Exploring Methods to Study the Relationship Between Motor and Declarative Memory Consolidation, Generalization, and Sleep Outside of the Laboratory.

A thesis submitted for the degree of Doctor of Philosophy.



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Abstract

Sleep has proven to be an important component of memory consolidation and generalization. Advancing our knowledge of the relationship between sleep and these important neural computations is advantageous to both basic and clinical neuroscience. Research suggests, however, that there may be important differences between sleep in the lab and in a naturalistic, at-home setting. In this thesis, we test the feasibility of conducting research into these topics remotely, in participants' homes. In chapter 2, we test online motor (serial reaction time task; SRTT) and declarative (paired-associates) memory tasks to test whether they can be learned in an at-home setting and whether they benefit from sleep compared to wake. We found that while these memory tasks could be learnt remotely and online, they showed no significant benefit of sleep over wake. In chapter 3, we asked whether task improvement correlated with features of sleep. Using a portable, low density electroencephalography (EEG) device (Dreemband) we explored the covariation between task performance and sleep features as measured by the EEG. We found a significant covariation between spindle-Slow Oscillation coupling and improvement on the SRTT, but no significant relationships between sleep features and performance on the paired-associates task. In chapter 4, we tested whether an underlying sequence structure could be generalized from one online task to another and whether this benefitted from sleep. We found no evidence of generalization and so developed a novel task to probe sleep-associated generalization in the future. In summary, this thesis established online motor and declarative tasks which can be learned remotely, it found that sleep features collected at-home mirror in-lab findings and set up a generalization task that can be learned remotely, although whether it benefits from sleep is yet to be tested.

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Abbreviations

ACC	Accuracy
CB	Contextual Binding (Theory)
CCA	Canonical Correlation Analysis
EEG	Electroencephalogram/Electroencephalography
GLM	Generalized Linear Model
nREM 1,2,3 /N1,2,3	Non-REM stages 1,2,3
CCA	Canonical Correlation Analysis
GLM	Generalized Linear Model
M1	Primary Motor Cortex
MPFC	Medial Prefrontal Cortex
NIBS	Non-Invasive Brain Stimulation
PSG	Polysomnograph/Polysomnography
RT	Response time
SHY	Synaptic Homeostasis Hypothesis
SO	Slow Oscillation
SRTT	Serial Reaction Time Task
SSL	Sequence-Specific Learning
SSS	Stanford Sleepiness Scale
SWA	Slow Wave Activity
tDCS	Transcranial Direct-Current Stimulation
TST	Total Sleep Time
TMR	Targeted Memory Reactivation

1. General Introduction

It is an often-touted fact that we spend approximately a third of our lives asleep. The fact that we spend such a sizeable portion of our time in this vulnerable and seemingly idle state suggests that it confers an evolutionary benefit. This notion is underlined by the fact that sleep is not unique to humans but rather is highly evolutionarily conserved (Lesku & Ly, 2017; Miyazaki et al., 2017; Ungurean et al., 2020).

Indeed, research has found that sleep offers a wide array of benefits and protections. For instance, sleep has been implicated in processes as diverse as glymphatic waste clearance (Iliff et al., 2013) energy restoration (Vyazovskiy & Harris, 2013), and problem solving (Sio et al., 2013). Furthermore, the link between sleep disturbances and various cognitive and health detriments has been well established. Even acute sleep deprivation can lead to heightened blood pressure, cognitive deficits, and immunosuppression (Zielinski & Krueger, 2011). When present chronically, abnormal sleep patterns and architecture are hallmarks of many psychiatric and neurodegenerative disorders and as such are often part of the diagnostic criteria (Wulff et al., 2010).

A key benefit of sleep is its beneficial effect on recently learned information. In the lab, it has been consistently found that sleep is a crucial component of optimal memory consolidation (Rasch & Born, 2013). Memory consolidation broadly refers to the process of transferring newly learnt information from a labile, short-term, memory trace to long-term storage in which the memory trace is less susceptible to distortion and forgetting. As we will discuss later in the introduction and throughout the thesis, consolidation may be different depending on the type of information being consolidated. For instance, the way consolidation effects learnt behaviour, the brain areas involved and the roles they play in consolidation may be different depending on the type of information being consolidated. In

this thesis, I aim to better understand the role of sleep in learning and memory consolidation outside of a lab setting.

1.1. Memory

Memory can be broadly broken down into two types:

- Declarative memories are those that are explicitly learnt and remembered; this includes memories of facts, dates, and events.
- Implicit memories or knowledge are typically acquired unconsciously or reflexively, and often difficult to explain to others (Graf & Schacter, 1985).

An example of implicit knowledge would be the rules of grammar of one's native language (we are often unaware of the rules behind this but have an intuitive sense of whether the structure of a sentence sounds right or wrong). Motor or procedural memories, on the other hand, can be implicit or explicit memories that involve movement and skill acquisition. For instance, a motor memory may initially be explicit before becoming an implicit in nature. Take learning to riding a bicycle – the movements involved therein will at first require explicit effort. However, with practice and time they will become increasingly reflexive and implicit and will not require conscious effort.

A detailed examination of the extensive literature surrounding different subtypes of memory is beyond the scope of this thesis. The remainder of the thesis will focus on motor and declarative memory. The following section is intended to provide some background on these memory types and explain the rationale for focusing on these forms of memory in the context of sleep.

1.2. Motor memory, the serial reaction time task, and consolidation

Motor memory covers a wide range of processes – from the mundane (e.g. picking up a teacup) to the extraordinary (e.g. a gold winning performance at the Olympics). However, in this thesis I will focus on motor sequence learning which, as the name suggests, involves the performance of discrete motor actions in a sequential fashion.

While rapidly executing a series of ordered movements may seem like a task without real-life applications, in fact many movements require this. Consider, for example, navigational directions, cooking recipes, or sport manoeuvres – these are procedures that we often communicate, teach, and learn in a sequential fashion (at least until they become implicit through repetition). An inability to execute such processes, such as is seen in the condition apraxia (from Greek "without action"), can be disruptive and affect an individual's independence and quality of life (Hanna-Pladdy et al., 2003). A key symptom of ideational apraxia (a rare form of apraxia) is carrying out sequential tasks in the wrong order (Poeck, 1983). Studying the mechanisms behind motor sequence learning therefore not only advances our basic understanding of the processes in health but could also help us better understand and address clinical conditions such as these.

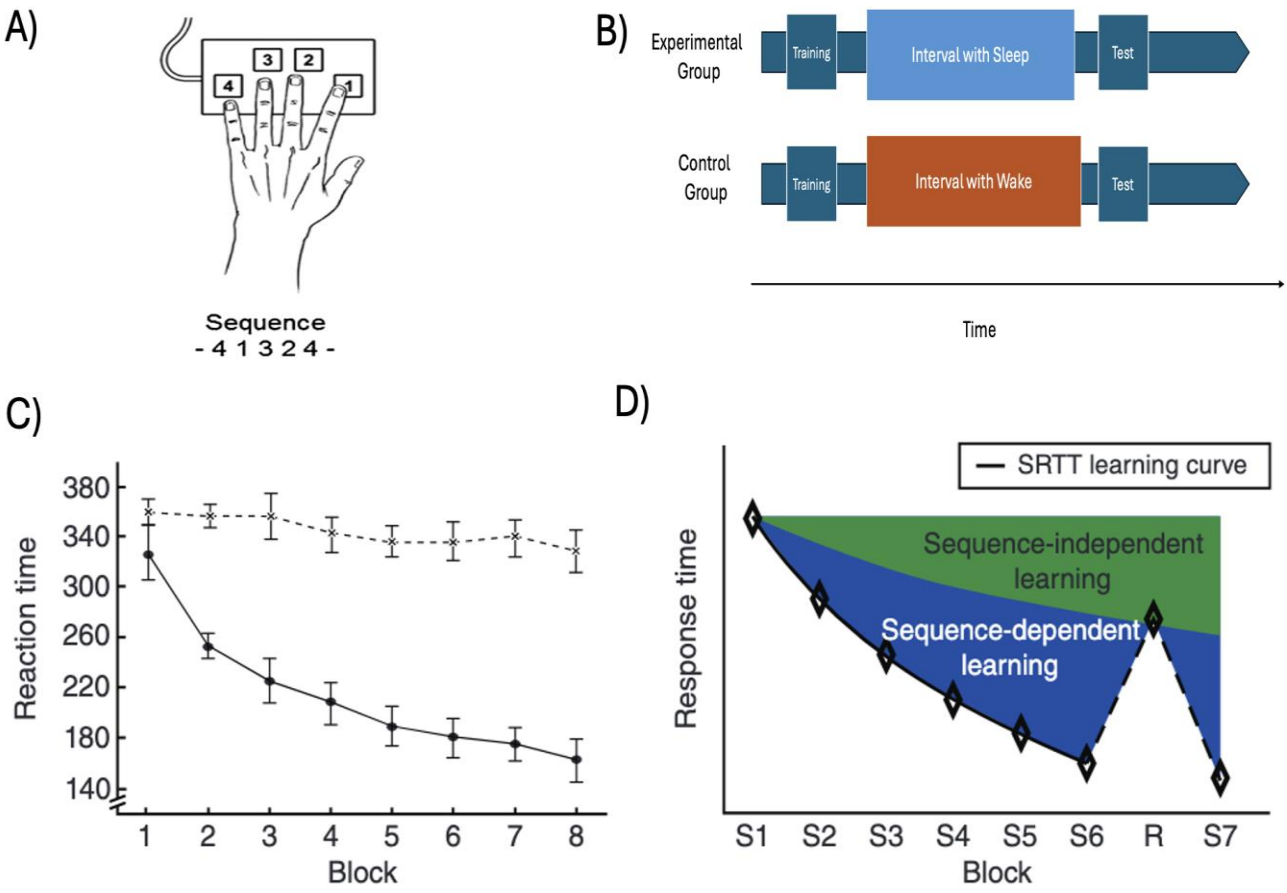


Figure 1.1 Outline of a Basic SRTT Paradigm in the Context of Sleep Consolidation and Example Learning Curves.

- **A. Example of a basic SRTT set-up**: Participants carry out sequences of button presses as quickly and accurately as they can. Variations in sequence features, fingers used, and uni vs bimanual tasks are common depending on the research question. Figure from(S. Fogel et al., 2017).
- **B. Example of a standard paradigm to test the effect of sleep on SRTT performance:** Participants are trained on the SRTT and are tested on the task after a time-matched interval that includes either sleep or wake. The change in SRTT performance from training to testing is then compared between groups.
- **C. An SRTT Learning Curve** demonstrating the change in response time across blocks during SRTT training. The black markers and solid line denote performance on a sequence that is fixed across blocks, while the grey markers and dotted line denote task performance on a sequence that changes between blocks. Figure from (Krakauer et al., 2019).
- **D. Sequence-Dependent vs Sequence Independent Learning**: This shows a typical SRTT learning curve and demonstrates how sequence-dependent and sequence-independent learning can be disentangled in an SRTT using a block of a random sequence (penultimate marker). Figure from (Krakauer et al., 2019).

I will be probing motor sequence learning using a common research paradigm called the **serial reaction-time task (SRTT)** which involves pressing a repeating sequence of buttons as quickly and accurately as possible (Figure 1.1A). With repeated practice on the task, response times typically reduce over time (Figure 1.1C) due to a combination of sequence-independent and sequence-dependent learning (Figure 1.1D).

There are several theories to explain how the SRTT is learnt – it was initially proposed that each action in the sequence is learnt in a chain-like fashion with the execution of each trial priming the individual for what the next trial is likely to be. This was previously proposed to occur through means of operant conditioning through trial repetition. However, motor sequences can be learnt in the absence of feedback (which challenges the proposed operant conditioning mechanism) and the fact that it is more difficult to execute the motor sequence when beginning in the middle of the sequence rather than at the start makes the chain-like priming of subsequent trials unlikely (Krakauer et al., 2019). An alternative hypothesis posits that, once the sequence is learnt, segments of the sequence are combined to form a higher-order representation of the complete sequence. This is supported by the findings that the response time (RT) of the first trial in a sequence varies depending on the length of the sequence, with longer sequences and higher numbers of unique items resulting in longer RTs (Harrington & York Haaland, 1987; Henry & Rogers, 1960). This suggests the sequence is being represented in full rather than item by item – in the latter scenario, the participant would only be thinking of the next item in the sequence and thus sequence length would be inconsequential. This abstract representation of the sequence allows for some flexibility in the execution of the sequence, for instance allowing a learnt sequence to performed better by the untrained hand (in unimanual SRTT) than a new sequence (Gabitov et al., 2016; Grafton et al., 2002). This a feature of the SRTT that will be explored in more depth in chapter IV when we discuss the concept of generalization. Based on this higher order representation of the SRTT sequences, and studies that have failed to find associated changes in primary motor cortex (M1) following SRTT learning, it has been argued that the SRTT is not so much a motor task as it is a sequence learning task (Krakauer et al., 2019). However, studies of non-invasive brain stimulation delivered to M1 show an improvement in learning by increasing M1 excitability, indicating that M1 is playing an important role (Bachtiar et al., 2018; Buch et al., 2011). Therefore, while abstract sequence representations seem to be part of how the SRTT is learnt, there is also evidence to suggest that this is interwoven with motor learning.

Improvements in performance are apparent not only during initial skill acquisition, but can also be observed after practice has ceased. These offline improvements in performance are thought to occur due to motor consolidation. Independently of how motor skills are acquired, there is evidence to suggest that sleep plays an important role in motor memory consolidation and in the consolidation of the SRTT specifically, demonstrated by comparing offline periods with and without sleep (Figure 1.1B). For instance,(Walker et al., 2002) found that participants who slept immediately after learning an SRTT and were tested in the morning showed a significant task improvement in terms of both speed and accuracy. Participants who learnt the SRTT in the morning, remained awake for an equivalent period of time, and were tested in the evening did not show this improvement. They did, however, show a comparable improvement in SRTT performance when they eventually had a period of sleep following the test in the evening. Additionally, it was found that this sleep-dependent improvement was significantly correlated with time spent in NREM2, particularly in the later stages of sleep (Figure 2). The finding that sleep enhances performance on the SRTT has been widely reported with both full nights of sleep and nap studies (Gudberg et al., 2015; Nissen & Bullemer, 1987; Walker, Brakefield, Hobson, et al., 2003; Walker, Brakefield, Seidman, et al., 2003) and this seems to occur regardless of sequence length (Kuriyama et al., 2004). Later, I will discuss candidate biological mechanisms that might underly this behavioural phenomenon.

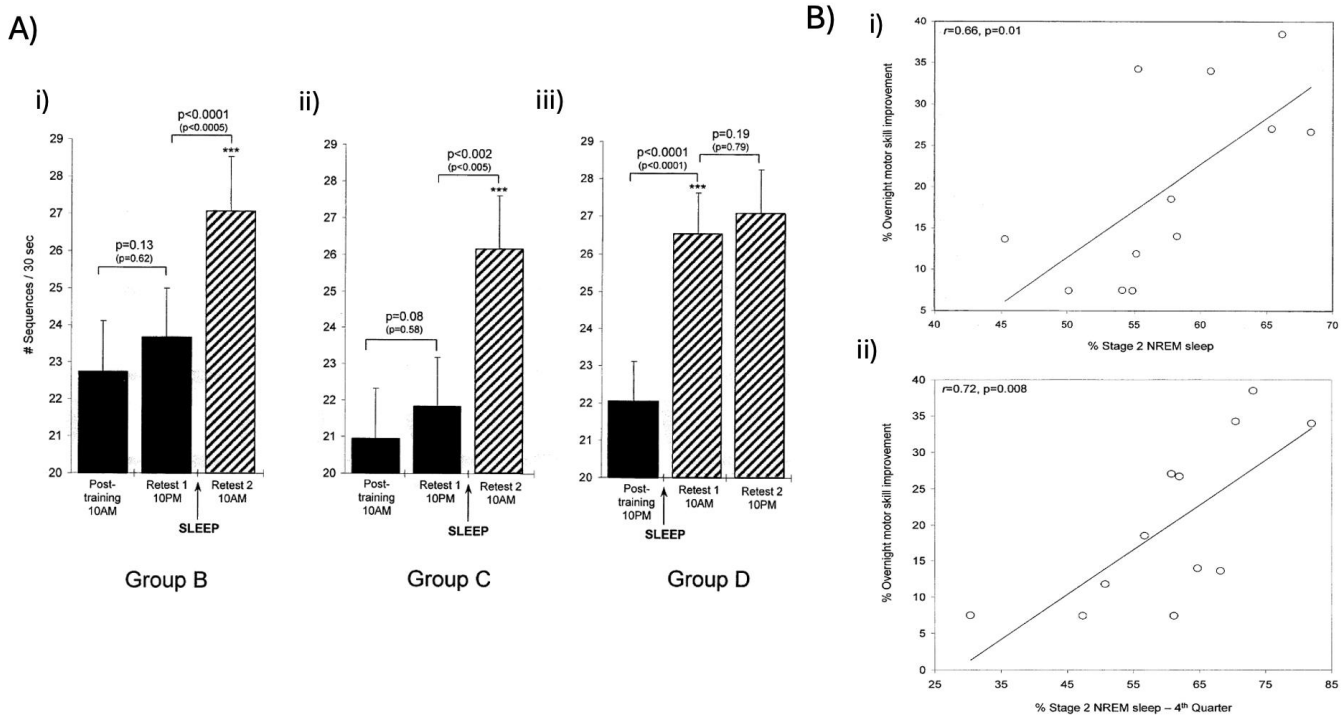


Figure 1.2 Summary of Findings by (Walker et al., 2002)**: Motor Learning is Enhanced by Sleep and This Enhancement Correlates With Time Spent in NREM2, Especially that in the 4th Quarter of the Night**

Sleep-Dependent Improvement in Motor Learning: No significant effect was observed for participants tested after a period of wake as shown by the black bars in both i) and ii) which were tested with and without a control for motor interference respectively. A significant improvement was only observed after the participants slept. This significant effect was also observed in participants who slept directly after post-training rather than after a period of sleep (iii).

The Sleep-Dependent Motor Improvement Seen in Panel A is Correlated with Time Spent in NREM2 i) particularly in the final quarter of the night ii).

Figures from (Walker et al., 2002)

1.2.1. Declarative Memory, the paired associates task, and consolidation

Like motor memory, declarative memory covers a wide range of exemplars. Its definition is so wide, in fact, that declarative memory is often what we are referring to when we use the umbrella term "memory". Declarative memories are further divided into episodic and semantic memories (Tulving & Others, 1972). Episodic memories are autobiographical memories of events or episodes experienced first-hand, such as having breakfast this morning or one's wedding. Semantic memories are those related to information that is learnt without first-hand experience, such as learning the capital cities of different countries. Through declarative memories, therefore, we are able to learn about the world and people around us as well as remember our own past experiences. Loss of declarative memory can

be accordingly devastating. For instance, declarative memory is often affected by dementia which can prevent both the acquisition of new declarative knowledge (such as the meaning of new words or the identity of a recent acquaintance) and the loss of previously learnt declarative knowledge (such as the layout of one's home). Expanding our knowledge of the mechanisms behind declarative learning would therefore place us in a better position to address these maladies, as well as be better able to understand and enhance declarative memory in healthy individuals.

In this thesis we are interested in episodic, declarative memory in particular. We will explore this through a commonly used behavioural paradigm called the **paired-associates task**. Sets of word pairs are presented to participants repeatedly and they are instructed to learn the associations. Participants are later presented with one of the words in the pair and asked to recall the corresponding missing word. They are thus tested on their ability to recall the paired words.

The encoding of the paired-associates task is likely the result of associative learning. In this view, when a given word-pair is presented, the hippocampus (specifically CA3) encodes the context of the memory while the language areas of the neocortex encode the presentation of the words themselves. Over multiple repetitions of the word-pairs, with these brain regions being activated simultaneously in response to each repetition, the connections between these areas are strengthened through Hebbian plasticity. As a result of these strengthened connections, when the participant is cued with a single word of the paired-associate, it should activate the neural representation of the corresponding word (Hasselmo, 1999).

Like motor memories, the consolidation of declarative memories is highly dependent on sleep. Studies have consistently found that declarative memories benefit from a night of sleep compared to wake (Diekelmann & Born, 2010). In contrast to motor memories like the SRTT, however, declarative memories tend to show maintenance in performance following sleep (Payne et al., 2012) although

some studies have found enhancement. In the case of the paired-associates task, this means fewer words are forgotten if participants undergo a period of sleep following encoding rather than an equivalent period of wake.

1.2.2. Neuroanatomy of consolidation of motor and declarative memories during sleep

Several studies have attempted to elucidate the neuroanatomical circuits involved in consolidation of motor and declarative memories. For example, functional imaging of two groups that either slept or stayed awake following SRTT learning found that during SRTT testing the group that had slept showed increased activity areas previously associated with motor improvements such as the hippocampus, M1, and cerebellum compared to the group that had stayed awake for the same amount of time (Walker et al., 2005).

The initial formation of sequence segments during learning and their eventual integration as a complete, abstract sequence is reminiscent of the consolidation processes discussed in the previous section on the roles of different sleep stages in consolidation (1.2 and 1.2.1). Indeed, there is evidence to suggest that sleep enhances motor learning through a similar process, which initially involves the hippocampus and striatal-cortical networks later in the consolidation process (Albouy et al., 2008, 2013). The hippocampus is involved in the initial encoding of the motor sequence and its activity predicts offline memory consolidation (Albouy et al., 2008; De Lucia et al., 2011; Moroni et al., 2008). During motor sequence learning the hippocampus and striatum show competitive activity – that is, if one area shows an increase in activity the other will show a decrease and vice versa. This relationship is important for successful encoding, and cooperative rather than competitive relationships between these areas have been associated with less efficient learning in both young and elderly participants (Albouy et al., 2008; Rieckmann et al., 2010). Cortical regions, including MPFC and dorsolateral

prefrontal cortex are believed to modulate the activity between the two areas during wake (Albouy et al., 2013). During sleep, however, this relationship becomes cooperative in nature with animal and human studies demonstrating that replay of learnt information co-occurs between these two areas (Lansink et al., 2009; Pennartz et al., 2004)). This change in their relationship, furthermore, predicts overnight enhancement of motor learning (Albouy et al., 2008). The coactivation and consequent functional strengthening (Euston et al., 2012; Pennartz et al., 2004) of these brain regions is believed to underlie motor sequence consolidation and the transfer of motor memories from the hippocampus to striatal and neocortical structures (Albouy et al., 2013).

Overnight increase in activity has been observed for both the hippocampus and the medial prefrontal cortex (MPFC) (Albouy et al., 2013) and an increase in their functional connectivity correlated with sleep-dependent enhancement of a motor sequence task (Albouy et al., 2008).

The striatum is involved in the formation of stimulus-response associations (Poldrack et al., 2001; Rieckmann et al., 2010; Schendan et al., 2003a, 2003b) and has been implicated in the chunking mechanism discussed earlier. Furthermore, striatal activity during sleep has been associated with sleep spindle activity (Barakat et al., 2013; S. Fogel et al., 2017) which, as we will discuss later, has been strongly linked to sleep dependent memory consolidation.

Much like motor memory, there is strong evidence to suggest that declarative memories are consolidated through information transfer from the hippocampus to other areas for stable, long-term storage, particularly the neocortex (Gais et al., 2007; Rasch et al., 2007). This is in accordance with the active systems consolidation theory (which was originally developed as a model to explain declarative memory).

It appears that the neural structures involved in the encoding of motor and declarative memories overlap, particularly when it comes to the importance of the role of the hippocampus for early memory formation and its interaction with task-relevant neocortical areas (Albouy et al., 2013). Indeed, studies have found that learning procedural and declarative tasks in the same session can result in interference unless the encoding session is followed by a period of sleep soon afterward (Brown & Robertson, 2007b, 2007a). This is presumably due to the interference caused by the usage of the same systems for both tasks.

1.3. A Physiological Introduction to Sleep

Sleep is behaviourally defined as a reversible loss of consciousness, characterized by heightened arousal thresholds, species-specific sleep posture, drop in physical activity levels, rebound following deprivation of said state, and is mediated by a circadian cycle (Campbell & Tobler, 1984; Rial et al., 2010).

In order to understand how memory consolidation is impacted by sleep, we will have to consider how we characterise sleep beyond behaviour. It is useful to define sleep behaviourally, when the aim is to establish whether an organism is asleep as opposed to resting, comatose, or in some another state that resembles sleep. However, if the goal is to explore something about sleep itself, an electrophysiological definition can be more informative. In order to define sleep in this way, polysomnography (PSG) – a word which means “multiple sleep measurements’- is often used. PSG is usually a combination of electroencephalography (EEG) and electrooculography, electromyography, and electrocardiography, although other measures can be added depending on the research or diagnostic question.

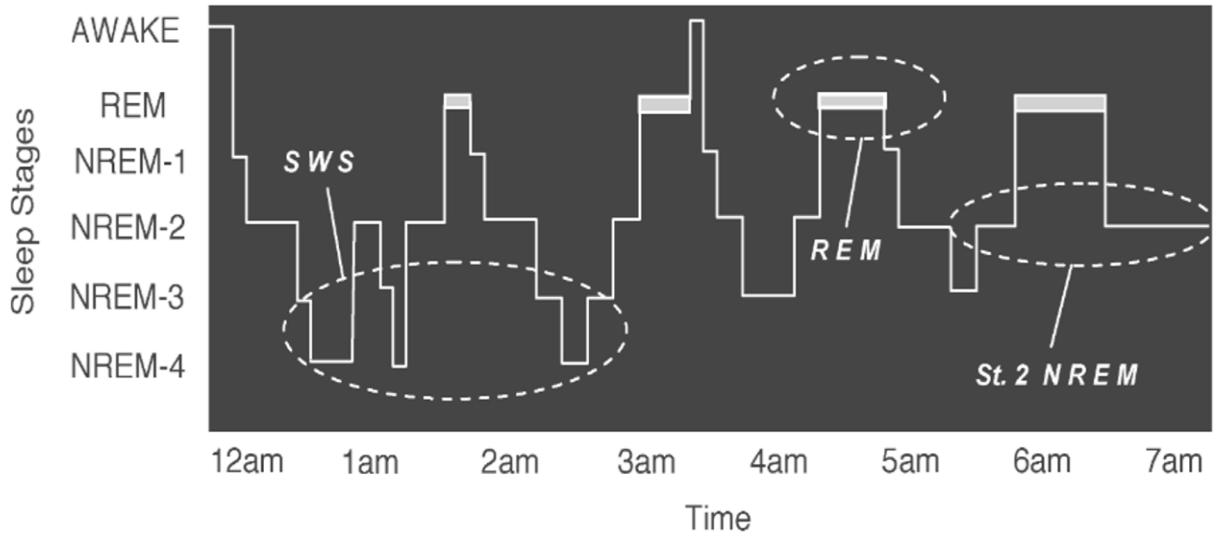


Figure 1.3 A Hypnogram Depicting the Different Sleep Stages in Standard Night of Sleep
Sleep stages during a standard 7hr night of sleep with 4 cycles. SWS, REM, and NREM2 (st. 2 NREM) highlighted. (Walker, 2008 b)

Using this approach, sleep can be roughly divided into rapid eye movement (REM) and non-rapid eye movement (NREM) sleep (Aserinsky & Kleitman, 1953, 1955). REM, through PSG, is characterized by fast, unsynchronized, low amplitude EEG, absence of muscle tone, high arousal thresholds, and the eye movements that give the sleep stage its name. NREM comprises of three sub-stages; NREM1, NREM2, and NREM3 (previously NREM3 and 4) with each ascending stage of sleep getting progressively deeper (that is, harder to rouse the sleeper from). NREM1 is a short period (1-5 mins) of light sleep that is characterized by alpha (7-12 Hz) and theta (4-7 Hz) activity, it makes up about 5% of total sleep time (TST). NREM2 is characterized by sleep spindles (distinct train of waves of around 12-16Hz, 0.5-1.5 secs in duration, show maximum amplitude in central derivations) and k-complexes (delta waves that last about 1 second, delta waves = 1-3 Hz), and makes up about 45% of TST. NREM3 is characterized by slow-oscillations (SOs) or slow-waves (SWs) which are 0.5-2 Hz in frequency and show a maximum amplitude of > 75 μV in the frontal derivations (Berry et al., 2017; Patel et al., 2022).

During a night of sleep, the sleeper typically progresses through NREM1-3 in order and then to REM before beginning the cycle again. Each cycle lasts approximately 90 minutes, with cycles in the earlier part of the night containing a higher percentage of NREM sleep and cycles in the later part of the night containing more REM sleep. The cyclical transitions between stages throughout the night and their differences in prevalence as the night progresses suggest that the stages may be performing distinct but complimentary functions. Indeed, there is theoretical and empirical evidence to suggest that this is the case.

1.4. The Role of Sleep in Memory Consolidation

Several theories have been proposed to explain how brain physiology during sleep could support memory consolidation. These are discussed below.

1.4.1. The Sequential Hypothesis

The Sequential Hypothesis posits that sequential state transitions during sleep are integral to memory consolidation, with the memory processing that occurs in each cycle setting up the foundation for the processing to be done in the subsequent cycle (Ambrosini & Giuditta, 2001; Giuditta et al., 1995). This contrasted with many of the models at the time which suggested that memory consolidation during sleep predominantly occurred during REM. The rationale behind the sequential hypothesis was that, ontogenetically, REM dominates sleep in early development and NREM appears in later development alongside wake. REM is thought to be heavily involved in forming genetically encoded neural circuits in early development while NREM adds and modifies these circuits based on waking experiences (Giuditta, 2014). Based on this, the sequential hypothesis model suggests that during NREM, adaptive memories acquired during the preceding waking period are strengthened whereas less relevant or adaptative memories are weakened. Then, during REM cycles, the gestalt of the adaptive memory

engram is integrated into existing memory frameworks and cemented into existing memory circuits for more permanent memory storage.

There is some empirical evidence to support the sequential hypothesis. In rodents, the number of NREM-REM cycles correlated with better memory consolidation and disrupting the cycles by waking them up before REM deteriorated consolidation (Rasch & Born, 2013). In humans, disruption of the cycles resulted in lower word-pair retention and visual texture discrimination (Ficca et al., 2000; Stickgold et al., 2000) compared to controls. Given the cyclical nature of sleep, however, it is difficult to manipulate a single stage or cycle in isolation and therefore difficult to assign them functions. Equally, it is difficult to disentangle measures like number of cycles with sleep duration, drive, or efficacy. Nevertheless, subsequent research and sleep models have built upon and refined this hypothesis and thus lent more credence to the notion that it is the combined functions of different sleep states that allow consolidation to occur.

1.4.2. The Sequential Hypothesis Refined

The Active Systems Consolidation Theory (Diekelmann & Born, 2010) is arguably the leading model of sleep-dependent consolidation. This model builds upon standard models of systems consolidation (Frankland & Bontempi, 2005; McClelland et al., 1995; Squire & Alvarez, 1995; Squire & Bayley, 2007) as well as the Sequential Hypothesis and proposes biological mechanisms for the functions that each stage plays in memory consolidation. The model suggests that during NREM2 and 3, when the hippocampus is active and the cortex is silent, the hippocampus reactivates cell assemblies that were active during the encoding of the day's learnt events. At this stage in sleep, the hippocampus sends this information to the cortical circuits involved in the memory engram being replayed. Because the cortex is a slow learner and requires multiple repetitions of an engram, the engram is replayed multiple times during NREM. Eventually, over several NREM cycles and perhaps various nights (Gais et al., 2007;

Payne & Kensinger, 2011; Peigneux, 2015; Stee & Peigneux, 2021) the information is transferred to the cortex for long term storage. During subsequent REM cycles, when the direction of communication is reversed and the cortex is active while the hippocampus is not, the cortex integrates the newly learned knowledge into existing cortical frameworks. We will discuss the biological mechanisms through which the brain achieves this in more detail below.

Previous authors (Vyazovskiy & Delogu, 2014) have proposed some mechanisms through which sleep physiology might support plasticity and learning. They propose that during NREM the brain undergoes a process of restoration from the strains of wake, with the up-state of the SO serving as a means to enable plasticity and the down-state allowing several cellular restoration and maintenance processes to take place. This is supported by the fact that SOs show distinct features over different brain areas, which suggests that SO are not homogenous across brain regions and are perhaps tailoring their function to the needs of specific brain areas. When enough SOs have transpired for the recovery of the involved brain areas to be complete, the sleep stage then transitions to REM. This offers an explanation as to why NREM is more prominent in the early stages of the night and why the percentage of TST spent in NREM increases with longer wake time (Cirelli & Tononi, 2008). The authors suggest sleep spindle density acts as a tagging mechanism to signal the NREM-REM transition, as sleep spindles are particularly prevalent prior to this transition (Vyazovskiy et al., 2004). REM sleep, on the other hand, is proposed to function as a "selection" mechanism that tests whether the preceding NREM cycle has indeed resulted in sufficient plasticity and cellular restoration. They propose that the similarities of REM sleep to the waking state provide a good testing simulation. If the previous NREM cycle the has not resulted in sufficient plasticity and restoration, then another cycle of NREM begins. This continues until all brain areas have undergone sufficient restoration or the sleeper is woken up by external stimuli.

More recently Singh et al., (2022) have provided in-silico support for the sequential hypothesis. They used models of the cortex and hippocampus to investigate how these brain regions interacted with each other while performing a category learning task, during subsequent simulated NREM and REM sleep, and how these interactions affected performance on their task. Because these experiments were done in-silico it was easier to manipulate and isolate different sleep stages to probe their function and effect on task performance. They found that error-driven learning, which calculates the difference between predicted and actual outcomes and then adjusts synaptic weights to minimize this difference, is supported by synaptic depression caused by oscillations in NREM. This synaptic depression, over time, weakens (and can eventually eliminate) weak synaptic connections within a given engram leaving behind the connections that were most strengthened during wake. Thus, the hippocampus maximizes signal to noise ratio and allows for error driven learning to occur in the absence of external input as ground truth. Then, during REM, the cortex is able to replay what was relayed to it by the hippocampus during NREM and this replay activates related attractor networks in the cortex. This has the dual function of integrating recently learned information with related information in long-term storage and replaying related engrams which prevents relevant but not recently experienced information from decaying. Finally, they found that the model performed best at the task when the simulated NREM and REM cycles were alternated, as opposed to simulating a sleep bout of half the duration but composed of exclusively NREM or REM sleep. More specifically, it allowed the model to improve upon recently learned information without doing so at the expense of remotely learned information.

1.4.3. Synaptic Homeostasis Hypothesis

Another prominent hypothesis is the Synaptic Homeostasis Hypothesis (SHY) (Tononi & Cirelli, 2003, 2006), which proposes that sleep allows for consolidation of learnt information through means of synaptic homeostasis. SHY proposes that during wake synapses associated with learnt information are strengthened through means of a net synaptic upscaling which, if no downscaling occurs, will

eventually lead to saturation and prevent further learning. They suggest that this net downscaling should occur during sleep for optimal consolidation. This is because during wake the brain is biased towards immediate experience due to external input from the environment. However, during sleep when there is a relative absence of external input, the spontaneous replay of recently learned information is able to elicit related memories. This more holistic sampling allows the information to be integrated into existing frameworks and addresses the dilemma of consolidating new information while maintaining learnt information. Importantly the synapses that have been potentiated during wake are less likely to be erased during downscaling, while weaker synapses are more likely to be pruned. Thus, the nocturnal downscaling allows for an increase in signal to noise ratio. It is worth noting that while the SHY hypothesis predicts a net upscaling during wake and a net downscaling during sleep, that does not negate the possibility that some upscaling occurs during sleep and some downscaling occurs during wake (Tononi & Cirelli, 2014). SHY is therefore not necessarily in opposition with other models that we have considered thus far, such as the active systems consolidation theory or Singh et al's., (2022) work. SHY is supported by findings that indicate that SWS, which most models (including SHY) agree is essential for consolidation, results in net downscaling compared to wake (Diering et al., 2017; Nagai et al., 2017; Vyazovskiy et al., 2008). However, this downscaling clearly occurs in conjunction with functional and structural upscaling (Chauvette et al., 2012; Vyazovskiy et al., 2009; Yang et al., 2014). Whether downscaling is the primary driving force behind sleep-dependent consolidation, as SHY argues, remains an open question.

1.4.4. An Alternative Theory

Finally, the Contextual Binding (CB) Theory (Yonelinas et al., 2019) proposes that during learning, the hippocampus binds information about the focus of the memory – called "item information" (such as the stimulus in a laboratory task) - with information about the context in which it was experienced. Context in this model can mean any aspect of the memory that is associated with the item information,

this can include the location of the experience, sensory information, or emotional state to give a few examples. The cortex, on the other hand detects familiar elements of these memories – i.e. where the information being encoded overlaps with previous knowledge. This is in accord with the other models we have considered thus far. Where this theory deviates from the others in terms of the role of sleep in consolidation is in its proposal that the key benefit that sleep endows over wake is an absence of interference, as there is less sensory input during sleep than during wake. This theory, unlike the others considered thus far, is agnostic about REM's role in consolidation. Furthermore, while the CB theory agrees that reactivation (or replay) of recently learned information reflects a process of consolidation from hippocampus to cortex, it sees reactivation as a phenomenon independent of sleep. This is because replay is not only observed during sleep but can also be observed during quiet wakefulness, therefore the CB theory sees replay as something that occurs post-encoding regardless of state. Unlike other models, therefore, this model does not differentiate between wake and sleep replay. Most research has focused on replay in sleep and the research that exists on wake replay is not without methodological limitations, however, some differences between these two forms of replay have been observed. Perhaps most notably the fact that wake reactivation occurs in the absence of SWA and spindles (Findlay et al., 2020; Tang et al., 2017), these differences suggest that the two forms of replay may not be interchangeable. It is also worth noting that this theory's primary objective is to provide an alternative explanation to the leading systems consolidation theory in the context of episodic memory. It is therefore unclear how other types of memory, such as motor memory, fit into CB. Regardless of the degree to which this theory is accurate, it highlights an important confound in sleep research – namely the difficulty in controlling for the inherent differences between wake and sleep. For instance, in this case it is difficult to design an experiment which would allow us to determine whether lack of interference is indeed the primary benefit of sleep over wake as it would be difficult for participants to spend an equivalent amount of time with similar interference in both sleep and wake. In summary, there is no definitive explanation for the roles that the different sleep stages play in memory consolidation and there are many gaps in our understanding of the biological mechanisms

behind this process. Nevertheless, there is some general consensus in the view that NREM's role likely involves replaying recently learned information in the hippocampus which transfers it to long term memory in the cortex, while REM sleep integrates said information within existing frameworks to create more holistic and gist-like representations in the cortex.

This thesis will focus primarily on the role of NREM sleep in memory consolidation. We have chosen this stage of sleep as our main focus because several studies (Diekelmann & Born, 2010), and the models described above, suggest that this stage of sleep is most associated with consolidation in the early stages of learning which we are exploring in this thesis. Again, as we narrow down our focus to what will be most relevant to our experiments, it is important to keep in mind that any consolidation occurring in SWS is likely being consolidated with the help of REM sleep and that consolidation is not an all or none process. As information is consolidated and practiced over time, the kind of processing that occurs during sleep and the degree to which each sleep stage is involved in the processing will likely vary depending on the learner's needs. It is equally important to remember that the time-scale of consolidation can be longer than the experiment and that what is captured in the beginning stages of learning, particularly in the motor/procedural domain, is only part of the learning process (Rakowska et al., 2021) .

1.5. Tagging Mechanisms on Motor and Declarative Memories

It is clear that we do not consolidate all learnt information equally. This begs the question - which mechanisms are at play during the learning phase to determine which information is preferentially replayed (and thus strengthened) during sleep? Some potential mechanisms through which this might be achieved were touched upon earlier in the thesis when we discussed models of memory

consolidation during sleep. For instance, the SHY hypothesis proposes that this is, at least in part, achieved by Hebbian strengthening through neural coactivation during the learning phase. This synaptic strengthening during learning means that when the brain enters a state of net downscaling during sleep, the strengthened synapses are more likely to be preserved while weaker synapses are more likely to be erased. This can result in a heightened signal to noise ratio of the engrams associated with the task. In the case of the SRTT, for instance, over many repetitions and feedback, the neural association between a given sound/image cue and the movement associated with it would be could more strongly associated and less likely to be downscaled during sleep. Thus, enhancement in performance may be observed following sleep.

An alternative, but not necessarily opposing, view is that memories or aspects thereof which are labelled as "important" are preferentially consolidated. There are several ways in which information may be labelled or tagged for further consolidation during sleep. There is evidence, for example, that information that is expected to be important or relevant in the future is better consolidated during sleep. Wilhelm et al., (2011), for example, found that participants who were told that they would be re-tested on a task, performed better after a period of sleep than those who were not made aware that they would be retested. This effect was found for both a paired-associates task and a motor sequence learning task and is associated with increased SO activity and spindle density compared to those who were not made aware of the retest. However, this behavioural effect has not always been replicated (Ashton & Cairney, 2021; Reverberi et al., 2020; Wamsley et al., 2016). Other features that have been found to increase offline consolidation are reward (Lansink et al., 2009; Sterpenich et al., 2021; Studte et al., 2017), weakly learnt information (Denis et al., 2021), and heightened emotions or stress associated with the information (Kim & Payne, 2020), to name but a few. One (of various) biological mechanism through which tagging for future offline consolidation is perhaps achieved is through theta oscillations (Hasselmo & Stern, 2014; Heib et al., 2015; Sederberg et al., 2003).

In summary, the mechanisms through which the brain preferentially selects certain information for further consolidation are not fully understood. However, there is evidence to suggest that how the information is learnt during wake can influence the degree to which it is subsequently consolidated in sleep.

1.6. NREM Sleep Oscillations and Memory Consolidation

Thus far we have discussed some of the evidence to suggest the importance of sleep, as whole, in memory consolidation. In the context of motor and declarative learning, it is NREM and its signature oscillations that have been most strongly implicated the early stages of learning. Previous studies have found beneficial effects of time spent in NREM on the consolidation of both declarative (Gais & Born, 2004; Plihal & Born, 1997; M. A. Wilson & McNaughton, 1994) and motor (Tamaki et al., 2013; Tucker & Fishbein, 2009; Wilhelm et al., 2011) tasks. Additionally, upregulating oscillations associated with NREM sleep using Non-Invasive Brain Stimulation (NIBS) techniques such as tDCS and TMR has resulted in improved performance in motor tasks (Cousins et al., 2016; Koopman et al., 2020) and declarative memory tasks (Marshall et al., 2004, 2006; Prehn-Kristensen et al., 2014; Rasch et al., 2007). While these results have not always been replicated (motor: Marshall et al., 2004; declarative: Bueno-Lopez et al., 2019; Marshall et al., 2006; Sahlem et al., 2015), this hints at a causal relationship between NREM markers and consolidation of motor tasks. Furthermore, declarative and motor memory consolidation deficits have been observed in patients with primary insomnia, which reduces the amount of time spent in NREM (Declarative: Backhaus et al., 2006;Motor: Cellini et al., 2014;). It is therefore this stage of sleep that we will be focusing on in this thesis, particularly stages N2 and 3. So, how does NREM sleep help to consolidate recently learnt motor information?

A key reason why NREM sleep is thought to be of particular importance during the early stages of learning is because replay appears to be most prevalent in this sleep state in both rodents and humans (Pavlides & Winson, 1989; Peigneux et al., 2004; Wilson & McNaughton, 1994). Replay refers to the process of reactivation of neurons that were active during the encoding of a memory during times of low cognitive load. During wake, in the early stages of memory formation, the hippocampus is proposed to unite information from several brain areas that process the different sensory aspects of a memory into a single memory or engram (McClelland et al., 1995). During subsequent sleep, the cortex momentarily releases tonic inhibition of subcortical structures during the up-states of the slow-oscillations (SOs) that characterize SWS. During this depolarizing phase the memory is repeatedly reactivated in the hippocampus, which recruits the brain areas originally involved in the memory formation as well as associated cortical regions (Ji & Wilson, 2007; Qin et al., 1997). This reactivation is correlated with the presence of sharp-wave ripples in the hippocampus and spindles which are generated by the thalamus (Rasch & Born, 2013). When these co-occur, the sharp-wave ripples become embedded in the trough of the spindle, forming what is called a spindle-ripple event (Steriade, 2006). These spindle-ripple events are, in turn, nested in the up-state of the cortical SO and thus feedback to the cortex which requires many repetitions of high amplitude input for LTP to occur (Lisman et al., 2001). Spindle activity in the neocortex is associated with an increase in calcium influx to the dendrites of the neocortical cells which in turn activates calcium-sensitive kinases which prime cells to fire. It is thus that the transfer of information from short-term storage in the hippocampus to long-term storage in the neocortex that underlies consolidation is proposed to occur.

In the following sections, we will discuss the signature oscillations of NREM sleep and their relevance to declarative and motor memory.

1.6.1. Evidence Linking Spindle Features and Memory

Sleep spindles are a hallmark oscillation of NREM sleep. They are oscillatory bursts between 11-17Hz (although definitions can vary between studies) lasting $\geq$ 0.5 s, that wax and wane in amplitude and which are particularly prevalent during NREM2. They are generated in thalamocortical regions and are thus in a prime location to facilitate communication different sensory areas (which integrate in the thalamus) to higher-order cortical regions. Spindle events are initiated in the thalamic reticular nucleus which induces spindle oscillations in thalamocortical regions through a mechanism of GABAergic inhibition and subsequent rebound excitation. While the thalamic reticular nucleus is able to generate spindles without the cortex (eg in vitro), feedback from the cortex allows for longer spindle duration and greater coordination with other oscillations (such as SOs) which allows for greater propagation of spindles to regions outside the thalamus (Fernandez & Lüthi, 2020).

As mentioned, spindles have been found to both induce sharp-wave ripples, which are associated with replay, and play a crucial role in the communication of these replay events to other brain areas through spindle-ripple events (Staresina et al., 2015). The high frequency associated with spindles have also been strongly associated with synaptic potentiation (Peyrache & Seibt, 2020; Rosanova & Ulrich, 2005; Werk et al., 2005). Spindles have therefore been proposed as a mechanism for sleep-dependent memory consolidation, particularly in the NREM stage.

Correlational evidence has lent support to the importance of spindles in memory consolidation. For instance, an increase in spindle density following learning has been observed in both humans and rodents and has been linked to post-sleep improvement in both motor (Fogel et al., 2017; Fogel & Smith, 2006) and declarative (Clemens et al., 2005; Gais et al., 2002; Ruch et al., 2012) tasks in general. An increase in spindle count has also been found to correlate specifically with word-pair retention

(Clemens et al., 2005; Lustenberger et al., 2015; Schönauer, 2018; Studte et al., 2017) and performance on the SRTT (Astill et al., 2014; Laventure et al., 2016; Morin et al., 2008; Nishida & Walker, 2007).

Further evidence for the relationship between spindles and replay comes from studies that have found that these spindle-related changes occur in the same brain areas that were involved in the learning of the task in question. For example, (Nishida & Walker, 2007) found increase in spindle power and density over the contralateral hemisphere to that of the hand used to learn an SRTT in a nap study. Furthermore, this increased spindle activity was significantly correlated with overnight improvement in task performance. Equally, (Cairney et al., 2018) had participants form an association between a visual cue (of an object or a scene) and an auditory cue. They then presented these auditory cues during a nap with the aim of cueing reactivation – a protocol which saw an increase in fast spindle count for task-relevant compared to novel cues. Importantly, they also found that by using a representation similarity analysis on the cue-elicited spindles, they were able to correctly decode the category of the image associated with auditory cue presented during the nap. Furthermore, the extent to which the cue elicited the correct image response predicted the degree of behavioural improvement on the task upon retest. In a similar study, but in the motor memory domain, (Belal et al., 2018) used a modified SRTT with faces and objects as visual stimuli, each associated with a sound and a key press. They cued reactivation using the auditory stimuli during NREM and used classifiers to determine whether the auditory cue was able to elicit a reactivation in the associated motor and visual EEG signatures associated with the sound. They found that this was indeed possible and that, like (Cairney et al., 2018), the extent to which the auditory cue elicited the correct EEG response predicted the degree of performance enhancement at retest.

Studies using more spatially sensitive approaches than EEG have supported these findings. For instance, simultaneous EEG and fMRI recordings have found that increases in spindles (and spindle amplitude) occur in task-related topographical areas during NREM sleep following a declarative

memory task (Bergmann et al., 2012). Furthermore, performance on the learning of the task predicted the degree to which these cortical spindles were coupled to the hippocampus. This lends support to the notion that spindles play a role in memory reactivation and that this often involves an interplay between the cortex and hippocampus. Similarly, in motor learning, strong evidence comes from research that has investigated motor learning and sleep-dependent consolidation in patients with intracranial electrode implants. For example, Johnson et al., (2012) trained patients to control a computer cursor by modulating the power of a high frequency wavelength in motor associated areas. They found that following training, spindle activity increased in the brain regions involved in the learning of the task. Together these studies demonstrate that the areas involved in the learning of a given task tend to show increased spindle activity during subsequent sleep.

The manipulation of sleep spindles has shed further light on their role in sleep-dependent memory consolidation. Experimental manipulations of sleep spindles suggest they play a similar role in the context of motor memory consolidation. (Rasch et al., 2009), for example, gave reboxetine to healthy participants. Reboxetine is an anti-depressant, specifically a serotonin norepinephrine reuptake inhibitor, which increases spindle density and increases the number of fast spindles during SWS. They found that reboxetine administration resulted in better performance on a motor sequence learning task compared to controls and that this improvement was positively correlated with spindle density and increased fast spindles. Presumably this effect was achieved through the indirect effects of serotonin, norepinephrine, or the REM suppression being studied in that experiment. Similar results were found by (Lustenberger et al., 2016) who used transcranial alternating current stimulation to boost spindle activity during sleep and observed an improvement in motor memory consolidation compared to a sham condition. Conversely, dampening of spindle activity following learning using auditory stimulation decreases the benefit of sleep on subsequent task performance (Schreiner et al., 2015).

Therefore, the literature has generally found that increases in spindle activity during post-learning NREM sleep benefit task performance at post-sleep retest. These spindles have been found to be higher in brain areas that were involved in the learning of the task. Furthermore, experimental manipulation of the spindles points to a positive relationship between spindles and memory consolidation. In sum, the combination of these findings support the notion that spindles are correlated with reactivations of recently learnt information and that their role in sleep-dependent memory consolidation is a positive one.

1.6.2. Evidence Linking SO-Spindle Coupling and Memory

It may not, however, be changes in spindle activity alone that predict consolidation. The high frequency nature of sleep spindles (and ripples embedded within) disallows communication with spatially distant areas which limits the extent to which they can consolidate information on their own. Conversely, SOs have the ability to carry information across long distances in the brain which has been proposed to aid in the transfer of information between the hippocampus and cortical long-term storage sites. A facet of NREM oscillatory features that has therefore begun to be explored in recent years is the coupling of spindles and SOs and its relationship to memory consolidation.

In support of this premise, studies have found increases in SO-spindle coupling following learning (Mikutta et al., 2019; Mölle et al., 2004) and there is mounting evidence to suggest that this coupling leads to greater consolidation (Bastian et al., 2022; Hahn et al., 2022; Helfrich et al., 2019; Schreiner et al., 2021; Staresina et al., 2015). Like the changes in spindle activity discussed previously, there is some evidence to suggest that post-learning changes in spindle-SO coupling are more likely to occur in brain regions involved in the learning of the task (Bastian et al., 2022; Huber et al., 2004; Yordanova et al., 2017). More specifically, it has been found that spindles that are coupled to the depolarized upstate of the SO increase the likelihood of consolidation (Mikutta et al., 2019; Ngo et al., 2013;

Rothschild, 2019), whereas coupling to another phase of the SO results in increased likelihood of forgetting (Helfrich et al., 2018; Nir et al., 2011; Tononi & Cirelli, 2006). Indeed, post-learning increases in spindle activity tend to be coupled to the SO up-state (Mikutta et al., 2019; Mölle et al., 2006, 2009). Finally, (Niethard et al., 2018) found that dendritic calcium activity in mouse pyramidal cells was most elevated when spindles occurred in the SO up-state as opposed to another phase of the SO. This suggests that, in addition to allowing for distant communication, this cross-frequency coupling enhances conditions which are conducive to plasticity and LTP which are important components of memory consolidation.

Lastly, and importantly for this thesis, the positive relationship between SO-spindle coupling and consolidation has been specifically observed in both the motor (Hahn et al., 2022; Huber et al., 2004; Mikutta et al., 2019; Rakowska et al., 2021) and declarative memory domains (Mölle et al., 2004; Ngo et al., 2013; Schreiner et al., 2021). This cross-frequency coupling relationship is therefore of interest to us.

1.6.3. Sleep-Dependent Memory Consolidation Outside of a Lab Setting

This thesis aims not only to explore the relationship between sleep and memory consolidation, but to do so in an at-home setting. Sleep-dependent memory consolidation has, thus far, been primarily explored in controlled lab settings. While the experimental control and equipment in a lab setting are essential in optimising signal to noise ratio and isolating variables of interest, it is also far removed from the conditions under which learning, and sleep, occur in every-day life. This represents a gap in our understanding of this phenomenon. Potential differences in sleep-dependent memory consolidation between these two settings are useful and important to explore at the level of basic research. Doing so ultimately provides us with a better understanding of sleep-dependent memory consolidation in conditions with varying degrees of experimental control and ecological validity. This,

in turn, allows us to identify the variables that affect sleep-dependent memory consolidation and characterize the ways in which they do so.

Existing evidence does suggest that environment influences sleep. One well-established finding is that participants sleep differently in familiar compared to unfamiliar settings, such as the home and the lab. Agnew Jr et al., (1966), for instance, found that participants experience significantly more awakenings and less time spent in REM during the first night of sleep in a lab setting. Their sleep architecture then returned to normal in succeeding nights of sleep in the lab once they became habituated to the setting. This finding, called the "first night effect", has since been replicated consistently, with studies finding sleep abnormalities of various kinds -usually related to heightened levels of alertness/vigilance and difficulty reaching the deeper states of sleep- when participants sleep in unfamiliar environments (Bruyneel et al., 2011; Tamaki et al., 2016). It is partly for this reason that habituation nights are protocol in many sleep studies (although this was already routine at the time of the Agnew et al publication).

Equally, there is evidence to support the notion that the environment in which learning takes place can affect the efficacy of learning. For example, research on social facilitation suggests that having someone observing the participant while they perform a task results in better task performance if the participant is already familiar or skilled at the task but will result in a poorer performance if the task is still in the process of being learnt (Strauss, 2002). Participants may therefore experience different baseline performance levels in the lab - where the experimenter is likely observing them during the task- compared to at home. Equally, research has shown that distracted learning and multitasking – which are presumably more likely to occur at home than in the lab where care has been taken to prevent this- have an adverse effect on learning (Schmidt, 2020). If participants are indeed more easily distracted in one environment than the other, then encoding differences might affect the degree to which a given memory representation is subsequently replayed (Schapiro et al., 2018). Therefore, it is

likely that the environment in which learning takes place not only impacts behaviour but offline consolidation as well. There are many other such environmental differences between the lab and the home that might influence learning and therefore sleep-dependent memory consolidation and without empirical testing it is unclear how they might do so.

1.6.4. Remote, At-Home, Sleep Studies: A Step Towards At-Home TMR

Another reason for our interest in studying sleep-dependent consolidation outside of the lab is that this thesis is intended, in part, as a steppingstone to conducting further at-home sleep research. More specifically, our hope is that the knowledge gained from this thesis will allow us to use Targeted Memory Reactivation (TMR) in a remote, at-home setting. TMR is a non-invasive method of biasing the content of replay during sleep. TMR protocols commonly involve presenting a sensory stimulus that has been associated with a behavioural task and re-exposing said stimulus to a sleeping participant in subsequent SWS. Research has found that TMR is able to drive next-day improvements in tasks of various kinds, including motor and declarative learning (Motor: Belal et al., 2018:Declarative: Cairney et al., 2018). As with sleep-dependent memory consolidation in general, most of what is known about TMR and its mechanisms comes from studies conducted in lab settings. Complementing such studies with research conducted in more naturalistic settings would provide us with a more comprehensive understanding of the effects of and mechanisms underlying TMR. Work in this thesis was therefore designed with potential future TMR applications in mind, although tests of TMR were outside the scope of the thesis.

Some studies have conducted TMR in at-home settings with minimal experimenter intervention. Göldi & Rasch (2019) was the first study to attempt TMR thus, they had participants learn Dutch-German word-pairs and played audio of the learnt words to participants using an mp3-player and found that TMR had a beneficial effect on word retention compared to sham stimulation but was not as effective

as an undisturbed night of sleep. Therefore, remote, at-home TMR appears feasible, but more research is needed to understand the neural mechanisms at play and how they relate to those observed in the lab.

Additionally, TMR has garnered a lot of interest as a clinical tool. The goal would be to tag a task in the affected memory domain with a sensory stimulus, and then use TMR to bias the replay of this task during sleep. For instance, Johnson et al., (2021) found that TMR helped post-stroke patients improve on a motor task and they were better able to generalize their acquired skills to other motor tasks. Given that a lot of clinical populations have difficulty travelling to a hospital or lab to receive TMR, it would make such treatment more accessible to both participants and treatment providers if it were able to be conducted remotely and in the patient's homes. Therefore, understanding potential differences in sleep-dependent consolidation and TMR effects in a lab compared to an at-home setting is equally important in the context of clinical applications.

1.7. Sleep and Generalization

Based on the research discussed thus far, it is clear that sleep is a critical component of the process of consolidation. That is, in the transformation of labile short-term memories into stable long-term ones. Thus far, however, we have considered the benefits of sleep in the context of a single task. Most of the key studies discussed compare performance on a task of interest before and after a night of sleep (with an equivalent period of wake as a control). These studies have demonstrated that, at least in the case of certain tasks, sleep can lead to overnight improvements in task performance.

Outside of the lab, however, there is often some degree of similarity between individual life experiences, and it is to our advantage to take the knowledge gained from previous experiences and apply it to new ones where appropriate. Applying appropriate past information to present and future

situations allows us to make predictions, learn from prediction errors, and creatively problem solve - to give just a few examples. Arguably, a key reason we consolidate information – why we remember past events accurately and integrate them into existing frameworks - is to be able to carry out these crucial computations. Therefore, given that sleep is important to consolidation a lingering question is: to what degree are sleep's benefits task-specific? Could sleep aid in the application (generalization) of knowledge from one task to another?

Evidence indeed suggests that generalization is, at least partially, a sleep-dependent process. Behavioural studies have found that sleep benefits generalization (or abstraction, as it is sometimes referred to in the literature) in a diverse range of tasks. For example, sleep (in the form of either a full night or a nap) has been found to aid in the generalization of grammar (Gómez et al., 2006; Hupbach et al., 2009) and word meanings (Horváth et al., 2016) in children, statistical patterns (Lerner & Gluck, 2019), motor learning (Cohen et al., 2005; Witt et al., 2010), and transitive inference (Barron et al., 2020; Gluck et al., 2002) to name a few. Furthermore, sleep seems to be important for multiple processes that can aid in generalization such as the detection of overarching rules and insight (Verleger et al., 2013; Wagner et al., 2004).

So, how does sleep achieve this? It seems that many of the same biological processes that enable sleep-dependent consolidation also support generalization. As previously discussed, during NREM sleep the engrams associated with information learnt during the day are replayed multiple times in the hippocampus, using representations that allow for pattern-separation. The shared features of these replay sequences are then processed in the cortex during REM. This overlap in replay sequences is thought to enable gist extraction and the building of cognitive schemas (or categories) (Lewis & Durrant, 2011). Thus, it seems that healthy sleep, as conceptualized in the Active Systems Consolidation Theory, is able to achieve balance between specificity (through replay in the

hippocampus during NREM) and generality of knowledge (through gist extraction in the cortex during REM).

To our knowledge, however, there is little research into the effect of sleep on the generalization of motor/spatial sequences to non-motor domains. In the final chapter of this thesis we aim to address this gap in the literature by developing such a task with the long-term aim that it can be tested as part of a sleep study in the future. Such a study would allow us to determine whether this type of cross-modality generalization benefits from sleep and, if so, what aspects thereof.

1.8. Summary and Thesis Objectives

Given the evidence that sleep plays a role in consolidation of different types of memories, combined with a lack of understanding of how these processes differ in naturalist, at-home, settings, the first two experimental chapters of this thesis aim to address the shortage of at-home, remote studies into sleep-dependent consolidation. We aim to do this by first establishing online declarative memory and motor learning tasks that can be readily carried out and learnt in a remote setting, and that demonstrate benefit from sleep. Once these tasks are established, we aim to probe the relationship between sleep features that have been implicated in sleep-dependent memory consolidation in the literature and overnight changes in performance in our tasks. In the third experimental chapter, we do this by using low-density, portable EEG devices mailed to participants' homes. This will allow us to investigate whether the relationships between these sleep features and measures of sleep-dependent consolidation mirror those seen in the lab-based literature. It is our hope that the outcomes of these two chapters will help set up the groundwork for future studies involving at-home TMR (which are outside the scope of this thesis). Finally, for future studies to more thoroughly probe the impact of sleep (and of modulation of sleep through TMR) on generalisation of learning, the third experimental chapter reports pilot data on the development of a task to test generalisation from a motor task.

1.8.1. Thesis Aims

Chapter II

- To establish online declarative and motor memory tasks that can be learnt remotely.
- To determine whether the declarative and motor tasks show evidence of sleep-dependent consolidation.

Chapter III

- To investigate which EEG features of NREM sleep correlate with overnight consolidation on the declarative and motor memory tasks from chapter 2 in an at-home setting.

Chapter IV

- To develop a task to test generalization from a motor sequence to a non-motor sequence.

2. Quantifying Motor and Declarative Learning and Its Relationship to Sleep Outside of the Sleep Lab

2.1. Introduction

Sleep plays an important role in learning and memory consolidation. However, differences in sleep have been observed in unfamiliar compared to familiar settings (Agnew Jr et al., 1966; Tamaki et al., 2016). This is a fairly well accepted phenomenon, and it is therefore standard to have one or more nights of habituation prior to conducting an in-lab sleep study to allow participants to familiarize themselves with the sleep lab. However, this still may not be truly reflective of participants' habitual sleep. Given that skill learning typically occurs outside of the lab, it is important to better understand how sleep impacts on learning and memory at home. Understanding relationships between sleep and learning outside the lab is also key to thinking about potential sleep interventions targeting consolidation, for example in rehabilitation or skill learning contexts.

We therefore aim to study sleep dependent consolidation remotely, in the participants' homes, which will allow us to study sleep and learning in a naturalistic setting. We intend this to be a steppingstone to at-home targeted memory reactivation (TMR), that is, a technique in which a sensory stimulus associated with a learned task is delivered to a participant during SWS in order to bias reactivation in favour of the learned task (Cellini & Capuozzo, 2018; Lewis & Bendor, 2019; Rudoy et al., 2009)). This technique allows us to non-invasively manipulate reactivation to better understand memory consolidation and potentially benefit skill learning over time.

To achieve our eventual goal of at-home TMR, we first need to ensure that we have behavioural tasks that can be studied in the context of sleep in a remote and at-home setting. As stated in the previous

chapter, we are particularly interested in motor and declarative memory. Thus, our first goal is to establish motor and declarative tasks that can be learned by participants online, in their own homes, and with minimal interaction with the researcher. Furthermore, in order to ensure that we are indeed studying consolidation, that is – the strengthening and stabilization of already learnt information – we aim to establish a set of tasks that can be learnt in a single session.

For our motor task we chose to employ the serial reaction time task (SRTT) as there is ample research to suggest that it benefits from sleep-dependent consolidation (Fischer et al., 2002; Korman et al., 2007; Walker et al., 2002), it can be learnt in a single session (Belal et al., 2018), and can be easily completed in an at-home setting using a computer keyboard . The SRTT usually consists of a set of visual stimuli that when presented in different spatial locations signal different motor responses, usually presses on a button-box or a keyboard. Participants are told to respond as quickly and accurately as possible (Nissen & Bullemer, 1987). Improved performance on the SRTT is defined by a decrease in response time (RT) and, to a lesser extent, increase in accuracy. Performance on the experimental sequences is also commonly compared to a random sequence to quantify the degree to which the improvements in performance relate to the learnt sequences as opposed to simply becoming more familiar with the task requirements or faster at pressing the buttons involved in the task.

We were also interested in studying the relationship between sleep and the consolidation of declarative memory. Like motor memory, there is ample evidence that declarative memory undergoes consolidation during sleep in the lab (Axmacher et al., 2008; Ellenbogen, Payne, et al., 2006; Gais & Born, 2004; Jenkins & Dallenbach, 1924). We chose to employ a paired-associates task, whereby participants are shown sets of word pairs (in our case, words that were unrelated to each other) and then are subsequently cued with one of the words in the pairs and asked to recall the word that corresponds to the cue.

In order to probe the role of sleep in these tasks we compared the changes in performance from learning to testing sessions in a group that underwent nocturnal sleep after learning and a group that underwent diurnal wake for an equivalent period of time. This is common in the field of sleep-dependent consolidation research. Previous studies have found that while both experimental groups improve on the task during the learning session at a similar rate and to a similar degree, the group that sleep after learning typically show a greater improvement from the learning session compared to the group that stayed awake (Brown & Robertson, 2007a; Fischer et al., 2002; Walker et al., 2002).

It is worth keeping in mind, however, that these findings have not gone unchallenged – particularly in regard to motor learning. Several studies have questioned whether sleep actually enhances motor learning, suggesting instead that it may simply help stabilize the RT learning curve and prevent decay in finger-tapping tasks such as the SRTT (Cai & Rickard, 2009; Genzel et al., 2009; Sheth et al., 2008) For instance, Pan & Rickard (2015) carried out a meta-analysis of 34 studies and identified several factors that that are commonly used in the literature including; data averaging of pre and post interval performance measures (eg across blocks or trials) which may obscure changes in performance that occur during these times rather than during the interval, time of testing, and training duration. They found that when these were controlled for, the sleep-dependent enhancement disappeared, and the results were more akin to those seen in the declarative memory literature – where learning is often merely stabilized following sleep. Despite these contradictions in our understanding of sleep-dependent consolidation it is nevertheless difficult to find studies that have outright failed to replicate a beneficial effect of sleep compared to wake post-learning in the SRTT literature. This potentially reflects a publication bias against negative findings and these kinds of results are probably more common than the literature would indicate. Equally, it is worth noting that the theoretical framework for sleep-dependent consolidation is far from completely developed. There are still many open questions regarding, for example, the mechanisms through which sleep is able to confer this beneficial

effect, to what extent is sleep unique in its ability to do so, and how it interacts with other states of consciousness to achieve this (Dastgheib et al., 2022). Therefore, while it might seem intuitive, at this stage in the field's research, that sleep plays an important role in learning and memory consolidation, it is important that we continue to investigate the extent to which it is important and under which conditions this is true.

Based on the majority of the literature, we therefore hypothesised that we would see a greater decrease (improvement) in RT, particularly in sequence-specific measures of performance, following a period of sleep compared to an equivalent period of time spent awake. In the case of the paired-associates tasks, previous findings have found that sleep slows down the decay of learnt declarative information compared to a period of wake (Ellenbogen et al., 2006; Payne et al., 2012). We therefore predicted that the group that slept following learning would show a lesser decay in word-pair recall than the group that stayed awake.

Thus, this first experimental chapter aims to replicate previous findings relating to the effects of sleep on the SRTT and paired-associates tasks and extend these to an at-home, online setting. This is achieved across two experiments.

2.2. Aims

1. To establish completely remote, online, motor and declarative memory tasks that can be learned in a single session.
2. To determine whether consolidation of task performance depends on sleep.

2.3. Methods

2.3.1. Participants

Participants were recruited through Prolific, an online research platform (https://www.prolific.co; both experiments) and through word-of-mouth, advertisements, and social media (experiment two only). The study was approved by University of Oxford Central University Research Ethics Committee (Ref R71108).

Inclusion criteria for this study were: 1) 18-60 years of age, 2) fluent in English, 3) have basic computer literacy, 4) have access to a working laptop with reliable internet and a QWERTY keyboard, 5) consent to not taking naps for the duration of the study and 6) consent to abstain from alcohol or drinks high in caffeine (such as coffee or energy drinks) during and 24 hrs prior to the study. Exclusion criteria were: 1) being deaf or hard of hearing, 2) being a skilled musician, 3) habitually playing action video games, 4) having a sleep disorder diagnosis, 5) having a history of or current psychiatric illness, 6) having a past or current neurological condition (e.g. epilepsy), 6) having a medical condition which impedes mobility of the hands and fingers (e.g. arthritis), 7) currently taking medication that is known to affect sleep or sleep architecture, 8) currently taking medications or recreational drugs that are known to affect sleep quality or cause drowsiness, 9) partaking in shift work or are currently jetlagged.

The key aims of these inclusion/exclusion criteria were to ensure that participants were physically able to complete the tasks, that they had not received more finger-focused training than the average person (e.g. through video games or high-level musical training), and that their sleep was not disturbed (e.g. through drugs, travel, or shift work).

The participant information sheet and the consent form were presented online, and participants were asked to confirm that they met all inclusion criteria and did not meet any exclusion criteria. Electronic informed consent was then obtained before participants were able to participate in the study. For participants recruited through Prolific, they used a numerical code generated by Prolific as their ID for

purposes of both consent and study participation. For participants recruited from Oxford they were provided with a study ID by the researchers which they used for consent (through Jisc) and for study participation. All participants were financially compensated for their time.

Following consent, participants were randomly assigned to the AM or PM (either automatically as part of the online consent or via a coin toss in the cases where consent was done through Jisc) conditions which determined the timing of each aspect of the study (see Figure 2.3 and LINK).

2.3.1.1. Experiment One

49 healthy adults consented to participate (mean age= 26.19 $\pm$ 5.78, 11 males).

Of the 49 participants, however, 1 participant was missing demographic information as a result of their Prolific profile expiring before our demographic data was compiled.

2.3.1.2. Experiment Two

50 healthy adults consented to participate (mean age= 27.57 $\pm$ 6.95, 15 males).

Of the 50 participants, however, 3 participants were missing demographic information. One was the result of their Prolific profile expiring before our demographic data was compiled and two were due to the experimenter neglecting to collect this data.

2.3.2. Behavioural Tasks

All tasks and questionnaires were administered online, in the participants' homes. The online tasks were designed using JsPsych-6.1.0 and hosted on a Bluehost server. Participants were asked to use Chrome as their browser to complete the behavioural sessions as this allows for optimal JsPsych performance. They were also asked to complete the tasks on a computer (as opposed to a phone or tablet) to minimize differences in keyboard size.

2.3.2.1. Paired Associates Task

The first task in all sessions was a paired-associates task which tests declarative learning. In this task (Figure 2.1), participants are first exposed to words in pairs and then cued with a single word from a pair – the aim is to recall the word associated with the cue. The unrelated words were chosen so that a learned association could be formed between the words, in contrast to related words where the relationship is already known to the participants. This learned association would therefore be more likely to benefit from sleep-dependent consolidation.

20 sets of word pairs were randomly selected from the unrelated word pairs used by Payne et al (2012), (Appendix 1). At the start of session one (S1) participants passively viewed all word pairs at a rate of 5000ms per word pair. The word pairs were separated by an inter-trial-interval (ITI) of 300 ms with a fixation cross at the center of the screen. Each word pair was presented once.

After the passive viewing, participants were immediately presented with a cue word from each pair and asked to type in the corresponding word. If their response was incorrect, they were told the correct word and asked to retype their answer. Cues were presented one at a time and the participants were unable to continue to the next trial until they had given the current response. Each word pair was presented twice. This first test was intended to be an opportunity for active leaning as opposed to a test of word retention.

In the second paired-associates test of S1 (following the motor learning task), they had to type in the word that corresponded to the cue and were not given any feedback. They did not undergo another passive viewing of the word pairs immediately prior to this. It is this second test that was used as measure of word-pair learning. In subsequent sessions, participants were tested in this same manner only. That is, they were cued with one of the words in a pair and were asked to type in the

corresponding word without feedback and without passive viewing of the word pairs. This was used as a measure of word-pair retention.

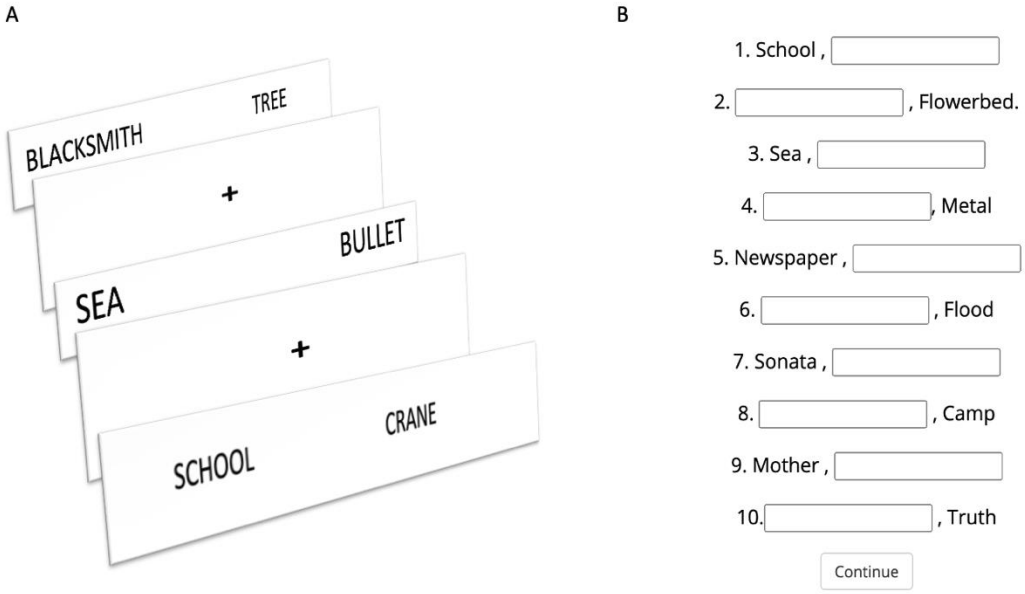


Figure 2.1 The Paired-Associates Task, Learning and Re-Test Examples
A) An example of the word pairs presented at the start of S1 as part of the learning stage of the study. Words were presented for 5000ms followed by fixation cross at the center of the screen which was presented for 300ms.
B) An example of the manner in which word-pair learning was retested at the end of S1, and in subsequent session(s). Participants had to type the corresponding word pair into the blank spaces.

2.3.2.1.1. Quantification of performance

Performance on the paired-associates task was calculated by the number of correct word pairs recalled out of a total of 20.

2.3.2.2. Serial Reaction Time Task (SRTT)

The second task in each session was a bimanual SRTT (Figure 2.2). Before the start of the task, participants were first presented with a video that played through the all the different sounds associated with the SRTT and were told to adjust the volume so that they could hear all of them before proceeding with the task. They were then given a short practice session (4 button presses) to familiarise themselves with how the stimuli would be presented on the grid and keys that needed to

be pressed in response to each cue. In this short practice session, participants could not proceed to the next trial until they had pressed the correct key.

At the start of each trial a sound was played and an image of either an object or a human face appeared at one of four possible locations on a grid. The location on the grid in which each image appeared denoted which key should be pressed in response. The participants were instructed “press the correct key as quickly as you can” in response to the stimuli, by pressing the keys “A”, “S”, ”K”, or “L” on a QWERTY keyboard. Participant response ended the trial regardless of whether the response was correct or not. The trial lasted for a minimum of 200 ms (the duration of the auditory stimulus) to allow for the sound to play in full.

The task involved two sequences each 12 key presses long (sequence A: ASALSKLAKSLK, sequence B: SLKSKALSKALA). Each sequence had its own set of visual stimuli and its own set of auditory tones. The sequences were adapted from (Cousins et al., 2014) which were in turn adapted from (Nissen & Bullemer, 1987). While the underlying sequence structures remained identical to that in (Cousins et al., 2014) which were matched for difficulty and were successfully used for TMR, we modified the response keys to facilitate bimanual responses. In experiment one, participants were trained on both sequences, whereas for experiment two participants were only trained on one sequence. The task was split into blocks composed of three repetitions of a single sequence. Random blocks were presented to probe sequence-specific learning during different stages of the sessions.

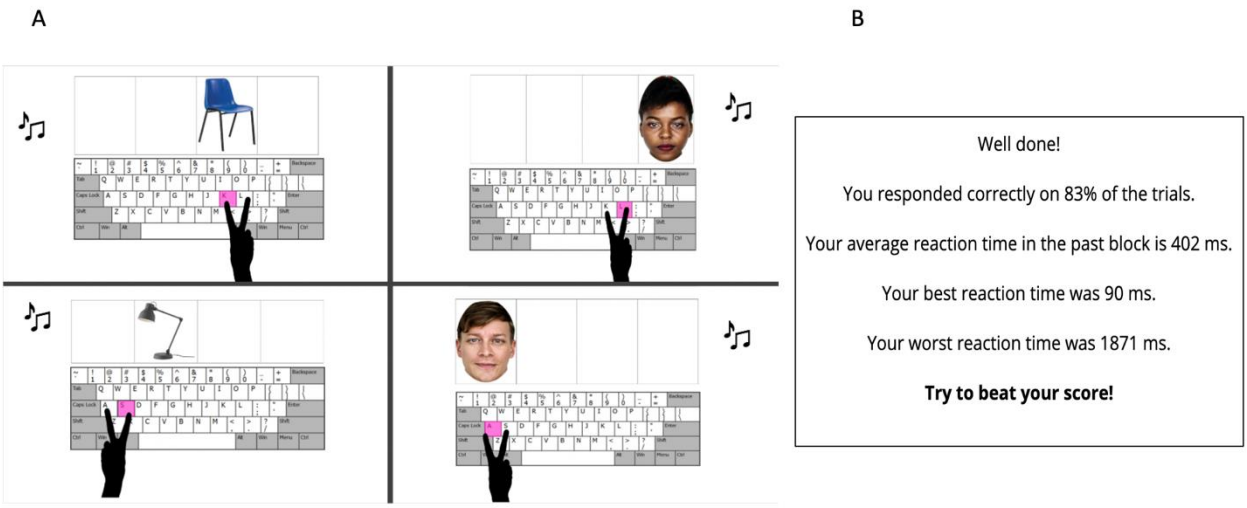


Figure 2.2 SRTT
The four possible visual stimuli presented at each trial of the SRTT. The image and its position on the grid determine the motor response to be made by the participant on the keyboard. The bottom portion of each image depicts the key to be pressed in response to the stimulus, as well as the finger and hand with which the button should be pressed. Note that the bottom part of the image has been added here for clarity and was not seen by participants during the task.
An example of the feedback given to participants between blocks.

As mentioned in the introduction, experiments in this thesis were designed with potential applications to future TMR experiments in mind (though TMR is outside the scope of the thesis). For the current chapter, adding an auditory component to the SRTT, such as a tone that accompanies each of the different visual stimuli in space, allows for future TMR experiments (Cousins et al, 2014; 2016). Further, using separate sets of auditory tones for each sequence enables future studies to employ TMR on one sequence and not the other, thus testing the effect of TMR on SRTT sequence learning. In terms of the motor responses, we chose a bimanual task using two fingers from each hand (index and middle). A bimanual task was chosen (a) because skill learning frequently incorporates co-operation between hands, and (b) so that the motor responses would elicit activity in both hemispheres that could later be classified and used to predict which cue was presented during TMR (Belal et al., 2018). The stimuli, which consisted of objects and faces, were also chosen for their ability to potentially be useful to an EEG classifier, as the faces would activate the fusiform face area while the objects would not (Calder, 2011) and potentially induce gamma oscillations in the same area (Lachaux et al., 2005).

2.3.2.2.1. Quantification of performance

We quantified performance on the SRTT using response times (RT) and a calculated composite score (CS) whereby both RT and accuracy are taken into account (CS = RT/accuracy (%)) to better account for speed/accuracy trade-offs.

Individual SRTT trials in which the RT (interval between visual cue and button press) was slower than 1 second were excluded. If the number of trials that had to be removed exceeded 50% of the total trials in a given block (>18/36 trials), that block was removed from analysis. After this initial data cleaning was done, if a participant had to have more than 50% of the total blocks removed in this way, then the participant was removed from the data pool.

To calculate measures of sequence specific learning (SSL) for RT (SSL_{RT}) and CS (SSL_{cs}), we compared RT or CS values between sequence and random blocks. To do this we averaged over 2-4 blocks of the sequence (see 2.3.6.2.1 and 2.3.6.2.2 for details) and subtracted this value from the value for the random sequence.

2.3.3. Questionnaires

Participants were asked to fill out a short questionnaire at the end of every session. In order to gauge self-reported measures of sleep quality and alertness during the task, participants were asked to report the time they went to sleep the previous night, the time they woke up that morning, complete the Stanford Sleepiness Scale (SSS) (Hoddes et al., 1973), and rate their experienced sleep quality the previous night on a 5-point Likert scale (5:"Very well", 4:"Well", 3:"Unsure", 2:"Poorly",1:"Very Poorly").

2.3.4. Experiment One specific study details

The study structure is in Figure 2.3. Participants were randomly assigned to one of two conditions, hereafter referred to as the AM and PM groups. Group affiliation determined the start time of the first session and therefore the two subsequent sessions. The AM group were instructed to begin S1 between 8-10am, S2 between 8-10pm that evening, and S3 between 8-10am the following morning. The PM group were instructed to begin S1 between 8-10pm, S2 between 8-10am the following morning, and S3 between 8-10pm the following evening.

Session 3 served as a control for circadian effects on task performance. Sleep studies that attempt to define the effect of sleep on a task by comparing the change in task performance after a period of nocturnal sleep and an equivalent period of diurnal wake run into circadian confounds. That is, if improvement is seen after a period of sleep compared to a period of wake (as is often hypothesised) it would be impossible to determine whether this was driven by sleep dependent consolidation or the fact that the group who slept likely experienced a decrease in sleep-pressure after a period of sleep whereas the wake group would experience an increase in sleep-pressure. Having a third session 12 hours after retest helps to mitigate this confound to some extent. If between-group differences in task performance over the first 12 hrs were driven by circadian factors we might expect to see a decrease in task performance in the following 12 hrs for the sleep group and an increase in task performance for the wake group.

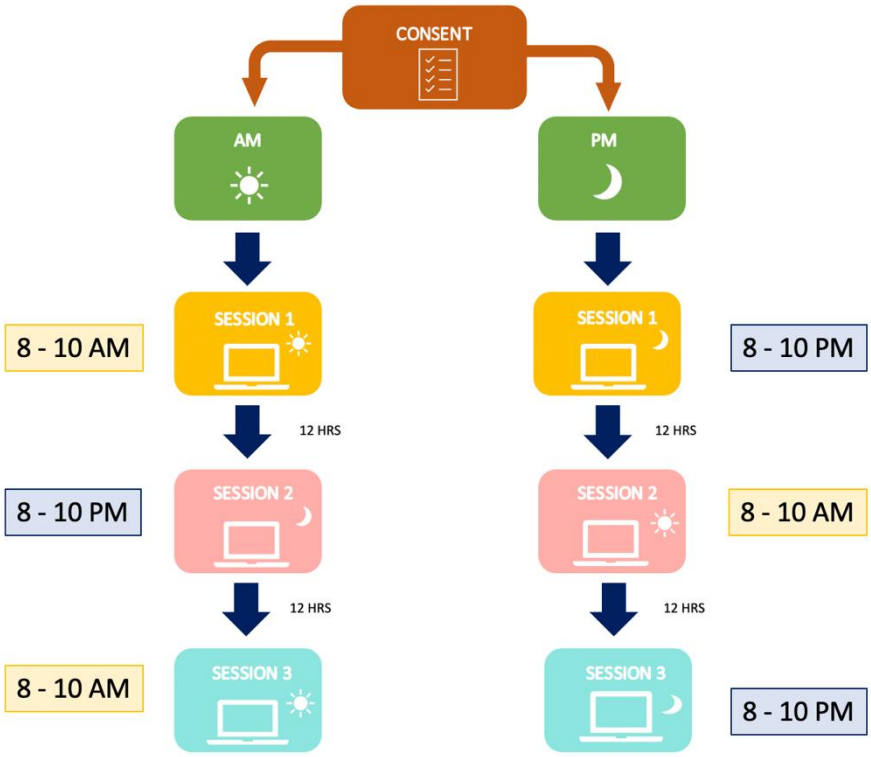


Figure 2.3 Schematic of Study I Structure.
Participants are randomly allocated to either the AM or PM group following the signing of the consent form. Group affiliation determines the time of day that participants complete each session. The study was composed of three sessions (hereafter referred to as sessions 1, 2, and 3), each took no more than 45 minutes to complete, spaced 12 hours apart.

The SRTT in S1 consisted of a total of 42 blocks (20 blocks per sequence) interspersed pseudorandomly such that no more than two blocks of the same sequence were presented consecutively. When the online session was initiated by the participant, the task structure was randomized so that half of participants were exposed to sequence A first and the other half to sequence B first. Two blocks of a random sequence were added to the SRTT in the first session at blocks 31 and 40 (the last random block in S1 was in the 40th position in the SRTT so that the participants could complete a final block of each of the experimental sequences before the end of the task. This way the experimental sequences, rather than the random one, were the last thing the participants experienced on the SRTT). There was a 10 second rest period between each block in which participants were given feedback (mean RT and accuracy (% of correct button presses)) and reminded to respond as quickly and accurately as possible.

Subsequent sessions (S2 and S3) consisted of 5 blocks (2 blocks of sequence A, 2 blocks of sequence B, and a random block). These sessions were intentionally kept brief as they were intended to probe memory consolidation as opposed to induce further learning. In both S2 and S3, the random block was the last block of the SRTT. Unlike S1, S2 began with sequence A and S3 with sequence B.

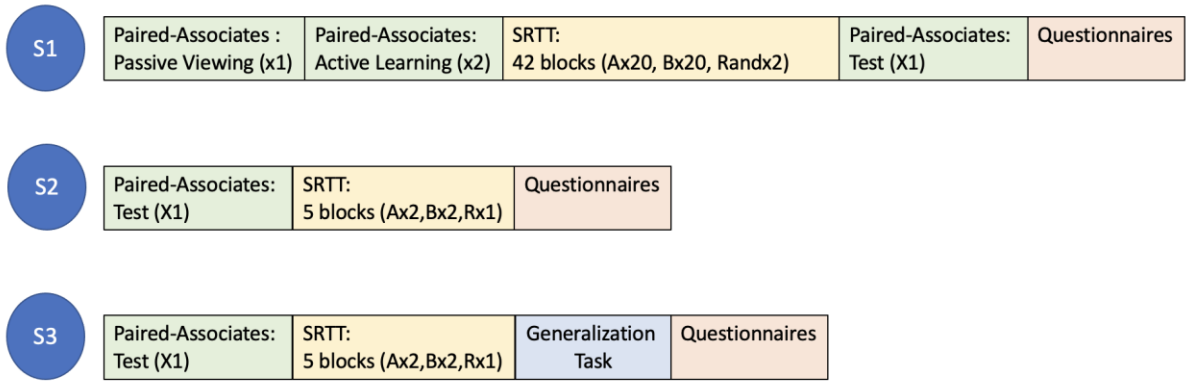


Figure 2.4 Schematic of Tasks and Their Order within Sessions 1-3.
* The generalization task is not discussed in this chapter, for details of this study see chapter 4 study I.

2.3.5. Details Specific to Experiment Two

In Experiment One, participants were exposed to two sequences. This raises the possibility of interference between the two sequences, which might influence learning and consolidation effects. In Experiment Two, participants were exposed to a single sequence, to reduce the possibility of interference effects. The experimental design was also simplified so that experiment two consisted of two sessions rather than three. S1 and S2 were separated by 12-hour intervals, as for experiment one. The participants in the AM group were instructed to complete S1 between 8-10am and S2 between 8-10pm the same day while the PM group did the reverse, being instructed to complete S1 between 8-10 pm and then S2 between 8-10am the next morning.

For the SRTT, participants were randomly allocated to train on sequence A or B (with equal probability). Session one consisted of 20 blocks of the experimental sequence (A or B) and 1 block of the random sequence. The random sequence block was the penultimate block seen by participants. In S2 the first two blocks consisted of the experimental sequence that they had trained on in S1, and the last block consisted of the random sequence.

The random sequences for the SRTT were generated using a twelve-cell random number generator on Excel. Generated sequences were manually checked for repeating items (e.g. the chair stimulus in two consecutive trials) and for motifs longer than 4 items that overlapped with either sequence A or B. A total of 20 random sequences were generated thus, and 3 of these were randomly selected by the behavioral code for the random block in sessions 1 and 2. The code ensured it was not possible to select the same random sequence twice in one block, therefore each of the sequences in the random block is novel to the participant. The pool of 20 sequences therefore generates a large selection of possible combinations, making it unlikely that many participants will encounter the same three random sequences in the same order within a block.

2.3.6. Analysis

For each performance measure, in order to calculate the change in performance between sessions we subtracted the session that we hypothesised would have a smaller value from the session we hypothesised would have a bigger value, such that improvement on a given task over time is expected to be denoted by a positive value.

2.3.6.1. Paired-Associates

Higher scores indicate better retention of word-pairs. To calculate the change index, we therefore calculated S2-S1 (both experiments) and S3-S2 (experiment one only).

2.3.6.2. SRTT

Change measures were calculated for RT and CS, as well as sequence specific learning metrics - SSL_{RT} and SSL_{CS}. For both RT and CS, better performance is indicated by lower scores, so scores were predicted to decrease over time and therefore change scores were calculated as S1-S2 (both experiments) and S2-S3 (experiment one only). For SSL, better sequence specific learning is indicated by higher scores, so scores were predicted to increase over time and therefore change scores were calculated as S2-S1 (both experiments) and S3-S2 (experiment one only).

2.3.6.2.1. Experiment one specific analyses

For SSL, we calculated sequence RT as the mean of the last 4 sequence blocks before the random sequence in each session. This was done so that, given that there were two experimental sequences, each sequence could be averaged over two blocks. As with other experiments, change values rather than raw scores were used to quantify change between pre to post sleep (or wake) SSL on the task.

Due to an error in an early version of the code, 39 of the 49 participants were missing one block in S1 (block 39). This block was therefore removed from analysis for any participants who did have block 39. Furthermore, 4 of the 49 participants had at least one missing value (block) which was removed due to poor performance (see 2.3.2.2.1). Missing blocks in the middle of the session were replaced with the average of the values before and after the missing block. Missing blocks at the start of the session (block 1) were replaced by the average of all the remaining participant's values at block 1. In the one instance where there were two consecutive missing blocks in a single participant, a weighted mean was calculated using the values of the preceding and proceeding blocks. The mean was weighted 0.75:0.25 towards the preceding value in the first block and 0.25:0.75 towards the proceeding value in the second block to reflect each block's relative proximity to these values in the average.

2.3.6.2.2. Experiment two specific analyses

For SSL, we calculated sequence RT as the average of the last 2 sequence blocks before the random sequence in each session.

2.3.7. Statistical Analyses

2.3.7.1. Question(s) 1: Do participants learn the tasks in a single session? Is the degree of learning in S1 comparable between groups?

2.3.7.1.1. SRTT:

In order to determine whether participants showed evidence of learning in S1 we conducted a mixed repeated measures analysis of variance (rmANOVA) using RT and CS values per block for both the AM and PM groups.

In order to determine whether participants learnt the task in a sequence-specific manner, we compared to SSL_{RT} and SSL_{CS} values to a test value of zero (no difference between sequence and random) using a two-tailed, one-sample t-test.

In order to determine whether non-sequence specific motor learning was affected by time of day we asked whether performance on the random blocks improves in a comparable manner between the AM and PM groups. To do this we compared the difference between AM and PM groups for RT in the two random blocks in S1 through a two-tailed, independent samples t-test.

For experiment two only, to ascertain whether the sequences were comparably difficult to learn we conducted a mixed rmANOVA on the RT per block in S1 for the two sequences (A and B) across the AM and PM groups.

2.3.7.1.2. Paired-Associates:

To determine whether the participants in the AM and PM groups learnt the task in S1, we ran two two-tailed, one-sample t-tests (one per group) to compare the percentage of words recalled per group to a test value of zero (no words recalled correctly).

To determine whether the AM and PM groups learnt the task to a comparable degree in S1, we ran a two-tailed, independent samples t-test using the percentage of words recalled by the AM and PM groups.

2.3.7.2. Question 2: Is there evidence of sleep-dependent consolidation?

2.3.7.2.1. SRTT

We conducted two-tailed, independent-samples t-tests on each of the change indexes (between S1 and 2) for RT, CS, SSL_{RT} and SSL_{CS} across the AM and PM groups.

2.3.7.2.2. Paired-Associates

A two-tailed, independent-samples t-test looking at the change in percentage of words recalled from S1 to S2 was conducted across the AM and PM groups.

2.3.7.3 Question 3: *Do Participants Generalize Between the SRTT and The Paired Associates?*

We conducted a two-tailed Pearson correlation on the SSL_{CS} and the percentage of word pairs recalled in S1.

2.4. Results

2.4.1. Experiment One

2.4.1.1. Characterizing learning in session 1 : Do Participants Learn Over the SRTT Training Session (S1) and is this Learning Different Between the AM and PM Groups?

2.4.1.1.1. Response time (RT):

The mixed rmANOVA on the block-wise RT in S1 (with sequences A and B pooled and the random blocks excluded) showed a significant main effect of block ($F(6.5, 332.6) = 37.8$ $p < 0.001$) indicating that the response times were different across blocks which suggests learning occurred during practice in S1 (Figure 2.5A).

There was a significant interaction between group and block ($F(6.5, 332.6) = 2.5$, $p = 0.02$) suggesting change in RT between blocks was significantly different across groups. Post-hoc comparisons across S1 blocks with Bonferroni correction, demonstrate that it was the blocks at the start of the session that were significantly different from the later blocks. This suggests that most learning took place in the first few blocks of S1. In terms of the interaction between the block-wise RTs and groups, the Bonferroni correction showed that it was the first seven blocks that showed significant differences between the two groups, this suggests that time of day had a significant effect on the early stages of learning.

Therefore, both groups showed evidence of learning in the first session of the SRTT, as evidenced by the decrease in RT across blocks, Block-wise comparisons showed that the early blocks were most different from the later blocks, suggesting the most learning occurred in the early blocks of the task. When block-wise RTs were compared across the AM and PM groups, the groups showed most significant differences in the first 7 blocks of the session, which suggests circadian effects such as sleepiness may have affected these initial learning stages.

2.4.1.1.2. Composite Scores (RT/accuracy):

The mixed rmANOVA on the CS per block in S1 for both AM and PM groups (Figure 2.5C) also showed a significant main effect of block ($F(7.469, 380.932) = 21.045$, $p < 0.001$) indicating that the composite scores were significantly different across blocks which suggests learning occurred during practice in S1. There was no significant interaction between group and block ($F(7.469, 380.932) = 1.843$, $p= 0.073$) and no main effect of group ($F(1,51) =.693$, $p = 0.409$) which suggests there was no significant difference in CS between AM and PM groups in session 1.

Therefore, there is also evidence for learning in both groups when the speed accuracy trade-off is taken into account through the use of a composite score.

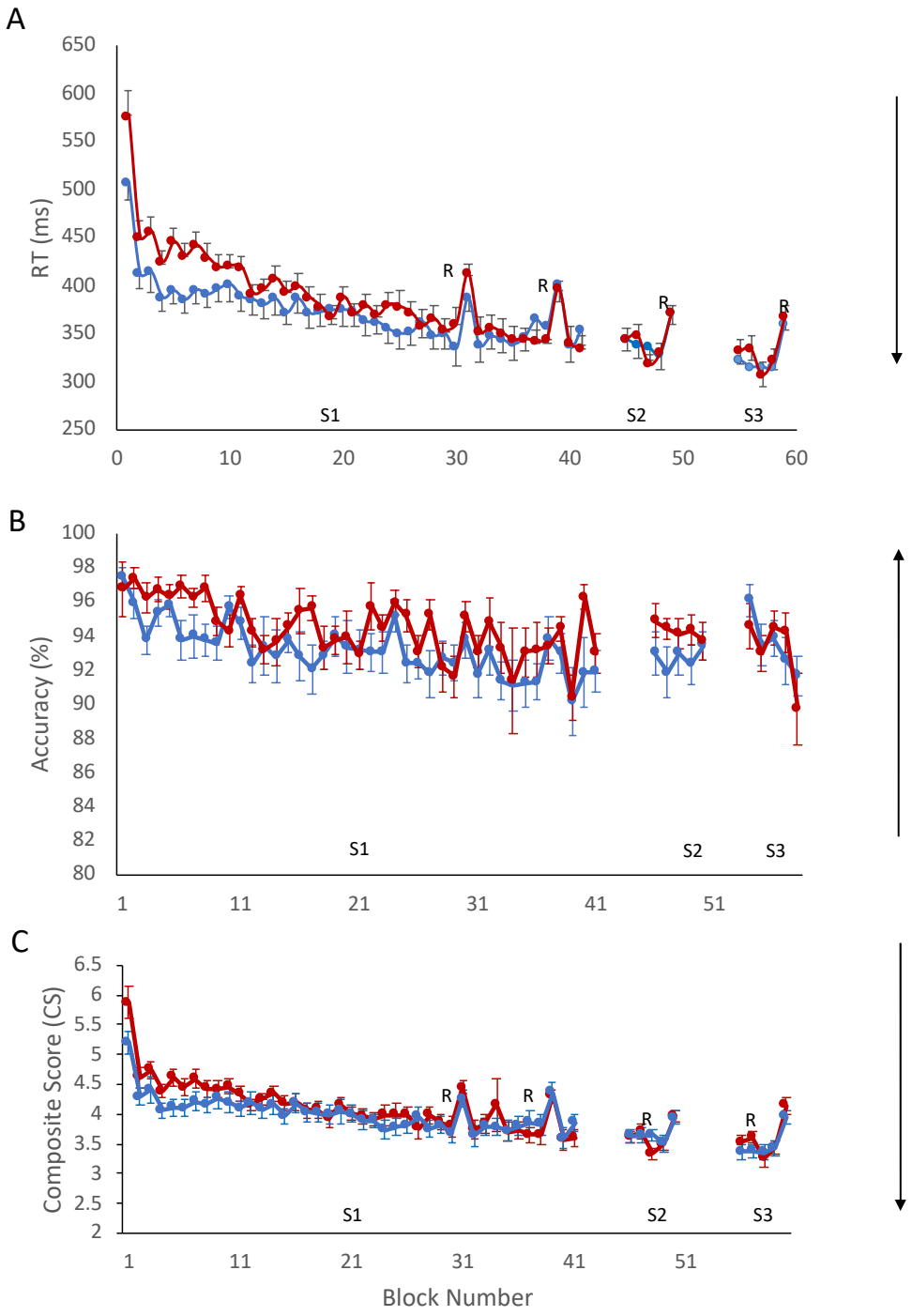


Figure 2.5 A Comparison of SRTT Performance Across Sessions as Measured by RT (A), Accuracy (B) and CS (C).
The PM group is represented in blue and the AM group in red. Sessions 1, 2, and 3 are labelled on all plots as S1, S2, and S3 respectively. Random blocks within a session are marked with an "R." Data points are the block mean with SEM error bars. Arrows denote the direction of task improvement. N=49, AM=27, PM=26

2.4.1.2. Do Participants Learn the SRTT Training Session (S1) in a Sequence-Specific Manner?

The SSL_{RT} and SSL_{CS} scores compared the RT or CSs of the random sequence and the experimental sequences. Since an output of zero would indicate that performance on the random and experimental sequences was equal, a one-sample t-test versus zero was used to assess whether any sequence specific learning had occurred.

2.4.1.2.1. Response time

For both groups SSL_{RT} was significantly different to zero (AM: mean difference = 52, SEM =9.1, t(26) = 5.739, p = <.001, PM: mean difference = 48.4, SEM= 7.7, t(25) = 6.397, p = <.001) . This suggests that both groups learned the SRTT in a sequence-specific manner. Additionally, an two-tailed independent samples t-test showed that there was no significant difference in SSL_{RT} between the AM and PM groups, t(51) = -.301, p = 0.765. Therefore, both groups showed sequence-specific learning to a comparable degree.

2.4.1.2.2. Composite Scores (RT/accuracy):

SSL_{CS} was significantly different to zero for both groups (AM: mean difference = 0.6, SEM = 0.1, t(26) =5.739, p= <.001, PM: mean difference = 0.6, SEM= 0.09, (t(25) = 6.397, p = <.001). This suggests both groups learned to perform the task in a sequence-specific manner. A two-tailed independent-samples t-test also showed that there was no significant difference in SSL_{CS} between the AM and PM groups (t(51)= -.174, p= .863). Therefore, both groups learned the SRTT in a sequence-dependent manner to a similar extent even when the speed/accuracy trade-off was accounted for.

2.4.1.3. Paired Associates Task: Do the AM and PM groups learn the paired-associates task to a comparable degree in S1?

We first needed to confirm that both groups were able to learn the task. To do this, we used two one-sample t-tests to compare the average accuracy (%) of word pair recall per group to a test value of

zero. Both the AM (t(25)= 12.46, p= <.001) and PM (t(25)= 10.6, p= <.001) groups had accuracy values that were significantly greater than zero (Figure 2.6) .

An independent-samples t-test showed that the AM and PM groups did not differ significantly in their learning of the paired-associates task as measured by the percentage of words recalled at the test in S1 (t(50)= -.812 , p= .420) (Figure 2.6A). Therefore, both groups were able to learn the paired-associates task in S1 and learned it to a comparable degree.

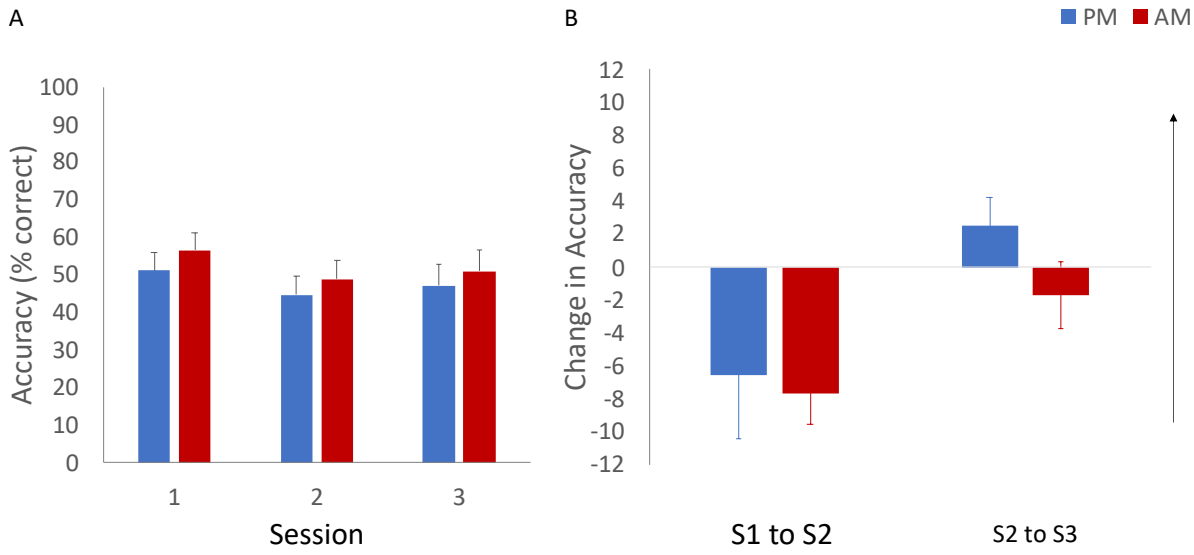


Figure 2.6 Between Subjects Comparison of Performance on the Paired-Associates Task
A) Accuracy of word pair recall across the 3 sessions for AM and PM groups. B) Change in word pair recall across sessions for AM and PM groups, the arrow indicates that bigger numbers denote improvement in recall performance between the relevant sessions. All values are the group average, the error-bars are the SEM. N=49, AM=27, PM=26

2.4.1.4. Characterizing sleep-dependent consolidation

2.4.1.4.1. SRTT: Is the Degree of Consolidation Greater After A Period of Wake Compared to A Period of Sleep?

In order to test the hypothesis that a period of sleep following learning of an SRTT results in better consolidation of learned information than an equivalent a period of wake, we compared the change in all performance measures (ΔRT, ΔCS, $\Delta SSL_{RT,}$ ΔSSL_{CS}) between sessions one and two between the AM and PM groups.

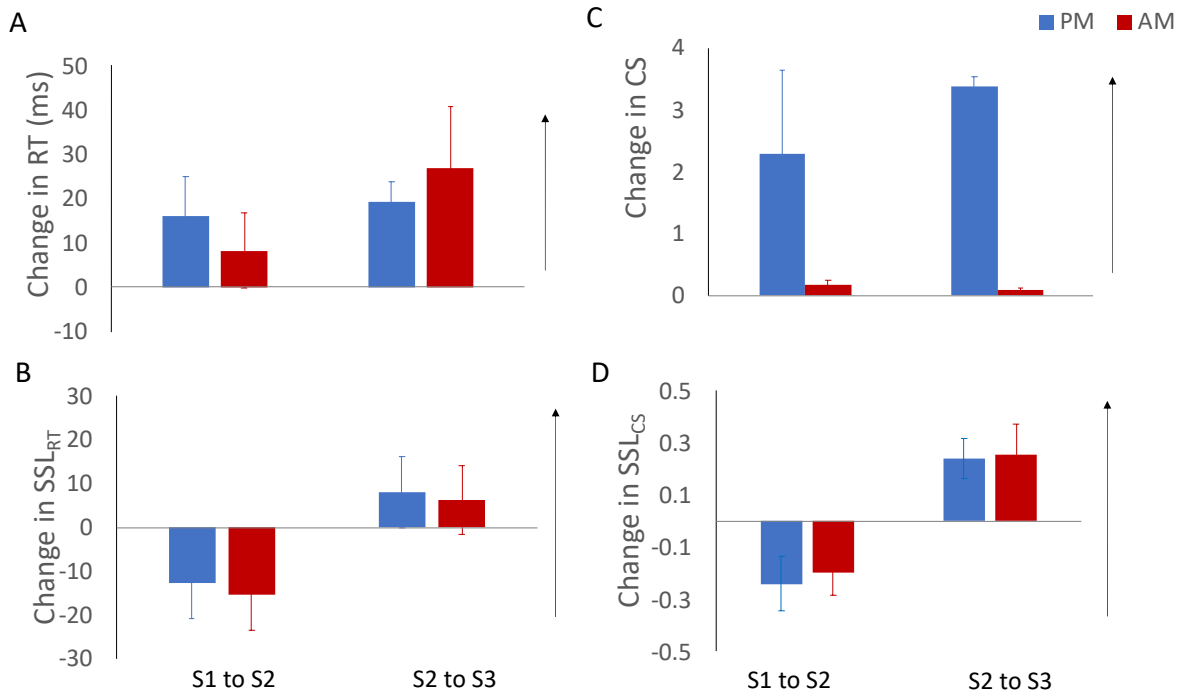


Figure 2.7 Comparison of the Change in Various Measures of SRTT Performance Across Sessions
A) Change in RT (ms) across S1 to 2 and S2 to 3 across the AM and PM groups. B) Change in SSL_{RT} across S1 to S2 and S2 to S3 for the AM and PM groups. C) Change in CS across S1 to S2 and S2 to S3 for the AM and PM groups. D) Change in SSL_{CS} from S1 to S2 and S2 to S3 for the AM and PM groups. The PM group is represented in blue and the AM group in red. The arrows indicate that bigger numbers equate to task improvement. All values are the group average, the error-bars are the SEM. N=49, AM=27, PM=26

An independent-samples t-test showed that there were no differences between groups for ΔRT ($t(51)= 0.64$, $p= 0.26$), ΔSSL_{RT} ($t(51)= .26$, $p= .4$), ΔCS ($t(49)= 1.52$, $p= .07$) or ΔSSL_{CS} ($t(49)=-.31$, $p= .38$) suggesting that consolidation of learning did not differ between the group that slept after learning, and the group that stayed awake for an equivalent period of time (Figure 2.7). Therefore, a period of sleep did not confer a significant benefit to participants' SRTT performance.

2.4.1.4.2. Paired-Associates: Is the Degree of Consolidation Greater After A Period of Wake Compared to A Period of Sleep?

We also aimed to test the hypothesis that a period of sleep following learning would benefit a declarative memory task. An independent-samples t-test looking at the change in percentage of words recalled from S1 to S2 re-test showed no differences between the groups ($t(50)=-.27$, $p= .4$; Figure 2.6B).

2.4.1.4.3. Does Sleep Benefit Consolidation Regardless of Whether it Occurs Immediately After Learning?

An exploratory question was whether the timing of sleep had an effect on the degree of consolidation that it conferred. That is, whether it was more beneficial to have a period of sleep immediately after learning (as in the PM group) or after a period of wake (as in the AM group). To do this, we compared all performance change scores between S1 and 2 for the PM group versus S1 to 3 for the AM group – the intervals where sleep occurred for each of the groups. We also compared the change in word-pair recall accuracy across the same sessions.

For the SRTT There were no differences between groups for ΔRT ($t(49)= -.36$, $p= .72$), in ΔSSL_{RT} ($t(49)= -.03$, $p= 1$), ΔCS ($t(49)= 1.46$, $p= 0.15$) or ΔSSL_{CS} ($t(49)= -2$, $p= .06$; Figure 2.8). Therefore, sleep immediately after learning did not confer significantly different benefits than a period of sleep following a period of wake, regardless of the behavioural measure of SRTT performance examined.

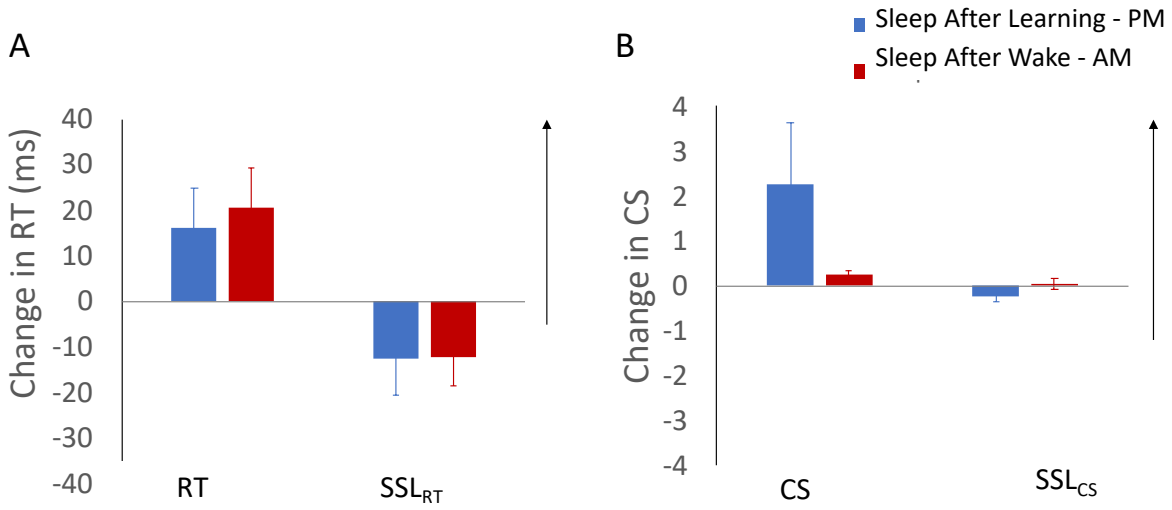


Figure 2.8 Change in SRTT Performance Over An Immediate of Delayed Sleep Period
A) Change in RT (left) and RT Normalized to the Random Sequence (right) from S1 to the post-sleep re-test for the AM and PM groups. B) Change in CSs (left) and CSs Normalized to the Random Sequence (right) from S1 to the post-sleep re-test for the AM and PM groups. The PM group is represented in blue and the AM group in red. The arrows indicate that bigger numbers equate to task improvement. N=49, AM=27, PM=26

For the paired-associates, an independent samples t-test showed that sleep timing in relation to learning did not significantly affect performance (t(48)= .029, p= .977; Figure 2.9).

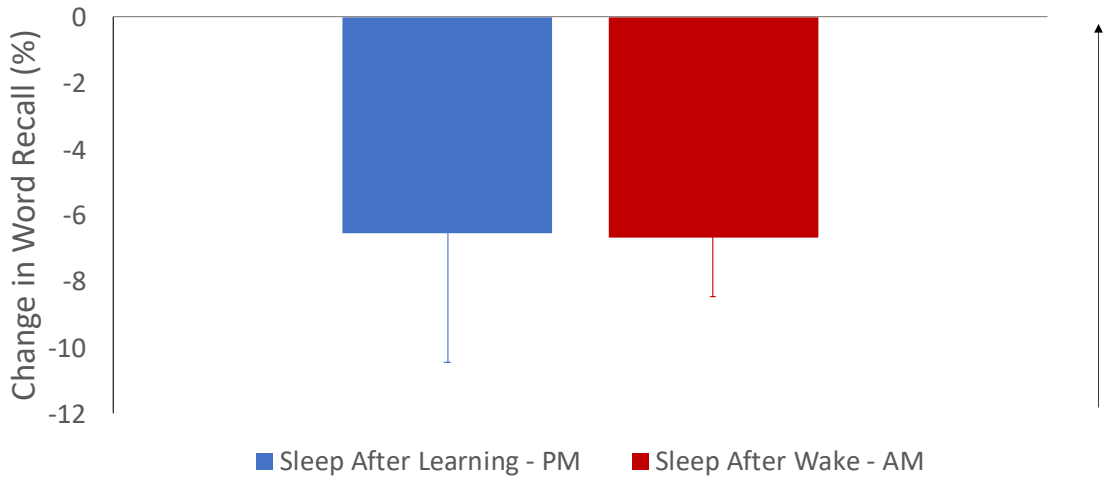


Figure 2.9 Change (%) in Word Recall Following Either Immediate or Delayed Sleep
The PM group is represented in blue and the AM group in red. The arrows indicate that bigger numbers equate to task improvement. N=49, AM=27, PM=26

2.4.2. Experiment Two

2.4.2.1. Do Participants Learn Over the SRTT Training Session (S1) and is it Different Between the AM and PM Groups?

2.4.2.1.1. Serial Reaction Time Task (SRTT)

In order to ensure that this revised version of the SRTT was also readily learned in a single session, an rmANOVA was run on RT and CS in S1 for both AM and PM groups. The random block was excluded from this analysis. There was a significant effect of block on RT (F(19, 6.775) = 37.634, p = <0.001), and there was no significant interaction between block and group which suggests that the groups learned the SRTT to a similar degree in S1 (F(19, 6.775) = .520, p=.955; Figure 2.10A). Similarly, there was a significant effect of block on CS (F(19, 8.961) = .23.163, p= <.001) and no significant interaction between group and block (F(19, 8.961), p= .784; Figure 2.10C).

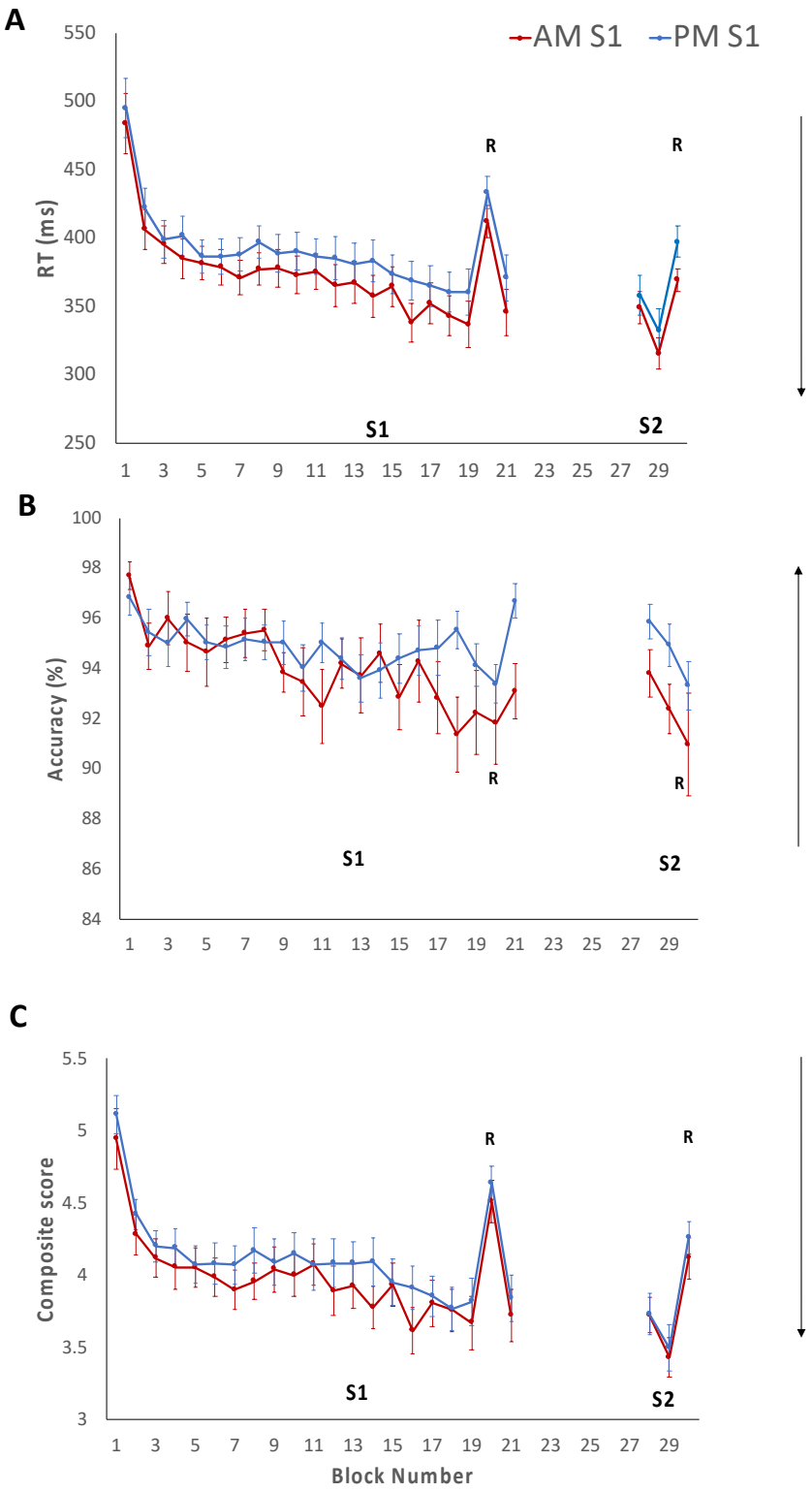


Figure 2.10 A Between Subjects Comparison of SRTT Performance Across Sessions as Measured by RT (A), Accuracy (B) and CS (C).

The PM group is represented in blue and the AM group in red. Sessions 1,2, and 3 are labelled on all plots as S1, S2, and S3 respectively. Random blocks within a session are marked with an "R." Arrows denote the direction of task improvement. N=50, AM=23, PM=27.

2.4.2.2. Are the experimental sequences equally challenging to learn?

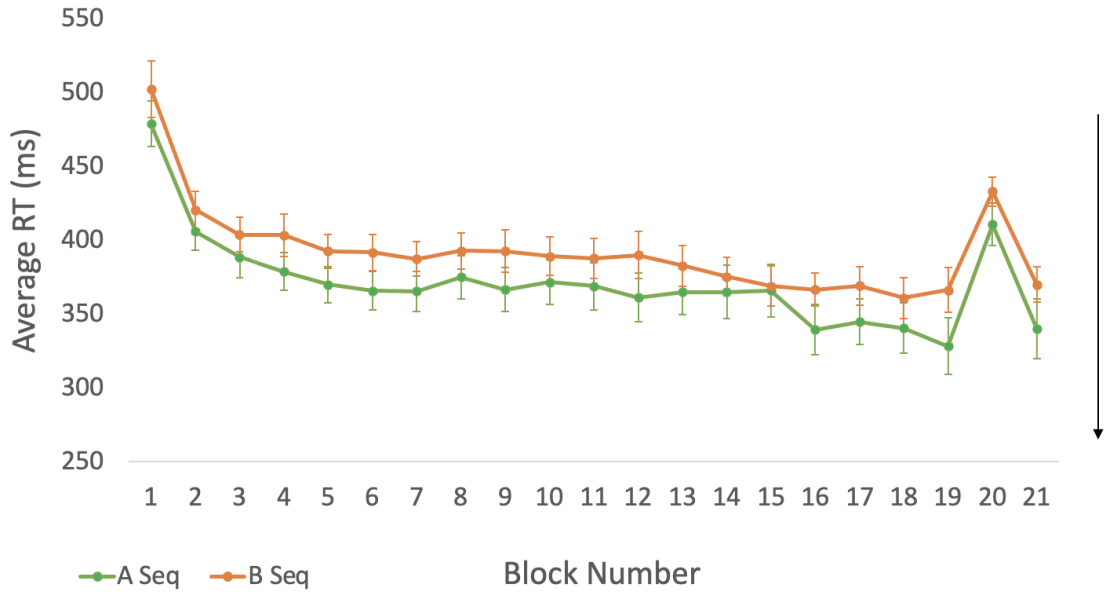


Figure 2.11 Block-wise Comparison of RT in Session 1 for A (Green) and B (Orange) Sequences.
The arrow denotes the direction of task improvement, the error bars are the SEM. N=50, A= 22, B=26.

In contrast to experiment one where all participants learned both experimental sequences, participants in the current study only learned one sequence – either A or B. The distribution of the two sequences across groups was AM - A: 45% , B:55%, PM - A: 46% , B: 54%.

We needed to know whether participants learned the two sequences equally well. The mixed rmANOVA on the RT per block in S1 for the two sequences (A and B) (excluding the random block) found no interaction between block and the sequences which suggests that the two sequences were similar in terms of how challenging they were to learn ($F(6.518) = .585$, $p = .757$; Figure 2.11).

2.4.2.3. Do Participants Learn the SRTT Training Session (S1) in a Sequence-Specific Manner?

Both groups showed a significant difference from zero for the SSL_{RT} (AM $t(22) = 6.166$, $p = <.001$; PM $t(26) = 7.164$, $p = <.001$) and SSL_{CS} (AM ($t(22) = 5.511$, $p = <.001$; PM $t(26) = 8.089$, $p = <.001$) suggesting that both groups learned the SRTT in a sequence-specific manner. Similarly, the independent samples

t-tests showed no differences between groups for SSL_{RT} (t(48) = -.050, p =.961) or SSL_{CS} (t(48) = -.290, p =.773)

2.4.2.4. Is non-sequence-specific RT in S1 comparable between the AM and PM groups?

Next, we checked whether RTs were affected by time of day, this is important to check as energy levels and levels of alertness are affected by time of day and this could affect RT and thus drive the direction of changes between sessions 1 and 2. An independent-samples t-test showed that there was no significant difference in RT of the random block between the AM and PM groups (t(48)= -1.276, p= .208). This suggests that response speed in the SRTT in S1 was not significantly affected by time of day.

2.4.2.5. Paired-Associates Task: Do the AM and PM groups learn the paired-associates task to a comparable degree in S1?

An independent-samples t-test compared the percentage of words recalled at the S1 retest between the AM and PM groups. A significant difference was found in the degree to which the two groups recalled the word pairs in session 1 (t(48) = 2.034, p= .024; Figure 2.12A). This demonstrates that the AM and PM groups did not start at a comparable baseline and suggests that there may be a circadian effect on the performance of this task in the short term.

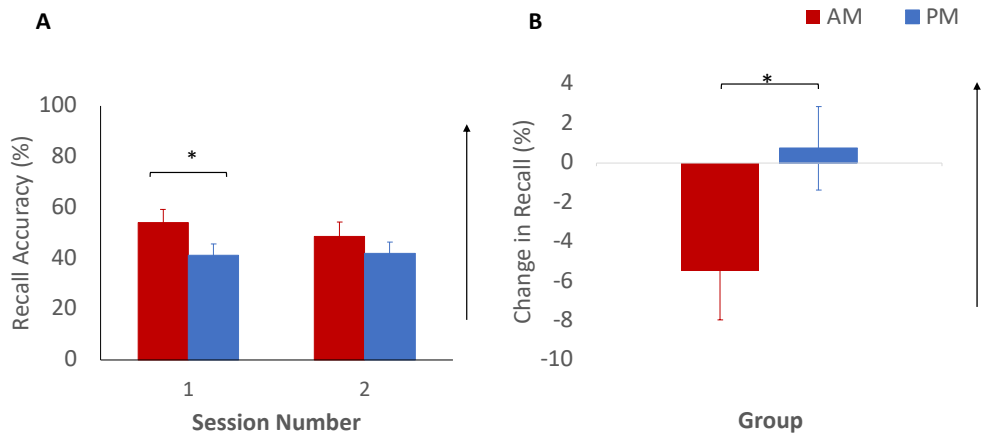


Figure 2.12 Between Subjects Comparison of Performance on the Paired-Associates Task
A) Accuracy of word pair recall across sessions for AM and PM groups. B) Change in word pair recall across sessions for AM and PM groups. The arrows denote direction of improvement. N=50, AM=23, PM=27.

2.4.2.6. Is the Degree of Consolidation of the SRTT Greater After A Period of Wake Compared to A Period of Sleep?

2.4.2.6.1. Serial Reaction Time Task (SRTT)

Independent-samples t-tests found no significant difference between the AM and PM groups for ΔRT (t(48) = -.562 , p= .288; Figure 2.13A), ΔSSL_{RT} (t(48) = -1.158 , p= .126; Figure 2.13B), ΔCS (t(48) = -.303 , p= .381; Figure 2.13C), or ΔSSL_{CS} (t(48) = -.384 , p= .351 Figure 2.13D) as metrics of consolidation. This suggests that there was no statistically significant benefit to participants' performance on the SRTT if they slept after learning or stayed awake for an equivalent time.

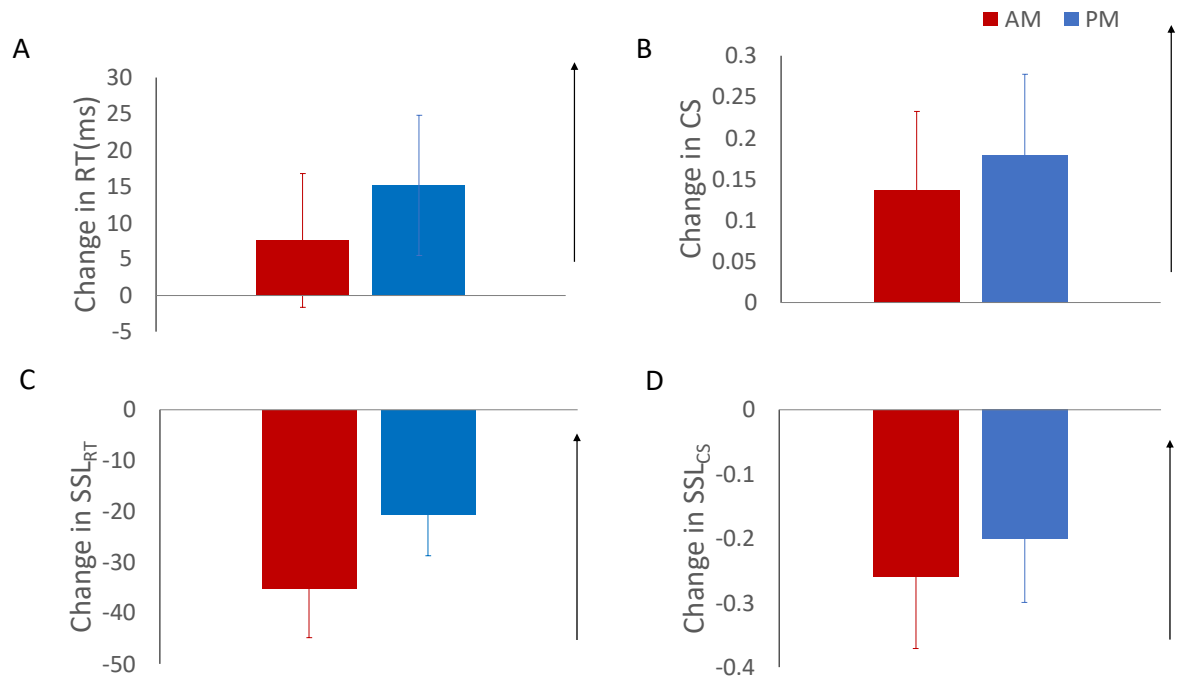


Figure 2.13 Subjects Comparison of the Change in Various Measures of SRTT Performance Across Sessions
A) Change in absolute RT (ms) from S1 to 2 and S2 across the AM and PM groups. B) Change in SSL_{RT} from S1 to S2 across the AM and PM groups.C) Change in absolute CSs across S1 to 2 and S2 across the AM and PM groups. D) Change in SSL_{CS} from S1 to 2 and S2 across the AM and PM groups. The PM group is represented in blue and the AM group in red. The arrows indicate that bigger numbers equate to task improvement. N=50, AM=23, PM=27.

2.4.2.6.2. Paired Associates Task

An independent-samples t-test showed a significant difference in the change in word pair retention between the AM and PM groups (t(48)= -1.761 , p= .042; Figure 2.12B), suggesting that sleep had a protective effect on word-pair decay over time. However, since performance at S1 was significantly different between the two groups we also conducted a one-way ANCOVA with performance at S1 as a covariate and found no significant difference in the change in performance between the two groups (F(1) = 1.986, p=.165). The differences in word-pair retention at S1 were therefore driving our previous result to some extent (Figure 2.14).

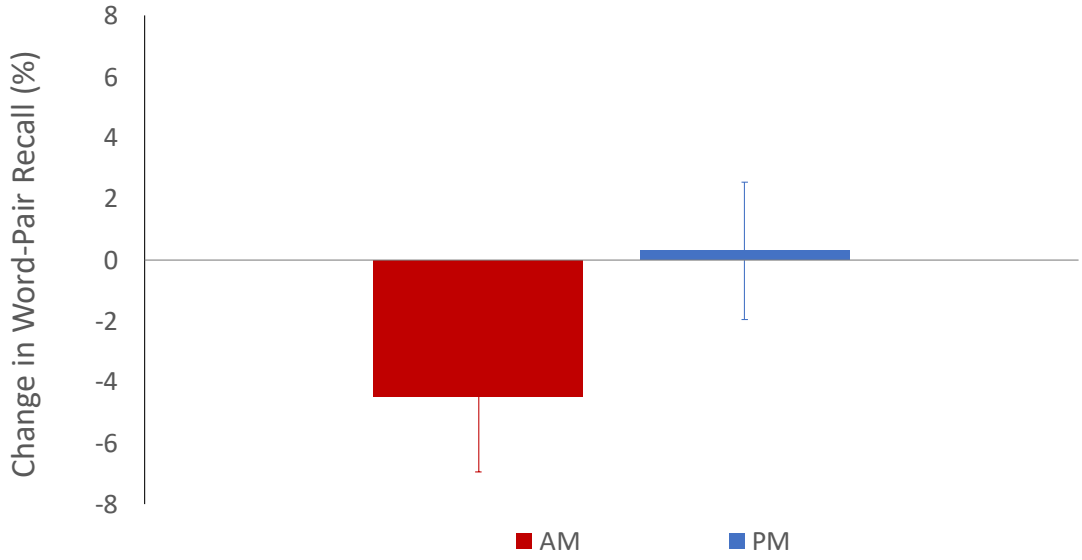


Figure 2.14 Change in Word-Pair Retention (adjusted for S1 word-pair recall)
Arrow denotes direction of improvement, the error bars are the SEM. N=50, AM=23, PM=27.

2.4.2.6.3 *Do Participants Generalize Between the SRTT and The Paired Associates?*

One final exploratory question we wanted to address was, given our later focus on generalization, whether there was any evidence of this between tasks – that is between the SRTT and the paired associates. While there are many related questions that could be asked – is it affected by sleep? Is there an effect of time of day (AM vs PM groups)? Does the relationship between the two tasks change after a period of sleep/wake? – we will simply ask whether performance on one task correlates with performance on the other. This test also serves to probe whether these two tasks are sufficiently independent of each other to be able to be used as a control in future TMR studies, as we originally envisioned. We decided to use the data at the end of session 1 to avoid the added variable of sleep vs wake. A two-tailed Pearson correlation showed no significant correlation between performance on the SRTT and paired associates (N=50, r = .15, p = .3, fig 2.15). This may be due to a lack of overlap or connection between the two tasks.

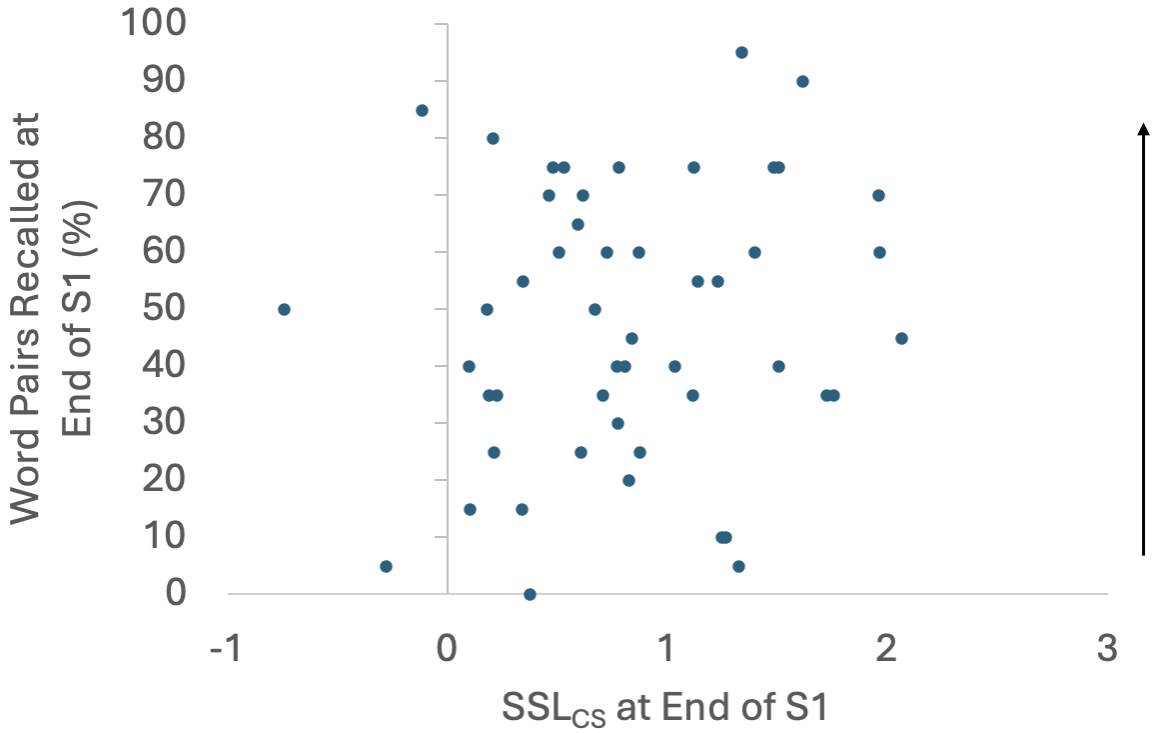


Figure 2.15 The Relationship Between Performance on the SRTT and Paired Associates Task in S1
The relationship between the SSL_{CS} and the percentage of word pairs recalled at S1.
Arrow denotes direction of improvement, N=50.

2.5. Discussion

Across two experiments we posed two key questions. First, we asked; **can we establish completely remote, online, motor and declarative memory tasks that can be learned in a single session?** To address this question, we asked participants to learn motor and declarative memory tasks online. We found, across both experiments, that both the AM and PM groups were able to learn the SRTT in one session using speed (RT) as a metric of task performance. We next asked whether the AM and PM groups learned the task in a comparable manner. This was done to ensure that the learning of our tasks was not readily affected by time of day. In experiment one, we found a significant difference in the learning curves between the two groups, however, this was driven by blocks at the beginning of the session and was not evident in experiment two.

This begs the question; are participants learning the sequences or simply getting faster at pressing the keys associated with the task? We found that both groups, in both experiments, were indeed able to learn the task in a sequence-specific manner and that this sequence-specific learning was comparable between the two groups.

The instructions for the SRTT place emphasis on both speed and accuracy, however, looking at Figure 2.5 and Figure 2.10 we can see that there is a trade-off in performance between these. Taking the speed-accuracy trade-off in the SRTT into account through the use of composite scores (RT/accuracy) we confirmed that participants learn in a single session and did so in a sequence-dependent manner that was comparable between groups. Notably, we did not observe the between-groups differences in early block performance that we observed in the analysis with RT as a performance metric in experiment one. This suggests that these differences were driven by speed rather than accuracy.

Similarly, participants were able to learn the paired-associates (declarative memory) task in one session regardless of group. Therefore, we successfully established a task that could be learned in a single session, in a remote, online setting.

Having established this, our second question for this chapter was; **is the consolidation of these tasks sleep-dependent?** In order to test the hypothesis that our motor and declarative memory tasks benefit from a period of sleep – i.e. show sleep dependent consolidation- we tested their performance of the aforementioned tasks after either a period of sleep or an equivalent period of wake. We hypothesized that the group that slept immediately after learning (S1) would perform better at retest (S2) than the group that was awake for an equivalent period of time. In order to control for circadian effects, we also retested them at S3 - 12 hours following S2 in experiment one.

We compared the change in the SRTT performance from the end of the learning session (baseline) to the first retest. Contrary to our hypothesis, we found no significant effect of sleep compared to wake in the change of any of the performance metrics. Equally, we found no effect of sleep compared to wake in the word pair retention for the paired-associates task.

2.5.1. Why don't we observe sleep dependent consolidation in the SRTT?

There are many potential reasons why, in contrast to previous studies (Ellenbogen et al., 2009; Payne et al., 2012; Walker, Brakefield, Seidman, et al., 2003; Walker et al., 2002) , we did not observe sleep dependent consolidation in the current study. One methodological explanation that experiment one posed was that it may be that learning two SRTT sequences is too demanding. While two sequences may be useful for a TMR protocol, such that one is the experimental sequence and the other the control, there are not many studies that look at sleep-dependent memory consolidation using two sequences simultaneously. In TMR studies, the change in performance of the targeted sequence and

the control sequence can be compared at post-sleep testing. In contrast to memory consolidation research where, more commonly, one sequence per learning session is used and change in performance is compared after a period of sleep compared to wake.

However, some sleep consolidation studies with more than one learned sequence have been carried out. For example, Walker et al., (2003a) asked participants to learn two sequences consecutively and tested them following a sleep or wake period. Participants in the sleep group showed significantly improved RT for both sequences and improved accuracy only in the latter sequence that was learned. This is in contrast to a different sub-study where only one sequence was learned, and sleep-dependent consolidation was observed in both RT and accuracy metrics. This demonstrates that it is possible to observe sleep dependent consolidation with two sequences, at least in terms of RT which is the standard metric in accessing SRTT performance. However, it should be noted, that this study differed from ours in that the test took place 24 hours after the learning session which means that a period of both sleep and wake had occurred for both groups. Furthermore, the learning session occurred at 1pm in both sub-studies and so the sleep group did not sleep immediately after the learning session. The finding that the second sequence they learned benefitted more from sleep also highlights the fact that there may be a recency effect when more than 1 sequence is used. However, it is also difficult to consider this finding in the context of our study because in their study participants learned the sequences one after the other as opposed to learning them in parallel as our participants did. Their experimental structure may therefore be more conducive to recency effects.

All of this raises the question; could sleep consolidation be observed if we simplify the SRTT? We therefore repeated the experiment but required participants to only learn one sequence. Similar to experiment one, we found no clear evidence for a benefit of sleep on consolidation of the SRTT. The fact that we did not find see any sleep-dependent consolidation suggests that this was not the reason we failed to see it in study I. While there was no significant effect of sleep compared to wake on task

performance, we did find that the group who slept after learning performed better on all measures of SRTT performance at retest (in terms of the average value being quantitatively higher - albeit not statistically so) and showed less forgetting of the word-pairs at recall. This, as before, suggests that perhaps the patterns that have been suggested by the literature are also present in our data but have not reached significance, perhaps due to low power. It also highlights the considerable variability across individuals, which is perhaps magnified in a home-setting compared to a more strictly controlled lab environment. An important future study would therefore be to repeat this study with a larger number of participants.

Another possible explanation for the lack of sleep-dependent consolidation is the finding that sleep confers a greater benefit to the SRTT if the task requires explicit learning of the sequences rather than implicit learning (Robertson et al., 2004). This may be because explicitly learning the sequences while maintaining low RTs is more cognitively demanding and sleep tends to benefit difficult (Spencer et al, 2017) and incompletely learned tasks (Denis et al., 2021). While it is well accepted that the SRTT inherently requires both explicit and implicit learning mechanisms (Robertson, 2007) we never asked participants to explicitly learn or demonstrate explicit knowledge of the sequences. Therefore, perhaps making the SRTT more explicit in nature would have yielded a clearer benefit of sleep to the SRTT.

Although our tests of sleep dependent effects did not reach significance in any instance, SRTT performance between the two groups did generally change in the directions that sleep dependent consolidation would predict. That is, the PM group showed quantitatively greater improvement on average than the AM group from S1-S2 (albeit not statistically so) on all performance measures other than the change in sequence-specific CSs. It is therefore also possible that the current study was underpowered, and a significant change might have been observed with a larger sample size. A related factor that might have contributed to the fact that we did not observe sleep dependent consolidation was that the study was conducted remotely. This is directly related to the low sample size because we

had a high drop-out rate, and several participants were excluded due to non-compliance with the study schedule. Furthermore, it is difficult to control experimental conditions across online participants as one would in laboratory conditions, and any number of factors (such as distractions) can influence performance on the tasks. A more controlled experimental environment than that offered by our remote testing set-up, might have therefore yielded different results.

With that being said, one of the goals of this study is to set up some groundwork for future remote sleep studies. The lack of control over experimental settings yielded by this study structure is therefore something that we would have to contend with if our aim is to study sleep and eventually conduct TMR in an at-home setting. In this we have addressed our goal as to our knowledge there is no research looking at sleep dependent motor consolidation using the SRTT in a remote setting. This study is therefore not only informative in studying the relationship between sleep and task consolidation in this setting but is also helpful in establishing future experimental protocols.

2.5.2. Why don't we observe sleep dependent consolidation in the Paired-Associates Task?

One plausible explanation is interference between the two tasks. That is, when two tasks are learned in close temporal proximity to one another it can result in one or both tasks being consolidated to a lesser extent than if they had only learned a single task. This seems to be true of motor/procedural and declarative memory during wake. Brown & Robertson (2007b) found that participants who learned a list of words after learning an SRTT deteriorated on the SRTT after a period of wake and the degree of motor deterioration correlated with their improvement on the word list task. This in contrast to participants who counted the number of vowels in a set of letters (a non-declarative task) and saw improvement in the SRTT over the wake period. The same effect was observed when, in a separate experiment, participants performed a declarative memory task and the SRTT functioned as the

interference task. This effect, however, was not observed after a night of sleep – in this case both groups improved on the SRTT. Similar effects have been found by Ertelt et al.,(2012). We therefore do not suspect interference to be the reason for the lack of sleep dependent consolidation. Nevertheless, the relationship between the SRTT and the paired-associates task is something to keep in mind when considering whether to use a declarative paired-associates task as a control in future TMR studies.

While not statistically significant, the direction of the group average changes in the results of our paired-associates task support what has been observed in the literature. For instance, (Ellenbogen, Hulbert, et al., 2006a) found that a period of sleep after learning prevents decay of word-pair recall (in the first 12 hours, ie during the sleep period) and protects from interference the day after learning (in the second 12 hours, ie during the wake period) compared to wake after learning. Similar results have been found by Payne et al., (2012) using 30 min, 12 hr, or 24 hr testing intervals and are supported by several others (Benson & Feinberg, 1975, 1977; Gais et al., 2006). As with the SRTT, it may therefore be that sleep-dependent consolidation is occurring but that we are underpowered to detect it.

2.5.3. Limitations

Finally, we must consider the flaws and limitations of our study. As discussed above, we experienced high levels of drop-out after participants had consented to participate. Further, conducting a study in an at-home setting introduces noise and variability as it is not possible to control factors that can be tightly controlled in the lab. In addition, during analysis of experiment one we found overlap in motifs between the experimental and random sequences. This means that there were elements of the random sequence was not completely novel to the participants and the random sequence may therefore not have been a true probe of motor or sequence-independent performance. In the second experiment this was rectified, and we found evidence for sequence specific learning, though still failed ot find evidence of sleep dependent effects.

Lastly, while perhaps not a limitation of the study per se, it is worth mentioning that the experiments were conducted during the COVID-19 pandemic. We did our best to control for factors that might influence normal sleep routines in the short-term (such as shift work and jet-lag), however chronic stress, lack of routine, illness, and grief can all effect both learning and sleep. Indeed, the International COVID-19 Sleep Study (ICOSS), an online survey-based study conducted across 14 different countries during the lockdown, found an increase in worsened sleep quality compared to pre-pandemic times in 20% of respondents – which included problems with sleep maintenance, nightmares, and excessive daytime sleepiness (Morin et al., 2021; Partinen et al., 2021).

2.6. Conclusions and Next Steps

We began this chapter by discussing the importance of conducting research outside of the lab in conditions which more accurately reflect the nature of the phenomena that we are studying. This is particularly true of sleep, as research suggests people sleep differently in novel settings (such as the sleep lab). We therefore set out to develop motor and declarative learning tasks that could be learned online in the participants' homes. Throughout this chapter we have developed such tasks, and the second study of this chapter has given us confidence that our task is applicable to remote, online setting to which we want to apply it. However, we have thus far not observed any behavioural evidence of sleep-dependent consolidation.

Our next step will be to take a different approach to exploring relationships between sleep and learning, by investigating variation across individuals. Rather than testing for between group effects comparing sleep and wake, as we have done in the current chapter, we will instead test whether electrophysiological sleep features correlate with learning. We hypothesise that this could allow for a more sensitive test of the relationship between sleep and learning, that leverages variation in sleep

across individuals. The next chapter will therefore allow us to explore whether we can observe these features in an at-home setting and whether they correlate with learning behaviour outside of the lab. Identifying and characterizing the roles of these features will also allow us to identify physiological traits of sleep that could be targets for at-home manipulations in the future.

3. Exploring the EEG correlates of memory consolidation in a naturalistic setting

3.1. Introduction

In the previous chapter, we established motor and declarative tasks that could be learnt at home, in a single session, with the aim of investigating whether these tasks benefit from sleep and exhibit sleep-dependent consolidation. To answer this question, we carried out between-subjects comparisons of the change in performance on our tasks over a 12-hour period that either included or excluded sleep and found no clear evidence of sleep-dependent consolidation using this approach.

In this chapter we aim to take a different, hopefully more sensitive, approach to investigating the relationship between sleep and sleep-associated improvement on these tasks in an at-home setting. Rather than asking whether sleep confers offline benefits to task performance compared to an equivalent period of wake, we asked whether any features of sleep correlate with changes in task performance. More specifically, we asked whether changes in sleep macro or microarchitecture correlate with behavioural improvements in our tasks. In this context, sleep macroarchitecture refers the duration of each sleep stage in a given sleep session – in short, the information that is represented on a hypnogram. In contrast, microarchitecture here refers to features of the oscillations within the stages of sleep and their features.

To address these questions, we asked participants to perform the same online tasks as the second study in the previous chapter. As before, all tasks were completed in the participants' homes without an experimenter present. In order to quantify their sleep features, we also sent participants portable EEG devices (Dreem) to wear during encoding, sleep, and retest.

3.1.1. Sleep Macroarchitecture

As discussed in previous chapters, sleep's general benefits are likely the result of the interaction of all the different sleep stages. However, previous studies have found consistent beneficial effects of time spent specifically in SWS on the consolidation of both declarative (Gais & Born, 2004; Plihal & Born, 1997; M. A. Wilson & McNaughton, 1994) and motor (Tamaki et al., 2013; Tucker & Fishbein, 2009; Wilhelm et al., 2011) tasks. Additionally, upregulating slow oscillations (SOs) during SWS using Non-Invasive Brain Stimulation (NIBS) techniques such as tDCS and TMR has resulted in improved performance in motor tasks [(Cousins et al., 2016; Koopman1 et al., 2020)] and declarative memory tasks (Marshall et al., 2004, 2006; Prehn-Kristensen et al., 2014). It is therefore this stage of sleep that we will be focusing on in this chapter, specifically stages N2 and N3. Based on the above findings, we hypothesised that participants who spent a greater percentage of the night in SWS would show a significantly greater degree of improvement on our declarative memory and motor tasks. This begs the question; what mechanisms take place during SWS to confer this benefit in memory consolidation?

3.1.2. Recap of Replay

One possible mechanism is replay – whereby cell assemblies that were active during the encoding of a task are reactivated during sleep. The relationship between this reactivation and neural oscillations results in the strengthening or weakening of a given memory. This phenomenon occurs most prominently in SWS (although it has also been observed in wake and, to a lesser extent, in REM) and is believed to be the mechanism through which SWS associated oscillations implement memory consolidation during sleep. A brief recap of replay will be presented below, see chapter 1 section 1.6 for a more detailed overview.

During wake, in the early stages of memory formation, the hippocampus is proposed to unite information from several brain areas that process the different sensory aspects of a memory into a single memory or engram (McClelland et al., 1995). During subsequent sleep, the cortex momentarily releases tonic inhibition of subcortical structures during the up-states of the slow-oscillations (SOs) that characterize SWS. During this depolarizing phase the memory is repeatedly reactivated in the hippocampus, which recruits the brain areas originally involved in the memory formation as well as associated cortical regions (Ji & Wilson, 2007; Qin et al., 1997). This reactivation is correlated with the presence of sharp-wave ripples in the hippocampus and spindles which are generated by the thalamus. When these co-occur, the sharp-wave ripples become embedded in the spindle's troughs, forming what is called a spindle-ripple event (Steriade, 2006). These spindle-ripple events are, in turn, nested in the up-state of the cortical SO and thus fedback to the cortex which requires many repetitions of high amplitude input for LTP to occur (Lisman et al., 2001). It is thus that the transfer of information from short-term storage in the hippocampus to long-term storage in the neocortex that underlies consolidation is proposed to occur (Figure 3.1).

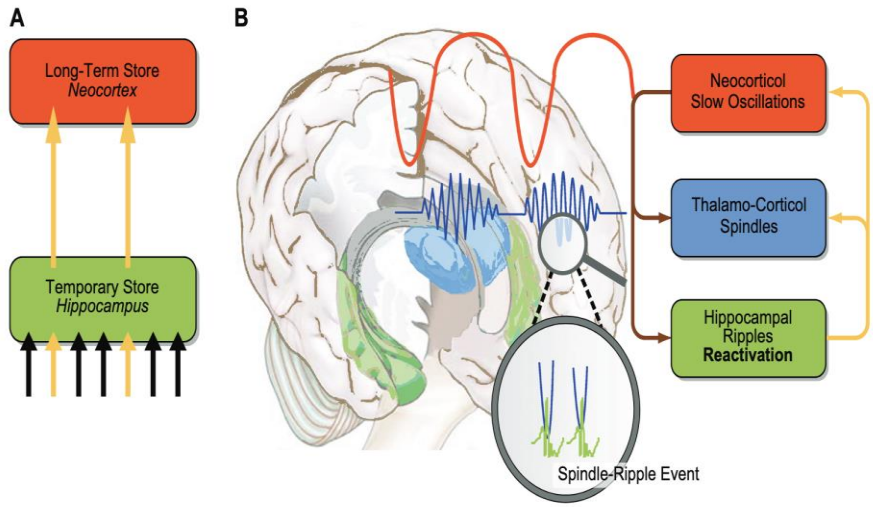


Figure 3.1 Cartoon Visualization of the Active Systems Consolidation Hypothesis (Rasch & Born, 2013)
A: A diagram of the flow of information during sleep, where replayed information is communicated from the temporary store that is the hippocampus to the cortex where it is consolidated for stable, long-term storage.
B: A diagram of the top-down and bottom-up oscillatory processes and their topographies.

3.1.3. Sleep Microarchitecture

Therefore, in addition to investigating the relationship between change in sleep macroarchitecture and change in task performance, we also aim to investigate the oscillatory features within SWS that correlate with behavioural performance. We chose our features of interest based on those which coincide with replay – namely SOs and sleep spindles. While it is not possible to measure replay or sharp-wave ripples directly using EEG, we can measure features of both spindles and SOs. Based on the mechanisms described above, we are interested in the number of spindles in the time spent in non-REM stages 2+3, henceforth N2+3, (spindle density), the strength of spindle power, and the degree to which SOs and spindles are coupled.

Previous literature suggests that of the EEG features in N2+3 that correlate with replay, spindle frequency and power are the most robustly correlated with sleep-dependent memory consolidation (Kumral et al., 2023).

3.1.4. Novelty of Research

As mentioned previously, most of our knowledge of sleep and consolidation in humans comes from studies conducted in laboratory settings where conditions are tightly controlled, multi-modal, high-density recordings are the standard, and sleep researchers are at hand to tackle any technical issues in real time. Recent efforts have been made, however, to study sleep-dependent consolidation in a more naturalistic home-setting, usually at the cost of experimental control and signal quality (Göldi & Rasch, 2019). It remains unclear, therefore, to what extent EEG markers of sleep-dependent consolidation in a home setting are associated with SRTT and paired-associates task performance in the same way that they are in a lab setting. That is, to what extent is the sleep we see in these artificial settings the same as the sleep that occurs in everyday life. And, if it is comparable, will we be able to

detect the same relationships between EEG markers of sleep-dependent consolidation and task performance as we do in the lab? Not only is the experimenter's control over the behavioural tasks diminished in these remote settings, but their control over the equipment is reduced when recording dimensions are low and therefore crucial. We hope that the following study contributes to this open question in the field.

3.1.5. Chapter Aims and Predictions

In summary, the key aim of this chapter is to investigate whether EEG features of NREM sleep correlate with overnight consolidation of motor and declarative memory in an at-home setting. More specifically,

- To examine the feasibility of measuring sleep microarchitecture, sleep spindles and slow oscillations using low-density EEG in a home setting
- To test for correlations between changes in sleep features and consolidation of motor learning
- To test for correlations between changes in sleep features and consolidation of word-pair learning

Based on the research discussed here and in the first chapter, we hypothesise that:

- **Sleep Macroarchitecture:** Increase in total time spent in N2+3 from baseline to post-encoding sleep will be positively correlated to improvement in performance on our behavioural tasks.
- **Sleep Microarchitecture**: Increase in spindle density, power, and SO-spindle coupling from baseline to post-encoding sleep will be positively correlated to improvement in performance on our behavioural tasks.

3.2. Methods

3.2.1. Participants

Participants were recruited via University of Oxford departmental and collegial mailing lists, social media, and physical posters/flyers posted around the University and Oxford at large. Study participants had to be 18-60 years of age, be fluent in English, have basic computer literacy, have access to a working laptop with reliable internet and a QWERTY keyboard, consent to not taking naps for the duration of the study, consent not to deviate from their normal caffeine or alcohol intake during the study, reside within the UK (ideally in Oxford or the surrounding areas to facilitate delivery of equipment), and have access to a device (e.g. smartphone or tablet) in order to download the Dreem "Alfin" app for Dreemband data collection. Potential participants were excluded if they were deaf or hard of hearing, a skilled musician, habitually play action video games, have been diagnosed with a sleep disorder, have a history of or current psychiatric illness, have a past or current neurological condition, have a medical condition which impedes mobility of the hands and fingers (e.g arthritis), were taking medication known to affect sleep or sleep architecture, were taking recreational drugs that affect sleep quality or cause drowsiness, were partaking in shift work, or were jetlagged.

Those who expressed interest in the study were emailed the Participant Information Sheet and a link to the online consent form (on JISC – an online survey platform). Once consent was obtained, participants were given a unique participant ID and sent links to another JISC questionnaire which asked for demographic information (age, sex assigned at birth, and self-reported handedness) and their contact information for purposes of mailing/delivering the equipment and sending study reminders.

Initially 44 participants were consented, but 2 withdrew and 1 did not follow the study schedule and was therefore excluded. Therefore, 41 participants (mean age= 27$\pm$6.55 years, 13 male, 2 left-handed)

took part in the study and were used for the behavioural analyses. Of those 41 participants, a further 19 were excluded due to noisy EEG data during the subsequent EEG preprocessing pipeline described in section 3.2.6.1, leaving a total of 22 participants (mean age= 28.95±9.5, 5 males, 2 left-handed).

3.2.2. Behavioural Tasks

The behavioural tasks (SRTT and paired-associates task) and surveys (SSS, self-reported sleep quality, and self-reported sleep and wake-up times) were the same as experiment two in Chapter 2. That is, the SRTT consisted of 20 blocks of the experimental sequence and 1 block of the random sequence. Participants learned either sequence A or B in the evening (instructed to perform the task between 8-10pm) and were tested 12 hours later (instructed to perform the task between 8-10am). They also were instructed to learn the same 20 word-pairs as in the previous studies (in the evening) and were tested on their word pair retention 12 hours later (in the morning).

3.2.3. Equipment

3.2.3.1. Low Density Electroencephalography

The portable, low density EEG headband used in this study was the Dreemband III (Dreem, France). This EEG headband is commercially available and was developed with the aim of providing an accessible means of monitoring sleep in a home setting. While the EEG device can be purchased for personal use – to monitor and improve one's own sleep using their various tools- it can also be purchased for use in sleep research to enable simple recordings of brain activity during sleep.

3.2.3.1.1. Hardware

The EEG headband is designed to be comfortable to sleep with as well as quickly and easily applied to the head (Figure 3.2A). Velcro size-adjusters of various sizes can be used to enlarge the circumference

of the headband as needed to ensure good contact with the scalp. Participants were also supplied with an elastic headband to place over the device if needed to ensure it was secure on the scalp.

The Dreemband makes use of Bluetooth for data transfer (as such the device is wireless) and uses dry, silicone electrodes to avoid the need for conducting gel. For EEG, it uses 2 frontal electrodes at F7 and F8, two occipital electrodes at O1 and O2, and a ground at FP2 according to the international 10-20 system. An accelerometer located on the band that sits on the top of the head measures movements, head position, and respiration rate (figure 3.2).



Figure 3.2 The Dreemband
A: A photograph demonstrating the build of the Dreemband III and how it is to be worn.
B: Specifications of the Dreem III headband.
Images from the Dreemband manual.

In order to compare the Dreemband to the gold-standard PSG, (Arnal et al., 2020) conducted simultaneous Dreemband and PSG recordings (n=25). They found that mean percentage error (MPE) of the relative spectral power between the Dreemband and PSG measurements was approximately 15% for alpha, beta, and gamma frequencies and 10% for theta which was deemed acceptable.

A

	DH/PSG F3–O1
Alpha	15 ± 3.5
Beta	16 ± 4.3
Delta	16 ± 6.1
Theta	10 ± 1.4

B

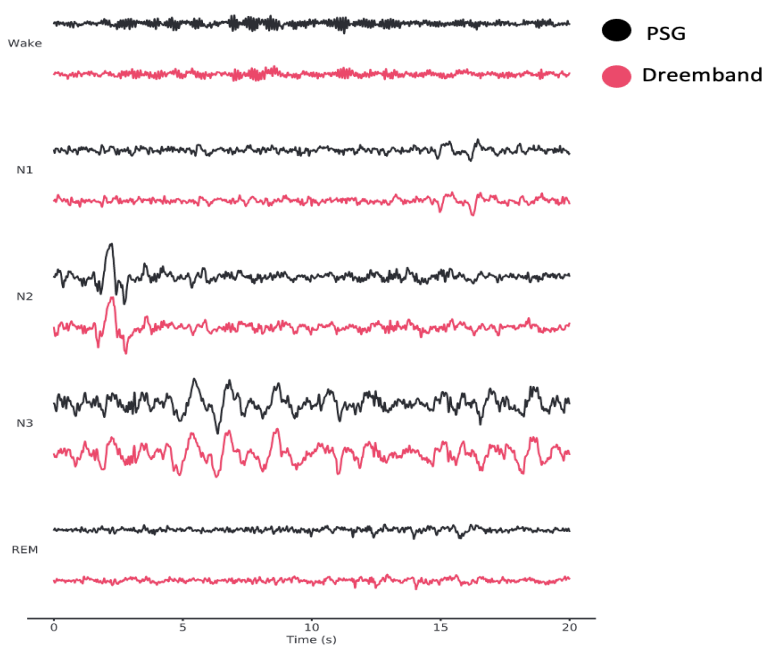


Figure 3.3 A Comparison of Dreemband and PSG EEG Recordings
A: MPEs of the relative spectral power across frequencies,
B: A 20s comparison of the EEG data acquired via PSG and the Dreemband across states of arousal.
Image from the Dreem General Guide which was adapted from Arnal et al (2020)

3.2.3.1.2. Software

Broadly speaking, the Dreemband uses a deep-learning algorithm which carries out real-time sleep scoring according to the American Association of Sleep Medicine (AASM) guidelines. A feature extraction algorithm first extracts features from the EEG and the other sensors in 30s epochs as is standard in PSG sleep scoring. It makes use of all its sensors and thus considers movement and breathing rate in addition to brain activity. A classifier is then used to determine the probability of the current epoch belonging to a given sleep stage based on the features of the previous 30 epochs. The classifier was trained using backpropagation and 423 recordings conducted using the Dreemband

hardware. Throughout the recording, individual channel quality is assessed based first on spectral and temporal distributions and then on the degree of correlation with the other channels. The algorithm works best with at least two EEG channels of acceptable quality from which it can extract features, but if this is not the case it will only use features extracted from the other sensors to score the data (Arnal et al., 2020; Chambon et al., 2019). The algorithm has been previously validated against 5 professional sleep technologists from three different institutes, the results of which are shown in figure and show a high degree consensus in our sleep stages of interest (Arnal et al., 2020).

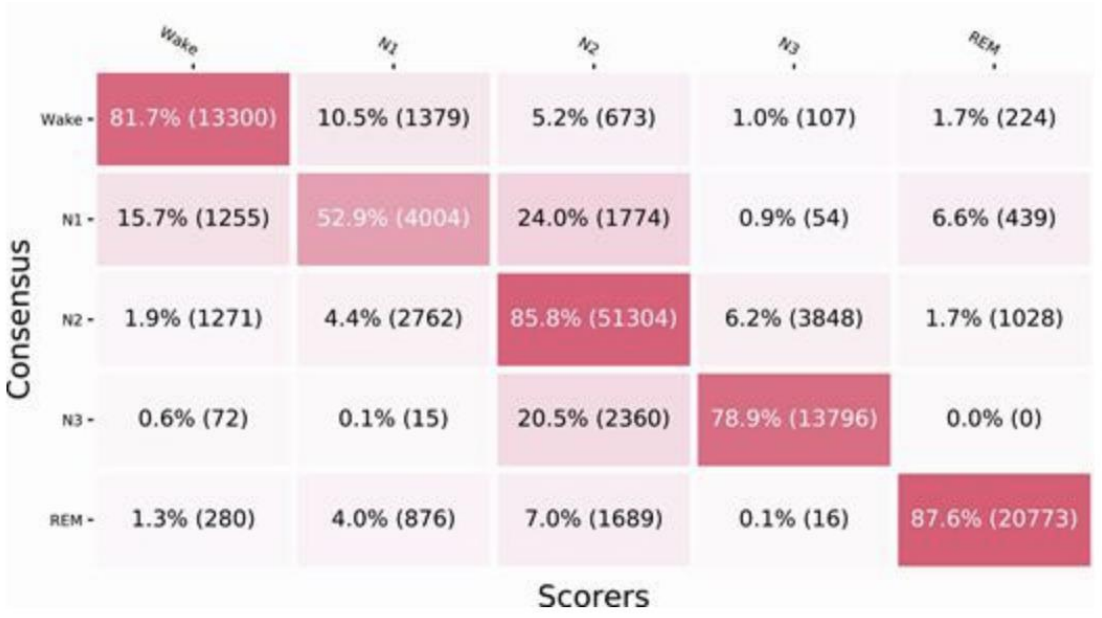


Figure 3.4 Confusion Matrix Showing the Degree of Consensus in Sleep Scoring Between Human Scorers and The Dreem Algorithm
The number of epochs scored for each sleep stage are shown in parentheses.
Figure (Arnal et al., 2020)

3.2.4. Study Procedure

3.2.4.1. Preparation

Once consent was obtained, participants were emailed to arrange a study start date and delivery of the Dreemband (along with size adjusters, charging cable and elastic headband). Participants were also given a set of instructions (in both paper and digital forms) which outlined how to set up and use the Dreemband, the schedule they needed to follow, and troubleshooting advice for common difficulties

with the Dreembands. Since the Dreemband needs to be paired to a phone app, a Dreem email (winstudy_SX@dreem.com) and password were generated for each participant in order to de-identify their data. This login information was included both in the instruction sheet and emailed to them.

Between participants, all of the study equipment that was to be given to the participants were cleaned using antiseptic wipes which contained at least 80% alcohol.

Given that our previous study found a tendency towards sleep dependent consolidation on the SRTT, this study did not include an AM group as in previous iterations of the study. Rather, all participants followed a single study schedule, which is outlined in Figure 1 and largely mirrors the schedule followed by the PM group in Chapter 2. This design allows us to address the key questions of interest for the current study, which concern the relationship between sleep characteristics and learning.

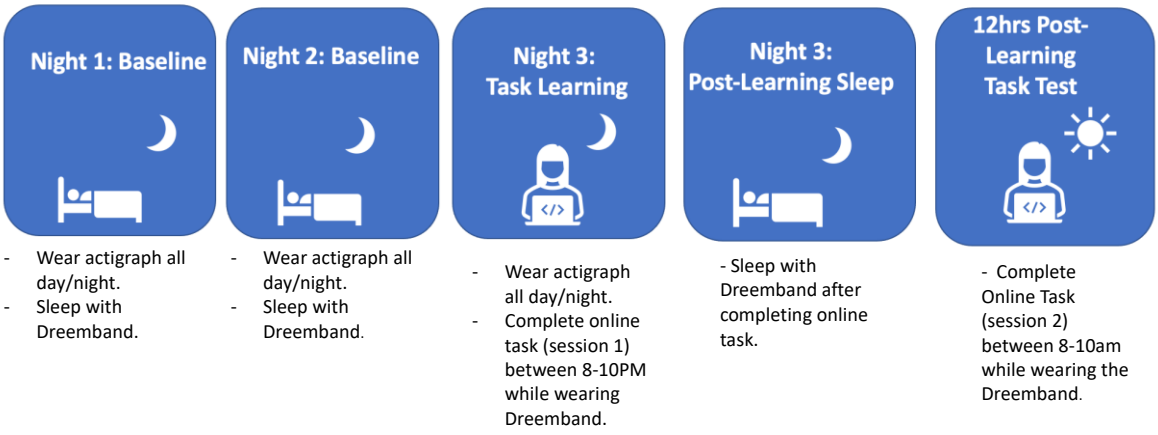


Figure 3.5 Study Outline
Graphical representation of the study schedule and the tasks required of participants at each stage of the study.

3.2.4.2. Baseline Nights

During the first two nights of the study, participants wore the Dreemband during overnight sleep. This allowed the participants to get habituated to sleeping with both the actigraph and Dreemband and to ensure that they knew how to set up the Dreemband prior to the post-learning night. This period also

allowed us to give participants feedback on the data quality generated by each night of sleep and suggest ways in which the quality could be improved (e.g. adjusting headband sizing). These two nights combined were subsequently used as a baseline measure of sleep EEG activity prior to task exposure.

3.2.4.3. Learning Night

On the third night, participants were instructed to begin the first (learning) session of the task online between 8-10pm. They were instructed to wear the EEG headband during task performance in order to capture task-evoked EEG activity for potential future analyses. The night that followed this first behavioural session was considered the post-learning sleep night. Finally, participants were instructed to perform the second (test) session of the task the following morning between 8-10am while wearing the Dreemband.

To ensure participants completed the tasks at the correct times, they received email reminders an hour before the start of the window of time to complete the task. Text reminders could be sent instead or in addition to the email reminders at the participant's request. Furthermore, the links to the tasks were only made available to them through the reminder email so that they could not access them too far ahead of time.

3.2.5. Behavioural Analysis

The behavioural analyses for both the SRTT and the Paired-Associates Tasks were the same as Chapter 2, experiment two. In brief, we measured performance on the SRTT through differences in sequence specific learning as quantified by the change in RT (SSLRT) and Composite Scores (SSLCS) normalized to the random sequence as well as raw change in RT and CS. In this instance, a Composite Score was calculated as a composite of the RT and accuracy (RT/Accuracy(%)). In the case of the paired-associates task, we measured performance as the change in word pairs accurately recalled.

3.2.6. EEG Analysis

3.2.6.1. Pre-processing

Our experimental design yielded between 2-4 full nights of data per participant, this was because some participants misunderstood when the learning session was scheduled to occur or delayed the start of learning session (S1) and so had an extra habituation/baseline night before S1. The decision tree shown in Figure 3.6 was used to determine which recordings were of sufficient recording quality to be analysed, which could not, and which required further inspection before being sorted into one of the two categories. The EEG recordings that were visually inspected had undergone some automatic preprocessing via the Dreem algorithm. This pre-processing was composed of an order 2 Butterworth bandpass filter between 0.4-18Hz, and a three notch (50Hz, 60Hz, 62.5 Hz) filter order 3.

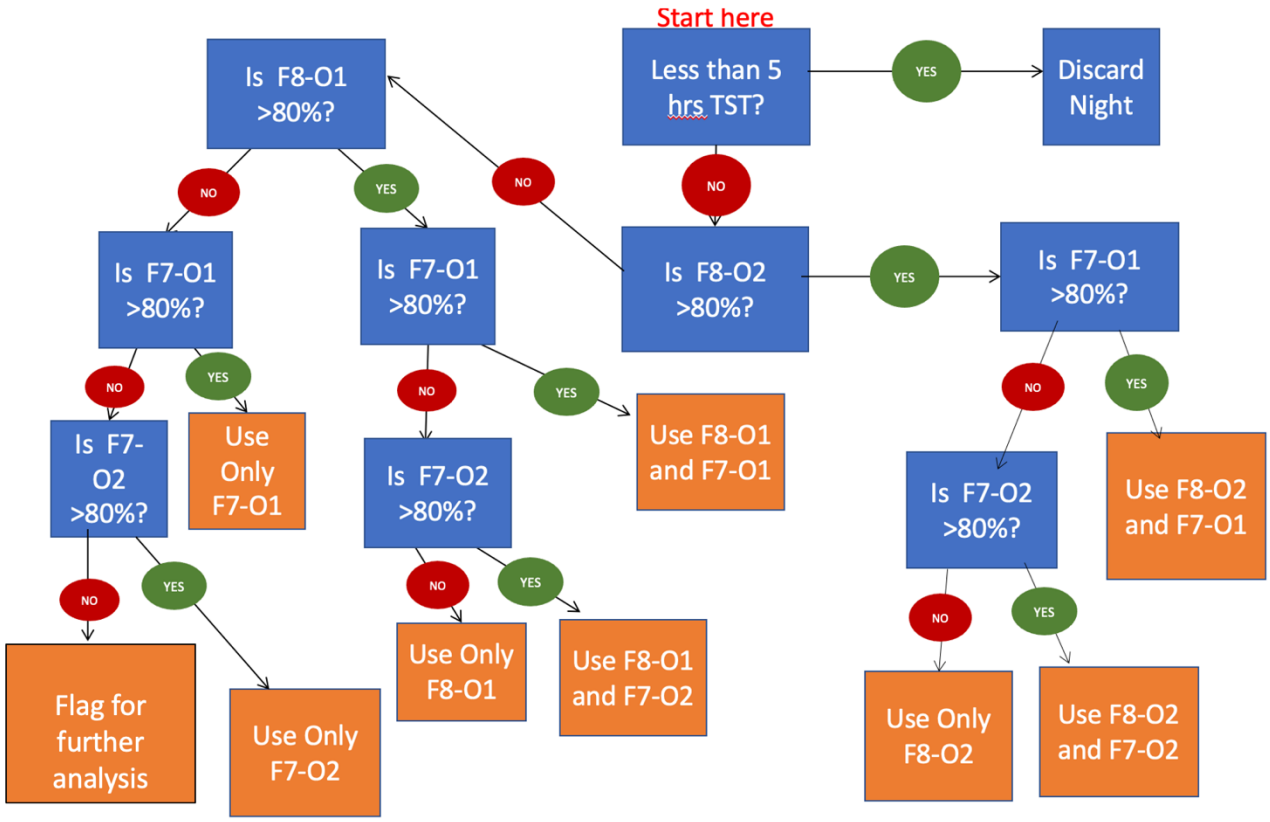


Figure 3.6 The Pre-processing Decision Tree
The decision tree that was used to decide which recording data to exclude from analysis, which channel derivations to use, and which data was in need of further consideration before deciding to include/exclude it from analysis. The 80% threshold here refers to the channel quality metric determined by the Dreem algorithm, it reflects the percentage of the recording for

which the quality of a given channel was deemed usable by the Dreem algorithm. This process was repeated for each night of data.

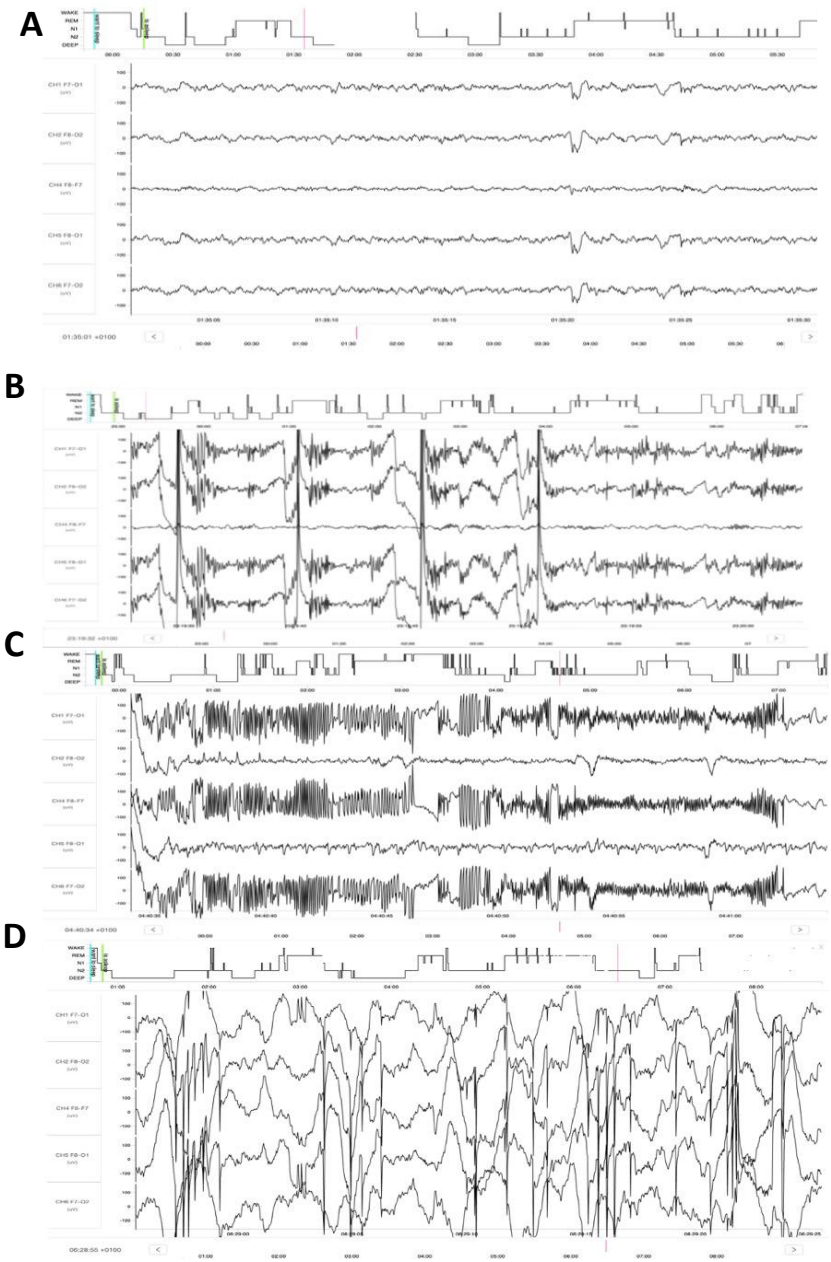


Figure 3.7 Examples of Clean (L) and Noisy (R) Sections of EEG Recordings

Screenshots of the raw EEG as shown on the Dreem Portal with the full recording's hypnogram at the top of each screenshot. Missing sections of the hypnogram denote parts of the recording that were too noisy for the Dreem algorithm to score.

A: An example of a clean section of a night's recording.

B: An example of noise in the occipital channels.

C: An example of single channel noise from F7.

D: An example of non-oscillatory noise of unknown origin.

Table 3.1 Summary of Outcomes of the Pre-Processing Decision Tree For Each Study Night

	Extra Baseline Night	Baseline Night 1	Baseline Night 2	Post-Learning Night
Total Participants	5	39	41	41
Discard Because <5 hrs TST	1	3	4	4
Use F8-O2 and F7-O1	2	10	13	9
Use F8-O2 and F7-O2		4	5	7
Use F8-O2 only	1	1	1	
Use F8-O1 and F7-O1			1	1
Use F8-O1 and F7-O2				
Use Only F8-O1				
Use Only F7-O1		3	1	
Use Only F7-O2		1		2
Flagged for Visual Inspection	1	18	15	18
Usable Sessions	3	15	18	22

All recordings with a duration of less than 5 hours were excluded. The rationale behind this was that across several sleep studies, 7-8hrs of sleep is considered normal sleep duration, >8hrs is considered a long duration, and <5-6 was considered short sleep duration or sleep deprivation (Cappuccio et al., 2011; Irwin et al., 2016). Dreem's channel quality metric was then used to determine channel referencing, shown in blue in the decision tree (Figure 3.6), channels with >80% quality metrics (the percentage of the recording that a given channel was deemed usable by the Dreemband algorithm) were confirmed to be usable based on visual inspection by an experienced EEG sleep scorer. Table 3.1 also shows the number of participants lost at each stage in the decision tree per night. If none of the channel derivations could be used, the recording was then visually inspected using the Dreem Portal, Dreem's online EEG display platform, with the hypnogram widget to determine whether our sleep stages of interest, N2 and 3, were of sufficient quality to use. Examples of what were deemed "noisy" and "clean" sections of the EEG recordings are shown in Figure 3.7.

Table 3.2 Summary of Baseline Night Status of Participants with a Useable Post-Learning Night

Participants with Useable Post-Learning Night	**32**
Participants with No Useable Baseline Night – Discarded from Analysis	10
Participants with 1 Useable Baseline Night	11
Participants with 2 Useable Baseline Nights	9
Participants with 3 Useable Baseline Nights	2
Total Participants Used in Analysis	22

Of the 41 participants who completed the study, 22 met the criteria of having at least one baseline night recording and the learning night recording of a high enough quality that they could be confidently used without an artifact rejection pipeline. This criterion was necessary in order to calculate the changes in the EEG-derived sleep features from baseline to post-learning sleep. For participants who

had more than one useable baseline night, those baseline nights were averaged in our calculations. For those that only had one baseline night, that night alone was used for our calculations of change in sleep features.

3.2.7. Analysis

3.2.7.1. EEG Analyses

Data from 22 participants were included in the EEG analysis pipeline. To calculate the change in percentage of the night spent in N2 and N3, the relative sleep metrics generated by the Dreemband for each recording were used. Since the Dreemband calculates the N2 and N3 values separately, these were averaged to generate a nightly percentage of the two combined. To compute the difference, the baseline change in N2+3% was subtracted from that of the learning night, such that positive values are indicative of more time spent in slow wave sleep for the post-learning night compared to baseline.

Sleep microarchitecture detection was completed using YASA (a Python toolbox for sleep data analysis, Vallat & Walker, 2021), and MNE python (Gramfort et al., 2013) was used for visualization. Using EEG recordings in (edf format) and hypnograms generated by Dreem, sleep stages of interest (N2 and N3) were firstly extracted. The edf format of the EEG data means that some preprocessing was already done to the EEG data. This was an order 2 Butterworth bandpass filter between 0.4-18Hz, and a three notch (50Hz, 60Hz, 62.5 Hz) filter order 3.

The YASA “spindles-detect” algorithm was then used to detect spindles within these stages across all channels (Fig) and calculate individual spindle features such as density. Spindles were defined as 12-15 Hz with a duration of ≥ 0.5 s (Berry et al., 2017).

We then used the “sw_detect” algorithm to detect slow oscillations and calculate coupling features with the spindle frequency. The spindle frequency range was defined as 12-16 Hz which are in the higher frequency range for spindles and the slow oscillation frequency range was defined as 0.3 - 1.5

Hz (Berry et al., 2017). The duration range for the down-state of the oscillation was between 0.3-1.5 seconds while the range for the up-state was 0.1- 1 seconds. The microarchitecture features of interest were then averaged within nights and the baseline subtracted from the learning night, such that positive values are indicative of increased coupling for the learning night compared to baseline. Based on the outcomes of the decision tree in figure 3.6 in which 19 participants were removed due to noisy data, all subsequent analyses were limited to the frontal-occipital derivates as has been done in other studies using the Dreemband (Birch & Zammit, 2021; Kings & Lewis, 2022). The derivates in question are summarized in table 3.2.

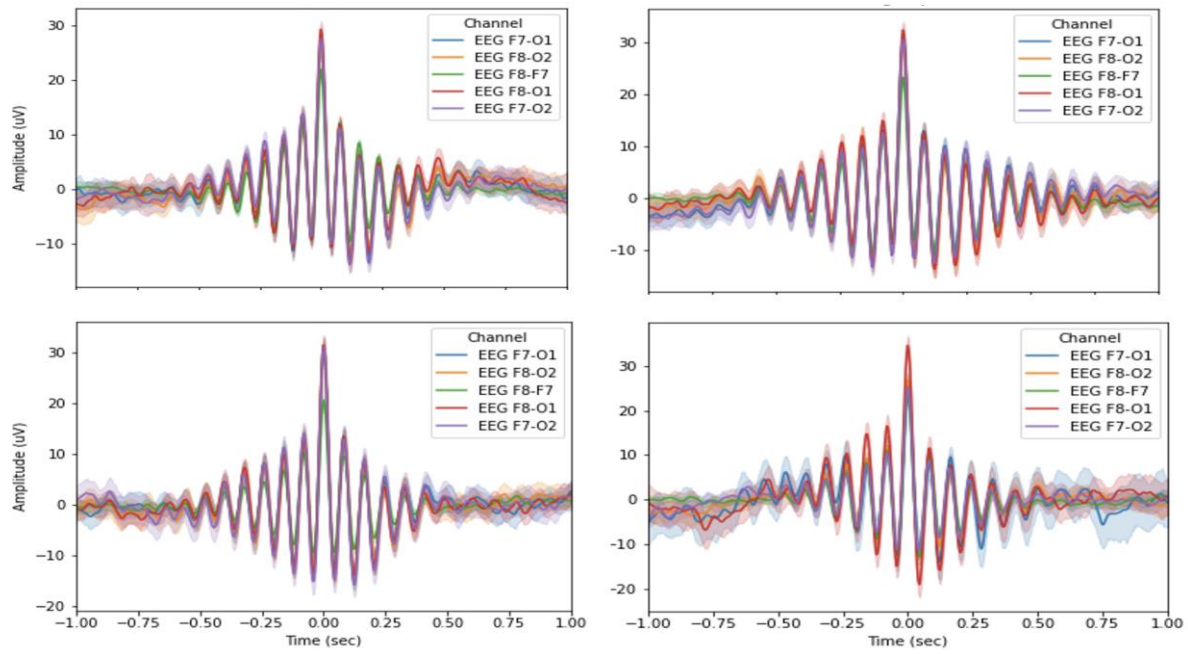


Figure 3.8 Examples of Average Spindles Detected by the YASA Spindle Detection Algorithm Across Channels in Stages N2+3
Example of spindles detected by the YASA algorithm, examples taken from different participants and across different nights.

3.2.7.2. Quantification of performance

Performance on the paired associates task was quantified by the absolute value of the number of word pairs participants accurately retained (max 20).

For the SRTT, preprocessing included exclusion of trials in which the RT was slower than 1 second. If the number of trials that had to be removed exceeded 50% of the total trials in a given block (>18/36

trials), that block was removed from analysis. After this initial data cleaning was done, if a participant had to have more than 50% of the total blocks removed in this way, then the participant was removed from the data pool.

Measures of performance based on RT (ms) alone were then calculated by averaging the last two blocks before the random sequence block. In order to quantify the level of sequence specific task performance (SSL_{RT}) at the end of the leaning session (S1), we averaged the RT on the last two blocks before the random sequence block and subtracted this average from the random block. The same was done to quantify performance level at retest (S2).

The composite scores were calculated by dividing the RT (ms) by the accuracy (%). Similar to RT, a sequence specific measure (SSL_{cs}) was calculated by averaging the CS over the last two blocks before the random sequence block and subtracting this average from the CS of the random block. The same was done to quantify performance level at retest (S2).

3.2.7.2.1. Change Indices

As in Chapter II, in order to calculate the change in performance between sessions we subtracted the session that we hypothesised would have a smaller value from the session we hypothesised would have a bigger value, such that improvement on a given task over time was always denoted by a positive change index value.

For the SRTT, change measures were calculated for RT, CS, SSL_{RT} and SSL_{CS}. For both RT and CS, better performance is indicated by lower scores, so scores were predicted to decrease over time and therefore change scores were calculated as S1-S2 (both experiments) and S2-S3 (experiment one only). For SSL, better sequence specific learning is indicated by higher scores, so scores were predicted to increase over time and therefore change scores were calculated as S2-S1 (both experiments) and S3-S2 (experiment one only).

For the paired-associates task, higher scores indicate better retention of word-pairs. To calculate the change index, we therefore calculated S2-S1 (both experiments) and S3-S2 (experiment one only).

3.2.8. Statistical Analyses

3.2.8.1. Question 1: Do participants show learning in a single session? In the case of the SRTT, is this learning sequence dependent?

SRTT: In order to determine whether participants showed evidence of learning in S1 we conducted a rmANOVA on the RT and CS over the training blocks. To determine if this learning was sequence dependent we ran one sample t--tests on the $SSSL_{RT,}$ and SSL_{cs} measures in S1.

Paired-Associates: In order to determine whether the participants' performance on the paired-associates task at S1 was significantly different from zero, we ran a one-sample t-test on the absolute words recalled in S1.

3.2.8.2. Question 2: Do participants show evidence of overnight consolidation of the tasks?

SRTT: To test whether there was a significant change in performance on the SRTT we ran paired t-tests comparing the RT, CS, $SSSL_{RT,}$ and SSL_{cs} in S1 to S2.

Paired-Associates: To test whether there was a significant change in performance on the SRTT we ran paired t-tests comparing the absolute number of words recalled in S1 to S2.

3.2.8.3. Question 3: Are changes in performance between S1 and 2 sleep-dependent or the result of differences in sleepiness due to different times of day?

To test whether there was a significant difference in SSS scores between S1 and 2, we conducted a paired t-test. To determine whether our behavioural results correlated with this difference, we ran Pearson correlations on the change in SSS scores and changes in all performance measures for the SRTT and paired-associates task.

3.2.8.4. Question 4: Do our sleep features of interest correlate with our chosen behavioural performance metrics?

To address this question in the context of the SRTT we ran Pearson correlations between change in our chosen sleep features (spindle density, spindle power, SO-spindle coupling, and total sleep time (TST) spent in nREM2+3) from baseline and ΔSSL_{RT}. To address this question for the paired-associates task, we ran a Pearson correlation between the change in above sleep features and the change in absolute number of correct word-pairs recalled.

3.2.8.5. Question 5: What is the relationship between our sleep features of interest and all of our behavioural measures of SRTT performance rather than only RT*?*

To answer this question, we ran canonical correlation analysis (CCA) with the change indices of our sleep features of interest against those of our SRTT performance measures.

3.2.8.6. Question 6: What is the relationship between our sleep features of interest and performance on the paired-associates task when the sleep features are considered in a single analysis, rather than analysed individually as with the correlation analyses?

To address this, we ran a GLM with all of the change indices of our sleep features of interest and the change in absolute number of word-pairs recalled.

3.3. Results

3.3.1. Behavioural Analysis

3.3.1.1. SRTT

We first wanted to ensure that the participants learned the SRTT in the first session as in previous studies. The rmANOVA revealed a significant effect of block on RT ($F(4.541, 741) = 39.964$, $p = <0.001$). This effect was also significant for CS ($F(6.377, 741) = 32.824$, $p = <0.001$). This suggests that participants improved performance over the blocks in the pre-sleep session of the SRTT.

We next wanted to confirm that participants had learned the SRTT in a sequence-specific manner. A one sample t-test showed that SSL_{RT} ($t(40) = 9.815$, $p = <.001$) and SSL_{CS} ($t(40) = 9.809$, $p = <.001$) were significantly different than 0. This suggests that participants learned the SRTT in a sequence-specific manner.

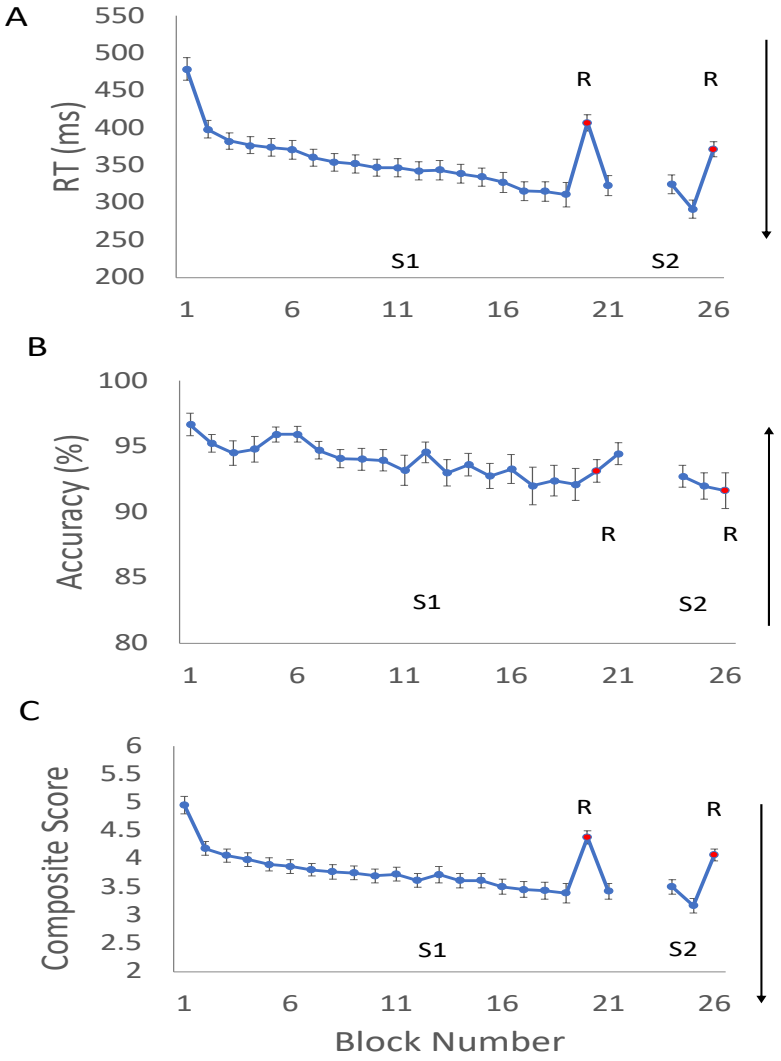


Figure 3.9 Comparison of SRTT Performance Across Sessions as Measured by RT (A), Accuracy (B) and Composite Scores (CS) (C).
Sessions 1,2, and 3 are labelled on all plots as S1, S2, and S3 respectively. Random blocks within a session are marked with a red marker and an "R."
Arrows denote the direction of task improvement.

The next question that we wanted to address was whether the participants showed overnight consolidation of the SRTT (Figure 3.10). The paired t-test showed no significant difference between S1 (end of learning session) and S2 (retest) for RT (t_{40}= 1.12 , r= .13) nor CS (t_{40}= 1.3, r= .107). However, there was a significant difference for SSL_{RT} (t_{40}= 4.69 , r= <.001) and SSL_{CS} (t_{40}= 2.7 , r= .005) from end of learning in S1 to retest in S2. This suggests that there was an improvement in sequence-specific task performance over the sleep period.

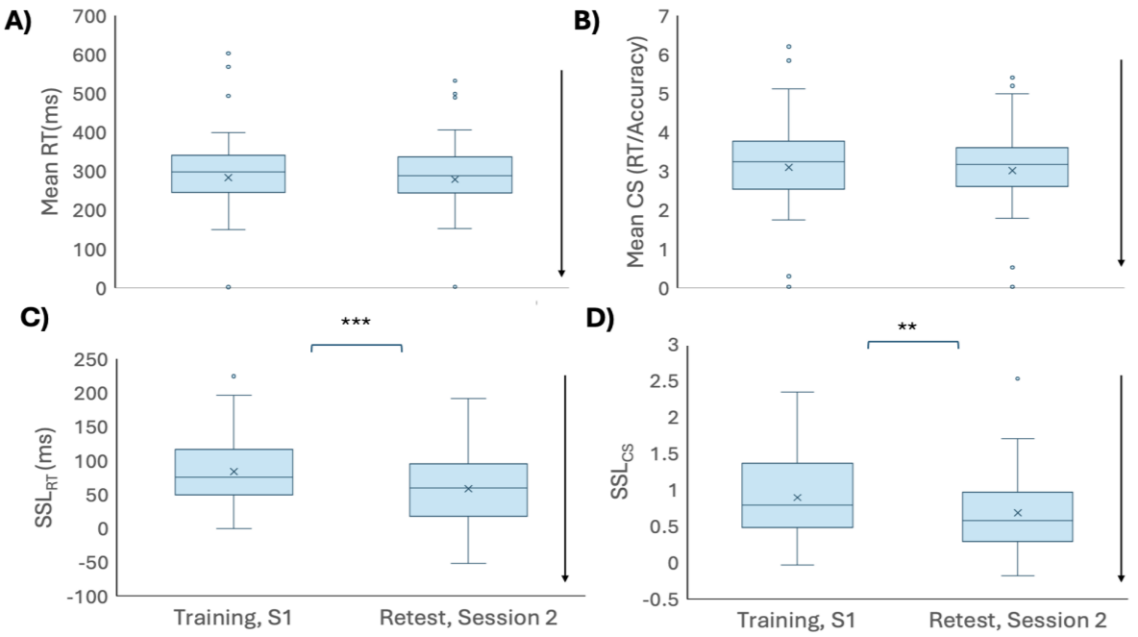


Figure 3.10 Comparison of End-Of-Session Performance at Session 1 (Pre-Sleep) and Session 2 (Post-Sleep)
A) Comparison of mean RT (ms) in Session 1 and Session 2. No significant difference was found in performance, as measured by RT, between pre and post sleep sessions.
B) Comparison of mean Composite Scores (RT/accuracy) in Session 1 and Session 2. No significant difference was found in performance, a measured by CSs, between pre and post sleep sessions.
C) Comparison of mean SSLRT in Session 1 and Session 2. A significant difference (r= <.001) was found in performance, as measured by SSLRT , between pre and post sleep sessions.
D) Comparison of mean SSLCS in Session 1 and Session 2. A significant difference (r= .005) was found in performance, as measured by SSLCS , between pre and post sleep sessions.
Task improvement is denoted by the direction of the arrows, as such, a smaller average performance measure means better performance. The mean is denoted by a cross on all box and whisker plots.

3.3.1.2. Paired-Associates Task

As with the SRTT, we wanted to test whether participants had learnt the task in S1. A one-sample t-test on S1 of the paired-associates task similarly found task performance (as measured by absolute number of words correctly recalled) to be significantly different than 0 ($t(40) = 12.11$, $p = <.001$). We also asked whether performance on the paired associates task benefited from the sleep period. Figure 3.11A, shows the change in word pair retention from S1 to S2 in individual participants. On average there a slight decay of performance rather than improvement. This is supported by the results of a paired t-test which show a small but significant reduction in the number of words recalled before and after sleep ($t_{40} = 1.9$, $p= .035$) (Figure 3.11B). Despite the wide range in absolute words recalled pre-sleep, there is no relationship between the number of words recalled pre-sleep and the change in the number recalled post-sleep (Figure 3.11C).

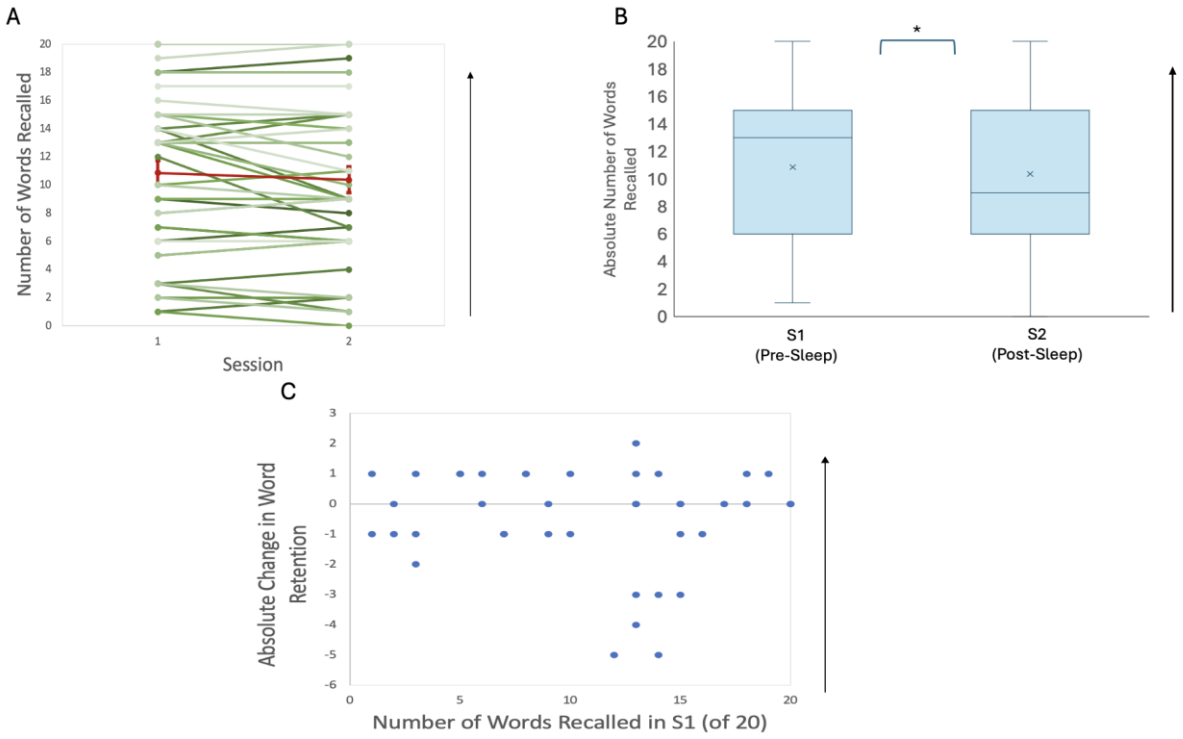


Figure 3.11 Paired Associates Task

A: Participant-wise change in number of words recalled (of 20) at the end of session 1 and at retest in session 2. The mean is shown in red and error bars represent the SEM.

B: A within-group comparison of words recalled at S1 (pre-sleep) and S2 (post-sleep). A t-test revealed a small, significant difference in performance between the two timepoints ($t_{40} = 1.9$, $r = .035$). The mean is denoted by a cross, and the median is denoted by a line.

C: The relationship between the number of words recalled at the end of session 1 and the change in word retention from S1 to retest at S2 (S2-S1). Negative change denotes a deterioration of word-pair retention overnight and positive change denotes an improvement of word-pair retention.

The direction of the arrow in each panel shows the direction of improvement on the task.

3.3.2. Stanford Sleepiness Scale – The Relationship Between Sleepiness and Task Performance

A key confound in this dataset is that we cannot determine from the above results whether any changes observed from pre to post sleep were due to the effects of sleep or simply differences in sleepiness due to the time of day (morning vs night). Our next steps were therefore to investigate how sleepiness affected performance on our tasks.

To address this issue, we compared the average SSS scores across S1 and 2 in order to determine whether there was any difference in sleepiness due to the time of day. A paired-samples t-test showed that participants were significantly sleepier in S1 than S2 ($t(40) = 3.45$, $p = .001$; Figure 3.12). This suggests that this change in alertness could contribute to overnight improvement in SRTT performance, rather than (or in addition to) the effects of sleep as we hypothesized.

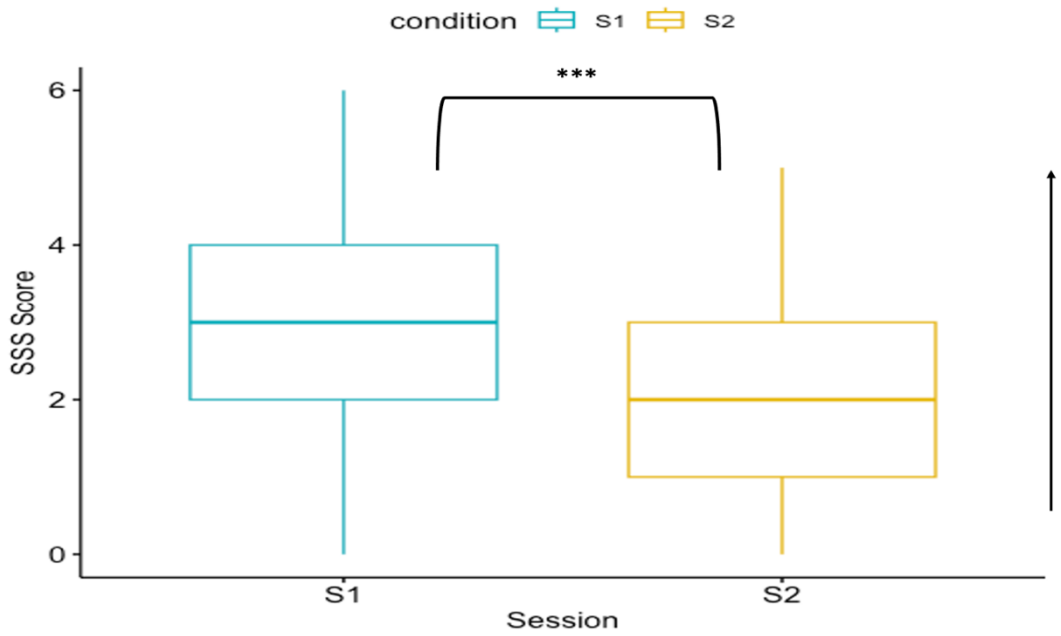


Figure 3.11 Comparison of the SSS Scores in Sessions 1 and 2
Boxplot comparing the SSS scores across the two sessions, S1 (8-10PM) mean=2.90, sd=1.13 and S2 (8-10AM) mean=1.85, sd=1.13. N=41.

Given that participants were sleepier in S1 than S2, we next wanted to determine whether there was a correlation between the change in SSS scores and the change in our main performance metrics for our two tasks. A Pearson correlation between the change in SSS scores and ΔSSL_{RT} showed no significant correlation ($r = -.243$, $p = .063$; Figure 3.12A). Equally, no significant correlation was found between the change in SSS scores and the change in word pair retention ($r= -.192$, $p = .230$; Figure 3.12B). This suggests that the change in alertness due to the time of day is unlikely to drive overnight changes in task performance.

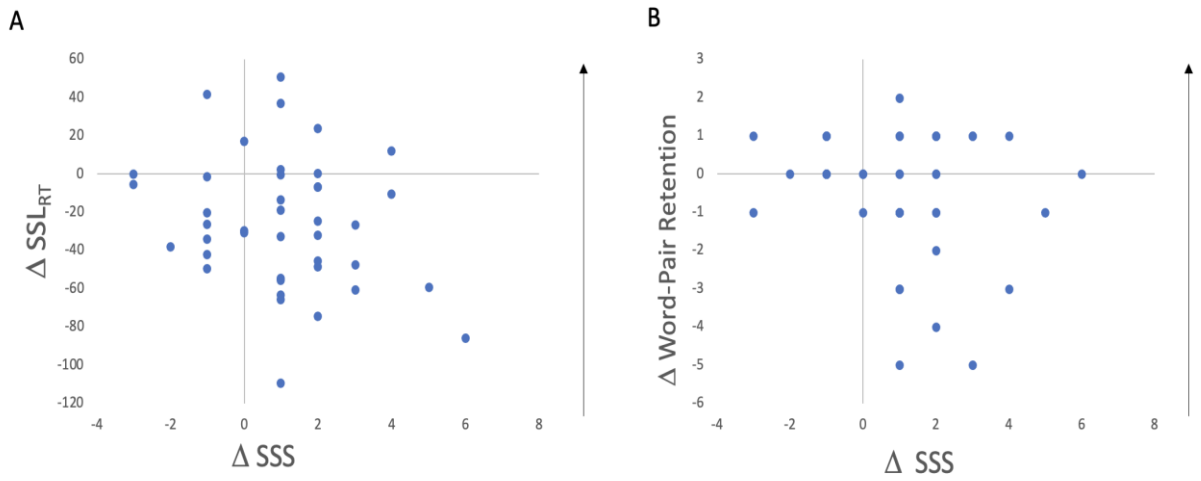


Figure 3.12 The Relationship Between the Change in SSS Score and Change in RT (A) and Change in SSS Score and Change in Word Pair Retention (B)
N=41, arrows denote the direction of task improvement (y-axes). On the x-axes, positive numbers denote an increase in alertness from baseline, while negative numbers denote a decrease in alertness from baseline.

3.3.3. Sleep Features

3.3.3.1. SRTT and Sleep Parameters

		Total Sleep Time (hrs)	Sleep Onset Latency (min)	Wake After Sleep Onset (min)	N1 (min)	N2 (min)	N3 (min)	REM (min)	Sleep Efficiency
Habituation Nights	Average	6.76	15.80	18.74	21.47	174.01	99.61	105.16	3.18
	SEM	0.14	2.19	3.05	0.91	6.28	3.75	4.4	1.55
	MIN	3.18	1.5	3	2.5	0.5	1.5	22.5	0.23
	MAX	9.18	57	140	40	312	174	199.5	96
Post-Learning Night	Average	6.83	14.09	16.09	22.26	172.34	103.74	117.37	0.92
	SEM	0.58	3.28	3.02	1.55	4.24	4.01	3.95	0.08
	MIN	1.59	0.5	4	8.5	35.5	0	16.5	0.55
	MAX	9.92	65	61.5	42	289.5	219.5	209.5	0.97

Figure 3.13 Summary of Sleep Macroarchitecture for the Habituation Nights and Post-Learning Night
Sleep efficacy is expressed by the ratio between sleep duration and time spent in bed.
Measures are averaged across nights for habituation nights.

Next, we wanted to determine the extent to which each of our sleep features of interest correlates with change in performance on our tasks. Our sleep features of interest were spindle density, absolute spindle power, SO-spindle coupling, and percentage of TST spent in N2 and N3. We chose SSL_{RT} as our behavioural measure of performance on the task because this is a commonly used performance metric

in the SRTT literature and thus would allow us to better compare our results to those of previous studies. This is particularly relevant to our goal of testing whether we observe similar findings in an at-home setting as have previously been found in lab-based studies. We are also particularly interested in task-specific improvement rather than gross motor learning and improving on the task in a sequence-specific manner will also be relevant to our experimental questions in Chapter IV. An increase in ΔSSL_{RT} denotes a greater change in RT normalized to the random sequence, which means that the participant is becoming faster relative to the random sequence which suggests they are completing the task in a way that takes the sequence structure into account.

Pearson correlations showed no significant relationships between ΔSSL_{RT} and change in spindle density (N=22, r = .142, p = .264, fig 3.11 A) nor between the change in absolute spindle power and ΔSSL_{RT} (N=22, r = .2, p = .19, fig 3.11 B). A significant correlation was found between the change in SO-spindle coupling and ΔSSL_{RT} (N=22, r = .404, p = .031, fig 3.11 C), such that increased SO-spindle coupling was associated with improved sequence-specific speed on the SRTT. No significant correlation was found between the change in percentage of TST spent in N2 and N3 and ΔSSL_{RT} (N=21, r = .495, p = .011, α=0.013, fig 3.11 D).

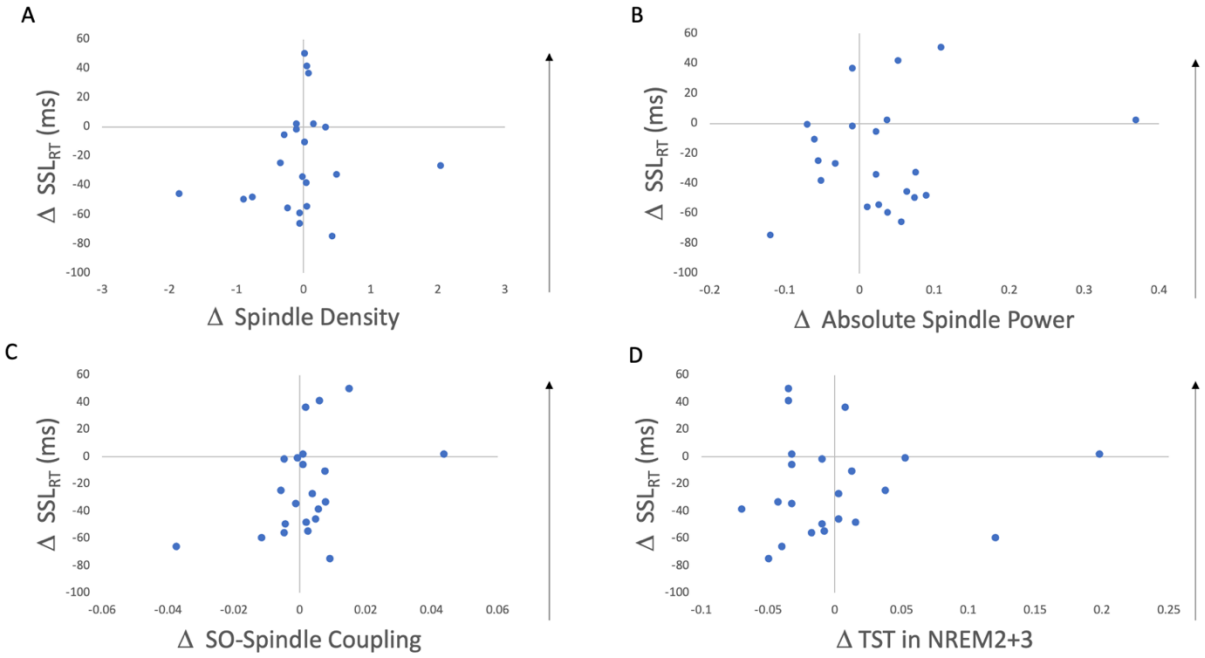


Figure 3.14 Relationships Between the Change in SRTT SSL$_{RT}$ and Sleep Features of Interest
A) Relationship Between the Change in Spindle Density and Change in SSL$_{RT}$.
B) Relationship Between the Change in Spindle Power and Change in SSL$_{RT}$.
C) Relationship Between the Change in SO-Spindle Coupling and Change in SSL$_{RT}$. D) Relationship Between the Change in Percentage of Sleep Time Spent in N2+3 and Change in SSL$_{RT}$T. Arrows denote the direction of task improvement (y-axes). On the x-axes, positive numbers denote an increase in the measure from baseline, while negative numbers denote a decrease.

3.3.3.2. Declarative Memory Task and Sleep Parameters

We also asked whether our selected sleep features had any relationships to the overnight consolidation of the paired-associates task. There was no correlation between the change in spindle density and change in word retention (r = .035, p = .443) nor between the change in absolute spindle power and change in words recalled at re-test (r = - .017, p = .470) nor between the change in SO-spindle coupling and change in word-pair recall (r = -.099, p = .339). Finally, there was no significant correlation between the change in percentage of the night spent in N2 and 3 and the change in word pairs recalled at re-test (r = -.214, p = .176).

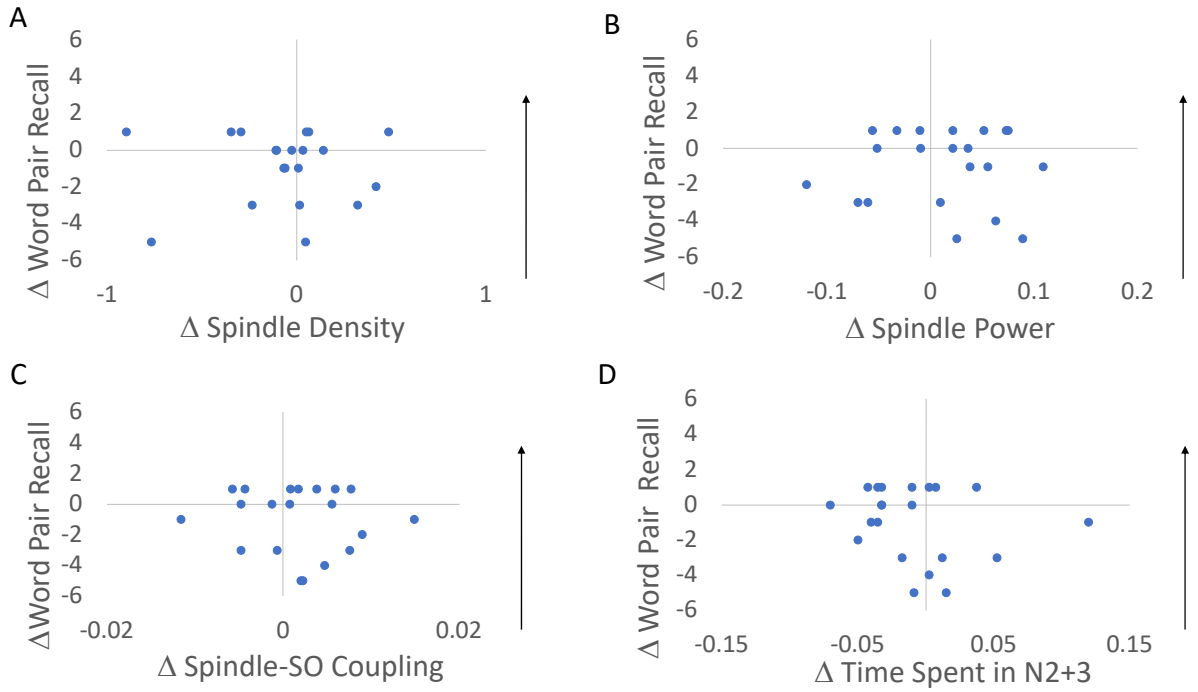


Figure 3.15 Relationships Between the Change in Word Pair Retention and Sleep Features of Interest
A) Relationship Between the Change in Spindle Density and Change in Word Pair Retention. **B)** Relationship Between the Change in Spindle Power and Change in Word Pair Retention. **C)** Relationship Between the Change in SO-Spindle Coupling and Word Pair Retention. **D)** Relationship Between the Change in Percentage of Sleep Time Spent in N2+3 and Word Pair Retention. Arrows denote the direction of task improvement (y-axes). On the x-axes, positive numbers denote an increase in the measure from baseline, while negative numbers denote a decrease.

3.3.4. Canonical-Correlation Analysis (CCA) of Sleep and Behavioural Change Indices

In order to further explore the relationship between our sleep and behavioural measures we performed CCA. CCA allows us to define the degree and nature of covariance between two arrays of variables, in our case changes in (1) measures of behavioural performance and (2) sleep features. We used the same sleep features we explored in the previous correlations; however, we also investigated their relationship to the other measures of SRTT performance (composite scores and sequence specific learning metrics). Prior to CCA, the sleep and SRTT data were standardised using a rank-based inverse

normal transformation (Smith et al., 2015). This process uses ranks to move the outliers closer to the mean with the aim of creating a more Gaussian data distribution. The strength and direction of the correlations between the sleep and behaviour variables before and after the standardisation are shown in Figure 3.16. The latent correlations between the sleep and behaviour variables become more apparent following this step.

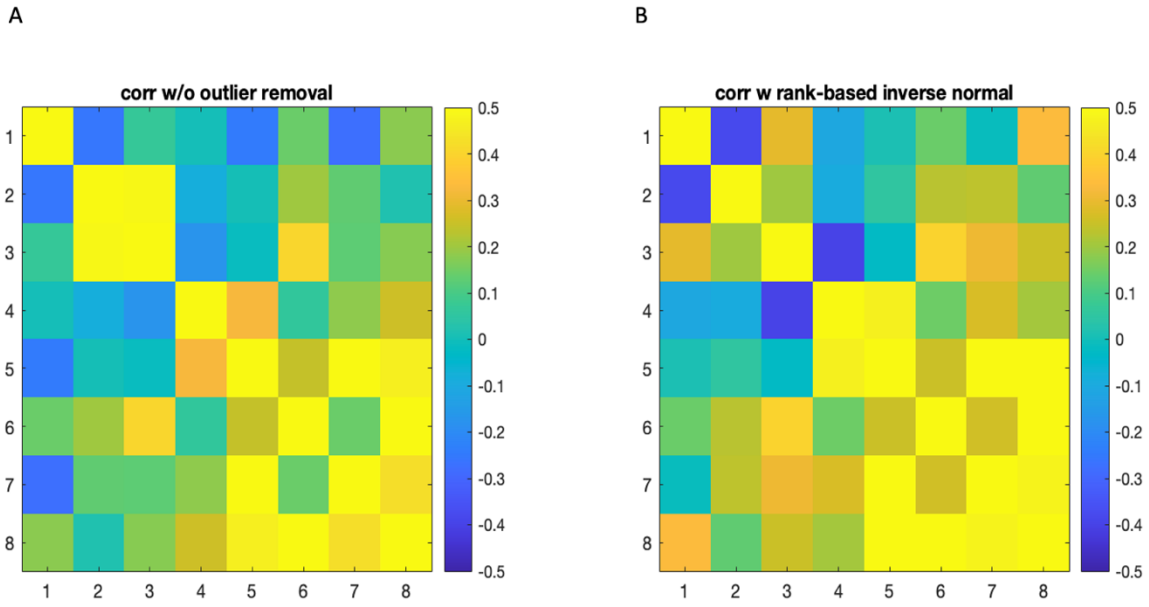


Figure 3.16 Heatmaps of the Correlations Between the Sleep and Behavioural Variables With (A) and Without (B) Outliers. Behavioural Variables (1- 4): 1 = change in RT, 2= change in RT normalized to random, 3=change in CSs, 4= change in CSs normalized to random.
Sleep Variables (5-8): 5= change in spindle density, 6=change in spindle power, 7=change in SO-spindle coupling, 8= change in % of night spent in N2+3. N=22.

The data with the rank-based transformation was then used to conduct CCA with 10,000 permutations (N=22). The CCA revealed one significant mode of covariation between the sleep and behavioural variables (r= 0.82, p= 0.0326; Figure 3.17). This mode of covariation was most strongly influenced by an increase in SO-spindle coupling (0.76), by an increase in absolute spindle power (0.43), and by a decrease in % of the night spent in SWS (-0.18). The influence of spindle density was minimal (0.06). Across SRTT performance variables, the covariation mode was most influenced by an increase in CS (0.42) and an increase in normalized CS (0.42). This indicates that a high scoring participant along this axis of covariation, that is - a participant who explains high proportion of the variance, would be one

that improves on the SRTT as measured by normalized RT and CSs and shows an increase in SO-spindle coupling and spindle power.

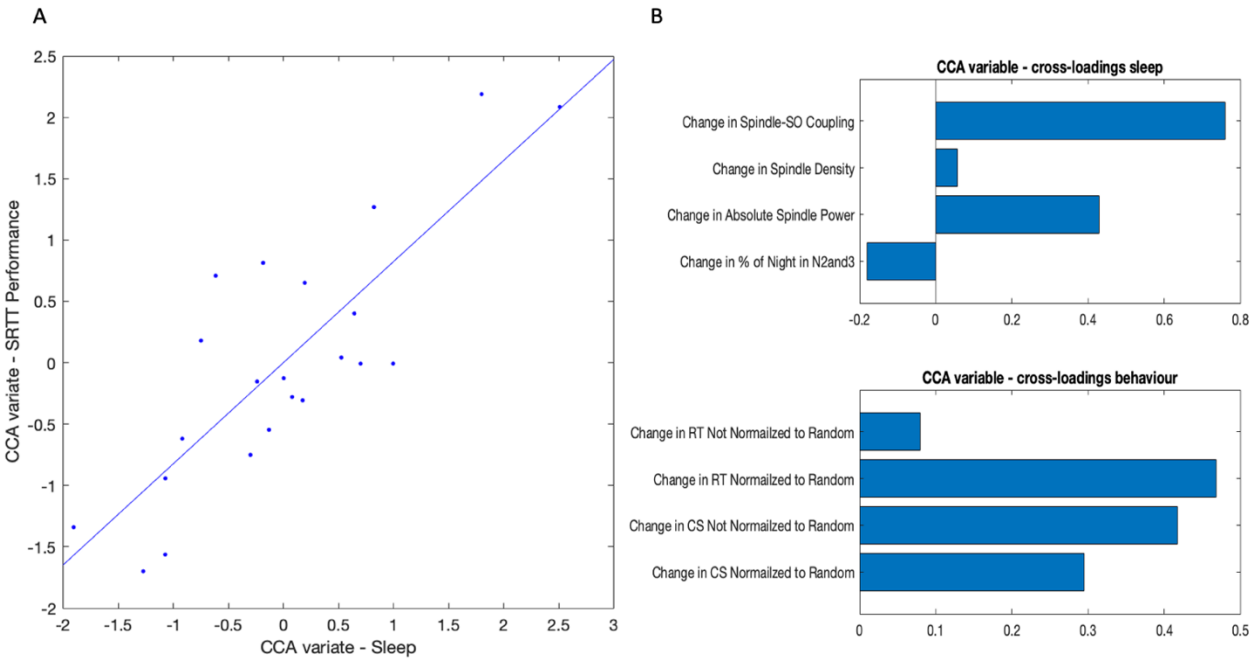


Figure 3.17 CCA (Canonical-Correlation Analysis) of Sleep and Behavioural variates (A) and Cross-Loadings (B).
N=22.
A) The correlation between SRTT Performance and Sleep. A positive correlation means that as the sleep features increase from S1-2, the SRTT performance measures improve from S1-2.
B) The CCA variables-cross loadings for the sleep (top) and behaviour (bottom). The x axes shows the p values for each of the variables shown on the y axes.

3.3.5. Generalized Linear Model (GLM) of Sleep and Paired Associates Performance Change Indices

We then wanted to explore the relationship between our sleep features of interest and overnight change in performance on the paired-associates task. We had predicted that our sleep features of interest would be associated with improvement in performance in the paired-associates task. Given that there was only one task performance metric, a CCA was not appropriate and therefore a GLM was used instead. We therefore conducted a GLM using the change in sleep features as predictors and the change in word pair retention as the response.

Prior to conducting the GLM, the sleep features were standardised via rank-based inverse normal transformation (Figure 3.18). This allowed us to normalize our data distribution without discarding outliers. Furthermore, this allows us to compare the effect sizes of our predictors because the input variance has been standardised.

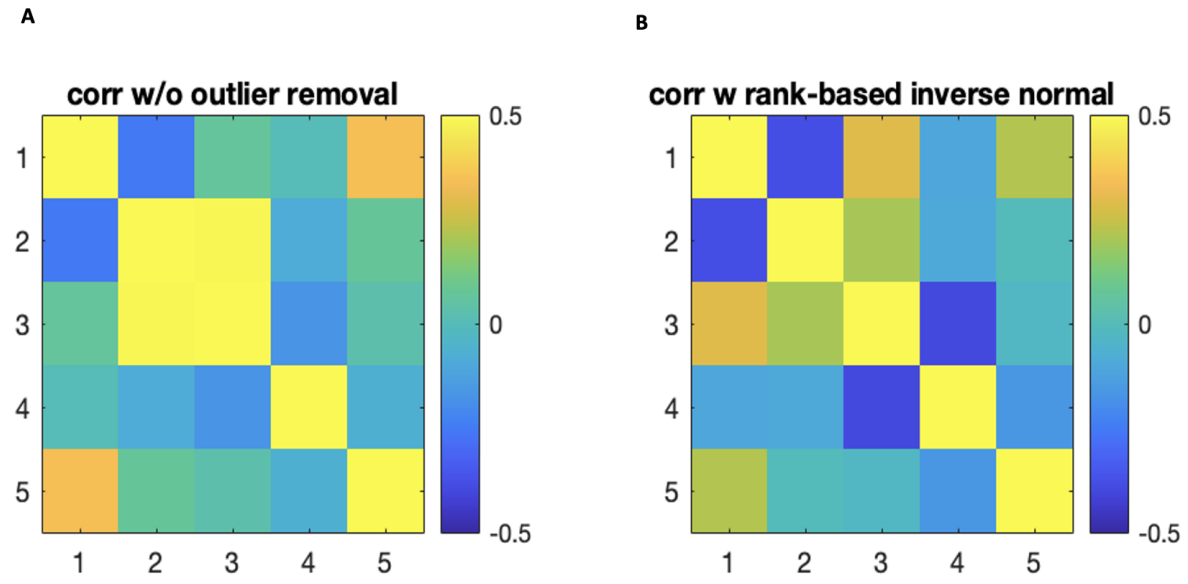


Figure 3.18 Heatmaps of the Correlations Between the Sleep and Word Pair Behavioural Variables With (A) and Without (B) Outliers.
Sleep Variables (1- 4): 1 = change in spindle density, 2= change in spindle power, 3= change in SO-spindle coupling, 4= change in % of night spent in N2+3.
Word Pair Retention (5): 5= change in word pair retention. N=22.

The mode of correlation for each of the sleep variables and word pair retention are summarized in Table 3.3 and visualized in Figure 3.19. None of the associations between the paired-associates task and sleep features reached significance (F= 0.51 , p = 0.73).

Table 3.3 GLM Summary of Predictor Relationship with Change in Word Pair Retention

	Estimate	SE	tStat	pValue
Intercept	1.8572e-16	0.20665	8.9874e-16	1

Change in Spindle Density	0.30277	0.2594	1.1672	0.25926
Change in Spindle Power	0.13336	0.25324	0.52664	0.60525
Change in Spindle-SO Coupling	-0.21703	0.26213	-0.82794	0.41917
Change in % N2and3	-0.19468	0.23854	-0.81611	0.42572

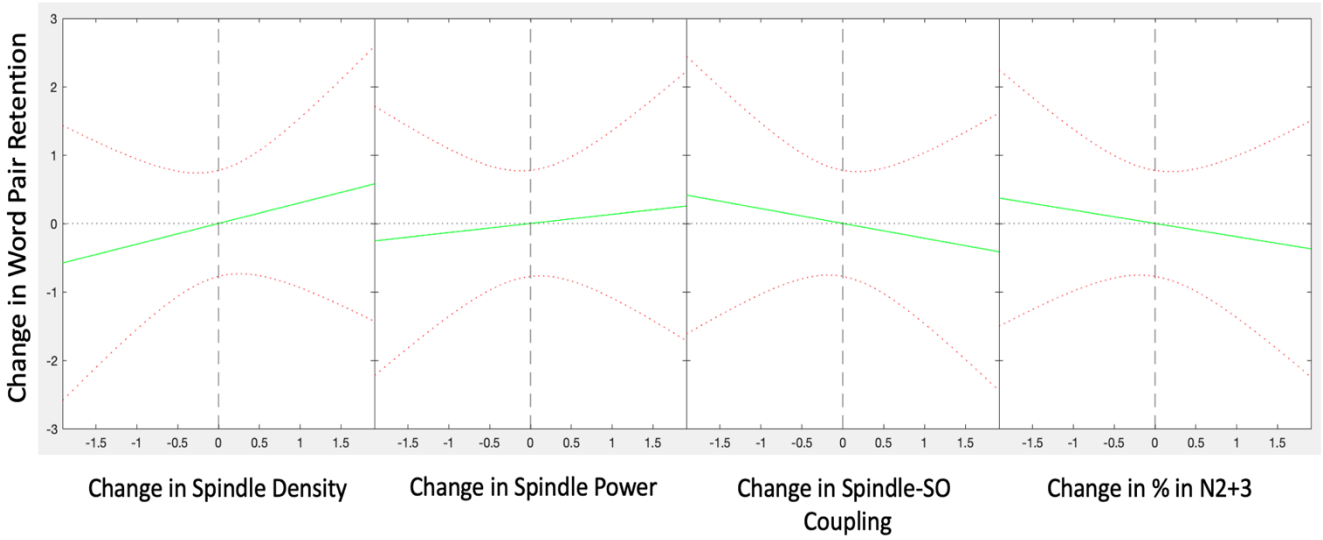


Figure 3.19 GLM Slices: Linear Regression Modelling of Changes in Sleep Features Fit to the Change in Word Pair Retention

3.5. Discussion

In this study, we investigated the link between motor and declarative memory measures and features of sleep which have previously been linked to sleep-dependent consolidation. Importantly, to address this question in a naturalistic environment, this study was carried out remotely in an at-home setting using portable EEG devices (Dreembands) and online behavioural tasks. In line with previous literature, our findings showed a positive relationship between changes in sleep and motor memory measures, whereby the change in coupling of spindles to slow-oscillations and the normalized metric of response time (SSL_{RT}) explained most covariance. Unexpectedly, no apparent links were observed between sleep measures and overnight changes in declarative word pair memory. Finally, no significant correlations were found between individual sleep and behavioural variables. The fact that a significant covariation was nevertheless found through the CCA suggests that it is the relationship across multiple variables of interest rather than any particular pairs of variables that best explain the data.

3.5.1. Sleep and motor memory consolidation

In this remote study, we found a relationship between sleep and motor memory, which is in line with previous laboratory studies using higher dimensionality EEG recordings (Gais et al., 2002; Schmid et al., 2020; Walker et al., 2005). This is encouraging, as it suggests that it is feasible to carry out at-home studies using low-density EEG to investigate the relationship between memory consolidation and sleep macro- and microarchitecture. Furthermore, considering the evidence suggesting differences in sleep characteristics when participants are in novel compared to familiar settings (Agnew Jr et al., 1966; Tamaki et al., 2016), corroborating laboratory findings with at-home studies adds valuable insights. Finally, the study provides evidence that it should be feasible to study sleep-dependent memory consolidation (or sleep in general) in clinical populations at home, thus also potentially reaching a population that may be less able to participate in laboratory studies. This study is, to our knowledge,

the first to demonstrate associations between sleep microarchitecture and SRTT consolidation using low density EEG in a naturalistic setting.

We investigated how sleep macro- and micro-architecture contributed to changes in procedural memory performance. While previous literature has found a positive relationship between macroarchitecture, such as time in N2 and SWS, and memory consolidation (Born, 2010), we found no such link (instead the was some indication of a potential small negative covariation). However, we found that certain brain rhythms *within* N2 and SWS positively covaried with improvements in SRTT performance. Specifically, SO-spindle coupling explained most covariance in this relationship. We also included spindle power and spindle density which contributed to the covariance in the data in descending order. This is in line with previous lab-based studies finding a positive relationship between procedural memory consolidation and SO-spindle coupling (Maingret et al., 2016; Navarrete et al., 2020; Staresina et al., 2015; Yordanova et al., 2017), spindle power (Kumral et al., 2023), and spindle density (Morin et al., 2008). Conversely, studies have found that memory consolidation takes a toll when spindles and SOs become uncoupled. For example, SO-spindle uncoupling in later life has been associated with memory impairment (Helfrich et al., 2018). On the other hand, upregulating SO-spindle coupling results in improved motor memory consolidation (Rakowska et al., 2021). Overall, our findings complement the literature suggesting that SO-spindles play a pivotal role in consolidation of motor learning.

What might these links between behaviour and sleep microarchitecture reflect? According to the active systems consolidation (ASC) hypothesis, markers of SWS are key to the transformation of labile short-term memories into more stable long-term memories. Specifically, SOs orchestrate spindles (along with hippocampal ripples and the reactivated memory) and together they facilitate the reorganisation of the new memories to the neocortex for long term storage as the SOs are better able to convey information across long distances in the brain (Buzsáki & Watson, 2012; Klinzing et al., 2019;

Rasch & Born, 2013). Our results may therefore reflect a mechanism through which participants are achieving overnight improvements in SRTT performance.

To investigate the specific measure of SRTT which is sensitive to sleep-associated changes, we included four SRTT outcomes in our analysis. The strongest mode of covariation in behavioural measures in our multivariate approach was the response time normalized to the random sequence (i.e., SSL_{RT}), followed closely by the composite score (CS and SSL_{CS}). Therefore, when observing memory consolidation using SRTT, it may be important to consider which measure of performance to analyse. For instance, change in absolute RT (Cousins et al., 2016; Walker, Brakefield, Seidman, et al., 2003; Walker et al., 2005) is a common measure of sleep-dependent consolidation on the SRTT, however, in our dataset this measure contributed the least amount of covariance to the sleep-behaviour relationship.

3.5.2. Sleep and declarative memory consolidation

We observed no significant relationship between our sleep features and declarative memory. Previous studies have reported positive relationships, with increased SO-spindle coupling and spindle density previously associated with improved declarative memory performance (Clemens et al., 2005; Mikutta et al., 2019; Schabus et al., 2004). Our findings were therefore unexpected. That said, many studies within the literature on sleep and declarative word-pair memory consolidation have either compared groups of wake or sleep (Payne et al., 2012), or compared non-invasive brain stimulation to sham stimulation (Ngo et al., 2013, 2015). Considering that the present study did not compare conditions, we may not be able to capture how declarative memory retention benefits from these brain rhythms during sleep. Furthermore, the pairing of words may also contribute to the absence of sleep benefits, as there have been some inconsistent reports of sleep intervention benefits for semantically unrelated word-pairs (M. O. Harrington et al., 2021).

Equally, our task was adapted so that it could be completed remotely, online, and in a timely fashion. This meant that the word-pair task had to be kept sufficiently short to avoid cognitive fatigue and to also allow sufficient time for the proper learning of the SRTT and completion of the questionnaires. As a result, our task had smaller sets of word pairs and less repetitions during the learning phase compared to other studies (Ellenbogen et al., 2009; Payne et al., 2012; Schabus et al., 2004; J. K. Wilson et al., 2012).These differences may have affected the results, for instance, the low number of repetitions during encoding may have reduced the extent to which they learnt the word-pairs in the encoding phase, thus in turn affecting the amount of information that could be consolidated during sleep.

Finally, a well-powered study (Cordi & Rasch, 2021) (Kumral et al., 2023)and a meta-analysis suggest that intraindividual associations between sleep measures and declarative memory are less robust than previously thought.

3.5.3. Strengths, limitations, and future directions

A fundamental strength of this study is that it was conducted outside of a laboratory setting. This was one of the aims of the thesis and it allowed us to study the relationship between sleep and memory consolidation in a more naturalistic setting. However, as with any experimental approach, it comes with associated disadvantages. Chief among them is that outside of a controlled laboratory setting and without direct contact with the participants we are much less able to exert experimental control over many aspects of the study. Therefore, the price of ecologic validity is that higher levels of noise are to be expected across the board for all study measures. This was indeed the case in our study, and a large proportion of data had to be excluded due to data quality issues, resulting in a lower powered study

than we had originally designed. Nevertheless, the similarity between our findings and previous lab-based studies provides reassurance that we are capturing some signal in the otherwise noisy data.

Due to the low-density nature of the device, it was not possible to observe any topographical features of the EEG data. Previous studies have suggested that sleep features associated with consolidation are, to an extent, local in nature and are more marked over areas involved in the encoding of the experimental task (L. A. Johnson et al., 2012; Nir et al., 2011; Petzka et al., 2022; Rakowska et al., 2021, 2022). Considering our focus on motor memory, it may be that other relationships would emerge if we had electrodes over task-relevant brain regions such as primary motor cortex. Future at-home studies that wish to look at the relationship between consolidation and sleep microarchitecture may benefit from having a higher density set-up to allow them to explore regional differences in consolidation. For example, (Ngo et al., 2013) were able to characterise the topography of SOs using 19 channels. However, higher density set-ups would require either the development of a new portable device or a traditional EEG set-up rather than a portable device which would require a researcher to travel to the participants' homes to set it up. It is, nevertheless, interesting that we have found an effect of sleep microarchitecture on behaviour using only frontal derivatives and low-density EEG.

Importantly, our findings are correlative. Without the use of experimental manipulations, it is not possible to draw causal conclusions about the relationships between our behavioural and biological measures. Therefore, a key next step would be to manipulate the oscillations of interest. For instance, TMR has been successfully used to increase spindle density and spindle-SO coupling (Ngo & Staresina, 2022; Rakowska et al., 2021) and this upregulation has been linked with improved SRTT performance (Rakowska et al., 2021). Thus, using a sleep intervention in a remote setting would provide valuable insights into whether these relationships between oscillations and memory benefits persist outside of the lab.

3.5.4. Conclusions

In summary, our findings suggest that oscillations in NREM sleep previously linked to memory consolidation positively covary with improved SRTT performance, with SO-spindle coupling and sequence specific response times as the key variables. These results are consistent with studies conducted in sleep laboratories using PSG, thus demonstrating that it is feasible to study sleep macro- and microarchitecture using low-density EEG remotely with minimal experimenter intervention. Our study therefore offers a promising foundation for similar studies in the future.

4. Developing an Online Task to Probe the Role of Sleep in Cross-Modal Generalization in an At-Home Setting

4.1. Introduction

Generalization refers to the ability to extend the knowledge gained in a specific instance to a broader context. When we successfully generalize past knowledge to new situations, it can allow us to make predictions about the future, apply creative solutions to new problems, acquire new skills more easily, and generally to better understand the rules and relationships that govern the world around us. Conversely, when generalization occurs in an aberrant manner, it can result in maladaptive cognition and behaviour. For instance, over-generalization of negative thoughts is a characteristic trait of depression, over-generalization of negative outcome predictions is a common trait in anxiety, and generalizing trauma to non-traumatic contexts is a hallmark of PTSD. Thus, understanding generalization can help us understand how the brain accomplishes this delicate balancing act and allows us to form accurate models of the world.

Thus far, this thesis has focused on the importance of sleep and its oscillations in memory consolidation. We have looked at how sleep can have beneficial effects on information that has already been learned, either by augmenting it in the case of motor learning or by protecting it from the decay as is the case with declarative memory. However, a key reason we consolidate information – why we remember past events accurately and integrate them into existing frameworks - is to be able to carry out future computations such as those described above. That is, to be able to apply past knowledge to future situations where relevant. Therefore, given that sleep is important to consolidation a lingering question is: **to what degree are the benefits of sleep task-specific? Could sleep aid in the generalization of knowledge from one task to a novel task?**

4.1.1. Possible Mechanisms

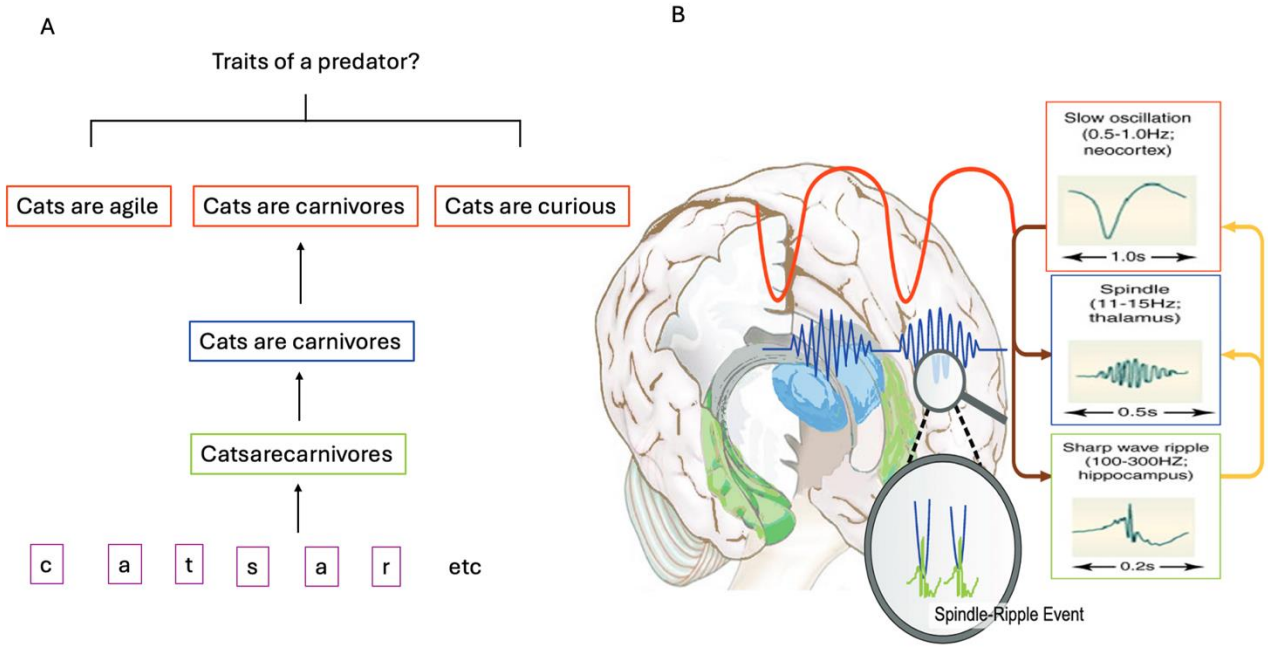


Figure 4.1 Brain Regions and Oscillations Involved in Generalization During Sleep
A: A conceptual illustration of the progression of information from low to higher order computations using the metaphor of reading. Initially, sensory information from different sensory areas is processed as separate, high-fidelity events. In the reading metaphor, these are separate letters which are shown in purple. The sensory events related to an experience are relayed from these sensory areas to the thalamus which relays the information to the hippocampus and other areas. In the metaphor, these are shown as a string of letters associated with a single experience or event and are shown in green. The hippocampus replays these multisensory experiences and processes them in a way that facilitates long-term storage. During sleep this is done through the replay of said experiences and the subsequent chucking of information through the coupling of spindle-ripple events. In the metaphor, these are shown as chunked strings of letters, or words, and are shown in blue. It is easier to detect relationships in the experience in this abstracted or chunked form. Thus, when they reach the cortex in this form, they are better able to be integrated with other, established schemas. In the metaphor, it is easier to read the sentence and make out the meaning of these letters, integrate them with other sentences or schemas regarding cats and execute computations about their relationship to each other. For instance, by noticing a pattern in these behavioural cat traits that are consistent with a predator. Equally, it allows for an update in schemas and allows for more accurate computations of the future, for instance by predicting how a cat might behave.
B: A schematic of the oscillations generated by each brain region and their interactions.
Adapted with permission from (Lewis & Durrant, 2011; Rasch & Born, 2013).

Evidence indeed suggests that generalization is, at least partially, a sleep-dependent process. Behavioural studies have found that sleep benefits generalization (or abstraction, as it is sometimes referred to in the literature) in a diverse range of tasks. For example, sleep (in the form of either a full night or a nap) has been found to aid in the generalization of grammar (Gómez et al., 2006; Hupbach et al., 2009) and word meanings (Horváth et al., 2016) in children, statistical patterns (Lerner & Gluck, 2019), motor learning (Cohen et al., 2005; Witt et al., 2010), and transitive inference (Barron

et al., 2020; Gluck et al., 2002) to name a few. Furthermore, sleep seems to be important for multiple processes that can aid in generalization such as the detection of overarching rules and insight (Verleger et al., 2013; Wagner et al., 2004) and gist extraction (Diekelmann & Born, 2010). How exactly sleep conveys these benefits is still an open question. The precise mechanisms involved are not well understood, it is unclear if all kinds of generalization benefit equally from sleep (Darsaud et al., 2011; Diekelmann et al., 2010; Fenn et al., 2009), and the roles different sleep stages play in this process are still being investigated (Durrant et al., 2011; Hennies et al., 2017; Lau et al., 2010; Pereira et al., 2023). Furthermore, there have been limited numbers of studies that have attempted to investigate the role of EEG oscillations in different stages of sleep and cross-modal generalization (Chatburn et al., 2021) especially outside of a lab setting.

While there is much that is still unknown about sleep's role in generalization, existing research suggests that many of the same biological processes involved in sleep-dependent consolidation are complementary to generalization. For instance, in previous chapters we discussed the active systems model of consolidation and the ways in which information is transferred from the hippocampus to the cortex for long term storage where it is integrated into existing schemas. In the context of motor sequence learning, consolidation and generalization seem to involve a process called "chunking' (Johnson, 1970; Krakauer et al., 2019). Chunking occurs when, instead of being represented in its entirety, parts of a sequence are grouped or chunked together (figure 4.1A). Thus, rather than being represented as the sequence truly is, the sequence is represented in a simplified, abstracted form. It has been suggested that this process is an important step in consolidating memories in a way that facilitates generalization.

An analogous process occurs when we read. Consider a page of writing as a sequence of letters. While the representation with the highest fidelity would involve representing individual letters, an arguably more useful representation is to chunk the letters into words, words into sentences, sentences into

paragraphs and so on. The higher the level of abstraction, the more we lose the granularity of those initial letters. However, it allows us to represent concepts and relationships between concepts in a way that the letters on their own do not (Figure 4.1A).

During NREM, and particularly SWS, learnt information is transferred from the hippocampus to the cortex for long term storage. In this exchange of information, the hippocampus is sometimes described as the "sender" or "transmitter" while the cortex is described as the "reader" or the "receiver" (Buzsáki, 2010). It is in the cortex, where the newly learnt information, in this abstracted state, is believed to be integrated with existing frameworks or schema. This integration has been proposed to facilitate the relational computations. That is, cognitive processes which require an understanding of the relationship between different knowledge frameworks or schema (Figure 4.1B).

Physiologically, chunking is believed to be driven by spike timing. Cell assemblies that fire in synchrony are perceived by upstream brain regions or "readers" as a single event. One key mechanism through which this is believed to be achieved is neural oscillations. In the context of the active systems consolidation theory, this is supported by the finding that when information is being transmitted from the hippocampus to the cortex it is done in the form of nested oscillations. That is, hippocampal ripples (which are associated with replay and are themselves a combination of the sensory inputs associated with a memory) are nested within spindles which are in turn nested in slow oscillations. Thus, when the information reaches the cortex it is no longer three different oscillations but one. Returning to the reading analogy, each slow oscillation might be the equivalent of a word composed of letters.

In addition to processing recently learnt information to facilitate generalization, sleep seems to provide optimal biological conditions for the integration of newly acquired information into established schemas. For example, SOs during NREM sleep create alternating states of hyperpolarization and depolarization. Overlapping sections, representing similar information, are more likely to be

potentiated in the depolarization stage while weakly encoded or nonoverlapping sections are less likely to survive successive bouts of hyperpolarization (Tononi & Cirelli, 2003). This overlap in replay sequences is thought to enable gist extraction and the building of cognitive schemas (or categories) (Lewis & Durrant, 2011). Additionally, in contrast to the waking state, sensory input during sleep is attenuated. This allows for the activation of schemas that are not directly relevant to immediate sensory experience to be reactivated (Tononi & Cirelli, 2003, 2006). This, in turn, allows for a more holistic sampling of experience and allows recently learned information to be integrated with past experiences. Finally, REM sleep is associated with uniquely low levels of norepinephrine which is, in turn, associated with synaptic depotentiation (while high levels of norepinephrine are associated with LTP) (Katsuki et al., 1997). This weakening of synapses has been proposed to weaken established schemas and thus make them more malleable to the integration of new information. Support for this comes from PTSD, in which sleep following a traumatic event often contains REM cycles with abnormally high levels of norepinephrine and low spindle power and count in NREM2. This has been proposed to contribute to the perpetuation of one of the key symptoms of PTSD: the inability to update schema associated with the traumatic event even when the traumatic conditions are no longer enduring (Sara & Poe, 2017). Similar conditions of low norepinephrine are only seen outside of REM in the seconds preceding spindles in NREM2 (Aston-Jones & Bloom, 1981). It has been proposed that this might act as a means to prime cortical schemas by making them more amenable to the incoming novel information in the SO-embedded spindles at this time when the hippocampus and prefrontal cortex are highly synched (Siapas & Wilson, 1998). If norepinephrine levels are experimentally augmented, spindles are likewise inhibited, and rodents are less likely to update established schema (Watts et al., 2012).

Finally, there is evidence that suggest that sleep may be particularly suited to the consolidation and generalization of sequences, such as those in the SRTT. Firstly, sleep seems particularly, but not exclusively, beneficial to motor tasks that recruit the hippocampus (Albouy et al., 2014)such as the

SRTT (Albouy et al., 2008, 2013; Laventure et al., 2016,). Since the hippocampus evolved to aid in navigation (Burgess, 2008; Moser et al., 2015) it is particularly well primed to process sequences of events in time – as might be seen with sequences of landmarks as one navigates a given environment. As a region that aided in spatial navigation it allowed for the detection of patterns and relationships in space. As humans evolved, however, the hippocampus also became a more general cognitive map allowing us to compute relationships between non-spatial concepts in a similar way to spatial ones. Such cognitive maps allow us to carry out computations involving abstract and non-spatial data similar to those that might be carried out in a spatial context (Tolman, 1948). For instance, in a spatial context, cognitive maps allow for the detection of shortcuts in previously experienced locations – a computation that relies on the understanding of relationships between points in space. Similar computations have been observed in non-spatial domains such as finding mathematical shortcuts (Wagner et al., 2004), and inferring latent connections between disparate events (Barron et al., 2020; Whittington et al., 2020) and there is evidence that these computations function using mechanisms similar to spatial ones (Behrens et al., 2018; Constantinescu et al., 2016; Garvert et al., 2017). Finally, these computations benefit from sleep and replay in particular, in a way that is consistent with the active systems consolidation model (Buzsáki & Fernández-Ruiz, 2019; Lewis & Durrant, 2011; Liu et al., 2019; Pereira et al., 2023; Wagner et al., 2004). Thus, it may be that the involvement of the hippocampus in sequential tasks like the SRTT makes it particularly amenable to sleep's benefits. Consolidation of motor sequences into a cognitive map that is effector and context independent may be a mechanism through which sleep-dependent generalization might occur.

4.1.2. The Present Study

In summary, while there are some differences in the proposed mechanisms for sleep-related generalization, most models subscribe to the above format. That is, that the hippocampus processes

the learnt information into chunks and the cortex, through repetition, is able to extract overarching patterns from these and integrate them into cognitive schemas, frameworks, or cognitive maps. It is then in the cortex, through cortico-cortical connections that cross-modal and other forms of generalization are made possible. Thus, it seems that healthy sleep, as conceptualized in the Active Systems Consolidation Theory, is able to achieve a balance between specificity (through replay in the hippocampus during NREM) and generality of knowledge (through gist extraction in the cortex).

However, as discussed, our knowledge regarding sleep's role in generalization is still incomplete. In order to investigate this further, a necessary first step is to establish a behavioural task that could be used to study cross-modal generalization in a naturalistic home setting. This chapter was therefore intended to generate a task that tests generalization and that allows us to compare degrees of generalization across a period of wake compared to sleep.

4.2. Chapter Aims

Study I: The aim of the first study described in this chapter is two-fold:

To determine whether participants are able to generalize a latent sequence structure from a motor to a non-motor domain.

We hypothesized that, if this was the case, participants would perform significantly better on a novel, non-motor task if its underlying sequence structure was the same one they had learned on the SRTT. Furthermore, we hypothesized that participants who perform better on the SRTT would perform better on the familiar sequence in the new non-motor task.

To determine if the ability to generalize is sleep-dependent.

We hypothesized that, given the literature discussed above, participants who underwent a period of sleep after learning the SRTT would perform better on the subsequent generalization task than those who underwent an equivalent period of wake.

These questions were addressed in parallel with those in chapter II. They were carried out on the same population of participants as chapter II, study II with the generalization task being completed in S2. For the sake of brevity, we have provided a brief outline of the methods that have already been described in this thesis and provided links to their more detailed counterparts in chapter II.

Studies II & III:

Study I exposed issues with the design of the generalization task and it became clear that we would have to take a different approach in order to address the questions we set out to answer in study I. In studies II and III, we therefore took a step back from the higher-order questions in study I in order to ask more practical questions related to a task that could be used to answer these questions. The aim of studies II & III was therefore simply to develop and test a task that could be used as a probe for generalization in a remote, online setting in the future.

Our key question was: **Does an embedded sequence structure improve task performance over time?**

We hypothesised that, if participants were learning the latent structure of the experimental sequence(s) they would show better performance on said sequence(s) compared to a random sequence. In study II we tested this hypothesis using a single sequence and, having established a working task with one sequence, we tested it with two sequences in study III.

4.3. Study 4.I

4.3.1. Study 4.I Methods:

4.3.1.1. Participants

The participants in study 4.I of this chapter were the same as those in chapter II, study II – study 4.I is a sub-study of chapter II, study II. The inclusion and exclusion criteria were therefore the same as in chapter II, study II. In brief, participants were recruited through Prolific (https://www.prolific.co). The key aims of these inclusion/exclusion criteria were to ensure that participants were physically able to complete the tasks, that they had not received more finger-focused training than the average person (e.g. through video games or high-level musical training), and that their sleep was not disturbed (e.g. through drugs, travel, or shift work). The study was approved by University of Oxford Central University Research Ethics Committee (Ref R71108).

The participant information sheet and the consent form were presented online, and participants were asked to confirm that they met all inclusion criteria and did not meet any exclusion criteria. Electronic informed consent was then obtained before participants were able to participate in the study. All participants were financially compensated for their time.

50 healthy adults consented to participate. Of these, 14 (7 from the AM and 9 PM group) were excluded from the analysis due to an error in the link sent to them in S2 that affected the generalization task. 37 participants in total (11 males, mean age= 27.03 $\pm$ 6.36) were used for the following analyses 3 of these 37 participants are missing demographic information.

4.3.1.2. Study Structure

On submission of the electronic consent form, participants were automatically randomly assigned to the AM or PM conditions which dictated the timing of the study sessions (see figure 4.2). All tasks and questionnaires were administered online, in the participants' homes. They completed the tasks on a computer (as opposed to a phone or tablet) in order to minimize differences in keyboard size that might alter the motor performance of the sequences. Participants were sent the links to the session

tasks shortly before it was their time to complete them to minimize the chances of them performing the sessions at the wrong times.

In session one, participants trained on the paired-associates task and SRTT. Following a period of sleep (PM group) or wake (AM group) session two included retesting on the paired-associates task and the SRTT followed by the generalization task, thus the generalization was in the morning for the PM group and the evening for the AM group. The paired- associates tasks will not be discussed further in this chapter as it is not relevant to our current research questions, but it is described and discussed in detail in chapter II.

4.3.1.3. Behavioural Tasks:

4.3.1.3.1. SRTT:

As described in chapter 2, participants trained on a 12 item SRTT. There were two sequences which participants could learn - sequence A: ASALSKLAKSLK, sequence B: SLKSKALSKALA. The task was completed bimanually, using two fingers from each hand (index and middle). The task was split into blocks composed of three repetitions of a single sequence. Random blocks were presented to probe sequence-specific learning during different stages of the sessions. Participants were trained on a single sequence, either A or B.

Performance on the SRTT was quantified as in previous chapters, that is, using the sequence specific learning (SSL) scores for RT (SSL_{RT}) and CS (SSL_{cs}), which were calculated by subtracting the average performance score of the last 2 blocks in the session from the performance score of the random sequence block.

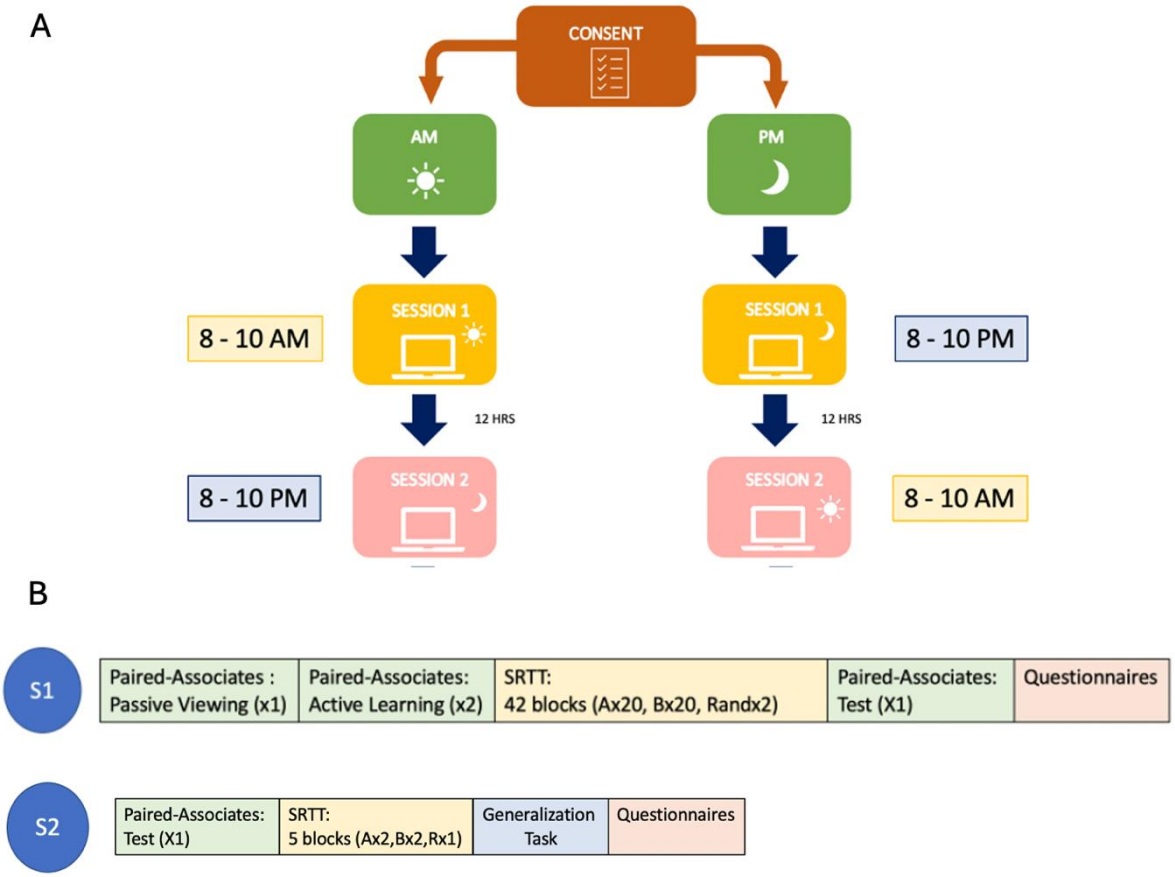


Figure 4.2. Flowchart of Study Structure and Breakdown of Within-Session Tasks For Studies I and II
A: Flowchart of Study I structure following consent.
B: Overview of the order of behavioral tasks and questionnaires in each of the three sessions of Study I.

4.3.1.3.2. Generalization Task

The aim of this task was to determine whether participants were able to generalize the sequence structure of one of the SRTT sequences to a novel task in a non-motor domain. We hypothesized that if this was the case, participants would be able to anticipate upcoming stimuli in the sequence, and therefore respond more quickly and accurately to sequence structures that were familiar compared to unfamiliar.

In order to test this, we used a familiar sequence, an unfamiliar sequence, and a random sequence. The familiar sequence was the one they had trained on during the SRTT in session 1. We translated the

underlying structure of the SRTT sequence into a series of images that correspond to each item in the sequence. That is, each item in the 12- item sequence was matched to a corresponding image as shown in figure 4.3. Finally, there was also a random sequence to quantify any non-sequence-structure dependent changes in performance. Whilst we envisioned using this random sequence in the same way the random sequence was used in the SRTT, in the absence of any clear difference between the familiar and unfamiliar sequence and given that we did not probe learning over the course of the many trials as we had done previously, we found it did not add any information that a comparison between the familiar and unfamiliar sequences did not. It is thus not reported further.

The visual stimuli were chosen because they had previously been used by Garvert et al (2017) in a similar visual orientation task. The images were originally generated by Rossion and Pourtois (2004) (Stimulus images courtesy of Michael J. Tarr, Center for the Neural Basis of Cognition and Department of Psychology, Carnegie Mellon University, http://www.tarrlab.org/) and were designed with the aim of being similar in terms of object familiarity and visual complexity. The familiar and unfamiliar sequences consisted of non-overlapping sets of images.

Participants were first asked to passively view a series of individual images appearing sequentially for the duration of a single block. They were instructed to "simply view each image attentively for a few seconds and then press the space bar to continue to the next image". Each image stayed on the screen until the spacebar was pressed. Participants were told they could take their time and that they could press the spacebar with any finger as this was not intended to be a response time or motor task. Individual items in all the passively viewed sequences were separated by a fixation cross. This passive viewing was intended to expose participants to the sequence structure and the correct orientation of each item in the sequence (Fig 4.3a).

Participants were then told that they would be shown images that they had seen before and that the images would either be facing in the same direction or a different direction as when they originally saw them. Their task would therefore be to determine whether images are facing in the same direction as before (during the passive viewing) or not. They were instructed to press the "Q" key if the image was facing the same direction as before or the "P" key if the image was facing in a different direction. They were asked to respond as quickly and accurately as possible and using whichever fingers they wished. A banner was displayed at the bottom of the screen which read "SAME (Q)" on the left side of the screen and "DIFFERENT (P)" on the right side of the screen (Fig 4.3b). This was to allow participants to focus on the task at hand without the added cognitive burden of remembering which button corresponds to which response. Participants were given trial-by-trial feedback, either "correct!" or "wrong!", on the accuracy of their response. The probability that each image was presented in the same or different orientation as the passive viewing was 50%.

Participants performed a practice orientation task prior to the actual task with the aim of helping them become better acquainted with what the task required them to do and the buttons they needed to press during the task. In this practice session, participants were shown a short series of pictures (different from those in the experimental task) passively. They were then shown the same pictures in the same or different orientation and were asked to indicate whether their orientation was the same or different from that in the passive viewing task.

For the actual task, each block was composed of three iterations of the 12-item sequence. There were three blocks of the familiar sequence and three blocks of the unfamiliar sequence. Participants saw both sequences passively once before completing the object orientation tasks. Since participants in this study were only exposed to either sequence A or B during the SRTT training in S1, we used whichever sequence they had been exposed to in S1 as the familiar sequence and that which they had not as the unfamiliar sequence. This ensured that the familiar and unfamiliar sequences were

counterbalanced across participants. Equally, we ensured that the set of images associated with sequences A and B in the generalization task were counterbalanced across participants, such that one set of images was never associated exclusively with the familiar or unfamiliar sequence across participants.

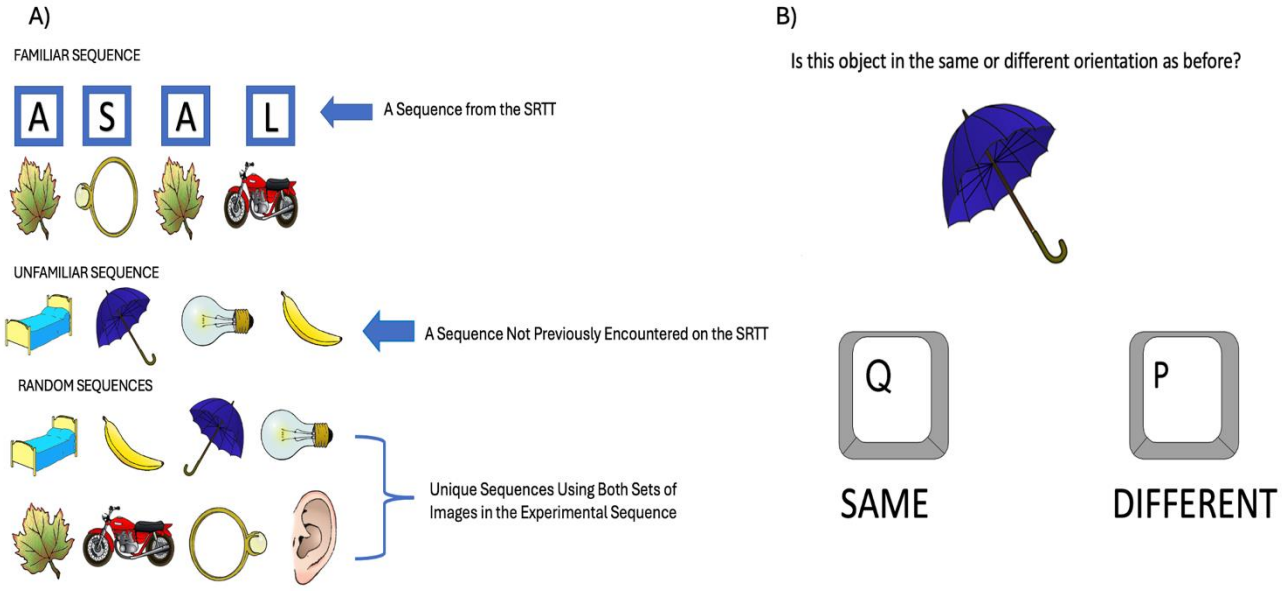


Figure 4.3 A Schematic of the Sequences Involved in the Study 4.I Generalization Task and The Task Objective
A) The different sequences involved in the generalization task and their characteristics.
B) The aim of the orientation task used to probe generalization and the buttons used to indicate each response.

4.3.1.4. Pre-Processing:

Trials with RTs above 2000ms were removed from the analysis, we did not impose an accuracy threshold in this study. Average RTs were averaged across blocks, which consisted of three repetitions of the sequence.

4.3.1.5. Statistical Analyses:

4.3.1.5.1. Do participants generalize a latent sequence structure from a motor to a non-motor domain independently of sleep or wake?

The first question we wanted to address was whether participants were able to generalise a latent sequence structure from a motor to a non-motor domain, regardless of experimental group. We hypothesized that if participants successfully generalized the sequence structure from the SRTT, they would show greater performance benefits over time with the familiar sequence than the unfamiliar sequence. We quantified this learning as the change in performance from the start of the task to the end, using RT, accuracy (ACC), and composite scores (CSs) as metrics. We then ran a two-way rmANOVA comparing each of these performance indices across the two sequences.

4.3.1.5.2. Is the ability to Generalize Sleep-Dependent?

We then asked whether being in the PM or AM group affected participants' performance on the generalization task, hypothesizing that the PM group would show greater generalization than the AM group. We compared performance across blocks for each sequence and group using a two-way rmANOVA. This was done for performance as measured by RT, ACC and CSs.

4.3.1.5.3. Does performance on the SRTT correlate with performance on the Generalization Task? Is this sleep-dependent?

We then wanted to determine the relationship between an individual's performance on the SRTT and their subsequent performance on the generalization task. To address this, we ran a Pearson correlation on SRTT performance at the start of S2 and the generalization task performance (in the same session). Performance on the SRTT was measured by the Sequence Specific Learning composite scores (SSL_{CS}) scores, which is the difference in the performance metric between the experimental sequence and the random sequence. A higher number indicates that participants have retained more sequence specific knowledge of the SRTT sequence. We quantified performance on the generalization task through the difference in the change in performance between the familiar and unfamiliar sequences from the first to last block in the session as measured by RT and CSs. To assess whether SRTT performance correlates with Generalization Task performance, we ran a correlation analysis on performance metrics across all

participants independently of groups. To assess whether the relationship between the SRTT and generalization task performances differed between the AM and PM groups we ran correlations on these groups separately and then compared the correlation strengths between groups using Fisher's r-to-z.

4.3.2. Results: Study 4.I

4.3.2.1. Do participants generalize a latent sequence structure from a motor to a non-motor domain independently of sleep or wake?

We first asked whether participants were able to generalize a latent sequence structure, regardless of whether they slept or stayed awake following learning of the SRTT in S1. We addressed this by comparing the performance between the familiar and unfamiliar sequences across blocks. The rmANOVA comparing RT of the two sequences (familiar, unfamiliar) across blocks revealed no significant effect of sequence on performance as measured by RT ($F(1, 35) = 0.88$, $P = 0.36$), and no significant interaction of block and sequence ($F(2, 70) = 0.13$, $P = 0.83$). However, a significant effect of block was found ($F(2, 70) = 19.45$, $P = <0.001$). This suggests block-wise improvement in RT irrespective of whether the sequence structure was familiar to the participant at the start of the task (Fig 4.4a). In terms of accuracy, no significant effects of sequence ($F(1, 35) = 0.12$, $P = 0.73$), block ($F(2, 70) = 1.36$, $P = 0.26$), or interaction of sequence and block ($F(2, 70) = 2.2$, $P = 0.12$) were found. This suggests that any improvement in performance is driven by RT rather than by accuracy which remains fairly stable across blocks and sequences (Fig 4.4b). Finally, for the CS we found no effect of sequence ($F(1, 35) = 0.79$, $P = 0.38$) or sequence by block interaction ($F(2, 70) = 1.08$, $P = 0.35$) but there was a significant effect of block ($F(2, 70) = 9.95$, $P = <0.001$; Fig 4.4c). Given the findings concerning the other performance metrics this result is likely driven by the significant effect of block on RT. In summary, participants get faster at determining whether the object orientation is the same or different independently of sequence structure and show a high, steady accuracy across blocks without evidence of a speed/accuracy trade-off.

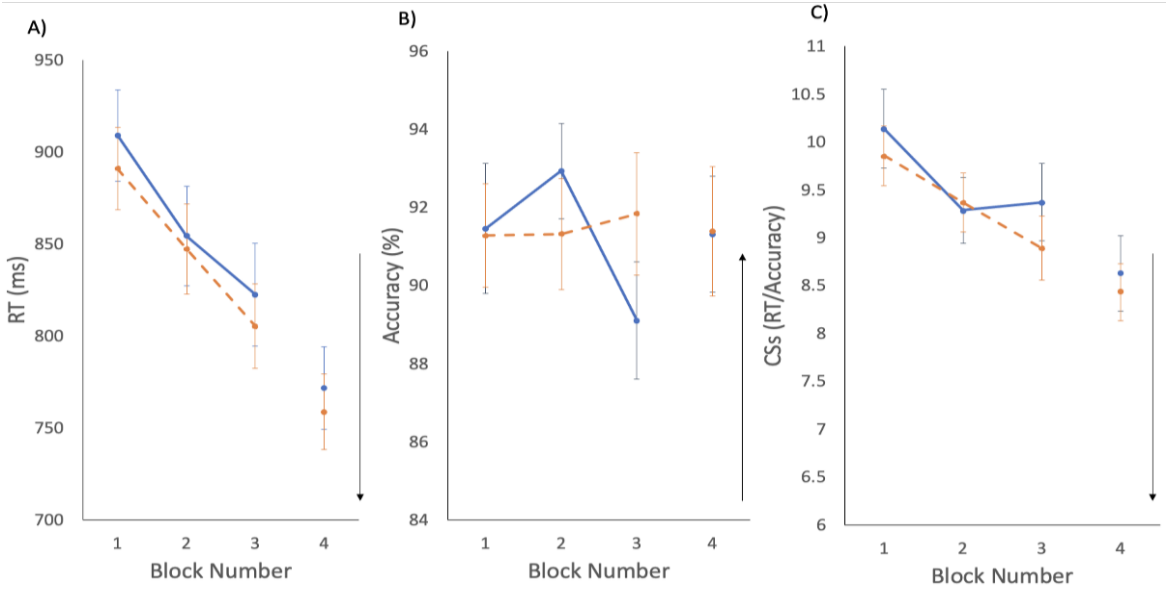


Figure 4.4 . Block-wise performance on the generalization task with participants pooled across the AM and PM conditions. Blocks 1-3 are the experimental sequences, and the fourth block is the random sequence. The familiar sequence is represented in blue and the unfamiliar sequence in orange. Arrows indicate the direction of improvement on the task.
A) Change in task performance across blocks as measured by absolute RT.
B) Change in task performance across blocks as measured by accuracy.
C) Change in task performance across blocks as measured by CSs.

4.3.2.2. Is The Generalization of Sequence Structure Sleep-Dependent?

Next, we asked whether any generalization of sequence structure from the motor to non-motor domain benefitted from sleep, by comparing AM and PM groups. The rmANOVA on RT across blocks for the familiar and unfamiliar sequences across the AM and PM groups found a significant interaction between group and block number ($F(2, 68) = 5.17$, $P = .008$), but no significant effects of group and sequence ($F(1, 34) = 0.28$, $P = .60$), or group, block number, and sequence ($F(2, 68) = .32$, $P = .73$) (Fig 4.5a). Post-hoc comparisons with Bonferroni correction showed that these significant interactions occurred in the PM group in the first block when compared with second ($P = <.001$) and third ($P = <.001$) blocks in the task, but did not occur in the AM group (Fig 4.5a). This may reflect circadian effects on performance, perhaps due to different levels of alertness between the AM and PM groups as the AM group may have been less alert (perhaps experiencing sleep inertia after waking up). The lack of significant interactions between group and sequence, however, suggests there was no significant difference in performance between familiar and unfamiliar sequences across the AM and PM groups.

The rmANOVA on accuracy across blocks for the familiar and unfamiliar sequences across the AM and PM groups found no significant interaction between group and block number ($F(2, 68) = 0.17$, $P= 0.74$), group and sequence ($F(1, 34) = 0.02$, $P= .90$), nor group, block number, and sequence ($F(2, 68) = 0.74$, $P= 0.48$) (Fig 4.5b). This suggests that group affiliation had no effect on performance across blocks or sequences when performance was measured by accuracy.

The rmANOVA on CS across blocks for the familiar and unfamiliar sequences across the AM and PM groups found a significant interaction between group and block number ($F(2, 68)= 4.13$, $P= .02$), but no significant interactions between group and sequence ($F(1, 34)= 0.44$, $P= 0.84$), nor group, block number, and sequence ($F(2, 68)= 0.1$, $P=0.34$). Post-hoc comparisons with Bonferroni correction revealed that significant improvements in RT occurred in the PM group in comparisons between block 1 and blocks 2 ($P= <.001$) and 3($P= <.001$), (Fig4.5c). This suggests that this interaction is driven by the aforementioned effects on RT rather than by accuracy.

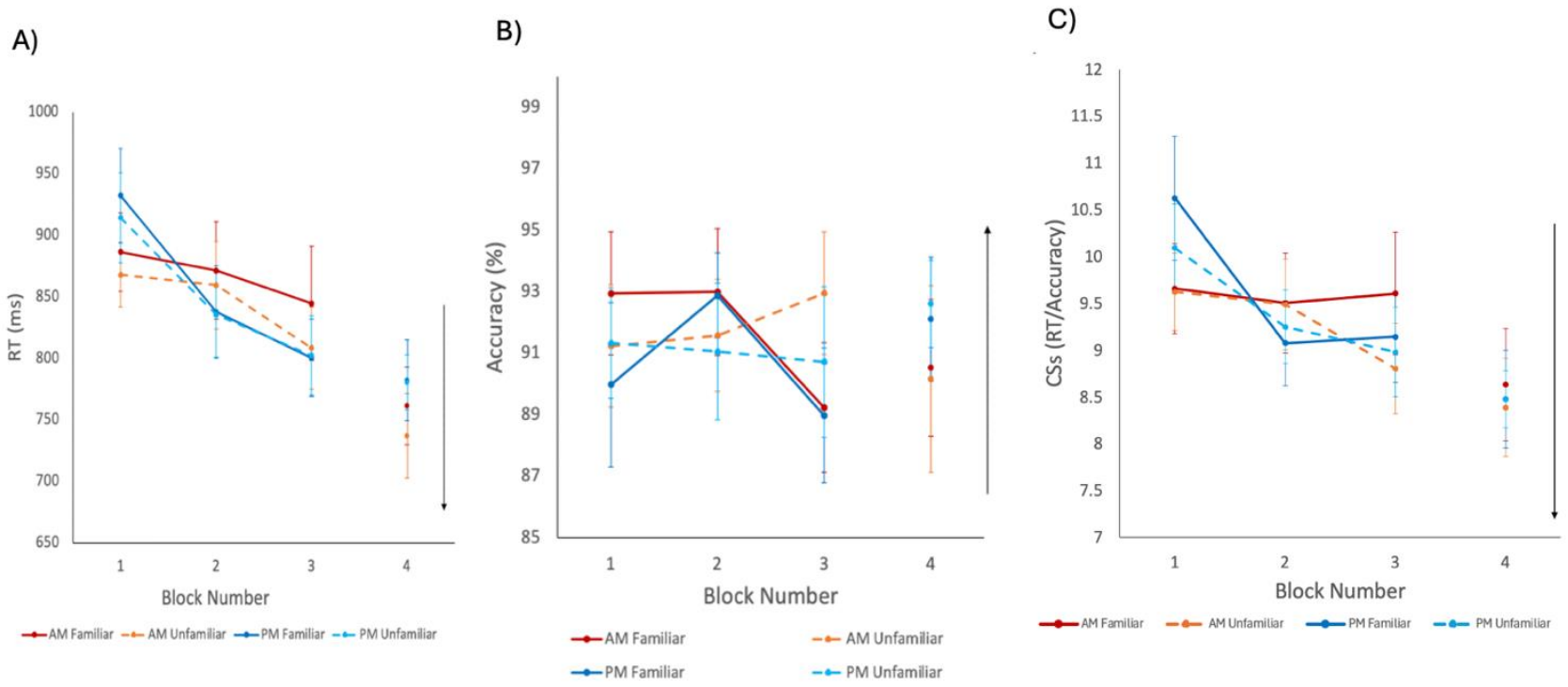


Figure 4.5 Block-wise performance on the generalization task as measured by RT, Accuracy, and CSs.
Arrows indicate the direction of improvement on the task.

A) Performance of the AM and PM groups across sequences as measured by RT. Blocks 1-3 are the experimental sequences, and the fourth block is the random sequence. The PM group's performance is shown in cool colors while that of the AM group is shown in warm colors. The familiar sequences are shown in filled lines and the unfamiliar sequences are shown in dashed lines.

B) Performance of the AM and PM groups across sequences as measured by accuracy. Blocks 1-3 are the experimental sequences, and the fourth block is the random sequence. The PM group's performance is shown in cool colors while that of the AM group is shown in warm colors. The familiar sequences are shown in filled lines and the unfamiliar sequences are shown in dashed lines.

C) Performance of the AM and PM groups across sequences as measured by CSs. Blocks 1-3 are the experimental sequences, and the fourth block is the random sequence. The PM group's performance is shown in cool colors while that of the AM group is shown in warm colors. The familiar sequences are shown in filled lines and the unfamiliar sequences are shown in dashed lines.

4.3.2.3. Does performance on the SRTT correlate with performance on the Generalization Task? Is this different across groups (AM vs PM)?

Finally, we wanted to know whether the degree to which participants had learnt a sequence structure in the SRTT related to the degree to which they generalized that sequence in the non-motor task. To do this, we used the Sequence Specific Learning composite scores (SSL_{CS}), which is the difference in CSs between the experimental sequence and the random sequence, as our measure of SRTT performance at the start of S2 (when the generalization task was presented). We chose to quantify performance on the generalization task through the change in performance between the familiar and unfamiliar sequences from the first to last block in the session as measured by CSs. We chose this measure of generalization task performance in order to try and characterize whether previous knowledge of the SRTT sequences affected speed or accuracy on the subsequent generalization time. This generalization task performance measures will henceforth be referred to as the sequence-specific difference (SD) as measured by CSs; SSD_{CS}. First, we looked at these correlations independently of group (AM or PM). There was no significant correlation between the performance on the SRTT as measured by SSL_{CS} (Fig 4.6a) and the generalization task as measured by SSD_{CS} ($r= 0.2$, $p= 0.9$) (Fig 4.6b).

We then asked whether the relationship between SRTT and generalization task performance differed between the AM and PM groups. As this is another means to measure of the degree of cross-modal generalization, we wanted to explore whether sleep strengthens the relationship between the SRTT and the generalization task. Given our hypotheses regarding sleep's role in generalization, we reasoned that sleep might strengthen the relationship between the two tasks. This strengthening might in turn facilitate the ability to generalize from one to the other. We therefore hypothesized that the correlation between the SRTT and the generalization tasks would be stronger after a period of sleep compared to a period of wake. To test this, we conducted Pearson correlations on the aforementioned performance

variables and found no significant correlations between SSL_{CS} a and SSD_{CS} (r= - 0.05, p= 0.84) in the AM group (Fig 4.6a). Equally, we found no significant correlations between SSL_{CS} and SSD_{CS} (r= 0.02, p= 0.96) in the PM group (Fig 4.6b). Therefore, we found no significant correlations between the performance on the SRTT and the subsequent generalization task as defined by any of our measures. We then asked if the correlations between the performance on the SRTT and the generalization task were different between the AM and PM groups, using Fisher's R to Z test but found no differences (all p > 0.05) (Fig 4.6a,b).

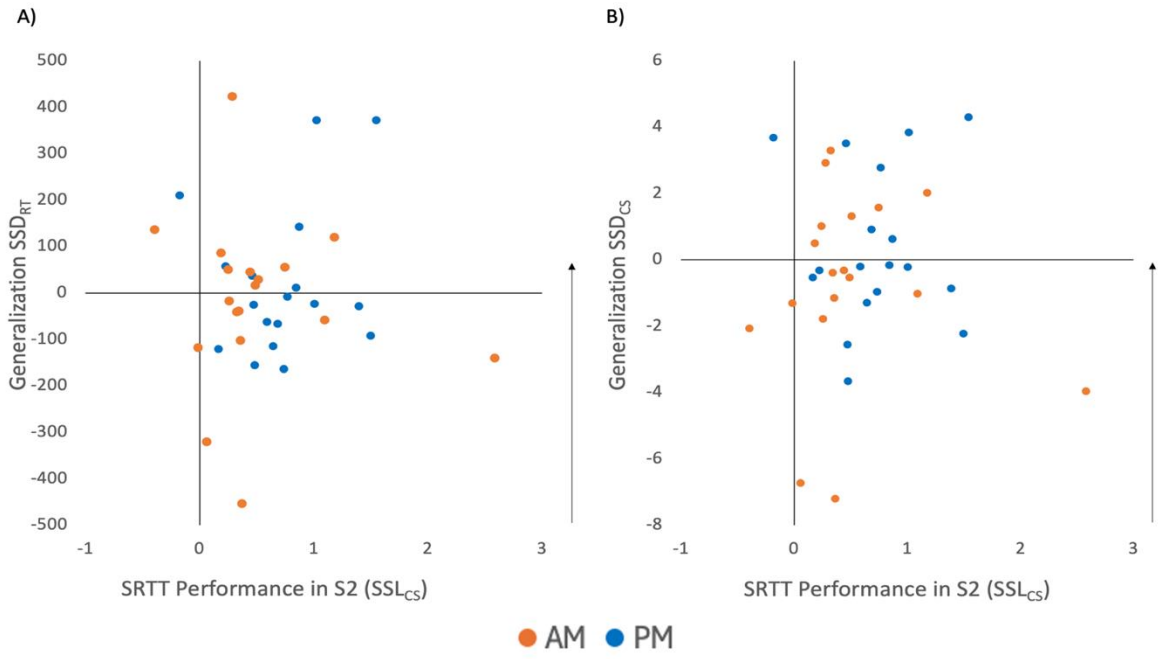


Figure 4.6 The relationship between performance on the SRTT at S2 and performance on the generalization task.
Data points corresponding to the AM group are shown in orange and those corresponding to the PM group are shown in blue. Arrows indicate the direction of improvement on the task.
A) The relationship between the performance on the SRTT as measured by SSL_{CS} and performance on the generalization task as measured by $SSD_{RT.}$
B) The relationship between the performance on the generalization task as measured by SSL_{CS} and performance on the generalization task as measured by $SSD_{CS.}$

4.4. Study 4.II

4.4.1. Methods: Study 4.II

As we did not find evidence of sequence-structure generalization in Study I, we decided to step back and ask whether participants are able to learn a sequence structure embedded within picture tasks similar to those used in study 1, but irrespective of cross-modal generalization or sleep-dependent consolidation. We therefore ran studies using the picture task only (without any preceding SRTT task), first including a single sequence (study 4.II) and then expanded to include two sequences (study 4.III). These two studies were otherwise identical. It is important to note that no sequences were familiar in these studies. Rather, they are designed to assess whether an embedded repeating sequence structure can improve performance over time. It was our intention to integrate the picture task with the SRTT in the future if we found evidence of learning in this study, in order to probe generalization.

4.4.1.1. Participants:

19 new participants (9 males, mean age = 28.06 ± 8.24,), who had not participated in any prior studies in this thesis, were recruited. 2 participants were missing demographic information. Due to a technical error, one participant was 72 years old which was above the specified age range. All participants were recruited remotely through prolific. Inclusion criteria were that participants were between 18-60 years of age, fluent in English with basic computer literacy, access to a working laptop with reliable internet and a QWERTY keyboard. As in previous studies, the exclusion criteria included being Deaf or Hard of Hearing, a skilled musician, a habitual video game player, or having a medical condition that restricted the mobility of their hands or fingers. Since we were not investigating sleep-dependent consolidation in this study, we did not include sleep-relevant criteria such as medications that might affect sleep or shift work.

4.4.1.2. Study Structure:

As with previous studies, recruitment, consent, and the study itself were completed remotely and online. Consent and all subsequent tasks took place in a single session.

4.4.1.2.1. Generalization Task:

Participants appeared to struggle with the rotation task in study I so in studies II and III we attempted to implement a simpler task that probed encoding of underlying sequence structure. In this task, participants were shown images in a sequential order (12-item sequence) and instructed to press the spacebar with the index finger of their dominant hand when a target image (presented to them at the start of each block), appeared on the screen. They were asked to respond as quickly and accurately as possible.

Unlike the previous generalization task in study 4.I, they were not presented with the sequences passively beforehand but we hypothesised that they would learn the embedded sequence structure over time as the task progressed. As such, participants were not made aware of the different sequences they would encounter during the task, nor were there labels on the screen to indicate what kind of sequence (experimental or random) they were presented. The target image changed between blocks such that different locations within the sequence were probed allowing us to test participants' familiarity with the underlying sequence structure as a whole. Trials including the target image lasted until the participant pressed the spacebar and the trials without the target image (where no response was expected) lasted for 750ms regardless of whether the spacebar was erroneously pressed or not. Each block consisted of three repetitions of the sequence, with each sequence repetition punctuated by a fixation cross. The session was composed of 22 blocks in total: 20 blocks of the experimental sequence and 2 blocks of the random sequence in the middle and end of the session (blocks 10 and 20 respectively).

The experimental sequence had the same underlying structure as sequence B from the SRTT (though participants did not complete the SRTT and so had no prior exposure to the sequence). Each random block consistent consisted of a different sequence to prevent any potential learning of the random sequence. The random sequences were created manually to ensure minimal overlap between them

and the experimental sequences. Thus, these sequences were random in the sense that there was no over-arching structure, not in the sense that they were created to have a completely random structure. Both random and experimental sequences were composed of the same set of images.

Feedback on the average speed (as measured by RT) of participants' performance was presented at the end of each block. Accuracy was not measured in this version of the task. Participants received detailed instructions at the start of each block early in the session but received shortened instructions as the task progressed and they became familiar with what was required of them in each block (Fig 4.7).

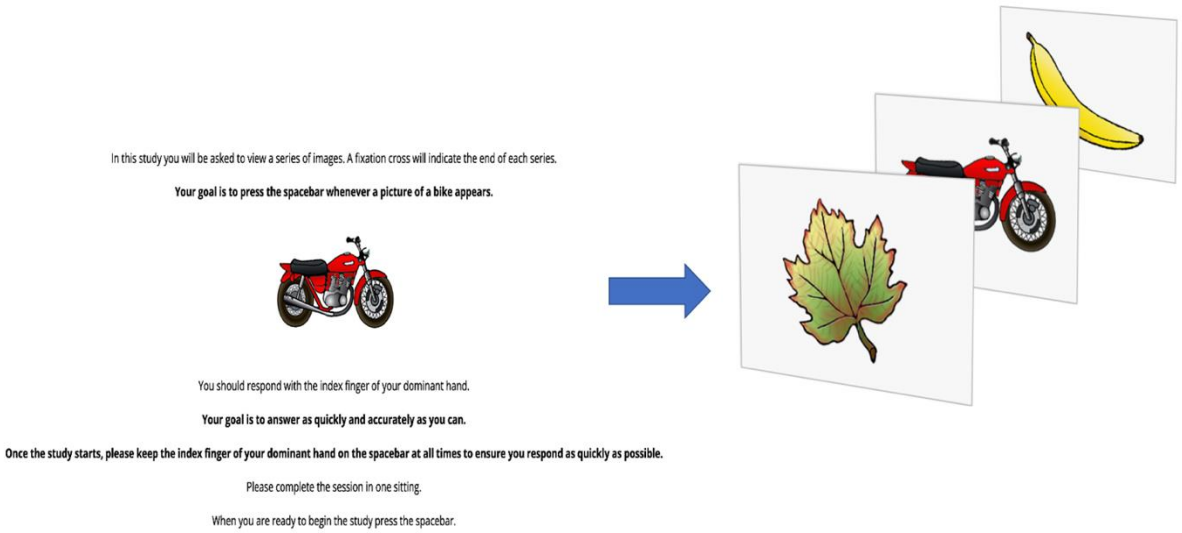


Figure 4.7 The Instructions for the Generalization Task in Studies 4.II and 4.III and Sample Start of A Sequence
In this example the participants would have to press the spacebar whenever the motorbike was presented. In this example, that would be in the second trial.

4.4.1.3. Pre-processing

We removed blocks in which mean RT was more than 3 standard deviations from the mean (across all blocks) from the analyses. Here we removed one such block. In order to impute these missing values, we took the average of the block before and after the missing block. There were no cases where the missing blocks were next to each other.

4.4.1.4. Statistical Analyses

The goal of studies II and III was to determine whether the participants were able to learn the new versions of the generalization task. We broke this down into two key questions:

4.4.1.4.1. Do participants learn to respond more quickly to the experimental sequence blocks over time?

More specifically, we asked whether there was a significant change in RT across the relevant blocks. To answer this question, we ran a two-way rmANOVA with Greenhouse-Geiser corrections using block-wise RT as a measure of task performance (with the random sequence blocks excluded from the analysis). We used Bonferroni correction for post-hoc comparisons to determine significant block-wise changes in RT.

4.4.1.4.2. Does performance improve in a sequence-specific manner?

More specifically, we wanted to know if the difference in performance between the experimental and random sequences increased in a way that demonstrated greater improvement for the experimental (repeated) sequence. To address this, we ran a two-tailed, one-sample, t-test to test whether SSL_{RT} (difference in mean RT between the random sequence block and the preceding sequence block) differed from zero. We performed this test separately for each timepoint when a random block appeared (blocks 10 and 20).

To test whether participants continued to improve on the task from the middle to the end of the session, we ran a paired t-test comparing the SSL_{RT} in the middle of the task (block 10) to the last block in the task (block 20). The change indices were calculated such that a bigger value denoted better performance on the experimental sequence.

4.4.2. Results: Study 4.II

4.4.2.1. Do participants learn to respond more quickly to the experimental sequence blocks over time?

The rmANOVA using block-wise RT as a measure of task performance (with the random sequence blocks excluded from the analysis) found that there was a significant effect of block ($F(4.56, 82.1) = 5.14$, $P = < 0.0001$) (Fig 4.8a), suggesting participants learnt the task and therefore got faster during the session.

4.4.2.2. Does performance improve in a sequence-specific manner?

To determine if this improvement in RT was sequence-specific we conducted one-sample t-tests at the two points at which the random sequences occurred in the session comparing the difference between the RT in the random block to that of the preceding block (i.e. SSL_{RT}) with zero (no difference). The SSL_{RT} was significantly greater than zero (first random block: $t(18)= 3.95$, $p= >0.001$; second random block: ($t(18)= 3.35$, $p= 0.004$) (Fig 4.8b). These results suggest that participants improve (get quicker) in a sequence specific manner.

To test whether performance continues to improve from the middle to the end of the task, and therefore whether the length of the session was appropriate, we ran a paired t-test comparing the SSL_{RT} in the middle of the task (block 10) to the last block in the task (block 20) (Fig 4.8a,b). The t-test found no significant difference ($t(18) = 1.3$, $p = 0.10$). These results that the benefits of the sequence structure on performance do not increase from the middle to the end of the session, where performance seems to plateau.

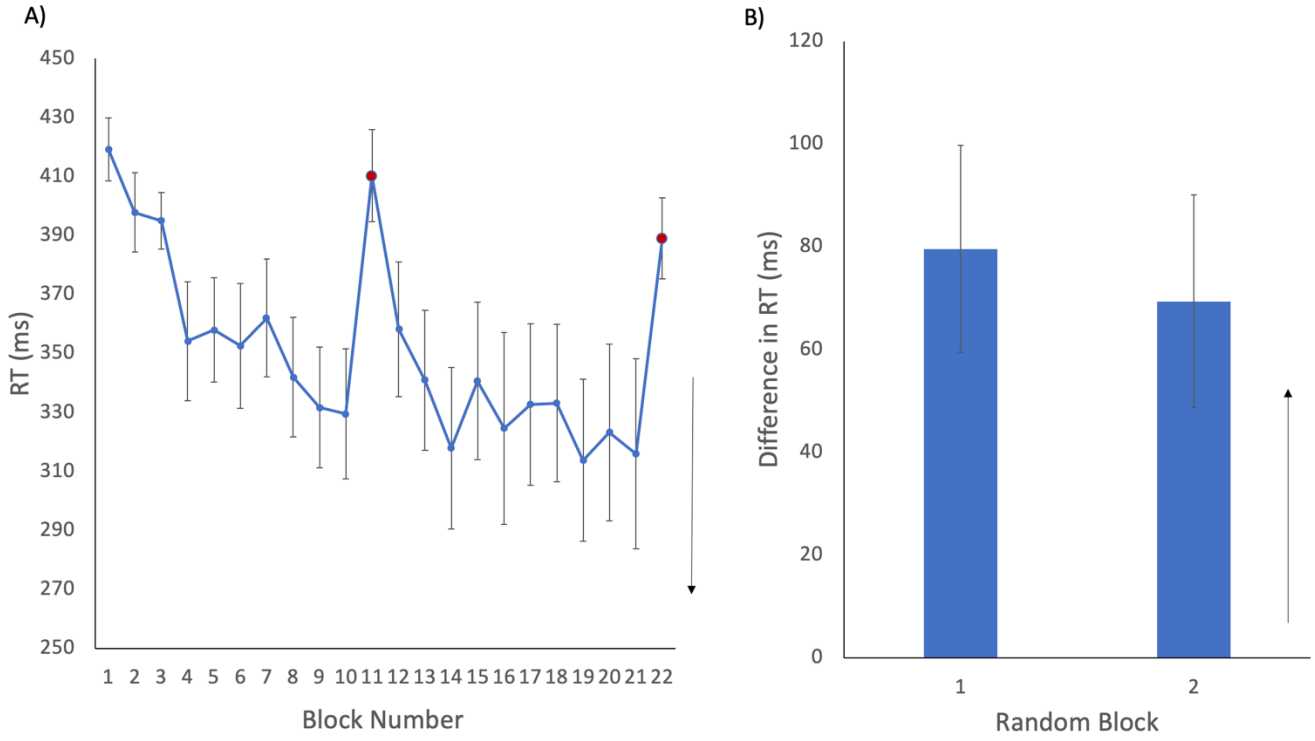


Figure 4.8 Study 4.II Learning Measures: Learning curve as measured by RT and difference in RT between experimental and random blocks SSL_{RT}
Arrows denote the direction of task improvement, error bars represent the SEM.
A) The learning curve of the study III generalization task as measured by RT. Experimental sequence blocks are shown in blue and random sequence blocks in red.
B) SSL_{RT} measured in the middle and end of the task.

4.5. Study 4.III

4.5.1. Methods: Study 4.III

4.5.1.1. Participants:

38 new participants (20 males, mean age = 28.47±9.86) were recruited through Prolific using the same inclusion and exclusion criteria as study 4.II.

4.5.1.2. Generalization Task:

The task consisted of two sequences with the underlying structures of sequences A and B from the SRTT described previously (though participants did not complete the SRTT so neither sequence was

familiar). There were 40 blocks per sequence with 4 random blocks adding up to a total of 84 blocks. There were 8 possible timeline structures (excluding random sequence counterbalancing), such that the interleaving order in which the sequences were presented, the sets of images associated with each sequence, and the target images were counterbalanced across participants. One of the 8 timelines was automatically selected at random at the start of the task.

The random blocks were presented every twenty blocks. Each random block consisted of sets of images associated with either sequences A or B.

4.5.1.3. Pre-processing

We removed blocks in which mean RT was more than 3 standard deviations from the mean (across all blocks) from the analyses. Here we removed 3 such blocks. In order to impute these missing values, we took the average of the block before and after the missing block. There were no cases where the missing blocks were next to each other.

4.5.1.4. Statistical Analyses

We approached this third study with the same set of questions we had in study 4.II, but aimed to determine whether our previous findings could be extended to a task with two experimental sequences, rather than one.

Do participants learn to respond more quickly to the experimental sequence blocks over time?
We ran a rmANOVA with Greenhouse-Geiser corrections on the RT across blocks the to answer this question. Post-hoc comparisons with Bonferroni correction were used to determine significant block-wise changes in RT.

4.5.1.4.1. Does performance improve in a sequence-specific manner?

To test whether participants were faster in the experimental sequence blocks compared to the random sequence blocks, a sign of sequence-specific learning, we ran two-tailed, one-sample t-tests for each random block in the session. In these, we compared the difference between the RT in the random block to that of the preceding block (i.e. SSL_{RT}) with zero (no difference).

To determine whether participants showed improvement in a sequence-specific manner, we conducted a rmANOVA with Greenhouse-Geiser corrections on SSL_{RT} at each quarter of the task, where a random block was presented. Post-hoc comparisons with Bonferroni correction were used to determine significant block-wise changes in $SSL_{RT.}$

4.5.2. Results: Study 4.III

Given that we observed some evidence of benefits to performance from an embedded sequence structure in study II using a single experimental sequence, we then carried out the same task using two experimental sequences instead of one. This is a necessary step as two sequences would be necessary to compare familiar and unfamiliar sequences in future experiments (and hence test for generalization). As it would likely take more effort to learn two sequences compared to one, we increased the number of blocks and therefore repetitions of the sequences. The data in 4-block bins are shown in Figure B, to allow a clearer view of the data given the large number of blocks in this version of the task.

4.5.2.1. Do participants learn to respond more quickly to the experimental sequence blocks over time?

Once again, we wanted to ask whether performance improves, that is- gets quicker, over time in sequence blocks. The rmANOVA with Greenhouse-Geisser correction found a significant effect of block for RT ($F(11.72, 422.01) = 2.19$, $P = 0.01$). Post-hoc comparisons with Bonferroni correction showed significant differences at block 7 compared to 25 ($p= 0.016$) and 11 compared to 25 ($p= 0.036$) (Fig 4.9a, b).

4.5.2.2. Does performance improve in a sequence-specific manner?

To determine if this improvement in RT was sequence-specific we conducted one-sample t-tests at the four points at which the random sequences occurred in the session comparing the difference between the RT in the random block to that of the preceding block (i.e. SSL_{RT}) with zero (no difference). This revealed that there was no significant SSL in random block1 ($t(37) = 0.22$, $p= 0.83$), but block 2 ($t(37)= 3.47$, $p= 0.001$), block 3 ($t(37) = 2.89$, $p=0.006$, and block 4 ($t(37)= 3.86$, <0.001) showed significant SSL. This shows that participants improve across the session, with the first block showing no significant

difference between the experimental and random sequence and the last block showing a highly significant difference.

To test whether sequence-specific benefits accrue over the course of the session, we ran a rmANOVA on the SSL_{RT} at each quarter of the task to determine whether there was a significant difference in sequence specific learning from the start to the end of the task. There was a significant effect of block ($F(2.41, 111) = 4.46$, $P = 0.01$) which suggests that participants are improving on task performance in a sequence-specific way. Post-hoc comparisons with Bonferroni correction showed a significant difference between the first and last blocks only ($p = 0.023$) (Fig 4.9a,b). Together these results suggest that while RT may plateau around block 25, sequence-specific learning takes longer to become evident.

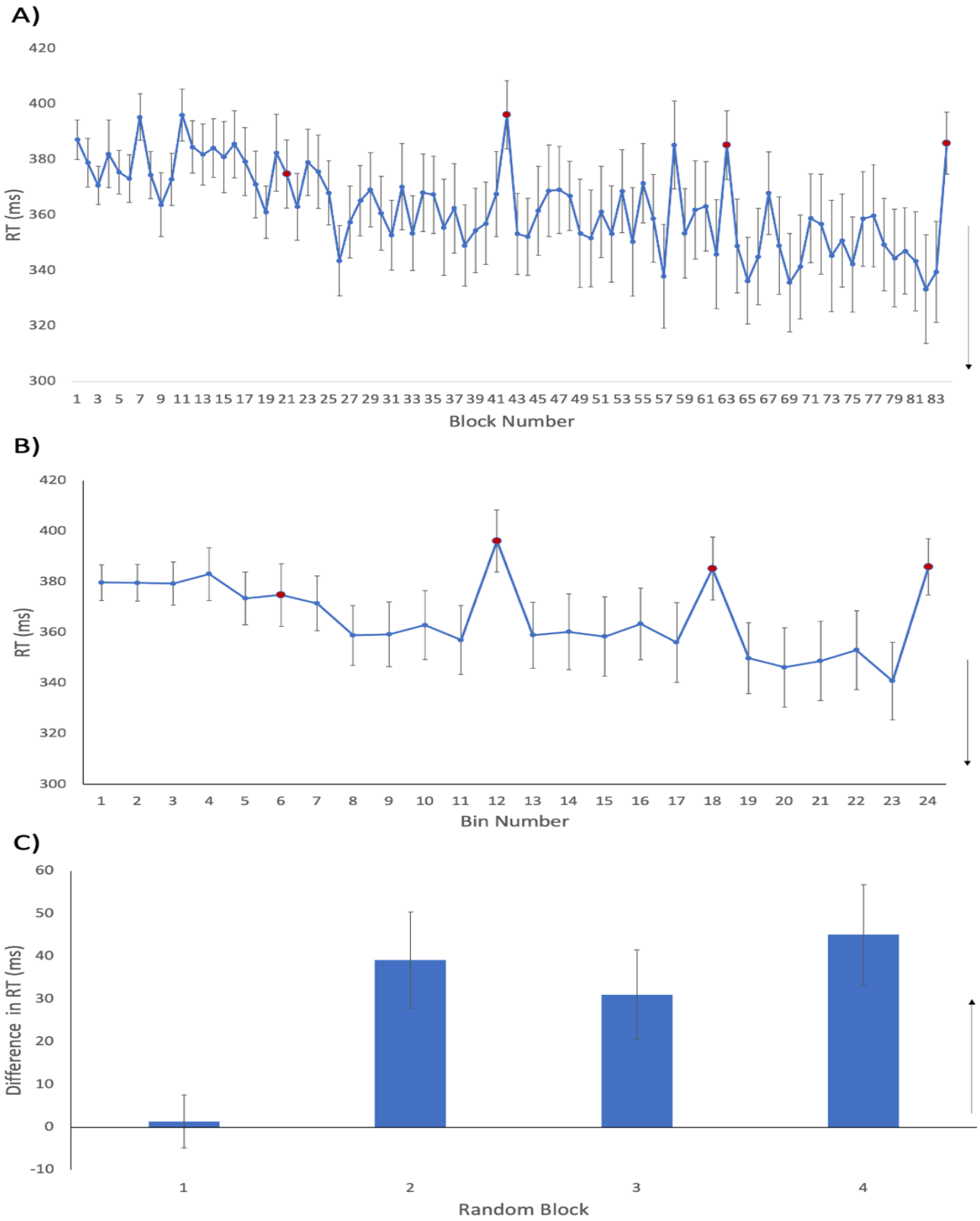


Figure 4.9 Study 4.III Learning Measures: Learning curves as measured by RT and difference in RT between experimental and random blocks SSL_{RT}

Arrows denote the direction of task improvement, error bars represent the SEM.

A) The learning curve of the study III generalization task as measured by RT. Experimental sequence blocks are shown in blue and random sequence blocks in red.

B) The learning curve of the study III generalization task as measured by RT in 4-block bins (except for the random sequence blocks, which were not binned). Experimental sequence blocks are shown in blue and random sequence blocks in red.

C) SSL_{RT} measured in quarters across the task.

4.6. Discussion

In this chapter we aimed to establish a task to test cross-modal generalization in an at-home setting with the aim of probing sleep's role in generalization. In the first iteration of the task (study 4.I), we found that while participants got faster at the task, they showed no differences in learning based on prior exposure to the sequence, suggesting a lack of evidence of generalization of sequence structure from the motor to non-motor domain. We also found that sleep did not appear to play a significant role in whether participants were able to generalize or not. Given these findings, in study II we created a new task and tested it using a single sequence, but without a previous SRTT session or sleep/wake manipulation. We found that participants improved their response time over the blocks and showed sequence-specific learning when compared to a random sequence. We also found that participants' performance began to plateau before the end of the session which suggests that longer sessions may not be necessary for participants to learn the task. Finally, we tested the new task using two sequences. We found that participants were able to maintain the sequence-specific learning in relation to the random sequence, as seen in study II, despite the added sequence. This suggests that the task is appropriate for future studies and could be used in combination with a prior motor task to probe generalization and sleep's relation to it.

The ability to generalize knowledge to appropriate future situations is an important part of memory and learning. Correspondingly, there seems to be overlap between the processes involved in the consolidation and generalization of knowledge – including sleep. However, there are still many open questions regarding how sleep and its mechanisms relate to generalization. While we began the chapter with the aim of addressing some of these questions in an at-home setting, the lack of evidence of generalization from the motor to non-motor domain in the task we designed for study I led us to focus instead on developing a different non-motor task. Therefore, while the studies in this chapter do

not themselves contribute to the previously discussed gap in our knowledge of generalization they will, hopefully, facilitate the eventual implementation of studies that can address these types of questions.

Our findings suggest that the task developed in study III can be learnt in a single session, online, in an at-home setting. Participants showed that they were able perform the task in a sequence-specific manner which suggests that participants are able to benefit from an underlying sequence structure. Furthermore, many generalization tasks used previously in a lab setting (Garvert et al., 2017) require many repetitions during task encoding to ensure familiarization with underlying sequence structure (for example, 1,596 repetitions in a single day of testing in Garvert et al., 2017). This is less feasible in a remote, at-home setting. It is therefore promising that participants were able to learn the task used here in a sequence-specific manner with less repetitions (249 repetitions in under an hour of testing). Therefore, the behavioural experiments presented in this chapter are an important first step in adapting the kinds of experiments previously run in the lab for use in a significantly less controlled setting.

A key limitation is that beyond the above methodological advances these studies do not advance our understanding of generalization and sleep's relationship to it. Furthermore, without integrating the non-motor task with the SRTT, whether this task will probe generalization remains an open question. There are therefore many future steps that must be taken in order to address the questions posed at the start of this chapter. The most obvious next step would be to integrate the study III task with the SRTT to test whether participants can generalize sequence structure from the SRTT to this new non-motor task. If a future behavioral study were to find evidence for sequence generalization, then a next step would be to probe sleep's role in generalization in a naturalistic (at-home) setting. This could involve integrating the task with the Dreemband or some other portable EEG device to study the relationship between sleep characteristics and generalization. As mentioned in the introduction, there

is a lot that is yet unknown about the mechanisms involved in sleep-dependent generalization. Future questions in this vein could therefore aim to understand how consolidation mechanisms such as SO-spindle coupling relate to generalization or whether NREM and REM play different roles in generalization. Eventually, experimental manipulations using techniques such as at-home TMR could be used to better understand mechanisms and test whether their role in generalization is causal.

5. Thesis Discussion and Conclusions

5.1. Thesis Summary

The goal of this thesis was to address gaps in the literature surrounding sleep-dependent memory consolidation and generalization in a naturalistic setting. In order to do this, we needed to develop a behavioural task that could be learned remotely and in a timely fashion. We also needed to establish feasible approaches to measure sleep in participants' homes. We used the Dreemband to characterise sleep macro and microarchitecture. On a practical level, this thesis was also intended as a first step in the development of an at-home TMR protocol. Conducting TMR at home would give us a more ecologically valid understanding of TMR, the signals we manipulate, and allow us to reach a broader participant pool (including clinical populations) than if we ran studies in a laboratory environment. A secondary aim of the first two chapters was therefore to assess the feasibility of the behavioral and EEG frameworks necessary for this to take place.

To achieve this, we began by conducting a series of online behavioural studies with the aim of developing a task that probed declarative and motor sequence learning in an at-home environment. We wanted to develop a task that could be that can be learned by participants online, in their own homes, and with minimal researcher intervention. Additionally, it was important that the tasks be learnable in a single session so that we could compare performance on the task after a period of sleep compared to a period of wake and thus investigate the effect of sleep on task performance.

In the Chapter 2 we developed a task that could indeed be learnt in a single session, online, and in a remote setting. There were no significant circadian effects on the learning of the SRTT or paired-associates tasks. Contrary to our hypotheses, we found no significant effect of sleep compared to wake on task performance.

We next, in Chapter 3, aimed to investigate which oscillatory features of NREM sleep, as measured by low-density EEG, correlate with overnight consolidation of motor and declarative memory in an at-home setting. Our findings suggest that oscillations in NREM sleep previously linked to memory consolidation positively covary with improved SRTT performance, with SO-spindle coupling and sequence specific response times as the key variables as determined by CCA. These results are consistent with those of studies conducted in gold-standard experimental settings, sleep laboratories using PSG, thus demonstrating that it is feasible to study sleep macro- and microarchitecture using low-density EEG in an at-home setting. Our study therefore offers a promising foundation for similar studies in the future that may wish to study sleep in a naturalistic setting.

Finally, in Chapter 4, we developed a non-motor task that could be learnt in a single session and that could be used to probe generalization of underlying sequence structures from the SRTT to a different task domain. We had hypothesised that such a task would benefit from sleep given that some degree of abstraction is inherent in the process of consolidation as explained by the two-step model of sleep. While we were not ultimately able to assess this, we developed a task to probe generalization and found that participants were able to learn the task in a sequence-specific fashion.

5.2. Strengths and Limitations

A key strength of this thesis is the ecological validity that it offers. As previously mentioned, research suggests that sleep can differ in significant ways in a lab setting (Agnew Jr et al., 1966; Tamaki et al., 2016). Conducting sleep research in participants' homes therefore gives us a snapshot of sleep that might be more representative of the way they habitually sleep. In terms of our long-term goals of potentially applying sleep interventions, such as TMR, to a clinical population, such as stroke survivors, an at-home protocol allows us to better evaluate clinical potential. Delivery of sleep interventions in

patients' homes would make such interventions more accessible and would allow more participants to be able to participate in such studies as they would not have to leave home to participate.

This same strength is also one of the biggest limitations of this thesis as the remote nature of all of our studies ultimately limited the control we had over our experimental variables. This means that both our behavioural and EEG measures are affected by noise. Where in a laboratory study an experimenter would ensure the participant stays focused on the behavioural task at hand and is not multi-tasking or completing the task in a distracting environment, this was not something we could do in a remote setting. Equally, where in a sleep lab an experimenter would ensure good signal quality from the PSG before starting to record and would intervene should that quality degrade at any point, we had no such opportunity in a remote setting. While we did our best to minimize the effect of such extraneous variables by providing detailed instructions and giving feedback on signal quality after baseline nights in an attempt to ensure optimal fit of the Dreemband, noise is part of the cost of conducting research in environmentally valid conditions.

5.3. Future Directions

5.3.1. Investigation of Self-Reported Sleep Measures and their Relationship to Task Performance

In Chapter 2, we collected self-reported measures of total sleep-time, sleep onset, wake onset, sleep quality, and sleepiness (SSS) at every session. However, this data and its relationship to variance in task performance were not fully explored in this thesis. While self-reported data is not as informative as objective measures of sleep quality or architecture such as EEG measures, exploring the relationship between self-reported measures of sleep and performance on our tasks might yield some answers as to why we did not find an effect of sleep on task performance. In other words, it might be the qualities of sleep (such as self-reported duration, quality, etc), rather than the presence or absence of a sleep

session that affect consolidation. Therefore, a useful future step might be to analyse this existing data further.

5.3.2. Investigation of the Relationship Between Actigraphy and EEG Sleep Measures:

Although this dataset was not analysed in this thesis, we collected actigraphy measures on all participants in chapter III. Future work could compare actigraphy measures of sleep and wake with those acquired by the Dreemband. Eventually, with the sleep-staging of the EEG data as potential ground truth, future studies could train a machine learning algorithm to differentiate between sleep-stages based on actigraphy data. This would allow us to explore the feasibility of actigraphy as a means of measuring sleep stages. Although somewhat tangential, this is relevant to the thesis objective of studying sleep in an at-home setting. As is the case with the Dreemband, actigraphy as a means of sleep staging could be a useful tool to study sleep in naturalistic settings and in a way that is more accessible to clinical populations.

5.3.3. Development and Application of a More Comprehensive Pre-processing Pipeline

In the interest of time, we carried out minimal pre-processing on the EEG data collected in chapter 3. We visually inspected our EEG datasets, manually removed noisy channels, and excluded participants with noisy data, leaving only those datasets we were confident would not require significant artefact rejection. As a result, we lost 19 of the 41 participants who completed the study. A key future goal would therefore be to run artifact rejection on all data. This would not only give us more confidence in our results but would allow us to make better use of our dataset.

5.3.4. Manipulation of Sleep Microarchitecture Through TMR

An important limitation of our findings in chapter 3 is that they are correlative: we tested for correlations between features of sleep and overnight changes in motor and declarative learning. Without experimental manipulations, we cannot attempt to draw causal conclusions about the relationships between our behavioural and biological measures. A key next step would therefore be to manipulate SOs and spindles to better probe their relationship to consolidation outside of the lab.

One possible way to achieve this is through targeted memory reactivation (TMR). TMR is a technique in which a sensory stimulus associated with a learned task is delivered to a participant during sleep (usually nREM) in order to bias reactivation in favor of the learned task (Cellini & Capuozzo, 2018; Lewis & Bendor, 2019; Rudoy et al., 2009)). As discussed in Chapter I, several studies have thus far employed this technique in a lab environment and have gained insights into TMR's relationship to reactivation (Belal et al., 2018; Bendor & Wilson, 2012; Cairney et al., 2018), how it benefits consolidation (Antony et al., 2012; Cousins et al., 2014; Shimizu et al., 2018), and where, perhaps, the limits of its effect on consolidation lie (Hutchison et al., 2021; Pereira et al., 2023). TMR has been successfully used to increase spindle density and spindle-SO coupling (Ngo & Staresina, 2022; Rakowska et al., 2021) and this upregulation has been linked with improved SRTT performance (Rakowska et al., 2021). TMR protocols have the benefit of being safe, non-invasive, and feasible to carry out in an at-home setting with minimal equipment. A manipulation of this type would therefore be a fitting means of accomplishing this next step at home.

It is our eventual goal to conduct TMR using a closed-loop stimulation device to allow for precise targeting of sleep stages. This would utilize simultaneous EEG recordings similar to the Dreemband (described in detail in chapter II) and a TMR auditory stimulus delivery system (potentially through bone conduction as previously done by Dreem). This would allow us to both manipulate and record

brain activity in a naturalistic sleep setting which, to our knowledge, has not been done previously. Not only would this be very useful as a tool for basic science, allowing researching to apply TMR in a variety of conditions, it could also potentially be used to improve memory consolidation in both healthy and clinical populations – particularly those who might otherwise have difficulty participating in a study or therapy session outside of their home. The analysis of the low-density EEG data we collected is also a first step in familiarizing ourselves with the kind of data the closed-loop would rely on to determine the sleep-stage.

5.3.5. Electrodes Corresponding to Our Areas of Interest

The Dreembands used in Chapter III have electrodes positioned over F7 and F8 and O1 and O2 and we did not attempt any source localisation of signals recorded. Given our interest in motor learning and memory, a reasonable next step in our research might involve expanding the low-density EEG set-up to allow us to conduct some source estimation of our signals of interest. Previous research indeed suggests that oscillations associated with reactivation and consolidation may be more pronounced in the brain regions involved in their encoding (L. A. Johnson et al., 2012; Ngo et al., 2013; Ngo & Staresina, 2022; Nir et al., 2011). Increasing the number of electrodes and adjusting their placement such that it corresponds to our areas of interest such that more precise analysis could be conducted would give us added confidence that our signal of interest is indeed related to behavioural task, particularly if coupled with TMR or some other manipulation of the signal.

5.3.6. Use of EEG Recordings During Task Encoding and Performance

While this dataset was not analyzed in this thesis, we recorded EEG using the Dreemband while the participants encoded (S1) and were subsequently re-tested on (S2) the tasks. This data could be used in future to understand how waking experience influences subsequent sleep. For instance, it has been

suggested that theta power, which is associated with increased focus or attention, acts as a tagging mechanism for subsequent replay. Examining the relationship between theta power during S1, or even subsections of S1 such as the SRTT, and subsequent oscillatory markers of consolidation would therefore further our understanding of the relationship between wake and sleep-dependent consolidation in an at-home setting.

More ambitiously, task related signals could perhaps be used to train an algorithm to detect these in subsequent sleep as an indirect measure of memory reactivation (Belal et al., 2018; Cairney et al., 2018).

Both endeavours would require significant pre-processing as the wake EEG data is likely extremely noisy. Nevertheless, given that the data has been collected, I believe it would be valuable to at least determine whether this dataset can be used to conduct further analyses such as those described above.

5.3.7. Integrate Latest Generalization Task with an SRTT

Unfortunately, while in chapter 4 we eventually developed a non-motor task that might be a feasible candidate to probe generalization, we did not have the time to integrate it with SRTT. A key next step in this study would therefore be to have participants learn the SRTT and investigate whether they can generalize underlying sequence structures to this new non-motor task.

5.3.8. Interrogate the Relationship Between Generalization and Sleep

If the results of the above study are positive, the next step would then be to test whether the ability to generalize was sleep-dependent. This would require an experimental structure similar to that used

in Chapter II, where we compared between-group performance on the task of interest after a period of sleep and an equivalent period of wake.

5.3.9. Sleep Correlates of Generalization

Finally, if cross-modal generalization was indeed sleep-dependent, the next step would be to determine what features of sleep are associated with the task. This could be done with an experimental design similar to that in chapter III, in which we explore the correlation between sleep stages and sleep oscillations and task improvement.

5.4. Conclusions

Our declarative and motor learning tasks can be learnt online, in a single session. Contrary to our predictions and much of the literature, however, we found no evidence of sleep-dependent consolidation when change in task performance following sleep was compared to that following an equivalent period of wakefulness. Using a low-density, portable EEG device we were able to sleep-stage and identify oscillatory markers of sleep-dependent consolidation. The relationship between SO-spindle coupling and task improvement mirrors that which has been found in laboratory settings with more sensitive equipment. Without manipulation of these oscillations, however, we cannot draw conclusions about the causality of these relationships. Future studies should therefore endeavour to manipulate these oscillations, perhaps by using TMR. We also developed a task that appears promising in its ability to be used as a probe for cross-modal generalization. Future studies should integrate this task with the SRTT to probe whether generalization of underlying sequence-structures can be achieved cross-modally and whether this generalization is sleep-dependent.

6. References

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7. Appendix

7.1. Appendix I: Word Pairs Used in the Paired-Associates Task

- SCHOOL, CRANE
- TOWER, FLOWERBED
- SEA, BULLET
- FAMILY, METAL
- NEWSPAPER, OPERATION
- BANNER, FLOOD
- SONATA, INCREMENT
- TENDENCY, CAMP
- MOTHER, STEAM
- INSECT, TRUTH
- RIVER, HALL
- COAST, SOUL
- BLACKSMITH, TREE
- DECREE, ROOM
- HOME, SCARF
- BUILDING, CHILD
- RAIN, MARRIAGE
- AVENUE, JOY
- DECENCY, FACTORY
- DIAMOND, SHIP