SRTT, and future work should establish how these regions interact during TMR to facilitate consolidation of procedural memories. Most importantly we must properly characterise the role of the hippocampus across the life-time of procedural memories, given its strong association with reactivation, but also its important role in learning and consolidation of tasks such as motor sequence learning (MSL), which were previously characterised as hippocampal-independent (e.g., Rasch et al., 2007).

Summary

TMR has allowed us to uncover the mechanistic role of reactivation in sleep-dependent consolidation of procedural memories, as well as the brain plasticity underlying this process. Beyond this initial consolidation period immediately following acquisition, skill memories undergo slower incremental improvements and a shift from explicit to implicit 'automatized' performance (Doyon & Benali, 2005), and the role for reactivation in this later transition remains to be explored. Lastly, we should not assume from our data that other procedural tasks share the same association with reactivation, especially for non-hippocampal tasks. A goal of future research is to uncover the complex relationship between reactivation and consolidation of many different forms of procedural learning.

Reactivation in sleep and wakefulness

There is now overwhelming evidence that slow-wave sleep is critical to consolidation of many declarative and procedural memories, via the selective reactivation of memory traces. The experiments reported in Chapters 3 and 4 support this prominent role for reactivation during SWS, although the finding of TMR related plasticity modulated by REM sleep (Chapter 4) hints at potential interactions between these stages during consolidation. Offline consolidation processes have also been associated with stage 2 sleep and wakefulness. This section assesses the contribution of these separate sleep stages and wakefulness to memory consolidation, to determine the potential role of reactivation across these separate states of consciousness in light of our own findings.

Slow-wave sleep and memory reactivation

The observed relationship between slow oscillation power during TMR and behavioural cueing effects (Chapters 3 & 4) fit neatly with Active Systems Consolidation, which posits slow oscillations to orchestrate hippocampal neuronal reactivation via thalamo-cortical spindle activity (Diekelmann & Born, 2010). A large number of studies show an association between slow-wave activity (SWA) and consolidation of declarative (e.g., Marshall et al., 2006) and procedural memory (e.g., Tamaki et al., 2013). In addition, the modulation of TMR related plasticity by SWS (Chapter 4) further implicates this

sleep stage in the neural reorganisation of representations. It should be noted however that the Synaptic Homeostasis Hypothesis (SHY) (Tononi & Cirelli, 2003, 2014) is potentially compatible with this finding. This model suggests SWA drives a process of global downscaling that selectively preserves strongly connected memory traces, and reduces the signal-to-noise ratio in preserved traces. Under this framework, TMR is thought to protect the cued memory from downscaling (Nere, Hashmi, Cirelli, & Tononi, 2013). Thus, the uncued sequence would have been downscaled and the cued sequence preserved, which might account for observed behavioural and neural changes. However, SHY does not fully account for the dynamic changes to activity and connectivity observed within motor networks after sleep (for review see Albouy et al., 2013), therefore components of both systems consolidation and synaptic downscaling may account for our findings.

Chapters 3 and 4 also found fast spindles close to primary motor cortex in the learning hemisphere were correlated with the procedural cueing effect, which builds upon previous findings of their role in consolidation (Fogel & Smith, 2006; Nishida & Walker, 2007), and specifically fast spindles (Barakat et al., 2011), by suggesting they interact with reactivation during TMR. It should be noted that spindles, like SWA, have also been associated with declarative learning in a number of studies (Gais, Mölle, Helms, & Born, 2002; Schabus et al., 2004) therefore both features appear to support consolidation across many forms of memory.

Interestingly, we also found a dissociation between spindles and slow oscillations (Chapter 3 and 4), where slow oscillations were correlated with explicit sequence knowledge, and spindles were associated with procedural skill. This was unexpected, given that both features have been associated with consolidation of procedural and declarative memories, and such a dissociation has not previously been observed with MSL. This relationship may be specific to TMR, perhaps showing differential effects of auditory cues on the two features. We found no evidence that cues enhanced SWA or spindles, but our procedure was not specifically designed for this purpose. Others have shown that TMR of declarative memories with odours can enhance SWA (Rihm et al., 2014) and localised spindles (Cox et al., 2014). Further work is needed to properly characterise how auditory TMR influences these features, since sound stimulation alone is known to effect oscillatory activity (Ngo et al., 2013), while spindles transiently block incoming auditory stimuli (Dang-Vu et al., 2010).

REM sleep and memory reactivation

REM sleep was not directly manipulated with TMR in this thesis, although as already discussed we did show TMR related plasticity was modulated by REM sleep in some regions, supporting interactions between the two stages in consolidation (Diekelmann & Born, 2010; Giuditta et al., 1995). The evidence for reactivation occurring during REM sleep is less consistent than it is for SWS. Spontaneous reactivation during REM sleep has been observed in rodents (Louis & Wilson, 2001) and humans (Laureys et al., 2001; Maquet et al., 2000; Peigneux et al., 2003), but it is fair to say this is outweighed by examples of similar processes during SWS (e.g., Peigneux et al., 2004; Wilson & McNaughton, 1994). In addition, declarative memory consolidation has been observed in the virtual absence of REM sleep (e.g., Plihal & Born, 1997), and TMR studies without REM sleep still demonstrate cueing effects for declarative memories (Diekelmann et al., 2011; Antony et al., 2012). Also, other attempts to cue REM sleep reactivation with odours have been unsuccessful (Cordi et al., 2014; Rasch et al., 2007). However, TMR during REM sleep was recently shown to increase false alarms in recognition testing, suggesting reactivation during this stage may play a part in the generalisation of memories (Sterpenich et al., 2014). This may account for our failure to induce false memories with TMR during NREM sleep (Chapter 5), because REM sleep is more crucial to the generalisation of episodic memories that lead to false memories. Further support for this potential role of REM sleep reactivation is provided by studies using the compound remote associates task (RAT) (Cai et al., 2009; Sio et al., 2012). This task entails studying three unrelated words to uncover the single common associate that links them, and the emergence of this associate after a nap was found to be REM-dependent (Cai et al., 2009). Arguably this type of generalisation is very similar to the formation of false memories in the DRM task, and an obvious extension of our work would be to attempt cueing the DRM during REM sleep.

Wakefulness and memory reactivation

There is a wealth of evidence for wake reactivation to occur in rodents (O'Neill et al., 2010), but its role in consolidation is debated. TMR during wakefulness failed to influence any measure of consolidation (Chapter 3), but it does influence consolidation under some circumstances. Diekelmann et al. (2011) showed that reactivating spatial

memories with an odour during wakefulness makes them vulnerable to interference, in contrast to the stabilising effect of reactivation during SWS. This is consistent with Reconsolidation Theory, which states that reactivated memory traces are destabilised during wakefulness and must be reconsolidated to avoid being altered or erased (Nader & Hardt, 2009). However others have shown wake reactivation to improve some spatial memories (Oudiette et al., 2013), and listening to learned piano melodies while awake can improve performance (Lahav, Katz, Chess, & Saltzman, 2013). A challenge to TMR studies that include wake control groups is to surmise whether cues instigate conscious rehearsal or unconscious consolidation processes. For example, verbal cues (Chapter 5) are often so closely associated with the learning experience that it is difficult to present them to awake participants without them gaining additional learning, rather than the intended covert reactivation.

Other sleep stages and memory reactivation

The remaining sleep stages 1 and 2 have received limited support for a role in reactivation and consolidation. Stage 1 hypnogogic dreams have been associated with the replay of the computer game Tetris (Stickgold, 2000), although this has not been convincingly linked to consolidation of the game per se. Stage 2 has been associated with procedural memory consolidation (Maquet et al., 2003; Walker et al., 2002, 2003) and also spindles that occur predominantly during stage 2 have been associated with consolidation of both declarative (e.g., Tamminen et al., 2013) and procedural memory (e.g., Nishida & Walker, 2007). A recent review also points out that reactivation of neuronal ensembles during 'SWS' identified in rodents most likely represents a homologue of light sleep reactivation in humans (i.e., stages 1 and 2), because rodent research classifies all of NREM sleep as SWS (Genzel et al., 2013). They also suggest the network physiology of stage 2 is more conducive to memory consolidation via hippocampal reactivation. On a more general note, Stage 2 accounts for nearly half the amount of naturally occurring nocturnal sleep, therefore it is crucial for future work to explore whether reactivation during this stage also supports memory consolidation.

Summary

Our findings provide further indication that the neurophysiological substrates of SWS are crucial to the reactivation and consolidation of many forms of memory. However we must acknowledge our methodological bias of only attempting TMR of the SRTT during SWS and wakefulness, therefore we cannot speak directly to the specificity of reactivation to SWS only. There are indications in the literature that TMR with sounds during REM sleep is effective under certain conditions, therefore further research using this approach will assist in our understanding of the interactions between SWS, REM sleep and reactivation in memory consolidation.

TMR methodology and future directions

The technique of targeted memory reactivation has contributed a great deal to our understanding of how reactivation undergirds sleep-dependent memory consolidation. It is important to acknowledge that this is a relatively young and untested technique, and there are still a great number of questions regarding how it works, what exactly it is reactivating, its limitations, potential practical applications, and other ways it might help answer questions regarding reactivation as a mechanism for different forms of memory consolidation.

When does TMR bias or interfere with consolidation?

An assumption made by TMR studies is that cues are enhancing or biasing natural consolidation processes. In most instances this is well supported, based upon observations of sleep-dependent consolidation of a task, alongside the manipulation of consolidation by TMR of the same task. However, Chapter 5 indicated that different features of semantically associated episodic memories can be enhanced or interfered with by TMR during NREM sleep depending on the cue used. The musical note cues biased consolidation of spatial associations of studied words, while conversely the verbal cues interfered with episodic memory of studied words. The fact that interference was specific to the cued words suggests that individual representations were reactivated in some way, but for unknown reasons this reactivation impaired consolidation. One other study has demonstrated forgetting after TMR, where extinction of a fear memory

was achieved by presentation of a contextual odour during SWS (Hauner et al., 2013). By contrast, odour cues during NREM sleep have also enhanced fear memories when using subtly different procedures (Rolls et al., 2013). This demonstrates a complex relationship between cues and the learning material, and we have yet to determine the conditions under which different tasks or cueing procedures bias or interfere with consolidation.

Under which states of consciousness does TMR work?

TMR has effectively enhanced consolidation of specific memories when applied during SWS (e.g., Rudoy et al., 2009) or throughout NREM (e.g., Schreiner et al., 2014), while during REM sleep it may support generalisation of memories in certain circumstances (Sterpenich et al., 2014). The effect of TMR during wakefulness has produced mixed findings (Diekelmann et al., 2011; Oudiette et al., 2013). An important related question is what exactly can be concluded when TMR fails? This is most pertinent for interpreting findings for wake control groups utilised in TMR sleep studies, where cues are usually presented during performance of a distractor task in order to prevent active rehearsal. Also, this distraction limits conscious processing of the cues which is considered a rough homologue to the unconscious processing of cues during SWS. When TMR during wakefulness has no effect on consolidation (e.g., Chapter 3), it could mean that consolidation of the task is sleep specific, but equally it could be that cues presented during wakefulness cannot influence reactivation because of the different ways sensory information is processed during wakefulness and sleep (Dang-Vu, 2012). Indeed, cues were shown to influence hippocampal replay events in rodents during sleep (Bendor & Wilson, 2012), but this has yet to be demonstrated during the replay events that are also common to wakefulness (O'Neill et al., 2010), therefore TMR may be an inappropriate method to modify these processes.

How does TMR interact with the selective mechanism of sleep-dependent consolidation?

Models of sleep-dependent consolidation posit a selective mechanism that must tag memories either during or after encoding in the awake state for subsequent processing during sleep, based on various factors such as reward, emotion and awareness (Stickgold & Walker, 2013). It is unclear whether TMR overrides this selective mechanism, or whether the two interact and TMR directs resources toward memories

that have already been selected. A recent study may indicate the former, whereby TMR rescued low value memories for consolidation that presumably would not have been tagged previously (Oudiette et al., 2013). Awareness of regularities prior to sleep also appears to tag procedural memories for consolidation (Song & Cohen, 2014), even if the preceding encoding was entirely implicit (Drosopoulos et al., 2011), and all published TMR studies included this explicit awareness prior to sleep. Similarly in chapters 3 and 4 we ensured participants were aware that SRTT stimuli followed a sequence at encoding, therefore it may be that the awareness of regularity our participants possessed was a necessary prerequisite for TMR to be successful. Memories may also be tagged for directed forgetting during sleep (Saletin et al., 2011; Stickgold & Walker, 2013), raising the possibility that verbal cues in Chapter 5 somehow influenced this process, leading to forgetting of cued words. There are a number of new insights that may come from TMR in conjunction with these 'tagging factors'. For example, fMRI could be used to examine the neural correlates of tagged and untagged memories that are cued or uncued, to reveal whether TMR influences plasticity in the same way that the selective mechanism influences plasticity when it selects one memory over another.

What are the practical applications of TMR?

Throughout this thesis, there has been a tight focus on TMR as a technique to investigate reactivation and plasticity, but there are a number of potential practical applications worth mentioning. A recent study by Schreiner and colleagues (2014) provides the most tangible evidence that TMR could be used to assist learning. Foreign vocabulary was learned before being reactivated during sleep with verbal cues, enhancing vocabulary learning over and above normal consolidation levels. Prior to this study, TMR was thought to bias a finite consolidation resource (e.g., Antony et al., 2012) rather than provide "gains" as in this study. The potential "costs" of TMR to uncued memories must still be established, but if they are found to be minimal then the technique could be used to boost memory in ageing for example. An additional consideration is whether the benefits of cueing outweigh the potential disruption they cause to sleep. We found many participants were highly sensitive to auditory stimulation, particularly with repeating notes or verbal cues (Chapter 5), therefore odour cues may be more practical. Lastly, the benefits of cueing over a single night are relatively small, therefore future studies should establish the cumulative effects of TMR over many days or weeks.

There is also an indication that TMR could be used to treat post-traumatic stress disorder or phobias. As mentioned earlier, Hauner et al. (2013) extinguished conditioned fear memories with TMR, raising the possibility that traumatic episodes could be extinguished in PTSD, although the precise conditions under which extinction or enhancement of memories is induced must still be clarified (Rolls et al., 2013; Oudiette et al., 2014). There are also ethical considerations, for example given the vulnerability of people while asleep there is a potential for misuse of the technique (Diekelmann, 2014). A great deal of further work is needed before TMR is used in any practical context, to establish unintended effects, and to understand the functional role of reactivation with consolidation of different types of memory.

How can TMR be used in future to explore other forms of sleep-dependent memory consolidation?

Our replication of cueing effects utilising the SRTT in Chapters 3 and 4, and also by Schönauer and colleagues (2014), means that it is a well-established paradigm to take forward and explore the specifics of TMR via subtle design variations. This final sub-section will outline some of these potential manipulations, and suggest additional ways TMR could be used to tackle remaining questions in the field regarding the role of reactivation in various forms of memory consolidation.

TMR and the relationship between cues and learning: The auditory and odour cues used previously in TMR are effective only under certain conditions. TMR using continuous environmental sounds as cues during NREM sleep was found to have no influence on declarative memory for word-pairs (Donohue & Spencer, 2011). Also odour cues do not appear to influence REM sleep reactivation of declarative memories (Cordi et al., 2014; Rasch et al., 2007), but sound cues have been effective in one study (Sterpenich et al., 2014). Notably, odour cues during SWS failed to reactivate MSL (Rasch et al., 2007), while by contrast auditory cues have been successful with similar procedural tasks in prior work (Antony et al., 2012; Schönauer et al., 2014) and this thesis (Chapters 3 & 4). This suggests that the close temporal relation between visuo-motor learning and cues is a necessary prerequisite to stimulate reactivation of procedural memories.

To further establish which types of auditory cue are optimal for TMR studies, some subtle variations of stimulus timing could be attempted. We found SRTT cueing was

successful despite the fact that sleep replay tones were spaced evenly apart, while cues during learning contained inconsistent gaps between stimuli that depended upon response speed. These timing differences between sleep replay and the learning experience could be systematically altered to determine the conditions under which TMR is most effective. Also related to timing, the responsiveness of the brain to auditory stimulation during NREM sleep is less consistent or even absent during spindles and during the downward slope of slow oscillations (Schabus et al., 2012), suggesting that timing cues to coincide with the upward slope might optimise TMR. Similarly, sound stimulation at a slow oscillation frequency (0.75Hz) can enhance slow oscillations (Ngo et al., 2013), therefore TMR at this frequency might also enhance cueing effects. Additionally, Chapter 5 demonstrated that the relationship between cues and the learning material can dictate whether consolidation will be enhanced or interfered with by TMR, therefore future studies should explore the effects of a range of cues in relation to a single task.

A last remaining question regards the amount of cueing that is necessary in order to bias memory consolidation. Converging evidence from our lab (Cairney et al., 2014), and this thesis (Chapters 3 & 4), suggest TMR biases processes that continue throughout SWS. Admittedly the experiments in this thesis cued extensively throughout SWS to maximise EEG data points, but the suggested triggering of an on-going process may indicate that a small amount of cueing at the beginning of nocturnal sleep would be sufficient to bias consolidation throughout the whole night.

TMR and consolidation of implicit memory: To date, all TMR studies in humans have involved hippocampal-dependent tasks containing a spatial component (e.g., Rudoy et al., 2009), and/or learning of associations between items (Schreiner et al., 2014), and the same is true of experiments within this thesis. This provides strong evidence for reactivation being hippocampal-dependent, but to our knowledge there are no published attempts to cue entirely non-hippocampal tasks. Some hippocampal-independent procedural tasks show sleep-dependent improvements alongside indications of spontaneous reactivation, such as texture discrimination (Karni et al., 1994; Yotsumoto et al., 2009). Such memories may undergo a different type of processing during sleep that has yet to be properly characterised, and potentially this processing could be influenced by TMR. In addition, implicit/explicit distinctions could be explored with TMR of implicit probabilistic SRTT sequence learning. Learning in

this instance is also hippocampal-dependent (Schenden et al., 2003), therefore could be compared to experiments in this thesis to highlight differences between implicit and explicit consolidation mechanisms of the same task. TMR of such tasks during SWS and REM could directly test the idea that each stage is responsible for declarative and procedural consolidation respectively (Plihal & Born, 1997).

TMR and integration of memories: A central tenet of Active Systems Consolidation (Diekelmann & Born, 2010) is that reactivation drives the integration of new memories within existing cortical networks, but this has not been experimentally demonstrated with TMR. Manipulation of the SRTT design could explore properties of integration in the procedural memory domain. Previous work indicates that most cues are processed sequentially during TMR, since cue effects can be specific to portions of a sequence (Schönauer et al., 2014), raising the possibility that replaying elements of learned sequences during sleep might facilitate their integration. Also, compelling evidence for sleeps role in integration is provided from studies showing that newly integrated words interfere with the processing of semantic and lexical neighbours (Dumay & Gaskell, 2007; Tamminen et al., 2010; Tamminen et al., 2013), therefore TMR of these tasks is an obvious next step. Finally, TMR is most effective with spatial tasks, therefore an effective way to explore declarative integration might involve learning new locations to integrate into a known map (schema), and a newly learned map (non-schema), to investigate how TMR of these locations influences their integration across sleep.

TMR and generalisation of memories: Chapter 5 investigated the generalisation of episodic memories in relation to existing semantic knowledge, and as already suggested this should be explored in future work with TMR during REM sleep (Sterpenich et al., 2014). Similar principles have also been applied to procedural memories with the generalisation of performance to the opposite hand (Cohen et al., 2005; Witt et al., 2010). Sleep is thought to promote consolidation of the goal-based representation (i.e., abstraction of the general procedural sequence or schema) separate from the required movements (Robertson, 2009), but there is currently no direct evidence that reactivation plays a part in this form of motor generalisation. Thus, a very simple manipulation would be to repeat the procedure of Chapter 3 but retest participants using the opposite hand to learning.

TMR and the reorganisation of memories to enhance creativity: A fascinating area that has been touched on with TMR is sleeps role in creativity, in a study where an odour presented throughout the night was associated with more creative solutions to the task it reactivated (Ritter et al., 2012). This type of creative problem solving has been associated with REM sleep with the Remote Associates Task (RAT) (Cai et al., 2009; Sio et al., 2013), therefore TMR of these tasks during REM sleep with sounds could help clarify if this is the case.

TMR and the neural signature of reactivation: While the neural activity of reactivation has been accurately characterised in rodents via single cell recording (Wilson & McNaughton, 1994), a remaining challenge is to characterise the same activation in humans using neuroimaging techniques. Our correlations between cueing effects and slow oscillations/spindles during TMR (Chapters 3 & 4) provide partial evidence for the neural correlates of reactivation, and others show similar relationships (Antony et al., 2012; Cox et al., 2014; Rihm et al., 2014), but a crucial goal is to link the neural activity of a memory during wakefulness with subsequent reactivation during sleep, to be certain that memories really are 'replayed' during sleep in humans.

Pattern analysis techniques provide the clearest way forward to really image the fingerprint of a memory being reactivated during sleep. Multi-voxel pattern analysis (MVPA) has been used to predict the content of hypnogogic dreams based on observed brain activity (Horikawa et al., 2013), but this was not investigated in relation to memory consolidation. The activity patterns associated with a paired-associate learning task were shown to spontaneously reactivate during wakeful rest and sleep, and this reactivation correlated with subsequent memory (Deuker et al., 2013). A drawback of these approaches is the problems associated with sleeping in the noisy MRI scanner environment, therefore a remaining aim is to use EEG classifiers to identify reactivation during more normal sleep, and also utilise TMR to inform the time at which reactivation is most likely to occur. As outlined in Chapter 2, this possibility was a key consideration in design of studies in this thesis, and a slowed version of the SRTT is currently being utilised for this aim in a separate project. A goal for future research should be to apply such classifiers to natural sleep to establish whether the underlying neural mechanisms for TMR are the same as those for spontaneous reactivation.

Conclusion

This thesis began by asking one of the most challenging remaining questions of human biology: why do we sleep? By using targeted memory reactivation in concert with modern neuroimaging techniques we have shed light on the answer to a small part of that question. We have affirmed the idea that sleep is crucial to stabilise, enhance and reorganise memories within the brain, and provided converging evidence for the reactivation of specific memory traces during slow-wave sleep as a central mechanism underlying that process. Our findings show that TMR is a powerful technique for exploring the way in which reactivation during sleep can alter post-sleep brain function, and future work should use it to explore the role of reactivation within other memory networks.

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Appendix A: DRM list words and their associated critical lures utilised in Chapter 5, adapted from Roediger et al. (2001). Lists are numbered in order of their likelihood of generating false recall. Bold words are critical lures.

1	2	3	4	5	6	7	8
Window	**Doctor**	**Smell**	**Chair**	**Smoke**	**Sweet**	**Rough**	**Needle**
door	nurse	nose	table	cigarette	sour	smooth	thread
glass	sick	breathe	sit	puff	candy	bumpy	pin
pane	medicine	sniff	legs	blaze	sugar	road	eye
ledge	health	aroma	seat	pollution	bitter	tough	sewing
sill	hospital	hear	couch	ashes	good	sandpaper	sharp
house	dentist	see	desk	cigar	taste	jagged	point
open	physician	nostril	sofa	chimney	tooth	coarse	thimble
curtain	ill	whiff	cushion	fire	nice	uneven	haystack
frame	patient	scent	stool	tobacco	honey	rugged	hurt
view	Surgeon	reek	bench	pipe	chocolate	gravel	knitting

9	10	11	12	13	14	15	16
Anger	**City**	**Soft**	**Cup**	**Cold**	**Mountain**	**River**	**Slow**
mad	town	hard	mug	hot	hill	water	fast
fear	crowded	light	saucer	snow	valley	stream	lethargic
hate	capital	pillow	tea	warm	climb	lake	stop
rage	streets	plush	measuring	winter	summit	Thames	snail
temper	subway	loud	coaster	ice	top	boat	cautious
fury	country	cotton	handle	wet	molehill	tide	delay
wrath	London	fur	coffee	chilly	peak	swim	traffic
happy	village	touch	drink	heat	plain	flow	tortoise
fight	metropolis	fluffy	plastic	weather	glacier	run	hesitant
hatred	urban	feather	sip	freeze	goat	bridge	sluggish

17	18	19	20	21	22	23	24
spider	**car**	**foot**	**pen**	**black**	**music**	**girl**	**rubber**
web	truck	shoe	write	white	note	boy	elastic
insect	bus	hand	fountain	dark	sound	doll	bounce
bug	train	toe	leak	cat	piano	female	gloves
fright	automobile	kick	quill	charred	sing	young	tire
fly	vehicle	sandals	felt	night	radio	dress	ball
arachnid	drive	walk	scribble	funeral	band	pretty	eraser
crawl	ford	ankle	crayon	colour	melody	hair	springy
tarantula	race	arm	tip	grief	horn	niece	foam
poison	keys	boot	letter	blue	concert	dance	sole
bite	garage	sock	marker	death	instrument	beautiful	latex

25	26	27	28	29	30	31	32
bread	**flag**	**justice**	**shirt**	**high**	**army**	**man**	**lion**
butter	banner	peace	blouse	low	Navy	woman	tiger
food	symbol	law	sleeves	clouds	rifle	husband	circus
eat	anthem	courts	trousers	up	draft	uncle	jungle
sandwich	pole	judge	tie	tall	military	lady	tamer
jam	wave	right	button	tower	marines	mouse	den
milk	raised	liberty	shorts	jump	march	male	cub
flour	national	government	iron	above	infantry	father	Africa
dough	checkered	jury	polo	building	captain	strong	mane
crust	emblem	truth	collar	cliff	combat	friend	cage
slice	sign	blind	vest	sky	war	beard	feline