The effects of closed loop auditory stimulation on brain and behaviour in the short and long-term

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Thesis Summary

One of the key functions of deep sleep has emerged as the processing and storage of memory. A recent technique has been developed to use precisely timed sound, delivered during deep sleep to boost its slow oscillations. The technique is known as closed loop auditory stimulation (CLAS) and has, in some limited circumstances, been shown to improve memory. This thesis aimed to expand our understanding of the effect of CLAS on memory in human participants. In particular, whether the benefit of stimulation can be generalised to other memory types and tasks, never before tested using CLAS: In Chapter 2 two previously untested with CLAS behavioural tasks were utilised, a motor sequence learning task and a pattern separation task, while in Chapter 3 three declarative tasks were assessed, all following night(s) with CLAS delivered using EEG monitoring. The thesis also sought to be the first to expand understanding of the effects of repeated nights of CLAS on sleep and behaviour: In Chapter 3 and Chapter 4 I used a device to deliver CLAS at home for seven and eight nights, and assessed the impact on memory recall. Finally, I aimed to understand for the first time if CLAS affected the activity of brain areas involved in the tasks during stimuli recall, using functional MRI scans. Results showed comparable electrophysiological brain responses from one night and one week of stimulation. Stimulation also led to changes in brain activity during memory task recall. One night of CLAS led to a decline in pattern separation performance. However, neither one nor repeated nights of stimulation led to changes in measured behavioural performance on all other tasks. The thesis therefore indicates that CLAS can affect the brain during sleep in a way that interacts with memory tasks, but does not always produce measurable performance change.

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Chapter 1
General Introduction

1.1 Overview of thesis

At the time of writing this thesis I will have spent nearly 9 years of my life asleep, around 1/3 of my total lifespan. This is a huge amount of time which serves no obvious evolutionary advantage, while I am asleep I can't eat or drink and I can't carry out other behaviours vital for life. Indeed, as my conscious brain is asleep I am in a vulnerable state. These drawbacks of sleep suggest that it must serve some vital importance, which has led it to be evolutionary conserved in arguably all studied animals (Cirelli and Tononi, 2008). Scientists have spent years studying to understand the importance of sleep and while it appears to have many benefits for the brain, one emerging focus has been on sleep's role in the storage, protection and restructuring of memories (for a review see Rasch and Born, 2013). The formation of memories is a vital part of what makes us human, it allows us to retain huge quantities of information and retrieve it across our entire lifespan. Complex languages of communication and intricate social structures would be impossible without it. The broad aim of this thesis is to contribute to our knowledge of how deep sleep affects memory, via three experiments where deep sleep oscillations are boosted using closed loop auditory stimulation (CLAS) and the effects upon memory tested.

In this introduction I will first introduce sleep physiology in the context of sleep research, before exploring the role of sleep in memory. I will then introduce the idea of stimulating deep sleep to investigate and influence memory, focusing on a non-invasive auditory stimulation technique, CLAS. CLAS is a relatively new technique, but there is already a set of studies that have made use of it that I will review. I will then describe some of the theoretical models of sleep's influence on memory, and explore how these can be tested in human participants. This review will form the basis for the questions that will be addressed in this thesis.

In the first experimental chapter I explore the impact of CLAS on two memory tasks shown to be influenced by NREM sleep, never before tested with CLAS. I will also explore the response from the brain when the stimulation sound is adjusted in its timing. In the second experimental chapter I will utilise, for the first time in a research context, a device to deliver CLAS at home, and use it to deliver CLAS over seven nights. I will explore the impact of this novel 'long-term' stimulation on three new declarative memory tasks. Then in chapter 4 I will assess the impact of long-term stimulation delivered at home on declarative and procedural memory, and use state of the art functional magnetic resonance imaging to explore effects of

stimulation on brain function. Finally, I will discuss the contributions of the results from this thesis and how these form a picture within the current literature, to help further our understanding of the role of deep sleep in memory processing.

1.2 Sleep physiology

No matter how unique we are as humans, each night most people follow a relatively similar routine: lying down we close our eyes and relinquish our consciousness to sleep. Once we close our eyes we fall into a common pattern, falling deeper asleep through the four stages of sleep (see an illustration of the stages in Figure 1). From wake the brain quickly progresses through the first (N1) and second (N2) non-rapid eye movement (NREM) stages, before a longer stop in the third NREM stage (N3). From N3 the brain moves back through a period of N2 to the rapid eye movement (REM) stage. Throughout the night the brain will repeat this cycle three or four times as depicted in Figure 1, but the length of time spent in each stage will change: At the start of the night a larger proportion of a cycle is spent in N3 sleep than REM, but this gradually switches over such that in cycles at the end of the night more time is spent in REM. While each stage is distinct, it is thought that the repeating order of the stages of sleep has an important role in the functions of sleep, particularly in creativity (Lewis, Knoblich and Poe, 2018).

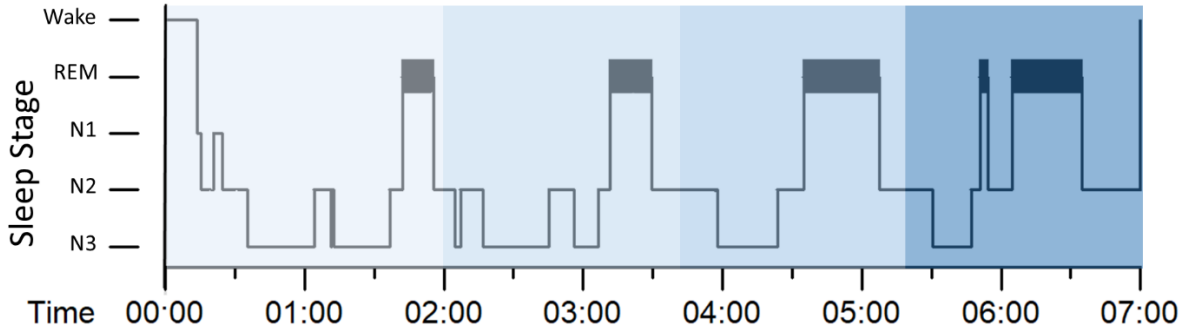


Figure 1 *An illustrative hypnogram showing the typical stages of sleep comprising one night. Shaded boxes indicate different sleep cycles.*

Each stage of sleep has its own set of characteristic oscillations in brain activity, changes in muscle tone and eye movements, which are used internationally to define and identify the stage, but that also indicate function. To assess these oscillations and study sleep, experimenters use a system of electroencephalography (EEG), electromyography (EMG) and electrooculography (EOG) combined to form

polysomnography (PGS). Studying the PSG can give an insight into the functioning of the brain whilst asleep.

N1 is passed through into and out of wake or surrounding brief arousals from sleep, and accounts for typically only ~5% of the night (Ohayon *et al.,* 2004). N1 generally represents a decrease in activity amplitude and an increase in synchrony of signals from across the brain. N2 however is responsible for at least 50% of a night's sleep (Ohayon *et al.,* 2004) and is where oscillatory activity only seen during sleep really emerges. One such oscillation is known as a K-complex; a sharp negative, then positive deflection in voltage; much larger than the surrounding ongoing activity and lasting at least half a second (Cash *et al.,* 2009; Berry *et al.,* 2018). K-complexes are often associated with near-arousal events: that is, something such as an external noise which could cause the sleeper to wake but is insufficient to actually disrupt sleep. It is thought that these K-complexes are linked to the arousal system in the locus coeruleus, and mark top-down control from the brain to maintain sleep (Cash *et al.,* 2009). Another key oscillation associated with N2 sleep is the sleep spindle: this is a series of fast oscillations at 11-16Hz, lasting more than half a second, which wax and wane in amplitude to form a spindle shape, see an example in Figure 2 (Berry *et al.,* 2018). Spindles are often sub-divided into fast and slow based on their frequency: In this thesis fast spindles are those within 11-15Hz and slow within 9-12Hz (Navarrete *et al.,* 2019). However, this division is not always made using the same frequencies and a functional difference for fast and slow spindles is debated (Cox *et al.,* 2017). Slow spindles are larger over frontal sites, like K-complexes; while fast spindles are largest in central and parietal regions (Anderer *et al.,* 2001; Mölle *et al.,* 2011). N1, N2 and N3 are often grouped together to form NREM sleep.

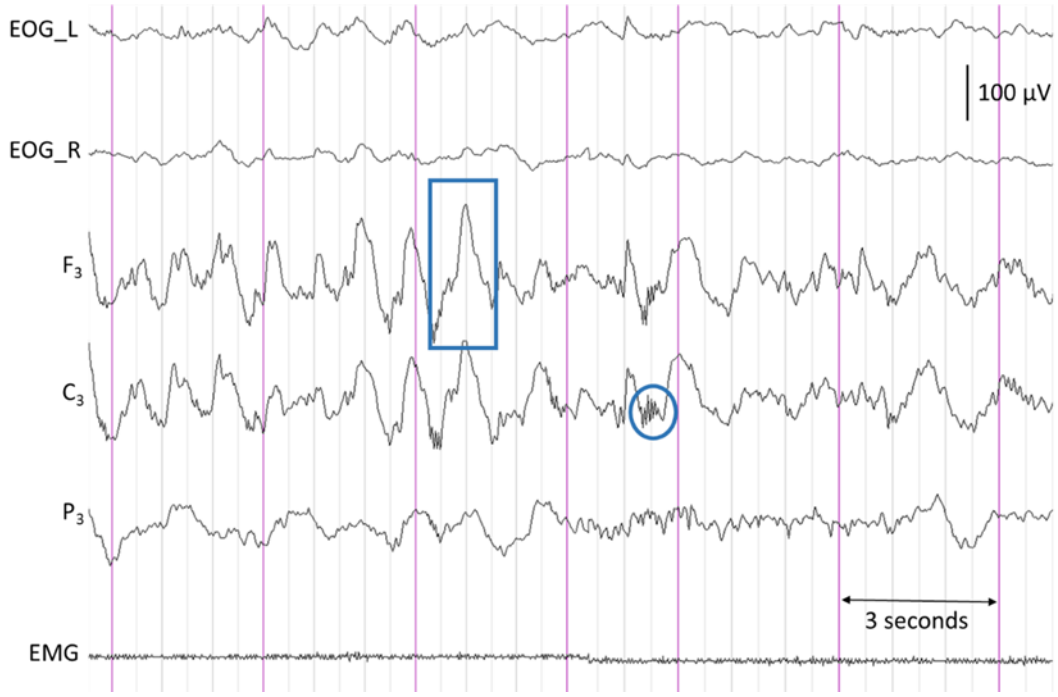


Figure 2: EEG during N3 slow wave sleep with characteristic oscillations. Square = slow oscillation, circle = spindle.

N3 sleep is also known as slow wave sleep (SWS) as it is characterised by near constant, large (>75uV), slow (~1Hz) oscillations (SO); largest over the frontal regions, see Figure 2. Similar to K-complexes SO stand out from the rest of the EEG activity, as the largest amplitude oscillations. SO can co-occur with spindles in N3 sleep, with fast spindles generally nested in the rising phase of the SO and slow spindles in the falling phase (Mölle *et al.,* 2011), see phase locations in Figure 6. It is thought that this pairing of oscillations plays a key role in memory consolidation, an idea that is discussed in sections 1.3.1 and 1.5.2. On average an adult spends around 20% of the night in N3 (Ohayon *et al.,* 2004). SWS has been functionally linked to many sleep processes, including the consolidation of memories from short to long term storage (for a review see Born, Rasch, & Gais, 2006, and further discussion in sections 1.3.1 and 1.5.2.), clearance of proteins which build up during the day (Xie *et al.,* 2013; Fultz *et al.,* 2019) and the regulation of hormones such as cortisol (for a review see Besedovsky *et al.,* 2012). SWS is under homeostatic control (Dijk, 2009); it is closely linked to the build-up of sleep pressure throughout the day urging us to go to sleep: Indeed the longer spent awake the higher the SWS power when we do sleep (Dijk, Beersma and Daan, 1987). SWS holds a prominent position at the start of the sleep cycle perhaps denoting its importance: Indeed because of this, shorter sleep opportunities tend to lead to less REM sleep, rather

than less SWS (Dijk, Beersma and Daan, 1987). Boosting SWS is often the aim of CLAS (described in section 1.5).

REM sleep is the final sleep stage, characterised by rapid and large movements of the eyes, visible in EEG. REM sleep is made famous by its association with dreaming, the often visual experiences many recall upon waking (Dement and Kleitman, 1957). REM sleep has also been associated to memory, particularly in associating shared information from different memories, known as the abstraction of gist (Durrant *et al.,* 2011; Lewis and Durrant, 2011; Friedrich *et al.,* 2015; Lutz *et al.,* 2017), and in the processing emotional memories (Hutchison and Rathore, 2015).

This thesis will employ several techniques (PSG and EEG) to study the brain activity during sleep with and without SWS stimulation using CLAS. Particular attention will be paid to the effects of stimulation on SO and spindles during N3, but the impact of stimulation across the macrostructure of the sleep cycle will also be assessed.

1.3 Memory

The idea that memories are formed and held in the brain as patterns of activity which pass between specific neurones forming local circuits has a venerable history (Ramon Cajal, 1894; Hebb, 1976, 2005). As individual neurones can form part of several circuits, and encode further information in the pattern in which they deliver action potentials, this means there is an almost infinite number of unique arrangements which can be used to encode memories across the lifetime of the human brain without needing to continue increasing the overall volume of the brain itself. In the interests of simplicity, memory is commonly split into 3 stages (1) Encoding; the receiving of new information, (2) Consolidation; storing that information, and (3) Recall; accessing the information when relevant.

Memories are also often divided into two categories (1) Declarative and (2) non-declarative. Declarative memories can be further divided into episodic and semantic memory: Episodic memories are with a linked to a time and place (i.e. In the science museum last year I met astronaut Tim Peak), while semantic is often describes as ‘knowing’, such that the memory lacks the time/place of learning (i.e. I know that the Earth rotates around the sun). While non-declarative encompasses implicit memory, such as procedural memory, conditioning and skill learning. Declarative and non-declarative memories differ in the brain regions they rely upon: For example, the consolidation of declarative memories relies heavily on the hippocampus and the medial temporal lobe (mTL), while procedural memories do not require input from

the mTL, they are instead more reliant on the striatum and motor cortex (Brown and Robertson, 2007).

A particular conundrum in the memory field had been how the brain is able to quickly acquire new information and recall it, undisturbed years later. This prompted the formation of the two stage model of memory: Stage (1) the encoding of memories into a *short term store,* then stage (2) the transfer/replication of these into a *long term store* as a consequence of consolidation (Walker, 2005). According to this model, following initial encoding memories are quickly written into the short term store which involves the hippocampus (Diekelmann and Born, 2010), where they are in a labile state which makes them sensitive to loss or interference (Wixted, 2004; Rasch and Born, 2013). Over time, through a process or processes of consolidation memories are integrated to a longer term store involving the cortex (Diekelmann and Born, 2010). Here, memories are more stable and can be incorporated into existing schemas of knowledge (Klinzing, Niethard and Born, 2019).

Initial evidence for these two stages came from studying patients with lesions in either their hippocampus or cortical regions: lesions in the hippocampus impaired new memory learning but did not affect their ability to recall much older memories (Scoville and Milner, 1957). People with retrograde amnesia caused by lesions in the hippocampus can often recall memories for things learnt a long time prior to the onset of their amnesia, but not things learnt more recently (McClelland, McNaughton and O'Reilly, 1995), indicating a decreased reliance on the hippocampus for these older memories. This interpretation is reinforced by lesion studies in rats: Winocur *et al.,* (2001) found that rats with hippocampal lesions made 1 or 2 days following learning (of a food preference), led to impairment in memory performance. However, lesions made 5 or 10 days post-learning, had no effect on food preference. They hypothesised that this was an indicator of the reduced role of the hippocampus in memory storage over time. While further studies in humans have provided supporting evidence through studies showing decreased BOLD activity in the hippocampus during memory recall over time, alongside increased BOLD activity in neocortical regions linked to the task (Payne and Kensinger, 2011; Durrant, Cairney and Lewis, 2013).

1.3.1 Slow wave sleep and memory

Sleep was initially believed to benefit memory by simply providing a period of time when no new information was encoded which may interfere with previously learned

information (Wixted, 2004; Rasch and Born, 2013). However, it soon emerged that the difference in performance seen following a period of sleep could be greater than that which would be expected by simply not forgetting any information, and instead was more in line with continued rehearsal (for a review see Rasch and Born, 2013). Sleep has been shown to improve performance on a variety of memory tasks, more so than an equivalent period of wake (see for a review Born, Rasch and Gais, 2006). Selective disruption of SWS has led to greater memory impairments than REM sleep deprivation: Studies selectively restricting SWS or REM by allowing only 4h sleep early in the night (rich in SWS) or late in the night (rich in REM) have shown memory impairment is greater with SWS loss (Yaroush, Sullivan and Ekstrand, 1971; Barrett and Ekstrand, 1972). Indeed a short period of SWS rich sleep can be equivalent to a normal night in terms of its effect on memory recall (Tucker and Fishbein, 2009; Cedernaes *et al.,* 2016). Studies aiming to disrupt SWS by waking participants when they enter N3 have yielded more mixed results (Genzel *et al.,* 2009; cf. Casey *et al.,* 2016), but this technique has also been criticised for the stress repeated awakening causes participants which could also affect memory recall (Born and Gais, 2000). More recently Fattinger *et al.,* (2017) used auditory stimulation during the SO down-phase (see Figure 6 for phase) to selectively disrupt oscillations without awakening participants. They found this impaired learning of a new motor tapping sequence and increased reaction time variability, and there were indications that the disrupted SWS led to a decrease in plasticity in motor areas. This all suggests that SWS plays a vital role in memory processing.

1.4 Stimulation of Slow Wave Sleep

In light of the important roles of SWS it has become a popular target for enhancement to boost these processes (for a review see Bellesi *et al.,* 2014). Different methods to specifically enhance the theorised memory role of SWS have been evaluated: including aiming to directly increase the oscillations of SWS via direct magnetic or electrical stimulation (Marshall *et al.,* 2006; Massimini *et al.,* 2007). It may be assumed that the most obvious of SWS enhancement would be simply sleeping longer. However, due to the highly controlled homeostatic nature of sleep, simply sleeping longer will not reliably result in longer in SWS, even in a healthy individual.

Transcranial magnetic stimulation at <1Hz, during NREM sleep has been shown to induce SO comparable to naturally occurring SO, in rats (Vyazovskiy *et al.,* 2009) and in humans (Massimini *et al.,* 2007). While transcranial application of oscillating

potentials at 0.75Hz has been shown to not only induce SO but significantly improve the recall of word pairs (Marshall *et al.,* 2006). However, these techniques do hold a number of practical limitations: the requirement of a large amplifier to be carefully positioned over a participant's head which therefore must remain fixed during sleep and, how the long term effects of such brain stimulation are unknown (for a review see Bellesi *et al.,* 2014). This restricts its use in a healthy research population. As I am primarily interested in how sleep can be manipulated to improve memory in healthy adults, this technique is not optimal.

Efforts have been made to utilise more naturalistic stimuli to affect SO, such as sound, that could be used more widely than electrical or magnetic stimulation. Sound rousing from sleep is a long-agreed phenomenon: indeed, it forms the basis of alarm clocks. However, sounds which do not lead to an awakening can still be processed by the brain (Atienza, Cantero and Escera, 2001). Mechanisms for these processes likely arose as sleep makes humans vulnerable to insult and scanning the acoustic environment whilst asleep is a good way to monitor for threat (Velluti, 1997). Loomis *et al.,* (1935) were using EEG to record sleep, when an experimenter slammed a door. The noise from the door caused a reaction from the brain of the sleeping person visible in the EEG, but it did not wake them. They continued to explore and found that sound reliably elicited a response from the sleeping brain, consisting of a train of SO. More recently, SO have been shown to be induced via rhythmic acoustic stimuli applied at the SO frequency (<1Hz): resulting in a transient increase in SO power (Tononi *et al.,* 2010; Ngo, Claussen, *et al.,* 2013). Acoustic stimulation can be delivered simply using speakers which do not need to be fixed to the participants, and are an example of a natural stimuli with no known negative effects in the short or long term (Tononi *et al.,* 2010). This made acoustic stimulation a promising avenue for SO enhancement. This lead to the development of CLAS the technique of stimulating SO this thesis focuses on.

1.5 Closed loop auditory stimulation

CLAS is a form of SWS stimulation that uses short bursts of sound to augment the oscillations in SWS and has been shown to improve declarative memory: Ngo, Martinetz *et al.,* (2013) showed how two 50ms bursts of pink noise, timed to coincide with the up-state of the SO, could improve memory for pairs of words learnt the day before. They studied the behavioural and electrophysiological responses of eleven participants to their phase specific stimulation over two nights. Stimulation was applied during NREM (N2 and N3) sleep for 210 minutes: The method consisted of

detecting a SO using a threshold of less than -80µV, waiting a fixed delay period, such that the first sound occurred during the rising phase of the SO, then a delay of 1.075ms occurred before the second sound occurred to also coincide with the up-state of the following SO. Stimulation was then paused following the second sound for 2.5seconds before detection began again. The same procedure was applied during a second night, but no sound was played (SHAM). They found that the stimulation affected the oscillatory activity of SWS: There was an increase in power in part of the SWS band (0.5Hz to 1Hz, SWA) during stimulation time; longer trains of SO (i.e. more SO occurred in a row) coupled with greater probability of three subsequent SO following the initially detected SO; and greater co-occurrence of fast spindles and SO; although no increase in spindle power. Importantly they also found that induced SO had many of the same characteristics (topography, shape, etc) as endogenous SO. However, there were differences in the later shape of the evoked SO, which could indicate a different mechanism to endogenous SO. It is difficult to determine exactly if the changes seen following CLAS are due to changes in the endogenous SO, or another response of the brain to sound such as K-complexes representing a suppression of arousal due to the sound (for a review see Halász, 2005). They also did not find any overall changes in sleep structure across the whole night, or any changes in power outside of the time stimulation was played. This could imply that stimulation was having only a very short term, reactionary, effect on oscillations. This also suggests that as some SO measures increase during stimulation that they then decrease following stimulation as no global effect is seen. This would imply that SWS is still bound by homeostatic controls despite stimulation. This could call into question the benefit of driving SO only during a period of the night.

In light of the importance of SO and spindles in the overnight consolidation of memories (for review see Walker and Stickgold, 2004), Ngo, Martinetz *et al.,* (2013) assessed the impact of CLAS influence upon memory. Specifically, they used a word pair (WP) associates task, likely due to the strong links between improvements on paired associates tasks and SWS (see section 1.6.1 for further discussion of declarative tasks). In their version of the WP task participants were taught 120 semantically related word pairs. Following learning participants underwent either CLAS stimulated sleep or non-stimulated sleep, before a test the following morning on all pairs. Ngo, Martinetz *et al.,* (2013) found a significant increase overnight in words recalled. They also went on to link their behavioural and electrophysiology

results: They found a positive correlation between the percentage of time spent in SWS and performance, although this was only significant for stimulation nights, which is odd as they did not show that stimulation changed the percentage of time in SWS. They also showed a correlation between fast spindle amplitude and performance increase. They hypothesised that due to these correlations, but an overall lack of increase in SO or spindle power, that it was the increased co-occurrence of spindles and SO, that lead to the memory benefit of stimulation. Bellesi *et al.,* (2014) theorised that the sound used in CLAS causes depolarisation of a large number of cortical neurones, which then results in a large hyperpolarisation thus increasing SWA. This increase in oscillatory power linked with increases in spindle power and co-occurrence of SO and spindles, is thought to drive an increase in memory consolidation. Thereby facilitating the transfer of memories from their short to long term store, resulting in improved recall. This experiment was the first time that auditory stimulation was used to improve declarative memory and provided a simple and non-invasive ways of manipulating SWS and effecting memory, thus it is utilised in this thesis.

Ngo, Martinetz *et al.,* (2013) also provided data from a small (n=7) control test, where they targeted stimuli during the SO down phase. They showed that there was no difference in the performance on the same memory task between SHAM and STIM nights. Interestingly in this cohort, performance in both nights was comparable to that of STIM nights from the main experiment, indicating that participants performed better overall. This could lead to questions whether the difference in the first experiment was really due to improvement in STIM or a decrease in SHAM. Both experiments used a very small number of participants, which could lead us to question the link between stimulation and improvement to WP memory. Was it just chance that the eleven participants in the first experiment performed worse on the SHAM night? This highlights the need for further examination of the influence of CLAS on declarative memory. Indeed, in Chapter 4 of this thesis I utilise a WP task very similar to this to assess the influence of CLAS on behaviour.

A CLAS definition for this thesis

For the purpose of clarity, I shall outline the definition of a closed loop auditory stimulation (CLAS) study used in this thesis. This is necessary as there are several variations of this technique which are often included or excluded from the definition. I consider a CLAS study to use a short burst of noise, targeted at a specific phase of the SO, with the aim of affecting the ongoing oscillatory activity of SWS. The mechanism of SO detection and sound placement can vary. The sound must not be previously connected to any memory or else this falls under targeted memory reactivation (TMR), nor should the sound be meaningful with the intention of creating novel memories. In this thesis I am focused on uses of CLAS to compliment and boost the ongoing stimuli, with the intention of improving memory.

Figure 3: *Thesis definition of CLAS*

Precise timing of the stimuli is important in CLAS, as stimulation appears to boost memory only when delivered in phase with the SO (Ngo, Martinetz, *et al.,* 2013; Weigenand *et al.,* 2016; Navarrete *et al.,* 2019). Navarrete-MejÍa *et al.,* (2019) found that the optimal time to deliver the stimulation was at the peak, or very beginning of the descending phase of the SO (see Figure 6 for illustration of SO phases), and that this varied with age. Most work that has found an increase in memory consolidation following CLAS, targeted the up phase or peak of the SO (Ong *et al.,* 2016; Papalambros *et al.,* 2017; Debellemaniere *et al.,* 2018). Indeed, stimulation during the down phase can disrupt SO (Cox *et al.,* 2014), and affect memory as Fattinger et al. (2017) showed that learning a new sequence was impaired. Timing of the stimulus is therefore an important factor in effectively delivering CLAS, to influence memory. Indeed, as outlined by Figure 3 this thesis will focus on CLAS studies aiming to boost SO.

Ngo *et al.,* (2015) also showed that breaks between the sounds, despite the presence of SO, led to greater influence of stimuli on spindles, possibly due to repeated stimulation falling during spindle refractory periods. They showed that slightly longer breaks were more effective at eliciting spindles than shorter breaks. In Chapter 2 of this thesis I will explore the optimal timing of the stimuli to see if the effect on the electrophysiological response can be optimised further.

1.5.1 Developing CLAS and word pair memory

The ability to manipulate memory using CLAS is also an exciting tool to better understand how memories are influenced during normal sleep. Many researchers (Cox *et al.,* 2014; Ngo *et al.,* 2015; Santostasi *et al.,* 2016; Ong *et al.,* 2016, 2018; Besedovsky *et al.,* 2017; Leminen *et al.,* 2017; Papalambros *et al.,* 2017; Debellemaniere *et al.,* 2018; Grimaldi *et al.,* 2019; Santiago *et al.,* 2019) went on to replicate and extend the findings of Ngo, Martinetz *et al.,* (2013). Leminen *et al.,* (2017) used a similar detection method to Ngo, Martinetz *et al.,* (2013) and also aimed to use CLAS to improve memory recall. They replicated the WP task used by Ngo, Martinetz *et al.,* (2013) and found very similar recall numbers, including a significantly higher recall following STIM nights. Two further studies extended the use of CLAS to improve WP memory, one into a nap (Ong *et al.,* 2016) the other into an older population (Papalambros *et al.,* 2017). Both employed a slightly different SO detection mechanism to Ngo, Martinetz *et al.,* (2013): Instead of detecting SO based on a pre-determined threshold, SO were identified through a phase fitting process developed by Santostasi *et al.,* (2016) termed the phase locked loop (PLL). In the PLL a sine wave is compared using the sum of least squares approach, to the detected EEG signal, once the detected signal enters SWS the signal will fit the shape of the sine wave much closer than during any other phase. The sine wave can then be manipulated to best fit the signal and used to predict exactly when the peaks and troughs of subsequent SO will occur. Ong *et al.,* (2016) tested WP memory for 40 semantically related word-pairs, in 16 participants, over two naps (SHAM and STIM). They again found a significant increase in memory performance following CLAS. However, unlike Ngo, Martinetz *et al.,* (2013) and Leminen *et al.,* (2017) who saw an increase in words recalled following sleep, they saw very little change in the number of words recalled. This could be due to the differences in the protocol of the word pair task or in their delivery of stimulation. Papalambros *et al.,* (2017) used the same PLL technique to deliver CLAS to 13 healthy elderly adults (mean age of 75.2 years). They calculated the percentage change over SHAM and STIM nights to assess the impact of CLAS on memory, and indicated a significant difference between performances as there was a greater improvement in STIM nights. They saw much smaller increases in the number of extra words recalled than previously discussed papers, however this is to be expected in an older cohort as memory performance and SWS declines with age (Mander *et al.,* 2013).

These studies replicating the findings of Ngo, Martinetz *et al.,* (2013) add evidence that CLAS is directly affecting memory recall on this task. This evidence highlights the potential of this technique to improve memory in normal healthy sleepers, which will be explored further in this thesis. Of note is that two of the three studies used a slightly different method to apply stimulation, yet yielded the same results, this also adds evidence that it is the sound itself that is impacting memory not some other aspect of Ngo, Martinetz *et al.,* (2013) method. The mechanism used to detect SO in this thesis will be the threshold method used by Ngo, Martinetz *et al.,* (2013) and Leminen *et al.,* (2017). As well as slightly changing the stimulation method, the WP tasks were not identical, again adding evidence of the generality of the task outside of the exact method used by Ngo, Martinetz *et al.,* (2013). WP tasks used in this thesis will lie within the range for tests already used and use 75 semantically related word pairs, to boost the chance of replicating these findings in my own experiments. But is there a plausible mechanism by which improving SWS using sound is influencing memory recall?

1.5.2 How may CLAS be influencing memory

To understand how boosting SWS could impact memory we need to explore the models of how sleep interacts with memory, particularly oscillations in SWS known to be affected by CLAS. One well-supported model of consolidation in SWS, which fits within the two stage model of memory (Walker, 2005), is the *active systems consolidation* (ASC) model (Rasch and Born, 2013). It is illustrated in Figure 4. According to ASC, systems consolidation is mediated via the interplay of sleep oscillations, particularly during SWS. As previously mentioned, there are two main oscillatory features of SWS detectable using EEG; the SO and the spindle. In addition to these, and un-detectable on EEG due to their source deep in the brain, are sharp wave ripples (ripples). SO are thought to be generated in the cortex and spread upwards and posterior across the cortex (Massimini *et al.,* 2004). Spindles are thought to be generated in thalamus and are sometimes referred to as thalamo-cortical spindles (Schönauer and Pöhlchen, 2018), while ripples are generated in the hippocampus (Mölle *et al.,* 2009). During sleep these oscillations can be seen to occur in synchrony; in the EEG we can observe the high frequency of spindles which occur in time with the up (fast spindles) or down (slow spindles) states of the SO, while deeper in the brain the ripples occur nested in the spindles (Rasch and Born, 2013, see Figure 4). It is proposed that the synchrony of these oscillations

facilitates memory transfer/translation from where it is initially encoded in the hippocampus (short term store) to the cortex (long term store), Figure 4.

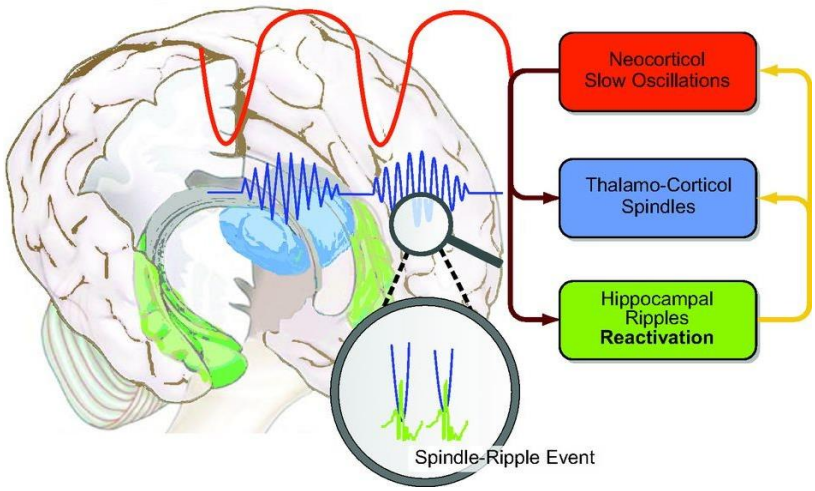


Figure 4 *Active systems consolidation. Adapted from Rasch and Born* (2013).

These oscillations are thought to create a brain state in which the circuitry involved in a memory can be reactivated. This hypothesis is supported by work in rodents which showed that the same pattern of neuronal firing could be observed in animals in post learning sleep (Lee and Wilson, 2002; Lansink *et al.,* 2009; Girardeau and Zugaro, 2011; Gulati *et al.,* 2014; Ramanathan, Gulati and Ganguly, 2015). In one study, Girardeau *et al.,* (2011) trained rats to run a long a track then recorded activity in pyramidal place cells during post running sleep, these cells fired in turn as the rodent progressed along the track. When the rodent then went to sleep they observed the same pattern of firing from these cells, albeit over a much shorter time frame, see the illustrated experiment in Figure 5.

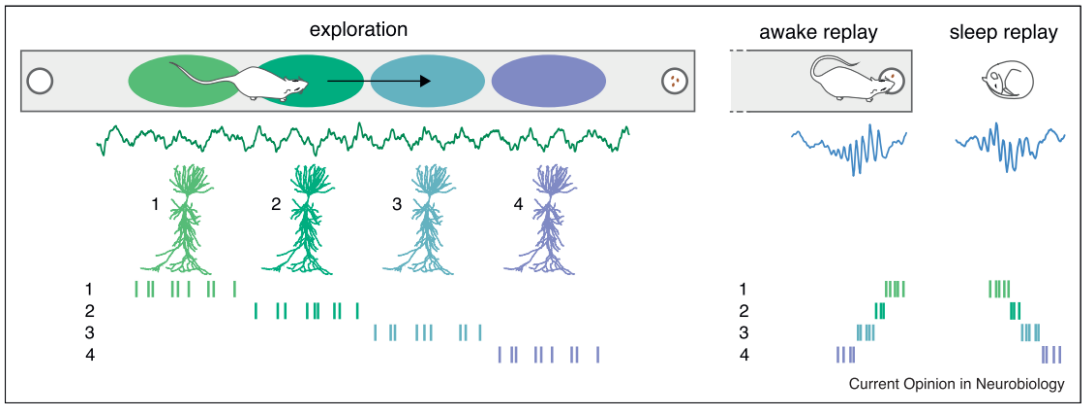


Figure 5 *Neuronal replay in the awake and asleep rat following motor activity. Adapted from Girardeau and Zugaro (2011).*

Replay in sleep has also been shown in humans using positron emission tomography (PET) imaging (Maquet *et al.,* 2000; Peigneux *et al.,* 2003) and intracranial EEG, inserted to treat epilepsy (Zhang, Fell and Axmacher, 2018; Eichenlaub *et al.,* 2020). Extensive research is now being carried out into recording such replay using non-invasive EEG, where often classifiers (computer algorithms that can detect patterns of activity in EEG) are being used to identify during sleep patterns of activity similar to those seen during learning (Schönauer *et al.,* 2017; Cairney *et al.,* 2018; Huang *et al.,* 2018; for a review see Schreiner and Staudigl, 2020).

Through boosting SWS oscillations, CLAS could be driving ASC and facilitating memory consolidation, thereby leading to greater memory recall. This will be assessed throughout this thesis via comparisons between memory recall following CLAS stimulated and non-CLAS stimulated sleep.

Another mechanism for how SWS facilitates memory management is via the regulation of synaptic strength to maintain synaptic homeostasis. This is described in the synaptic homeostasis hypothesis (SHY) detailed by Tononi and Cerelli (2003): Throughout the day as information is encoded, the pressure on synapses in the brain increases as new connections are formed requiring more and more space and energy. Huber *et al.,* (2007) showed in rats that increased learning was linked to increased expression of genes linked to plasticity such as BDNF, indicating the formation of new synaptic connections. SHY hypothesises that this leads to a decrease in signal to noise ratio of information storage in the brain, which must be addressed if the efficient storage of information is to be maintained. During SWS the synaptic strength globally downscales (Tononi and Cirelli, 2003, 2006). Huber *et al.,* (2007) also showed that increased learning during the day led to an increase in SWA during subsequent sleep. During SWS slow entry of calcium into synapses can lead to long term depression, and thus synaptic pruning via synaptic dephosphorylation regulated by NDMA receptors (Walker *et al.,* 2005). However, the pruning of synapses is not universal: some synapses are protected and even strengthened during this time (De Vivo *et al.,* 2017). SHY posits that the up and down scaling of synapses depends on the utility of the synapses for memory, such that synapses vital for the information encoded are up scaled while non-specific and peripheral synapses are downscaled (Tononi and Cirelli, 2014; Seibt and Frank, 2019). This therefore leads to the strengthening of memories during SWS, while

lowering the global synaptic strength ready for more encoding the following day. Thus synaptic homeostasis is maintained.

A further model has been proposed that offers a possible mechanism for the selection of synapses to up and down scale (Seibt and Frank, 2019). Seibt and Frank, (2019) proposed that memories are tagged for remembering, or forgetting, during wake shortly following learning, building upon the synaptic tagging and capture hypothesis by Redondo and Morris (2010). Then during subsequent SWS sleep these tagged synapses acquire plasticity related products (PRP), it is thought that PRP capture is facilitated by spindles during SWS. Spindles which have been shown to be boosted via SWS stimulation (e.g. Ngo, Martinetz, *et al.,* 2013). Then, during primarily REM sleep, these PRP allow protein translation that aids synaptic downscaling and upscaling (Seibt and Frank, 2019).

Therefore, there is the potential that boosting SWS via CLAS could facilitate these process and therefore lead to higher signal to noise ratio, better cognitive processing and better post-sleep recall. Memory recall for stimuli encoded prior to sleep, following one and repeated nights of CLAS, will be tested across the chapters of this thesis. However, SHY could also imply that as synaptic strengths are held in homeostatic balance by SWS, that driving SWS using CLAS may not be sufficient to lead to a change in memory behaviour as the natural homeostatic mechanisms will act to balance the synaptic strengths in the optimum manner. Particularly as CLAS has not been shown to lead to longer in SWS or an increase in SO power across the whole night (e.g. Ngo, Martinetz, *et al.,* 2013). However, as it stands the mechanisms through which CLAS could influence memory are unclear. While this thesis does not aim to elucidate these mechanisms it is essential any benefit or detriment to memory found via CLAS be placed within a plausible context via which stimulation could be directly leading to such effects of memory.

1.5.3 Failures to replicate CLAS memory benefit

Despite evidence showing the benefits of CLAS in improving WP memory and recent evidence supporting the hypothesis that boosting the action of SWS and its oscillations could boost overnight memory consolidation, not all studies involving CLAS have replicated the early results. Indeed, a more recent attempt to replicate the findings of Ngo, Martinetz *et al.,* (2013) failed to find the same memory benefits (Henin *et al.,* 2019). Despite a lack of behavioural results, Henin *et al.,* (2019) did show an increase in spindle and SO power during stimulation time and an increase

in fast and slow spindle amplitude time locked to SO, although fast spindle amplitude was not correlated with memory. This is at odds with Ngo, Martinetz *et al.,* (2013) theory that these manipulations in electrophysiology underlie the behavioural changes they saw, and perhaps bring doubt to whether CLAS directly improves memory. The SO amplitudes found by Henin *et al.,* (2019) were lower than those seen in previous studies and they hypothesise this may be due to participants slightly slower SO peak frequency leading the sound to be less well placed. They also theorised that the translation of Ngo, Martinetz *et al.,* (2013) original stimuli may have affected the semantics of the memory task, although in both experiments participants completed the test in their native language and the task has been used to successfully improve WP recall in other languages (Ong *et al.,* 2016; Leminen *et al.,* 2017).

Indeed, Schneider *et al.,* (2020) applied the same protocol as Ngo, Martinetz *et al.,* (2013) to a cohort of middle aged subjects (mean age 55.7 years ±1.0) and found that their memory for WP became significantly worse on STIM nights compared to SHAM. Their WP task was also the same as Ngo, Martinetz *et al.,* (2013) except they had reduced the word list to 80 to reduce the cognitive burden on the older cohort. They theorise that the lack of improvement was due to the differing characteristics of SO and spindles in younger and older people, such that the older cohort was less susceptible to electrophysiology changes by the stimulation. However, Papalambros *et al.,* (2017) showed CLAS led to a memory improvement in an even older cohort. An investigation by Navarrete *et al.,* (2019), which included Schneider *et al.,* (2020) data set, concluded that older people have a smaller window for optimal stimulation, thus making stimulation in these people more difficult as it is easier to apply the sound at the wrong time and not improve SO or indeed negatively impact SO. Therefore, stimulation may have fallen at a less favourable phase in the SO in Schneider *et al.,* (2020), than in Papalambros *et al.,* (2017) leading to the difference in results. This again highlights the importance of sound timing, which will be addressed in Chapter 2.

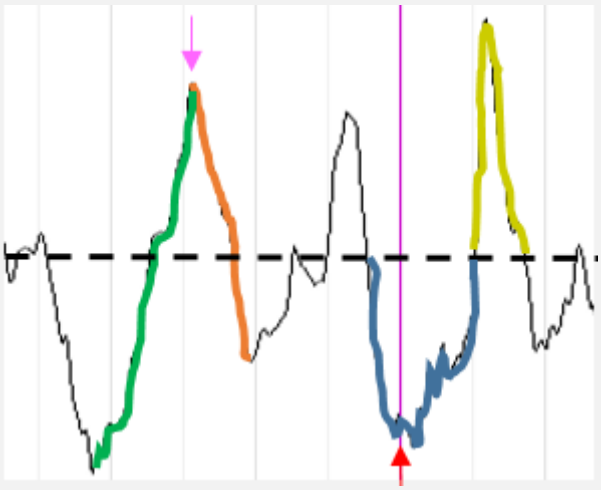
CLAS has been applied to a few other memory tasks aside from the WP task, including encoding of pictures and their recall (Leminen *et al.,* 2017; Diep *et al.,* 2019; Schneider *et al.,* 2020), finger tapping tasks (Leminen *et al.,* 2017; Schneider *et al.,* 2020) visuospatial navigation (Henin *et al.,* 2019) and executive function tasks such as the n-back task (Diep *et al.,* 2019). None of the investigated tasks showed a benefit from the application of CLAS.

Diep *et al.,* (2019) suggested that CLAS did not impact all subjects equally and split their population into *responders* and *non-responders* using the percent change in slow wave energy (SWE). They defined slow wave energy as: $\mathrm{SWA} * \#\mathrm{minutes\ N2} + \mathrm{N3\ for\ each\ sleep\ cycle}$ (Diep *et al.,* 2019, p4) and showed that 65% of their subjects saw a positive increase (>3%) in SWE with stimulation, but 30% did not (< -3% increase). When they excluded these *non-responders* they showed a correlation between the percent change in SWE from SHAM to stimulation nights and change in performance on an n-back task and a verbal fluency task. However, it is unclear if they had a prior hypothesis about which individual participants would be split using a pre-defined value of SWE, or if they performed these analyses post-hoc. As such caution must be applied when comparing their subset population to balanced and pre-defined whole subject analysis, particularly as the measure participants was split upon was the same measure linked to performance change.

Therefore, as can be seen from the literature reviewed there are many unanswered questions in how CLAS influences memory performance: Does CLAS reliably lead to an improvement in WP memory? Can CLAS improve memory on tasks other than the WP, that have been shown to rely on SWS mechanisms? These are some of the questions that will be addressed in the upcoming chapters of this thesis. Specifically, in Chapter 2 and Chapter 4 I explore the effect of CLAS on a motor sequence task and a pattern task, then in Chapter 3 I assess the impact of CLAS on three declarative tasks and in Chapter 4 I assess the impact of CLAS on the WP task.

Where is the 'up-state'?

When reading papers centred on CLAS one can start to feel a little like 'Alice in Wonderland' forgetting which way is up. With so many references to Up Phases, Down Phases, rising phase, falling phases, up-to-down transitions down-to-up and everything in between. Therefore, I have detailed the commonly used phrases and mapped their location onto the SO:



Falling Phase/Down state: Anything from the peak to the trough. Rising Phase/Up State: Anything from the trough to the peak. Positive phase: Anything above the zero crossing. Negative Phase: Anything below the zero crossing. Positive Peak/Up-to-down transition = Highest positive point on the wave. Negative Peak/Down-to-up transition = Lowest point/trough. It would help reader understanding if a convention was decided upon when referring to the different locations on the ongoing slow oscillation signal. Perhaps Peak and Trough are adequate along with Rising (time after the trough and before the peak) and Falling (time after the peak and before the trough) phase. In this thesis I aim to use only peak, trough, rising and falling phase for clarity.

Figure 6 *Where is the SO up-state.*

1.6 Testing the role of sleep in memory

To test sleep's effect on memory consolidation over one or multiple nights in humans, subjects are taught some stimuli and tested following a retention interval containing one or multiple night's sleep. There are hundreds of tasks which test memory which could be explored, a subset of which have been shown to be improved by sleep. Three categories of such SWS dependent tasks that this thesis

will focus on in relation to CLAS are: (1) declarative tasks, (2) procedural tasks and (3) tasks that assess qualitative changes to memories linked to the hippocampus. For a review on sleep dependent memory consolidation and testing see Diekelmann Wilhem and Born (2009). Using such tests will allow us to test the boundaries of the effect of CLAS on sleep dependent memory.

1.6.1 Declarative memory

One popular test to assess declarative memory consolidation, is the Word Pair (WP) task. WP is a paired associates learning task where participants are taught a list of word pairs (e.g. FOX-FUR or BRAIN-CONCIOUSNESS). Then participants are tested on their knowledge of the pairs, via cuing using one word of the pair (e.g., FOX-) and being asked to type or report the second word. Pairs in this task can be semantically related, or not (Payne *et al.,* 2012); be real words (Gais *et al.,* 2007); or nonsense words or words from a foreign language (Schreiner and Rasch, 2015). Recall on the WP task has been shown to be improved if a retention interval contains sleep, compared to a decrease in recall across wake (Gais *et al.,* 2007; Payne *et al.,* 2012; Wilson *et al.,* 2012). Sleep was found to protect against losses seen across wake, particularly if sleep immediately followed learning (Payne *et al.,* 2012; Wilson *et al.,* 2012). SWS in particular has been shown to be beneficial to WP recall (Plihal and Born, 1992; Tucker *et al.,* 2006; Backhaus *et al.,* 2007). Playing of sounds, previously (prior to sleep) linked to stimuli, during NREM sleep has also been shown to benefit recall of WP, but not when played during wake (Schreiner and Rasch, 2015). Sleep lacking in SWS does not hold the same benefit to memory as sleep rich in SWS (Plihal and Born, 1992). The percentage of total sleep time in SWS has also been shown to be correlated with overnight improvement in WP memory (Backhaus *et al.,* 2007). Schreiner and Rasch (2015) also linked improvements in WP memory following targeted memory reactivation (TMR) cueing, where sounds previously linked to stimuli are repeated during NREM sleep, to increases in SO and spindles following the sound. This adds evidence that these oscillations are involved in WP memory consolidation specifically.

It could be argued that some of these benefits to memory are provided by the time of day stimuli were learnt and recalled; as sleep versus wake experiments often train and test at opposite times of day to capture sleep and wake. However, sleep has been shown to be more beneficial than wake, irrespective of the time of day of encoding or testing (Barrett and Ekstrand, 1972; Payne *et al.,* 2012). Indeed, the

time of day sleep occurs does not affect impact on declarative memory, so long as there is sufficient SWS (Koulack, 1997; Ong *et al.,* 2020).

It is thought that WP consolidation during sleep could occur via the ASC theory previously discussed. Gais *et al.,* (2007) provided evidence for this when they showed that sleep or wake following learning impacted hippocampal activity two days following learning: Higher BOLD fMRI signal from the hippocampus during WP recall was shown when sleep followed learning, and connectivity between the hippocampus and the medial pre-frontal cortex (mPFC). They also showed that after six months', retrieval of pairs in the sleep group led to less activation in the hippocampus (than at 2 days) and more activation in the mPFC, potentially indicating memories relied less on the hippocampus and more on the cortex after this time.

Alongside this evidence that WP memory is improved by SWS, it is the only task, where it has been shown that CLAS can lead to an increase in recall (Ngo, Martinetz, *et al.,* 2013; Ngo *et al.,* 2015; Ong *et al.,* 2016; Leminen *et al.,* 2017; Papalambros *et al.,* 2017). As such it is a key task to aid understanding of the effects of boosting SWS using CLAS on memory, and shall be utilised by this thesis. As recent work has failed to replicate CLAS benefits to WP (Henin *et al.,* 2019; Schneider *et al.,* 2020) this highlights the need to explore this task further to understand what about the task is or isn't improved by CLAS. In Chapter 3 I will examine the impact of CLAS on three tasks closely related to the WP task, while in Chapter 4 I will use fMRI to understand the impact of CLAS on WP recall.

1.6.2 Motor memory

There is an extensive body of work investigating the impact of sleep on performance on a procedural motor tasks where participants are taught a short sequence (~5 items) of finger presses. This task is known as the finger tapping task (FTT), where participants tap out a sequence of finger movements as many times as possible in a given time window. FTT has been shown to be improved by sleep: Performance after a delay including sleep led to an improvement in the number of sequences accurately completed, while the same delay not including sleep, led to no change in performance (Walker *et al.,* 2002; Nishida and Walker, 2007). This performance change was also positively correlated with the time spent in N2 sleep. Nishida and Walker (2007) found a positive correlation between performance increase following sleep (90min nap) and the difference in spindle density in motor cortex between

hemispheres. As their task was performed solely using the left hand they could link the performance directly to the activity seen in the right hemisphere motor cortex during sleep. Walker *et al.,*(2005) went on to show increased activity in areas of the brain related to the task following sleep, compared to a wake only retention interval. Specifically; the right primary motor cortex, medial pre-frontal lobe, hippocampus and the left cerebellum, with a decline in activity in the parietal lobe and left insula. Brown and Robertson (2007) also found an increase in procedural motor learning after sleep, compared to wake. Together evidence strongly implicates NREM sleep in the overnight consolidation of this task leading to performance improvements, post sleep.

Further research then went on suggest that the way in which the task is learnt, either via explicit learning of a known sequence, or implicitly, has a significant impact on the effect of sleep upon performance. Implicit learning of a procedural sequence can be induced using a task similar to the FTT, called the serial reaction time task (SRTT). In this task participants are shown on screen a number of locations where visual cues can appear, each location is associated to a button on the participant's keyboard (Robertson, 2007). Participants are tasked with pressing the correct button as quickly as possible when the visual cue appears in each location (for an image of an SRTT task see section 2.3.4 Behavioural task procedure: Serial reaction time task, Figure 9). Unbeknown to the participant the visual cues follow a set sequence of locations, which is repeated across sequence blocks of the task. This is why this task is often reported as an implicit learning task (Robertson, 2007), unlike the FTT. Therefore, as the participant progresses through the task, repeating the sequence, they become faster at reacting to the visual cues as they learn the sequence and became able to anticipate the location of the next cue (Nissen and Bullemer, 1987). Unlike the FTT this allows the participant to gradually acquire knowledge of the sequence and can lead to improvements in RT over a longer time period (Verstynen *et al.,* 2012) than the FTT which can reach a performance plateau much faster (Bönstrup *et al.,* 2019). At the end of the task, a period of time is spent reacting to cues that do not follow the sequence, but instead use a random location order. This allows for assessment of the participants gain in motor ability, sometimes termed as visuo-spatial mapping gain (Robertson, 2007): This gain reflects the ability to react to the random appearance of a visual cue and press the corresponding button. The difference in time taken to respond to sequence and random cues can therefore give an indication of task skill. The skill has components of procedural, perceptual and

declarative learning, as the participant uses the sequence knowledge to better perform the motor procedural task. Explicit knowledge of the sequence can also be tested by asking the participant to recount the sequence. How and when this explicit knowledge arises in the standard SRTT task is debated (Fischer *et al.,* 2006; Cousins *et al.,* 2014), and as such variations on this task have been utilised to better understand the explicit and implicit components. This ability to extract out the different aspects of SRTT performance are part of what makes it an attractive tool for assessing the impact of sleep on motor memory.

Sleep is also thought to facilitate the consolidation of SRTT memory (Maquet *et al.,* 2000; Peigneux *et al.,* 2003; Morin *et al.,* 2008). Spindles in post-learning sleep have been shown to increase compared to sleep after a control task (Morin *et al.,* 2008). Post sleep SRTT performance has also been improved by boosting consolidation during sleep via TMR procedures (Cousins *et al.,* 2014, 2016; Schönauer, Geisler and Gais, 2014; Koopman *et al.,* 2020). Questions have been raised around the particular aspects of the SRTT that sleep preferentially improves. Robertson *et al.,* (2004) showed that sleep only improved SRTT skill on an explicitly learnt task. As when the task was implicitly learnt both wake and sleep intervals saw participants gain in skill. However, the extent to which their explicit task was truly explicit is debatable, as they simply told participants that there was a sequence and indicated the start of each repeat of the sequence during learning. Whereas in their implicit task they did not mention a sequence. Many versions of the SRTT lie in between these two tasks as participants are told of the presence of a sequence but not what the sequence is or given indication of when each new sequence starts. Spencer *et al.,* (2006) showed that implicit SRTT skill could be improved by sleep if the cues used held contextual information. In their task the context came from assigning different colours to the sequence as well as location. The SRTT procedure used in this thesis borrows from this in that instead of the same image appearing in each location, each location has its own unique image. Participants will also be informed of the presence of the sequence. Therefore, the SRTT task procedure in this thesis is likely to be influenced by SWS such that it is a prime target for CLAS improvement. This may give insights into the influence of SWS oscillations on SRTT performance and allow generalisation of the benefit of CLAS outside of the WP task. In Chapter 2 the task will be tested following one night of CLAS, to see if stimulation influences performance, while in Chapter 4 fMRI will be utilised to see if CLAS influences the brain areas involved in SRTT recall.

1.6.3 Qualitative changes to memories

While much of the research focus has been on how sleep can quantitatively change memories (i.e. increase recall) there has been recent arguments that sleep can also qualitatively change memories (see Landmann *et al.,* 2014, for a review). Examples of qualitative memory changes are when links are drawn between information encoded separately (Durrant *et al.,* 2011; Lutz *et al.,* 2017), or memories encoded implicitly can be explicitly recalled (Nieuwenhuis *et al.,* 2013; Cousins *et al.,* 2014). Cousins *et al.,* (2014) showed that TMR cueing benefited the explicit recall of a motor sequence taught implicitly. While sleep has been shown to increase the RT to identifying nonsense words when these words are closely related to real words i.e. cathedruke, allegedly due to their integration into the schema delaying their identification as nonsense (Tamminen and Gaskell, 2008).

Stark *et al.,* (2019) developed a task, called the mnemonic similarity task (MST) which allows two forms of memory restructuring to be assessed: pattern separation and pattern completion. Pattern separation is where similar items are held in the hippocampus as distinct entities; for example, the pattern AX could be recalled from the presentation of an A but not confused with the pattern BX. Whereas pattern completion allows us to draw connections and conclusions from incomplete information, such that presentation of the X would activate both memories of AX and BX. In the MST subjects learn a set of object images, they then are tested on the recall of these images following a delay. In the test they are presented with 1/3 new images (not seen before), 1/3 old images (identical to learning) and 1/3 similar images (images of the same objects as learning but a different image, i.e. they learnt the image of a red pair of socks but during the test saw a blue pair of socks). This allows for the calculation of not only recognition memory (how well they tell new from old images) but also pattern separation (how well they tell similar from old images). This task can provide insights into the reorganisation of the memory for these images and whether they have undergone pattern separation. As pattern separation and completion have been ascribed as functions of the hippocampus (Rolls, 2013) and the hippocampus is a key part of the ASC, thought to be influenced by CLAS, it is an interesting task to probe the effect of CLAS on sleep memory restructuring. This task was used in Chapters 2 and 4 to assess the role of CLAS in these restructuring processes.

1.7 Expanding CLAS beyond the lab

As a relatively easy to administer technique, using external, natural stimuli (Bellesi *et al.,* 2014) with the potential to improve memory (Ngo, Martinetz, *et al.,* 2013; Ngo *et al.,* 2015; Ong *et al.,* 2016; Leminen *et al.,* 2017; Papalambros *et al.,* 2017) CLAS is an attractive technique for commercial use. Recent advances in electrical engineering have reduced the production costs of portable EEG devises and auditory stimuli can also be generated and produced by small processing units (Debellemaniere *et al.,* 2018; Garcia-Molina *et al.,* 2018; Ferster, Lustenberger and Karlen, 2019). This has led to the development of headsets which can administer CLAS outside of the lab setting (Arnal *et al.,* 2017; Debellemaniere *et al.,* 2018; Garcia-Molina *et al.,* 2018; Ferster, Lustenberger and Karlen, 2019). As the electrodes used in these systems do not require specialist preparation of the scalp or precise measurement to place them correctly over known brain areas, as with conventional lab based EEG systems, they can be utilised by consumers and research participants alike in the home setting. This opens a range of avenues not only for commercial devices but also to expand CLAS research outside of the lab. To investigate the technique in more naturalistic sleep settings for the participant, reducing the effect sleeping in the strange environment of the sleep laboratory can have particularly on the first visit (Agnew, Webb and Williams, 1966; Newell *et al.,* 2012). It also removes a lot of practical barriers to repeated nights of stimulation, as participants do not need to be supervised in the sleep lab.

One such device is the Dreem headband (Arnal *et al.,* 2017). This device was produced to deliver CLAS to consumers outside of a research context, for casual use. The device was verified by Debellemaniere *et al.,* (2018) who showed that it could reliably identify sleep stage, and deliver CLAS at a time near to the SO peak during N3 sleep. Recent updates to the scoring algorithm of the device have also been validated as having equal agreement as that between expert human sleep scorers (Arnal *et al.*, 2020). Debellemaniere *et al.,*(2018) also found that stimulation led to an increase in SO amplitude and power. This device is utilised in Chapters 3 and 4 to deliver CLAS in a home setting over repeated nights.

1.8 MRI analyses

Since its inception functional magnetic resonance imaging (fMRI) has become a powerful tool for understanding the structure and function of the human brain. By allowing imaging in not only alive, but awake and behaving humans, the technique

allows questions to be raised about the brain areas involved in tasks that likely underlie the impact on behavioural performance. Takashima *et al.,* (2006) showed using fMRI that recall of images across one, thirty, sixty and ninety days after encoding led to a decrease in BOLD activity in the hippocampus and an increase in activity in the mPFC. They also showed that recall on day one, which was separated from image encoding by a nap, positively correlated with the time spent in SWS during the nap. Ong *et al.,* (2018) used fMRI to show that when images were encoded following a night of CLAS there was a positive correlation between the magnitude of SO increase, and activation in the hippocampus. So far none have utilised fMRI to understand how CLAS affects blood flow in the brain areas involved in memory recall. Therefore, there is call for the effect of CLAS on the recall of memories to be assessed using fMRI as it will enhance our understanding of the influence of stimulation on memory. It will also help to uncover some steps between the electrophysiological impact of CLAS and changes in memory performance. In Chapter 4 fMRI will be utilised to do just this; recall of procedural and declarative memory will be tested following CLAS and SHAM sleep.

1.9 Longitudinal studies

The majority of sleep and memory studies focus on the immediate effects on memory recall of one night of sleep, but the role of sleep over subsequent nights, and its long-term impacts are important issues. Therefore, one of the primary aim of this thesis was to understand the effect of several nights of CLAS on memory.

Only two CLAS studies have previously applied stimulation for more than one night (Debellemaniere *et al.,* 2018; Garcia-Molina *et al.,* 2018) and both were using this as a way to assess the effect of a novel CLAS device to stimulate SWS. Neither assessed the impact of repeated nights on behaviour. Debellemaniere *et al.,* (2018) delivered CLAS over 10 nights and compared the difference in ERP between CLAS trials on the 1st and 10th night, and found that there was no difference in the effect of stimulation. This implies that the brain does not habituate to multiple nights' stimulation, but it also implies that the effects of stimulation do not sum to create a larger effect after 10 nights. This thesis will describe experiments in which CLAS has been applied for seven and eight nights, this will allow the role of stimulated sleep oscillations in long term memory consolidation and potentially reorganisation. As described in section 1.5.2, theories link reactivation of memories in SWS to consolidation and memory plasticity over days and weeks (Pereira and Lewis, 2020).

1.10 Research Objectives

This thesis aims to explore the impact of CLAS on sleep-dependent memory, using tasks and conditions as yet untested, to find the boundaries of the impact of CLAS on memory. In Chapter 2 I begin with a traditional CLAS study, investigating the effect of stimulation delivered in a sleep laboratory, on memory recall across one night. Two tasks previously untested with CLAS tasks are utilised, assessing procedural and qualitative changes in memory overnight, which will allow us to see if the benefit of CLAS on memory can be generalised beyond the WP task. This may also aid understanding of what types of memory are affected by CLAS. An attempt will also be made to optimise the timing of CLAS sounds, to boost the brain response. The thesis in Chapter 3 will then move beyond the lab utilising a new CLAS device that enables stimulation to be delivered at home. The device will allow a longitudinal study of CLAS and its effects on memory. Memory recall following stimulation will be tested using three declarative WP linked tasks after one and seven nights of stimulation. This will allow me to see if the benefits to the WP task of CLAS can be expanded to other related tasks and, if the mechanisms leading CLAS to affect memory are active over subsequent nights of sleep. Finally, in Chapter 4 the thesis will explore the impact of stimulation beyond behavioural performance scores, specifically using fMRI to see if stimulation leads to changes in brain activity during memory recall. This may shed light on the intermediate steps between boosting SWS and performance changes. I also again assess the impact of long-term CLAS upon 3 tasks: (1) procedural skill memory as it has been shown to develop more slowly than declarative, (2) WP closely related to that shown to be improved by one night of CLAS and (3) the qualitative restructuring of pattern memory. Finally, I consider the implications of the results for our understanding of the effects of CLAS and sleep on memory in Chapter 5.

Chapter 2

Assessing the impact of closed loop auditory stimulation on motor and pattern separation tasks

2.1 Abstract

Closed loop auditory stimulation (CLAS) has been shown to improve post-sleep recall of word pair (WP) memory. The stimulation has been shown to increase slow oscillations (SO) and spindles, which are integral for overnight consolidation of memory during slow wave sleep (SWS). By boosting these oscillations, stimulation is thought to lead to greater consolidation of WP memory resulting in better recall. In this chapter I wanted to understand if CLAS would affect other memory tasks in the same manor. I selected tasks which have been shown to be dependent upon sleep, and SWS in particular: the serial reaction time task (SRTT) a motor sequence learning task and a mnemonic similarity task (MST) which is thought to draw on the hippocampus.

Seventeen participants underwent polysomnography (PSG) recording in the sleep laboratory on two nights. CLAS was delivered during stage 2 (N2) and Stage 3 (N3) sleep on one night (STIM), while on the other night the timing of sounds was marked but no sounds were played (SHAM). Prior to sleep participants completed encoding and testing phases of both SRTT and MST tasks, then following sleep they were tested again. There was no significant difference between performance change across either the stimulated night or the SHAM night. Event related potentials however, indicated a significant reaction to the sounds, as expected from assessment of previous literature. Further measures of oscillation density and power also indicated the expected response to stimulation. CLAS did not influence overnight performance change on the SRTT nor MST tasks, leading us to question the generality of this stimulation in improving memory.

2.2 Introduction

Slow wave sleep (SWS) is thought to play a key role in the overnight consolidation of memory (see Rasch and Born, 2013, for a review). This process stabilises the memory, makes it less vulnerable to interference and integrates it within existing knowledge. Due to its importance for memory, the idea of boosting the action of SWS is a popular one, with several techniques arising to do just this (see section 1.3.1 for discussion). One technique which influences the ongoing oscillatory activity of SWS is closed loop auditory stimulation (CLAS) (Ngo, Claussen, *et al.*, 2013; Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017;

Papalambros *et al.*, 2017). CLAS has been shown to increase the number of word pairs recalled, when applied during SWS between training and testing (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017). However, recent attempts to generalise this positive impact on memory to other tasks, thought to be consolidated in a similar manor to WP memories, have failed (Leminen *et al.*, 2017; Schneider *et al.*, 2020). Leminen *et al.,* (2017) completed perhaps the most extensive testing of CLAS and memory, as they assessed the impact of one night of CLAS on four tasks; (1) the WP task, (2) an image encoding task, (3) a finger tapping task (FTT) and (4) a name and face association task. They found that stimulation only led to an improvement in the WP task, while all other tasks saw no difference in performance following stimulated or non-stimulated nights. This requires further investigation to establish the impact of CLAS on other memory tasks which have been shown to be dependent on the activity of SWS oscillations affected by CLAS. This chapter will further pursue this issue, by utilising two tasks novel to CLAS research but established in their dependence on SWS; the serial reaction time task (SRTT) and the mnemonic similarity task (MST).

I was also interested in whether small changes to the timing of stimulation would affect the brain response. Particularly whether the duration of the stimuli could be extended and if the inter-stimulus interval (ISI) utilised in CLAS could be optimised.

All published CLAS experiments utilised 50ms of pink noise to stimulate SWS (Ngo, Claussen, *et al.*, 2013; Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Weigenand *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017). This is a somewhat arbitrary starting point, but it is useful as this duration is a short enough sound to fit within the SO, allowing for precise targeting of the SO. No studies have used a different stimuli duration or compared stimuli of different duration. Neurones involved in the auditory response are sensitive to different sound characteristics, such as volume, tone, and duration. While the majority of neurones respond to the beginning of a sound, there are those that respond to the cessation of a sound, particularly longer sounds (Phillips, 1993; Alain, Woods and Covarrubias, 1997). Therefore, the duration of the auditory stimuli could lead to variations in the number of neurones affected by the sound and thus affect the brain response of the SO. In this chapter I investigate the effect of doubling the length of the sound to 100ms on the event related potential response from the brain.

The timing of the CLAS sound has been shown to be critically important to the response of the brain. Studies have shown that the optimal time for the sound to arrive is in the up-phase of the SO near the peak (Navarrete *et al.*, 2019). Indeed, each CLAS study showing an improvement in memory following stimulation has targeted this phase of the SO (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017). Stimulation directed at the falling phase of SO has been shown to disrupt the ongoing oscillations (Cox *et al.*, 2014; Fattinger *et al.*, 2017). Ngo *et al.,* (2015) demonstrated that both memory and electrophysiological benefits from stimulation were comparable whether every SO was targeted or only two SO in a row were targeted before a delay. These authors also found that no matter the stimulation protocol, the increase in spindle activity only occurred in association with the first click. They also showed a greater response to stimulation by fast spindles for an ISI of 2-5s, than a shorter ISI of 0.125-0.5s, and thus suggested that the inability of every click to elicit a spindle phase locked to the SO is due to the refractory period of the spindles. This raises the question of how sounds could be spaced to optimise the spindle response. Many published studies using CLAS-like stimulation use a fixed delay between sounds. This delay varies between published experiments; For example, Ngo *et al.* (2015) used an ISI of 2.5 seconds, Weignand *et al.* (2016) used between 5 and 9 seconds, while Debellemaniere *et al.* (2018) used at least 9 seconds. Instead of a fixed duration, some CLAS protocols use a technique called the phase locked loop (PLL) to determine when to play stimulation (Santostasi *et al.*, 2016; Papalambros *et al.*, 2017; Garcia-Molina *et al.*, 2018). This method fits a sine wave to the SWS EEG to determine the phase of the SO and therefore decide when to deliver sound such that it falls during the desired phase (Santostasi *et al.*, 2016).

In addition to testing different durations of applied sound, I set out to optimise the spacing of these click sounds. To examine this, I utilised the fixed delay method to place the sound in the SO (Ngo, Martinetz, *et al.*, 2013), but counted the number of SO that occur during the ISI. Measuring the ISI in SO instead of seconds or sine waves could also provide a more tailored approach to CLAS as it does not rely on participants SO having the same periodicity. I thus varied the ISI between click stimuli from one to three SOs to determine the optimal spacing. One to three SO should fit within the optimal ISI timing of 2-5s indicated by Ngo *et al.,* (2015).

Many memory tasks have been shown to be SWS sensitive (for a review see Born, Rasch and Gais, 2006). For example, the learning of a sequence of motor movements, such as finger tapping (FTT) or serial reaction time task (SRTT) (Walker *et al.*, 2002, 2005; Robertson, Pascual-Leone and Press, 2004; Cohen *et al.*, 2005) discussed in greater detail in introduction section 1.6.2. SRTT is a test of procedural motor memory and declarative sequence learning that can be used to show memory consolidation and reorganisation from implicit to explicit memory (Fischer *et al.*, 2006; Cousins *et al.*, 2015).

Performance on motor sequence tasks has been shown to improve with sleep, participants get faster at completing the required number of finger movements to complete the sequence, when the retention interval includes sleep (Robertson, Pascual-Leone and Press, 2004; Walker *et al.*, 2005). Robertson *et al.* (2004) found that across a retention interval containing sleep, reaction time (RT) and skill on the SRTT increased further than when the retention interval only contained wake. They described the version of the SRTT used as an explicit version of this task, but did not actually tell participants the sequence, but instead informed participants of the presence of a sequence and indicated its start. In the version of the SRTT used in this chapter the procedure is not dissimilar, my participants were informed of the presence of the sequence. Robertson *et al.* (2004) also showed that overnight skill improvement correlated positively with the time spent in NREM sleep, indicating the importance of this stage for the improvement in performance. This raises the question of whether CLAS as it targets the action of SWS could lead to improvements on this task. Indeed, other forms of auditory sleep stimulation have been shown to boost SRTT task performance; Cousins *et al.,* (2016) showed that targeted memory reactivation (TMR), a technique designed to specifically increase the reactivation of certain memories paired to the cue sounds, led to an overnight increase in implicit and explicit knowledge of the SRTT sequence compared to an un-cued sequence. They also showed that improvement in cued SRTT sequences was dependent on spindle activity in brain regions associated with the task. Koopman *et al.,* (2020) also used TMR to improve overnight performance on the SRTT task, and they found that only TMR applied during SWS, not REM, led to this improvement. This makes the SRTT task a prime candidate for influence via CLAS, as it relies upon the sleep stage, and oscillations known to be influenced by stimulation.

There has been some debate over the explicit and implicit nature of the SRTT task and some have suggested the task must be explicitly learnt to benefit from sleep (Robertson, Pascual-Leone and Press, 2004). It has been suggested that sleep could preferentially improve the declarative memory of the sequence itself, leading to improved SRTT skill as participant's knowledge of the sequence increases so too does their speed at completing it. Cousins *et al.,* (2014) found that following a night of TMR where one of two learnt SRTT sequence was cued; explicit knowledge of the cued sequence was greater than the un-cued sequence. It could be hypothesised that if stimulation were applied following learning of this task, it would increase the participant's explicit knowledge of the implicitly learnt sequence. Therefore, if this consolidation was boosted by CLAS I would hypothesise that there would be greater explicit knowledge of the sequence than following SHAM nights.

Another type of memory process thought to be improved by sleep is pattern separation and completion (see intro for definition section 1.6.3). According to O'Reilly and McClelland (1994) the hippocampus must be good at both of these actions to successfully process our memory, as both completion and separation are required for accurate memory recall. Both processes are thought to rely heavily on interaction with different areas in the hippocampus (see Rolls (2013) for a review). As the hippocampus is thought to be important in how memories are processed overnight (Born, Rasch and Gais, 2006; Rasch and Born, 2013) effort has been made to understand what happens to the representations of memories held there during sleep (Rasch and Born, 2013). Most investigations have concentrated on pattern completion or gist abstraction (Lutz *et al.*, 2018; Pereira and Lewis, 2020), while more recent research implicates sleep in supporting pattern separation too (Hanert *et al.*, 2017). Hanert *et al.,* (2017) used a task called the Mnemonic Similarity task (MST) to assess pattern separation after a period of wake and a period of sleep. In this task pattern separation is assessed via the correct discrimination of similar images from the exact images encoded before sleep or wake. Hanert *et al.* (2017) found performance on the task decreased in both groups, but performance after sleep fell by significantly less then wake. They also showed a positive correlation between spindle density and pattern separation performance. This could imply that sleep has a protective effect on the performance of this task and links task performance with SWS oscillations, such that this task is an interesting candidate for improvement via

CLAS. In this chapter I will assess the impact of one night of stimulation on the pattern separation ability measured in this task.

To our knowledge the MST and SRTT have not yet been tested using CLAS, this chapter aimed to understand the effect of one night of CLAS upon these tasks, and see what this might teach us about the impact of CLAS on the process of memory consolidation in the brain.

2.2.1 Experiment summary

This was a within-subject crossover design in which participants attended the sleep laboratory for 2 nights of monitored sleep. During one (STIM) night they received CLAS consisting of six different trial types (two durations of sound and three durations of ISI), during the other (SHAM) night timing for CLAS was marked but no sound was delivered. On both occasions participants were tasked with learning and testing in two memory tasks (SRTT and MST) prior to sleep, and were tested on these tasks the following morning. The change in their performance between immediate and delayed testing on STIM (CLAS) and SHAM (No –CLAS) nights was assessed. As all six trials types were played in one night we could not test their relative impacts on behavioural performance. Checks were made to ensure that stimulation led to the expected electrophysiology changes despite the varied duration of sounds and ISI. Assessment of the impact of duration and ISI changes on the electrophysiological brain response was also assessed. Participants also underwent assessment using subjective and objective forms of arousal testing and questionnaires assessing their perceived sleep quality.

2.3 Methods

2.3.1 Participants

17 participants aged between 18 and 30 (6 Male, mean age 24.1 years old ±0.9 SEM), screened via a questionnaire, and determined to be free from psychological disorders and sleep disturbances, right handed, and with normal (or corrected to normal) vision and hearing were recruited. Participants were also excluded if they had travelled across two time zones within the two months of the experiment, had undertaken night-shift work, consumed any substances known to affect sleep, or if they regularly napped during the day. This study was granted ethical approval by Cardiff University

Psychology School Ethical review panel under number EC.17.12.12.5187A8. participants were paid £60 for their time.

2.3.2 Materials

Polysomnography

Participant's sleep was monitored using polysomnography via a Brain Vision system (Brain Products GmbH, 2006). 12 silver/silver chloride electrodes were applied to the scalp in the following positions (10-20 international EEG system): FPz, F3, F2, F4, C3, C2, C4, P3, P2, P4, O1 and O2. Two electrodes were placed around the eyes (one above the left eye and the other below the right eye, EOG) to collect data on eye movements and two electrodes were placed on the chin (EMG). Electrode locations were determined using an EEG cap with 10-20 locations marked. First the circumference of the participant's head at the widest point, was measured and a suitable sized EEG cap selected. The location of the centre point of the participant's head was determined by measuring the distance from the naison to the inion, as well as from ear to ear, with the intersection marked as Cz. The chosen cap was placed over the head and Cz lined up with the marked location. Each of the locations of the desired electrodes was then marked before the cap removed. *Nuprep* gel was used to prepare the scalp for electrodes and EC2 conductive paste used to improve impedance. The ground electrode was placed on the forehead to the right of FPz, while the reference was the mean of the signal from electrodes placed over the two mastoids. Care was taken to ensure that the reference electrodes were placed over bone and not muscle which could introduce noise to the reference channel. Impedance was checked in all electrodes before proceeding (EEG electrodes were re-applied until impedance was less than 5 kΩ, EMG less than 10 kΩ). Electrodes were connected to an amplifier and battery which sent recordings from the participant to a computer where the ongoing EEG trace would be recorded and viewed live by the experimenter.

Stimulation equipment

Auditory stimuli were delivered through in-ear 'bud' earphones (Sony). At the determined times (outlined below), the recording PC sent the stimuli sound to the participant via the earphones and also sent a trigger signal via an auxiliary channel to the EEG system to allow the EEG trace to be marked with the time the sound played. This allowed for precise synchronisation between the stimulation and recorded EEG.

Sleep laboratory behavioural testing

Behavioural testing, was carried out in the same room as participants slept, using a desktop PC. The SRTT and PVT tasks were run using MATLAB and Cogent version 1.32 (Romaya, 2000), while the MST was delivered using PsychoPy version 0.96. Participants used the PC keyboard and mouse to record responses to all tasks, they listened to sounds in the SRTT using on-ear headphones. They were allowed to sit at a comfortable distance from the screen.

Questionnaires

A written Stanford sleepiness scale (SSS) was used to assess participant alertness, where participants wrote down the number corresponding to their perceived level of alertness. See appendix section 7.1 for SSS. A sleep quality questionnaire was also used to assess participants perceived sleep quality. Based on the SF-A-R (Gortelmeyer, 2011) see appendix section 7.1 for questionnaire.

2.3.3 Experiment design

Participants slept in the Cardiff University Brain Research Imaging Centre (CUBRIC) sleep lab on two separate occasions, 7 nights apart: an experimental (STIM) night; and a control (SHAM) night (Figure 7), night order was counterbalanced. Participants arrived at the laboratory between 7pm and 8pm, first they completed the SSS before completing the completed 3 tasks: MST, SRTT, and the Psychomotor Vigilance Task (PVT) to test alertness and arousal. Task order was also counterbalanced, using a Latin square, whereby each task was shifted one place so it appeared first, second, third and fourth for different participants. The sequence of the tasks did not change: MST, SRTT then PVT. Task order was the same for each visit made by a participant. Following task completion participants changed into bedclothes and had EEG electrodes applied. Participant's sleep was monitored using polysomnography (detailed above section 2.3.2 Polysomnography). Participants were then put to bed with lights off between 10pm and 12pm, participants were screened prior to the experiment to ensure this fell within their usual bedtime. Upon waking participant's EEG electrodes were removed and participants were given at least thirty minutes and the opportunity to shower to overcome sleep inertia. Following this time, they completed the SSS again and all three tasks (in the same order). They then completed

the sleep quality questionnaire (see section 2.3.2 Questionnaires), before leaving the laboratory.

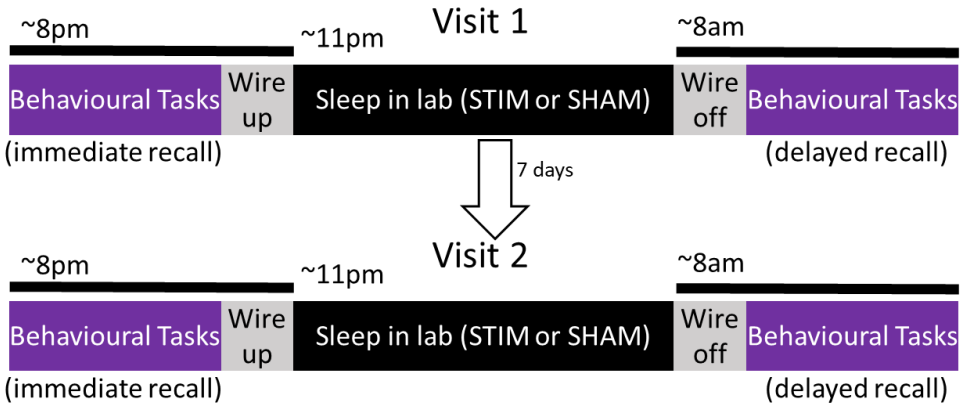


Figure 7 *Experimental protocol. Participants make 2 visits to the lab 7 days apart. One visit for 1 night of CLAS (STIM), one visit for 1 night of no sounds (SHAM). Behavioural tests are encoded before sleep and tested upon waking. The PSG equipment was applied after behavioural tasks and before bed, and removed before tests were completed in the morning. The order of visits was counterbalanced between participants.*

Stimulation procedure

Wake Pilot: To determine the most effective duration of sound stimuli a pilot was carried out in awake participants. Ten participants (1 male, age range 22-29) had their brain activity monitored using EEG while six durations of *pink noise* (1/frequency Hz), centred around the 50ms stimuli commonly used in CLAS protocols (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Weigenand *et al.*, 2016; Papalambros *et al.*, 2017), were played: 10ms, 25ms, 50ms, 100ms, 200ms and 400ms. Following application of EEG in same layout as described in section 2.3.2, participants were instructed to sit quietly with their eyes closed whilst they listen to sounds played through the in-ear phones. Two ten min blocks each consisting of 120 sounds (each sound repeated 20 times), with a short self-timed break between (up to five min). Sounds were split into two blocks to reduce the chance of the participant becoming distracted. EEG was analysed to assess the amplitude of resulting event related potentials (ERP), variation in response between participants and topography for each sound. Both 100ms and 50ms tone durations were selected to be tested in the main experiment.

Stimulation algorithm: In the main sleep experiment stimulation was managed and delivered via a custom MATLAB algorithm (Navarrete *et al.*, 2019). The algorithm was based on the Ngo, Martinetz *et al*., (2013) CLAS method: SO were detected when the signal from the reference electrode (Fz) fell below -80μV. A delay, initially set at 0.5s was implemented before the sound was delivered, such that the sound occurred just before the peak of the SO. The algorithm was started once I had determined (by scoring the live EEG stream) that the participant had been in stable SWS for 10min and allowed to run for 4 hours (irrespective of sleep stage). During the first hour; stimulation was paused if the participant woke until stable sleep was re-established; I could also adjust the delay in the system (by adding or subtracting time from the algorithm's delay time), so that the stimulation fell as close to the peak of the ongoing SO as possible. During SHAM nights the stimulation program was the same as on STIM nights (see Figure 7) but the volume output for stimulations was set to zero. Volume was still adjusted prior to sleep with the participant, so they were not aware that they would not hear any sounds that night.

Stimulation conditions: As I was interested in the impact of varying the duration of the stimuli sound and the ISI between sounds, six different trial conditions were tested. The stimulus duration was either 50ms or 100ms (as determined in above described *wake* pilot) and the interval between stimuli consisted of 1, 2 or 3 SO as this number of SO account for the optimal ISI of two to five seconds determined by Ngo *et al.,* (2015). All durations were combined with all intervals, thus creating six unique conditions. Stimulus order was randomised. As I wanted to measure ISI using SO which were consecutive, a cut off was applied for each duration ISI, determined by the length of time it was likely to take to get one, two or three consecutive SOs. To determine these cut-offs, ten sleep recordings were examined and the number of SOs that occur in 2-5 s established (Ngo *et al.*, 2015). The recordings were collected on healthy young participants, using the Emblar N7000 system and F3, F4, Fz, C3, C4, O1 and O2 electrodes (Tsujimura, 2018, full details of the experiment this data was collected for can be found in Chapter 3, Section 3.3, P88). Using Brain Vision Viewer each of the ten nights were examined, N3 was visually identified (using American Association of Sleep Medicine (AASM) scoring system, Berry *et al.*, 2018). SO were identified by eye using the criteria outlined in the AASM, on channel F3 or F4, half the participants were assessed on each channel. Tools within Brain Vision used to measure the length of time from the zero

crossing prior to the negative peak to the zero crossing subsequent to the positive peak. Ten of each SO train length (one, two or three) were timed in each participant, the mean for each participant found, before calculation of a group mean. The cut off used for each ISI was 1SO: 2 seconds, 2 SO: 3 s and 3SO 4seconds, see Figure 8. The algorithm determined the number of SO in each length of ISI using the same procedure as it identified the SO to stimulate, using a -80µV threshold.

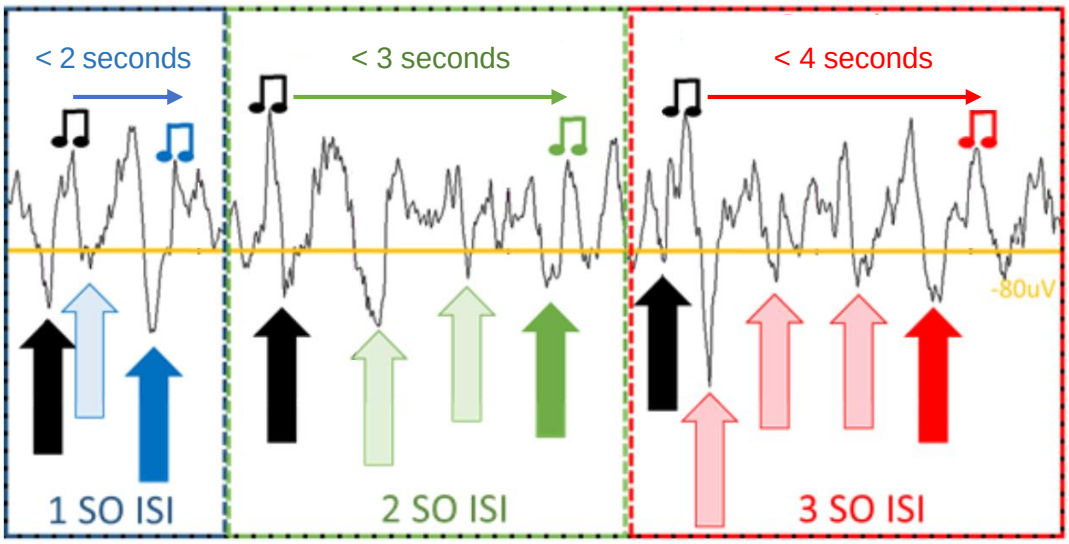


Figure 8 *Schematic of how the algorithm would measure an ISI of 1, 2 and 3 SO. Black line shows slow wave sleep. Musical Notes indicate when sound was played. Pale vertical arrows show when SO was detected but not stimulated, bright vertical arrows show when the SO was detected and stimulated. Yellow line shows the algorithm threshold of -80uV. Top horizontal arrows indicate cut off time for each ISI.*

The volume of stimulations was adjusted prior to sleep to an audible level that the participant felt comfortable sleeping with. This was determined by setting the volume to 40dB and playing the pink noise stimuli to participants through headphones, if they felt that the volume was too loud to sleep they indicated this to the experimenter who turned the stimuli down by 5% and repeated the sound.

2.3.4 Behavioural task procedure

Serial Reaction Time Task

The SRTT is a visual, procedural, motor task wherein participants are taught a 12-item sequence of button presses using four buttons linked to certain location-image – sound stimuli presentations. Custom MATLAB scripts were written to run this task, adapted from scripts by Belal *et al.,* (2018), utilising the Cogent 2000 toolbox

(Romaya, 2000), and the procedure was based on that used by Cousins *et al.,* (2014). First participants were given on-screen instructions for the task (see these in appendix section 7.3.1); Participants were then given the chance to ask questions before the PC volume was set to 12% and they put on headphones to begin the task. The starting screen consisted of a grey background with four locations marked out with white lines across the horizontal centre of the screen (see Figure 9). Each trial was made up of presentation of an image in one of the four locations, and a sound. The images presented were greyscale images of a male face, a female face, a tap and a lamp. Each image always appeared in the same location (Male face far left, lamp second from left, female face second from right, tap far right) (Cousins *et al.*, 2014). Each image was accompanied by its own 200 ms, unique tone (lower octave C, D, E and F), delivered through on-ear headphones. Each position was assigned a corresponding key on a standard 'qwerty' English keyboard (far left = 1, second from left = 2, second from right = 3, far right = 4). As instructed, participants were tasked with pressing the button corresponding to the location of the image/tone, as quickly as they could after it appeared. As soon as participants pressed the correct key the image returned to the start screen (white lines but no images), for a duration of 300ms before the next trial started. Trials followed each other in a set 12-trial sequence, and was one of two counterbalanced sequences A (1 2 1 4 2 3 4 1 3 2 4 3) or B (2 4 3 2 3 1 4 2 3 1 4 1) (Cousins *et al.*, 2014). All participants completed sequence A on their first visit and sequence B on their second. Participants were made aware via task instructions that there was a sequence but not how long it was, or that recall would be tested. Trials of the sequence were grouped together into *sequence blocks;* such that one block consisted of three repeats of the sequence followed by a 2 second fixation cross, then 3 more repetitions of the sequence (72 cues in total). Following each block participants were given feedback on their speed (mean trial response and fastest response), and accuracy (how many errors made) in the pre-ceding block, for 25 seconds. The beginning of the next block was preceded by a five second count down.

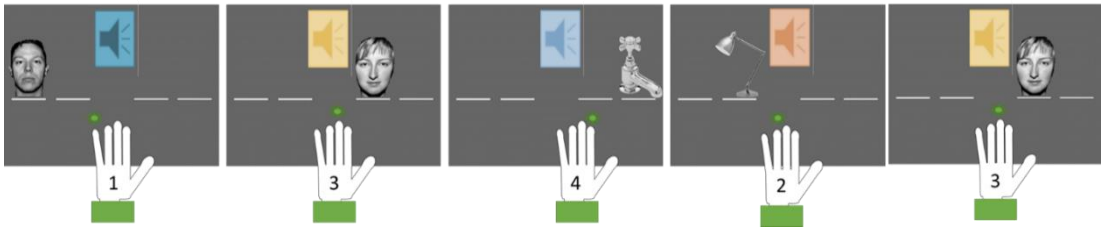


Figure 9 *Serial Reaction Time Task. Five trials of the SRTT where participants used solely their left hands. Coloured speakers indicate different tones and the number on each hand indicates the finger used to respond to that stimulus set. Fingers were numbered 1-4 pink finger to index finger.*

Participants were also given *random blocks* where the presentation of trials did not follow the sequence, but instead followed a random sequence. The same number of trials made up a random and sequence block. The random trial sequence was generated prior to the experiment and set the same for all participants. It was ensured that no more than five consecutive trials in the random blocks followed the sequence. Participants were made aware that some of the blocks would contain no sequence (these blocks were distinguished by a central 'R'). Feedback was also given following random blocks.

In the evening before sleep participants completed ten sequence blocks followed by two random blocks. In the morning participants completed six sequence blocks and six random blocks. Explicit recall was also tested in the morning by asking participants to mark down the 12-item sequence on a sheet of paper as per Cousins *et al.,* (2014).

Mnemonic Separation Task

The MST is a task of image discrimination where participants must discriminate between images previously taught, images very similar to these and new images. The version used in this chapter was based on that employed by Hanert *et al.,* (2017), scripts were downloaded from Stark Lab (2013) version 0.96 PsychoPy, first used in Kirwan and Stark (2007). The MST task downloaded consisted of six independent image sets each consisting of 384 images. Each set had 192 unique objects, each of which has two similar images. Four image sets were used for this experiment, the order in which they appeared counterbalanced between participants.

Encoding: See Figure 10 A. Pre-sleep, participants were presented with a series of 128 colour images of objects from one set of task images. Which images were presented was determined by a random seed based off the participant's unique ID

number. When the participant saw the image, they were tasked with classifying the object as indoor or outdoor. Indoor was represented by pressing the *V* key on a standard 'QUERTY' keyboard and outdoor by pressing the *N* key. Images appeared on screen for 2s with 0.5s interval (fixation cross). The words 'Indoor or outdoor?' appeared at the top of the page (see Figure 10 A) and participants were given a card reminding them which button to press for which response. Participants were given no explanation of what constituted an indoor or outdoor object. This was repeated twice so that participants were exposed to 128 stimuli from two different image sets Stark *et al.,* (2013).

Immediate recall: See Figure 10 B. An immediate recall task was then performed using images only from one of the image sets from encoding. 64 previously seen (target/old) images, 64 new images never seen before (foil/new), and 64 images that are similar to the images learnt in the first part of the task (lure/similar) were shown to the participant. As each image appeared on screen participants were asked to classify the images as 'old', 'similar', or 'new' using the *V*, *B* and *N* keys respectively. Instructions for this part of the task were relayed to participants via a video produced by Stark *et al.,* (2013). Images stayed on screen for two seconds with a one second ISI (fixation cross).

Delayed recall: The same procedure as immediate recall was followed, but using the other set of images from encoding.

Figure 10: *Mnemonic similarity task.* ***A*** *Encoding: Participants were presented with a series of images and asked to classify them as indoor or outdoor.* ***B*** *Recall: Participants were presented with a new group of images, 64 'targets' as seen before, 64 new 'lure' images similar to the images from the encoding, and 64 new 'foil' images that have not seen before. They are asked to classify images as 'old', 'similar', or 'new'*

Psychomotor Vigilance Task

The PVT is an objective test of arousal using a reaction time task, modified from a script originally written by Petzka (2016). Following instructions participants were presented with a black screen with a white central fixation cross. They were informed that at random intervals, the fixation cross would disappear and be replaced by a counter, counting up in milliseconds. They were tasked with pressing the spacebar as soon as they saw the cross disappear. Their response would stop the counter and give a RT. If participants took longer than 1s to respond, then the words *'Please pay*

attention' would appear in red text at the centre of the screen, before the next trial started with a new fixation cross. Participants completed this task for 10min in the evening, and again following the same protocol in the morning.

2.3.5 Analysis

EEG Analysis

First, raw sleep EEG was pre-processed using the MATLAB toolbox [Field Trip]. Signals were band-pass filtered (using an IIR Butterworth filter) between 0.3 Hz and 35 Hz. Initial filtering allowed the removal of signals not generated by brain activity, such as electrical interference from mains electricity (50Hz). This initial filtering was carried out on unsegmented data to minimise any distortion affects which may occur at the edges of the recording. Had this been conducted on segmented data these distortions could occur at the start and end of each trial. When the six sound conditions were played by the stimulation algorithm (see section 2.3.3 Stimulation procedure) it labelled each trial with the duration of the sound it used (50ms or 100ms) and the ISI it was going to leave before it played the next sound. Therefore, during analysis, the part of the label indicating the ISI before the next sound needed to be moved to the following sound, such that the label indicated the ISI before that trial. This would allow assessment of the impact of the different intervals on the response of the brain to the sound following the interval. To do this a custom MATLAB script was written to appropriately adjust the labels using the [Field Trip] structures. Then the raw EEG was segmented into 3 second trials with the sound stimuli being delivered 0.5 s into the trial. The timing of trials was compared to the scoring for the night such that any trial occurring outside of N2 or N3 was removed.

Sleep data was scored using an offline automatic scoring system (Z3 Max, Oracle, Neurobit Technologies, 2019), it was also scored by myself and another trained sleep scorer. Agreement between these three scores was assessed by finding the percentage of epochs (30 second intervals), within each night where two of the three scorers agree. Agreement is assessed for each epoch. Scores were compared, and on average out of participants tested (n=14, 3 participants excluded due to missing scores for one night) all three agreed mean=79.7% (SEM=0.88), while the other scorer and Z3 mean=78.7% (SEM=1.240), and myself and Z3 mean=81.4% (SEM=1.45). Statistical analysis indicated no difference between the comparisons of the two human scorers, and Myself and Z3 ($z(27)=500.00$ $p=.078$), or the two human

scorers and the other scorer and Z3 ($z(27)=372.00$, $p=.749$). Thus analysis utilised the Z3 automatic scoring, as it was deemed the most consistent in its pattern of mistakes, and thus provides the most consistent score across all nights making any SHAM and STIM differences more likely to occur due to the stimulation itself not scoring errors.

Trials containing arousals, defined using the AASM (Berry *et al.*, 2018) as a sudden change in EEG above 16Hz for more than three seconds, preceded by at least ten seconds of stable sleep and accompanied by more than one second of elevated EMG if in REM, were identified visually through scoring. These trials were then removed from analysis along with any trials not in NREM stages S2 and S3. Channels were visually checked for any that had become detached during the night. These channels and the trials in which the signal was lost were marked as errors and interpolated using neighbouring channels.

Event Related Potentials

First, all trials within each night were time locked to the sound onset, and a mean signal across all three second trials, calculated. The signal across all SHAM nights was then averaged to give a mean SHAM ERP, and trials averaged across STIM nights to give a mean STIM ERP. The trials within each STIM night were then segregated to give mean signals for 50ms sound trials and 100ms sound trials, as well as mean signals for 1SO ISI trials, 2SO ISI trials and 3SO ISIS trials.

Cluster permutation analysis was then conducted to explore any time periods where there was a significant difference in signal voltage between stimuli conditions (i.e. 50ms Vs 100ms, and 1SO vs 2SO vs 3SO), using the Monte-Carlo method. In this test, first the difference between SHAM and STIM signal is assessed: at each time point a t-test is conducted on the mean signal from all participants to see if there is a significant difference between conditions. For neighbouring (in time) significant time points the t-statistic is summed to give the cluster statistic. Then in a number of the participants the signals for SHAM and STIM are switched and the t-tests run again to give new cluster statistics for this shuffled data. This is repeated for 10,000 permutations within each a unique shuffle of the data is made. Then the cluster statistics for the real data (correctly labelled SHAM and STIM) are compared to the cluster statistics for the shuffled permutation data. Real cluster statistics are only considered significant if they are smaller than 2.5% (not 5% as tests are two-tailed) of

the cluster statistics from the shuffled data. These significant clusters are presented in the results.

Sleep Oscillation Features

A custom MATLAB program was used to identify the location of all of the SO (0.5 to 4Hz), fast (11-16Hz) and slow (9-11Hz) spindles in each recording (Navarrete *et al.*, 2019). I split the spindle frequency band into fast and slow as Schneider (2020) indicated different responses of each to CLAS. This script also determined the trough amplitude of all SO. I developed a custom MATLAB script to quantify the number of SO, fast and slow spindles in each night, as well as use the times when stimulation was live for each participant to calculate the number of these oscillations during stimulation. This was also used to extract the amplitude of SO troughs within stimulation time. The script then went on to calculate the density (number per second) of each of these waves inside, and outside stimulation time, and the mean amplitude of SOs.

Calculations were made for each channel in each participant for both SHAM and STIM conditions. The mean density was calculated for frontal channels (F3, Fz, F4) for SO density and trough amplitude, and central channels for spindles (C3, Cz, C4). SO and Spindles are often not global events and therefore not expected to occur with equal frequency and size across the whole brain (Happe *et al.*, 2002). Therefore, if the mean of these events was taken from all channels it would likely skew results to make it appear as if fewer events were occurring than in reality. SO largely occur frontally and thus means were taken from frontal electrodes, whereas spindles occur more parietal so means were taken from central electrodes. These values were compared for each participant in each condition night. SHAM and STIM densities were then compared using appropriate paired t-tests (after normality testing).

The percentage change from SHAM to STIM nights was then calculated for each participant: $\left(\frac{(STIM-SHAM)}{SHAM}\right) \times 100$. This gives a normalised value for each participant that showed how much their density of SO or spindles, or SO amplitude, changed when they experienced CLAS, which can be compared across participants. This is particularly important when considering the variation between participants in normal sleep oscillations (for a review on inter-individual sleep differences see Van Dongen, Vitellaro and Dinges, 2005), if I did not normalise the oscillatory values it would be difficult to compare between participants.

Time Frequency Analysis

Time frequency analysis was conducted for trials that occurred during N3 sleep. A multi-taper approach was used, applying a sliding Hamming window to calculate the power of frequencies from 0.1 to 30 Hz across the trial window from 4 s before the sound, to 5 s after the sound. A longer time window was applied than that for ERP analysis as I was particularly interested in the effects of simulation upon low frequency oscillations, and a large time window was needed to analyse at least 3 waves within the trial window. The time window examined varied for each frequency assessed, to fit 3 oscillations of that frequency, such that at 1Hz the time window was 3s while at 0.5Hz it was 1.5seconds. Grand mean was calculated across participants for SHAM and STIM conditions using [Field Trip] functions and a custom MATLAB script. The difference between conditions was also calculated and visualised.

To allow for direct comparison between nights and participants Power Spectral Density (PSD) was calculated for each participant and condition. This allowed the power in each frequency for the given time (stimulation to 2s) to be normalised by the area under the curve of the absolute power. Time from the onset of the sound to two seconds after was assessed to highlight the effect of the sound on the signal.

The power for each participant at given frequencies was then calculated: slow wave activity (SWA): 0.5 to 4Hz, slow spindle: 9 to 11Hz and fast spindle power: 11 to 17Hz (Navarrete *et al.*, 2019).

The percentage change $\left(\frac{(STIM-SHAM)}{SHAM}\right) \times 100$ from SHAM to STIM was then calculated for each channel in each participant, before a mean of central (spindle and sigma power) or frontal (SWA) was calculated. The power values for each participant SHAM and STIM was visualised to illustrate how it changes for each participant. The change in values from SHAM to STIM was tested using the appropriate paired t-test following normality testing.

Behavioural tasks

Serial reaction time task

The analysis was conducted in the same way as in Cousins *et al.,* (2014). First, RT for each of the trials were calculated, and any that fell outside of ±2 standard deviations (SD), determined for each participant, were removed. Mean RT for each participant in each of the evening sequence learning blocks were plotted and a polynomial curve fitted using MATLAB. Participants were removed if the change in

their RT over the task did not have a negative slope (n=4), see appendix section 7.3.2. Not possessing a negative slope indicates that the participant likely did not grasp the sequence and thus their RT did not decrease across sequence blocks, and that they did not acquire greater skill in the task which would have also resulted in shorter RT in response to trials.

Pre-sleep: The mean RT for the last 2 sequences, and the 2 random blocks was calculated. A score of 'Sequence Specific Skill' (SKILL) was then calculated by subtracting the mean sequence RT from the mean random RT. This signifies the RT influenced by the sequence itself, with the speed at which the participant can react to a cue (random RT) removed (Robertson, 2007; Cousins *et al.*, 2014). Such that an increase in the RT attributed to the sequence is shown by a larger SKILL. *Post sleep:* The difference in the mean RT of the last two sequence blocks and first 2 random blocks was calculated as the post-sleep SKILL. These sequence and random blocks are closest to each other, so are less likely to be influenced differently by factors such as fatigue (Cousins *et al.*, 2015). Participants scores were compared to the group mean and those outside ±2 SDs from the mean were removed (n=1). The overnight percentage change in SKILL was also calculated for each participant: $\left(\frac{(Post\ sleep\ SKILL - Pre\ sleep\ SKIL)}{Pre\ sleep\ SKIL}\right) \times 100$, such that a positive percentage change indicates that the sequence increases its influence on SKILL overnight. This was calculated for SHAM and STIM nights, for each participant. Appropriate paired significance testing (paired two tailed t-tests for normally distributed or paired Wilcoxon signed rank tests for non-normally distributed data) was performed following checks for normality (Shapiro Wilke test), using Prism v.8 (GraphPad, 2019), to assess differences between SHAM and SITM scores.

Explicit recall scores were calculated by summing the correct items given in the paper test. An item in the 12-item sequence was only considered correct if it fell within a group of ≥2 correct items. i.e. if the sequence was 1 2 1 4 2 3 4 1 3 2 4 3 and the participant wrote 1 2 1 4 3 3 2 1 1 3 2 2 they would score 4 as despite getting 3 and 1 correct, as neither neighbouring number was correct they were not credited, as per Cousins *et al.* (2014). A Wilcoxon Signed Rank Test was performed to determine if there was a significant difference between scores in SHAM and STIM. A non-parametric test was chosen as scores failed normality testing (Shapiro Wilke).

Mnemonic similarity task

Two metrics were extracted from the raw scores calculated from MST behavioural results, using a custom written MATLAB script. (1) The pattern separation score (PSS) was calculated using the following formula:

$$PSS = correct\ 'similar' response\ to\ similar\ image - false\ 'similar' response\ to\ new\ image$$

and (2) the recognition memory score (RMS) was calculated using the following formula: $RMS = correct\ \ 'old' response\ to\ target - false\ \ 'old' response\ to\ foil$ (Hanert *et al.*, 2017). Both scores were calculated for each participant, for immediate recall (scores in the evening before sleep) and delayed recall (scores in the morning post sleep). Scores for each individual were calculated as a percentage of the evening score: $\frac{\text{morning score}}{\text{evening score}} \times 100$. Participants whose overnight change scores fell outside ±2 SDs from the mean were removed (PSS n=3, RMS n=3).

Each of the similar images (at testing) in this task could be attributed to one of five similarity bins, divided upon how similar the image was to its original image (the image learnt at encoding). The similarity between the two images had been rated by participants in Yassa *et al.,* (2011), bin 1 consisted of the most similar images while bin 5 consisted of the least similar images. An example of a highly similar image may be two images of the same frying pan with the viewing angle of the pan rotated slightly between images, while a less similar pair might be images of two different frying pans. PSS was calculated separately for objects belonging to each similarity bin (Hanert *et al.*, 2017), and overnight change calculated as: $Evening\ PSS - Morning\ PSS$. Change was calculated as an absolute change instead of a percentage change as there were many zero scores in the morning and it is without meaning to calculate a percentage change from zero.

Paired student-t tests and Wilcoxon signed rank tests were used to compare PSS and RMS respectively, dependent upon whether data indicated a normal distribution (normality testing using Shapiro Wilke). While a 2-way ANOVA was performed on similarity bins.

Psychomotor vigilance test

To assess objective arousal, the RT to PVT tests in the evening prior to sleep, and morning post-sleep were assessed. Primary analysis was performed using a custom

written MATLAB script. First outlier trials were removed from each participant's RT, using ±2 SDs from the mean as thresholds. The mean RT was then calculated for the participant. Next the fastest and slowest 10% of RT for each participant was extracted and the mean calculated (Basner and Dinges, 2011). As these have been shown more susceptible to changes brought on by sleep disruption than overall RT (Basner and Dinges, 2011). For each of the overall, fast and slow means, the overnight change was calculated: $Morning\ RT\ mean - Evening\ RT\ mean$. Using Prism (GraphPad, 2019), group means were calculated for each category (overall, fast and slow, for SHAM and STIM) and student t-tests were used to compare SHAM and STIM conditions, as normality testing indicated a normal distribution.

Questionnaires

To assess subjective arousal, the score on the SSS was assessed. The overnight change in SSS score was calculated as $Evening\ SSS\ score - Morning\ SSS\ score$. Wilcoxon signed rank tests were performed to assess the difference between SHAM and STIM in the evening morning and the overnight change, due to normality testing indicating a non-normal distribution to the data.

To assess participant's perception of the night's sleep, their responses to the sleep quality questionnaire following SHAM and STIM nights were assessed. For each participant a score was calculated for sleep quality (SQ), and the feeling of relaxation by general effect score (GES). These were then compared between SHAM and STIM nights using paired t-tests, as data indicated a normal distribution.

2.4 Results

As the main objective of this chapter was to assess the impact of one night of CLAS delivered in the sleep laboratory, on the behaviour in the SRTT and the MST tasks, it must first be established that the CLAS had the expected impact on sleep. Thus I will first consider the impact of electrophysiological measures in the STIM nights' verses SHAM nights and see if they match what is expected following CLAS (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017).

2.4.1 Effects of CLAS on sleep macrostructure and arousal

First, I shall consider the effect of stimulation on the overall macrostructure of sleep, which can be observed from inspection of Table 1. Inspection of the table shows that

stimulation appeared to cause no change in time spent in any sleep stage nor total sleep time (TST). This is what was expected as previous published works have not indicated that CLAS delivered during SWS leads to a change in whole night macrostructure (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Papalambros *et al.*, 2017).

Measure		***SHAM***	***STIM***	***Statistics***
Time in stage /min	*TST*	*413.84 ±12.76*	*427.69 ±29.25*	*t*(15)=-1.59 *p*=.132
	N1	*16.31 ±3.13*	*19.13 ±2.58*	*z*(15)=31.00 *p*=.187
	N2	*228.31 ±8.72*	*237.09 ±7.17*	*t*(15)=-1.21 *p*=.245
	N3	*88.25 ±7.61*	*83.56 ±6.96*	*t*(15)=0.88 *p*=.393
	REM	*80.97 ±6.35*	*87.91 ±6.04*	*t*(15)=-1.22 *p*=.240
	WASO	*25.70 ±6.07*	*22.47 ±4.39*	*z(15)=63.00 p=.530*
Arousals		*16.19 ±2.40*	*16.13 ±2.10*	*t*(15)=0.034 *p*=.973
%TST	*N1*	*3.98 ±0.71*	*4.47 ±0.60*	*t*(15)=-0.06 *p*=.951
	N2	*55.30 ±1.50*	*55.38 ±1.29*	*t*(15)=-0.06 *p*=.951
	N3	*21.08 ±1.58*	*19.57 ±1.61*	*t*(15)=1.23 *p*=.236
	REM	*19.64 ±1.41*	*20.58 ±1.37*	*t*(15)=1.23 *p*=.236

Table 1: *Time in each sleep stage in minutes and as a percentage of TST. Time in sleep stages as a percentage of total sleep time (TST). Automatically scored by Z3Score* (Neurobit Technologies, 2019)*.*

Second, I assessed (using paired two-tailed t-tests or Wilcoxon signed rank tests) the impact of stimulation on subjective and objective arousal measures. Inspection of Table 2 shows that stimulation appeared to have no effect on subjective sleep quality (GES), or arousal (SSS score). Objective arousal, measured via the PVT task, was also not affected by stimulation. Again this is not unexpected as Ngo, Martinetz *et al.,* (2013) also showed that one night of CLAS did not affect subjective or objective arousal, measured using the SSS and PVT respectively.

Measure		SHAM	STIM	Statistics
Sleep Quality	GES	5.20 ±0.56	5.56 ±0.53	*t*(16)=-0.91 *p*=.379
	SQ	3.56 ±0.20	3.31 ±0.22	*t(16)=1.04 p=0.317*
SSS	Evening	2.53 ±0.24	2.65 ±0.30	*z(16)=10.00 , p*=.516
	Morning	2.41 ±0.19	2.59 ±0.19	*z(16)=12.00, p*=.383
	Overnight	0.18 ±0.26	0.06 ±0.23	*z(16)=-4.00, p*=.883
PVT /ms	Overall	1.87 ±5.46	-14.95 ±15.31	*t*(11)=1.08 *p*=.299
	Fastest	-0.83 ±4.04	-5.74 ±9.30	*t*(11)=0.50 *p*=.626
	Slowest	-2.03 ±17.02	-18.32 ±21.63	*t*(11)=0.66 *p*=.521

Table 2: *Questionnaire and PVT scores. Sleep quality questionnaire sleep quality (SQ) score and General Effective Score (GES) Mean for SHAM and STIM. Stanford sleepiness scale (SSS) overnight change (Evening - Morning). Psychomotor vigilance task (PVT) mean overnight change (Morning - Evening) in reaction time (ms) across all trials (overall), the fastest 10% of trials and the slowest 10% of trials.*

2.4.2 Effect of CLAS on sleep oscillations

Next I wanted to study the effect of CLAS as a whole on the oscillations of sleep, first I calculated the event related potential (ERP) for SHAM and STIM as a grand mean for all participants, see Figure 11. It is clear from inspection of Figure 11, that there was a difference in the shape of the response between SHAM and STIM. A Monte-Carlo cluster analysis indicated the two signals were significantly different during several periods of time. Indeed, the two signals are statistically distinct for the entire time window tested, apart from where they cross as STIM crosses from positive to negative voltage. In Figure 11, pink boxes indicate time when STIM voltage was greater than SHAM voltage (0.04 - 0.31 s: cluster=564.48 *p*=.020; 0.85 - 1.424 s: cluster=2727.40 *p*<.001) while blue boxes indicate time when SHAM was greater than STIM (0.38 - 0.79 s cluster=-1441.00 p<.001; 1.55 — 1.99 seconds: cluster=-849.07 *p*=.004). This difference in voltage as a result of stimulation is precisely that which was expected following CLAS as indicated in previous studies using this stimulation over one night and seeing an impact of stimulation on behaviour (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017).

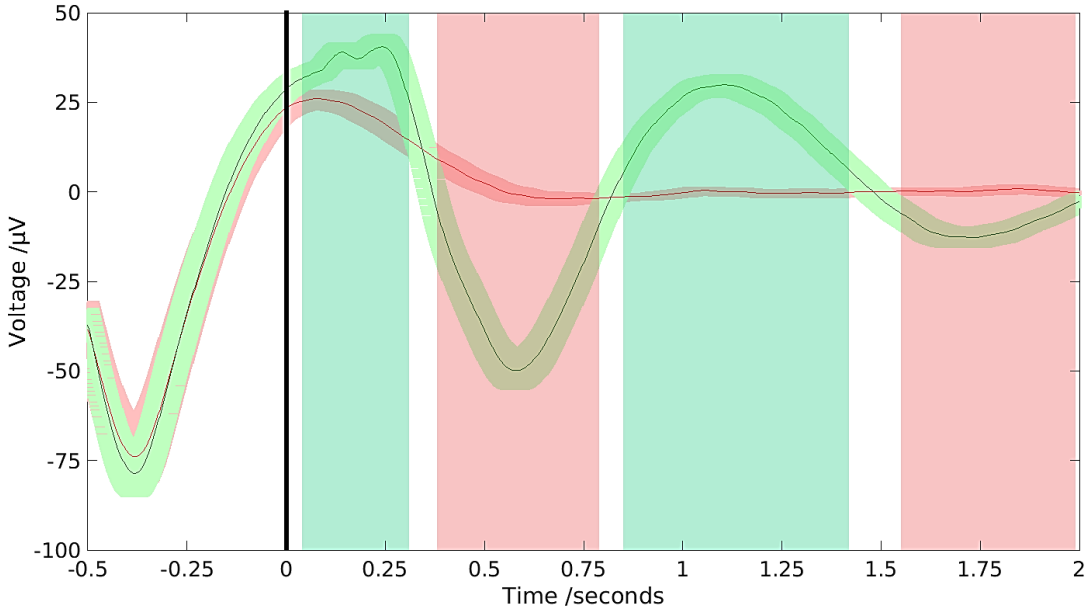


Figure 11: Grand mean of participants event related potential (ERP) response to SHAM (red) and STIM (green) trials. Mean of frontal channels (F3, Fz, F4), shaded areas show SEM. Sound presented at time=0 (solid bar). Shaded boxes indicate times of significant difference in signal, red SHAM>STIM, green SHAM<STIM.

To assess the impact of stimulation on sleep oscillations, I examined the SO trough amplitude and SO, fast and slow spindle density and power, see Table 3. This showed a significant increase in the mean SO trough amplitude during STIM nights. This fits with the voltage difference seen in the SHAM and STIM ERP in Figure 11. There was also significantly larger SWA in STIM than SHAM, but no significant difference in SO density, although it fell slightly from SHAM to STIM, this is consistent with the findings of Ngo, Martinetz *et al.,* (2013). These results would suggest CLAS led to larger, potentially less frequent SOs, in keeping with previous literature (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Santostasi *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017; Henin *et al.*, 2019; Navarrete *et al.*, 2019; Prehn-Kristensen *et al.*, 2020).

Spindle density and power was also assessed, split into fast and slow spindles: Slow spindles showed a significantly higher density in SHAM nights compared to STIM, and a significantly higher power, see Table 3. Fast spindles did not differ in density but did exhibit higher power in SHAM, see Table 3. It is more difficult to compare such results with previous studies as many don't report spindle measures or use one

frequency band for all spindles or different bands from those used here for fast and slow spindles.

The increase in SO amplitude and SWA was expected, and as there was no significant increase in the density of SO, this implies that the increase in power is driven by the increase in amplitude. The decrease in slow spindle density and power was unexpected. Also unexpected was the decrease in fast spindle power although the density decline was not significant and could thus be driven by a decrease in the amplitude of fast spindles. This decrease was unexpected as previous CLAS studies have reported increases in fast spindle power following CLAS (Schneider *et al.*, 2020), or spindle power generally (Henin *et al.*, 2019). Although others reported no change (Ong *et al.*, 2016), while many correlate spindle increases only at specific times in the SO (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015). Not all studies showing spindle increases have seen memory benefits (Henin *et al.*, 2019; Schneider *et al.*, 2020). It has been proposed that the coupling of spindles to SO is more important to consolidation than the frequency of spindles (Ngo *et al.*, 2015).

Measure		***SHAM***		***STIM***		***Statistics***
SO trough Amplitude		*-75.30*	*±4.66*	*-91.40*	*±11.20*	***z(13)=85.00 p=.042***
Density per min	*SO*	*13.38*	*±0. 70*	*11.07*	*±0.59*	*t(13)=1.734 p=.107*
	Slow spindles	*1.07*	*±0.11*	*0.79*	*±0.09*	***t(13)=2.38 p=.034***
	Fast spindles	*1.84*	*±0.17*	*1.60*	*±0.18*	*t(13)=1.42 p=.178*
Power	*SWA*	*0.75*	*±0.02*	*0.78*	*±0.02*	***t(14)=-3.33 p=.005***
	Slow spindle	*0.03*	*±0.003*	*0.03*	*±0.002*	***z(14)=120.00 p<.001***
	Fast spindle	*0.02*	*±0.01*	*0.02*	*±0.01*	***z(14)=109.00 p=.003***

Table 3: *CLAS effects on sleep oscillations. SHAM and STIM mean ±SEM. Significant measures highlighted in bold. All measures assessed during stimulation time only. SO measures are a mean of frontal electrodes (F3, Fz, F4) while spindle measures are a mean of central electrodes (C3, Cz, C4).*

2.4.3 Effects of CLAS on behaviour

As stimulation had the expected effects on sleep despite the changes to stimuli duration and ISI I proceeded to investigate the effects of one night of CLAS on behaviour in the SRTT and MST tasks.

Serial reaction time task

To give a descriptive overview of responses across the entire task the mean RT for all participants at each block in SHAM and STIM tests was assessed, see Figure 12. As can been seen from inspection of Figure 12 the SEM overlaps between SHAM and STIM at each block, significance testing using a Monte-Carlo method also indicated no clusters of significant difference between RT. This shows that CLAS did not influence RT performance on any block in this task.

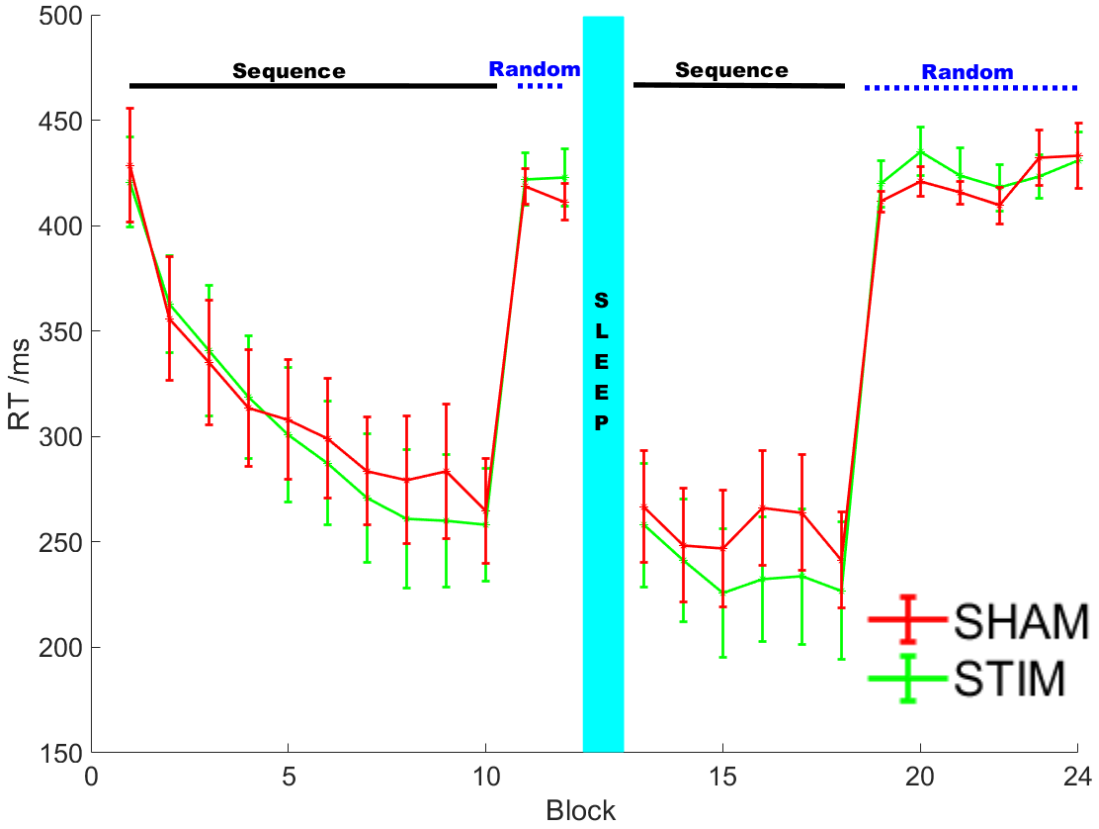


Figure 12: *Mean RT at each SRTT block. Pre sleep: blocks 1-12 and post-sleep: blocks 13-24. Random blocks at 11, 12 and 19-24. Mean ±SEM.*

To investigate changes in SKILL across the experiment I calculated the overnight percentage change in SKILL: Both conditions showed an improvement in SKILL overnight (SHAM mean=27.25% SEM=11.86%, STIM mean=18.80% SEM=11.86%), as can be seen from inspection of Figure 13. However, there was no significant difference between conditions in SKILL improvement ($z(10)=-8.00$ $p=.765$). Overnight percentage difference in the mean RT from the final two sequence blocks of each task falls, under SHAM (mean=-8.64% SEM=4.73%) and STIM (mean=-17.90% SEM=7.61%). However tests indicate no significant difference ($z(10)=-10$, $p=.424$).

Overnight the RT of the final two random blocks does not change in SHAM (mean=0.55% SEM=1.63%) or STIM (mean=1.47% SEM=2.00%, $t(10)=0.50$ $p=.626$).

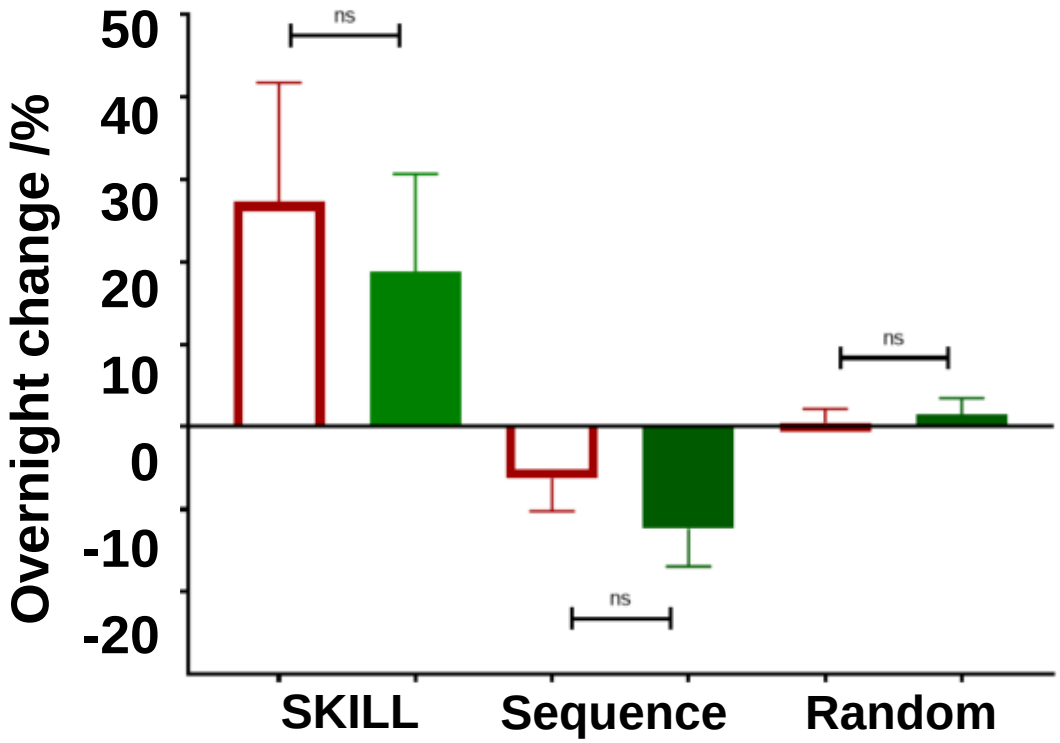


Figure 13: *SRTT performance. Red=SHAM, Green=STIM. Group mean ±SEM. Sequence specific skill (SKILL).*
Overnight change =(Morning-Evening)/Evening×100.

At the end of their SRTT participants were asked to write out the sequence as an explicit recall test. As inspection of Figure 14 reveals, scores showed no difference between SHAM (mean=9.91 SEM=0.98) and STIM (mean=8.818 SEM=1.31, z(10)=-6.00, $p=.750$), it could be the case that there were ceiling effects in this test as many subjects scored 100% (12/12).

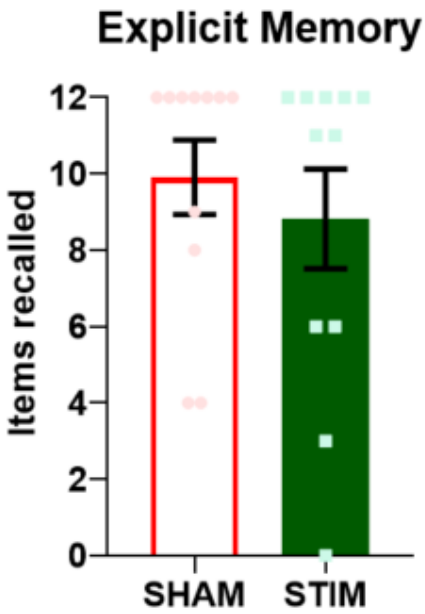


Figure 14: *SRTT explicit recall scores. Mean ±SEM. Maximum score=12.*

Mnemonic similarity task

Next I shall consider whether CLAS had any effects on the performance on the MST. Scores were calculated for pattern separation (PSS) and recognition memory (RMS), and normalised as a percent of the learning score for each participant, thus if participant's performance stayed the same the morning after sleep their score would be 100%. As can be seen from Figure 15 A, on both SHAM nights (mean=66.16% SEM=7.52%) and STIM nights (mean=70.57% SEM=7.95%), mean PSS performance became worse overnight, as scores were less than 100%. Statistical testing indicated no significant difference between conditions ($t(12)=0.33$ $p=.744$). Similarly, inspection of Figure 15 B shows that RMS scores in SHAM (mean=76.82%, SEM=4.90%) and STIM (mean=80.88%, SEM=3.64%) declined overnight to a similar degree ($z(12)=29.00$, $p=.340$). For both scores this appears to be more pronounced in the SHAM group compared to the STIM but there is no significance difference.

Target stimuli were then divided into similarity bins, based on how similar they were to their lures and therefore how difficult they were to accurately discriminate (1 most similar to 5 least similar). PSS scores and their absolute change overnight were then calculated. Inspection of Figure 15 C, does not reveal a clear trend from similar to dissimilar images, but it does appear that STIM led to a smaller decrease in bins 2 (SHAM mean=-2.33 SEM=0.64, STIM mean=-0.75 SEM=0.76) and 4 (SHAM mean=-

2.92 SEM=0.75, STIM mean=-1.25 SEM=1.11). However, an ANOVA with stimulation, and similarity bin as within participant factors indicated there was no significant main effect of stimulation ($F(55)=1.45$ $p=.234$) nor bin ($F(55)=0.16$ $p=.959$), or interaction ($F(4, 55)=0.78$ $p=.546$).

Altogether this implies one night of CLAS delivered in the sleep laboratory did not affect performance on the MST task as measured through PSS and RMS.

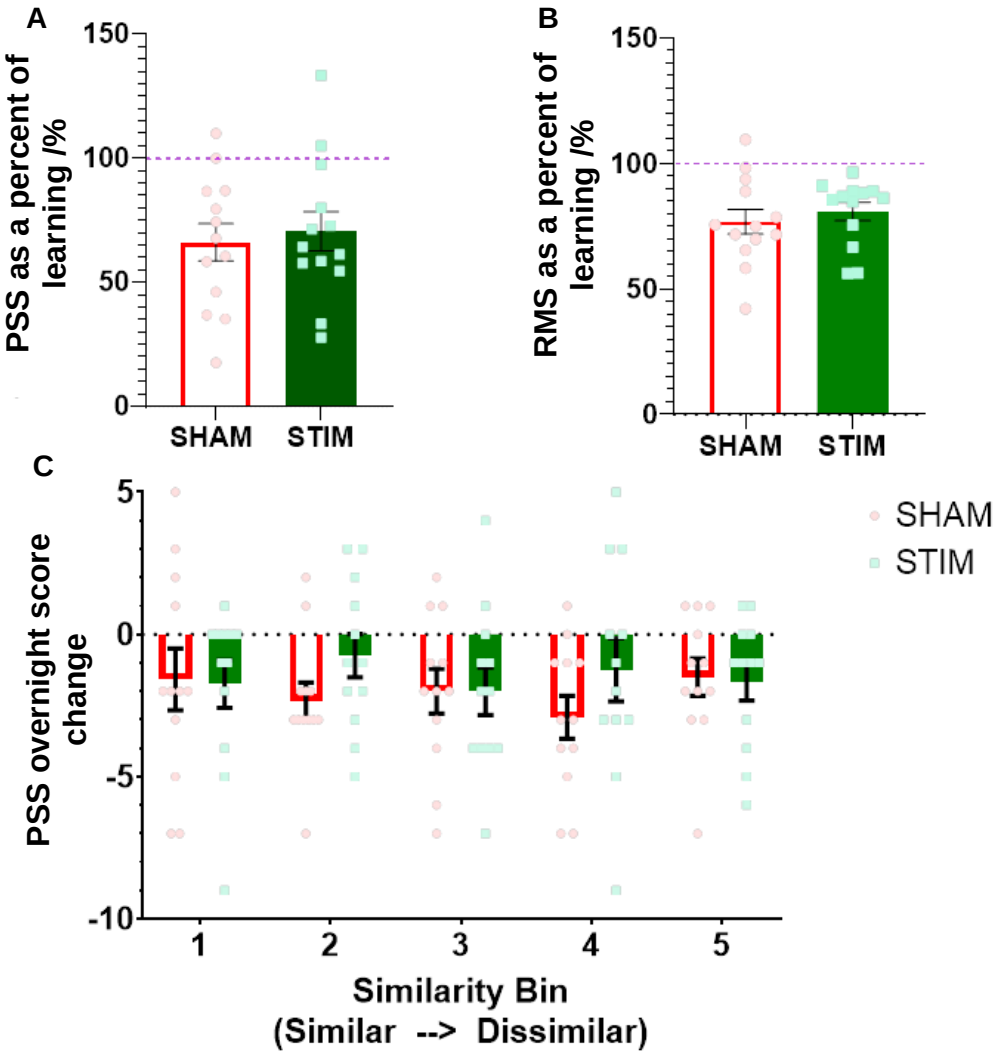


Figure 15: *Pattern separation and recognition memory score from MST.* **A;** PSS change overnight as a percentage of learning mean ±SEM, **B;** RMS change overnight as a percentage of learning mean ±SEM and **C;** PSS change overnight broken down by similarity bin, absolute change.

2.4.4 Effect of adjusting sound duration and ISI

A secondary question posed in this chapter was if the ISI between sounds could be optimised by counting the duration in the number of passing SO instead of the number of passing seconds or sine waves, as per the Ngo, Martinetz *et al.,* (2013) threshold method of CLAS and the PLL method (Santostasi *et al.*, 2016) respectively. As such I wanted to understand the impact of varying the duration of the ISI by one, two and three SOs on the electrophysiological response from the brain. I was also interested if increasing the duration of the sound itself from 50ms to 100ms could affect the brain response. Section 2.4.2 explores the combination of all ISI and stimuli durations tested and resulted in the expected brain responses, but what of the differences between conditions tested?

Duration ERP

Two durations of stimuli were tested 50ms and 100ms, the effect of each duration on the resulting ERP is displayed in Figure 16 A. Inspection of Figure 16 A, shows that the application of 50ms and 100ms stimuli both elicit comparable ERP responses from the sleeping brain. The only significant (cluster=-439.26, p=.010) divergence in response to each duration sound comes between 1.162s and 1.444s after stimuli onset. In the first trough after the stimuli there appears to be a greater influence of the 100ms stimuli, leading to a deeper trough. However, this was not statistically significant using a cluster analysis. As the ERP rise into the second peak the influence of the two different stimuli seem to become indistinguishable again before separating around the time of the second peak. To see if the difference in signal was caused by an increase in the presence of spindles during this time (indicated in the yellow box in Figure 16 A), the normalised fast and slow spindle PSD during this time was calculated, shown in Figure 16 B. As inspection of Figure 16 B shows, there is little difference between duration conditions, in fast (50ms: mean=0.06 SEM=0.01, 100ms: mean=0.06 SEM=0.01) or slow (50ms: mean=0.03 SEM=0.003, 100ms: mean=0.03 SEM=0.003) spindle power during this time for 50ms or 100ms trials (fast: z(14)=48.00 p=.525; slow: z(14)=46.00, p=.454).

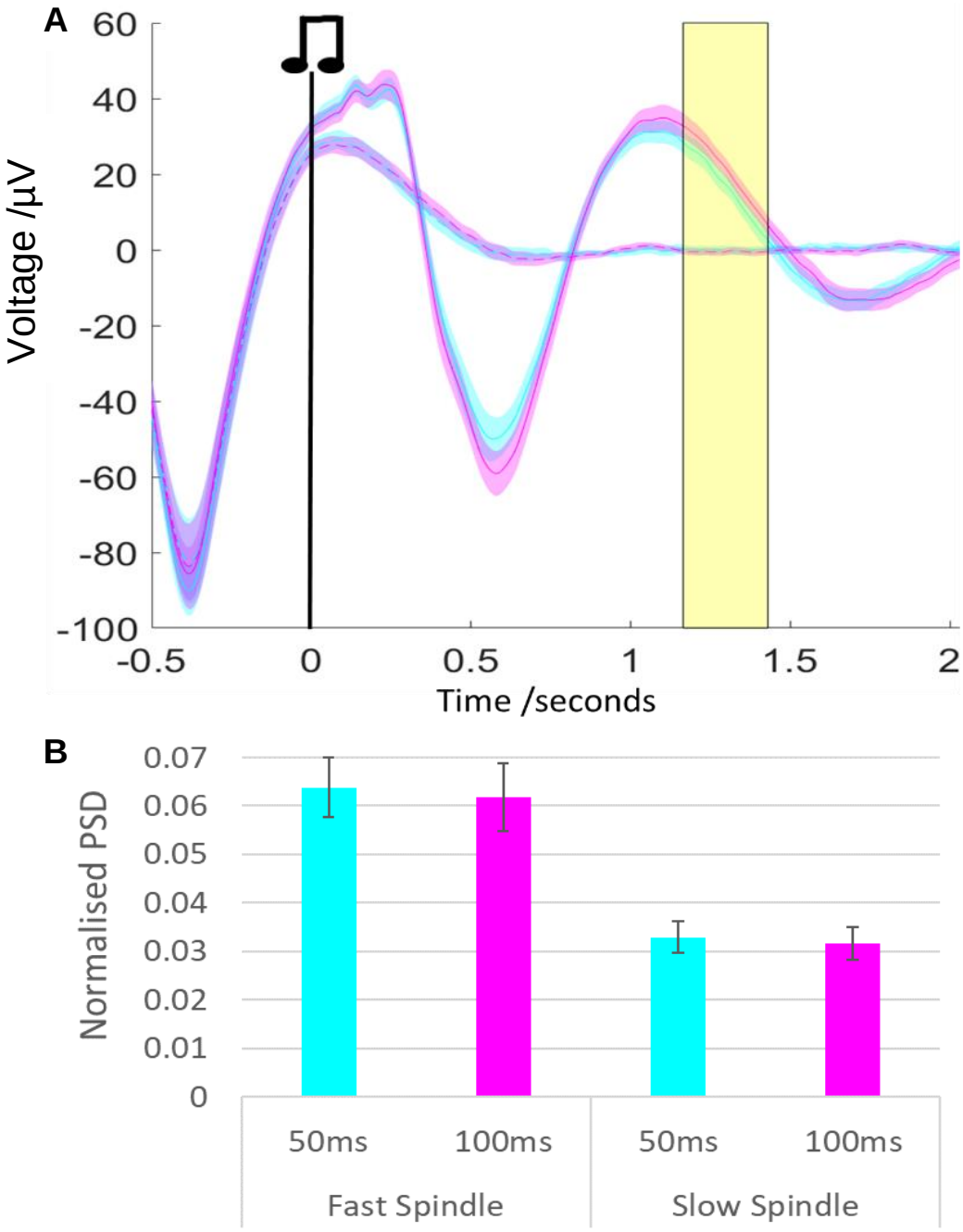
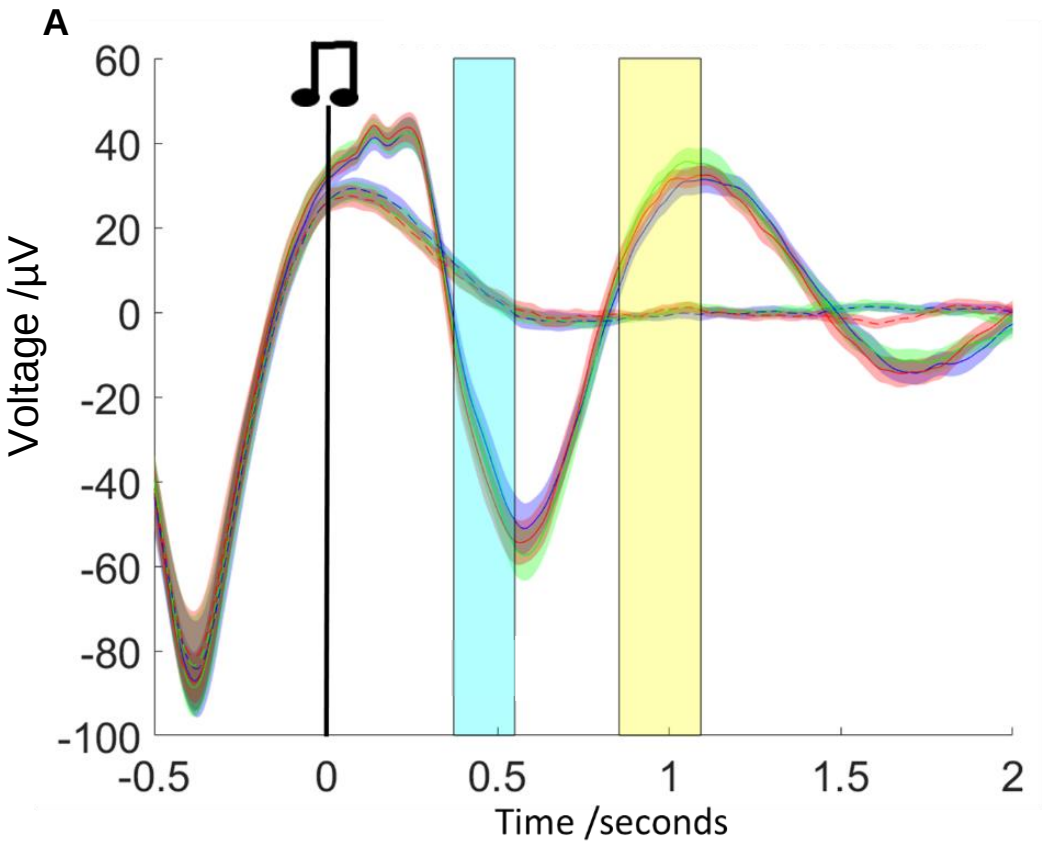


***Figure 16**: ERP response to 50ms and 100ms duration sounds and spindle power. A: Stimuli presented at time=0. Blue=50ms stimuli and pink=100ms stimuli. Dashed lines show SHAM trials, while solid lines show STIM trials. The yellow box indicates the signal from 100ms>50ms in STIM trials only, during this time. B: Normalised power in fast and slow spindle bands during significant cluster in A. Mean ±SEM.*

ISI ERP

Three ISI were tested: 1SO, 2SO and 3SO, as can be seen from observation of Figure 17 A, all three ISI elicit comparable ERP. Statistical analysis showed significantly greater activity in 1SO than 3SO trials, between 0.37s and 0.55s (cluster=311.21 *p*=.013, Figure 18 A, blue box). Analysis also showed greater activity in 2SO trials than 1SO during 0.85 s to 1.11 s (cluster=-418.12 *p*=.010, Figure 17 A, yellow box).

Again to see if significant difference in ERP were influenced by spindles I assessed the power of fast and slow spindles in both of the significant clusters indicated in Figure 17 A. Inspection of Figure 17 B indicated the normalised power in fast and slow spindle bands during trial time indicated to show 1SO>3SO in ERP voltage. As can be seen from the figure there was a small increase from 1SO (fast: mean=0.03 SEM=0.003, slow: mean=0.03 SEM=0.002) to 2SO (fast: mean=0.03 SEM=0.003, slow: mean=0.03 SEM=0.002) to 3SO (fast: mean=0.03 SEM=0.003, slow: mean=0.03 SEM=0.003) in fast and slow spindles. However, SEM overlap between ISI and an ANOVA with ISI (1SO, 2SO and 3SO) as within participant factors, indicated no significant main effect of ISI (fast: $F(28)=1.75$, $p=.192$; slow: $F(28)=2.79$, $p=.078$). A similar trend is apparent in the second significant cluster, shown in Figure 17 C for fast spindles (1SO mean=0.06, SEM=0.01; 2SO: mean=0.06, SEM=0.01; 3SO: mean=0.06, SEM=0.01). Closer inspection of Figure 17 C indicates that slow spindle power in 2SO (mean=0.02 SEM=0.002) ISI trials contained less power than 1SO (mean=0.03 SEM=0.003) or 3SO (mean=0.03 SEM=0.003) trials. Inspection of Figure 17 C shows that there is twice as much power in the fast spindle band, as the slow spindle band for that second cluster, across all ISIs. This is also twice as much power than in the fast or slow spindle bands in the first cluster (see Figure 17 B). Again statistical testing using an ANOVA of power in both spindle bands in the second cluster (Figure 17 C) indicated no significant main effect of ISI (fast: $F(28)=0.38$, $p=.685$; slow: $F(28)=1.64$ $p=.211$).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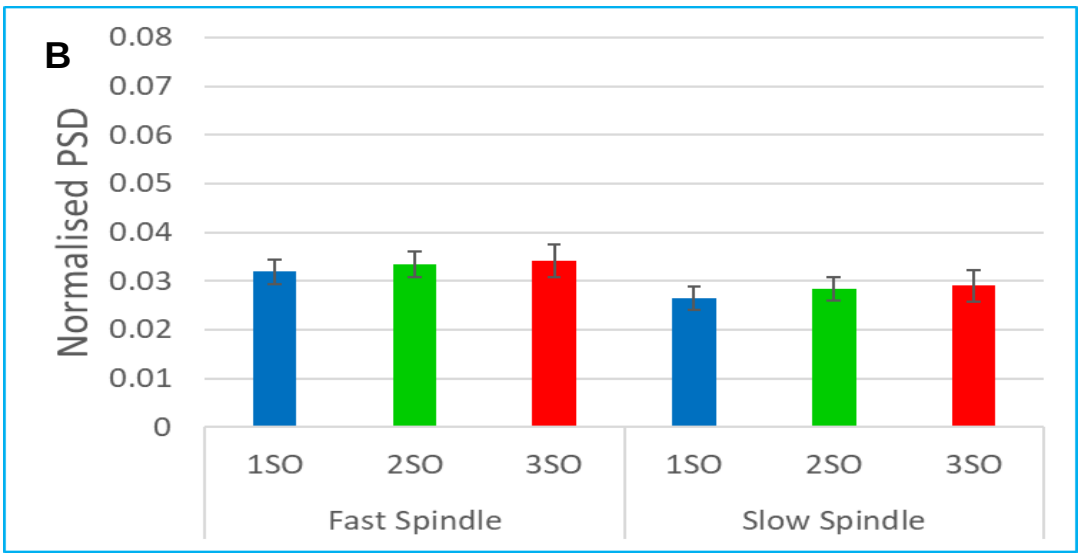
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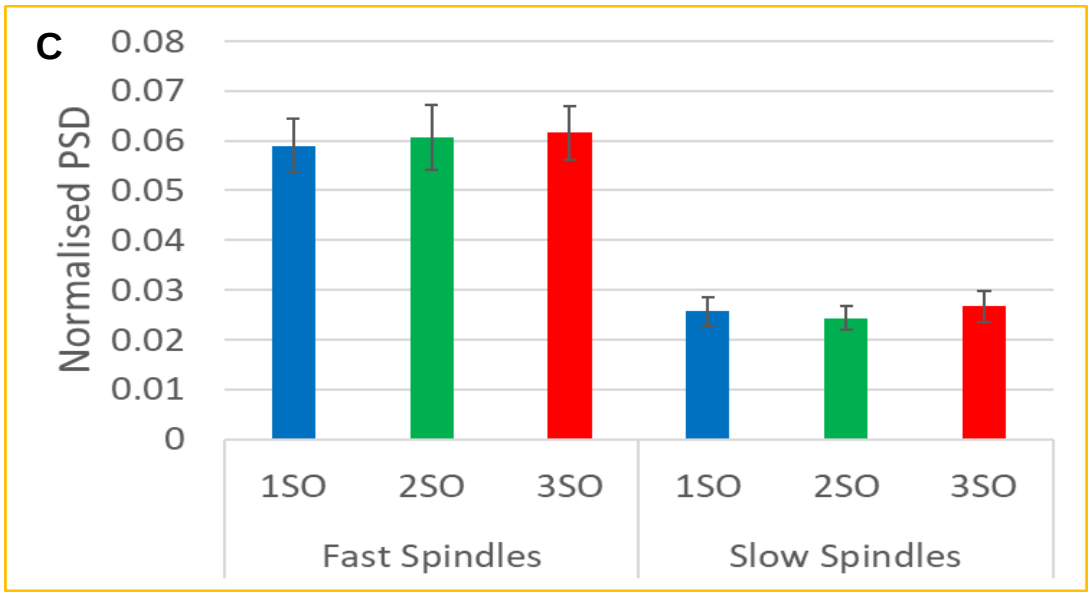


Figure 17: *ERP response to ISI variation and spindle power in significant clusters* ***A:*** *Stimuli presented at time=0. Dashed lines indicate SHAM solid indicate STIM. blue=1SO ISI, green=2SO ISI and red=3SO ISI. Blue box indicates 1SO>3SO signal while yellow box indicates 2SO>1SO signal. P values for significant differences presented.* ***B*** *and* ***C:*** *fast and slow spindle power in significant cluster time form A. Mean ±SEM. B: First cluster indicated In A by blue box, C: Second cluster indicated in yellow box in A.*

2.5 Discussion

In this chapter I aimed to understand the effect of one night of CLAS delivered in the sleep lab, on the overnight change in performance in the SRTT and MST tasks. Results indicate that stimulation did not significantly affect performance changes over one night in either task. I will discuss some theories as to why one night of CLAS was insufficient to boost SRTT or MST memory.

I was also interested in how changing the duration of the ISI and sound, would affect the response from the brain. Results indicated that despite a short duration where 100ms trials showed higher voltage than 50ms trials, there was no major difference in the ERP elicited by the sound. Indeed, there was no difference in the power of fast or slow spindles during this time despite my hypothesis this could have been driving the ERP voltage difference. Results were similar for ISI, where there was a short duration where 1SO ISI lead to larger response than 3SO, and another duration where 2SO led to a larger response than 1SO. However, as with sound duration the

differences were not sufficient to alter the ERP drastically and power calculations indicated no significant difference between ISI trials.

2.5.1 Effect of CLAS on behaviour

Despite having the expected effects on the microstructure of sleep, such as increased SO amplitude and power, on this occasion CLAS has no discernible impact on the performance of the tested memory tasks. I had hypothesised that due to the dependence of SRTT on SWS (e.g. Spencer, Sunm and Ivry, 2006; Cousins *et al.*, 2014, 2015), boosting SWS oscillations using CLAS would lead to task improvements. However, results indicated no difference between SHAM and STIM nights in terms of SKILL, sequence block RT or random block RT on the SRTT. Previous studies testing the impact of one night of CLAS on a finger tapping task (an explicitly learnt procedural task similar to the SRTT, see General introduction section 1.6.2) found no impact of stimulation on the task (Leminen *et al.*, 2017; Schneider *et al.*, 2020). There has been some implication that only explicitly learnt motor sequences, are improved by sleep (Robertson, Pascual-Leone and Press, 2004). However, even if the SRTT procedure used here is considered implicit, this is not likely a reason why I did not see influence of CLAS as results showed participants had explicit knowledge of the sequence, indeed many participants could recite the entire 12-item sequence. Also the FTT, which is usually considered more explicit in its learning than the SRTT, did not show an improvement following CLAS either (Leminen *et al.*, 2017; Schneider *et al.*, 2020). Early work did link performance on motor learning tasks to REM sleep rather than NREM sleep (Maquet *et al.*, 2000; Fischer *et al.*, 2002; Peigneux *et al.*, 2003), as CLAS only affects NREM sleep (Ngo *et al.*, 2015; Leminen *et al.*, 2017; Papalambros *et al.*, 2017) this could result in the lack of effect. Although more recent work has highlighted the importance of NREM in this task so this is unlikely. Therefore, it could be argued that CLAS does not improve performance on such motor procedure tasks. Results could imply that despite the declarative sequence influence on the SRTT the memory for the sequence is not consolidated in a way that it can benefit from CLAS. Or at least any changes in memory are not apparent in the motor RT responses.

Participants did, as expected, become faster overnight, as shown by the continued decrease from learning in sequence blocks, the increase in skill (SKILL) and decrease in overnight percentage change in RT of sequence blocks. This fits with the hypothesis that sleep is benefiting SRTT performance, such that a retention interval

containing sleep leads to a greater improvement in performance than an interval without sleep (Robertson, Pascual-Leone and Press, 2004; Brown and Robertson, 2007; Cousins *et al.*, 2015).

Explicit memory did not differ significantly between SHAM and STIM nights, although most participants scored very high on both occasions, so results could be limited by a ceiling effect. Cousins *et al.,* (2014) showed that TMR of an SRTT sequence during SWS led to significantly greater explicit recall of the sequence following sleep. However, participants in Cousins experiment showed much poorer explicit recall of the sequence than in our experiment, scoring on average <5 out of 12 (cued sequence) and <2 out of 12 (un-cued sequence). This also adds evidence that in this chapter any explicit performance gains were blocked by a ceiling effect. To test this further, fewer training blocks of sequence could be used, or participants could be taught two sequences simultaneously, as they were in Cousins *et al.,* (2014) as this may impede explicit learning.

In this chapter I also assessed the impact of one night of in-lab CLAS on PSS and RMS scores in the MST task. I had previously hypothesised that due to previous work implicating the hippocampus and SWS in pattern separation that CLAS would improve PSS performance but not RMS. While results showed no improvement in RMS they also showed no improvement following stimulation in PMS. As no other work has been published assessing the impact of CLAS on pattern separation it is difficult to compare this result to any previous work. Leminen *et al.,* (2017) did investigate the effect of one night of CLAS on image memory, and like RMS in this chapter they found no difference in recall following stimulation. As for PSS; Hanert *et al*., (2017) who compared performance in this task between wake and sleep groups, found that sleep led to a smaller decrease in PSS score overnight compared to wake. However, as highlighted by Poh and Cousins (2018), the significantly larger decrease in PSS score seen in the Hanert *et al.* (2017) wake group, could imply that the group difference was driven by across-wake forgetting. Some have argued that pattern separation is mainly involved with encoding of the new information into the hippocampus (Hunsaker and Kesner, 2013) not the process of consolidation which leads memories to be stored in the cortex (McClelland, McNaughton and O'Reilly, 1995). Indeed, the mechanisms in the hippocampus involved with pattern separation and completion are unknown in sleep. Thus it is perhaps unsurprising that CLAS did not affect PSS memory.

It would appear that CLAS in one night is insufficient to cause significant changes in the performance of either of these tasks.

2.5.2 CLAS influence on sleep macrostructure and microstructure

Stimulation did not affect sleep macrostructure or arousal when measured either objectively or subjectively, this is in line with all other studies of CLAS which did not find overall changes to sleep following stimulation (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016, 2018; Santostasi *et al.*, 2016). I also assessed the microstructure of oscillations when the duration of the sound and ISI was altered.

I tested two durations of sound; 50ms and 100ms and showed that 100ms led to greater amplitude in the ERP response during the falling phase of the second SO following the sound. This falling phase of the SO indicates a time when the neurones involved are hyperpolarising, and when slow spindles are most likely to occur (Mölle *et al.*, 2011). However, further investigation of the power in fast and slow spindle bands during this time did not indicate an increase a difference between durations. As the peak amplitudes of the SO are not different between sound durations it could be that the 50ms is causing neurones to depolarise faster than 100ms which leads to a significant difference at this time. Visually it appeared that there is a non-significant difference at the peaks and troughs of the SO following the sound that 50ms is smaller. This could indicate why hyperpolarisation is happening slightly faster in this instance as fewer cells have been excited. However, there is no significant difference at the peaks of the ERP between sound durations. To further investigate this, experiments could be conducted in rat models or using intracranial electrodes to assess cell recruitment and depolarisation rate.

Overall analysis indicates that the response from the brain is comparable despite doubling the sound duration. This could benefit the use of meaningful sounds in the CLAS procedure to increase the specificity of the memories targeted without compromising on sound placement, as the longer sound could hold more meaning but still be placed at the optimal time in the SO. Such as in closed loop TMR (Göldi *et al.*, 2019).

Similar results arose when I varied the duration of the ISI by counting the passing of one two and three SO. These three durations were designed to cover the ideal ISI (in

seconds) proposed by Ngo *et al.,* (2015) for induction of fast spindles. ERP results showed a significant difference between ISI durations during the rising phase of the SO, the most likely time in a SO to find coupled fast spindles (Mölle *et al.*, 2011),and high fast spindle power. However, the assessment of fast spindle power during this time did not indicate any difference between the tested ISI. The second significant cluster in the ERP which indicated a difference between ISI was during the falling phase, but power assessments of slow spindles at this time again did not indicate any difference.

Together these results could indicate that any of the three ISI tested are sufficient to lead to the desired ERP and spindle effect from the brain. The greater personalisation of the stimulation ISI by counting in SO instead of seconds or sine waves could have led to the small increases in ERP as participants had the ISI more tailored to their ongoing SO rhythm than that imposed by second timing or a fixed wave. As precise sound timing has been shown to be important (Weigenand *et al.*, 2016; Navarrete *et al.*, 2019) this could be significant, particularly in populations with greater differences in SO timing (Navarrete *et al.*, 2019). Results do show that measuring the ISI in SO rather than seconds or sine waves could offer a simpler protocol for CLAS as it utilises the threshold mechanism used to detect which SO to stimulate. Thus this could aid the production of wearable ambulatory CLAS EEG systems.

2.5.3 Conclusions

In this chapter I have shown for the first time that one night of in-lab CLAS does not improve recall performance on the SRTT nor MST tasks. It could be concluded that the mechanisms that confer benefit to these tasks from SWS are not the same as those which are affecting the WP task which is boosted by CLAS. Or that the protocols employed in this experiment were insufficient to record performance changes. Also, as in other studies, the short one-night window for stimulation might be insufficient to boost the activity on these, or similar, memory tasks. Further experiments should be conducted to understand the impact of repeated nights of stimulation on memory recall in these and other SWS linked memory tasks. The word pair task would be a particular task to focus on to investigate if the benefits shown in one night can be continued or even increased with further nights of stimulation.

In terms of longer stimuli duration this experiment shows that 100ms are comparable in their brain response to 50ms which could aid the use of more meaningful sounds

into CLAS without losing the accuracy shown to be important in sound placement. Measuring the ISI in SO also proved to lead to little difference in brain response such that it could be utilised as a simple tool to measure the ISI in future.

Chapter 3
Exploring the effects of repeated nights of auditory stimulation on declarative memory tasks

3.1 Abstract

Slow wave sleep (SWS) is held to be an essential pillar of memory functioning, but much is still unknown about how this sleep stage influences memory. Since its inception in 2013, closed loop auditory stimulation (CLAS) has swiftly become a popular tool to investigate SWS; initially, as a promising technique to boost SWS and improve declarative word pair (WP) memory. However, recent work has cast doubt on the existence and extent of the memory benefits of this stimulation. To increase our understanding of the influence of this stimulation on WP memory this chapter investigates second order elements of the WP task, e.g. motivation, pair association and task procedure, to understand their effects. Also, the impact of CLAS on memory has been restricted to its application on a single night. I sought to answer what influence stimulation may have on declarative memory if applied for seven nights. The Dreem headband, a mobile EEG device, was used to deliver CLAS. 20 young healthy participants received 7 consecutive nights of CLAS and 7 consecutive nights of sham simulation in a counterbalanced order. A WP memory task, an image pair task, and a creative verb generation task, were assessed after one night of CLAS and one night of sham stimulation, and then after 7 nights of these two forms of stimulation. There were no effects of CLAS on behaviour following one or seven nights of stimulation. The results of this experiment adds to a growing body of evidence suggesting that CLAS does not always improve declarative memory.

3.2 Introduction

Slow wave sleep (SWS) sleep has been shown to be integral to the overnight consolidation of memories (see Section 1.3.1). A theory for the mechanism of SWS involvement is Active Systems Consolidation (ASC), wherein memories are transferred from short term storage in the hippocampus to longer term storage in the cortex (for a review see Rasch & Born, 2013). This idea builds on evidence showing that memories become less reliant on the hippocampus with increased time since encoding, which suggests that memories are being transferred from the hippocampus to the cortex (O'Reilly Randall *et al.*, 1994; Winocur, McDonald and Moscovitch, 2001; Payne and Kensinger, 2011; Durrant, Cairney and Lewis, 2013). It has been proposed that this transfer is facilitated by the synchronisation of cortical slow oscillations (SO), thalamo-cortical spindles and hippocampal sharp wave ripples during SWS (Rasch and Born, 2013). CLAS has been shown to influence SO, entraining them, increasing their amplitude, and increasing their coupling with spindles (Ngo, Martinetz, *et al.*,

2013; Ngo *et al.*, 2015; Ong *et al.*, 2018; Grimaldi *et al.*, 2019; Schneider *et al.*, 2020). CLAS has also been shown to improve memory for word pairs (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Leminen *et al.*, 2017; Papalambros *et al.*, 2017; Ong *et al.*, 2018), and it has been hypothesised that stimulation boosts the action of consolidation of memories via the coupling of SO and spindles (Ngo, Martinetz, *et al.*, 2013). However, more recent experiments have failed to observe differences in WP performance as a consequence of stimulation (Diep *et al.*, 2019; Henin *et al.*, 2019). Indeed, one experiment from our lab even indicated a decline in memory following CLAS (Schneider *et al.*, 2019). Moreover, studies that have attempted to examine the generality of the improvements in WP memory to related tasks have also failed (Leminen *et al.*, 2017; Ong *et al.*, 2018; Diep *et al.*, 2019; Henin *et al.*, 2019).

The principal aim of Chapter 3 was to investigate the impact of CLAS on declarative tasks linked to the WP task, to understand the conditions under which (associative) memory processes could be improved by CLAS. To do so, this chapter examines the influence of CLAS on three tasks: (1) a WP task in which different stimuli were tagged with differing levels of importance through reward (WPr); (2) an image based paired associates learning (iPAL) task; and (3) a verb generation task (VGT) in which upon presentation of a noun a verb has to be generated.

The study also investigated how the consolidation of memories changed over multiple nights following encoding, and examined whether or not the influence of CLAS on memory recall changed if stimulation was applied for more than one night following learning. It has been proposed that the benefits of sleep upon memory are initially through reactivation, but longer term, benefits are derived through more structural plastic changes in synapses and white matter tracts which occur over subsequent days and weeks (review see (Pereira and Lewis, 2020) or (Almeida-Filho, Queiroz, & Ribeiro, 2018). The impact of CLAS on the brain's initial response to the sound is undiminished when delivered over up to ten nights (Debellemaniere *et al.*, 2018), and this suggests that CLAS might continue to promote consolidation over consecutive nights. To the best of my knowledge, however, no other study has investigated the effects of CLAS on memory performance over more than one night. Here, training on the three tasks occurred on day one prior to seven nights of CLAS delivered at home using a portable EEG device (Dreem headband). Memory for items encoded on day one was then assessed on day two and day eight. This procedure enables a comparison with the previous literature on the effect of one night of stimulation on

memory performance, while also allowing exploration of any changes in the impact of CLAS between night one and the subsequent six nights.

3.2.1 The three tasks

Using reward to tag important stimuli (WPr): There is a finite amount of time during sleep to replay and thus support the consolidation of memories; and it has been proposed that memories can be tagged according to their importance and these tags determine which memories are prioritised for consolidation (Born and Wilhelm, 2012; Stickgold and Walker, 2013; Cairney *et al.*, 2018): Important memories are marked out as such, and undergo more reactivation than non-tagged memories. I investigated whether CLAS preferentially boosted memories that had been marked as of higher importance in the WPr task. The importance of each word pair was denoted by instructing participants that their correct recall would result in a monetary reward. The preferential improvement of WP memory for pairs associated with a monetary reward following a night of CLAS, has been demonstrated in healthy children by Prehn-Kristensen et al., (2020). They found that on stimulation nights the word pairs associated with future reward were better recalled than those that were not associated to reward. This observation is consistent with the idea that CLAS can impact memory for words associated with monetary reward preferentially over words not associated with reward. They also showed that participants forgot (slightly) fewer reward associated words after CLAS than SHAM stimulation, and forgot more non-rewarded words under STIM than SHAM conditions. This pattern of results could imply that the improved recall of reward associated items was at the expense of the non-reward items during STIM night. To the best of my knowledge no one has replicated this finding in an adult population. Therefore, I added a monetary reward to some of the WP stimuli used in this experiment to examine if CLAS preferentially lead to better recall of these word pairs. Here, participants received three WP lists: For one list, they were informed prior to learning that correct recall of the word pair would be associated to a 5p/correct pair reward upon recall (Prehn-Kristensen *et al.*, 2020); for the second list they were informed after learning that correct recall would be associated with the same reward (Fischer and Born, 2009); and a third list that was not associated with instructions concerning future rewards. The list with reward notification following learning was added to remove potential increased encoding attention on stimuli with prior knowledge of reward at learning, leading to increased recall independent of consolidation (Fischer and Born, 2009).

Memory for paired images (iPAL). Paired associate learning involving images (iPAL) is also thought to rely on declarative memory (Bergmann *et al.*, 2012; Leminen *et al.*, 2017). While there is evidence showing that CLAS does not improve encoding (Ong *et al.*, 2018; Schneider *et al.*, 2019) or consolidation (Leminen *et al.*, 2017) of un-paired images, there have been no studies of the effect of CLAS on paired-associate learning involving images. Here, I studied the impact of CLAS on this task to examine the generality of the effects observed with the word pair task.

Verb generation (VGT): The final task was one that shared some of the features with the WP task, but instead of involving the generation of a learned associate of a given word, the task required the generation of a creatively related verb when prompted with a noun (e.g., responding *sip* to the word *mug*; Prabhakaran, Green, & Gray, 2014). This task was originally designed to test participant creativity, a behaviour thought to rely on REM sleep, which is not affected by CLAS (e.g. Ngo et al., 2015). For the present purposes, the verb generation task (VGT) was considered potentially useful because it shared many features with the WP task, but involved no consolidation of learned associations. Therefore, I did not expect it to be improved by CLAS, which has not been shown to influence REM.

3.2.2 At home closed loop auditory stimulation

Recent development of ambulatory EEG devices, has allowed the delivery of CLAS in the home environment (Debellemaniere *et al.*, 2018; Garcia-Molina *et al.*, 2018; Ferster, Lustenberger and Karlen, 2019). These new devices are designed to be utilized by participants themselves and avoid the need for participants to come into sleep laboratories to have polysomnography applied in order to deliver CLAS. This opens up avenues for low-cost testing over several nights within a participants own environment. This study utilized the Dreem headband as one such ambulatory dry EEG device which can deliver CLAS, along with online behavioral testing (Pavlovia) to allow for sleep stimulation over a long-term and continued data collection during a global health crisis (Covid-19).

3.2.3 Experiment outline

To investigate the impact of CLAS over one and multiple nights on the performance of the three tasks, participants slept at home for 14 nights, and used the Dreem headband, a mobile EEG device capable of delivering CLAS. On seven consecutive nights the headband delivered CLAS (STIM) and on seven consecutive nights it

delivered no stimulation (SHAM, see Figure 18). The order in which participants completed the SHAM and STIM weeks was counterbalanced and they were blind to the condition in each week. Between the two weeks of testing, participants had a break from wearing the headband of at least five days. Participants completed the three behavioural tasks (WPr, iPAL and VGT) online from home on days one, two and eight (see Figure 19). This allowed behavioural performance with and without CLAS to be compared within participant, across the first and the subsequent six nights of the experiment. The entire study was carried out remotely so as to comply with national Covid-19 restrictions during July 2020 to June 2021.

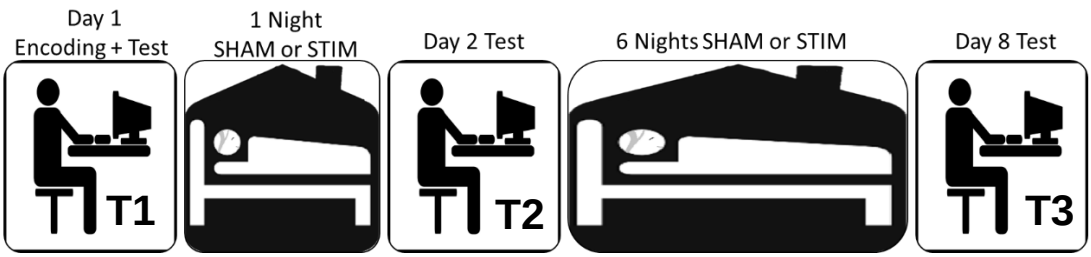


Figure 19: *Experiment outline*

3.3 Methods

3.3.1 Participants

30 healthy individuals were recruited and completed the experiment (14 males; mean age = 24.6; range = 19-30). Participants were screened using an online questionnaire, and were excluded if they indicated any conditions or medications which could affect their sleep, visual or auditory impairments, or learning disabilities. Seven participants were removed due to the first night of STIM being missing from headband recordings due to technical issues with the Dreem headband; two additional participants were removed as they failed to use the headband correctly (leading to multiple missing nights of sleep recording); and a further participant was removed due to no stimulation being sent in the first night of the STIM week. The final analysis included 20 healthy young adults (13 males; mean age = 25.0; range = 20-30). The experiment was carried out under approval from Cardiff University School of Psychology ethics committee (ethics approval number: EC.19.07.16.5657R2A9). Procedures were also adapted to remain compliant with the Covid-19 precautions devised by Cardiff University School of Psychology during July 2020 to June 2021. Participants were paid £48 for their time spent participating in this study (plus extra dependent upon WPr performance of up to £10).

3.3.2 Materials

Sleep Monitoring and Closed Loop Auditory Stimulation

CLAS was delivered at home to participants using the Dreem™ Headband: A dry ambulatory EEG device described in Debellemaniere *et al.,* (2018), henceforth known as the headband.

Figure 19*: The Dreem Headband. The left image shows the device itself and right image shows how it is worn by participants. The headband is fitted with a range of sensors: an accelerometer to measure movement of the headband, a pulse oximeter to measure participant hart rate, bone conduction speakers to deliver sounds and five dry conductive polymer EEG electrodes. Three of the electrodes are placed over the forehead and are close to the electrode 10-20 notation positions of F7, Fpz and F8. The 2 rear electrodes are close to O1 and O2. Left image adapted from Arnal et al.,* (2020).

The device shown in Figure 19, was fitted with 6 electrodes, approximately in the equivalent positions of the 10-20 system of F7, F8, 2 Fpz (one referenced to O1, one referenced to O2), O1 and O2. The device also housed an accelerometer to measure movement, and a pulse oximeter to measure heart rate. Sounds were delivered via bone conduction at a volume of approximately 40dBA, which is around the same as a quiet library. EEG was sampled at a rate of 250Hz, and band-pass filtered at 0.4-18Hz; while the accelerometer sampled at 50Hz (Debellemaniere *et al.*, 2018). During the night the headband used a 2s sliding window to adaptively select the cleanest

channel to detect SWS, and used these selections to create a virtual detection channel (Debellemaniere *et al.*, 2018). The phase of the SO to target was then estimated on this virtual channel using phase fitting, where five sinusoidal waves at a frequency of 0.8 to 1.2 Hz were fitted to the detected N3 signal (Cox *et al.*, 2014). The algorithm aimed to play the sound such that it coincides with the time at which the phase angle of the target SO is at 45° (during the rising phase of the SO, Debellemaniere et al., 2018). The headband also had in place a number of restrictions which would prevent it from playing a sound at the wrong time: (1) It must wait 15 min from the first detection of N3; (2) sounds could not be sent less than 3 min since a major body movement; and (3) it would not play sounds 4h after the first detection of N3 sleep. When the headband determined it was the optimal time to play the sound, it sent two 50ms pulses of pink noise, via bone conduction at 40dBA, each aimed at the rising phase of consecutive SO. Following two bursts of 50ms noise the algorithm waits 9s before sending another two sounds. Stimulation therefore used a longer inter-stimulus interval (ISI) than those investigated in Chapter 2. Approximately 50% of stimulations were sent with no sound, to act as control conditions, randomly interspersed amongst sound trials. If a change of sleep stage was detected, then the algorithm paused stimulation for 30s.

The sleep scoring algorithm implemented in the headband has been compared by Arnal *et al.,* (2020) to the scores of five human scorers on polysomnography (PSG) measurements collected concurrently. They found that agreement between the headband and scorers was 74.0% for Wake, 47.7% for N1 sleep, 82.9% for N2, 82.6% N3 and 84.5% for REM. The low agreement with N1 sleep is consistent with my observations that there were very few epochs of N1 sleep detected by the headband. As such, headband detection of N1 was not deemed reliable and is not reported in this chapter.

Online behavioural testing

Due to the ongoing Covid-19 pandemic, study protocols and operating procedures were adjusted to reduce risk of disease transmission, including minimising face-to-face contact with experimenters and permitting participants to undertake behavioural testing online from home. Care was taken to provide participants with detailed instructions to minimise the impact of completing tests unsupervised. For example, participants were instructed to put their phone on silent and out of sight during testing and to find a quiet space where they would not be disturbed. Tasks were coded using

custom scripts in PsychoPy3 version 2020.2.4 (Peirce *et al.*, 2019) and hosted online using Pavlovia, Version 2020.2 (Pavlovia.org). Participants were emailed links to access tasks on the day they needed to complete them. They were asked to start learning tasks between 3pm and 6pm and then start all subsequent testing sessions at the same time on the correct day. Participants were instructed to complete the tasks in the order presented (1. WPr, 2. VGT, 3. iPAL), in a quiet place without music or their mobile phone.

Questionnaires

Prior to starting the experiment participants were asked to complete an online questionnaire regarding their sensitivity to sound. A questionnaire used in the assessment of Hyperacuasis (high sensitivity to sound) was used, taken from Khalfa *et al.,* (2002), see appendix section 7.1.

Participants completed a sleep diary for each experimental night of the experiment. The diary included time to bed and to rise, estimates of wake after sleep onset (WASO), whether the headband fell off during the night or played any audible sounds, the Stanford sleepiness scale (SSS), space to record any dreams and space to record any caffeinated or alcoholic drink consumption, see appendix section 7.1. Only SSS is reported here.

At the end of their participation, participants completed an online questionnaire asking about their experiences with the headband including; comfort, ease of use, and reasons for missing nights wearing the headband (see appendix section 7.1). Participants were also asked to indicate which week they felt the sounds were played overnight, as well as whether they felt like they completed the memory tasks better in the first or second week. Participants were asked to summarise the experiment in their own words, so this could then be used to text mine instances related to the experiment from the dream reports collected daily in the sleep diary. These were completed before participants were then debriefed by the experimenter and paid. Results from this questionnaire will be discussed in the General Discussion section 5.4.

3.3.3 Experiment design

The experiment was conducted with a blind, within-subject, counterbalanced design. Participants wore the headband for seven consecutive nights with either SHAM (no CLAS) or STIM (CLAS, see section 3.3.2 for stimulation procedure) settings. Prior to

these experimental nights, participants had two adaptation nights where they slept with the headband but it was switched off. Each participant repeated this core protocol, such that they had a SHAM week and a STIM week, with at least five nights break without the headband in-between. Condition order was randomly assigned, with nine participants completing STIM first and eleven SHAM first. See schematic of design in Figure 20.

Following recruitment and screening, participants were mailed the headband and charger. They were instructed to quarantine the headband for 72 hours to reduce risk of transmission of Covid-19 between experimenters and participants. Once this was complete participants slept with the headband for two adaptation nights (night -1 and night 0 in Figure 20) during which the headband was turned off. Participants were given a link to bespoke online instructions for the experiment including how to put on the headband and start the recording: These instructions also included a video link to a practical demonstration of the headband and its use by the experimenter (instructions link: https://cardiffunipsych.eu.qualtrics.com/jfe/form/SV_3pxfqV7lp0ZdLgN, video link: https://youtu.be/c-2THpVcBfk). They were asked to begin completing the sleep diary questionnaire each morning, and continue this for the duration of the experiment (excluding the break between SHAM and STIM conditions, see questions in appendix section 7.1).

Figure 20 provides a schematic for the study. On Day 1, participants were emailed instructions to complete three behavioural tasks on their laptop or PC; WPr, VGT and iPAL (see section 3.3.4 for procedure descriptions). During their first run through completing tasks they were asked to begin between 3pm and 6pm, and informed that the time they completed tasks would be recorded. Once participants completed the tasks (encoding plus test one, T1) they were instructed to sleep with the headband switched on and recording. The following day they were sent instructions to a testing phase for the three tasks (T2), and asked to complete the tasks at the same time as the previous day. They were then asked to continue to sleep with the headband switched on for a further six nights. On the eighth day they were emailed instructions to repeat the testing for the three tests (T3), the same as on day two.

Participants were then given at least five days rest from wearing the headband or completing any forms for the study. During this time the headband was returned to the experimenter who sterilised it using a UV light chamber for 5 minutes. The sleep data was then downloaded from the headband via the Dreem website (https://dreem-

viewer.rythm.co/login, only anonymized data was accessible by Dreem as per the study GDPR agreement). Participants repeated the whole experiment again with the opposite sound condition. At the end of the experiment participants completed the feasibility questionnaire online.

Day	Night	Participant task
-1		Headband finishes quarantine
	-1	Wear headband to sleep at home. Headband switched OFF.
0		Sleep diary questionnaire
	0	Wear headband to sleep at home. Headband switched OFF.
1		Sleep diary questionnaire. **Encoding + T1:** 3pm – 6pm start. 1. WPr, 2. VGT, 3. iPAL 
	1	Wear headband turned ON. STIM OR SHAM.
2		Sleep diary questionnaire **T2:** 3pm – 6pm start. 1. WPr, 2. VGT, 3. iPAL 
	2	Wear headband turned ON. STIM OR SHAM.
3		Sleep diary questionnaire
	3	Wear headband turned ON. STIM OR SHAM.
4		Sleep diary questionnaire
	4	Wear headband turned ON. STIM OR SHAM.
5		Sleep diary questionnaire
	5	Wear headband turned ON. STIM OR SHAM.
6		Sleep diary questionnaire
	6	Wear headband turned ON. STIM OR SHAM.
7		Sleep diary questionnaire
	7	Wear headband turned ON. STIM OR SHAM.
8		Sleep diary questionnaire **T3** 3pm – 6pm start. 1. WPr, 2. VGT, 3. iPAL 

***Figure 20**: Experimental design. Experiment protocol for one week. Yellow sections indicate tasks participants carried out during the day, while blue sections indicated the status of the headband at night. Adaptation to the headband (switched off) occurred over the 2 nights prior to Day 1. On Day 1, 3 and 8 participants completed behavioural tasks (T1, T2 and T3 respectively). On nights 1 to 7 participants slept*

wearing the Dreem headband with sleep monitoring on and either SHAM (no CLAS) or STIM (CLAS).

3.3.4 Behavioural task procedure

Participants completed three behavioural tasks which explored the effect of one week of CLAS on their memory and creativity. On the evening of day 1 (see Figure 20), following adaptation, participants underwent the encoding phase for all three tasks including a test (T1).

At the same time on day 2 (see Figure 20) participants were tested on all three tasks (T2), which took around 30min, before being retested 6 days later on day 8 (T3). Repeat testing allowed for investigation of the impact of one night of stimulation, seven nights of stimulation, and analysis of whether the impact seen after seven nights was greater than the impact after one night.

Task order was fixed: First, participants completed the WPr followed by VGT, then finally iPAL. Due to the similarity in the tests between WPr and iPAL they were separated by the VGT. To further aid participants to distinguish between the tasks the background and text colour of each task was different: WPr = Black text on a white background; VGT = White text on a black background; and iPAL = black text on a grey background. Altogether the tasks took participants between 1 and 2 hours to complete, the VGT took 10 min while both WPr and iPAL took between 25 and 45 min depending on the speed at which participants met the required criterion (see section 3.3.4 below). The words used in the VGT were removed from the possible list of words used in the WPr task, again to reduce interference between tasks, see words used in appendix sections 7.2 and 7.4.3.

Word pair associates task with reward

Participants were informed at the beginning of the task that they would learn three lists which would be associated with different rewards: They were told that each list would have its own border, but that they did not need to recall this. The three lists each had 25 word pairs (75 pairs in total) and words within a pair were semantically associated (i.e. FOX and FUR). The word pairs were taken from Ngo, Martinetz *et al.,* (2013) and translated from German to English. The three levels of monetary reward were conveyed to the participant via the instructions appearing before and after encoding of each list (encoding; presentation of all pairs, test with feedback and test without feedback). The three levels of reward were as follows: (1) List associated with

no reward (nR), none of the instructions associated with this list mentioned reward. (2) List associated with a reward of 5 pence per correctly recalled pair at testing (T2, T3), of which the participants were notified in pre-learning instructions (B4). (3) List associated with 5 pence reward for each correctly recalled pair at testing (T2, T3), participants were only notified of this reward in instructions after learning (Af). The order in which the three reward conditions were presented was randomised between participants. The borders for each list differed in colour and shape (see appendix section 7.4): Six borders were used, and all participants saw each border with one list, which list was randomised between participants. The borders were removed for the final test to keep it as similar to the subsequent tests (see Figure 21). Prehn-Kristensen *et al.,* (2020) used similar borders to help participants distinguish between rewarded and non-rewarded words at learning.

Encoding: The encoding phase consisted of viewing all word pairs once, (Figure 21 A). Pairs appeared on screen for 2s with an inter stimulus interval of 2s . Participants were instructed to remember the pair, and informed that this would be tested. Then participants were shown the first word from the pair and asked to type in its pair word (word order was randomised across participants). The correct answer was briefly shown for 2s, irrespective of whether the participant answered correctly (see Figure 21 B). Participants either completed 3 rounds of recall with feedback, or fewer rounds if they achieved 50% correct by the end of the round. A final testing phase then occurred in which participants were again shown the first word in the pair and asked to type its pair word, this time no feedback was given and all pairs were used once (this test is referred to as T1, see Figure 22 C).

Testing: The next day, participants were tested on all the pairs of words (T2). The first word was presented on screen and the participant was required to type in the pair word, no feedback was given and no borders were used (see Figure 21 C). All 75 word pairs learnt were presented in a random order (with the 3 lists mixed together). Participants were informed at the beginning that the words from all 3 lists would be tested mixed together, but that the reward values were still being calculated. Once they had been tested on all pairs they were shown the reward they had achieved. Repeat testing at the end of the seven experimental nights was conducted in the same way (T3).

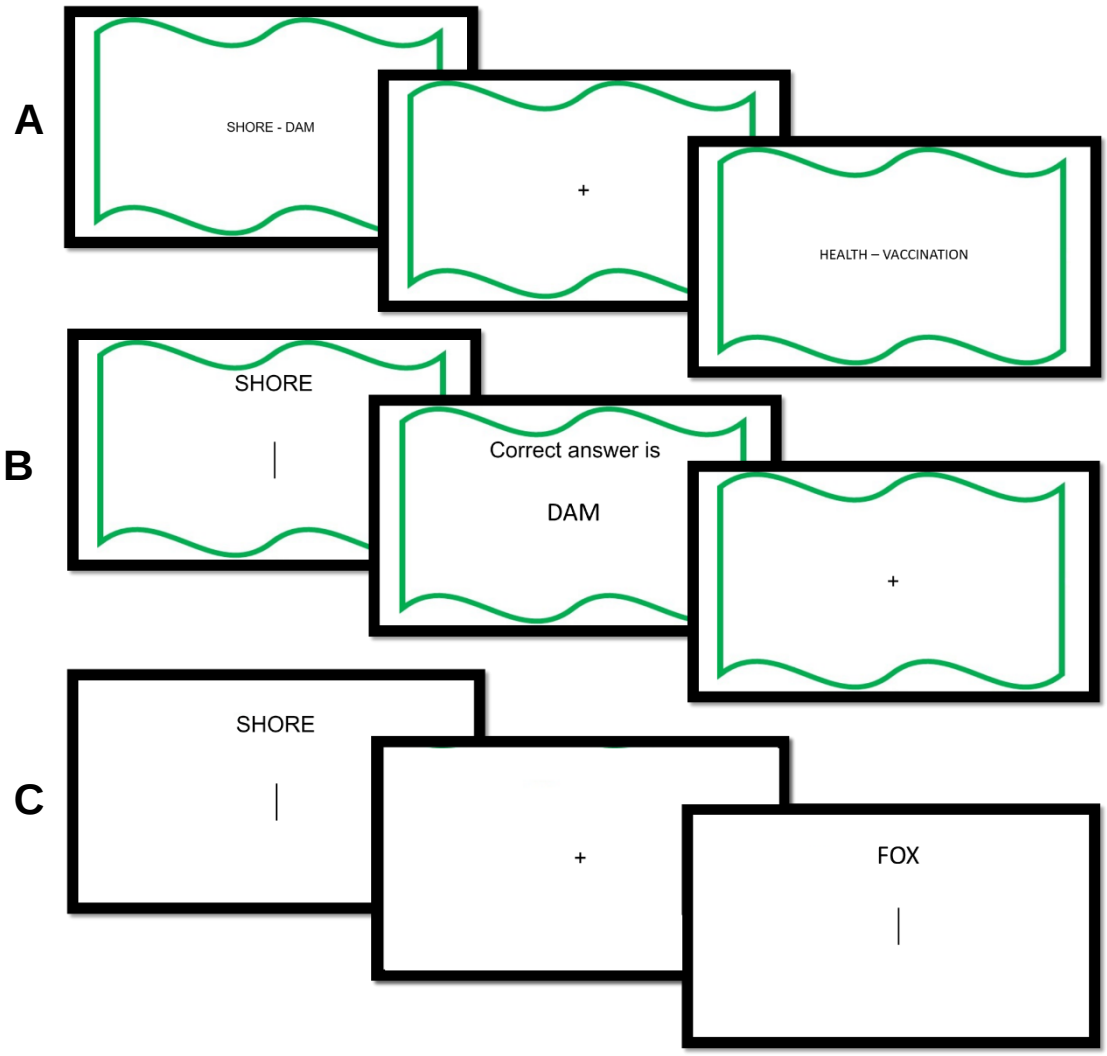


Figure 21: *Word pair task with 3 levels of reward.* ***A****: Learning. Borders were used to indicate the 3 different word lists with different reward associations.* ***B****: Test with feedback. Carried out straight after learning of each list (T1).* ***C:*** *Test without feedback, carried out on just one reward list immediately following B at T1, and of all reward lists randomly ordered on day 2 (T2) and day 8 (T3). Borders were removed for this test. At subsequent testing (T2 and T3) words from all 3 reward lists were shown in a random order.*

The second time participants completed the experiment (with the opposite stimulation condition) the procedure for WPr was identical except that 75 new pairs were used. All participant lists were drawn randomly from the same pool of 150 pairs, such that each participant's lists were unique, but all participants saw the same words across the whole experiment.

Image Paired Associate Learning Task

In the iPAL task participants learnt 20 pairs of images, adapted from Bergmann *et al.,* (2012). All images fell into one of four categories; female face, male face, rural scene, or urban scene (see appendix section 7.4.2 for images). An effort was made to match images to those used in Bergmann *et al.,* (2012); Faces were taken from The Karolinska Directed Emotional Faces database (Lundqvist, D., Flykt, A., & Öhman, 1998). The faces selected were centred on the screen with neutral expressions. Scenes were open commons licence images taken from Google images. Rural scenes showed avenues of trees, surrounding a road or path, leading away from the viewer, while urban scenes show a road leading away from the viewer. Scenes with obvious features such as legible signs were not selected. All images were shown in greyscale. Ten images were chosen for each image category (i.e. ten female faces), and paired as follows: two pairs from within the same category; two pairs with images from each of the other three categories (see illustration in Figure 22). Such that there were 20 unique pairs.

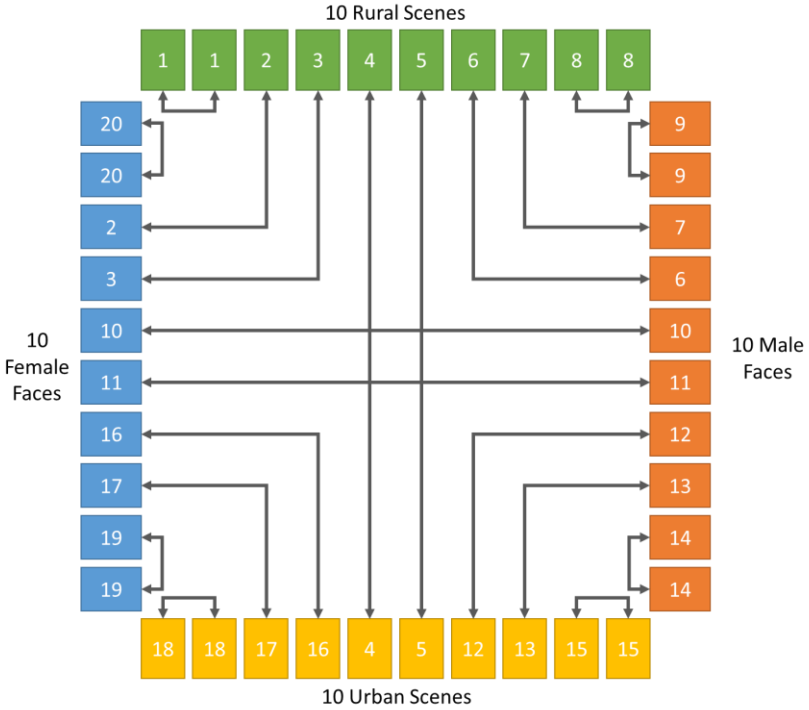


***Figure 22**: Pair assignment: Diagram illustrates the pairing of images used in iPAL task. Arrows connect the two images to form a pair. Square colour shows image category (green=rural, orange=male, yellow=urban and blue=female), numbers show the 20 unique pairs.*

Encoding: Training consisted of showing the two paired images in succession, with each image being presented for 1.5 s. A fixation cross was then presented for 2.5 s between each pair (see Figure 23 A). Participants were instructed that they would see all pairs in both orders (i.e. image a followed image b and then image b followed by image a, totalling 40 pair trials) and that their memory for the pair would be tested (Bergmann *et al.*, 2012). Pair memory was first tested with feedback. Participants were shown one image from the pair and asked first to select the category (female face, male face, rural scene or urban scene) of the pair image. They were then presented with all the images from that category and asked to select the correct pair image. The category of images displayed always corresponded to the participant's initial choice even if that choice was incorrect. The correct pair image was then displayed for 2s, (see Figure 23 B). Once participants reached 50% correct image pairs (not category choice) or had been tested on all 40 pairs three times, they were tested one final time on all 40 pairs without feedback (see Figure 23 C, T1). Asking participants to first select the image category then the image itself allows for 2 scores of memory: The correct category score and the correct image score.

Testing: On the following day, participants were tested on all 40 pairs without feedback (T2, see Figure 23 C), and this test was repeated on day 8 (T3). In the second week, participants were presented with a different list of images, belonging to the same categories as before. The two lists of images used in the study were counterbalanced with respect to the list that was presented in the SHAM and STIM weeks, and which was presented in the first and second weeks.

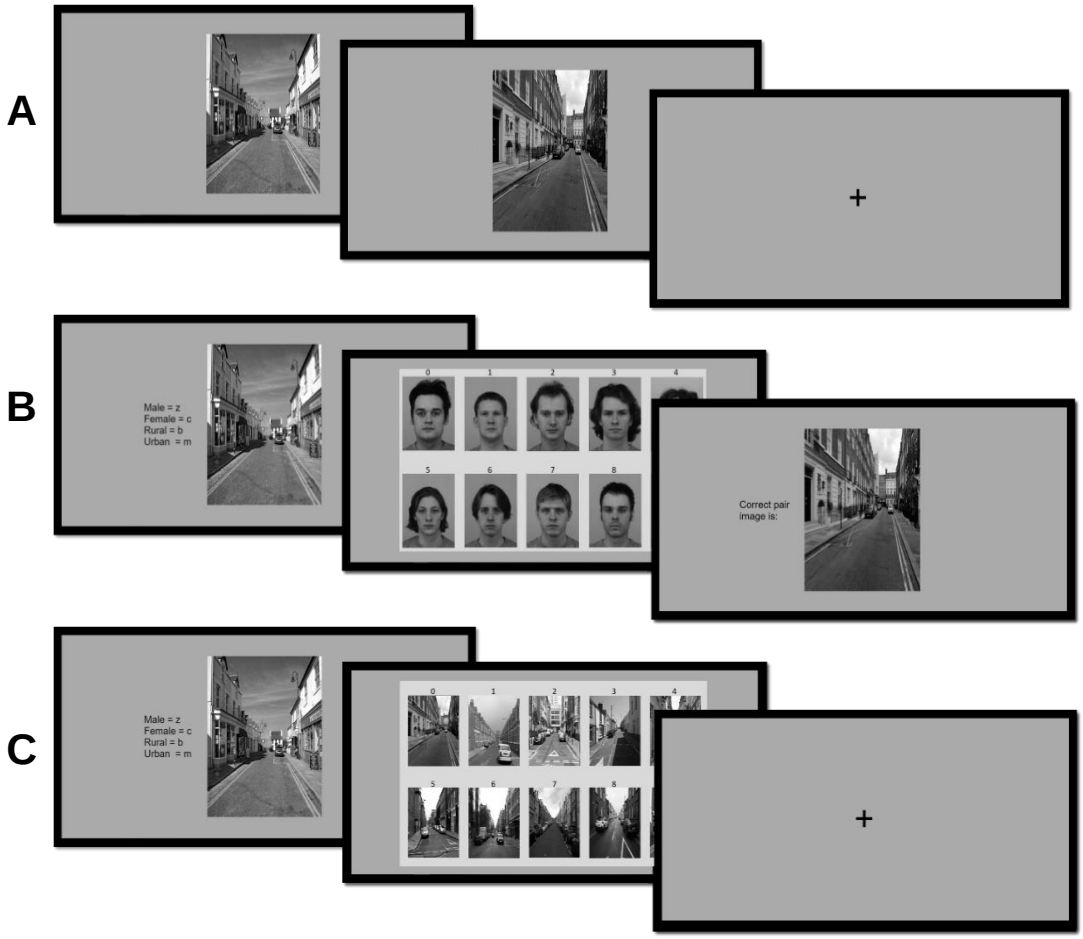


Figure 23*: Image paired associate learning task.* ***A****: Participants were first shown all pairs of images. Each of the images that belonged to a given pair was shown in succession, once in each of the two image orders. In between successive image pairs there was a 2-s fixation cross.* ***B:*** *Participants memory for the pairs was tested. First they were shown one image from the pair and asked to select the category of the pair image (male face, female face, rural scene or urban scene). They were then shown all images of the category they selected and asked to select the correct pair image. They then received feedback in the form of a brief presentation of the correct pair image.* ***C:*** *Recall was tested with no feedback on days 3 (T2) and 8 (T3). Participants were always shown the category they chose even if it was incorrect. For this target image the pair was an urban scene, however in B male faces was incorrectly selected and shown, while in C urban scenes was correctly selected.*

Verb Generation Task

A creative version of the VGT was developed in which participants were presented with a single noun and asked to type in a verb that was creatively associated with that noun (Prabhakaran, Green and Gray, 2014; Heinen and Johnson, 2018;

Koopman, 2020). Instructions were given on the definition of a noun and a verb, and also on how to generate a creative response. Creativity instructions were taken from Heinen and Johnson (2018): "*Give a very creative or original verb response to the noun. By "creative," we mean a verb that is clearly related to the noun, and also rarely used in association with the noun. A verb that would probably come to mind for very few other people."* No spell check was applied whilst participants typed their response.

Participants then provided, on a separate screen, a list of all the other verbs they were considering for that noun, (see Figure 24). 32 nouns were presented and participants moved through the task at their own pace by pressing 'return' when they finished typing. The task was repeated three times each week: on days one (T1), two (T2) and eight (T3). On the second week, participants saw a new list of 32 nouns. When seeing the same noun for a second or third time participants were left to decide whether using the same verb or a new verb best fit the instructions. This procedure was adapted from Koopmann (2020). Nouns were taken from (Prabhakaran, Green and Gray, 2014) who categorised them as high constraint or low constraint in terms of how likely participants were to give the same verb in response. Each list was made up of 16 high constraint words and 16 low constraint words. Each participant saw the same 64 nouns over the whole experiment, but which list the words fell into was randomised for each participant. Word order was always randomised within each test.

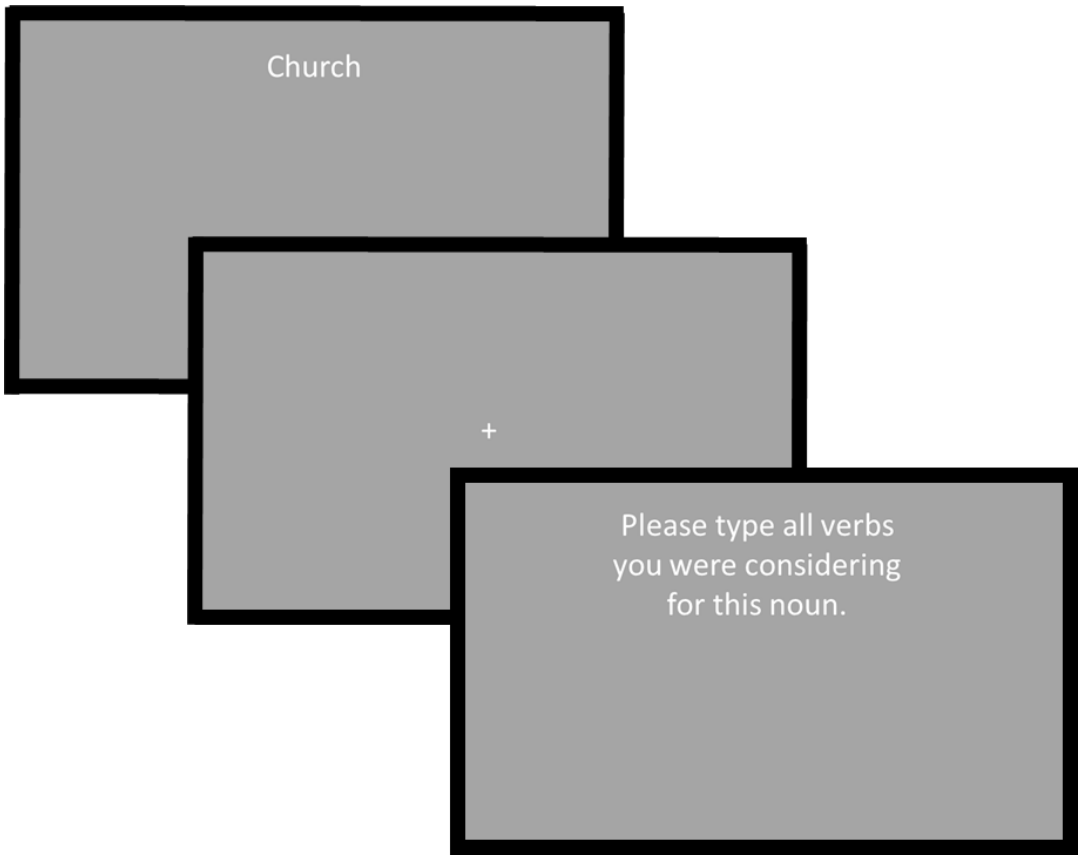


***Figure 24**: Verb generation task: Following instructions participants were shown 32 nouns (one at a time) and asked to type in a creative verb related to the noun. Then they were asked to type in any other verbs they were thinking about for that noun.*

3.3.5 Analysis procedure

Headband EEG data

The time spent each night in each sleep stage was calculated by the headband's own scoring system (Arnal *et al.*, 2020), this system indicated poor scoring of N1 resulting in very low time spent in this sleep stage (1 or 2 min per night) or no N1 scored for the majority of nights. Due to this, N1 is not reported (see discussion). Mean time in each stage were calculated for N2, N3, REM stages as well as total sleep time (TST), sleep onset (time from lying down with eyes closed to first sleep stage (Debellemaniere *et al.*, 2018; Arnal *et al.*, 2020), and WASO. Significance testing was conducted using either paired students t-test or a paired Wilcoxon signed rank test, depending upon the outcome of Shapiro-Wilke test for normally distributed data, to assess for differences in the means SHAM and STIM groups. Sleep onset for one participant on one night was removed from the analysis as it was an obvious outlier at 149 minutes.

EEG data from each night was first pre-processed: including band-pass filtering at 0.3-30 Hz and band-stop filtering at 0.1 Hz and 50 Hz, to remove device and line noise frequencies. The hypnogram and stimulation information from the headband's internal algorithm were converted so they could be matched to the EEG signal. The data was then segmented into 5s epochs before and after the sound (time sound played = 0s) and sham sound stimulations. Nights without at least 50 stimulations were discarded from further analysis because very low numbers of stimulation indicate poor recording quality. One night was removed for one participant on these grounds. The two channels with data from Fpz referenced to O1 or O2 were inspected for each trial; If either channel recorded signal above 400μV or below -400μV (indicating noise as this is outside the normal range for human brain activity), then that channel was discarded for that trial and the opposite reference channel was used. If both channels showed signal falling outside this range, then the trial was removed. If neither channel indicated data outside this range, then a mean of the two channels was used for that trial. This approach was used as participants showed a particular pattern of activity of poor signal from whichever reference they were not lying on. This was likely caused when the rigid headband became looser on the opposing side of the head, which would lead to poor signal, altering as the participant changed position.

Trials were further cleaned, as they were filtered in the slow wave frequency band (0.2Hz to 2Hz) to isolate SO activity. Both FPz channels (one with O1 reference and one with O2 reference) in each slow wave filtered trial were then sorted to exclude those trials/channels that did not show signal below -20μV in the two seconds before the sound. These trials were likely to have been triggered incorrectly; all trials should include at least one SO which was detected prior to sound stimulation. Trials not meeting this criterion were marked and removed from the broadband filtered EEG trials, so that the final clean trials were not filtered in the SO band.

Custom MATLAB scripts were written utilizing functions from the FieldTrip toolbox (Oostenveld *et al.*, 2011) and elements of scripts written by Navarrete *et al.,* (2019). Of the 20 participants, one missed one night of STIM due to forgetting to wear the headband (not first or seventh night), one missed two nights of STIM due to forgetting to wear the headband (not first or seventh nights), one participant through experimenter error only completed 6 nights of STIM before final tests, and one was missing the final night of recording however reported wearing the headband for this night. All are included in the below ERP calculations, and thus reported as first and last night's, indicating between five and seven nights of stimulation.

Once data was cleaned for all nights for a participant, a mean of the ERP trials within each night was calculated for that participant. Comparisons were made between SHAM and STIM within the same night (STIM night) to control for inter-night differences, such as TST or headband position. Then a grand mean for each participant was calculated from the night means. This grand mean was then combined with other participants to create the final grand average mean (GA ERP). Monte-Carlo cluster permutation (significance level of $p<.05$) was applied from 0 to 2.5s to compare the GA ERP in SHAM and STIM. This allowed comparison between the two groups whilst controlling for the large number of multiple comparisons that arise from multiple EEG channels and time-points. At each time point the t-statistic was calculated between SHAM and STIM, before 1000 permutations were calculated where data from the two groups was pooled and randomly assigned back into two groups. The difference in the means between the two groups was then tested to give the null distribution against which to compare the test statistic and determine if the difference in the data was significant.

The first and last night grand means for each participant, were also collated to provide a grand mean of first and last nights. This allowed the investigation of the effect of repeated nights of stimulation upon the ERP. For each participant, Monte-Carlo cluster permutation (significance level of $p<.05$) was applied from 0 to 2.5s to compare the first and last STIM night.

Behavioural task data

Raw participant results tables were downloaded from Pavlovia, before custom MATLAB 2017b scripts were used to calculate raw scores for each task at each test (T1, T2 and T3). Statistics were conducted using R studio, primarily rstatix, tidyverse, ggpubr, patchwork, packages, lme4, lmerTest, emmeans, AICcmodavg, plyr, LSAfun, toolboxes. All tests were two-tailed, with a significance value of $p=.05$, unless otherwise stated. Normality testing was conducted using Shapiro-Wilk test and the plotting of residuals, using quantile-quantile (QQ) plots, to show the distribution of the data against the expected data, to see if there was a normal distribution. To test for significant differences between STIM and SHAM two-tailed paired t-tests were used. If normality testing indicated deviation from normal distribution in the data, then a Wilcox Signed Rank test (Wilcox) was employed.

In instances with more than two groups of data a repeated measures analysis of variance was conducted (RM ANOVA). RM ANOVA has two key assumptions, one

that the variance between groups is relatively similar, such that sphericity is not violated, and that the data has a normal distribution (Vasey and Thayer, 1987). To assess any violations of sphericity, Mauchly's test was used, and if this indicated a violation then Huynh-Feldt epsilon (HFe) correction was applied and corrected *p* values reported and highlighted. If Shapiro-Wilks tests were violated, then the QQ plot was assessed. If this assessment revealed deviations from the expected normal distribution were small (i.e., one or two points deviating from expected range) then RM ANOVA was used to assess analysis of three or more groups. If any significant effects emerged from the RM ANOVA then the post-hoc tests were conducted using the appropriate test from paired *t*-test and Wilcoxon Signed rank. If deviations from a normal distribution were larger, then a linear mixed effect (LME) model was applied to the data, because this allows for tighter control of factors affecting variance and is robust against deviations from normality. Residuals from the LME were also checked using QQ plots to ensure they met the expectations for a normal distribution.

First, the raw scores at T1 were checked to ensure learning was equivalent on SHAM and STIM weeks. All tests indicated that learning was equivalent in SHAM and STIM: WPr: 2x3 RM-ANOVA with within-participant factors of condition (SHAM or STIM) and reward (nR, Af, B4), indicated no main effect of stimulation (F (17)=0.57, p=.460), or reward (F(34)=2.04, p=.146, and no interaction between reward and stimulation (F(2, 34)=0.79, p=.461). For the iPAL: Wilcoxon-Signed rank test indicated no significant difference between stimulation conditions (z(19)=113.00, p=.239), and there was no difference for the VGT test (t(19)=-1.40, p=.178).

As the intent of this research was to assess the impact of repeated nights of stimulation upon behavioural performance, the retention of word and image pairs from the memory tasks was assessed across Nights 1-7; $Nights\ 1-7\ retention = (\frac{(T3-T1)}{T1} \times 100)$. As previous literature has theorised a differing mechanism of consolidation across the first night following learning and subsequent nights (Almeida-Filho, Queiroz and Ribeiro, 2018), I also assessed the retention of stimuli across Night 1; $Night\ 1\ retention = (\frac{(T2-T1)}{T1} \times 100)$ and the subsequent six nights separately; $\text{Nights } 2-7 \text{ retention} = (\frac{(T3-T1)}{T1} \times 100)$. This also allowed results from this study to be compared more directly to previous work which assessed the impact of one night of CLAS upon memory retention. VGT was not assessed in this way as it is not a memory task and therefore I am not interested in stimuli retention. So in this task the

raw scores at each test were assessed and compared between SHAM and STIM conditions.

Word Pair task

WPr scores indicated the number of times the participant correctly gave the pair word when cued for each test and under each reward condition. Scores were assessed in two ways: (1) divided by the reward level (nR, Af, B4); and (2) averaged across reward to give pooled results more closely comparable to previous CLAS WP experiments. Two participants were excluded from the WPr (pooled or reward) analysis due to technical difficulties with the data collection on this task (i.e. they did not complete learning task as their computer crashed).

Image paired associates task

iPAL scores were the number of instances the participant selected the correct pair image when cued during each test. One participant used the incorrect number keys at both learning tests, so their running score used to determine when criterion was reached, was not accurate and they were over trained (completed 3 rounds of training). However, as this occurred at both SHAM and STIM learning for this participant their scores were comparable across stimulation conditions, and their scores were not excluded. This participant also used the wrong number keys at testing along with one test for another participant, so an extra script was written to re-calculate accurate performance scores for these participants.

Verb Generation Task

First VGT results were cleaned to ensure any obviously misspelled words were corrected (i.e. injest was corrected to ingest), as no spell check was present when participants completed the task. A custom R script (Koopman, 2020) utilised the LA corpus to calculate the semantic distance between the given noun and the participant's creative verb, using latent semantic analysis, as defined in Heinen and Johnson (2018). Semantic distance was calculated using the LSAfun toolbox: First the semantic similarity between the noun and the verb was calculated using the cosine of the two vectors generated by the co-occurrence of the noun and the verb in the EN100K corpus. The inverse of the semantic similarity was then found as the semantic distance, the resulting value lying between 0 and 1. On each testing occasion the participant's mean semantic distance was calculated. As data across all factors was normally distributed (Shapiro-Wilk normality test), a two-way RM ANOVA was used.

To assess further the impact of stimulation upon semantic distance scores in the VGT, a Bayesian RM ANOVA test was applied. Unlike the standard RM ANOVA, the Bayesian version will indicate not if the means are significantly impacted by any of the factors, but instead if there is sufficient evidence to support or reject the null hypothesis, that there is no impact of stimulation or time on semantic distance.

Sleep Diary

Stanford Sleepiness Scale (SSS) scores recorded by participants each morning following an experimental night were averaged to give means for SHAM and STIM on each week. Three participants failed to correctly record SSS each day and thus were excluded. Potential difference between means was then assessed using a paired *t*-test.

3.4 Results

3.4.1 Effects of repeated nights of CLAS on sleep structure

The time spent in each sleep stage was compared between SHAM and STIM nights for each participant, to see if CLAS impacted sleep structure, summary in Table 4. To statistically test for differences between nights, paired t-tests and Wilcox Signed Rank tests were performed. Investigations indicated the only significant difference between SHAM and STIM nights was a reduction in WASO on STIM nights (mean=6.67 min, SEM=0.95) verses SHAM night (mean=9.82 min, SEM=1.55, $t(19)=2.21$ $p=.040$). The number of arousals nor the subjective arousal (measured using the SSS) differed significantly between nights.

Measure	Sleep Stage	SHAM ±SEM	STIM ±SEM	Significance
Minutes	TST	418 ±13.10	403 ±12.60	*t*(19)=1.40 *p*=.177
	N2	192 ±9.10	182 ±8.07	*t*(19)=1.20 *p*=.060
	N3	113 ±4.60	111 ±5.45	*t*(19)=0.86 *p*=.398
	REM	116 ±7.10	114 ±5.98	*t*(19)=0.23 *p*=.818
	WASO	9.82 ±1.55	6.67 ±0.95	***t*(19)=2.21 *p*=.040***
	Sleep Onset	15.30 ±5.91	13.30 ±5.26	*t*(19)=1.82 *p*=.085
% of TST	N2	0.46 ±0.02	0.45 ±0.02	*t*(19)=0.43 *p*=.675
	N3	0.28 ±0.01	0.29 ±0.01	*t*(19)=-1.11 *p*=.281
	REM	0.27 ±0.01	0.271 ±0.01	*t*(19)=0.18 *p*=.859
Instances	Arousals	3.17 ±0.48	2.640 ±0.41	z(19)=124.50 *p*=.243
Stanford Sleepiness Scale		3.51 ±0.15	3.48 ±0.17	*t*(16)=0.38 *p*=.707

***Table 4**: Sleep macrostructure. Mean time in sleep stages, time in stage as a percent of total sleep time (TST), wake after sleep onset (WASO), sleep onset, number of arousals and subjective arousal rating from Stanford sleepiness scale. ±SEM, * significant at α<.05.*

To assess the impact of sound stimuli upon the ongoing oscillatory activity in N3, the GA ERP for SHAM and STIM was calculated. The ERP in Figure 25 indicates that when sounds were played (STIM), there was a significant deviation in the EEG voltage compared to when no sounds were played (SHAM). Statistical support for this conclusion can be found in Table 5. The difference between SHAM and STIM is significant at almost all time-points (excluding those surrounding zero crossings) until 2.3s following the sound, see Figure 25.

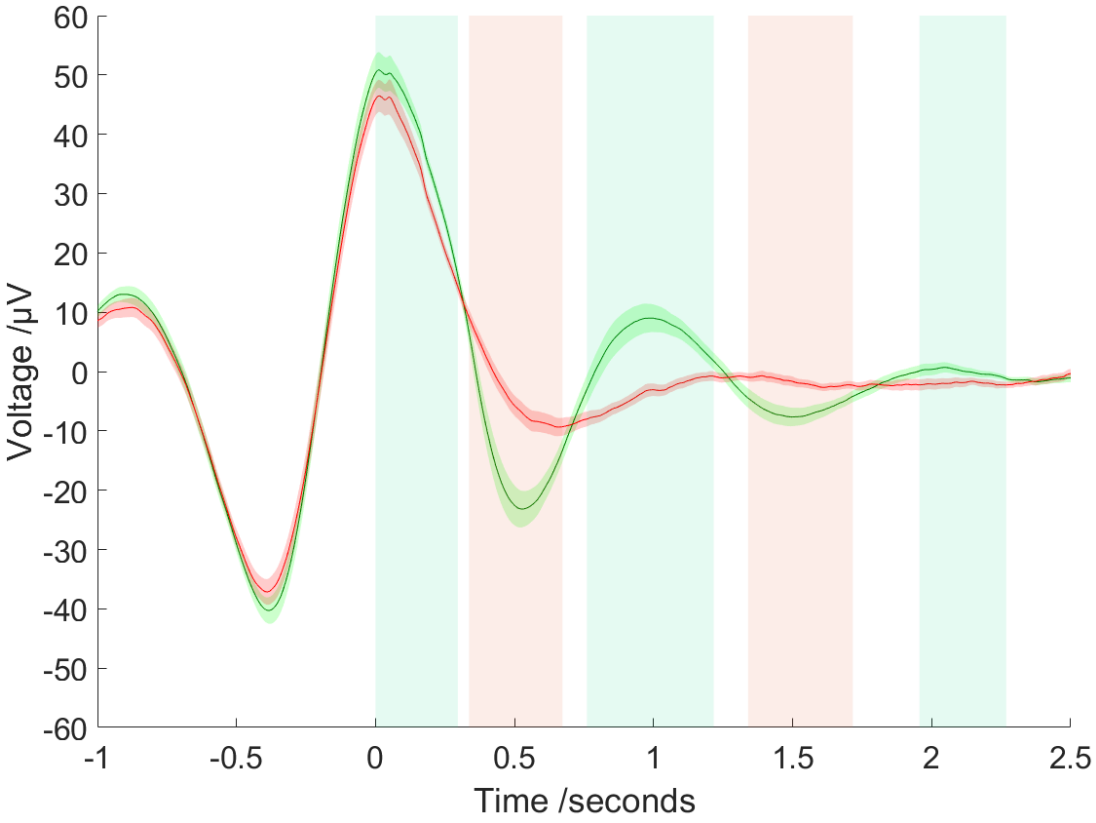


***Figure 25**: Grand mean event related potential SHAM vs STIM. Average voltage time locked to sound onset (0s), for SHAM red and STIM green trials. Shaded areas indicate SEM. Significant differences between SHAM and SITM voltage are indicated by green (STIM>SHAM) and red (STIM<SHAM) shaded boxes, α=.05.*

To investigate whether the ERP on STIM trials changed across the week, the STIM ERP including only trials from the first night of stimulation was compared to the STIM ERP containing only trials from the final available night of stimulation (see Figure 26). Inspection of Figure 26 indicates that there is very little difference in the shape and voltage of ERP generated from the first and final STIM nights (see summary in Table 5). Statistical testing using a Monte-Carlo permutation test, did however indicate a period of time (1.21s to 1.56s, pink shaded box in Figure 26) where the final night voltage significantly exceeded that of the first night (cluster statistic=-195.34 p=.036).

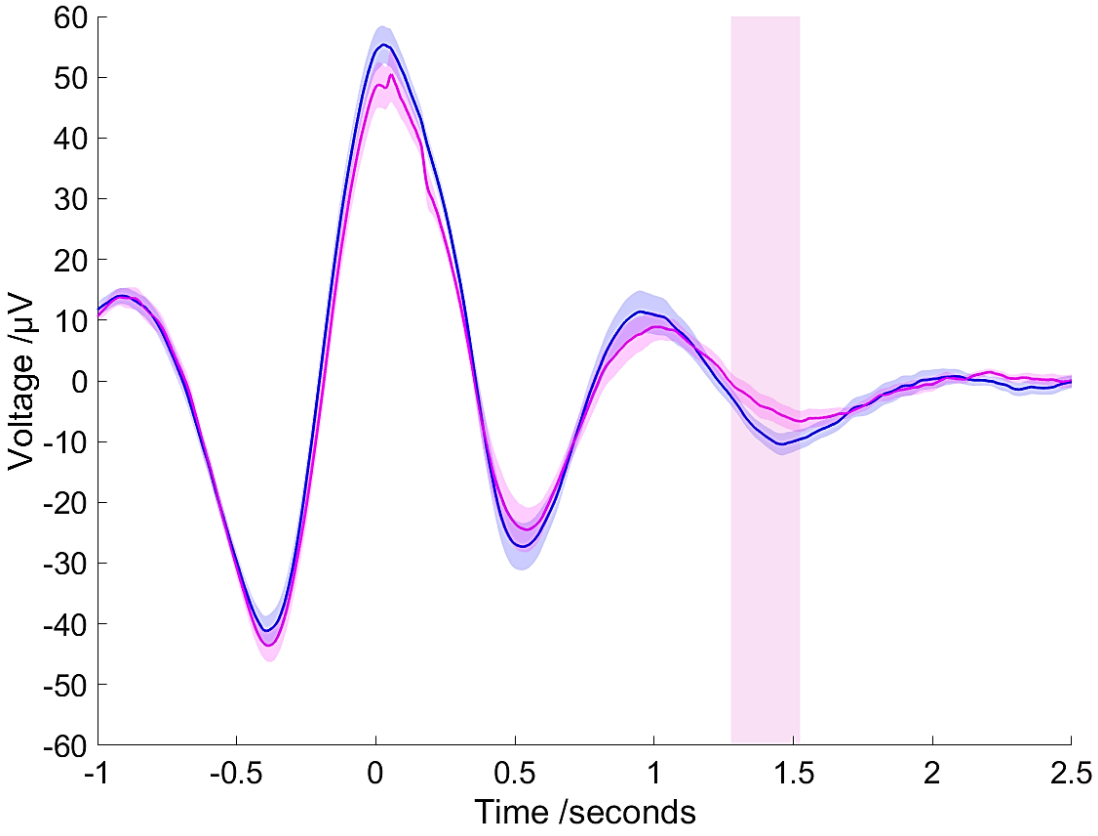


Figure 26: *First verses last night event related potential. Grand mean plot of participants first (blue) and last (pink) nights of stimulation. Shaded areas show SEM. Pink shaded box indicates the time when there was a significant difference in ERP between first and last night, as determined via a Monte-Carlo cluster permutation test.*

	Time /s	*p* value	Cluster	SD	CI range
SHAM vs STIM (Figure 25)	0 – 0.30	.007	-299.11	<0.001	0.002
	0.34 – 0.67	<0.001	464.05	<0.001	<0.001
	0.76 – 1.22	<0.001	-599.57	<0.001	<0.001
	1.34 – 1.72	.006	305.51	<0.001	0.002
	1.96– 2.27	.037	-199.38	0.002	0.004
First Vs Final night (Figure 26)	1.28 – 1.52	.036	-195.34	0.002	0.004

Table 5*: ERP Monte-Carlo Cluster permutation statistics for* Figure 25 *and Figure 26.*

3.4.2 Effects of repeated nights of CLAS on behaviour

The scores for each task were analysed across retention intervals. The raw scores for each task for each test and stimulation condition are presented in Table 6.

Test	Condition		Word Pair		image Paired Associate Learning	Verb Generation Task
T1	SHAM	nR	22.0	± 0.44	33.10 ± 1.03	0.54 ± 0.01
		Af	21.3	± 0.55		
		B4	22.2	± 0.37		
	STIM	nR	22.7	± 0.47	31.80 ± 1.02	0.56 ± 0.01
		Af	21.9	± 0.74		
		B4	21.8	± 0.57		
T2	SHAM	nR	18.6	± 0.71	28.40 ± 1.24	0.54 ± 0.01
		Af	18.0	± 0.85		
		B4	18.7	± 0.69		
	STIM	nR	18.0	± 0.76	28.00 ± 1.72	0.54 ± 0.01
		Af	17.6	± 0.95		
		B4	18.3	± 0.84		
T3	SHAM	nR	16.2	± 0.92	28.60 ± 1.37	0.55 ± 0.01
		Af	15.8	± 0.92		
		B4	16.7	± 0.83		
	STIM	nR	16.80	± 1.01	26.40 ± 1.57	0.57 ± 0.01
		Af	15.90	± 1.01		
		B4	15.80	± 0.97		

***Table 6**: Mean (±SEM) behavioural scores across testing.*

Word Pair with Reward

To probe any effects of sleep stimulation upon WPr performance, the retention of pairs across Nights 1-7 was assessed (see Figure 27 A). Inspection of this figure indicates very little difference in retention under SHAM or STIM on any of the three reward lists. Statistical analysis using a RM-ANOVA with reward (nR, Af, B4) and stimulation (SHAM, STIM) as within-participant factors, indicated no main effects

(stimulation $F(7)=0.16$, $p=.694$; reward HFe(1.57)=0.78, p[Hf]=0.761), or an interaction ($F(2, 34)=1.09$, $p=.770$).

As earlier discussed (in section 3.2), there is evidence that different processes may be at work consolidating information in the first night, compared to continued consolidation of the same information over subsequent nights (Almeida-Filho, Queiroz and Ribeiro, 2018). Here, two further retention intervals were assessed: across the Night 1 (T1 to T2) and across Nights 2-7 (T2 to T3). Analysis of these tests allow the current results to be better compared with previous research, which focussed on the impact of one night of CLAS on WP performance. WPr performance across these intervals is displayed in Figure 27 B. This figure shows that there is overlap between SHAM and STIM SEM in each reward list, in retention over Night 1 and over Nights 2-7, suggesting that there is no significant difference between stimulation conditions. This is supported by statistical testing (RM-ANOVA) with reward, retention interval (Night 1 or Nights 2-7) and stimulation, as within participant factors. The analysis indicated that only the retention interval had a main effect on performance ($F(17)=110.31$, $p=.005$; Post-hoc: z(17)=1793.50, $p<.001$, next lowest p value =.187).

The introduction of reward to the WP task was a novel manipulation, which has only been tested once before with CLAS by Prehn-Kristensen *et al.,* (2020) in a population of children, with and without attention deficit hyperactive disorder (ADHD). To assess if results in this experiment were comparable to previous CLAS experiments utilising the WP task, performance was pooled across the reward lists and any differences between SHAM and STIM assessed. The same tests were conducted as for lists divided by reward, and results were consistent. The only significant main effect was of retention interval. Participants forgot significantly more pairs across Night 1, than across Nights 2-7 ($F(17)=42.42$, $p<.001$, z(17)=650.00, p<.001 (see Figure 28). There were no other significant effects or interactions (smallest $p=.078$).

Inspection of Figure 27 and Figure 28 indicate that across all tests there was no consistent difference in each participants scores depending on condition (STIM or SHAM). From these analyses it appears that there is no impact of stimulation on performance on the WPr task, either over the first night or across the week.

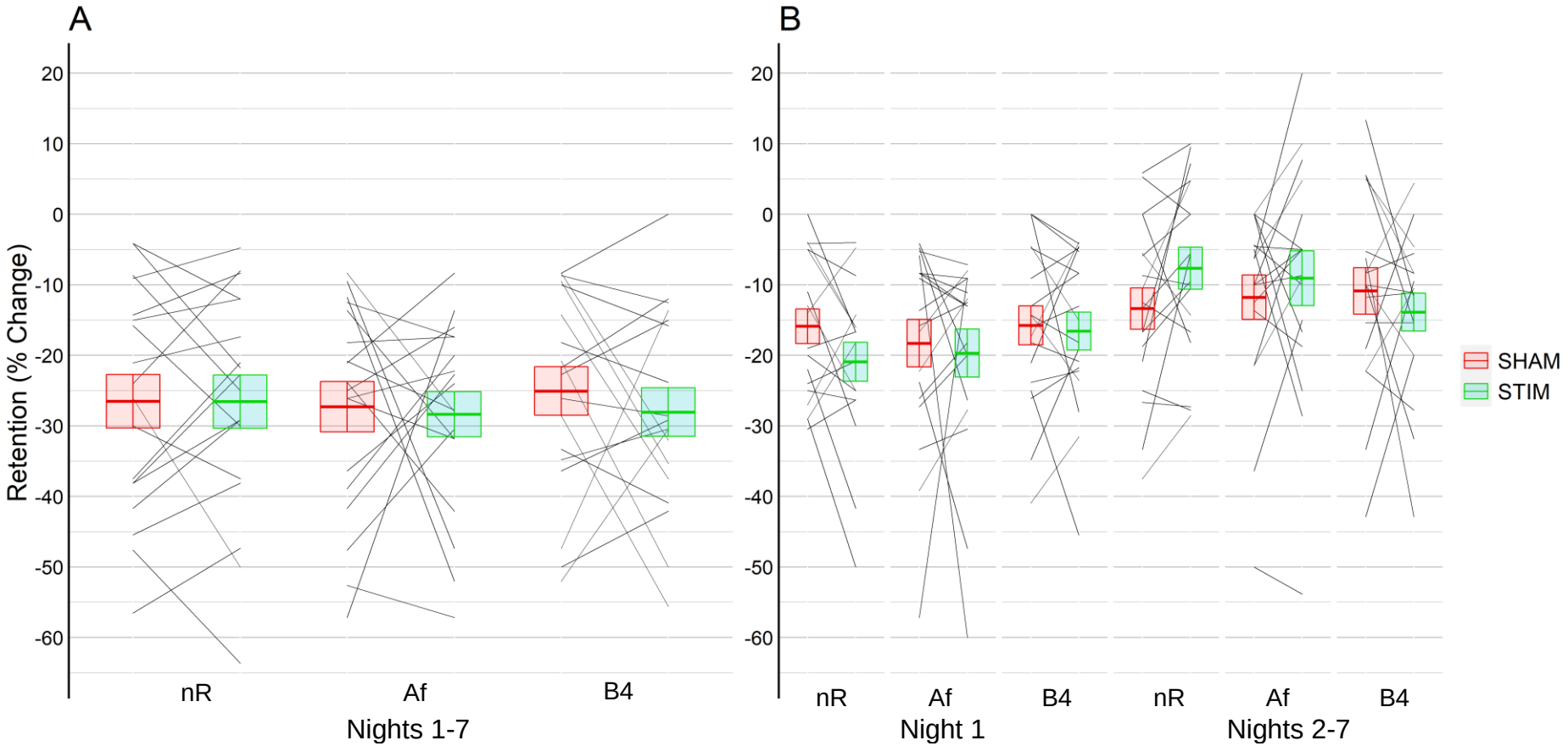


Figure 27: *Word pair reward task retention. The retention of word pairs as a percentage change over (A) Nights 1-7 and (B) Night 1 and Nights 2-7T1)/T1. Coloured boxes show mean ± SEM, black lines indicate individual performance. nR = No reward, Af=reward after learning, B4=Reward before learning.*

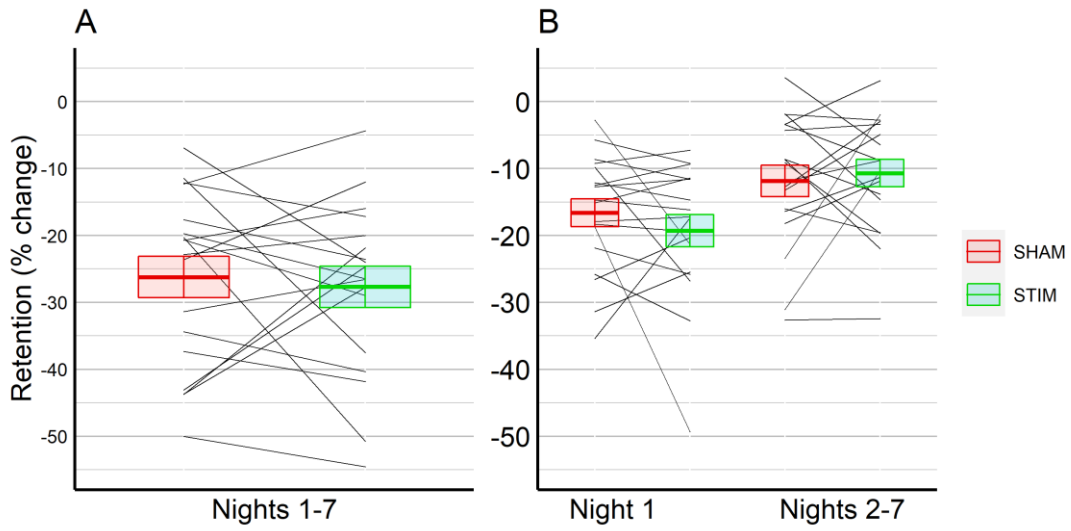


Figure 28: *Pooled word pair retention. Word pair reward pooled pairs retained presented as percentage change overnight (T2-T1)/T2, over six nights (T3-T2)/T2 and over week (T3-T1)/T1. Red boxes indicate mean ± SEM. Black lines indicate individual performance.*

Image Paired Associates Task

To investigate if CLAS is influencing iPAL scores over the course of the experiment, the retention of stimulus pairs was assessed across the week (Nights 1-7 retention). Inspection of Figure 29 A, indicates that there was very little difference between scores for SHAM (mean=-14.10%, SEM=2.69) and STIM (mean=-17.00%, SEM=3.92) across Nights 1-7. As expected, statistical testing indicated no significant difference between SHAM and STIM scores (z(19)=114.50, *p*=.738).

For the same reasons as previously mentioned, I was also interested whether CLAS influenced iPAL score differently over Night 1 compared to the rest of the week (Nights 2-7). So, retention scores were again calculated for the first night ($Night\ 1\ retention = \frac{(T2-T1)}{T1} \times 100$) and the subsequent six nights ($Nights\ 2-7\ retention = \frac{(T3-T2)}{T2} \times 100$).

Inspection of Figure 29 B indicates that there was more forgetting over the Night 1 than over Nights 2-7. This was supported by significance testing where a RM-ANOVA (and post hoc Wilcoxon signed rank test), indicated a main effect of retention interval (*F*(19)=6.72, *p*=.018, z(19)=267.00, *p*=.087). Further inspection of Figure 29 B indicates that across Nights 2-7 there is an increase in the number of pairs recalled in SHAM (mean=2.65%, SEM=5.86) compared to forgetting in STIM (mean=-3.05% SEM=5.72), whereas across Night 1 there was slightly less forgetting in STIM (mean=-13.00%, SEM=3.77) than SHAM (mean=-13.70%, SEM=3.15).

Unsurprisingly, statistical testing using a RM-ANOVA indicated that there was no main effect of condition ($F(19)=0.37$, $p=.552$) or interaction ($F(19)=0.25$, p=.622).

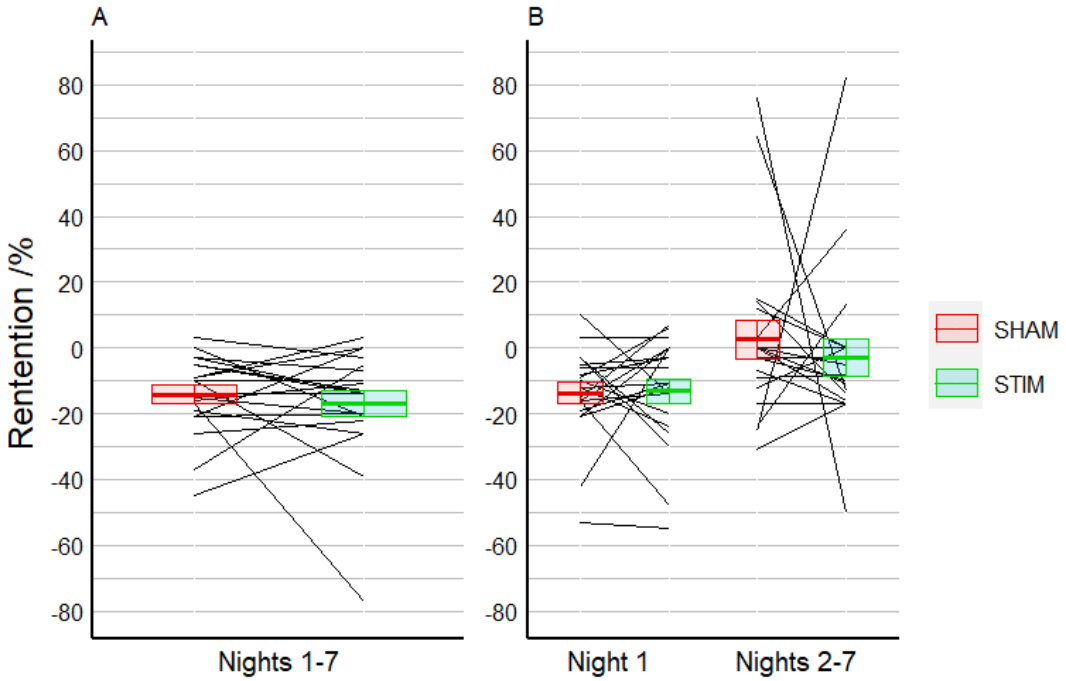


Figure 29: *Image paired associate task retention.* ***A****: iPAL percentage change in score across the week.* ***B****: iPAL percentage change in score across Night 1 and Nights 2-7. Boxes indicate condition mean ±, red = SHAM and green = STIM.*

Verb Generation Task

The results from the VGT are shown in Figure 30. Inspection of Figure 30 does not indicate any marked difference between SHAM and STIM during any test. RM-ANOVA confirmed that there was no significant difference between stimulation conditions ($F(19)=2.04$, $p=.170$), no effect of test ($F(19)=3.09$, $p=.057$), and no interaction between these factors ($F(2, 38)=1.23$, $p=.305$). The fact that the effect of test is close to the cut-off for statistical significance could be driven by slightly higher scores, under both conditions, at T3. Higher scores indicate that at T3 participant's verbs were less semantically related to the cue noun. However, scores across the three tests indicated a remarkably stable semantic distance score, particularly in SHAM (T1 mean=0.54 SEM=0.01, T2 mean=0.54 SEM=0.01, and T3 mean=0.55 SEM=0.01) but also in STIM despite a slight decrease in T2 (T1 mean=0.56 SEM=0.01, T2 mean=0.54 SEM=0.01, and T3 mean=0.57 SEM=0.01). This is unlike the two memory tasks where performance fell across the week and most significantly across Night 1. Scores around 0.5 indicate words which are neither related nor-

unrelated, as the same word would receive a semantic distance score of 0, while two completely un-related words would receive a score of 1. These scores are to be expected when participants were asked to give creative verbs (ones few people would associate together), but that were still related to the noun.

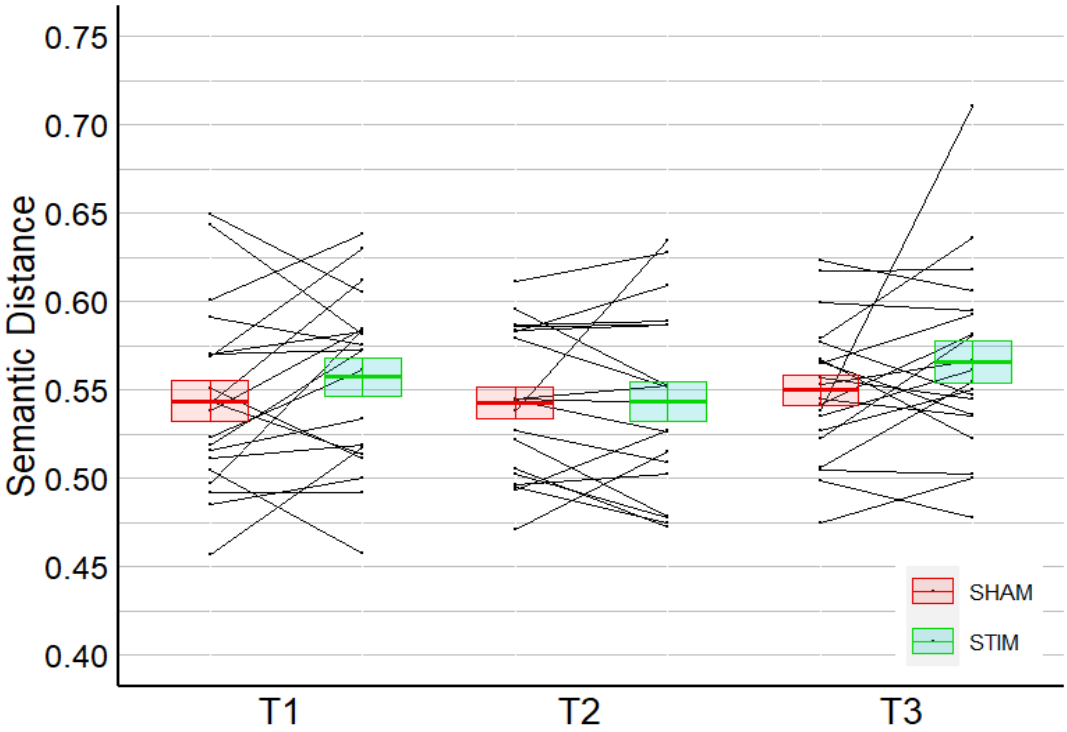


Figure 30: *Verb generation scores at each test. Mean semantic distance for each testing session for SHAM (red) and STIM (green) conditions. Error bars show standard error of the mean.*

A Bayesian RM ANOVA was also conducted to specifically test evidence for the null hypothesis (H_0) that CLAS does not affect VGT performance. The model:

$$(\text{anovaBF}(\text{Semantic Distance} \sim \text{Stimulation} * \text{Test}, \text{data}, \text{which Random} = \text{"Participant"}))$$

was applied so that the effect of condition (SHAM or STIM) and test (T1, T2, T3) could be assessed, with participant as the random factor. Results indicated mild evidence to accept the H_0 that neither CLAS (bf=0.358) nor test (bf=0.179) nor an interaction (bf=0.065) had an effect on VGT performance.

3.5 Discussion

This study confirmed that CLAS, delivered by the Dreem headband, elicited comparable effects on the ERP response over the course of seven nights. No negative influence of stimulation being present was detected in sleep macro-measures, indeed stimulation appeared to reduce WASO. Despite this influence on sleep, CLAS over repeated nights did not appear to have any affect upon task performance for stimuli learnt prior to stimulation, on any of the three tasks. This is

surprising given the similarity of the procedures used here (particularly the WP task) to those that have been improved by CLAS previously (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017).

In fact, other manipulations (e.g., the prospect of reward) did not affect the behavioural outcomes of the procedure in the anticipated fashion (cf. Prehn-Kristensen *et al.,* 2020). One of the differences between the study reported by Prehn-Kristensen et al., (2020) and the current study was that their participant group was children, unlike the current study that used healthy young adults. Children have been shown to have more SWS than adults (Diekelmann, Wilhelm and Born, 2009), and thus could have a different propensity for CLAS to affect reward pair recall. Indeed, Prehn-Kristensen *et al.,* (2018) found that rewarded pairs were only protected from forgetting during sleep (no CLAS), compared to wake, in children, but not adults following the same experimental protocol. One possible explanation for the fact that reward did not affect performance is that participants did not value the reward attributed to two thirds of the word pairs, and as such did not attribute higher importance to these pairs. Moreover, participants were aware that all pairs would be tested and therefore even the non-rewarded pairs were associated with some future-usefulness, and as such could all have been tagged for consolidation (Wilhelm *et al.*, 2011; Dongen *et al.*, 2012). However, Prehn-Kristensen *et al.,* (2020) did not find a difference between reward-associated and non-reward-associated pairs following the SHAM night. Clearly, there is a need for a further exploration of the boundary conditions of the effects reported by Prehn-Kristensen *et al.,* (2020).

Indeed, the fact that there was no effect of CLAS on the WPr task, even when scores were pooled across reward, adds to the emerging picture that early reports of the impact of CLAS on the WP task should be interpreted with additional caution. Perhaps the sole result that was predicted was that CLAS did not impact VGT. However, given the fact that the CLAS had no impact on memories acquired during the experiment, this finding becomes less interesting than it might have been (cf. Lewis, Knoblich, & Poe, 2018).

Across all three tasks, the response of individual participants to stimulation (i.e. whether they performed better or worse on STIM nights compared to SHAM), varied greatly: There was no consistent pattern. It has been suggested that CLAS affects people to varying degrees, for example it differs in older cohorts compared to young (Navarrete *et al.*, 2019; Schneider *et al.*, 2019). Diep *et al*., (2019) did show that while there was no impact of CLAS upon their declarative task when they examined

performance across their whole cohort, that when they subdivided the group based upon a measure of a change in SWA, that an improvement in the task positively correlated with the SWA measure. To the best of my knowledge, inter-participant differences have not been specifically investigated in healthy young populations, and the low numbers of participants in different studies would make it difficult to draw definitive conclusions (e.g., n=12, Ngo, Martinetz et al., 2013). See further discussion on individual responses to CLAS in general discussion section 5.8.

3.5.1 Repeated nights of stimulation did not improve memory performance

This is the first study to deliver CLAS over more than one night and assess this impact on behaviour. Results did indicate that even following repeated nights of stimulation the ERP response during SWS was comparable to that after one night (Ngo, Claussen, *et al.*, 2013; Henin *et al.*, 2019; Navarrete *et al.*, 2019; Papalambros *et al.*, 2019; Schneider *et al.*, 2020) and that seen in Chapter 2 of this thesis using in-lab PSG directed CLAS. As previously mentioned results did not indicate any effect of CLAS upon memory performance, this was true for the first night of stimulation and the subsequent six nights. As one night of stimulation did not affect memory performance compared to SHAM it is perhaps unsurprising that repeated nights of stimulation also failed to impact memory recall. Indeed, CLAS has been heavily implicated in the earliest stages of consolidation occurring closer to encoding (Ngo, Martinetz, *et al.*, 2013), via systems consolidation (Rasch and Born, 2013) or synaptic up and downscaling (Tononi and Cirelli, 2014), and not later stages of synaptic and neural plasticity associated to longer term consolidation (Takashima *et al.*, 2006; Pereira and Lewis, 2020).

Indeed, the only significant effect identified was a greater decrease in performance across Night 1 than the decrease seen across Nights 2-7, on both the WPr and iPAL tasks. This fits in with the Ebbinghaus forgetting curves first proposed in 1886, which indicate that the recall of memory gets worse with distance from the time of learning but not at a linear rate (Della Sala, 2010, Chapter 1). Instead, the rate of forgetting is much closer to a power curve where forgetting increases rapidly shortly after learning before decaying at a slower rate until it plateaus. This is consistent with the finding from this chapter that the number of word pairs and the number of image pairs correctly recalled was significantly worse over the first night than over the subsequent six nights combined. However, the fact that the majority of the forgetting that occurred

over the week appears to have occurred across this first night is ambiguous: It could also have been the result of seeing all of the pairs again at T2. It remains the case that there was no difference between SHAM and STIM performance, and we cannot conclude that repeated nights of CLAS had any effect on either early or late memory consolidation.

There was also some indication that stimulation led to less time awake during the night (WASO). However, as there was no increase in time spent in any sleep stage nor a decrease in the total sleep time (TST) in STIM, it is hard to understand where this extra time asleep is being spent. In a paper assessing the accuracy of the Dreem headband to score sleep, the manufacturers of the headband compared its internal scoring algorithm to the scores provided by five expert sleep raters using PSG of the same night (Arnal *et al.*, 2020). Their results indicated that the headband was least accurate at determining N1 sleep. Thus, in this study I have not presented the time in N1 from the headband as I do not consider it to have been accurately scored as in the vast majority of nights the headband indicated 0 minutes of N1 sleep, which is well below what I would expect (Berry *et al.*, 2018). As such, this small decrease in WASO could be caused by the headband misinterpreting N1 sleep as WASO. This is supported by the lack of difference in the subjective arousal rating (SSS) between SHAM and SIM weeks. If participants had experienced more WASO on SHAM nights it is likely they would have reported worse arousal ratings as increased WASO would indicate a more disturbed night sleep. It is perhaps safest to conclude, therefore, that not too much weight should be attached to the significant difference found. However, these results do suggest that when stimulation was applied there was no detriment to sleep overall, which could have masked any benefit of the stimulation on memory: disruption to sleep could have negatively impacted memory recall.

3.5.2 Conclusion

Despite the expected influence on sleep ERP, CLAS over one and seven nights did not influence behaviour on our WP linked tasks. It could be concluded that there is something in the procedure of previous experiments or in their study population, that has not been captured here. However, results from this study do add to growing evidence that CLAS does not improve WP memory or memory in other closely related tasks. The study was also the first to test the effects of CLAS on these specific behavioural tasks in an adult population. Of course, the lack of an improvement on the WP task, in particular, could have been due to the use of the headband in the

much less controlled home setting compared to previous work conducted in sleep laboratories. Nevertheless, the ERP results indicate the device seems to have worked as expected. On this basis, one needs to consider the possibility that the behavioural testing procedure might have disrupted the ability to observe positive effects. This conclusion, however, markedly undermines the view that CLAS could improve memory generally. In everyday life we rarely have a single mnemonic task to perform, and it rarely involves learning new word pairs.

This chapter gives us initial insight into the effects of repeated nights of CLAS upon two declarative memory tasks, the effect of reward on WP performance following CLAS in adults and the effect of CLAS upon the memory for paired images. This will allow further studies to build upon this knowledge to further understand the impact, if any, of CLAS upon overnight consolidation of memories.

Chapter 4

Closed loop auditory stimulation changes BOLD activity at declarative and procedural memory recall

4.1 Abstract

It remains unclear whether or not closed loop auditory stimulation (CLAS) affects memory. Early investigations showed the benefit to memory for pairs of words if CLAS was applied during post encoding sleep. However, this finding has not generalised to other memory tasks and recent attempts to replicate early findings have failed. In line with this, the studies reported in Chapters 2 and 3 did not find any influence of CLAS on declarative, procedural or pattern separation tasks. This was investigated with behavioural and EEG methodologies.

In this chapter fMRI is used to assess whether repeated CLAS impacts the neural processing associated with memory and behavioural performance using three tasks: (1) A version of the word pair (WP) task more closely aligned in procedure to those found to be positively influenced following one night of CLAS. (2) A procedural memory task (serial reaction time task, SRTT), as procedural memory has been shown to take longer than declarative memory to consolidate leaving more opportunities for repeated nights of CLAS to influence behaviour and brain activity. (3) A pattern separation task (mnemonic similarity task, MST), as pattern separation is a process that has been linked with the hippocampus. Indeed, the results of recent research suggests that that pattern separation benefits from sleep, and increased SO and spindles. The use of fMRI, in addition to the other modalities, gives spatial and functional neuroimaging data which has not been used previously to investigate the effects of CLAS on memory recall.

20 participants slept for two weeks wearing an ambulatory dry EEG device capable of delivering CLAS. During one week CLAS was applied during SWS, while during the other (SHAM) week no sound was applied. At the start of each week, participants were taught stimuli on all three tasks and tested on their memory for stimuli recall. Participants were then tested again following one-night of sleep with the headband and following a further six nights of sleep. Testing for the SRTT and one of the WP tests in the MRI scanner occurred following one and seven nights. Participants were then taught novel stimuli on all three tasks before a final night of sleep (SHAM or STIM), the day after which they underwent testing for the stimuli learnt the previous day.

While there was no effect of CLAS on performance in the WP or SRTT, fMRI data indicated changes in brain activity following CLAS: In the SRTT, cerebellum activity increased across the week following CLAS, while activity in the caudate and temporal

lobe were higher with CLAS. Activity in the putamen was also greater during WP recall following CLAS. Results also indicated a performance decrease in the MST over the first night following learning (at the start and end of a week of stimulation), indicating a decline in pattern separation performance following CLAS.

These finding are the first to consider the impact of CLAS upon brain activity during declarative or procedural memory recall. This opens questions on why there were changes in activity in brain areas related to the tasks following CLAS which did not result in changes in behaviour. These results are also the first to indicate an influence of CLAS on any task other than the WP task, and the first to indicate a decline in pattern separation performance following stimulation. This poses further questions as to why sleep can benefit pattern separation but CLAS sleep can lead to its detriment.

Together these results indicate that CLAS is affecting brain activity despite no change in performance on WP and SRTT tasks. However, also that CLAS leads to a decline in pattern separation performance over one night.

4.2 Introduction

In Chapter 3 I explored the effects of repeated nights of closed loop auditory stimulation (CLAS) on memory using three tasks (including a WP task). Neither one, nor repeated nights of stimulation, had any effect on behavioural performance in these tasks. These results contrast with the improvements on the WP task seen after only one night of CLAS (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017). I hypothesised that there might be differences in how the brain is performing the tasks following CLAS, but these differences might be too subtle to impact behaviour. This left open important questions: Does CLAS affect how the brain performs memory recall? Is this affected by repeated nights of stimulation? In Chapter 4, I aim to thoroughly investigate the effects of a week of CLAS on not only behaviour, but also any changes in BOLD activity through fMRI scanning when recalling task-related memories. To date, MRI scanning has not been used to understand how CLAS affects the recall of memories. Indeed, the only research that has used MRI in combination with CLAS involved assessing activity during image encoding following stimulated or non-stimulated sleep (Ong *et al.*, 2018).

In this chapter I examined the influence of repeated nights of CLAS on three tasks: the word pair task (WP), the serial reaction time task (SRTT) and mnemonic similarity

task (MST). I used a conventional WP task, which was more closely based (cf. Chapter 2) on those procedures shown to lead to a positive impact of CLAS (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017).

I also used the SRTT again. Despite Chapter 2 indicating no effect of one night of CLAS on SRTT performance there is evidence repeated nights might incur performance changes: Unlike declarative memories (e.g., those acquired during the WP task), procedural memories are often acquired more slowly, and can last for years (Pereira and Lewis, 2020). Finger tapping tasks have long been used to probe the consolidation of memories across sleep, and the skills developed during the SRTT (a procedural motor sequence learning task) has been shown to last for at least one year following training (Romano, Howard and Howard, 2010). Procedural memory has also been shown to be improved by sleep (Fischer *et al.*, 2002; Walker *et al.*, 2002, 2005; Albouy *et al.*, 2013; Landmann *et al.*, 2014; see also further discussion in sections 1.6.2 and 2.2). A single night of sleep results in improvements in finger tapping tasks, similar to the SRTT, relative to the same retention interval spent awake (Walker *et al.*, 2002). It has also been shown that repeated nights of sleep lead to larger improvements in task skill than one night of sleep, even without further testing or training (Walker *et al.*, 2002). This suggests that consolidation occurs in nights after the first post-training night, which might mean that those memories could be influenced by CLAS over a more protracted period. Therefore, repeated nights of stimulation might be expected to have beneficial effects on performance in the SRTT task, despite one night being ineffective (Chapter 2).

Human memory systems can hold distinct and overlapping memories. We are also able to take new information and integrate it into existing schemas of knowledge or update those schemas to account for new information (for a review see Landmann *et al.*, 2014). This requires the brain to represent how information overlaps and how it is distinct. The process of pattern completion refers to the idea that a fragment of a training stimulus (e.g., X within the pattern BX) can retrieve the whole training pattern (AX), and pattern separation refers to the process wherein memories of similar patterns (AX and BX) come to address distinct representations. The hippocampus has been heavily implicated in these processes, and it has become clear that different regions of the hippocampus appear to control different aspects of pattern separation (see review Rolls 2013) and pattern completion, although the two processes are complementary to each other (Ngo *et al.*, 2020). Recent studies have indicated that

pattern separation is benefited by sleep (Hanert *et al.*, 2017; Doxey *et al.*, 2019). Hanert *et al*., (2017) reported a positive correlation between assessments of pattern separation and SO and spindle density, and hypothesised that this relationship reflected the interplay of oscillations involved in active systems consolidation (ASC, see discussion in section 1.5.2), with the hippocampus helping to stabilise distinct representations. These processes are therefore a potential target for CLAS. However, sleep has also been closely linked to the abstraction of gist, linked to pattern completion (Lewis and Durrant, 2011; Stickgold and Walker, 2013; Lewis, Knoblich and Poe, 2018). If the hippocampus is performing both operations, it is unclear if stimulation will specifically boost one over the other, both or neither. Moreover, there is also the potential that CLAS might affect the processes of pattern completion and separation differentially across several nights, given the changing involvement of the hippocampus in memory consolidation across time (Vahdat *et al.*, 2017). This makes it especially interesting to assess the impact of CLAS over time.

4.2.1 fMRI and recall

The use of fMRI to assess the changes in blood oxygen levels enables brain correlates of memory processes to be assessed alongside the impact of CLAS on this relationship. CLAS has been implicated in boosting consolidation during SWS via the interaction of SO, spindles and hippocampal ripples to facilitate the long-term storage of memories beyond the hippocampus (Ngo, Martinetz, *et al.*, 2013). On this basis, it might be expected that hippocampal activation during memory retrieval would be reduced following CLAS to the extent that memories are less reliant on the hippocampus, following storage in the cortex (Rasch and Born, 2013).

In the SRTT one might expect changes in the level of activation across primary motor areas, the striatum and the cerebellum. Indeed, increases in activity in primary motor areas, the cerebellum and frontal areas have been linked to fewer errors in performance on a sequence finger tapping task following sleep, versus the same task following wake (Walker *et al.*, 2005). It has been shown that during learning and improvement on finger tapping tasks the brain engages cerebellar-cortical networks, while following the achievement of a performance plateau the striatum and cortex are more heavily involved (for a review see Doyon *et al*., 2009).

For the WP task, previous fMRI experiments using this task (not in the presence of CLAS) have highlighted activity in the precuneus, visual integration area, frontal cortex and anterior cingulate in retrieval of pairs (Mottaghy *et al.*, 1999). There is also

significantly higher activation in the hippocampus in older adults carrying out WP retrieval than younger adults (Mander *et al.*, 2013). This difference was linked to poorer performance in older adults on this task. Reduced BOLD activity in the hippocampus was also directly linked to an increase in SWA and a decrease in memory performance (Mander *et al.*, 2013). Therefore, in this experiment one might expect CLAS to reduce hippocampal activity to a greater extent than SHAM. I might also expect this relationship to increase over time. Other areas related to activity in the task may also differ in their activity following CLAS: Stimulation has been shown to improve the recall of word pairs and thus CLAS may reduce the activity in areas related to task encoding (i.e. hippocampus) and increased activity in areas related to post consolidation cortical memory retrieval. The use of fMRI imaging during memory recall allows this hypothesis to be explored for the first time.

4.2.2 First night consolidation

In Chapter 3, there was evidence of greater forgetting across the first night following encoding, than over the rest of the week. This implies that the first night of sleep has a particularly important impact on memory. It is accepted that poor sleep, such as in patients with insomnia, impairs WP memory consolidation over one night, leading to poorer recall in these groups than healthy controls despite equal encoding (Backhaus *et al.*, 2006; Nissen *et al.*, 2011). However, due to difficulties inducing more efficient sleep in healthy participants it is unclear what effect boosted sleep prior to this first night of consolidation will have. As discussed in the general introduction (section 1.5.2) it has been proposed that sleep leads to pruning of connections that are not relevant via synaptic downscaling (for a review see Tononi and Cirelli, 2006), this process can then free up space to consolidate more information. Could CLAS boost this process and free-up more space for subsequent memories to be consolidated?

Almost all of the previously cited literature has investigated memory consolidation across only the first night post learning. It has been shown that CLAS in the night prior to encoding does not benefit encoding (Ong *et al.*, 2018; Schneider *et al.*, 2019). However, no published work has asked what CLAS preceding this first night of memory consolidation may do to the memory. Does a week of CLAS boosted SWS lead to better consolidation across a subsequent first night? Thus in this experiment novel stimuli will be encoded on day 1 (preceding any stimulation) and day 8 (following a week of stimulation). Recall for both stimuli sets will be assessed one night following their encoding, such that I can assess the first night of consolidation with and without

preceding stimulation. As such I was interested in how repeated nights of stimulation might affect consolidation across this first night.

4.2.3 Experiment outline

This experiment focused on the impact of eight nights of CLAS on memory and brain function, measured using three behavioural tasks; (1) SRTT, (2) WP and (3) MST, and fMRI scans to assess recall in SRTT and WP. Participants used the same device as in Chapter 3 to undergo CLAS at home. Stimuli for the three tasks was encoded on day one and day eight; Stimuli taught on day one was be tested on days two and seven while stimuli taught on day eight was tested on day nine. To assess the impact of CLAS I examined consolidation across both the first and last nights (first night for stimuli taught on day one and day eight respectively), as well as consolidation across repeated nights of stimulation. MRI scans were performed during recall testing (of stimuli learnt on day one) on day two and seven. Analysis focused on the impact of CLAS on brain activity and performance at memory recall.

4.3 Methods

4.3.1 Participants

20 participants (13 females, mean age=22.3 years, range=19-30) were recruited who reported no sleep, physical or psychological disorders. They were paid £140 for their participation. An online screening form and screening appointments to check MRI safety established that they refrained from regular daytime napping, had a regular sleep cycle of more than 6 hours a night, and were safe to enter the MRI machine. Two participants were excluded from all analysis as their records for their first night of CLAS were lost due to technical error, and it could not be proven whether they underwent CLAS on this night. Three further participants were excluded as they did not receive stimulation on the final night of the experiment due to technical faults with the headband, which meant that their performance change on Night 8 could not be assessed. A further participant was removed as they withdrew from the experiment following their third MRI scan, while another participant was excluded as they did not complete follow up tasks to their second MRI scan due to a personal issue. Finally, another participant was removed as they did not complete their second scan due to illness. This left 12 participants for analysis (6 females, mean age=22.6 range=19-30). The experiment was approved by Cardiff University ethics review board (approval number: EC.19.07.16.5657R2A9) and procedures were also constantly adapted to

remain in line with the Covid-19 precautions devised by Cardiff University School of Psychology during October 2020 to June 2021.

4.3.2 Materials

Sleep Monitoring and Closed Loop Auditory Stimulation

The EEG device used in this chapter was the same as that used in Chapter 3: described in section 3.3.2. As in Chapter 3 participants sleep was monitored throughout the experiment from their homes using the Dreem headband a dry ambulatory EEG device capable of delivering CLAS (Arnal et al., 2017; Debellemaniere et al., 2018).

Magnetic Resonance Imaging

All scans were conducted on a modified Siemens 3 Tesla 'Connectome' scanner at the Cardiff University Brain Research Imaging Centre (CUBRIC). The experimenter completed 'MRI Operator Training' at CUBRIC and was the lead operator on all scans and was assisted by a second operator. For all scans a 32-channel head coil was used. However, due to an error the anterior portion of the coil was switched off in a number of scans, see appendix section 7.5.1. Careful consideration was given to the effect of this on results, but due to the within scan controls used in the fMRI image analysis and the random spread of the issue, no specific adjustment was made to the fMRI data. A T1 weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE), echo planar imaging (EPI), multiband scan was performed after localiser scans to give a structural scan of the participant.

For the fMRI scans themselves: First, reference scans were taken in the opposite encoding direction to the main fMRI scan. Second, two sets of EPI multiband functional scans were conducted. Both sets of scans consisted of the same settings (see Table 7), only differing in duration. EPI set one: a 15min scan taken while participants performed recall of the SRTT task (see procedure in section 4.3.3 SRTT). EPI set two: a 10-minute scan taken while the participants completed recall on the WP association task (for procedure see section 4.3.3 WP). Third, a B0 field map was acquired to assist with mapping of magnetic field inhomogeneity's. Fourth, a series of scans set up to allow Composite hindered and restricted modelling of diffusion (CHARMED) were acquired. Finally, a CHARMED reference scan was acquired in the opposite encoding direction. Conducted with the same parameters. CHARMED analysis will not be discussed in this chapter.

Order	Scan	TR /ms	TE /ms	Flip angle /deg	Voxel Size /mm	Direction
1	Localiser	8.6	4	20	NA	A>>P
2	T1 MPRAGE	3200	2	9	1 x 1 x 1	A>>P
3	fMRI ref	2000	35	70	2 x 2 x 2	P>>A
4	SRTT fMRI	2000	35	70	2 x 2 x 2	A>>P
5	WP fMRI	2000	35	70	2 x 2 x 2	A>>P
6	B0 field map	465	4.92	60	3 x 3 x 3	A>>P
7	CHARMED	3000	59	90	2 x 2 x 2	A>>P
8	CHARMED ref	3000	59	90	2 x 2 x 2	P>>A

Table 7: *MRI scan setup. A denotes anterior and P posterior.*

Online behavioural testing

As described in Chapter 3 section 3.3.2 behavioural tests were completed online from participant's homes via Pavlovia Version 2020.2 (Pavlovia.org). Tasks were coded using custom scripts in PsychoPy3 version 2020.2.4 (Peirce *et al.*, 2019). Four participants had to carry out post MRI tasks at CUBRIC on a provided laptop, due to a fire at the data centre for Pavlovia leading to the website being inaccessible for 48h. Two of these participants were required to spend an extra night with the headband to allow them to complete day 9 tasks the following day from home when the website again accessible, as participants completed this on both SHAM and STIM weeks they are analysed along with the main group.

Questionnaires

Participants completed a sleep diary for each experimental night of the experiment, (appendix section 7.1). The diary included time to bed and to rise, estimates of wake after sleep onset (WASO), whether the headband fell off during the night or played any audible sounds, the Stanford sleepiness scale (SSS), space to record any dreams and space to record any caffeinated or alcoholic drink consumption. Only SSS is reported here.

Finally, participants completed an online questionnaire asking about their experiences with the headband including; comfort, ease of use, and reasons for missing nights wearing the headband (see appendix section 7.1). Participants were also asked to indicate which week they felt the sounds were played overnight, as well as whether

they felt like they completed the memory tasks better in the first or second week. Participants were asked to summarise the experiment in their own words, so this could then be used to text mine instances related to the experiment from the dream reports collected daily in the sleep diary. These were completed before participants were then debriefed by the experimenter and paid.

4.3.3 Experimental design

First, participants were given instructions for headband use. Due to participant difficulties in the use of the headband in Chapter 3, more detailed instructions were given: Participants were instructed that the headband should be adjusted to make it as tight as possible while still comfortable to sleep with. Participants were also provided with written instructions for the experiment as a PDF, as well as via a custom written website which could be accessed on a mobile device; a 'How-to' video, and the contact details of experimenters to ask questions or raise issues during the experiment. Second, participants underwent three nights of adaptation to sleeping with the device at home. During this adaptation period no sounds were played through the headband, but participants were asked to turn it on to allow for checks into data quality and that the participant could operate the device correctly. Before the end of adaptation, the experimenter conducted a video call with participants to check headband use and address any issues. Following this adaptation, participants brought the headband into the laboratory so that the experimental condition could be set up on the headband (STIM = overnight CLAS, SHAM = no stimulation). Each night (including adaptation) participants were instructed to put on the headband and switch on the recording before they went to bed, turn off the recording when they woke, and place the headband on to charge until the following night. Each time the participant attended the laboratory for an MRI scan they were instructed to bring the headband so that the experimenter could upload sleep recordings. Anonymous EEG data was uploaded to Dreem servers before being downloaded locally by experimenters.

The design of the study was within-participants, with participants receiving the SHAM and STIM conditions in a counterbalanced order. Participants were blinded to which condition they were experiencing. Participants completed: 3 behavioural tasks in the following order; SRTT, MST and WP; five times T1-T5; online at home (see below 4.3.3 Experimental design sections for task procedures and illustration of whole experiment procedure in Figure 31). Participants were instructed to begin tests between 4pm and 6pm, and were informed that the time they began tests would be

recorded. As in Chapter 3 participants were emailed links to access tasks on the day they needed to complete them, and instructed to complete tasks in the order presented (SRTT, WP, MST). After the initial adaptation, participants completed encoding and test 1 (T1) on all three tasks. They then experienced the first experimental night with SHAM or STIM. The following afternoon (at 5pm or 7pm) they attended the lab for an MRI scan which consisted of structural, diffusion and functional scans where the SRTT and WP tasks were tested (T2, see below section 4.3.3 for MRI procedure). Participants then returned home and completed a follow-up test on the WP task and the test for the MST (T2). Participants then continued to wear the headband at home for a further six nights (Figure 31). At the end of the week participants returned to the lab for a second MRI scan, with identical procedure to the first (including the same stimuli for SRTT and WP tasks, T3). Following the scan, they returned home where they again completed a follow up WP test and the MST test (T3). They also underwent the same learning tasks from the start of the week once again (SRTT, WP and MST), this time with new stimuli (T4). The following day participants were tested at home on these new stimuli (T5). Participants then had at least 1-week rest, where they did not wear the headband, or undergo behavioural testing, before completing all of the testing again with the opposite condition (SHAM or STIM) and new stimuli at T1 and T4.

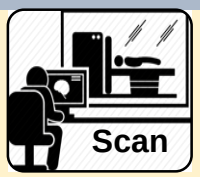
Day	Night	Participant task	
-2 to 0		Participant receives Dreem headband. Sleep questionnaire (each day) and video call with experimenter (once).	
	-2 to 0	Wear headband to sleep at home. Headband switched on. SHAM.	
1		Sleep diary questionnaire. **Encoding + T1:** 3pm – 6pm start. 1. SRTT, WP, MST	
	1	Wear headband turned ON. STIM OR SHAM.	
2		Sleep diary questionnaire **T2** MRI, 5pm – 6pm start: 1. T1 MPRAGE, 2. SRTT fMRI, 3. WP fMRI (T2a), 4. CHARMED (1hour total) Post MRI @Home. 1. WP (T2b), 3. MST	 
	2	Wear headband turned ON. STIM OR SHAM.	
3 to 7		Sleep diary questionnaire	
	3 to 7	Wear headband turned ON. STIM OR SHAM.	
8		Sleep diary questionnaire **T3** MRI, 5pm – 6pm start: 1. T1 MPRAGE, 2. SRTT fMRI, 3. WP fMRI (T3a), 4. CHARMED (1hour total) Post MRI @Home. 1. WP (T3b), 3. MST **Novel: Encoding + T4** Post MRI, 1. SRTT, 2. WP, 3. MST.	  
	8	Wear headband turned ON. STIM OR SHAM.	
9		**T5** Same time as encoding T4, 1. SRTT, 2. WP, 3. MST	

Figure 31: *Experiment design.*

MRI Procedure

Scans lasted for approximately one hour, and the scanning operation was led by Holly Kings with assistance from another experimenter to comply with safety procedures in

place at CUBRIC, which required two MRI trained personnel present. Participants were screened on arrival at each scan for any medical issues or metal items which might cause them harm in the MRI scanner, before changing into scrubs provided by CUBRIC. At T2 (first scan) they were shown an online video with instructions on how to complete the SRTT and WP tasks inside the scanner and given the chance to ask questions. They were offered the video again at each subsequent scan. Prior to placement in the scanner, participants were given MR safe in-ear earphones for sounds to be delivered during the SRTT task, a button box consisting of five buttons placed under each finger on their left hand and a panic alarm with which to alert experimenters of any issues during the scan. Participants were then placed in the scanner and a localiser scan was conducted to determine the location of each participant's head (11 seconds) and allow field of view (FOV) for subsequent scans to be determined.

Scanning parameters are shown in in Table 7. Following the localiser, a structural MPRAGE scan was conducted lasting 6 minutes and consisting of 192 sagittal slices. Following MPRAGE a reference scan was conducted for the fMRI scans lasting around 30seconds. During this scan the sounds for the SRTT task were played to participants through the earphones. The sounds were specific to the sequence they were about to hear, but played out of sequence with >1 second between sounds to avoid reinforcing the trained sequence. Prior to the sounds being played, on-screen instructions informed the participants of the sounds and asked them to alert the experimenter following the scan if they could not hear the sounds over the scanner noise: As each sound was played the word 'Sound' appeared centrally on a black screen to indicate to participants when sounds should play. Following the scan participants were asked via an intercom if they could hear the sounds. If they could then the MRI procedure continued to the next scan, if they could not then the volume was increased and the reference scan was repeated. Once participants were happy they could hear the SRTT sounds over the scanner noise, the fMRI scan to assess BOLD response during SRTT task was undertaken (see next section for task procedure). There was then a 10 minutes fMRI scan during which recall for fifty of the WP pairs was tested (see section 4.3.3 Word Pairs for task procedure). A B0 map was then taken (1 min) to allow fMRI images to be distortion corrected during analysis. Finally CHARMED microstructure scans were conducted lasting around 15 minutes. Participants were then removed from the scanner, given time to change before returning home to complete further behavioural tests.

Serial Reaction Time Task

Participants were first exposed to a sequence on the SRTT on Day 1 (T1, at home; see Figure 31), where they received training via Pavlovia, on a sequence of 12 button presses. They were then tested on the same sequence on Day 2 (T2) and Day 8 (T3) in the MRI scanner whilst undergoing fMRI scans, using MATLAB. On Day8, following the MRI scan, they were also taught a new sequence (T4) that was tested on Day 9 (T5); both tasks occurred at home and were delivered using Pavlovia. On their second run through the experiment, participants were taught two new sequences. Training sequences were counterbalanced between participants using a Latin square. I modified a script from Chapter 2 for use in the MRI, while new scripts for PsychoPy3 version 2020.2.4 (Peirce *et al.*, 2019) were developed collaboratively, to run on the online platform Pavlovia.

The SRTT task used in this experiment was similar to Chapter 2 but with a few differences: Inter stimulus intervals were reduced to 300ms from 1230ms, to allow participants to perform much faster on the sequence blocks. Random sequences were changed to follow the following rules: (1) No consecutive equal stimuli (i.e. 1, 1); (2) no sequences of five or more items that match any of the testing sequences; and (3) each item must repeat the same number of times across each test (i.e. five occurrences of item 4). This allowed random trials to represent a better control as they more closely matched conditions of the sequence blocks just without the sequence. Random sequences were generated before the experiment began and fixed for all participants, so that participants saw the same random stimuli associated to the same sequences as each other. Two new testing sequences were also introduced to allow each participant to conduct the complete test 4 times: C [3 4 3 1 2 1 2 3 4 3 4 1] and D [4 1 3 1 4 3 2 3 2 4 1 2]. These new sequences were constrained by the following rules: (1) 12 items long, using only the items 1 to 4; (2) contain 3 repeats of each item; (3) don't share sequences of five or more items with another sequence (i.e. A, B, C or D); (4) each item appears in each half of the sequence; (5) no two items repeat in a row (i.e. 2, 2). These were the same constraints used by Cousins *et al.,* (2014) to generate sequence A and B. One block of training was also modified so that it contained three repeats of a sequence (instead of six) and had no fixation cross break. Breaks between blocks were shortened to 15s with a 5s countdown. The images and sounds were also changed from the task used in Chapter 2 so that there were four separate image and sound sets, one for each sequence (A, B, C and D): As four repeat instances of the sequence were being used, it was thought

that having 4 unique stimuli sets would better allow us to track changes in memory overnight. Particularly in the random blocks which, without different images and sounds for each sequence, would be identical on each repeat of the test. Images were taken from the NOUN database (Horst and Hout, 2016), because the images are unusual and therefore more likely to form their own unique memory engrams (see images in appendix section 7.5.2). Sounds were generated by a computer to emulate a violin, a trumpet and a piano playing four musical notes (A, C, D and E). Sounds were still 200ms long with a 10ms fade in and out, and a central letter still indicated to participants which sequence (or random block) they were using.

Encoding: Participants saw 20 blocks with sequences and 2 blocks of random trials. As stated above, one block consisted of three repeats of the 12 item sequence (sequence block) or 36 stimuli following the random rules (random block). Feedback (15s) on speed (fastest and mean response) and accuracy (number of incorrect button presses) was also provided following each block.

MRI recall: two tests were made of this task: on Day 2 (T2, Figure 31) and on Day 7 (T3 Figure 31). These tests were completed in the MRI scanner, and lasted for ~15min. Tests consisted of 14 blocks of sequence and 2 blocks of random. Intervals between blocks in the MRI scanner were divided into *feedback time*, which consisted of 15s to read the same feedback as that provided in encoding, and *rest time*, where participants had between 15s and 20s (jittered) with a fixation cross. Participants made responses using button boxes that were compatible with the modified 3 Tesla Siemens MRI scanner. Buttons were placed under the four fingers on their left hand such that the buttons were labelled one to four left to right, as on a keyboard. While participants undertook this task they were undergoing fMRI scans detailed in 4.2.1 MRI Procedure.

At home recall: Testing on Day 9 was conducted online at home, following the same format as in the MRI scanner (i.e. 14 sequence blocks and 2 random) except that rest time was removed and participants again used buttons 1-4 on a standard qwerty key board.

Word Pairs

To probe declarative memory, a WP associates task was used to compare long-term effects of CLAS with short-term effects reported in the literature (Ngo, Martinetz et al., 2013, 2015; Ong et al., 2016; Leminen et al., 2017; Papalambros et al., 2017). The same word list was used as in Chapter 3. Pairs were semantically linked such as ‘Nail’ and ‘Varnish’, for a full list of words used please see appendix section 7.2. Word pairs

were then randomly assigned to four lists, each containing 75 pairs, for each participant, such that over the course of the experiment each participant learnt all 300 words via four unique lists. Custom scripts were written to run this task using MATLAB in the MRI scanner, and PsychoPy3 version 2020.2.4 (Peirce *et al.*, 2019) and Pavlovia, Version 2020.2 (Pavlovia.org) for at home online testing.

Encoding: Procedure was similar to Chapter 3 but with only one list and no reward instructions. After the participants had received instructions, each pair was displayed centrally on a screen for 2s, with 1s fixation cross between words. Participants were not asked to make any response but were informed that they needed to recall the word pair and told not to write anything down. After all pairs has been displayed, memory for the pairs was tested: The first word of the pair was presented on a screen and the participants were asked to type in the correct pair to that word. They were allowed to skip a trial if they did not know the pair word. Irrespective of whether their response was correct or incorrect, participants were then presented with the correct pair word. Testing continued until participants obtained >50% correct (38 words). If they did not reach 38 correct on the first run through of all 75 pairs, then they were shown only the words that they got wrong until they cumulatively reached 38/75 correct. In each round the order of the words was changed, to avoid participants recalling word order instead of the pair association. This criterion acted as a base level of performance required to ensure that participants had encoded an adequate number of the word pairs. Finally, participants received a test of all 75 word pairs without feedback. This test was used as their baseline for pairs learnt.

Recall: The recall of the WP task was conducted the day after encoding, first in the MRI scanner then again outside of the scanner once the participant had returned home.

MRI recall test: A list of 50 words was created for each participant based upon their answers in the final encoding test for use in the MRI scanner recall tests. Ideally, this list consisted of 30 words correct at encoding, and 20 words incorrect at encoding. However, if participants did not have 20 words incorrect at encoding (due to the 50% criterion they must have at least 30 correct) then all their incorrect words were used alongside enough correct words to make the list up to 50. During the fMRI scan participants were again presented with the first word of the pair and asked to silently recall the corresponding pair. They were given the opportunity to recall the pair word for 4 seconds (pilot data indicated that the mean reaction time for word recall was 2.61s, n=9). During this time, they were asked to indicate with a button press if they

recalled the pair word or not. They were then asked to select the correct second letter of the pair word. That is, if the pair was ‘Passion’ and ‘Kiss’ then they would select ‘I’. They were given 4 options of letters (i.e. I F E O), that appeared centrally on screen below the numbers 1 to 4 which indicated which button participants should press to select each letter (Muehlroth *et al.*, 2019). The location of the correct letter in the four letter order was randomised, so that it did not always appear in the same position. The three incorrect letters were randomly selected from a list of all the other second letters of pair words. The four letters were also checked for repeats, and another letter selected if repeats were found, such that there were always four unique letters to choose from. Participants were given 5s to make their choice before the next trial began. 14 null events were randomly interspersed amongst trials to provide more randomness in the fMRI task, and prevent any synchronisation between responses and other physiological factors such as heart rate. Null events consisted of a fixation cross for 9s. Responses were made using an MRI safe button box, positioned in the participants left hand. Prior to starting the task in the MRI participants had the chance to complete a 5-trial practice run before the scan was started, trials were identical to testing and contained five extra (not used in fMRI scan test), words they got correct at encoding final test. This set up was adapted from the WP type tasks used by Ngo, Martinetz *et al*., (2013) and Meuhlroth *et al*., (2019).

After the MRI scan was complete, participants were instructed to complete a further test: This was conducted at home on their own PC following the MRI scan. Participants were presented with the first word of all 75 pairs, one at a time, and asked to type its corresponding pair, no feedback was provided. Following submission of their answer, if the pair being tested was presented in the scanner, they were immediately asked if the word they just typed was the same as the word they were thinking of in the scanner. These results could be used along with responses in the scanner to attempt to determine if the participant had recalled the correct word in the scanner or not. Other ways of asking the participant to give the corresponding word pair in the scanner were considered, such as asking participants to speak out loud the word, or asking the participant to use two button boxes (up to 10 numbered buttons) to type the response. However, the use of verbal reporting was ruled out due to potential issues with mouth movement and speech generation leading to brain activity that obscured the activity from word recall. Typing was also discounted due to various artefacts potentially created due to different levels of movement per word and the increased time it would take for participants to make responses.

Encoding and testing for this task at the end of the week (Day 8/T4 and Day 9/T5), was completed online: Encoding followed the same procedure as the encoding on Day1, using 75 new pairs, and testing on Day 9 (T5) consisted of a single test of each pair without feedback.

Mnemonic Similarity Task

A similar protocol to that described in Chapter 2 was used for the MST task. The procedure followed that set out in Hanert *et al*., (2017) and used stimuli from Stark *et al.,* (2013). However, a new script was written to allow this task to be run online and completed by participants in their own homes. Four of the image stimuli sets created by the Stark Lab (Stark *et al.*, 2013) were used. Each participant learnt each of the four sets during the experiment (at T1, or T4, round 1 or round 2), which were counterbalanced using a Latin square. Each set contained 192 images, each with a similar pair image, 180 images were selected from each set. Each participant was trained on a unique combination (i.e. old similar or new) of the 120 images from within a set. Stark *et al*., (2013) images can be divided into five similarity bins depending upon how similar the two paired images are. The stimuli combinations learnt by participants contained twelve images from each similarity bin. Half of these images were tested immediately and half after a retention interval.

Encoding: Participants were shown each of the 120 colour object images, each on screen for 2s. As they viewed each image they were asked to classify the image as an indoor or outdoor object, pressing the *v* key for indoor and the *n* key for outdoor. Encoding occurred twice, once on Day 1 and once on Day 8, utilising the same procedure but completely different image lists.

Recall: Participants' recall was assessed immediately following encoding (T1 and T4). Their completion of the recall task was supported by a short instructional video (Stark, Kirwan and Stark, 2019). In the test, participants were shown 90 images and were asked to classify each image as *old* by pressing the *v* key (the same as one seen in the first section), *similar* by pressing the *b* key (a similar image to one shown in the first section) or *new* by pressing the *n* key (an image never seen before in the context of the task). This comprised the 'old similar new' task (OSN). In this test 30 images were the same as those shown in the first section (old), 30 images were totally new (new), while the remaining 30 were the pair image to that shown in the first section (similar). Participants were then tested again using the same protocol as the second phase (old, similar, new test) the following day (Day2 T2 and Day9 T5), but now on the other half of the images learnt but not previously tested in the recall phase.

Following a week sleeping with the headband the participants were again tested on the same stimuli as T2 following the MRI scan (Day 8, T3).

4.3.4 Data Analysis

Dreem headband

As described in Chapter 3 (section 3.3.5) the time spent each night in each sleep stage was calculated by the headband's own scoring system, without N1 (see Chapter 3 discussion section 3.5.), along with TST, sleep onset and WASO. Mean time in each stage was calculated for each participant and the group, before appropriate statistical testing to assess difference between SHAM and STIM. Time in each sleep stage was calculated for the first night (n=10 as two participants were missing the raw recording for one of their first SHAM nights), nights 1-7 (n=12) and night 8 (n=11 as one participant had to be excluded as they were missing the final raw recording on SHAM).

EEG data from the headband was analysed in the same way as described in Chapter 3 (section 3.3.5 Analysis) including filtering and cleaning of the signal resulting in grand mean ERP for the group representing sound and no-sound trials from the STIM nights.

The first and last night grand means for each participant were again collated to provide a grand mean of first and last nights. This allowed the investigation of the effect of repeated nights of stimulation upon the ERP. For each participant Monte Carlo cluster permutations (significance level of p<.05) was applied from 0s to 2.5s compare the first and last STIM night.

Behavioural tasks

As in Chapter 3 (section 3.3.5 Analysis: Behaviour) spreadsheets containing participant responses were downloaded from Pavlovia, and custom written MATLAB scripts used to extract raw scores. Raw scores at T1, T2, T3, T4 and T5 were then assessed for all behavioural tasks as well as retention (calculated as percentage change), over, Night 1 ($\frac{(T2-T1)}{T1} \times 100$) , Night 8 ($\frac{(T5-T4)}{T4} \times 100$) , Nights 2-7 ($\frac{(T3-T2)}{T2} \times 100$) and Nights 1-7 ($\frac{(T3-T1)}{T1} \times 100$) for WP and SRTT. In MST only, absolute change was used instead of percentage change in score over retention intervals; change overnight (Night1 = $T2 - T1$, Night 8 = $T5 - T4$) Nights 2-7 ($T3 - T2$) and Nights 1-7 ($T3 - T1$).

As I was interested in if repeated nights of consolidation would improve consolidation of newly learned material over one night, I needed to understand how performance

on tasks changed on Night 8 (seven nights of prior stim) compared to Night 1 (no prior stim).

Statistics were conducted in the same way as described in Chapter 3 section 3.3.5: using R studio, a significance threshold of p=.05, two-tailed tests, Shapiro-Wilk tests for normality, paired-t-test, paired Wilcox test, and repeated measures of analysis of variance (RM ANOVA).

Serial Reaction Time Task

As in Chapter 2 section 2.3.5: First, for each participant at each test; custom MATLAB scripts extracted the RT for each stimulus, any RT lying outside 2SD (SD calculated per block), were removed. The mean RT was then calculated per block for each participant. Second, group means were calculated for each block under both conditions. These group mean RT along with SEM, were plotted, to give a visual indication if there was any difference in the raw data between SHAM and STIM conditions during the experiment (see Figure 33). As this did not indicate any differences between SHAM and STIM, a measure of the influence of the sequence on RT (sequence specific skill, henceforth referred to as SKILL) was calculated. This allowed me to assess the influence of stimulation on the sequence knowledge, and how this affected RT (as per Chapter 2). SKILL indicates the proportion of the RT that can be attributed to sequence knowledge (i.e. how much faster the participant reacts to the stimuli when there is a sequence vs when stimuli are random). $SKILL = average\ RT\ to\ last\ four\ sequence\ blocks - average\ RT\ to\ random\ blocks.$ Four instead of two blocks (as in Chapter 2) were used as I have halved the number of sequence runs in each block such that this represents the same number of trials. First raw SKILL was assessed at each test (T1, T2, T3, T4, T5), to consider the impact of CLAS on performance. Then to further investigate the effect of repeated nights of CLAS, SKILL was calculated and assessed for each retention interval (Night 1, Nights 2-7, nights 1-7 and Night 8).

Word Pair

To assess the impact of CLAS on WP performance across the week, the raw data from T1, T2 and T3 tasks were assessed. Data was divided up based on the maximum possible number of correct pairs, due to the testing of a subset of pairs inside the MRI scanner; a = all 75 word pairs, b = 55 scan pairs assessed outside the MRI scanner, c = 20 pairs not assessed in the scanner and s = 55 scan pairs tested inside the MRI scanner. Thus tests were conducted on: (1) T1a, T2a and T3a, (2) T1b, T2b and T3b, and (3) T1c, T2c and T3c.

To assess the impact of stimulation over each retention interval the percentage change (as detailed above) was assessed. Intervals composed of: Night 1, Nights 2-7, Nights 1-7 and Night 8.

Mnemonic similarity task

The responses to images were extracted from Pavlovia results spreadsheets, using a custom MATLAB script. As in Chapter 2 I then calculated the pattern separation score (PSS) $PSS = correct\,'similar'response\ to\ similar\ image - false\,'similar'response\ to\ new\ image$

and recognition memory score (RMS) $RMS = correct\,'old'response\ to\ target - false\,'old'response\ to\ foil.$

Subtracting false similar responses from PSS and false old responses removed the bias of preferentially responding with these buttons according to Hanert *et al.,* (2017). PSS scores were also subdivided into five lure similarity bins, based on how similar the target lure image was to the target image (rated by Yassa *et al.*, 2011): B1 (most similar) to B5 (least similar). The least similar images should be easier to tell apart and lead to fewer old responses to similar images, improving PSS, while the most similar images B1 should lead to more old responses to similar images, worsening PSS. First raw PSS and RMS scores were assessed, before the absolute change between testing intervals was calculated. Unlike SRTT and WP, meaningful values for percentage change could not be calculated due to the large number of zero scores: there is no meaningful way to calculate a percentage change which starts at zero and ends with a non-zero value. A further participant had to be excluded from MST data analysis as they completed their final T4 and T5 tasks in the wrong order due to experimenter error (i.e. they completed the testing phase before the learning). Therefore, n=11 for the MST analysis.

fMRI

Pre-processing

fMRI pre-processing was carried out using statistical parametric mapping 12 (SPM12) software developed by University College London Wellcome Institute for Neuroscience (Penny *et al.*, 2007). Pre-processing and statistical analysis were the same for SRTT and WP fMRI scans, with only the design matrix (DM) specific to each scan. First, raw DICOM files from the scanner were converted to *.nifi*, using a custom written MATLAB script, to allow for processing. Second, images were passed into a custom built pre-processing pipeline. The pipeline consisted of several steps: (1) images were realigned to the first fMRI image in that scan to correct for any small

movement participants may have made during the scan. (2) Scans were then corrected for inhomogeneities in the magnetic field caused by the presence of the participant in the scanner bore, using a separately acquired field-map scan (Table 7 scan 6: 'B0 field map'). This field map indicated where inhomogeneities were largest and allowed the fMRI images to be corrected accordingly. In some scans the field of view used to acquire the B0 field map was too small, causing the image to wrap around in the phase encoding direction, along the Y axis. This was accounted for in the application of the field-map to the fMRI images, by informing SPM12 that wrapping in the Y direction had occurred. This allowed the program to find information missing from the back of the head at the front of the image, and therefore no image was lost. (3) fMRI scans were then co-registered to the T1 MPRAGE structural scan from that session, using the mutual cost function. (4) fMRI scans were then segmented into white matter, grey matter, cerebral-spinal fluid (CSF), skull, soft tissue and 'other'. (5) The functional and structural images were then normalised to fit a 'standard space' using the Montreal Neurological Institute mean of 140 structural scans, so that scans from different participants and sessions can be compared. (6) Structural scans were also normalised so that each individual's functional scan could be compared to their structural scan during pipe-line checking. (7) fMRI images were then smoothed using an 8mm full-width half maximum (FWHM) gaussian kernel. (8) Finally, the translations and transformations made by realignment of the fMRI images was checked to ensure no participant moved father than 3mm or 3degrees. One participant was removed from analysis of the SRTT fMRI images due to moving 4.32mm along the z axis, in the SRTT scan.

First-level statistical analysis

Once pre-processed, the first level of statistical analysis was performed on each participant's four individual scans (test two/T2 and test three/T3, SHAM and STIM). A DM was compiled for each scan (i.e., one for each participant, each task and each session), indicating the timings during the fMRI task when participants observed specific stimuli and made responses. For both tasks the times spent observing at a fixation cross (rest in SRTT and null trials in WP) was not modelled to allow statistical comparisons to be made against these times as a baseline (Pernet, 2014).

SRTT task: The DM was composed of four columns indicating the times when the participant was completing: (1) the sequence blocks, (2) the random blocks, (3) the time the participant was observing feedback on their performance, (4) times when buttons were being pressed. A further six columns were added to the DM which

indicated the translations and transformations (one in each vector; x, y and z, for translations and transformations) performed during realignment which derived from movements made by the participant during the scan. Statistical analysis at the first level in this task, was a one-sample t-test with *time in Sequence blocks* modelled against the baseline (rest blocks). This gave voxels where there was greater, or less, activity at the times when the participant was completing sequence blocks than during the baseline when activity which related to the other columns in the design matrix was removed.

WP task: The remembered and forgotten trials were first identified. Remembered trials were defined as those in which participants reported that they could remember the pair word upon seeing the cue word, and when they also selected the correct letter. The duration of the remembered trial ran from the presentation of the cue word to the end of letter selection screen presentation. Forgotten trials were when the participant indicated that they could not remember the pair word when presented with the cue word and also selected number four at letter selection (as instructed to do when they forgot the pair word). Again the duration of the forgotten trial ran from the presentation of the cue word to the end of the letter selection. As such the DM consisted of three columns which indicated: (1) timing of remembered trials, (2) timing of forgotten trials and (3) times of button presses. Similar to the SRTT DM a further six columns were added to indicate times when movement occurred. For the WP task the statistical comparison at the first level was a one-sampled t-test comparing remembered trials to baseline. The initial plan was to compare remembered to forgotten trials, however participants performed very well on the task such that very few forgotten trials emerged: Too few to allow for a statistical comparison.

Second level analysis

RM-ANOVA: The multivariate and repeated measures (MRM) MATLAB toolbox (McFarquhar *et al.*, 2016) was utilised to conduct a RM ANOVA upon the statistical output of the first level analysis from all participants. The 2x2 ANOVA included Time (Scan 1 and Scan 2) and CLAS (SHAM and STIM) as within-participant factors. The only supra-threshold clusters emerged when the approximate *p* values were calculated using no whole brain correction, a *p* value threshold of $p<.001$ and Wilkes-Lambda multivariate test statistic. No supra-threshold clusters were identified as interaction or main effects from the ANOVA at stricter levels of significance thresholding.

Post-hoc testing: Clusters that indicated a significant interaction in the ANOVA, were used to form regions of interest (ROI) masks which were used to identify the drivers of these clusters through post-hoc testing. For example, ANOVA testing indicated significant clusters active in the interaction of CLAS and Time in the SRTT task. An ROI mask was created consisting of only these clusters and this was then applied to the brain during post-hoc t-tests, such that only these areas were tested for significance. This allowed for an understanding of the drivers of the significant ANOVA result, while also reducing the burden of multiple-comparison correction: As tests were only conducted on the voxels indicated in the mask fewer multiple comparisons were made so the correction is less stringent than if testing had been conducted across all voxels in the brain. As such results were only considered if they passed FWE correction across all voxels in the mask at $p<.05$. One sample t-tests were used for post-hoc testing as they allowed for easy control of the within participant element of the experimental design, such that the difference between the factors being tested could be first established for each participant before significance testing applied. For example, if an interaction was significant between time and CLAS at the ANOVA, then post-hoc one sample t-tests were applied to two sets of scans: (1) SHAM vs STIM at Scan 1 ($\mathrm{STIM\ scan\ 1\ image} - \mathrm{SHAM\ scan\ 1\ image}$) and (2) SHAM vs STIM at scan 2 ($\mathrm{STIM\ scan\ 2\ image} - \mathrm{SHAM\ scan\ 2\ image}$). This would allow understanding of where the significant difference indicated in the interaction was strongest. Calculations, indicated in brackets, were conducted using SPM12 feature *ImCalc* which allowed for one image to be subtracted from another. This utilised the intensity value at each voxel and simply preformed the required subtraction on the value at each voxel. Figures are given indicating the overlap of activity indicated by ANOVA and post-hoc tests to be significant.

To understand if areas of activity correlate with behavioural or sleep factors, these were added to MRM RM ANOVA analysis as covariates, such that each measure could be correlated with activity in each particular contrast. The sleep covariates utilised were all participant week averages SHAM-STIM and composed of: (1) percent of TST in N3, (2) percent of TST in N2 and (3) the number of stimulations delivered per night. While for behaviour the covariates were; *SRTT:* SKILL at T2 ($\mathrm{STIM} - \mathrm{SHAM}$), (2) SKILL at T3 ($\mathrm{STIM} - \mathrm{SHAM}$), and (3) SKILL in $(\mathrm{ST\ T3} - \mathrm{SH\ T3}) - (\mathrm{ST\ T2} - \mathrm{SH\ T2})$ and *WP* all ($\mathrm{STIM} - \mathrm{SHAM}$); (1) T3s, (2) Night 1 score percentage change, (3) Night 8 score percentage change and (4) Nights 1-7 percentage change.

Regions of interest

There were specific ROI where I was particularly interested in the effect of CLAS on BOLD responses during WP and SRTT recall. There were common regions for both tasks: Regions involved in memory, including the hippocampus, para-hippocampus (for a review see DeJong, 1973; or Knierim, 2015), visual cortex and striatum (Packard and Knowlton, 2002). Historically, focus on the striatum has fallen on motor responses (Doyon *et al.*, 2009), but it has also been strongly linked to memory and learning, in particular cued learning, or stimuli and response learning (for a review see Packard & Knowlton, 2002). Regions involved in SWS: the medial prefrontal cortex (Mander *et al.*, 2013).

SRTT specific ROI centred around motor regions known to be involved in this sort of procedural task: The Striatum including the caudate and putamen as these are areas involved in motor responses and memory (Driscoll, Bollu and Tadi, 2021), the caudate has also been previously shown to be influenced by TMR in this task (Fogel *et al.*, 2014; Cousins *et al.*, 2016). The cerebellum as again this is a region of particular interest in motor tasks (Walker *et al.*, 2005), as well as primary and secondary motor areas. Areas around the temporal medial gyrus were also included in SRTT ROI due to the auditory components of the version of the task used and the location of the primary and secondary auditory cortices here.

The WP specific ROI were areas known to be specifically involved in this task: Pre-cuneus, anterior cingulate and medial prefrontal cortex (Gais *et al.*, 2007; Fandakova *et al.*, 2018; Muehlroth *et al.*, 2019).

4.4 Results

4.4.1 Sleep macrostructure

Table 8 indicates the mean time spent in each sleep stage, on Night 1, Nights 1-7 and Night 8. Stage N1 is excluded due to the poor scoring of this stage by the Dreem headband. Inspection of the statistical tests described in Table 8 indicates no significant difference between SHAM or STIM over any of the stages except for a significant decrease ($z(9)=46.50$, $p=.047$) in WASO on the first night in STIM (mean=5.90 SEM=1.35) compared to SHAM (mean=16.40 SEM=5.65) .

NIGHTS	STAGE	SHAM /MIN	STIM /MIN	PAIRED COMPARISON
NIGHT 1	*TST*	*436.15 ±18.68*	*449.30 ±14.93*	*t (9)=-0.61, P=.555*
	ONSET	*14.21 ±2.07*	*19.16 ±4.08*	*t (9)=-1.34 P=.214*
	N2	*199.25 ±11.84*	*200.15 ±20.76*	*t (9)=-0.04 P=.969*
	N3	*105.75 ±7.18*	*116.90 ±11.52*	*Z(9)=14.50 P=.202*
	REM	*136.45 ±11.28*	*140.40 ±8.77*	*t (9)=-0.23 P=.824*
	WASO	***16.40 ±5.65***	***5.90 ±1.35***	***Z(9)=47.50 P=.047***
NIGHTS 1 TO 7	*TST*	*413.93 ±16.78*	*428.61 ±10.93*	*t (11)=-0.89 P=0.39*
	ONSET	*20.42 ±3.25*	*23.14 ±5.7*	*t (11)=-1.60 P=0.14*
	N2	*183.17 ±37.36*	*189.10 ±9.60*	*t (11)=0.72 P=0.488*
	N3	*106.80 ±10.79*	*109.46 ±7.66*	*Z(11)=28.00 P=0.42*
	REM	*128.22 ±9.70*	*134.40 ±6.31*	*t(11)=-0.89 P=0.39*
	WASO	*7.95 ±1.55*	*7.60 ±1.01*	*t(11)=0.20 P=0.84*
NIGHT 8	*TST*	*421.73 ±24.44*	*408.05 ±18.82*	*t(10)=0.64 P=0.54*
	ONSET	*27.35 ±10.07*	*32.26 ±11.89*	*Z(10)=36.00 P=0.83*
	N2	*170.64 ±16.57*	*175.50 ±11.10*	*t(10)=-0.32 P=0.49*
	N3	*116.14 ±9.28*	*107.91 ±10.45*	*Z(10)=48.00 P=0.21*
	REM	*138.73 ±9.20*	*130.09 ±9.84*	*t(10)=0.85 P=0.42*
	WASO	*7.50 ±2.06*	*6.59 ±1.62*	*t(10)=0.45 P=0.66*

Table 8: *Time in sleep stages. Mean ±SEM time in each sleep stage for SHAM and STIM as measured by the Dreem headband. Night 1 n=10, Night 1 to 7 n=12, Night 8 n=11.*

4.4.2 Impact of stimulation on sleep oscillations

Signal time-locked to the onset of the stimulus were averaged across all participants on STIM nights during SHAM and STIM trials in channel Fpz, see Figure 32 top and Table 9. Statistical analysis indicated that the STIM signal deviated significantly from SHAM at all-time points except those surrounding STIM zero crossings. When the

STIM trials from the first night were statistically compared to the STIM trials from the final night (Night 8) there were no periods when one signal was statistically different to the other, see Figure 32 bottom. This implies that there was no habituation to stimulation across the week, or accumulation of response.

Test	Cluster	Start time /sec	End time /sec	Test statistic	P value <.05	SEM
All Nights	Positive	1.45	1.72	0.001	.008	63.03
		0.42	0.67	0.001	.008	62.95
	Negative	0.78	1.18	<0.001	<.001	-86.30
		0.11	0.32	<0.001	.008	-60.82

Table 9: ERP clusters. *Monte Carlo significant clusters for all night ERP.*

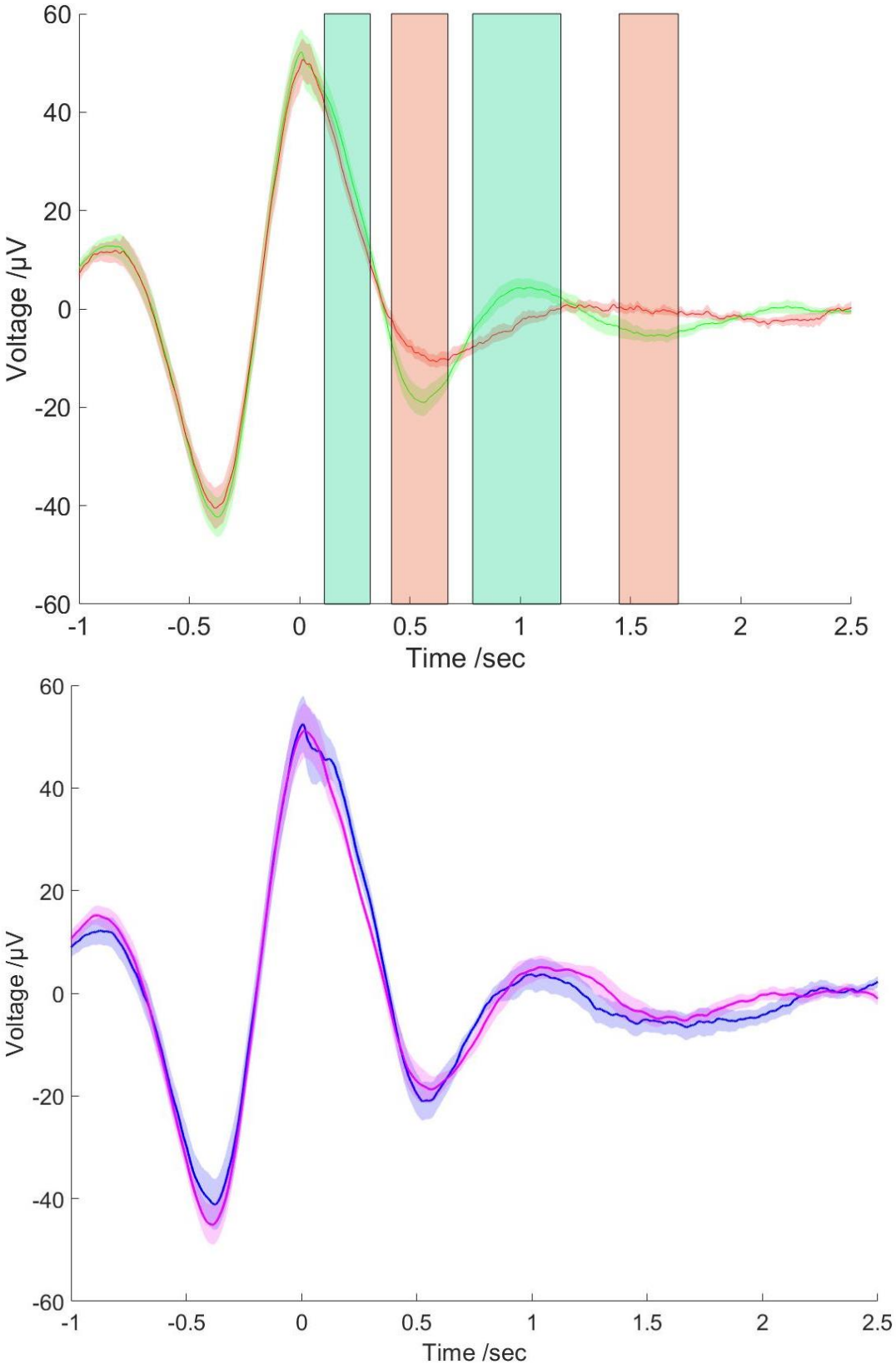


Figure 32: *Group average Event Related Potential*. ***Top****: All night ERP. Red = SHAM and Green = STIM, mean voltage ±SEM. Boxes indicate significance in Monte Carlo*

cluster permutation test p<.05, Red indicates SHAM>STIM, green indicates SHAM<STIM. ***Bottom****: First night vs last night ERP. Blue = STIM first night and Pink = STIM Night 8, mean voltage ±SEM.*

Once again (see Chapter 3 results) the results have shown that one week of CLAS leads to the expected ERP response, this time without any signs of habituation.

4.4.3 Effects of a week of stimulation on task performance

Serial reaction time task

The SRTT was used to indicate participants motor sequence learning performance across the week following repeated nights of STIM or SHAM. Inspection of Figure 33 illustrates that mean RT at each block were very similar between SHAM and STIM, with very few instances of separation of SEM across all five tests. Inspection of Figure 33 gives a good descriptor of how participants RT changed across the tests for this task: It can be seen that RT falls across each test's sequence blocks before rising at the random blocks (blue circles), this is likely to indicate that participants learnt the sequence. From T1 to T3, RT continues to decline following an initial rise in the first few sequence blocks, as the participant re-orientates with the task, indicating that with repeated testing participants are still getting faster at the task. The introduction of a new sequence at T4 appears to lead to an increase in RT, above that previously seen from random blocks, but below the initial RT in T1 early sequence blocks. RT at the start of T4 appears to be consistent with the random block RT at T1. This lower RT when learning sequence 2 than learning sequence 1 could indicate participants increase in skill / comfort with the task, particularly as it is only lower than the first block in T1. At T5 there is a similar pattern of activity to T2 where after an initial few blocks with high RT, the RT decreases over the test further than in T1. Random blocks in T4 and T5 appear to be very similar.

To quantify any differences between SHAM and STIM I first looked at SKILL: The RT difference between the final four sequence blocks and the random blocks, was calculated to determine the contribution of the sequence to the RT during sequence blocks. This is assumed to be a proxy measure of sequence learning. Inspection of Figure 34 indicates the changes in SKILL from T1 to T5. SKILL increases from T1 to T3, and T4 to T5, despite raw RT decrease (as seen in Figure 33), as SKILL indicates the amount of RT that is accounted for by the sequence, so this proportion is increasing not the block RT. There was a tendency for the SKILL in the STIM condition to be greater (T1: mean= 152.47 SEM=23.52, T2: mean=191.75, SEM=23.52, T3:

mean=221.12, SEM=16.60, T4: mean=167.87, SEM=61.52, T5: 210.66, SEM=21.39), indicating a larger impact of the sequence on RT, than the SKILL in SHAM (T1: mean=131.58 SEM=23.02, T2: mean=191.53 SEM= 19.25, T3: mean= 195.60 SEM=17.04 T4: mean= 158.14 SEM= 19.25, T5: mean= 195.59, SEM= 20.08). However, this did not prove to be statistically significant as a RM-ANOVA indicated no main effect of stimulation ($F(11)=1.05$, $p=.328$). Statistical tests also indicated no significant interaction effect of test or CLAS ($F(4, 44)=0.21$, $p=.932$).

Further inspection of Figure 34 indicates that SKILL in both conditions increases from T1 to T3 and T4 to T5, with a decrease from T3 to T4 when the new sequence was introduced. Statistical testing indicated that there was a main effect of test upon SKILL (HFe(31.71)=2.88, *p<.001*), post hoc analysis, adjusting for multiple comparisons, indicated a significant difference between T1 and T2 ($z(24)=23$, $p<.001$), T1 and T3 ($z(24),=19.00$, $p<.001$), T1 and T5 ($z(24)=45.00$, $p=.018$), T2 and T3 ($z(24)=51.00$, $p=.035$), T3 and T4 ($z(24)=276.00$, $p<.001$) and T4 and T5 ($z(24)=28.00$, $p=.002$). This confirms that sequence SKILL increased as participants were repeatedly tested, again indicating increased knowledge of the sequence. Noticeably there was no significant difference between tests T1 and T4 ($z(24)=103.00$, $p=1$, p value adjusted for multiple comparisons), T2 and T5 ($z(24)=124.00$, $p=1$, p value adjusted for multiple comparisons), indicating that the next day test of the second sequence was comparable and the next day test of the first sequence.

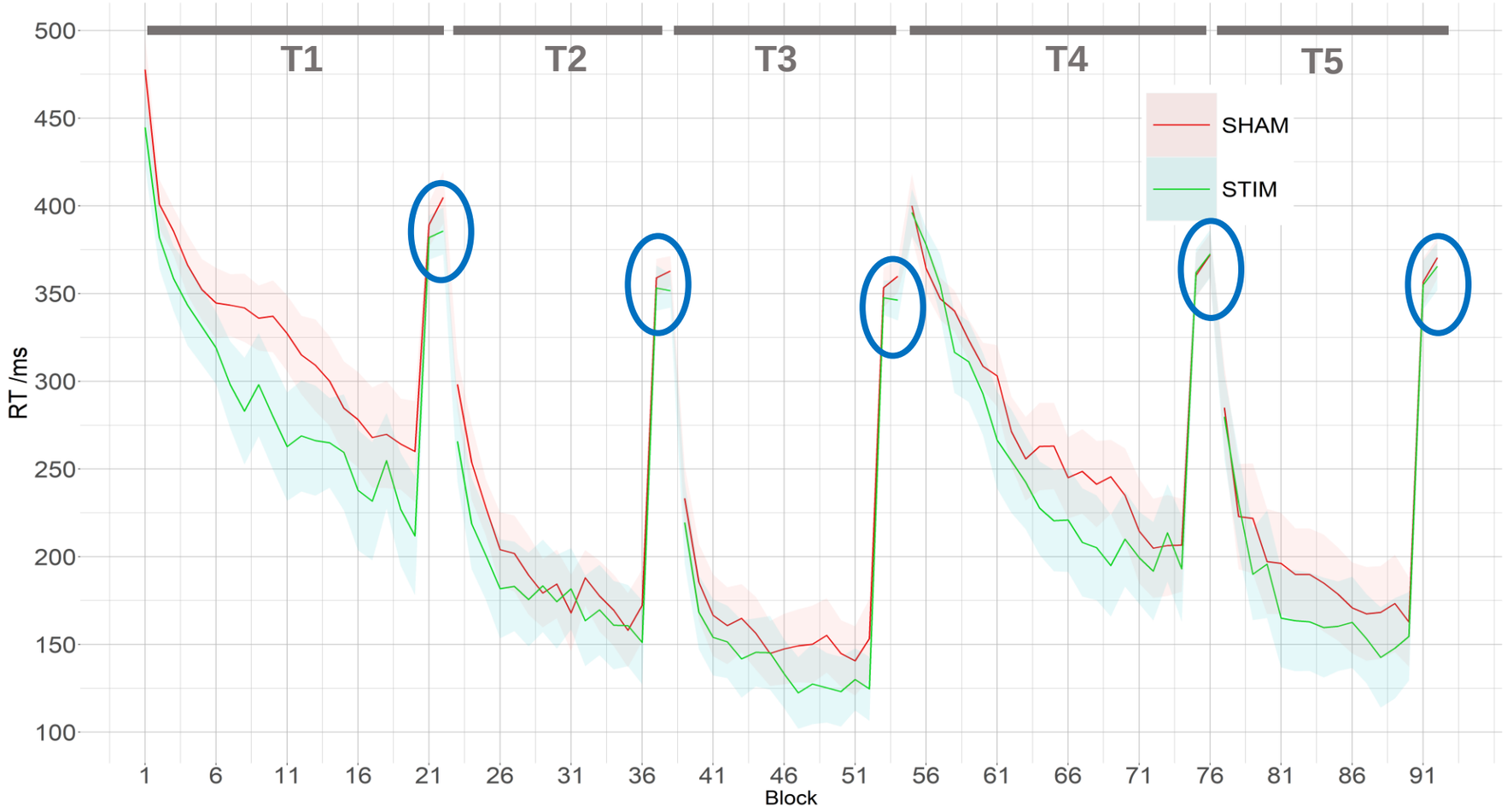


Figure 33: *SRTT block RT mean at each block. Shaded area shows ±SEM. Blocks 1-22 = T1, 23-38=T2, 39-54=T3, 55-76=T4, 77-92=T5. Blue circles indicate Random blocks.*

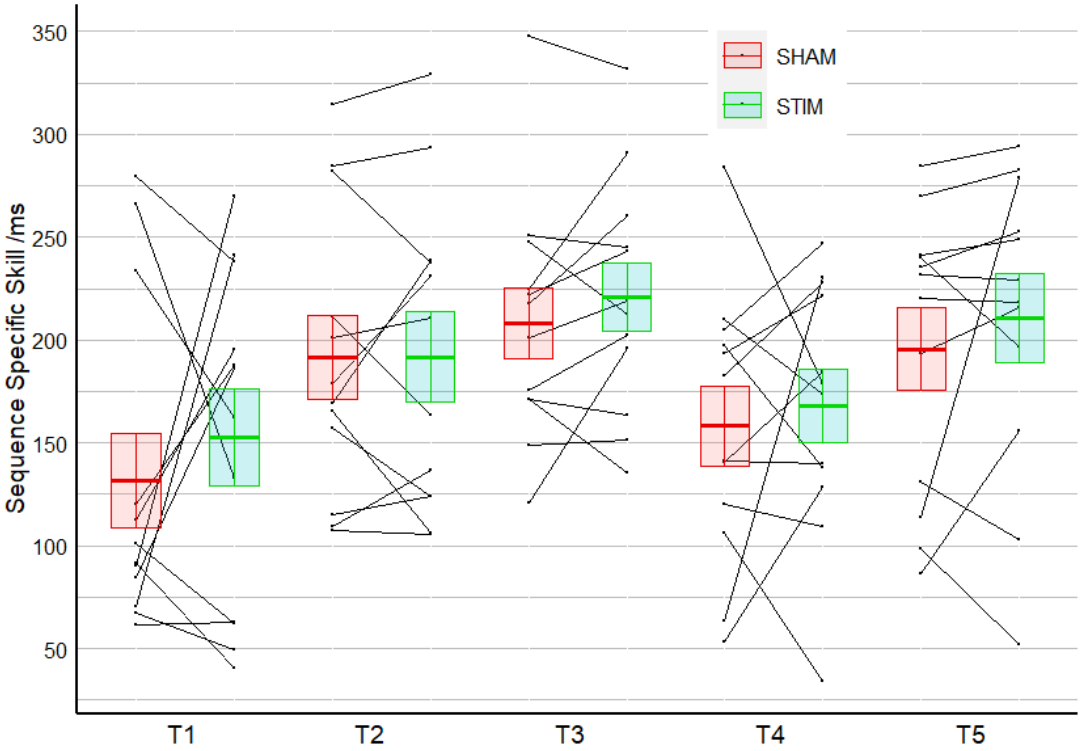


Figure 34: *SKILL for SRTT at each test. Boxes indicate mean ±SEM SKILL RT, red indicates SHAM while green indicates STIM. Black lines indicate individual participant performance.*

To assess further whether or not repeated nights of CLAS impacted performance change, I calculated the change in SKILL over the different retention intervals between tests: Night1, Nights 2-7 and Nights 1-7 (see Figure 35). First, percentage change in SKILL was calculated over both of these intervals in SHAM and STIM and compared statistically. Inspection of Figure 35, indicates an overlap in SEM between SHAM and STIM at both retention intervals, showing no significant difference in stimulation condition. Indeed there was no main effect of CLAS ($F(11)=0.09$ $p=.772$) or interaction between stimulation and retention interval ($F(110)=1.20$ p=.296). In Nights 2-7 STIM SKILL (mean=22.69, SEM=7.87) was greater than SHAM (mean=13.74, SEM=6.64) but testing of just this retention interval indicated no significant difference ($t(11)=27.00$, $p=.419$). Further inspection of Figure 35 indicates that the mean increase in SKILL over nights 2-7 is smaller than that seen over Night1 in both conditions ($F(11)=9.71$, $p=.010$) with post hoc ($t(24)=3.19$, $p=.004$). This shows that the impact of the sequence on the RT increased by significantly more over the first night compared to the subsequent six nights.

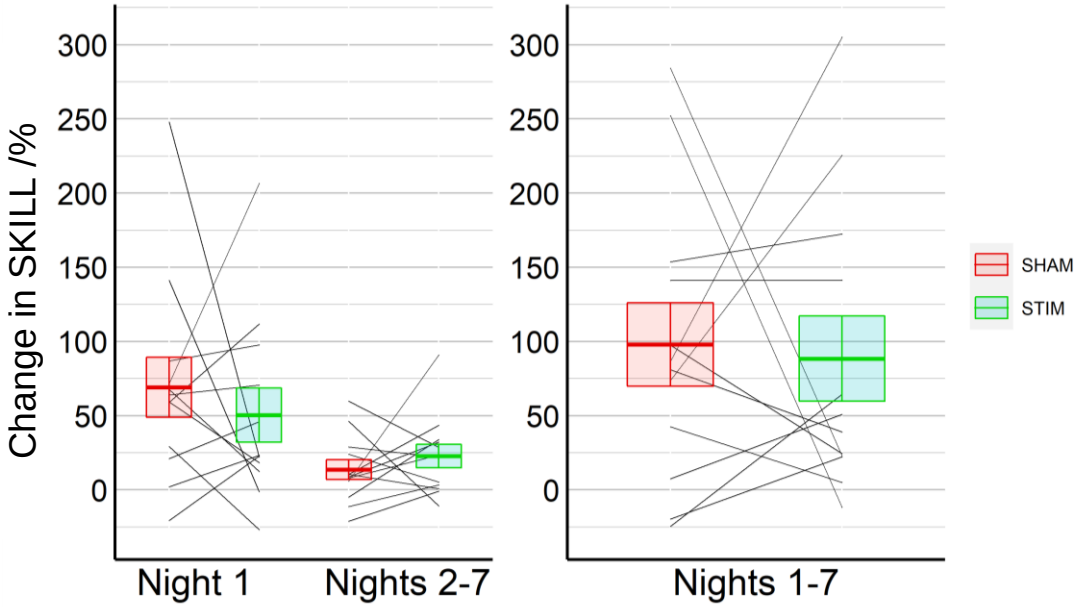


Figure 35: *SRTT final SKILL as a percentage change over different nights. Coloured boxes show SKILL RT mean ±SEM. Black lines indicate individual performance difference between SHAM and STIM.*

Assessment of Figure 35, indicates a change in SKILL over Nights 1-7 for both conditions, but with very little difference between SHAM (mean=97.97, SEM=28.13) and STIM (mean=88.47, SEM=28.83) conditions, ($z(11)=38.00$, $p=.980$).

In summary CLAS appears to have little effect on the performance of these participants on the SRTT task, irrespective the length of time for which stimulation was applied. But similar to other memory tasks tested in the previous chapter the first night appears to have a special impact as most of the gain in SKILL acquired across the week was gained in this first night.

fMRI

fMRI scans were taken during T2 and T3 recall of the SRTT while the motor sequence was being performed. A RM-ANOVA conducted on SRTT fMRI images at the group level, using scan (T2 and T3) and CLAS as within-participant factors (SHAM and STIM) revealed a main effect of CLAS in some of our ROI, see Table 10 and Figure 36. Inspection of parameter estimates in the left caudate cluster (see Figure 36) indicate increased activity in STIM during sequence blocks compared to baseline, but decreased activity in SHAM. The second cluster is located in the temporal lobe, in the middle temporal gyrus near the secondary auditory cortex, and may therefore be connected to auditory processing of the notes played in the task. Mean parameter estimates show (see Figure 36) that during SHAM there is a decrease in BOLD in this area while in STIM there is an increase. The secondary auditory area has been

implicated in auditory memory and as such, greater BOLD activity here could indicate activation of this auditory memory. It is curious that this difference in activity was unilateral as sound was delivered to both ears.

Effect	Location	Voxels	Test statistic	P value	Co-ordinates		
					x	y	z
Main effect of CLAS	*Temporal Middle gyrus -Left*	*5*	*F(10)=24.07*	*0.001*	*-50*	*-8*	*-14*
	Caudate - Left	*20*	F(*10)=60.09*	*<0.001*	*-12*	*10*	*16*
CLAS x test interaction	*Cerebellum Crus2 Left*	*25*	F(*1, 10)=44.14*	*<0.001*	*-22*	*-74*	*-38*

Table 10: *Significant clusters from RM-ANOVA interaction between CLAS and test and the main effect of CLAS, in the SRTT.*

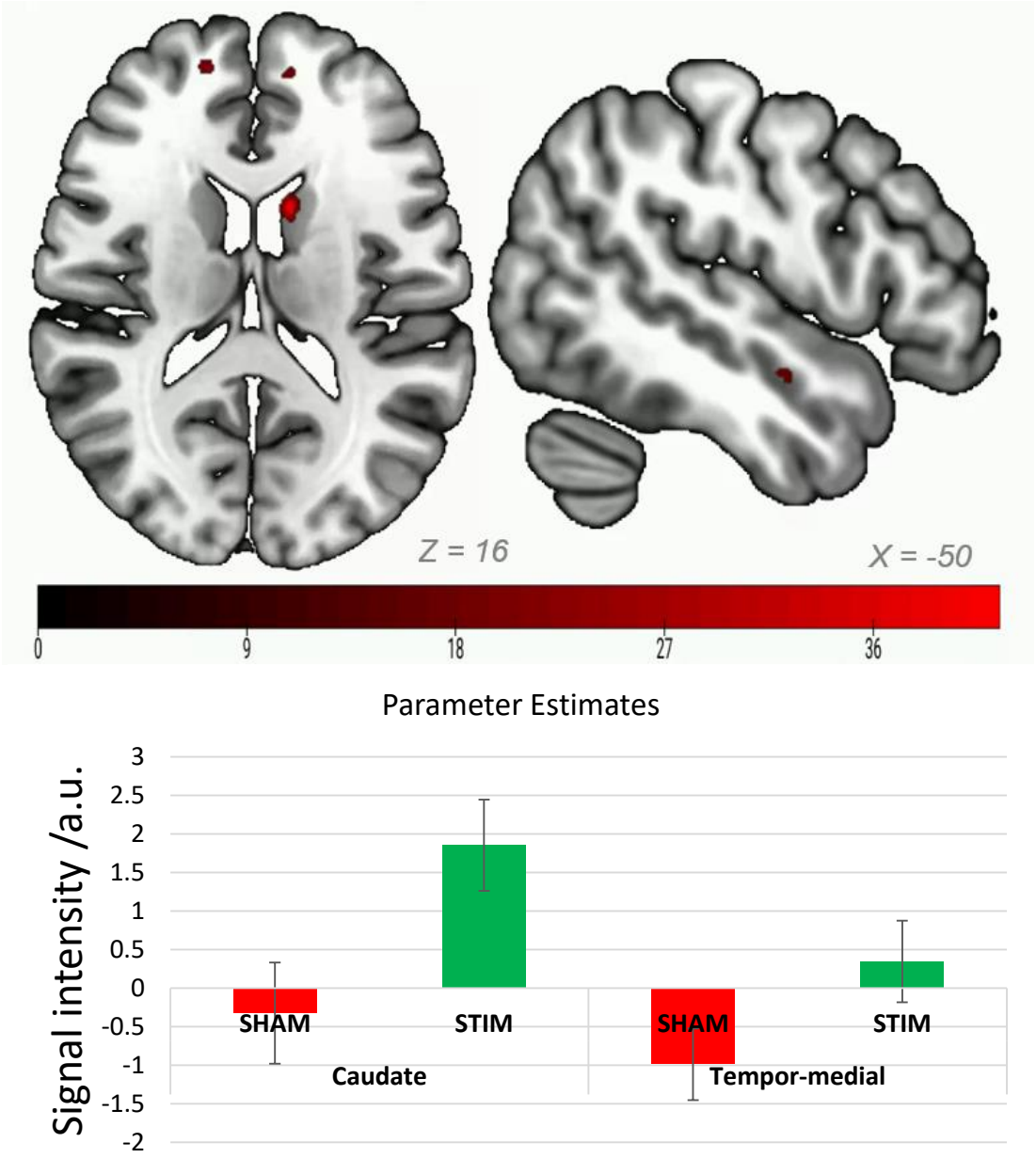


Figure 36: *Main effect of CLAS in SRTT. RM-ANOVA Unc p<0.001. Parameter estimates indicate mean ±SEM.*

The RM-ANOVA also indicated an interaction between time and CLAS in BOLD activity in the left cerebellum, see Table 10, and Figure 37. Parameter estimates indicate a double dissociation between time and stimulation: Such that from T2 to T3 in SHAM activity decreases while STIM activity increases. Thus, this area becomes less involved in sequence recall in SHAM and more involved in STIM across the week, and that even after one-night SHAM and STIM show different levels of activity in this area.

To further assess the driver of this interaction, significant clusters from the RM-ANOVA interaction were used to define a ROI for post-hoc testing of the difference

between SHAM and STIM ($\mathrm{STIM} - \mathrm{SHAM}$, see methods) at T2 and T3. When conducted on the SHAM vs STIM contrast at T2, this analysis revealed no clusters at FWE p<.05. However, the same analysis on SHAM vs STIM at T3 revealed activity in the left cerebellum crus 2, (t(1,10)=4.47, peak p=.026 (FWE), cluster p=.014, 6 voxels), Figure 37, indicating that the T3 difference between SHAM and STIM is driving the interaction. This is supported by the parameter estimates (see bottom image in Figure 37) as there is a larger difference between conditions at T3 than at T2.

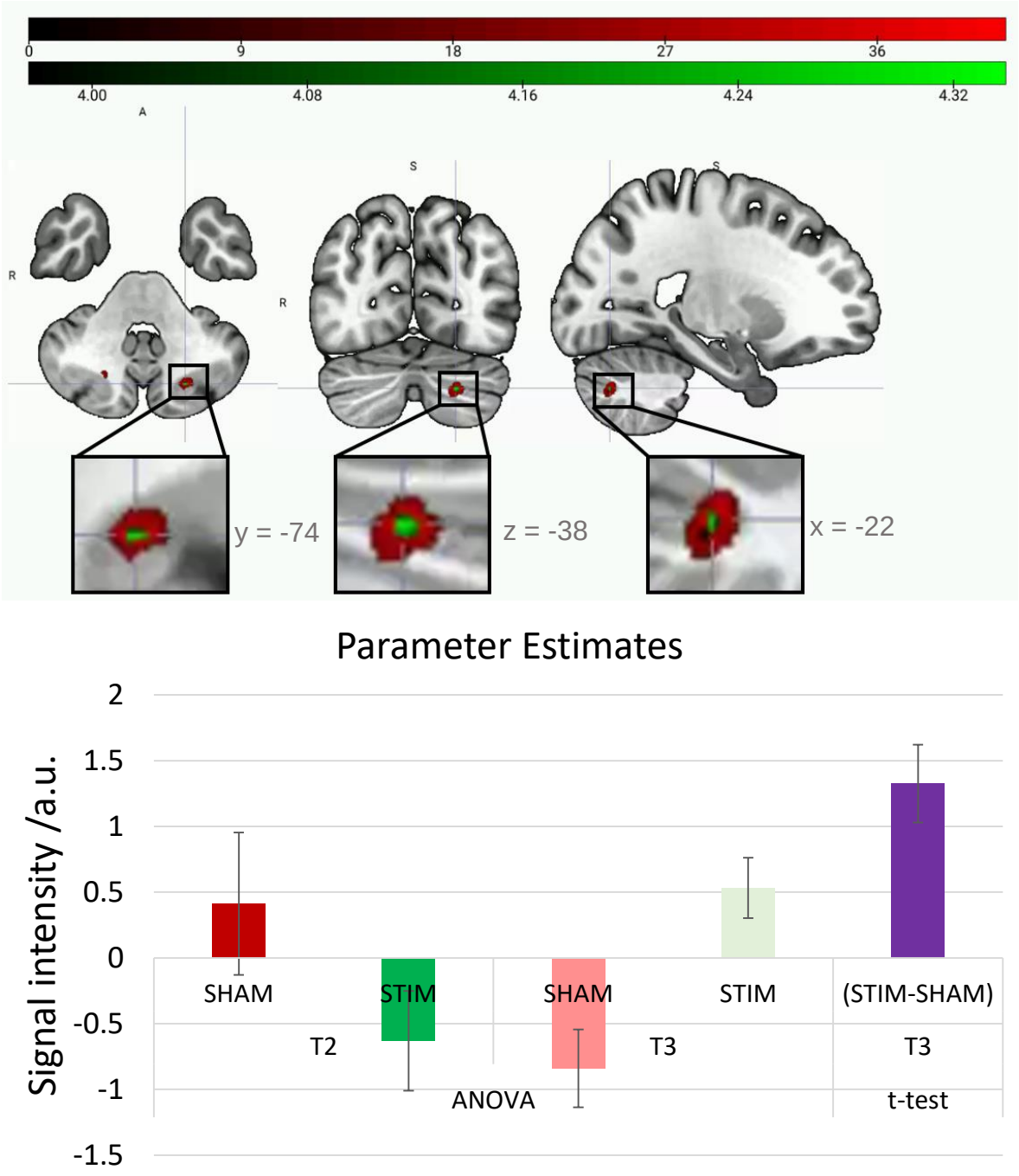


Figure 37: *Stim-Sham response in left cerebellum activity during SRTT changes over time.* ***Upper****: Red indicates the area significant at RM-ANOVA testing for an interaction between time and CLAS, unc. p<.001. Green indicates the area significant at single t-test in STIM-SHAM at T3, FWE p<.05, T threshold = 3.98. y, x and z coordinate of slices indicated. R=right, S=superior.* ***Lower****: Parameter estimates for peak of above clusters.*

There was also a main effect of time upon activation in a third set of clusters, see appendix section 7.5.3. As the effect of time on behaviour is not the main focus of this chapter these results are presented in the appendix (see section 7.5.3) for completeness, but will not be discussed in detail here.

In summary CLAS led to no change in behaviour but there was a change in the BOLD activity of the caudate, tempor-medial gyrus and cerebellum. Specifically, CLAS was associated with an increase in activity in the caudate and tempor-medial gyrus, and an increase with time in activity in the cerebellum. No areas were found to be significantly correlated to activity in any of the tested covariates (see methods section 4.3.4 fMRI: Second level analysis for covariates).

Word Pair

Behavioural data from T1, T2 and T3 was assessed based on the pairs which were tested inside and outside the MRI scanner (see methods section 4.3.4 Word Pair); Thus, I separately analysed (1) all 75 words: T1a, T2a and T3a, (2) only the 55 words tested in the scanner: T1b, T2b and T3b and (3) only the 20 words not tested in the scanner: T1c, T2c and T3c.

Scores decreased with repeated testing from T1 to T3 with no significant main effects of CLAS or interactions between CLAS and test. Statistics were also conducted on the percentage change over each of the retention intervals of the week; Night 1, Nights 2-7 and Nights 1-7, in each group. Analysis of Night 1 and Nights 2-7, using a RM-ANOVA with CLAS (SHAM and STIM) and retention interval (Night 1 and Nights 2-7) as within participant factors, indicated no significant effects on WP score change; no significant main effect of CLAS (75 words: $F(11)=1.02$ $p=.333$, 55 words: $F(11)=0.30$ $p=.593$, 20 words: $F(11)=2.16$ $p=.170$), no main effect of retention interval (75 words: $f(11)=0.78$ p=0.396, 55 words: $f(11)=1.00$ p=0.34, 20 words: $F(11)=0.61$ $p=.450$), and no interaction (75 words: $F(1, 11)=0.608$ $p=.452$, 55 words: $F(1, 11)=.035$ p=.855, 20 words: $F(1, 11)=4.76$ $p=.052$). When Nights 1-7 were considered together there was also no significant difference between SHAM and STIM in any word group (75 words: $z(11)=93.5$ $p=.225$, 55 words: $z(11)=93.5$ $p=.225$, 20 words: $t(11)=0.970$ $p=.344$). As results did not differ between word groups, only the scores for all 75 words are shown in Figure 38. As previously found in Chapter 3 and previous work (Henin *et al.*, 2019), our data thus indicates that there was no effect of stimulation on WP performance.

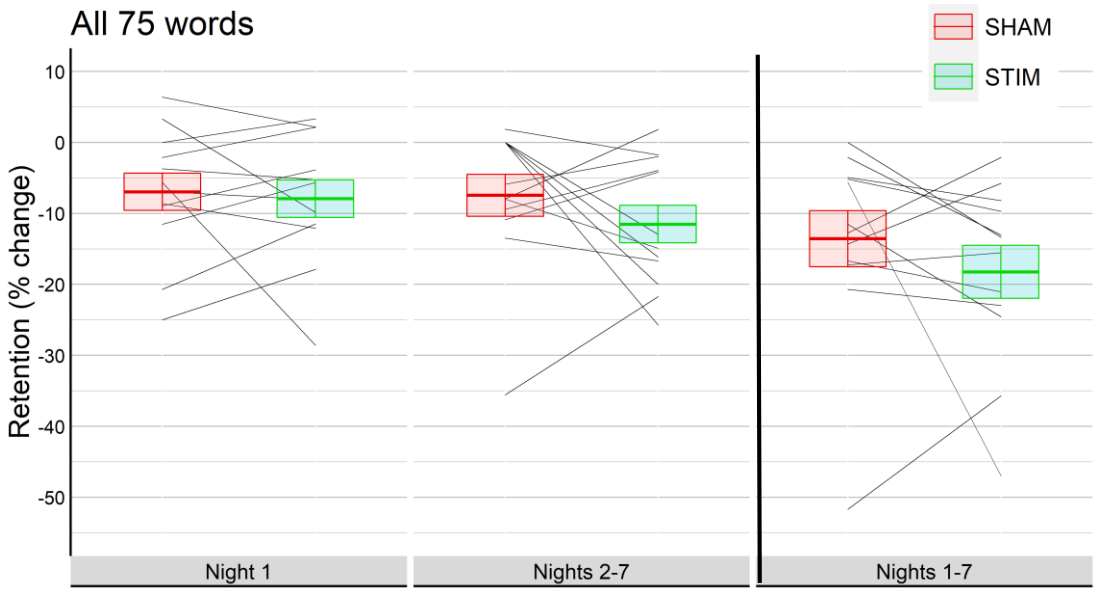


Figure 38: *Change over each retention interval in WP score.* Figure depicts all 75 words tested (including 55 tested in the scanner and 20 not tested in the scanner). Boxes indicate mean ±SEM. Separate statistical tests were conducted on Night 1 and Nights 2-7 than Nights 1-7 as they cover the same interval.

fMRI

Participants recalled a subset of the pairs learnt, inside the MRI scanner during T2 and T3, and the resulting fMRI scans were processed such that resulting clusters of activity indicated activity from *Remember trials (*see methods section 4.3.3 Word Pairs*)* compared to baseline (fixation). A RM ANOVA conducted on the group level WP fMRI results indicated that the only significant clusters of activity were found in the main effect of CLAS (see Table 11) and the main effect of time (see appendix section 7.5.3).

For the main effect of CLAS there was a cluster of activity in the Putamen, see Table 11 and Figure 39. Inspection of Figure 39 indicates a decrease in activity during *remember trials* in both SHAM and STIM, denoted by a decrease in parameter estimates, but a significantly larger decrease in SHAM compared to STIM, see Table 5 for significance values.

Location	***Voxels***	***Test statistic***	***P value***	***Co-ordinates***		
				x	***y***	***z***
Putamen -Right	*7*	*42.012*	*<0.001*	*32*	*-10*	*-2*

Table 11: *Significant clusters indicating a main effect of CLAS in the WP task from a RM-ANOVA*

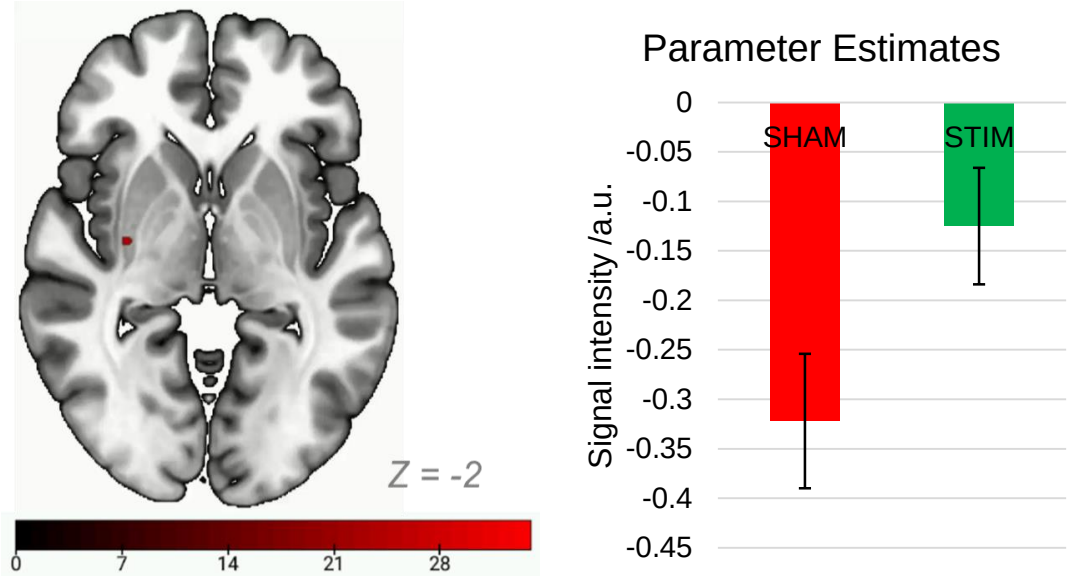


Figure 39: *Significant clusters indicating a min effect of CLAS in WP task. Significant activity indicates activity during remembered trials. Below plot indicates parameter estimated for both clusters in SHAM and STIM.*

The main effect of time however revealed activity in three distinct clusters, appendix section 7.5.3, (1) Frontal Superior Medial -Left, (2) Parietal cortex –Right, and (3) Temporal inferior –Left. Again as the change in behaviour across time is not the main focus of this chapter the results of this analysis are contained in the Appendix.

To summarise, despite the absence of any influence of CLAS on behavioural performance, stimulation did lead to greater BOLD activity in the putamen.

No areas were found to be significantly correlated to activity in any of the tested covariates (see methods section 4.3.4 fMRI: Second level analysis).

Mnemonic Similarity Test

Initial assessment of PSS raw scores indicated scores were higher in STIM (mean= 13.64 SEM= 1.75) than in SHAM (mean= 9.36, SEM= 1.88) at T1 before sleep ($t(11)=-2.37$, $p=.039$). Therefore, for PSS the change in score across the pre-determined retention intervals (Night1 Nights 2-7 and Night 8, see methods: 4.3.4 Mnemonic similarity task) was assessed instead of raw score. PSS scores were assessed both divided by similarity bin (see Figure 41) and pooled (see Figure 40), but as results were always the same, only those analyses on data divided by bin are discussed here.

PSS score over Night 1 and Nights 2-7 was assessed first, using a RM-ANOVA with the within participant factors of retention interval (Night 1, Nights 2-7), CLAS (SHAM, STIM) and similarity bin (Bin 1 to 5): There was a significant interaction between

stimulation and retention interval (F(1, 10)=6.13 p=.025). Post-hoc testing indicated this was driven by the difference between SHAM and STIM in Night1 (z(54)=896.00, p=.012, see Figure 40) as there was no significant difference across Nights 2-7 (z(54)=407.00, p=.303), see further discussion of this effect in section 4.4.4. There was also a main effect of bin, see Figure 41, (F(40)=6.49, p<.001), with post-hoc tests revealing a difference between B1 and B4 (z(43)=682.00, p=.002). See appendix section 7.5.4 for mean values of change in score per bin.

When Nights 1-7 were assessed as the only retention interval, the RM-ANOVA indicated no main effect of CLAS (F(10)=3.05 p=.111). Indeed the only significant effect in this subset of data was a main effect of bin, Figure 41, (F(4,10)=6.49, p<.001), which post-hoc testing indicated was again driven by the difference between B1 and B4 (z(21)=163.00, p=.008).

Overall, these results indicate that there is an effect of CLAS on this task over Night 1, suggesting an CLAS is detrimental to task performance following the first night. This effect is then outweighed by subsequent night's sleep despite continued stimulation, as the subsequent six nights of stimulation do not alter performance. See appendix section 7.5.4 for figure of pooled PSS scores.

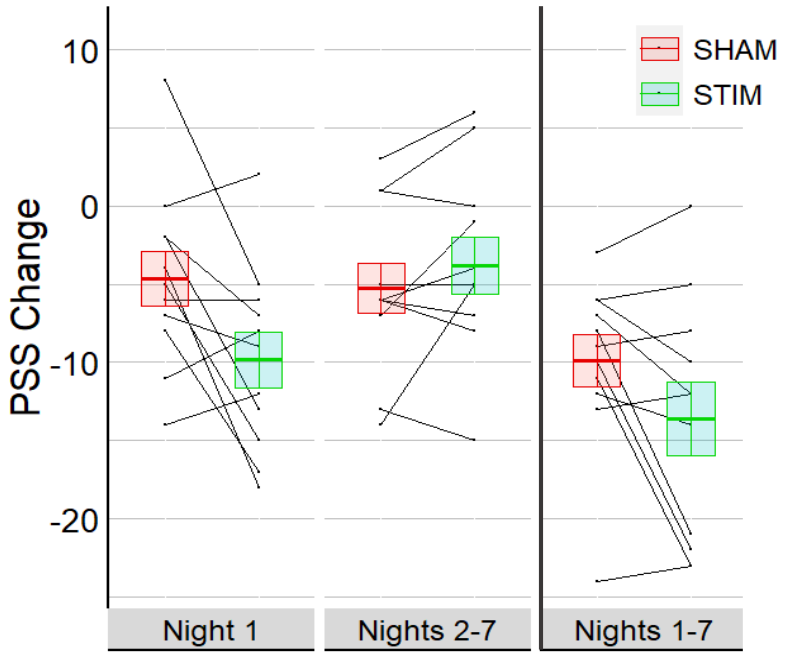


Figure 40: *Pooled (all similarity bins) PSS data across Night 1, Nights 2-7 and Nights 1-7. Coloured boxes indicate mean ±SEM.*

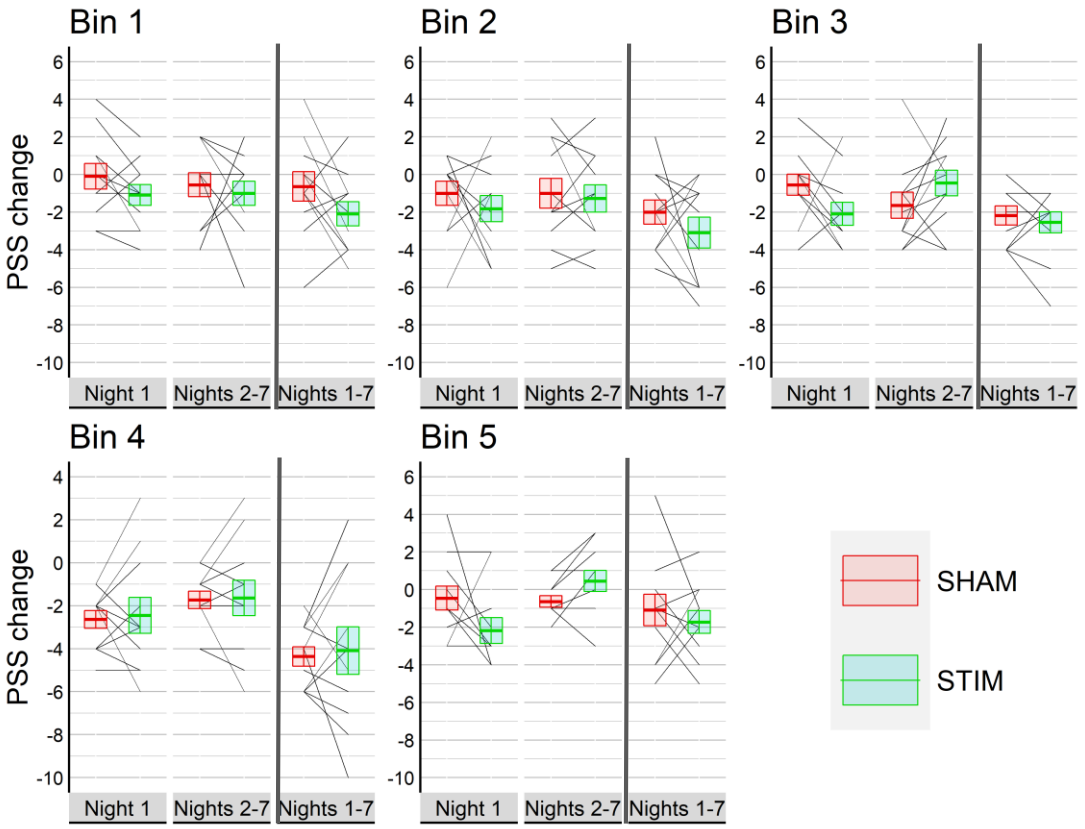


Figure 41: *Absolute change in PSS. X- axis indicates night intervals.*

As well as PSS, RMS can be calculated for this task, this gives a picture of participant's memory for the items irrespective of their similarity to other items, and therefore allows us to assess CLAS impact on this memory. Assessment of raw data indicated no difference at T1 ($z(10)=36.50$, $p=.789$) between SHAM (mean=25.46 SEM=10.68) and STIM (mean=25.00 SEM=1.06). As such raw scores were assessed as well as change across the three retention intervals. However, results did not differ between raw and absolute change, therefore only absolute change is discussed here, see Figure 42.

Assessment of Night 1 and Nights 2-7, Figure 42, (Night 1: SHAM mean=-13.09, SEM=1.38; STIM mean=-11.91, SEM=1.26; Nights 2-7: SHAM mean=-5.27 SEM=1.21, STIM mean=-4.36, SEM=1.55) indicated no main effect of stimulation ($F(10)=0.85$ p=.378) or an interaction ($F(10)=0.01$, $p=.941$). But a main effect of retention interval on RMS ($F(10)=25.64$ p=.005, post-hoc: $t(22)=-4.78$, $p<.001$), such that RMS score fell by a significantly larger amount over Night 1 than over Nights 2-7.

Assessment of RMS change over Nights 1-7, Figure 42, (SHAM mean=18.36, SEM=1.138; STIM mean=-16.27, SEM=1.61) also indicated no significant difference

between SHAM, and STIM ($t(10)=-0.92$, $p=.378$). Therefore, unlike PSS there is no effect of stimulation on RMS neither over the first night or subsequent six nights of stimulation.

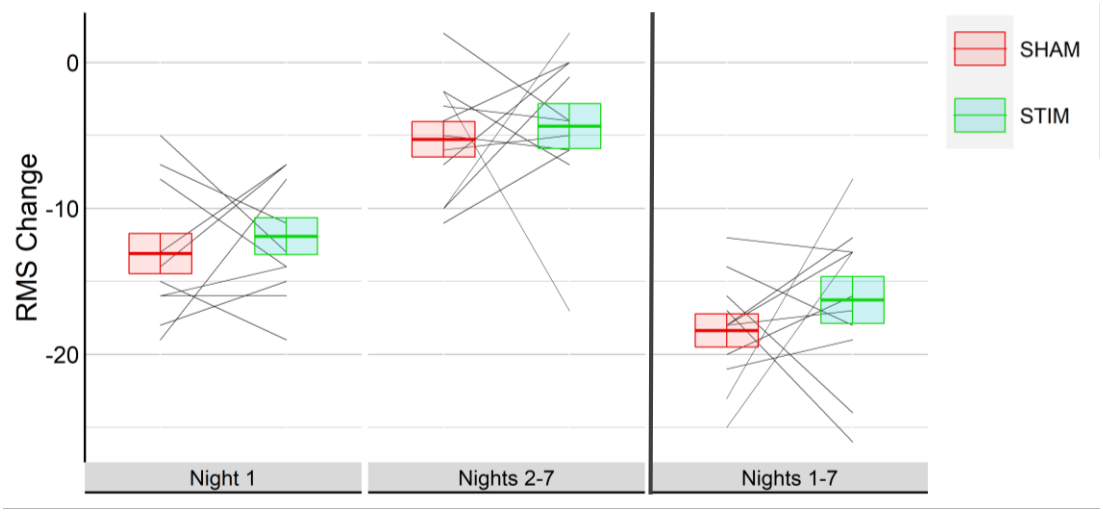


Figure 42: *Recognition memory score (RMS) change. RMS score change across all tested retention intervals. Boxes indicate mean ±SEM, Red = SHAM while Green = STIM. Black lines indicate individual participant performance. Nights 1-7 were assessed in a separate ANOVA.*

4.4.4 Effect of stimulation on *first night* consolidation.

The first night following learning seems particularly important for memory, and we have shown above and in Chapter 2 that this is where the majority of forgetting or RT gain occurs. In this experiment there are effectively two *first nights* Night 1 for stimuli encoded at T1, but also Night 8 for new stimuli encoded at T4. This design allowed me to observe the effect of one week of CLAS upon memory recall across the first night retention interval, to see if preceding that first night with repeated nights of stimulation changed consolidation on the first night post learning.

Serial reaction time task

For the SRTT the change in SKILL across Night 1 and Night 8 was calculated. Inspection of Figure 43, indicates that the mean SKILL increases over Night 1 by a greater percentage in SHAM (mean=59.05, SEM=20.25) than in STIM (mean=50.30, SEM=18.28), while over Night 8 there is very little to distinguish percentage change in SKILL between SHAM (mean=30.71, SEM=9.07) and STIM (mean=29.67, SEM=8.45) conditions. There was no main effect of CLAS found using a RM-ANOVA with stimulation, retention interval (Night 1 and Night 8) as within-participant factors:

(F(11)=0.42, p=.529), nor main effect of retention interval (F(11)=4.58, p=.056) or interaction of interval and stimulation (F(11)=0.34, p=.569). However as mean SKILL change is positive it does indicate that following both nights the sequence has a greater impact on RT in line with Figure 33 and Figure 34. This suggests that there is no impact of CLAS across the first night following encoding independent of whether that first night is preceded by seven nights of CLAS or not.

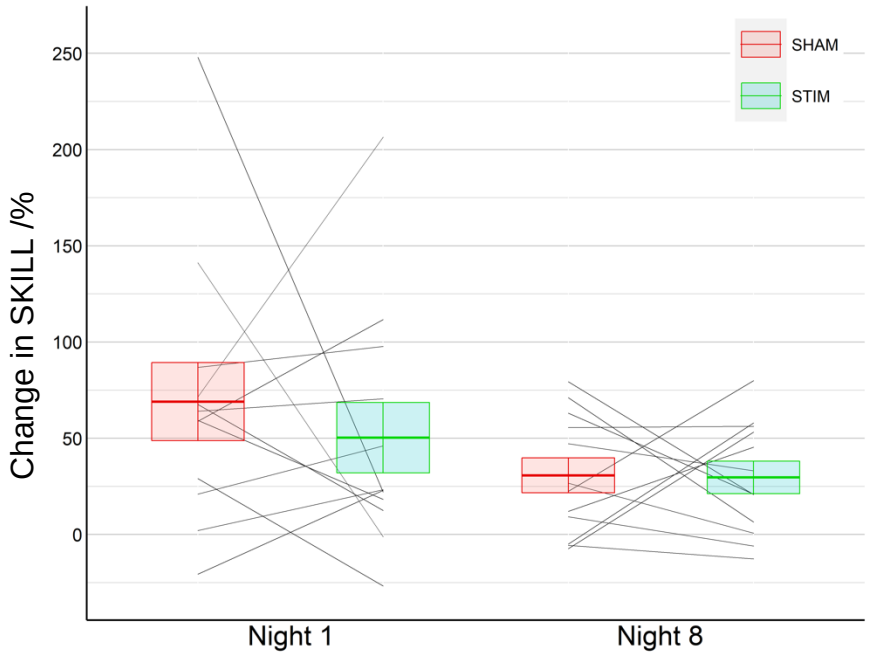


Figure 43: *SRTT SKILL Change over Night 1 and Night 8. Coloured boxes indicate mean ±SEM, red) SHAM, green) STIM, black lines show individual participant performance.*

Word Pair

In the WP task the raw scores of the 75 pairs learnt at T1a and T4 and tested at T2a and T5 respectively were assessed. Upon data inspection there was little difference between SHAM (T1a: mean=55.7, SEM=1.52; T2a: mean=51.7, SEM=1.82; T4: mean=57, SEM=52.2; T5: mean=52.2, SEM=3.09) and STIM (T1a: mean=55.20, SEM=1.64; T2a: mean=50.80, SEM=1.84; T4: mean=58.60, SEM=1.86; T5: mean=52.70, SEM=2.71) in each test. Statistical testing using a RM-ANOVA with stimulation and test (T1a, T2a, T4 and T5) as within participant factors supported this, indicating that there was only a main effect of test on score (HFe(19.51)=1.77, p=.026), with post-hoc indicating a significant difference between T1a and T2a (t(23)=4.19, p=.002), T2a and T4 (t(23)=-3.63, p=.008) and T4 and T5 (t(23)=5.22, p<.001), indicating a decline in score over both nights.

To further assess the impact of a week of stimulation on overnight performance change in WP, the overnight percentage change for Night 1 and Night 8 was calculated and is displayed in Figure 44. From inspection of Figure 44 it might appear that scores under STIM (Night 1: mean=-7.92, SEM=2.64; Night 8: mean=-10.5, SEM=2.84) conditions are decreasing by more overnight than that by which SHAM (Night 1: mean=-6.96, SEM=2.62; Night 8: mean=-9.20, SEM=3.16) decreases. However, overlap of the condition SEM and statistical testing using a RM-ANOVA with retention interval (Night 1 and Night 8) and stimulation as within participant factors, indicated no main effect of CLAS ($F(11)=0.17$, $p=.685$), or interaction with retention interval ($F(11)=0.88$, p=.369), or indeed a main effect of retention interval ($F(11)=0.01$, $p=.932$).

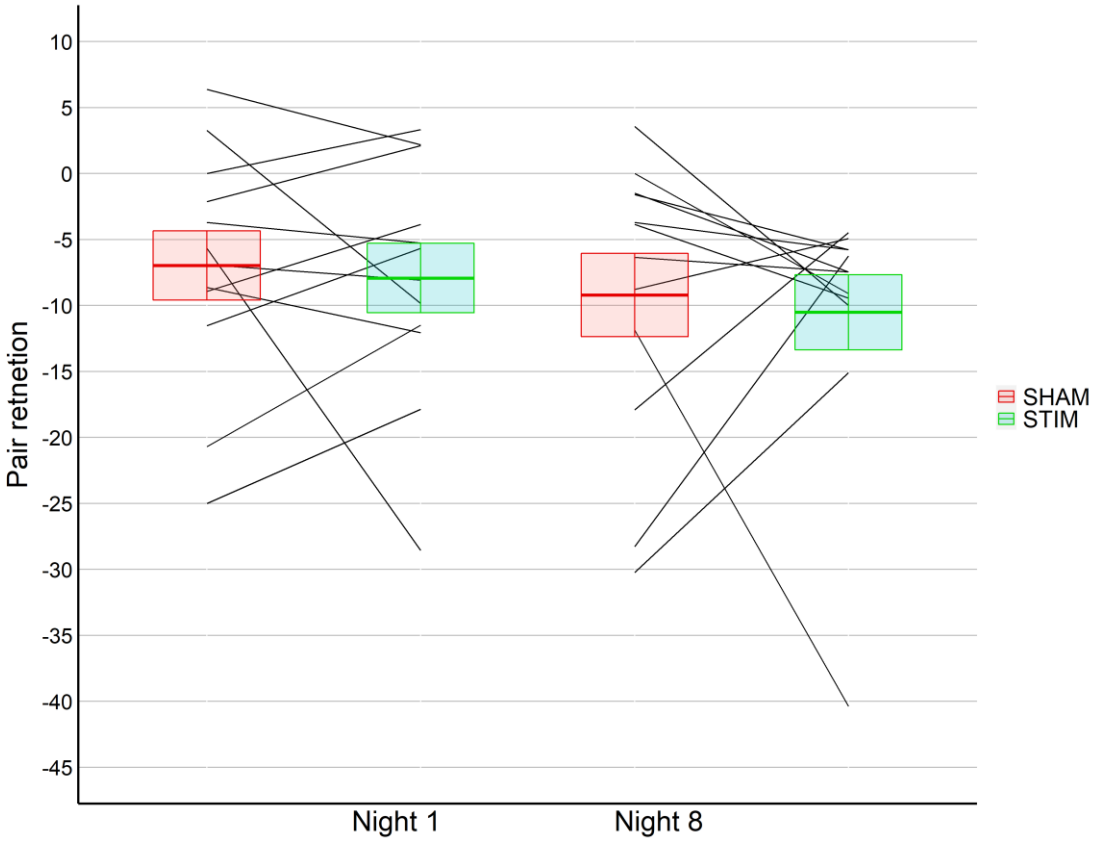


Figure 44: *Word Pair overnight change at the start and end of the week. Overnight percentage change in score from learning on Night 1 and Night 8. Error shows SEM.*

Mnemonic similarity task

When the retention intervals over Night 1 and Night 8 were assessed for the MST, see Figure 45, both intervals saw a larger overnight decrease in PSS under STIM conditions (Night 1: mean=-9.82, SEM=1.78; Night 8: mean=-6.73, SEM=1.83) than

SHAM (Night 1: mean=-4.64, SEM=1.77; Night 8: mean=-4.46, SEM=2.11). This was supported by a RM-ANOVA using within participant factors of retention interval (Night 1, Night 8), CLAS (SHAM, STIM) and similarity bin (Bin 1 to 5), which indicated a main effect of CLAS ($F(10)=8.17$, $p=.017$, post-hoc: $z(109)=3033.00$, p=.005). This suggests that PSS was less diminished overnight in SHAM than in STIM. There was no interaction between CLAS and retention interval: $F(1,10)=1.37$, $p=.268$ or CLAS and bin: $F(4,40)=0.54$, $p=.709$ or CLAS bin and retention interval: $F(4,40)=0.32$, $p=.866$).

The ANOVA also indicated a significant main effect of similarity bin, Figure 45, ($F(40)=6.56$, $p<.001$): Post-hoc testing indicated this effect to be driven by the difference between B1 and B4 (z(43)=626.00, p<.001) as this was the only significant difference between bins (next smallest p=.059). No other effects were significant in the ANOVA. Thus PSS was higher in Bin1 images (most similar) than Bin4 images (second least similar). For pooled PSS see appendix section 7.5.4.

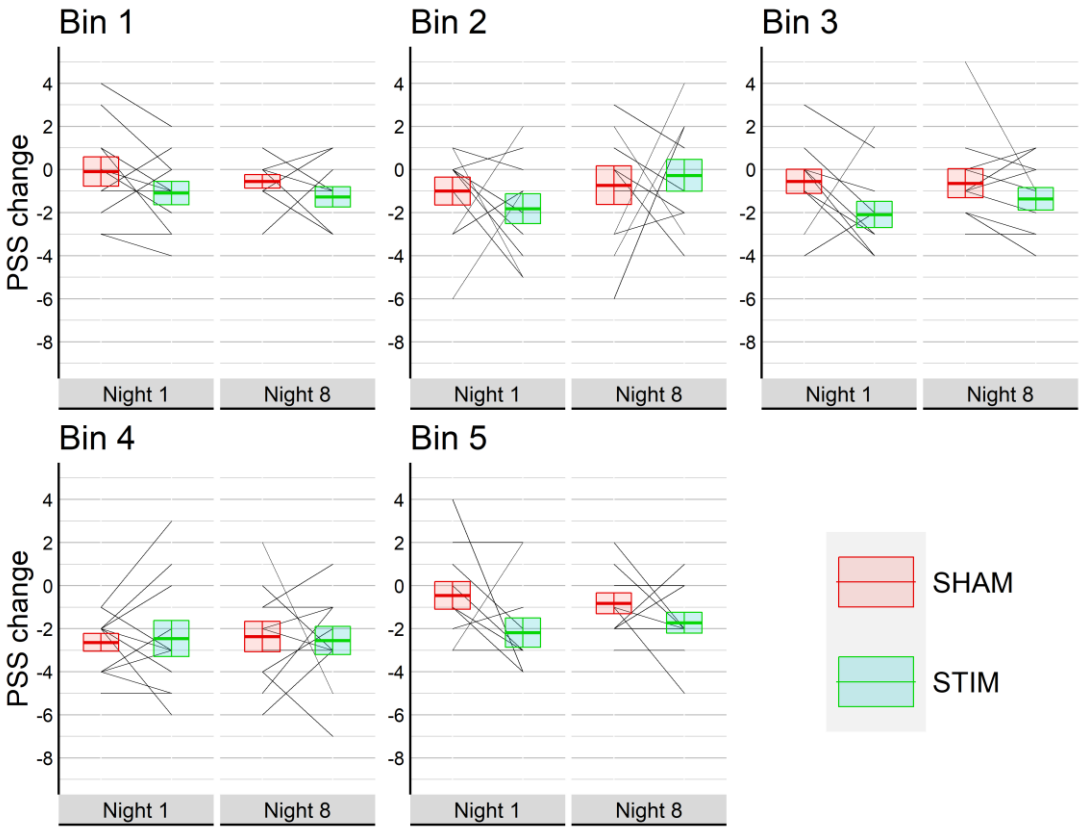


Figure 45: *Change across Night 1 and Night 9 in PSS. Boxes indicate mean ±SEM*.

The change in RMS score over Night 1 and Night 8 was also assessed, see Figure 46. As inspection of this figure shows over Night 1 there was a larger decrease in

RMS recalled in SHAM (mean=-13.09, SEM=1.38) than in STIM (mean=-11.91, SEM=1.26), whereas the opposite was true for Night 8, where the decrease in score was larger in STIM (mean=-12.09, SEM=2.01), than SHAM (mean=-9.82, SEM=2.43). Statistical testing using a RM-ANOVA with retention interval (Night 1 and Night 8) and CLAS (SHAM and STIM) as within-participant factors, indicated no interaction between retention interval and stimulation ($F(1, 10)=1.52$, $p=.246$), or main effect of stimulation ($F(10)=0.18$, $p=.684$) or retention interval ($F(10)=0.49$, $p=.500$).

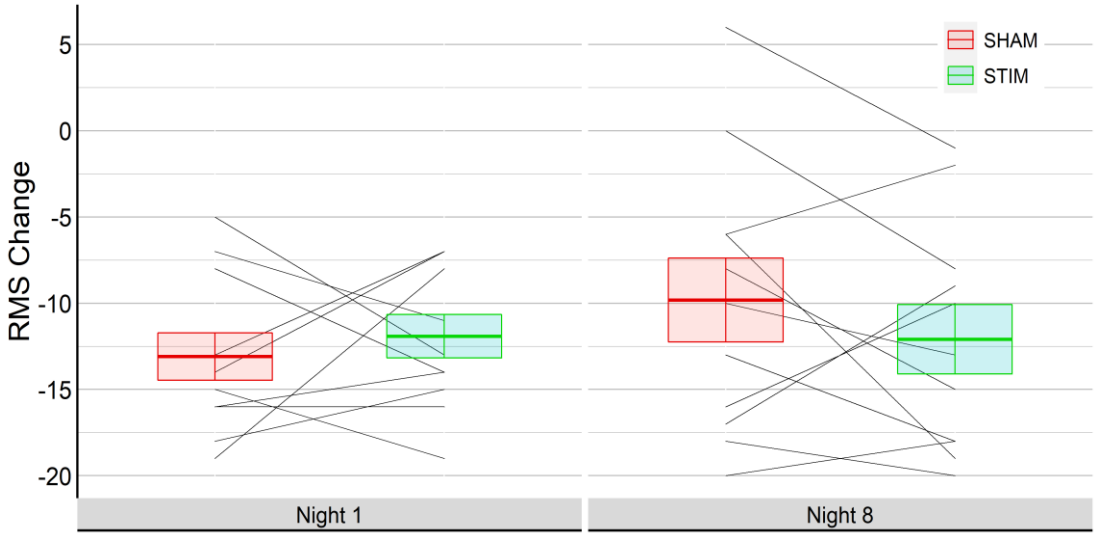


Figure 46: *Change in RMS in Night 1 and Night 8. Boxes indicate mean ±SEM*

4.5 Discussion

Key results in this chapter show that CLAS has led to BOLD activity changes in the brain during recall of the SRTT and WP tasks: In the SRTT CLAS led to an increase in in activity in the Caudate and temporal lobe, while CLAS and time led to an increase in activity in the cerebellum, particularly driven by greater STIM activity in T3. In WP CLAS led to a smaller decrease in activity in the Putamen. Behaviourally recall in the MST showed that following one night of CLAS, at the start and end of the week, pattern separation score was worse than SHAM.

Findings reported in this chapter could indicate the potential for future performance differences as a result of stimulation: TMR in one night has recently been shown to lead to increase SRTT SKILL performance ten days after TMR but not in the day after TMR (Rakowska *et al.*, 2021). The authors hypothesised that TMR led to preferential treatment of the cued memories which took time to result in behavioural changes.

This idea is discussed further in the context of the thesis as a whole in the general discussion.

4.5.1 CLAS and time led to increased BOLD activity in the cerebellum during SRTT

Results indicated that there was an interaction between time and CLAS in the BOLD activity in the Cerebellum (Crus 2 Left, x=-22), such that activity was greater in SHAM at T2 but greater in STIM at T3. Post-hoc testing indicated the driver of this interaction to be greater activity in STIM at T3. This Crus 2 region, also known as Lobule VII, in the left cerebellum is located between the Horizontal fissure and ansoparamedian fissure. Unlike the cortex, ascending and descending fibres to the cerebellum do not crossover, such that the left cerebellum is responsible for motor activity in the ipsilateral side of the body. Indeed, a similar task used by Walker *et al.,* (2005) had participants using their left hand and led to activation in the left cerebellum VII lobule. Therefore, this activity is likely to be linked to activity in the left hand which was completing the task. This area along with others in the cerebellum has been linked to training on motor sequence learning tasks (Walker *et al.*, 2005; Fogel *et al.*, 2014; Cousins *et al.*, 2016).

Parameter estimates suggest that there is an increase in activity in the cerebellum across the week in STIM. A similar area to that shown here was found to be active by Walker *et al.* (2005) when they used fMRI scans to assess brain activity whilst participants were tested on a sequence finger tapping task, much like the SRTT. They found this area to be more active when the test was separated from learning by a retention interval containing 12h sleep, compared to an interval containing 12h wake. They hypothesised that activity here, along with other motor regions also found to be more active following sleep, led to greater accuracy in the finger tapping task. This could suggest that the greater activation in this area in STIM scans at T3 only, indicates some improvement in the task conveyed by both time and CLAS. Cousins *et al.,* (2016) found greater activity in the left cerebellum when performing sequences of the SRTT which had been cued using TMR in SWS, when the time spent in REM was included as a covariate. An increase in activity in the left cerebellum correlated with spindle amplitude, has also been found when comparing testing on a motor sequence pre- and post-sleep (Barakat *et al.*, 2013). These spindles had also been linked to performance gains across sleep in speed of completing the sequence. Interaction between CLAS and spindles is a plausible route for stimulation to influence

brain activity as it has been shown in multiple CLAS studies (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Papalambros *et al.*, 2017; Henin *et al.*, 2019; Schneider *et al.*, 2020). Further assessment of the sleep EEG would be required to confirm this in this experiment, for example to see if CLAS led to an increase in spindles and if this correlated with activity in regions more active in STIM. However, as I did not see an increase in SKILL associated with CLAS or time, or a decrease in RT, it seems that the behavioural measure was not sufficiently sensitive to index this.

Parameter estimates also suggested activity in the cerebellum decreased with time in SHAM. Initially high activity in the cerebellum has been found during early learning of motor sequences followed by a decrease with increased practice (Doyon and Ungerleider, 2002; Leslie G. Ungerleider, Julien Doyon and Avi Karni, 2002). While this fits with results seen in SHAM the opposite is true for STIM. As there is less activity in STIM than baseline at T2 it could indicate that this process has already occurred in STIM. Unfortunately, MRI data was not collected during learning to see if cerebellar activity was higher in STIM, as this could indicate that the sequence has been consolidated more in STIM over the first night. The increase in the cerebellar activity in T3 seen in STIM could therefore be indicative of another process, or the increased effort to retrieve the more securely consolidated sequence memories in STIM.

4.5.2 CLAS leads to an increase in BOLD activity in the caudate during SRTT

A main effect of CLAS on SRTT was also found in the caudate nucleus, another motor area previously associated with motor sequence tasks (Fogel *et al.*, 2014; Cousins *et al.*, 2016). The caudate along with the putamen is a part of the striatum and is thus involved in many functions including movement, vision and memory (Packard and Knowlton, 2002; Mink, 2013; Hélie, Ell and Ashby, 2015). The caudate acts as a relay station for information crossing cortical and sub-cortical structures (Labadie, 2003). In this experiment the left caudate was recruited during STIM, and suppressed during SHAM. Albouy *et al.,* (2012) also showed increased activity in the caudate as learning stabilised (less errors were made) on a finger tapping task (FTT). Cousins *et al.,* (2016) also showed an increase in activity bilaterally in the caudate correlated with time spent in SWS, in SRTT sequences TMR-cued overnight. They hypothesised this is because the striatum is highly involved in motor skill learning, particularly in the later stages. The link between SWS and caudate activity is interesting as in this experiment

the caudate activity was higher following stimulation which is linked to enhancing SWS, however we saw neither increase in the amount of time in SWS nor a correlation between the activity in the caudate and the difference in time spent in SWS between SHAM and STIM. Odour TMR also has been shown to increase striatal activity linked with post-sleep increase in motor sequence performance (Laventure *et al.*, 2016).

It has been suggested that increased activity in the striatum during well practiced motor tasks could signify consolidation of motor sequence skill (Doyon *et al.*, 1996, 2009; Leslie G. Ungerleider, Julien Doyon and Avi Karni, 2002; Fogel *et al.*, 2017). Increased activity has been shown following sleep (Walker *et al.*, 2005; Debas *et al.*, 2010), and linked to spindles during NREM sleep (Barakat *et al.*, 2013; Fogel *et al.*, 2017). Therefore, increased activity shown in the caudate following CLAS could indicate CLAS is driving this consolidation.

Results also showed a non-motor region within which activity was effected by CLAS: An area in the left temporal lobe which has previously been identified as active in motor sequence tasks (Walker *et al.*, 2005; Fogel *et al.*, 2014). This area may be less tied to the motor aspects of this task but instead relate to the other elements such as the auditory cues, as this area is close to the secondary auditory area. Durrant *et al.,* (2011) showed that participants could significantly better recall a probabilistic sequence of tones following sleep, than following the same retention interval of wake. I propose that participants in my experiment are using the auditory cues in the SRTT to assist them in their prediction of the next button to press (indeed anecdotally participants reported using this strategy). Therefore, the increased BOLD activity in the temporal lobe could denote this auditory prediction is greater following stimulation, as stimulation has facilitated the usual sleep dependent process of abstracting the auditory cue rules.

All together this provides the first evidence that CLAS can affect BOLD activity during motor sequence recall.

4.5.3 CLAS led to a smaller decrease in BOLD activity in the putamen during WP recall

fMRI scanning showed that CLAS led to greater activity in the putamen following CLAS. The putamen, like the caudate is part of the striatum, and involved in many processes (Mink, 2013). The putamen has previously been found to be active in relation to the WP task (Liu *et al.*, 2014; Marin-Garcia, Mattfeld and Gabrieli, 2021) and also when a variation of this task is used with image word pairs (Fandakova *et*

al., 2018) particularly when assessing remembered words like in my protocol: Marin-Garcia *et al.,* (2021) showed putamen activity increase correlated with words remembered. My data shows that there is greater activity in STIM than SHAM in the left putamen which could indicate better recall in this task. Putamen activity and communication with other medial temporal lobe areas has been shown to be important in memory retrieval for declarative tasks learnt with feedback (Shohamy *et al.*, 2004; Agrawal, Sharma and Chinnadurai, 2021).

Activity in the putamen on declarative tasks has also been linked with motivation (Han *et al.*, 2010) and CLAS has been shown to specifically increase consolidation in WP stimuli associated to reward (Prehn-Kristensen *et al.*, 2020), although I did not find this in Chapter 3. There has been some indication that motivation and reward are not the same such that CLAS may be affecting motivation without the promise of reward. Such that there is the potential that CLAS in this experiment could be increasing the motivation of participants to perform well at this task, as putamen activity has been linked to motivation over reward (Miller *et al.*, 2014), and this may not translate to more words recalled. There is also some evidence that activity in the putamen is involved in ignoring irrelevant distractors (McNab and Klingberg, 2007); such as the incorrect letters presented in this protocol. Therefore, increased activity here could indicate an interaction with this choice. However, as no ROI associated with memory were found to be recruited differently following CLAS, it could reinforce that CLAS does not change WP memory (Henin *et al.*, 2019), particularly as I did not find stimulation led to a change in performance.

4.5.4 CLAS leads to poorer pattern separation performance

Despite (1) using tasks similar to those previously shown to have been impacted by sleep (Walker *et al.*, 2002; Backhaus *et al.*, 2006; Schreiner and Rasch, 2017), TMR (Cousins *et al.*, 2014, 2016; Rakowska *et al.*, 2021) and CLAS (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017) and (2) showing activity differences in the brain with stimulation in areas linked to these tasks, there was no relationship to performance changes in these tasks. It is likely that the tasks and procedures used here were not sufficient to detect any changes caused by these differences in activity brought about by CLAS.

However, CLAS did have an impact on the change in PSS on the MST over the first night following encoding: Stimulation lead to significantly poorer performance over both Night 1 and Night 8 than SHAM. The difference between SHAM and STIM was

not apparent in RMS indicating that memory for the images themselves was not affected by CLAS.

When presented with a similar image in the recall of this task, participants are faced with three possible answers: (1) *similar*, the correct choice, and evidence that this participant holds the old image in their memory in such a way it is distinct from similar images likely to indicate pattern separation; (2) *old* an incorrect response indicating that the participant does not hold a distinct enough memory of the old image such that this similar image fits close enough to their recollection to be mistaken for the image itself, likely to indicate pattern completion; and (3) *new* an incorrect response indicating the participant has forgotten the original image. Thus a fall in PSS score (indicating fewer correct similar responses) can arise from recognition errors or pattern completion. A fall in RMS overnight was observed, but did not differ between SHAM and STIM. It is therefore more likely that this stimulation difference is driven by an increase in pattern completion in STIM. While Hanert *et al.,* (2017) did find that sleep led to less decline in PSS than wake over the same interval, they also showed that PSS in sleep depended upon the degree of similarity between similar images, such that the most similar images led to poorer PSS while the least led to greater PSS. They hypothesised that this was an indication that both processes of pattern completion and pattern separation were occurring more during sleep than wake. The results presented in this chapter might suggest that under STIM conditions pattern completion is being favoured over pattern separation, thus causing the fall in PSS.

It has been hypothesised that sleep favours the abstraction of gist (Lewis and Durrant, 2011; Klinzing, Niethard and Born, 2019), drawing on the commonalities between memories (a form of pattern completion). This process is reinforced through repeated stimulation or reactivation of the same memories, particularly through NREM sleep (Lewis, Knoblich and Poe, 2018). It is possible that CLAS, by boosting slow oscillations and spindles, leads to more effective reactivation of memories in NREM sleep and thus leading to more pattern completion than under non-stimulated sleep, where pattern separation is higher (Hanert *et al.*, 2017; Doxey *et al.*, 2018). SWS TMR has been shown to negatively impact the ability to specifically recall unique qualities of stimuli (a form of pattern separation, Witkowski *et al.*, 2021).

Despite CLAS leading to lower PSS scores across Night 1 there was no difference between stimulation conditions over Nights 2-7. This could suggest that pattern separation declines across a week without stimulation, but it reaches this state quicker following CLAS, such that PSS score is worse following one night of STIM but

comparable to SHAM after seven nights. This implies that STIM is hastening the intrinsic sleep properties. It fits with the idea that gist abstraction is promoted over multiple night's sleep in non-stimulated sleep (Deliens and Peigneux, 2014).

However, some results have indicated that in the short term, i.e. over a nap, or the first night following learning, that sleep promotes strong interaction with the hippocampus which keeps memories distinct and encourages pattern separation (Klinzing, Niethard and Born, 2019). This fits with the finding by Hanert *et al.,* (2017) that sleep led to sustained pattern separation over one night verses a decline in wake. Although there has been an argument that this simply denoted forgetting in wake and maintenance of the memory across sleep, not improvement by sleep (Poh and Cousins, 2018). However, over several nights, sleep promotes the consolidation of memories in the neocortex away from the hippocampus and incorporates them into existing memories promoting pattern completion and gist abstraction (Klinzing, Niethard and Born, 2019). It could be hypothesised that by boosting SWS and ASC, CLAS is accelerating this process. Indeed some abstraction of memory has been shown for the context memories were learnt within, over just one night (Cairney *et al.*, 2011). Therefore, stimulation lead to a decline in pattern separation over the first night compared to SHAM.

This is the first evidence to suggest that CLAS influences performance on a task other than the WP task, and thus opens up an exciting new avenue for understanding of the influence of manipulating SWS oscillations with another form of memory. This finding also provides more evidence for some role of sleep in pattern separation and completion, giving more detail on how these memories are manipulated by sleep over time.

4.5.5 Eight nights of CLAS leads to electrophysiology results consistent with one night of stimulation

There were again no detrimental effects of stimulation on time in each sleep stage, indeed there was again a significant decline in WASO. However, this was only seen during Night 1 not across Nights 1-7 or Night 8. In Chapter 3 I suggested the detected decrease in WASO could be an error of the headband scoring algorithm, however, as in this chapter I only saw this result in Night 1 this makes this less likely; As a machine, the algorithm, would consistently make the same scoring mistakes. As this effect is only seen in the first CLAS night it could imply that stimulation as a novel addition to sleep is suppressing WASO. However, as in Chapter 3 there were no other significant

differences in other sleep stages or TST which might account for the extra time in STIM produced by less time in WASO. Therefore, it is difficult to determine where this time is being spent. It could be sufficiently spread between other stages such that it did not lead to a statistically significant difference. This is reflected in Table 8, as several stages are slightly longer in STIM although non-significant, including TST (SHAM mean=436.2 min, STIM mean=449.3 min). One possible mechanism for decreasing WASO is that the sounds from the headband elicit K-complex like events, known to be linked with the continued maintenance of sleep (Nicholas, Trinder and Colrain, 2002), such that there is actually less disturbance of sleep caused by other factors such as external noise which is disrupting sleep in SHAM nights. To investigate this further the number of K-complexes could be calculated to see if there is an increase in STIM nights. Or the number of micro arousals could be quantified to see if there were more in STIM which could indicate greater suppression of arousals. This is potential for future work as the focus of this chapter is on fMRI. If this were the case, then it would put forward the first evidence that CLAS can lead to a more restful sleep with less time spent awake.

Once again the results have shown that one week of CLAS leads to the expected ERP response, this time without any signs of habituation. Unlike Chapter 2, ERP analysis of the first and final night did not show any statistical difference, which implies that the stimulation was equally effective across the eight nights of stimulation. This fits with the results of Debellemaniere *et al.,* (2018) who showed that ten nights of stimulation with the same device did not lead to habituation of the brain response as measured by ERP. Thus it can be assumed that any lack of behavioural impact of CLAS after the first night is not due to the brain habituating to the stimulation across the week.

4.5.6 Conclusions

This chapter shows for the first time that CLAS can affect BOLD activity in the recall of declarative and motor tasks. Specifically, that activity increases across time and stimulation in the cerebellum; and stimulation in the caudate, potentially indicating an increase in consolidation. While striatum activity was also increased with stimulation in recall of word pairs. These findings do support the hypothesis that CLAS is affecting memory despite several recent failures to replicate early results.

This chapter also provides the first evidence that CLAS can affect pattern separation performance as measured by the MST. This is the first time a group level effect of

stimulation has been shown to affect task performance on any task other than a particular procedure of the WP task. This could lead to greater understanding of the effect of stimulation upon overnight consolidation and reorganisation of memories as well as provide insights into the effect of sleep on the memory processes probed by the task. It opens many questions on how exactly stimulation acts upon memories and if this can be replicated. The wider implication of the fMRI and behavioural impact of CLAS will be discussed in the wider context of CLAS in the following general discussion chapter.

Chapter 5
General Discussion

5.1 Overview of thesis findings

The aim of this thesis was to increase our understanding of how slow wave sleep (SWS) interacts with memory by boosting SWS using CLAS. Specifically, across the experimental chapters I expanded the testing of CLAS: into novel tasks, to probe declarative and procedural memory; across multiple nights, to understand long term influence of SWS on memory; and into MRI analysis, to understand the impact on activity in the brain.

In Chapter 2 I explored the impact of one night of stimulation upon two new tasks; the mnemonic similarity task (MST) and the serial reaction time task (SRTT) not previously tested with CLAS. Neither task exhibited a significant impact of CLAS. Stimulation in this experiment was delivered in the laboratory using standard PSG. I also explored the impact of increasing the duration of the auditory stimulation '*click*' as well as the inter-stimulus-interval between sounds. I determined that increasing the sound duration from 50ms to 100ms or measuring the ISI in SO had no profound impact on the resulting ERP.

In Chapter 3 I explored the impact of one and seven nights of CLAS on three declarative tasks each focussing on an element of the word pair (WP) task. The experiment was also the first to utilise a new dry EEG device, to deliver CLAS at home. Results indicated that neither one nor seven nights of stimulation led to a significant change in recall performance on the word pair with reward (WPr), image paired associates (iPAL) or verb generation (VGT) tasks. Although repeated nights of stimulation led to no habituation of the electrophysiological response. This indicates that repeated nights of stimulation do not increase the influence of CLAS on these tasks.

In Chapter 4 I utilised MRI scans to explore the impact of CLAS on how the brain recalls stimuli in the WP and SRTT tasks. Again I used the headbands to allow participants to receive CLAS at home during the experiment. Results showed that despite having no impact on behavioural performance, seven nights of CLAS led to significant changes in brain associated BOLD brain activity whilst recalling the SRTT and WP tasks. Results also indicated that one night of CLAS could be detrimental to pattern separation scores as measured using the MST.

In this discussion I aim to bring together some of the themes that have emerged from the experiments detailed in the previous three chapters and position these within the current body of literature. Particularly focussing on the insights these results across

behaviour, EEG and MRI, might reveal about the impact of CLAS on memory. Finally, I will look forward to how we can learn more by asking future research questions.

5.2 Does CLAS improve memory

The chapters in this thesis assess a number of behavioural tasks, all previously shown to be influenced by SWS and its oscillations, but never before tested with CLAS. Results indicated that neither one nor seven nights of stimulation led to significant differences in performance. WP is the most widely studied memory task with CLAS, but results have been mixed as to the influence of CLAS on performance, this is discussed further in section 5.6 Word Pair. The influence of CLAS on the SRTT has not been assessed before, but two studies have shown that stimulation had no effect on finger tapping performance (Leminen et al., 2017; Jules Schneider et al., 2020). The influence of CLAS on pattern separation, image pair consolidation and creative verb generation have never been tested before.

EEG results indicated that stimulation influenced SO in the expected manner, as per previous CLAS experiments (Ngo, Martinetz, *et al.*, 2013; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017; Henin *et al.*, 2019), even after eight nights (Debellemaniere *et al.*, 2018). But there were no correlations between EEG and behaviour. In Chapter 4 I utilised fMRI scanning to assess BOLD as an indirect measure of brain activity whilst participants recalled stimuli on WP and SRTT tasks. Here, CLAS led to a change in the BOLD signal, suggesting that stimulation might alter brain function (e.g., recall). However, BOLD changes did not correlate with behaviour. This leads me to question why CLAS is causing EEG and functional changes that are not altering the processes that underpin performance in my chosen tasks.

One possibility is that changes in EEG oscillations and BOLD are a pre-curser to future behavioural changes. This fits in well with the theory that CLAS leads to stimulated replay of memories, potentially denoted by the SO response to stimulation, which is influencing the synaptic up and downscaling of synaptic connections related to memories (Tononi and Cirelli, 2014; Seibt and Frank, 2019; Pereira and Lewis, 2020). These small synaptic changes may not be sufficient to lead to large enough changes in circuitry that would lead to more words recalled, or faster overall finger movements in the number of days assessed by behavioural recall in this thesis. But these changes may be sufficient to lead to changes in BOLD activity as measured during stimuli recall. This could indicate the start of a chain which will eventually lead

to plastic changes that would be large enough to result in performance improvements. Results supporting this view have been shown following SWS TMR (Cairney *et al.*, 2018; Rakowska *et al.*, 2021). Thus, Rakowska *et al*., (2021) suggested something similar when they applied TMR cueing of SRTT sequences during SWS, and found no behavioural influence of cueing the day following stimulation, but found a benefit to SKILL (difference in mean RT between sequence and random blocks) ten days later. While Cairney *et al*., (2018) found strong evidence of memory replay during SWS following TMR delivered in a nap, but no improvement post-nap in memory. However, when they tested participants following an additional night's sleep they found that cued stimuli were better recalled. They were confident in the presence of cued replay during the nap as they found increased spindle activity following sounds and that their algorithm could identify stimuli during this increased spindle activity. It could be said that in both of these experiments additional testing reinforced memory that later related in measurable performance changes. However, in TMR, non-cued stimuli act as within-night controls and are tested the same amount as non-cued memory, such that any difference in performance shown between cued and un-cued memory is more likely to arise as a result of initial cuing not re-testing. The same cell firing patterns seen during spindles coupled to SO have been shown in animal models to induce synaptic changes in the short and medium term (Timofeev *et al.*, 2002; Rosanova and Ulrich, 2005). Indeed, spindle activity was shown to cause short and long term potentiation at synapses (Rosanova and Ulrich, 2005). Thus if CLAS increases spindle activity in SO it could be causing differences in potentiation. To test this theory, CLAS experiments in future could include a follow up behavioural test on all tasks, ten days following stimulation. If performance changes do emerge, assessments should be made to see if they correlate with the brain regions I have shown to be influenced by CLAS over the shorter term. It would also be interesting to see if BOLD activity changes correlate with spindle changes following CLAS in the short and long term.

An alternative analysis is that the assessed behavioural measures (i.e. mean block RT and change in words recalled) were not sensitive enough to detect significant performance changes influenced by alterations in brain activity. Indeed, sleep does not influence consolidation of all aspects of a task equally (Robertson, Pascual-Leone and Press, 2004; Fischer *et al.*, 2006; Spencer, Sunm and Ivry, 2006). Thus, if we were assessing elements of the task that were not improved then we would see no difference in performance, while other unmeasured components may have been

improved. In the SRTT, for example, previous work has shown that sleep can have an effect on the variability of responses and errors made, but not the RT (Lutz *et al.*, 2018). RT as a measure can also be made more sensitive to effects of sleep: Using the PVT (employed in Chapter 2), Basner and Dinges (2011) showed that sleep loss led to increases in RT only in the slowest 10% of responses, while the fastest 10% were comparable to normal sleep. They hypothesised this indicated that sleep loss led to lapses in concentration, resulting in increased RT; but participants also experienced moments of normal arousal leading to the stability in the fastest responses. Similarly, by assessing SRTT using SKILL derived from block RT, similar lapses and moments of increased speed could be masked, hiding any influence of CLAS on these instances. Therefore, future experiments could assess the variability of RT in SHAM and STIM and the error rate to see if this correlates with BOLD changes. This particularly may correlate to BOLD activity in the cerebellum as it is known to play a major role in the prediction of the outcome of motor movements and adjustments following errors (for a review see Popa & Ebner, 2019).

As SRTT BOLD activity differed in the caudate, it is possible that a behavioural measure more closely related to activity in this area might have yielded a brain-behaviour relationship. For example, the caudate is known to be particularly involved in the spatial elements of motor tasks (Cook and Kesner, 1988), such that RT for each location in the SRTT may differ or correlate to caudate BOLD activity. The RT for each location could be assessed in isolation and SKILL calculated relative to random trials of that location, to see if this could tease out a behavioural difference between SHAM and STIM. In the WP task the primary performance measure was the number of words recalled. However, a different measure may have better correlated to the identified BOLD change in the putamen: Such as the confidence of participants in their response, as this participant motivation has been linked with activity in the putamen (Mizuno *et al.*, 2008; Miller *et al.*, 2014).

As this is the first study to utilise fMRI to investigate brain function whilst performing recall on any memory task following CLAS, it has highlighted the potential for this technique to uncover functional differences despite behavioural similarities. This provides further evidence that CLAS is affecting brain function, albeit in ways that did not correlate with behavioural measures. This leads me to question if any further brain changes, particularly following long-term stimulation, such as plasticity, could reveal more about the interaction with this stimulation technique and consolidation of memories.

In chapter 4 the fMRI analysis was conducted across the whole brain with multiple comparisons correction applied via the use of a *p*-value threshold for significant clusters of $p<0.001$ in a-priori ROI, without further correction via using family wise error rate (FWE) or false discovery rate (FDR). This method has been used in published fMRI studies, particularly when conducting exploratory studies, including in sleep and memory research (Van Der Werf *et al.*, 2009; Bergmann *et al.*, 2012; Fogel *et al.*, 2014; Shanahan *et al.*, 2018). For a review of fMRI thresholding methods see Woo, Krishnan and Wager, (2014) or Yeung (2018). This method of analysis would indicate that there is a 0.1% chance these clusters of activity may have passed the significance threshold by chance. The threshold chosen is important as in analysis of fMRI there is a high risk of Type 1 errors (false positives) due to the large number of statistical tests conducted as tests are performed for each voxel and time point (Penny *et al.*, 2007; Yeung, 2018). Thus, as multiple comparison correction was not conducted using FWE or FDR the threshold for significant *p* values was much lower than the usual value of 0.05, to lower the chance of false positives. When analysis in Chapter 4 was conducted with FWE multiple comparison correction (across the whole brain) at $p<0.05$ no clusters passed this threshold for significance. Therefore, this could cast some doubt on the robustness of the clusters presented in Chapter 4, and the conclusion that CLAS is affecting brain activity at recall in the SRTT and WP tasks. If we consider that CLAS may not affect BOLD activity significantly in these clusters, results fit closer to the behavioural analysis that indicated CLAS did not lead to a change in performance after one or multiple nights of stimulation in the SRTT or WP tasks. This would add to more recent evidence that despite robust CLAS effects on sleep oscillations, stimulation has no impact on memory recall (Henin *et al.*, 2019). However, as discussed, this method of using the stringent threshold of $p<0.001$, is not an unusual way of correcting for false positives, and as discussed in Chapter 4 and above, significant clusters were in areas of relevance to the tasks being performed despite whole brain analysis. To test this further the experiment could be repeated with a larger group of participants to increase the power in the sample and see if significant clusters could be replicated (Lieberman and Cunningham, 2009) and see if results could then withstand more stringent multiple comparison correction. This would allow more confidence in the extent of CLAS impact on BOLD activation in task recall.

5.3 Longitudinal CLAS

This thesis presents the first ever experiments delivering CLAS for more than one night and assessing this impact on task performance. Results testing memory recall over the week indicated there was a decrease in performance associated with time but not CLAS. Performance following one-night retention interval was also shown to be comparable if it was preceded by a week of stimulation or not. This indicated that CLAS did not affect consolidation over either one night or over a night following a week of stimulation.

fMRI results in SRTT indicated that there was an increase in BOLD activity in the cerebellum with both time and stimulation. As discussed in Chapter 4 this is a region implicated in motor skills. Word pair recall did not indicate an influence of time on the areas involved in remembering pairs. It is not unexpected that of the two tasks the motor task would indicate an impact of stimulation over several nights: As motor skill is expected to develop much more slowly than declarative memory (Kami *et al.*, 1995; Walker *et al.*, 2003). Perhaps, as previously discussed (in section 5.2 Does CLAS improve memory), CLAS is influencing the very beginning of the chain of events leading to consolidation. Walker *et al.,* (2003) showed three nights of sleep had further benefits to FTT than one night of sleep, even without retesting in between. Indeed, Kleim *et al.,* (2004) show that training rats on a reaching task only led to increased synapses after 7days of training, and evidence of reorganisation in the corresponding motor area after 10 days of training. If CLAS is interacting with the early stages of consolidation, namely the tagging of synapses for up or downscaling, then maybe this process is available for interaction longer in motor tasks, such that repeated nights of CLAS lead to changes in performance. Whereas in a declarative task the memories are consolidated faster and as such repeated nights of stimulation do not effect activity. So in the WP task CLAS is not affecting consolidation for as long as it is in SRTT.

Results do suggest that any effect of CLAS on memory performance does not accumulate over seven nights. But there is also the chance that seven nights is not enough to see any influence of stimulation on the latter plastic stages of consolidation (Seibt and Frank, 2019). It could be expected that CLAS through its influence on SWS would affect brain plasticity, particularly if delivered over repeated nights. Mander *et al.,* (2017) found that older participants performed worse on declarative memory tasks, they linked this decline in performance with a decline in SWS. They also linked this SWS decline to atrophy in frontal grey matter. More recently (Mander *et al.*, 2017)

they went on to show that spindle density declines in older adults and this was predicted by whiter matter volume in distributed tracts. They also showed that the decline in spindle density was linked to poor declarative memory performance. Therefore, by boosting SWS CLAS could lead to measurable plasticity in frontal grey matter and white matter tracts. Repeated nights of stimulation are more likely to influence plasticity as they could interact with the later stages of consolidation that cause plasticity (Seibt and Frank, 2019; Pereira and Lewis, 2020). If there were structural changes and these correlated with the time and stimulation related changes in SRTT this would give strong evidence that stimulation was involved in this consolidative process that starts with synaptic changes and ends with plastic changes. Microstructure could also be assessed using CHARMED scans to see if there are any diffusivity changes which might imply the same. The use of a device to deliver CLAS in the home environment is essential for the further study of questions relating to long term stimulation.

5.4 Expanding CLAS beyond the lab

Chapters 3 and 4 in this thesis utilised an ambulatory, dry EEG device, to deliver CLAS, unsupervised, in the home. Chapter 3 and 4 were the first CLAS studies to use these devices outside of the company that manufacturer them, and the first to assess the impact of their use on memory. The use of the headbands was considered a success; ERP results presented in Chapters 3 and 4 indicate stimulation was applied and led to the expected brain response, as per Debellemaniere *et al.,* (2018). The device also allowed stimulation to be delivered over repeated nights, and for participants to sleep in their own environment as opposed to the sleep laboratory, which is known to affect sleep (Agnew, Webb and Williams, 1966). Without these devices sleep data could not have been collected during the pandemic. However, there were a number of limitations: A large amount of data was lost through issues uploading the data to the server, whole nights did not arrive, despite participants reporting correct headband use. There was also a number of occasions when participants admitted not wearing the headband when instructed, 12 out of 52 admitted this in the final questionnaire, citing reasons such as ‘Forgot’ and ‘Not charged’. Although this only accounted for less than 2% of total experimental nights. There was also the potential that as stimulation was unsupervised, it could have led to more arousals as experimenters couldn’t reduce the stimuli volume as they could in the lab. However, as results in Chapters 3 and 4 indicate there was no significant difference in arousals, indeed I identified less wake after sleep onset in STIM in both

studies using the device. Breakthrough of the sound could have also led participants to know which week stimulation was applied, removing the blinding of the stimulation counterbalance. At the end of their participation in the studies described in Chapter 3 and Chapter 4, participants were given a questionnaire to complete asking questions about how they found wearing the headband (see appendix section 7.1 for questionnaire). One question asked which week they thought stimulation was applied (pre-ceded by a brief explanation of the SHAM and STIM set up in the study, as participants were ignorant of this during the study). Between both studies there were 47 answers, 57% of which were 'Don't know'. Of those that did select a week, only 40% were correct (17% of total sample), below the chance level of 50%. This indicates that participants were not aware of which week the stimulation was played, and therefore I can be confident the stimulation was delivered in such a way by the headband that blinding was not compromised. Schneider (2020) showed that telling participants that CLAS would be applied overnight but not applying any stimulation, did not lead to any significant improvement in memory or changes to electrophysiology compared to a control night. Thus we would not expect participant's knowledge of which nights were stimulated to affect performance even if they had known.

Participants may have found the headband uncomfortable to sleep with, as all answered positively or neutrally ('unsure') to: 'Did the headband disrupt your sleep', and none answered positively to: 'Did the headband improve your sleep'. Although comfort ratings (out of ten stars) stayed consistent across both weeks of both experiments using the headband (n=39, week1: mean=5.85, SEM=0.36; week 2: mean=5.95, SEM=0.31; paired t-test: $t(38)=-3.36$, $p=.722$), and did not differ between SHAM (mean=5.92, SEM=0.33) and SITM (mean=5.87, SEM=0.35, $t(38)=0.17$, $p=.860$). It is difficult to judge the discomfort or detriment to sleep caused by the headband, as no sleep measures were made on nights without the headband. However, total sleep times (TST) as reported in Chapter 3 and Chapter 4 are well within the expected range for healthy sleep (Ohayon *et al.*, 2004) and indeed are comparable to those in Chapter 2 where sleep was recorded in the lab using conventional PSG. One drawback with the sleep scoring of the headband was its inability to accurately detect N1, as I only saw one or two epochs of N1 sleep across the both experiments. In the paper validating the headband scoring algorithm (Arnal *et al.*, 2020), N1 had the lowest agreement between the algorithm and human scorers.

In terms of the headbands ability to accurately deliver CLAS this is not significant, however it could call into question the TST measures.

Altogether, the use of a dry ambulatory EEG device to deliver CLAS in this study was successful, however; more refinement of the scoring mechanisms would allow all measures to be fully utilised. I also would suggest as many nights as possible for participants to become comfortable sleeping and operating the headband to be included in any study design using such devices.

5.5 One night of CLAS is detrimental to pattern separation

Results from Chapter 4 indicate that CLAS leads pattern separation score to decline over the first night; This fits with the idea that sleep promotes the abstraction of gist and generalisation of memories (Cairney *et al.*, 2011; Durrant *et al.*, 2011; Lewis and Durrant, 2011), at the detriment to pattern separation. This is the first time CLAS has been shown to affect a memory task other than the WP task. Results can offer insights into how pattern separation and completion are influenced by SWS. There has been little previous investigation of the role of sleep in pattern separation and this finding suggests that it may be impaired by the increase in SO and spindles induced by CLAS. As there was no difference over the six nights it could imply that the biggest impact of SO and spindles (oscillations affected by stimulation) occurs on the first night following learning for pattern separation. Perhaps during this first night synapses for scaling are tagged, a process influenced by stimulated replay, but processes occurring later are more REM dependent are not influenced by these oscillations, such as translation of proteins and synaptic plasticity (Seibt and Frank, 2019; Pereira and Lewis, 2020).

It is surprising that results from Chapter 4 indicated a significant effect of stimulation over one night while results from Chapter 2 did not. There are a number of important differences in the procedure of both experiments that could account for differences. For example, the first experiment (Chapter 2) was carried out in the lab using regular PSG while the second experiment was carried out at home using the Dreem headband. There is the potential that participants slept deeper in their own homes which led to more efficient SWS with larger SO for CLAS to target. It has been shown that sleep in a new environment can alter the quality and structure of sleep (Agnew, Webb and Williams, 1966; Newell *et al.*, 2012). The stimulation differed slightly between Chapter 2 and Chapter 4: Chapter 2 used single click trials with two sound durations and three variations of inter-stimulus interval (ISI), while Chapter 4 used two

click trials with fixed ISI. As the timing of stimulation has been shown to be important (Ngo *et al.*, 2015; Weigenand *et al.*, 2016), this could have effected CLAS influence on MST score, however Chapter 2 did not indicate any large differences between duration and ISI trials. The small sample sizes in both experiments are also likely to influence results. This could be enough to incur the different results found.

To further probe this effect, future experiments could aim to directly assess pattern completion: The Deese-Roediger-McDermott paradigm is such a test, where words are encoded that fit into categories (i.e. moon and astronaut), then at testing participants have to recognise words previously taught from new words that would also fit the category (i.e. rocket, Pardilla-Delgado & Payne, 2017). Or a hierarchy could be implicitly taught using images where the participant learns via trial and error the order of images: Participants are taught that A>B, B>C, C>D (Ellenbogen *et al.*, 2007). Following the interval containing sleep and CLAS they would then be asked to order pairs not seen before such as A>D. If these tests implied pattern completion was stronger following CLAS this would add evidence that this process is affected by the oscillations strengthened by stimulation.

5.6 Word Pair

What of the discrepancies between studies in CLAS effect on WP? A recent meta-analysis was conducted by Wunderlin *et al.,* (2021) to assess all CLAS studies that tested stimulations effect on WP performance. I focused on their sub-analysis of 'phase-locked stimulation only' rather than their main analysis as it more closely aligned to what I consider CLAS studies (see General Introduction section 1.5 Figure 3). In this sub-analysis they removed two papers compared to the main analysis which do not conform to the definition of CLAS as used in this thesis; (1) Weigenand *et al.,* (2016) as they used an *open-loop* system to apply stimulation in which they deliberately re-set the ongoing SO rhythm by playing a sound to evoke a SO/K-complex then stimulated subsequent SO, and (2) Choi *et al.,* (2019) as they specifically targeted spindle activity not SO as with other CLAS procedures. In this sub-analysis Wunderlin *et al.,* (2021) found a small, effect of CLAS on WP memory (Hedges g'=0.36, 95%-CI=0.00; 0.72, z=1.98, p=0.047), this rose to a slightly larger, effect (Hedges g'=0.44, 95%-CI=0.09; 0.79, no z score given, p=0.01) when they removed those studies recruiting middle aged and older participants (such that the mean age of included paper participants was 23.6 years). This therefore suggests that when considering these papers CLAS has an effect on WP performance.

However, Wunderlin *et al.,* (2021) did note the small sample size in many of the studies. They described that with the given effect sizes, to achieve 80% statistical power would require sample sizes of at least 42, whereas the mean of studies was far short of this at only 16. This could cast doubt on their finding that overall CLAS impacts WP performance, as most studies are likely under powered.

As with many areas of research we have to also consider the impact of publication bias (for a review see Joober, Schmitz, Annable, & Boksa, 2012), such that papers which did not indicate CLAS had an impact on WP performance were not published. Indeed, Wunderlin *et al.,* (2021) assessed this, and while they found no evidence of publication bias (based on the Hedges g' size and error using a funnel plot), I am not convinced there is none associated with this question. As mentioned, I do not agree with all included papers in this meta-analysis and as such, removed two of six papers indicating a negative effect. Leaving only Henin *et al.,* (2019), which Wunderlin *et al.,* (2021) included both of their experiments as separate studies, and Schneider *et al.,* (2020); as included papers that showed no effect of stimulation on WP memory. Had the funnel plot used by Wunderlin *et al.,* (2021) to assess publication bias, only included these two studies on the negative side with the five positive studies, their conclusions may have been different. Indeed, if I set aside my definition of CLAS and consider the full meta-analysis conducted by Wunderlin *et al.,* (2021), there is no significant impact of stimulation on WP performance. Also since this meta-analysis there has been an additional paper published that also fails to see any benefit of CLAS on WP memory (Harrington, Ngo and Cairney, 2021), this time for semantically unrelated word pairs. Alongside my recent evidence of no impact of CLAS on WP with or without reward over one night or a week it is becoming more difficult to defend that CLAS can improve overnight recall in the WP task.

5.7 CLAS selectivity

In terms of boosting memory consolidation, one could consider CLAS a non-specific technique, in that it does not specifically target a particular memory, instead it aims to boost the endogenous mechanisms supporting memory. It has been suggested that there is limited capacity for consolidation on any given night (Feld, Weis, Born, & Weis, 2016; cf. Schechtman et al., 2021), and that some memories may be prioritised (Fischer and Born, 2009; Wilhelm *et al.*, 2011). This could lead to CLAS boosting some stimuli from the day before but not all, mechanisms behind any such prioritisation are not well understood. Participants in experiments will have experienced a whole day of other sensory input alongside memory tasks in the

experiment and as such there is likely a lot of information for them to consolidate. This information will be unique to each participant on each day. If memories from the experimental tasks are not naturally prioritised for consolidation, then boosting endogenous consolidation with CLAS may not improve their recall. But the recall of other memories may be improved. This may have contributed to different effects found on the MST task in Chapter 2 and Chapter 4. This would be very difficult to assess experimentally, but one method could be to restrict input for a day before stimulation, such that the experimental tasks were more prominent, or introduce some sort of questionnaire or task that could assess if participants performed better on memories for other aspects of their day. Another approach could be to combine CLAS with the TMR technique of assigning a memory to the auditory stimulus, to preferentially induce the replay of that specific memory, coined closed loop–TMR (CL-TMR).

CL-TMR combines the timing of CLAS with the memory specific precision of TMR. TMR has been shown to benefit a variety of memory types including: motor (Cousins *et al.*, 2016; Belal *et al.*, 2018; Rakowska *et al.*, 2021); declarative (Schreiner and Rasch, 2017); locations (Rasch *et al.*, 2007; Rihm *et al.*, 2014); and navigation (Shimizu *et al.*, 2018). Indeed, TMR studies have been able to identify the same patterns of activity following the sound in SWS as seen during stimuli encoding (for a review see Schreiner & Staudigl, 2020). Some studies have even been able to identify the sound matched stimuli from the pattern of activity in sleep (Schönauer *et al.*, 2017; Cairney *et al.*, 2018). Traditionally, TMR has only been targeted at a sleep stage not at a particular time in the ongoing oscillations. As discussed in Chapter 2 the timing of the sound in CLAS has been shown to be important: Playing sounds away from the optimal timing (during the rising phase of the SO) has been linked to smaller increases in memory performance, where they were shown (Weigenand *et al.*, 2016; Navarrete *et al.*, 2019), and sounds played during the trough of a SO have been linked with induced forgetting (Cox *et al.*, 2014; Fattinger *et al.*, 2017). It has been shown that when TMR cues occur during SO up-states they preferentially lead to a better memory outcome than cues that occur during SO down-states: Göldi *et al.,* (2019) showed that CL-TMR cues presented during the up-state of the SO led to better recall performance for memory linked to those cues, compared to memories associated to sounds not played.

In CL-TMR memory linked sounds are played at precise times aimed at the SO up-state. This therefore combines the best of TMR: (1) that you are confident that the stimulation is targeting the memories you are focused on; and (2) the precise timing

of the sound such that it is most likely to lead to a successful reactivation/replay. The inbuilt control of non-cued items in a TMR like protocol would also assist in controlling for any differences between nights (or days) in a CLAS protocol. CL-TMR would also be useful for long-term studies as the same sounds could be used over a series of nights to see if repeated targeting of the same memories leads to differences in their recall. This would allow for a much more controlled exploration of the change in consolidation of memories during sleep over time. There are likely still limitations to work out: The length of the sounds used in TMR are generally much longer than the 50ms CLAS standard, but in Chapter 2 I showed that doubling the sound duration to 100ms still lead to a comparable ERP response from the brain.

Recent work has begun to explore the utility of CL-TMR in boosting memory recall (Batterink, Creery and Paller, 2016; Shimizu *et al.*, 2018; Göldi *et al.*, 2019). Shimizu *et al.,* (2018) used CL-TMR to drive a reduction in the amount of time taken by participants to navigate between two points in a Virtual Reality environment. As well as a reduction in route time, they also saw an increase in fast spindles coupled to SO. This finding is consistent with the suggestion that the reduction in route time was linked to an increase in the replay of task related memories induced by stimulation. This could be a powerful technique for building upon the work of this thesis and further understanding the consolidation of memories overnight.

5.8 Individual responses

The application of CLAS resulted in high levels of individual variability in changes in behaviour following stimulation. There was often little consistency even in the direction of change between SHAM and STIM conditions. That is, for some participants there was an increase in SKILL on the SRTT overnight on SHAM nights and a decrease on STIM nights, whilst for others there was the opposite pattern. One possible cause of such differences is inter-participant variation in the responses to stimulation.

This may be a plausible reason that different experimenters found opposing results when utilising the same protocol: Ngo, Martinez *et al.,* (2013) found WP enhanced by one night CLAS while Henin *et al.,*(2019) found no change in behaviour following stimulation, despite using the same task and procedure. If the two cohorts were made up of different proportions of participants who respond to CLAS or not, then this could affect the significance of a difference between SHAM and STIM performance at a group level. This is likely to be exacerbated by the small cohort used in CLAS studies. As previously mentioned above, in their meta-analysis of CLAS studies, Wunderlin *et*

al., (2021) found the mean number of subjects in studies was 16. This means that the presence or absence of a few highly responsive participants could drive a significant result.

There have been only a few studies which have considered the effects of CLAS on the individual (Diep *et al.*, 2019; Garcia-Molina *et al.*, 2019). Both found individuals who did not follow the group trend when they considered SWA enhancement by CLAS. Garcia-Molina *et al.,* (2019) found 14% of their participants showed a decrease in SWA after CLAS while all other participants showed an increase. Diep *et al.,* (2019) found that 30% of their participants showed a decrease, of on average 12%, while the majority (60%) showed on average an increase of 28%. Their SWA measures differed: Garcia-Molina (2019) took the AUC of SWA, while Diep *et al.,* (2019) took a cumulative measure of SWA throughout the night normalised by the total SWA and total sleep time. This normalization was to take into account time spent in S2 and S3 sleep, which they termed this slow wave energy (SWE). Diep *et al.,* (2019) also went on divide participants into two groups based on their SWE change over the night with stimulation. The *Responder* group participants all indicated an increase overnight in SWE. They then reported a significant correlation between SWE change following CLAS and performance in verbal phonetic fluency and working memory (n-back test). However, it is not clear if their division of participants was decided *a priori*, using this novel SWE measure, or post-hoc. Papalambros *et al.,* (2019) also saw a positive correlation between percent change in SWA after stimulation and improvement in a declarative memory task in patients with amnesic mild cognitive impairment. Ngo, Martinez *et al.,* (2013) also found a positive correlation between memory and fast spindle RMS peak amplitude.

It is also worth considering the possibility that individual differences in sleep do not lead to individual differences in memory performance. Ackerman *et al.,* (2015) studied individual sleep differences and found no correlations with overnight memory performance; in SWA, spindle density or theta power in REM. This suggests that despite CLAS affecting participants' sleep differently, it might not relate to their individual memory performance. Taken together this analysis suggests that the makeup of study cohorts is certainly worth carefully considering in CLAS experiments going forward.

5.9 Conclusions

This thesis is the first to test the effects of CLAS on the SRTT, VGT iPAL and MST tasks. It is the first to assess the impact of CLAS on reward in the WP task in adults.

It is also the first to assess the effect of repeated nights of stimulation on memory and assess the impact of stimulation on brain activity during memory recall. The research described in this thesis has demonstrated that while CLAS may not always lead to performance changes, it can cause EEG and BOLD activity changes likely associated with memory consolidation. The research has also shown that one night of at home CLAS can impair pattern separation performance, potentially indicating an increase in pattern completion. Finally, the research has shown that an ambulatory EEG device can be used in an experimental setting to deliver CLAS at home to study the relationships between sleep, the brain, and memory performance. Taken together, the thesis suggests that CLAS has some effect on memory processing in the brain, and warrants further investigation, particularly utilising techniques able to assess brain activity and potential structural changes.

6
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7
Appendix

7.1 Questionnaires

Figure 47 shows the questions from the hyperacuasis questionnaire given to participants prior to their participation in the experiment described in Chapter 3.

Answer options: No, Yes a little, Yes quite a lot, and Yes a lot

1. Do you ever use earplugs or earmuffs to reduce your noise perception (Do not consider the use of hearing protection during abnormally high noise exposure situations)?
2. Do you find it harder to ignore sounds around you in everyday situations?
3. Do you have trouble reading in a noisy or loud environment?
4. Do you have trouble concentrating in noisy surroundings?
5. Do you have difficulty listening to conversations in noisy places?
6. Has anyone you know ever told you that you tolerate noise or certain kinds of sound badly?
7. Are you particularly sensitive to or bothered by street noise?
8. Do you find the noise unpleasant in certain social situations (e.g. night clubs, pubs or bars, concerts, firework displays, cocktail receptions)?
9. When someone suggests doing something (going out, to the cinema, to a concert, etc.), do
   you immediately think about the noise you are going to have to put up with?
10. Do you ever turn down an invitation or not go out because of the noise you would have to face?
11. Do noises or particular sounds bother you more in a quiet place than in a slightly noisy
    room?
12. Do stress and tiredness reduce your ability to concentrate in noise?
13. Are you less able to concentrate in noise towards the end of the day?
14. Do noise and certain sounds cause you stress and irritation?

    (Additional sleep questions added)
15. Do you often find noise makes it hard to fall asleep?
16. Are you often woken up by noise?

Figure 47 *Adapted Psychometric Normalisation of Hyperacuasis Questionnaire*

Figure 48 indicates the questions posed to participants each day via completion of an online sleep diary. Figure 50 and Figure 51 show the reference tables included with the questionnaire.

1. Please enter the time you went to sleep last night (use 24h clock i.e. 23:00)
2. Please enter the time you woke up this morning (use 24h clock i.e. 23:00)
3. Please enter the time it took (in min) for you to fall asleep
4. How many times did you wake up last night?
5. In total how long did these awakenings last (in min)?
6. Did the headband fall off last night? **YES/NO**
7. Did you hear any sounds from the headband last night? **YES/MAYBE/NO**
8. If yes (or maybe) please describe the sound:
9. Please indicate the Degree of Sleepiness that best describes how you felt when you woke this morning? **1/2/3/4/5/6/7**
10. Did you have any dreams last night? **YES/NO**
11. Please write down anything you recall from dreams last night
12. How many of the following drinks did you consume yesterday? **Slider 0-10**
13. How many Units of alcohol did you consume yesterday? Use the below table to estimate: (Table below)

Figure 48 *Sleep diary questions. Bold text indicates options for multiple choice questions.*

Degree of Sleepiness	Scale Rating
Feeling active, vital, alert, or wide awake	1
Functioning at high levels, but not at peak; able to concentrate	2
Awake, but relaxed; responsive but not fully alert	3
Somewhat foggy, let down	4
Foggy; losing interest in remaining awake; slowed down	5
Sleepy, woozy, fighting sleep; prefer to lie down	6
No longer fighting sleep, sleep onset soon; having dream-like thoughts	7
Asleep	X

***Figure 49**: Sleep diary reference table 1 of 2. Stanford sleepiness scale (SSS) from Shahid et al* (2011).

Type of drink	Number of alcohol units
Single small shot of spirits * (25ml, ABV 40%)	1 unit
Alcopop (275ml, ABV 5.5%)	1.5 units
Small glass of red/white/rosé wine (125ml, ABV 12%)	1.5 units
Bottle of lager/beer/cider (330ml, ABV 5%)	1.7 units
Can of lager/beer/cider (440ml, ABV 5.5%)	2 units
Pint of lower-strength lager/beer/cider (ABV 3.6%)	2 units
Standard glass of red/white/rosé wine (175ml, ABV 12%)	2.1 units
Pint of higher-strength lager/beer/cider (ABV 5.2%)	3 units
Large glass of red/white/rosé wine (250ml, ABV 12%)	3 units

*Gin, rum, vodka, whisky, tequila, sambuca. Large (35ml) single measures of spirits are 1.4 units.

***Figure 50**: Sleep diary reference table 2 of 2. Alcohol units table from NHS Alcohol Advice* (2013).

Figure 51 indicates the questions asked in the end of experiment headband feasibility questionnaire.

Figure 51: Questions from the headband feasibility questionnaire. Participants were only given this questionnaire at the end of their participation in the experiment once they had completed both SHAM and STIM weeks. Participants were only given this questionnaire at the end of their participation in the experiment once they had completed both SHAM and STIM weeks →

1. How much did you like wearing the Headband? **Slider 1-5**
2. What did you like most about wearing the headband?
3. What did you like least about wearing the headband?
4. Did you need to reduce the size of the headband (i.e. using an elastic band.)? **YES/NO**
5. How long did it take you to put on the headband and start the recording? (to the nearest minute) **Two sliders one for each week; 0-15 min**
6. On a scale of 1-10 how would you rate the Headband over the FIRST WEEK of the experiment? **1-10 stars for comfort and ease of use.**
7. On a scale of 1-10 how would you rate the Headband over the FINAL WEEK of the experiment? **1-10 stars for comfort and ease of use.**
8. On a scale of 1-10 how would you rate the Headband over the WHOLE experiment? **1-10 stars for comfort and ease of use.**
9. Did you wear the headband every night you were instructed to? **YES/MAYBE/NO**
10. If you did not wear the headband on every night. For what reasons did you not wear it? (Tick all that apply) **Uncomfortable / Forgot / Headband not charged / Wanted a 'night off' / Not sleeping at home / Other**
11. If you selected 'other' please add your reasons here:
12. How many nights did you not wear the Headband? **Two sliders one for each week 0-9**
13. Did the headband disrupt you sleep at all? **Definitely yes / Probably yes / unsure / Probably not / Definitely not / Yes but only for the first two nights of the experiment.**
14. Did the headband improve your sleep at all? **Definitely yes / Probably yes / unsure / Probably not / Definitely not**
15. Did the headband interfere with your usual routine? **Definitely yes / Probably yes / unsure / Probably not / Definitely not / Yes but only for the first two nights of the experiment.**
16. What changes would you make to the headband?
17. Would you like to continue wearing the headband? **YES / MAYBE / NO**
18. Do you think you performed better on the tasks on one week more than the other? **No / Yes. I think I performed better on the first week / Yes. I think I performed better on the second week**
19. If you selected yes please give the reason you think you performed better on that week.
20. Please can you write a detailed description of the experiment as if you were explaining it to an interested friend. Tell them what you had to do and what you thought of each stage.
21. As will be detailed further in the debrief information which you will receive. One week you wore the headband, sounds were played overnight while you slept. The other week no sounds were played. Which week was which varied for each person. Can you please indicate which week you think sounds were playing overnight for you? **Week 1 / Week 2 /Not sure**

7.2 Word Pairs

Figure 52 shows all word pairs used in this thesis, pairs consist of two semantically related words translated from the German word set used in Ngo et al (2015).

Word 1	***Word 2***	***Word 1***	***Word 2***
'INDUSTRY'	*'SECTOR'*	*'MUSCLE'*	*'FIBRE'*
'OWNERSHIP'	*'SHARE'*	*'COAST'*	*'DUNE'*
'NORM'	*'MORALE'*	*'INSECT'*	*'DRAGONFLY'*
'PERJURY'	*'HONESTY'*	*'OSTRICH'*	*'TULIP'*
'VIEW'	*'OPINION'*	*'FISH'*	*'SCALE'*
'PASSION'	*'KISS'*	*'LABORATORY'*	*'PIPETTE'*
'APPLE'	*'PEACH'*	*'RADIO'*	*'VOICE'*
'STEAM'	*'ENGINE'*	*'WORM'*	*'DARKNESS'*
'VALOUR'	*'BRAVERY'*	*'GLOVE'*	*'FROST'*
'DEMAND'	*'SALARY'*	*'HEDGEHOG'*	*'CREATURE'*
'PAPER'	*'LETTER'*	*'IRRIGATION'*	*'DROUGHT'*
'BUILDING'	*'HOTEL'*	*'TAXI'*	*'DISTANCE'*
'GENIE'	*'BOTTLE'*	*'DOCUMENT'*	*'BACKUP'*
'MEMORY'	*'ELEPHANT'*	*'RUNNER'*	*'TRAINER'*
'HEALTH'	*'VACCINATION'*	*'SOUND'*	*'WAVE'*
'LARVA'	*'CATERPILLAR'*	*'GROUP'*	*'ASSEMBLY'*
'PRIDE'	*'FAME'*	*'STUDENT'*	*'LECTURER'*
'TIME'	*'ORIGIN'*	*'SALAD'*	*'GARDEN'*
'POVERTY'	*'MISERY'*	*'OFFER'*	*'MARKET'*
'JUDGE'	*'FAIRNESS'*	*'MONK'*	*'NUN'*
'TWILIGHT'	*'UNDERWORLD'*	*'FOREHEAD'*	*'CHIN'*
'PROOF'	*'FACT'*	*'CANOPY'*	*'SKYLINE'*
'ALCOHOL'	*'OPIUM'*	*'LUCK'*	*'CHANCE'*
'BLESSING'	*'CREATOR'*	*'MOOD'*	*'HUMOUR'*
'FLAN'	*'SWEETS'*	*'GOAL'*	*'DIRECTION'*
'ACTION'	*'INTENTION'*	*'TASK'*	*'EXECUTION'*
'COMPARISON'	*'METAPHOR'*	*'THEORY'*	*'EXCEPTION'*
'OPPORTUNITY'	*'ENCOUNTER'*	*'NAIL'*	*'METAL'*
'CAR'	*'PRESTIGE'*	*'ADDITION'*	*'SUPPLEMENT'*
'FLOOR'	*'ATTIC'*	*'MOOR'*	*'BOG'*

'PROFILE'	'PHOTOGRAPHY'
'BOY'	'GIRL'
'WOOL'	'CLOTHING'
'DEFINITION'	'CONCEPT'
'SCREAM'	'PANIC'
'PLAN'	'CITY'
'FUR'	'FOX'
'MACHINE'	'APPARATUS'
'SILENCE'	'LONELINESS'
'GALE'	'BREEZE'
'JOB'	'RECOGNITION'
'CHAOS'	'STRUCTURE'
'NEWSPAPER'	'PRINT'
'CASH'	'VALUE'
'SHAPE'	'CIRCLE'
'AGREEMENT'	'CONTRACT'
'BRAIN'	'CONSCIOUSNESS'
'BENEFIT'	'COST'
'BALL'	'SQUARE'
'STAR'	'CHRISTMAS'
'MUG'	'COFFEE'
'BIRD'	'CAT'
'DUST'	'CLEANLINESS'
'TOAST'	'PROVERB'
'SOIL'	'STONE'
'INFORMATION'	'CONTENT'
'DEMOCRACY'	'SYSTEM'
'SOLUTION'	'PROBLEM'
'SINGER'	'ARTIST'
'NEED'	'ADVERTISEMENT'
'FUN'	'PARTY'
'SLAVE'	'KING'
'RIGHTS'	'CONSTITUTION'
'ORIGINATOR'	'CAUSALITY'
'LACK'	'ABSTINENCE'

'RESEARCH'	'PATENT'
'CLIFF'	'ABYSS'
'OBSESSION'	'DEVIL'
'AMOUNT'	'CHANGE'
'INSINUATION'	'SUSPICION'
'DIAMOND'	'GOLD'
'ROOM'	'CORNER'
'POEM'	'LOVE'
'LABYRINTH'	'SEARCH'
'BEGGAR'	'MISFORTUNE'
'COVERT'	'FOREST'
'TREASON'	'FAITH'
'GODDESS'	'PRAYER'
'LEADER'	'BOSS'
'MUSICIAN'	'ACCORDION'
'FEATURE'	'DETAIL'
'SERVANT'	'POSTURE'
'TALENT'	'HEREDITY'
'GOURMET'	'DELICACY'
'FLAG'	'CONQUEST'
'POLICEMAN'	'GUARD'
'FEAR'	'SNAKE'
'OXYGEN'	'AIR'
'HISTORY'	'DEVELOPMENT'
'INDULGENCE'	'CIGAR'
'LOOK'	'PERSPECTIVE'
'PAINTER'	'PIANIST'
'LOSS'	'DECLINE'
'CRITICISM'	'DOUBT'
'FRIEND'	'TRUST'
'GAIN'	'PROGRESS'
'GRASS'	'CATTLE'
'HILL'	'HUT'
'GHOST'	'APPEARANCE'
'ILLUSION'	'PERCEPTION'

'TERM'	'MEANING'
'WETNESS'	'STORM'
'INSPIRATION'	'IDEA'
'ARMOUR'	'ATTACK'
'RECOMMENDATION'	'ADVICE'
'PIECE'	'BOARD'
'SETBACK'	'PAST'
'ANECDOTE'	'JOKE'
'ABILITY'	'GENES'
'SHAME'	'BODY'
'DOLL'	'CHILD'
'SHORE'	'DAM'
'WINE'	'GRAPE'
'MOUNTAINS'	'HEATHER'
'WINTER'	'ACCIDENT'
'LOCOMOTIVE'	'RAILTRACK'
'DYNAMO'	'LIGHT'
'BARREL'	'RAIN'
'AIRPLANE'	'JUICE'
'OVEN'	'FIRE'
'FIR'	'BARK'
'CHESS'	'ROOK'
'ZEUS'	'HADES'
'DECREE'	'RULING'
'WEDDING'	'ALTAR'
'PRISON'	'OFFENCE'
'NIGHT'	'SEARCHLIGHT'
'REVOLVER'	'CALIBRE'
'FIGHT'	'VICTORY'
'THEATRE'	'ROW'
'RINGS'	'TREE'
'DOCTOR'	'SURGERY'
'ENERGY'	'PETROLEUM'
'SEAM'	'STITCH'
'TOY'	'PLASTIC'
'MARRIAGE'	'ENGAGEMENT'
'COMEDY'	'DRAMA'
'ANIMAL'	'FROG'
'SKIN'	'BLOOD'
'SEA'	'STEAMBOAT'
'CRITERION'	'CHOICE'
'DISGUISE'	'VEIL'
'BUTTERFLY'	'FLOWER'
'HOSTAGE'	'PRISONER'
'GREETING'	'FRIENDLINESS'
'DECENCY'	'CUSTOM'
'POWER'	'RULER'
'BUNGALOW'	'SETTLEMENT'
'GRACE'	'MERCY'
'DISCIPLINE'	'OBEDIENCE'
'QUESTION'	'OBJECTION'
'FATE'	'IRONY'
'CLOCK'	'CHURCH'
'VALLEY'	'MEADOW'
'LANGUAGE'	'ACOUSTICS'
'HARDSHIP'	'STRENGTH'
'DREAM'	'REALITY'
'SALVATION'	'HEAVEN'
'EXAMINATION'	'FAILURE'
'REQUIREMENT'	'DIFFICULTY'
'EVALUATION'	'RESULT'
'RUBBER'	'GLUE'
'DUMMY'	'CRASH'
'COLLECTION'	'BOX'
'NEON'	'FOCUS'
'FOOD'	'CANDY'
'MISSION'	'MESSENGER'
'TENANT '	'RENT'
'CIRCUS'	'CLOWN'
'LEAF'	'ROOTS'

'WATERCOLOUR'	'GALLERY'
'KITCHEN'	'BUCKET'
'MUSEUM'	'EGYPT'
'LIBRARY'	'SIGNATURE'
'BARBECUE'	'SUMMER'
'BOOK'	'AUTHOR'
'HURRICANE'	'SWIRL'
'BLACKSMITH'	'HORSESHOE'
'SHOP'	'SIGN '
'BED'	'SLEEP'
'CLOUD'	'SKY'
'WEST'	'COMPASS'
'HARBOUR'	'CRANE'
'HUSBAND'	'MISTRESS'
'FUNERAL'	'DEATH'
'STADIUM'	'RUGBY'
'OFFICE'	'MAIL'
'STOMACH'	'SUPPER'
'BOOTS'	'HEEL'
'OCEAN '	'BEACH'
'ELECTION'	'BALLOT'
'STEPS'	'MILE'
'FILM'	'CAMERA'
'KEY'	'HANDLE'
'BLACK'	'COLOUR'
'WEEK'	'THURSDAY'
'GLACIER'	'AVALANCHE'
'RIDER'	'TICKET'
'CURRENT'	'STREAM'
'CROWD'	'PEOPLE'
'FOUNTAIN'	'STATUE'
'FRAME'	'PAINTING'
'ROOF'	'GUTTER'
'PILOT'	'LICENSE'
'COCKTAIL'	'UMBRELLA'
'HAND'	'PALM'
'PUS'	'BACTERIA'
'SIREN'	'FIRETRUCK'
'PALACE'	'CROWN'
'UNIVERSITY'	'SEMESTER'
'EMOTION'	'HAPPINESS'
'TENDENCY'	'HABIT'
'AUTHORITY'	'STATE'
'POT'	'KETTLE'
'HUM'	'RECORDER'
'LANDSCAPE'	'CLIMATE'
'GEAR'	'CLUTCH'
'GARBAGE'	'WEEKDAY'
'CAPTAIN'	'WAR'
'ADMIRAL'	'NAVY'
'TEA'	'HERB'
'CHAPEL'	'MARY'
'CHICAGO'	'GANGSTER'
'MONEY'	'BANK'
'SEED'	'FEEDER'
'HATCHLING'	'EGGSHELL'
'ACHIEVEMENT'	'EFFECT'
'CHARGE'	'CARD'
'RESCUE'	'AMBULANCE'
'PAW'	'TRAIL'
'KETCHUP'	'LID'
'HYGIENE'	'NEEDLE'
'IMMIGRATION'	'FREEDOM'
'URGENCY'	'COUNTDOWN'
'PLANET'	'MARS'
'SHADOW'	'NIGHTFALL'
'ENTITLEMENT'	'LEGISLATURE'
'SCOUT'	'TENT'
'CARP'	'POND'
'MEAT'	'BUTCHER'

'ARTERY'	'PHYSIOLOGY'	'AWARD'	'ACCEPTANCE'
'TOE'	'VARNISH'	'DRIVEWAY'	'BUSH'
'MOUSE'	'HOLE'	'CALENDAR'	'HOLIDAYS'
'SQUIRREL'	'NUT'	'ASSIGNMENT'	'DEADLINE'
'HAY'	'CHICKEN'	'COLD'	'COAT'
'CONFERENCE'	'SUIT'	'PLANE '	'RUNWAY'
'NOTIFICATION'	'ALERT'	'TONGUE'	'MOUTHWASH'
'ACCOUNT'	'DEBT'	'BALLET'	'DANCE'
'SAND'	'SHOE'	'TROPICS'	'HUMIDITY'
'NATION'	'ANTHEM'	'SYMPHONY'	'BEETHOVEN'
'STOPWATCH'	'TIMER'	'EXPRESSION'	'EYEBROW'
'PARSLEY'	'SEASONING'	'LADY'	'BLOSSOM'
'BAGPIPE'	'HAGGIS'	'ROUGE'	'LIPSTICK'
'MAGPIE'	'SILVER'	'FATHER'	'RELATIVE'
'DIRT'	'FINGERNAIL'	'LEAD'	'CLIMBING'
'SLEEVE'	'BUTTON'	'INTOLERANCE'	'DIET'
'COURTESY'	'TACTFULNESS'	'DIVORCE'	'LAWYER'
'DISCUSSION'	'DISPUTE'	'HOUSE'	'CARDS'
'MAPLE'	'AUTUMN'	'BETRAYAL'	'LOYALTY'
'MOON'	'SPACESHIP'	'HEN'	'SHED'
'OPERATION'	'SCAR'	'TELEVISION'	'PROGRAMME'
'RHYTHM'	'GUITAR'	'TENDON'	'ACHILLES'
'TOP'	'SURFACE'	'ENDEAVOUR'	'STRESS'
'CHLORINE'	'POOL'	'WIND'	'FLOODING'
'HOME'	'DOORMAT'	'MELODY'	'SONG'
'BOAT'	'SAIL'	'WEIGHT'	'BATHROOM'
'OUTLET'	'DISCOUNT'	'HERD'	'FARMER'
'SOCKET'	'CABLE'	'TEAM'	'OUTCOME'
'SCISSOR'	'PENCIL'	'SONATA'	'JOY'

***Figure 52**: All word pairs.*

7.3 Chapter 2

7.3.1 SRTT task instructions

The written instructions presented to participants before the SRTT tasks were as follows: *You will be presented with a grey screen containing four horizontal lines.*

Visual cues will appear above each line. Your task is to press the key corresponding to the cues as quickly and accurately as possible. The keys are as follows: 1- Far left, 2 - second from left, 3 – second from right, 4 – far right. The cues will appear as pictures of faces or objects, but this is completely irrelevant to the task and can be ignored. You must only use your non-dominant hand. So if you write with your right hand please use your left. Each trials will contain several repeats of a sequence, however in trials marked with an ''R'' cues will be random. If you press the wrong key at any moment, or press too early, the cue will remain on screen until you have pressed the correct key.

7.3.2 SRTT learning curves

To assess if participants learnt the SRTT sequence their mean RT at each sequence block was plotted, see Figure 54. A polynomial curve was fitted to their change in RT across the sequence blocks for SHAM and for STIM visits (SRTT learning was pre stimulation). Those participants whose curve did not have a negative slope (n=4, marked on Figure 54 with an exclamation mark) were removed from further SRTT analysis as it was deemed they had not sufficiently learnt the sequence or improved on the task across the learning blocks.

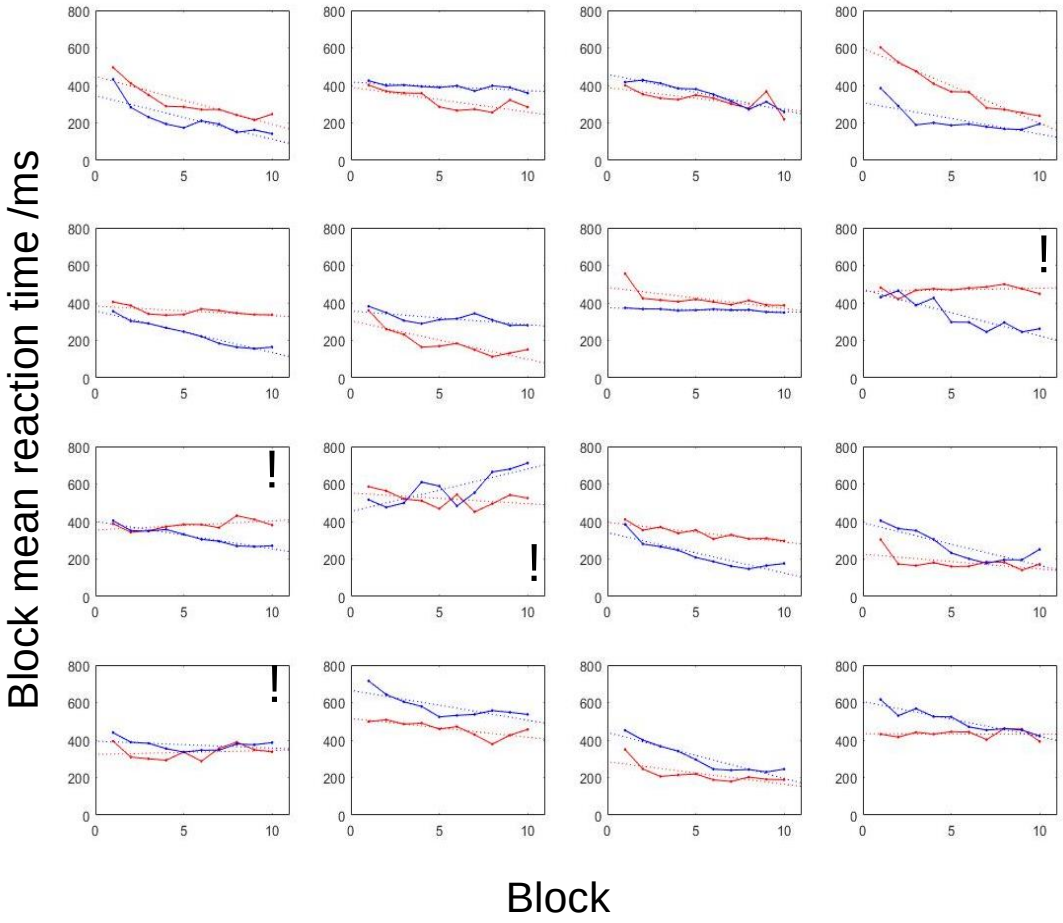


Figure 54 *Individual participant learning of SRTT sequences. Mean RT per sequence block, at learning, on SHAM (red) and STIM (blue) nights.*

7.4 Chapter 3

This section provides extra information regarding the stimuli used in Chapter 3.

7.4.1 Word Pair

Figure 55 shows the background shapes were used to aid participants in differentiating between the three word lists they learnt during the WPr task. Participants were informed not to recall these backgrounds

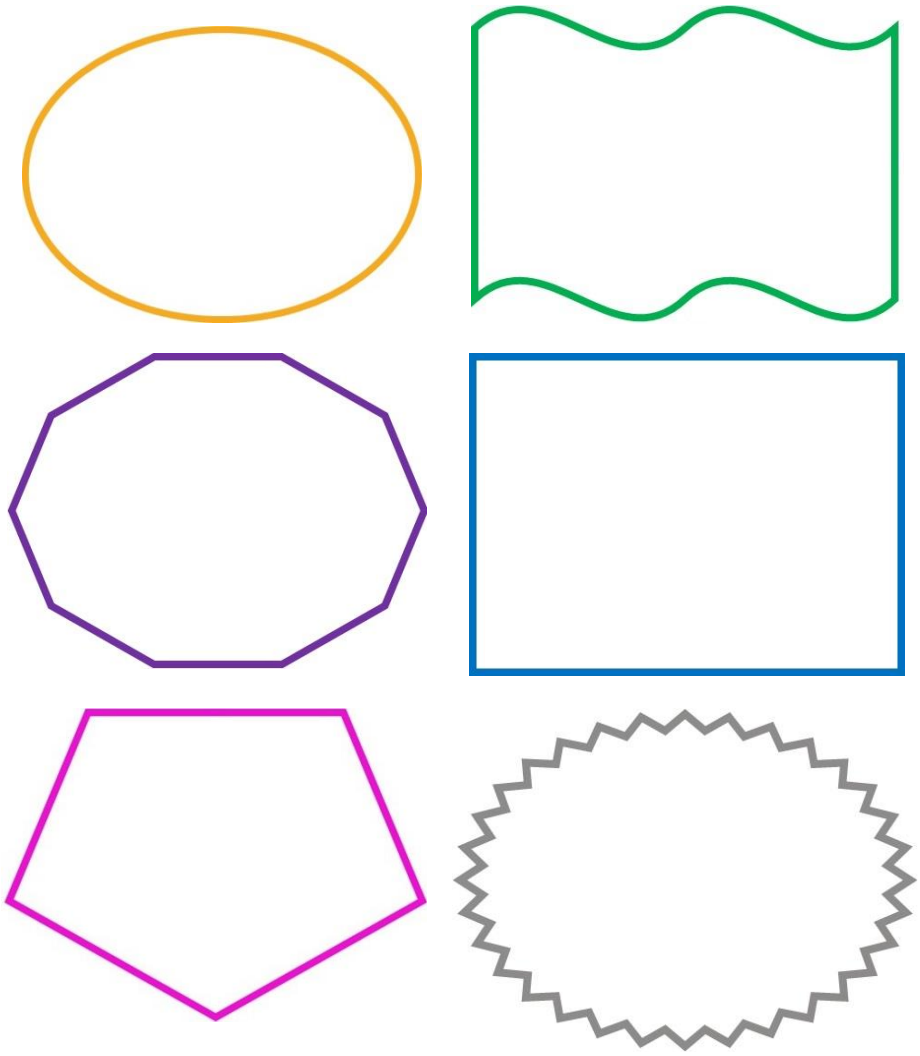


Figure 55: *Background shapes used in WP online learning to denote reward. Backgrounds were randomly assigned to reward lists. Chapter 3 methods section 3.3.4.*

7.4.2 Image paired associates task

Figure 56 and Figure 57 show Set A images used in the iPAL task while Figure 58 and Figure 59 show set B.

Figure 56 Top: *Female face images and* ***Bottom:*** *Male face images for set A of the iPAL task.. Face images were taken from the Karolinska Directed Emotional Faces database (Lundqvist, D., Flykt, A., & Öhman, 1998).*

***Figure 57**: **Top:** Rural scene images and **Bottom:** Urban scene images for set A of the iPAL task images were taken from Google images under creative commons licence.*

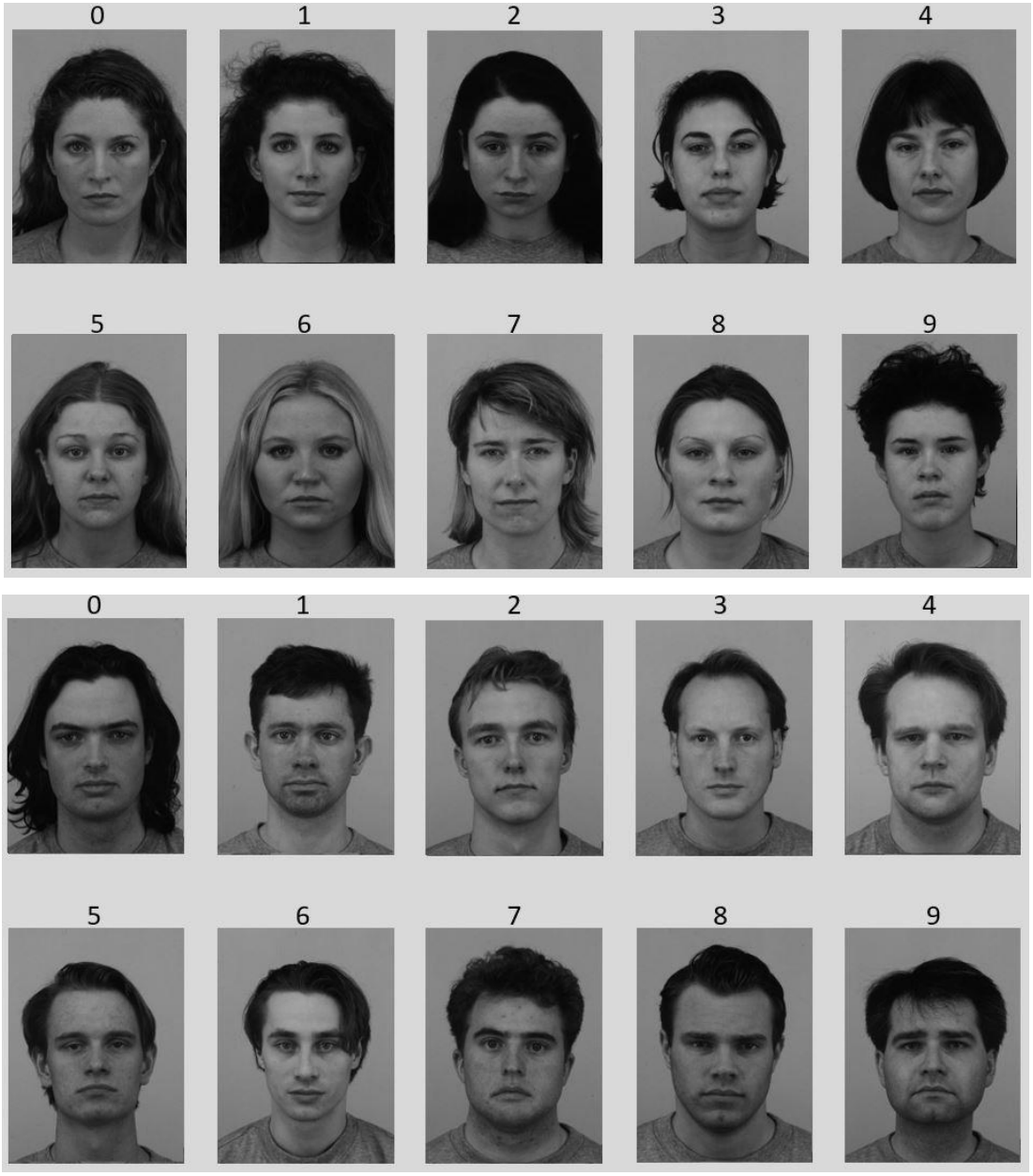


Figure 58: Top: *Female face images and* ***Bottom:*** *Male face images for set B of the iPAL task.. Face images were taken from the Karolinska Directed Emotional Faces database (Lundqvist, D., Flykt, A., & Öhman, 1998).*

Figure 59: Top: *Rural scene images and* ***Bottom:*** *Urban scene images for set A of the iPAL task images were taken from Google images under creative commons licence.*

7.4.3 Verb generation task

For the VGT task participants saw 32 words per round, made up of a random selection of the available words (64) half high constraint and half low constraint (Prabhakaran, Green and Gray, 2014), see Table 12 for all words.

High Constraint		*Low Constraint*	
rock	*poem*	*leaf*	*tune*
card	*soap*	*taxi*	*drum*
shoe	*couch*	*house*	*bucket*
oath	*drug*	*street*	*cafe*
office	*rose*	*lamp*	*store*
belt	*soup*	*canoe*	*muscle*
finger	*pillow*	*home*	*letter*
blade	*tool*	*oven*	*music*
cart	*debt*	*fist*	*ring*
pill	*hole*	*tongue*	*infant*
note	*horn*	*dish*	*clay*
feet	*golf*	*flower*	*hair*
artist	*manual*	*church*	*phone*
tree	*baby*	*boot*	*pan*
cannon	*money*	*snow*	*grass*
bread	*candle*	*paper*	*key*

Table 12: *Verb generation task words. Words from Prabhakaran et al.,* (2014)

7.5 Chapter 4

7.5.1 MRI Scanning coil issue

Towards the end of MRI data acquisition, it became apparent that during a number of the MRI scans the anterior coil of the head coil had been switched off. It was determined that the anterior coil was either on or off for all scans in a session, but that the distribution of which scans was random between participants and sessions. The number of each combination of scans with anterior coil on and off are listed in Table 14; Five of the participants either had the coil on or off for all four of their scans. While for five participants the anterior coil was only on for one scan, three for the first SHAM scan and two for the second SHAM scan. Two participants had two OFF and two ON scans both the same for SHAM and STIM and opposites. As almost all combinations equal out as both the opposite set up is present we did not reasonably think that any of the results acquired from MRI scans arose from this error.

ALL OFF	ALL ON	SHAM 1 ON only	SHAM 2 ON only	Equal SHAM/STIM
4	1	3	2	2

Note: In n=12 participants used in analysis. SHAM 1 ON only = SHAM 1ON, SHAM 2 OFF, STIM 1 OFF, STIM 2 OFF. SHAM 2 ON only = SHAM 1 OFF, SHAM 2 ON, STIM 1 OFF, STIM 2 OFF. Equal SHAM/STIM; n=1: SHAM 1 OFF, SHAM 2 ON, STIM 1 OFF, STIM 2 ON, n=1: STIM 1 OFF, STIM 2 ON, SHAM 1 OFF, SHAM 2 ON.

Table 13: *Status of anterior coil during MRI scans*

7.5.2 SRTT NOUN images

In this chapter the images previously used in the SRTTT in Chapter 2 (Cousins *et al.*, 2014) were replaced with images from the NOUN database (Horst and Hout, 2016), see images in Figure 60.

Figure 60: *Images used for SRTT task in Chapter 4. Each horizontal line indicates one set A, B, C and D top to bottom respectively.*

7.5.3 Chapter 3 fMRI main effect of time.

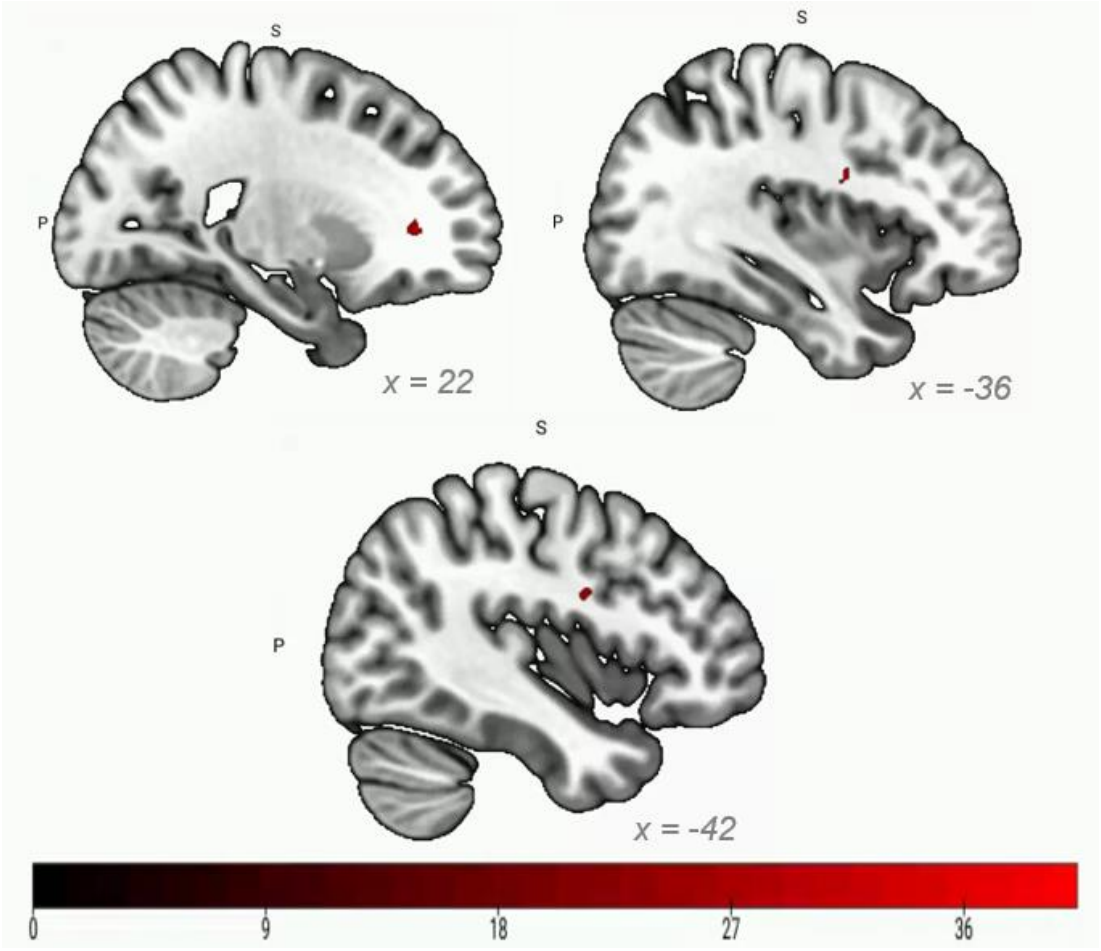
SRTT

The RM-ANOVA of SRTT indicated a significant main effect of time in the clusters listed in the below Table 14 and Figure 61. One cluster occurs in the left lateral ventricle, and is could be due to flow of CSF interfering with BOLD activations. One cluster is located in the right of the frontal lobe around the anterior corona radiate (x=22). Two of the peaks in the table represent the same frontal cluster (x=-36 and

x=-42), and both peaks indicate greater activity in STIM when performing sequence blocks but less activity than baseline in SHAM.

				Co-ordinates		
Location	**Voxels**	**Test statistic**	**P value**	**x**	**y**	**z**
Frontal -R	*18*	*f(1,10)=35.023*	*<0.001*	*22*	*38*	*4*
Lateral Ventricle -L	*21*	*f(1,10)=42.107*	*<0.001*	*-20*	*-38*	*16*
Frontal Cerebellum -L	*19*	*f(1,10)=32.409*	*<0.001*	*-36*	*0*	*26*
Frontal Cerebellum -L	*19*	*f(1,10)=30.537*	*<0.001*	*-42*	*-2*	*24*

Table 14: *Significant clusters from RM-ANOVA of SRTT fMRI, Unc p<0.001.*



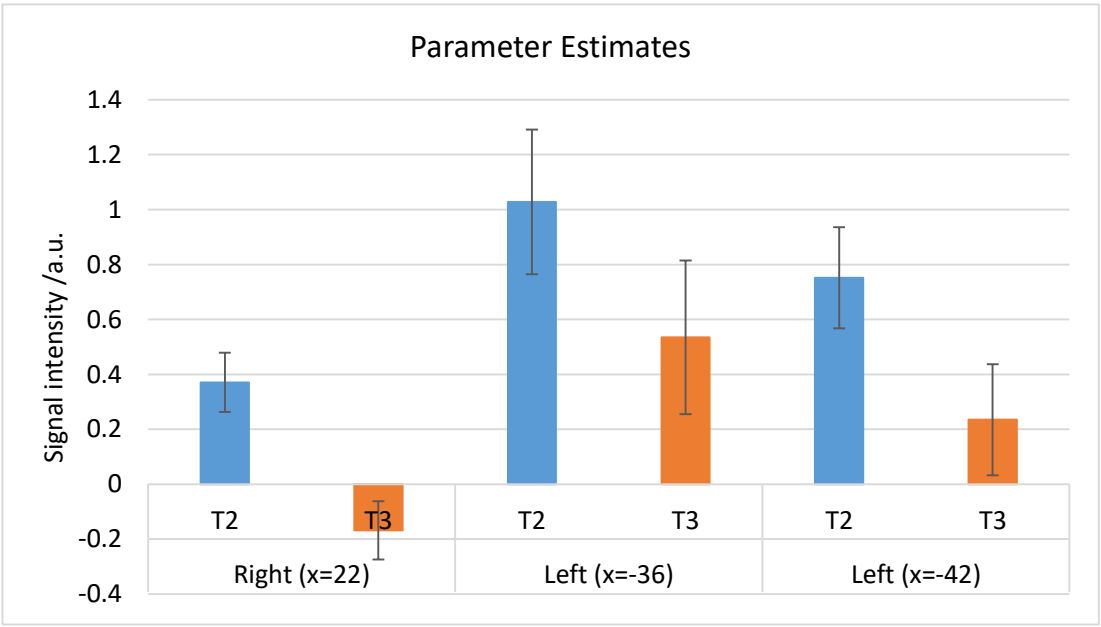


Figure 61: Areas active with the main effect of time on SRTT task. *Significant clusters indicating a main effect of time on activation during sequence blocks.*

Word Pair

RM-ANOVA analysis indicated a significant main effect of Time on the activity seen in the clusters indicated in Figure 62 and Table 15, in remembering pair words in the WP task. Inspection of Figure 62 parameter estimates indicates that all clusters saw more activity in SHAM than STIM, indeed all saw increased activity in *remembered trials* compared to baseline in T2 but a decrease in T3.

				Co-ordinates		
Location	***Voxels***	***Test statistic***	***P value***	***x***	***y***	***z***
Frontal Superior Medial –Left	*31*	*f(1, 23)=37.889*	*<0.001*	*-10*	*60*	*20*
Parietal cortex – Right -Right	*18*	*f(1, 23)=30.298*	*<0.001*	*18*	*-52*	*42*
Temporal inferior –Left	*6*	*f(1, 23)=23.655*	*<0.001*	*-52*	*-26*	*-20*

Table 15: *Significant clusters of activity in remembered trials indicating a main effect of time.*

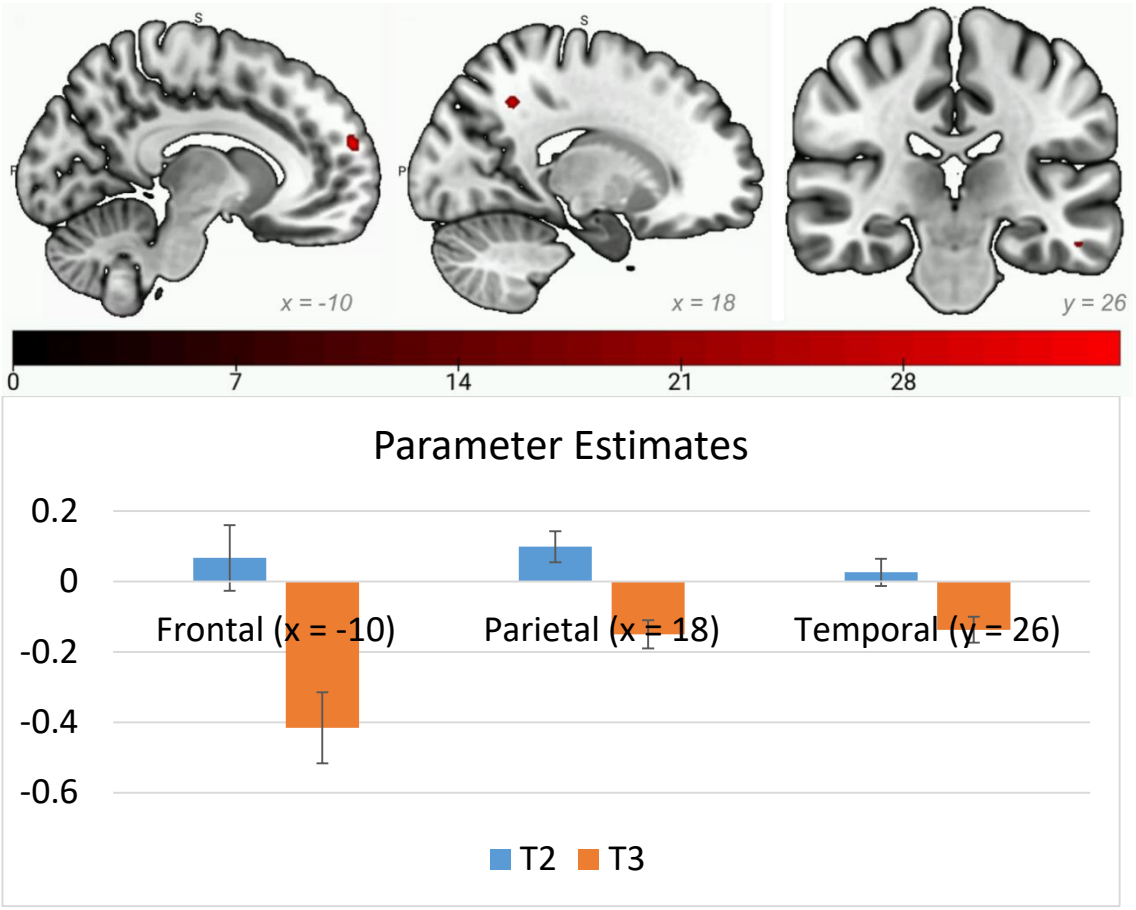


Figure 62: Significant clusters with the main effect of time – WP. *Significant clusters indicated by ANOVA main effect of time, Unc p<0.001*

7.5.4 Mnemonic similarity task

Table 16 shows the mean values for pattern separation score (PSS) in the MST task divided by similarity bin.

Stimulation	Similarity bin	Retention Interval	Mean	SEM
	B1	*Night 1*	*-0.09*	*0.68*
	B2	*Night 1*	*-1.00*	*0.65*
	B3	*Night 1*	*-0.55*	*0.56*
SHAM	*B4*	*Night 1*	*-2.64*	*0.41*
	B5	*Night 1*	*-0.46*	*0.64*
	B1	*Night 8*	*-0.55*	*0.31*
	B2	*Night 8*	*-0.73*	*0.90*

	B3	*Night 8*	*-0.64*	*0.68*
	B4	*Night 8*	*-2.36*	*0.70*
	B5	*Night 8*	*-0.82*	*0.48*
	B1	*Nights 2-7*	*-0.55*	*0.64*
	B2	*Nights 2-7*	*-1.00*	*0.8*
	B3	*Nights 2-7*	*-1.64*	*0.69*
	B4	*Nights 2-7*	*-1.73*	*0.41*
	B5	*Nights 2-7*	*-0.64*	*0.31*
	B1	*Nights 1-7*	*-0.64*	*0.79*
	B2	*Nights 1-7*	*-2.00*	*0.65*
	B3	*Nights 1-7*	*-2.18*	*0.5*
	B4	*Nights 1-7*	*-4.36*	*0.45*
	B5	*Nights 1-7*	*-1.09*	*0.84*
	B1	*Night 1*	*-1.09*	*0.55*
	B2	*Night 1*	*-1.82*	*0.70*
	B3	*Night 1*	*-2.09*	*0.61*
	B4	*Night 1*	*-2.46*	*0.84*
	B5	*Night 1*	*-2.18*	*0.69*
	B1	*Night 8*	*-1.27*	*0.47*
STIM	*B2*	*Night 8*	*-0.27*	*0.74*
	B3	*Night 8*	*-1.36*	*0.53*
	B4	*Night 8*	*-2.55*	*0.65*
	B5	*Night 8*	*-1.73*	*0.49*
	B1	*Nights 2-7*	*-1.00*	*0.65*
	B2	*Nights 2-7*	*-1.27*	*0.73*
	B3	*Nights 2-7*	*-0.46*	*0.68*

B4	*Nights 2-7*	*-1.64*	*0.82*
B5	*Nights 2-7*	*0.455*	*0.56*
B1	*Nights 1-7*	*-2.09*	*0.64*
B2	*Nights 1-7*	*-3.09*	*0.83*
B3	*Nights 1-7*	*-2.55*	*0.56*
B4	*Nights 1-7*	*-4.09*	*1.11*
B5	*Nights 1-7*	*-1.73*	*0.60*

Table 16: *Mean values for absolute change in PSS divided by similarity bin.*

MST analysis was conducted both on PSS scores divided into individual similarity bins and pooled across bins. The statistics provided in the text cover the bin separated data, but for completeness the pooled data in shown here in Figure 63.

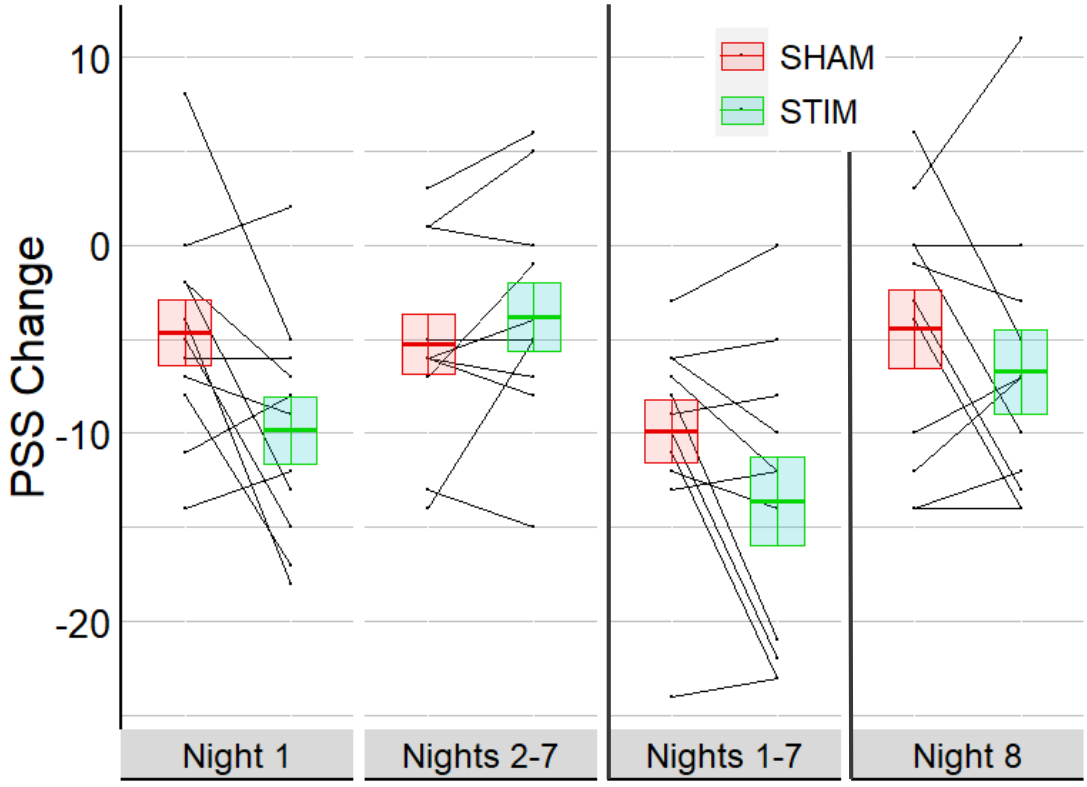


Figure 63: *MST data pooled across similarity bins. Data indicates percentage change over listed retention interval. Statistical tests were conducted on Night 1 and Night 8 together, Night 1 and Nights 2-7 together and Nights 2-7 alone.*

“There is a time for many words,

and there is also a time for sleep.”

— Homer, The Odyssey