CLOSING THE LOOP AND OPENING THE POSSIBILITIES: AUGMENTING SLEEP-DEPENDENT CONSOLIDATION TO IMPROVE LONG TERM MEMORY

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CLOSING THE LOOP AND OPENING THE POSSIBILITIES: AUGMENTING SLEEP-DEPENDENT CONSOLIDATION TO IMPROVE LONG TERM MEMORY

by

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DISSERTATION

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inspired me to become a scientist and encouraged me to think about the world in a critical way.
ABSTRACT

Memory consolidation occurs during slow-wave sleep. This is a complex process that can be studied using electroencephalography (EEG) and modulated with brain stimulation. Previous studies have shown that transcranial alternating current stimulation (tACS) can be used to entrain or disrupt endogenous oscillations in cortical networks and can be used for the manipulation of consolidation processes during sleep. In this series of experiments, sleep-dependent memory consolidation was modulated using closed-loop tACS (CL-tACS to improve memory performance for three tasks: a verbal paired associates task (PAT), an interference paired associates task (iPAT), and a visual category learning task. EEG was collected during testing to investigate the neural response to stimulation. Results suggest that CL-tACS improved memory performance and reduced retroactive interference. The behavioral effects were also associated with features (event-related spectral perturbation; ERSP) in waking EEG. These results provide further evidence for a relationship between slow wave oscillations and memory consolidation.
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1.0 INTRODUCTION

Humans have been curious about memory capacity and how sleep appears to confer a benefit to memory processes for centuries, dating from the historic Roman teacher of rhetoric, Quintilian, who recognized that a single night’s sleep served to strengthen memory (Butler, 1922). What are the processes during sleep that function to maintain or concretize memories? What brain systems are involved in such processes? Is there a potential to optimize or enhance sleep using external methods to improve memory or other cognitive performance? Non-invasive brain stimulation (NIBS) is one method by which these questions can be probed for answers by augmenting ongoing memory consolidation processes during sleep.

Though many excellent reviews have focused on sleep’s role in memory consolidation (Diekelmann & Born, 2010, Rasch & Born, 2013), none have been focused on augmenting sleep with external stimuli, specifically transcranial current stimulation (tCS), with the goal of understanding how sleep-dependent memory consolidation can be improved, accelerated, or optimized.

Categorization of Memory

Stickgold (2005) suggests a memory taxonomy including declarative, divided into sub-categories encompassing episodic (memories for specific events) and semantic (memories for facts), and non-declarative, divided into procedural memories, conditioning, non-associative memory, and priming, a taxonomy largely shared by Tulving (1984). Tulving (1995) suggests that there are three stages of memory: encoding, storage, and retrieval, and that having a working taxonomy is imperative for constructive discussion (Tulving, 1985; for an argument in relation to sleep, see Conte & Ficca, 2013). These demarcations suggest
independent processes, however, memory engages multiple interrelated processes in the nervous system, thus for example, it is difficult to discuss semantic memory without some conversation of episodic memory, as all information is learned with a contextual representation. Though this manuscript will mainly be focused on the semantic aspect of declarative memory consolidation, it is important to be cognizant of the interrelated nature of such processes.

1.1 Memory Consolidation

Defined by Dudai (2004) as “the progressive postacquisition stabilization of long-term memory,” consolidation involves many neurological, cellular, molecular, and systems-level processes that interact in a complicated way to produce long-term memories. Thus, this definition may not be adequate to describe such a complex phenomenon (Stickgold, 2005). At the neuronal level, memory formation is based on the change in the strength of synaptic connections in the network representing the memory. Encoding induces synaptic long-term potentiation (LTP) or long-term depression (LTD) as major forms of learning-induced synaptic plasticity. Activity reverberating in the neuronal representation following encoding is thought to promote two kinds of consolidation processes, termed “synaptic consolidation” and “systems consolidation” (Dudai, 2004; Dudai, Karni, & Born, 2015). Long-term memory appears to be separate and not entirely reliant on short-term memory processes, as drugs infused into the hippocampus (HC) and entorhinal cortex (EC) in the rat after training selectively block short term memory, leaving long-term memory unaffected (Izquierdo et al., 1998).

Muller & Pilzecker proposed the preservation-consolidation hypotheses of memory in 1900, based on data observed from humans showing that memory of learned information was
subject to interference from intervening information presented shortly after initial learning, suggesting that new memories are fragile and labile in nature, and consolidation occurs over time, not immediately, and involves many biochemical processes (McGaugh, 2000; Kandel, Dudai, & Mayford, 2014).

**Synaptic Consolidation**

Synaptic consolidation is achieved within minutes to hours after learning takes place, has been demonstrated in all systems of memory studied (Dudai & Morris, 2000; Dudai, 2004), and leads to remodeling of synaptic and spine morphology, resulting in changes in the efficacy of the participating synapses. Also referred to as cellular or local consolidation, it is thought to occur via stimulus-induced activation of intracellular signaling cascades, resulting in modification of the synapse, gene expression, and synthesis of compounds that alter synaptic efficacy (Rasch & Born, 2013; Dudai, Karni, & Born, 2015). Dudai & Morris (2000) suggest five criteria for synaptic consolidation that result in protein-mediated synaptic tagging influenced or driven by brain derived neurotrophic factor (BDNF; Bramham & Messaoudi, 2005), or by mechanisms related to LTP (Frey & Morris, 1997). Synapse-synthesized proteins may work in a feedback loop to strengthen tagging, and possibly serves as retrograde information, which travels to other cellular structures and alerts the nucleus about the change (Dudai & Morris, 2000). Recent evidence suggests that glial cells may also be important for memory consolidation. Steadman et al. (2020) show that in adult rats, spatial learning leads to de novo myelination and oligodendrogenesis in a water maze task. Importantly, interfering with the learning-related increases in oligodendrogenesis impair memory consolidation in the water maze as well as in contextual fear memories, potentially
by interfering with hippocampal sharp wave ripple/cortical spindle coupling. These results suggest a role for glia in plasticity and memory consolidation.

Systems Consolidation

Systems consolidation takes considerably more time than synaptic consolidation (Born, Rasch, & Gais, 2006), and involves the hippocampus in early, but not late, stages (Dudai, 2004; though see Nadel & Moscovitch, 1997 for a discussion of multiple trace theory). It builds on synaptic consolidation and refers to processes in which reverberating activity in newly encoded representations stimulate a redistribution of the neuronal representations to other neuronal circuitries for long-term storage (Rasch & Born, 2013). It appears to be selective, showing a preference for consolidation of information with relevance to future plans (Born & Wilhelm, 2012), or explicit or salient cues during encoding (Saletin & Walker, 2012), a process that Stickgold & Walker (2013) call “memory triage”.

Reconsolidation – evidence for the labile nature of memories

Consolidated memories do not appear to be protected against interference (Nader & Hardt, 2009). Reconsolidation has been studied in numerous species, and involves neurotransmitters, hormones, growth factors, transcription factors, and immediate early genes (Tronson & Taylor, 2007). Amygdalar anisomycin injection after reactivation of a fear memory in rats led to a failure of reconsolidation, suggesting that new protein synthesis is required in this context (Nader, Schafe, & Le Doux, 2000a), though the interpretation of reactivation creating new traces, rather than modifying an old trace, is contended (Nader Schafe, & Le Doux, 2000b). Zif268 mutant mice were unable to successfully reconsolidate reactivated object recognition memories (Bozon, Davis, & Laroche, 2003). Rats exposed to electroconvulsive shock (ECS) immediately after learning showed impairment, whereas a
delayed shock led to no such impairment in a conditional emotional response. However, if
the conditional stimulus (CS) was presented the day after consolidation then followed by
ECS, the memory was again impaired, whereas no impairment was observed in rats that did
not receive the CS presentation. This suggests that the CS reactivated the memory, which
was then subsequently disrupted in reconsolidation (Nadel & Land, 2000). Mactutus, Riccio,
& Ferek, (1979) showed that the degree to which an animal is physically cooled impacts the
degree to which memories can become reactivated, and this effect is variable depending on
the age of the memory. Hupbach, Gomez, & Nadel (2009) show that misremembering
reactivated memories in humans is contingent upon receiving a reminder about the nature of
the information initially presented. Deębiec, Doyère, Nader, & LeDoux, (2006) show that
directly reactivated memories become more labile, and require reconsolidation, compared to
indirectly reactivated or associated memories in a second-order fear conditioning task in rats.

Cellular processes for consolidation and reconsolidation appear to involve distinct
processes. Lee, Everitt, & Thomas (2004) showed a double dissociation between
consolidation and reconsolidation in rats. By injecting antisense oligodeoxynucleotides into
the hippocampi of rats, they showed that memory consolidation involves BDNF, but not the
gene zif268, whereas reconsolidation involves specifically zif268, and not BDNF. Others
have found similar evidence (Bozon, Davis, & Laroche, 2003). Experience modulates gene-
mediated memory, as rats exposed to enriched environments before sleep showed different
zif268 expression profiles during sleep compared to control animals (Ribeiro, Goyal, Mello,
& Pavlides, 1999).
1.2 Models of Memory Consolidation

*Standard Consolidation Model*

Hebb & Gerard (1949) proposed the dual-trace theory of memory, where the stabilization of reverberating neural activity underlying the manipulation and maintenance of short-term memories produces, in time, long-term memories. Building upon this idea, Alvarez & Squire (1994) suggest the Standard Model of Memory Consolidation (SC) whereby these memories are formed early by the medial temporal lobe (MTL), a structure that is implicated in memory by studies of human amnesiacs (Scoville & Milner, 1957), including the hippocampus (HC), and entorhinal (EC), perirhinal (PC), and parahippocampal (PHC) cortices, collectively known as the hippocampal complex (HPC), before migrating to cortical areas for long-term storage (Alvarez & Squire, 1994; Squire & Alvarez, 1995), though the HC may support storage of some long-term memories (Sutherland et al., 2001). SC predicts that memories become independent of the HPC and MTL over time, thus damage to the MTL will result in a temporal gradient of retrograde amnesia (RA), where more recent memories will be impaired, leaving remote memories unaffected. The severity and temporal extent of the amnesia varies with the extent of damage to the HPC (Alvarez & Squire, 1994; Squire & Alvarez, 1995, Frankland & Bontempi, 2005; Nadel & Moscovitch, 1997), and the gradient can last up to 15 years (Squire, 1986; Squire, 1992). The primary feature in the model is that the connections between MTL and cortical areas are much faster compared to the connections between cortical areas.

*Multiple Trace/ Trace Transformation Theory*
Multiple Trace Theory (MTT) was proposed by Nadel & Moscovitch (1997) as an alternative model to SC. MTT states that the hippocampus creates allocentric spatial representations or “maps”, which provide the context in which memories are embedded. It does not differentiate between recent or remote maps, and thus would predict that damage to the HPC would always result in memory impairment, regardless of the age of memory, especially for spatial (Nadel & MacDonald, 1980) or episodic (Frankland & Bontempi, 2005) information. Semantic memory is not mediated by the HPC, since it is context-free, and thus damage to the HPC should not produce deficits in semantic memory (Moscovitch & Nadel, 1998; Moscovitch, Nadel, Winocur, Gilboa, & Rosenbaum, 2006; Nadel & Peterson, 2013; see Nadel, Samsonovich, Ryan, & Moscovitch, 2000 for a computational model approach).

When lesions are confined to the hippocampus alone, retrograde amnesia is short lived, regardless of the type of information in question. However, when lesions extend beyond the hippocampus, the duration of memory loss is related directly to the extent of the damaged tissue, a finding that has support from connectionist neural network modeling simulations (Nadel, Samsonovich, Ryan, & Moscovitch, 2000). Lesions of the hippocampal formation, including the subiculum, result in autobiographical memory loss for approximately a decade. If the lesion is larger, extending to the HPC, memory loss extends for 20-30 years in several domains, including autobiographical, personal semantic, and even vocabulary in extreme cases. If the lesion extends beyond that, into inferotemporal cortex, memory loss for autobiographical information extends for the entire lifespan, a so-called flat gradient, something not predicted in SC (Nadel & Moscovitch, 1997; Moscovitch & Nadel, 1998).
Though this model has gained in popularity, there is evidence that its claims are not always supported, such as work by Sutherland (reviewed in O’Reilly, Bhattacharyya, Howard, & Ketz, 2011) showing that the degree to which fear conditioning is impaired in rats shows a flat gradient regardless of lesion size.

Trace transformation theory suggests abstraction and transformation of hippocampal-neocortical episodic information into semantic information. The resulting memories then interact with representations that retain their context/episodicity and dependency on the HPC, whereas semantic, gist-like memories are independent of HPC involvement (Winocur & Moscovitch, 2011). This theory can also account for evidence in the lesion literature that has pitted SC against MTT for years, as it suggests that it is the type of information in question that leads to the finding of flat or graded RA gradients, as episodic aspects of a memory are always reliant on the HC, but gist-like memories are independent of HC involvement.

Evidence for this theory comes from functional magnetic resonance imaging (fMRI) work showing that recognition accuracy of information about movie episodes was stable after an initial decline when measured three weeks after encoding, whereas blood oxygen level-dependent (BOLD) activity in retrieval related areas was positively associated with accuracy only after months. Hippocampal activity during retrieval was similar over time during recall but decreased in recognition. These data suggest that the memory trace becomes transformed to something more schematized over time, and that the hippocampus subserves retrieval of episodic memories long after encoding, in direct contrast to against SC (Dudai, Karni, & Born, 2015).

Trace transformation can involve the strengthening of memory traces through synaptic rescaling, the integration of new information into an existing memory trace, the
establishment of new connections between existing memory traces, and updating of existing memories. Weaker memory traces do not survive downscaling, and traces that survive are replayed to cortex for consolidation through LTP-like processes (Dudai, Karni, & Born, 2015). Slow-wave sleep (SWS) is thought to play a crucial role in downscaling (SWS; Tononi & Cirelli, 2003).

1.3 Brain Networks of Memory Consolidation

Memory is composed of multiple interacting systems (Voss & Paller, 2008), involving numerous brain regions, including the hippocampus, amygdala, neostriatum, neocortex, and the cerebellum (Squire, 2004), divided into two main streams, the dorsal “where” stream, and the ventral “what” stream. Multiple primary sensory pathways process information that is then sent to multimodal association areas, where this information is packaged together, and sent to the MTL. The PC and lateral EC are responsible for determining the familiarity of specific objects, and the PHC and medial EC are responsible for processing the spatial contexts in which memorable events occur. These areas interact via reciprocal connections, and the resulting memory is a computed combination of semantic and episodic information. The anterior portion of the HC has connections to the ventromedial prefrontal cortex (vmPFC), an area of the brain that accumulates information about the context of interrelated memories, biases or selects retrieval of information in the “what” stream, and generates schemas about memories, allowing for an abstract representation of semantic information (Preston & Eichenbaum, 2013). Moscovitch, Cabeza, Winocur, & Nadel (2016) propose that the vmPFC could act as a hub binding the global context of events represented in the aHPC and general knowledge, including that about the self, into a schema that captures what is common to all such events. These schema-dependent memories may be
more robust against forgetting, either due to time since acquisition (processes of decay or interference), or to physical damage to the brain (Dudai, Karni, & Born, 2015). Moscovitch, Cabeza, Winocour, & Nadel (2016) propose the global-local hypothesis of anterior-posterior HPC organization. Posterior HPC, with its connections to perceptual regions in cortex, as well as higher proportion of pattern-separation units (dentate gyrus (DG)/CA3 ratio) allows for a more precise encoding of information in terms of positions within a continuous dimension, while the aHPC, with larger CA1 proliferation and connections to lateral temporal cortex and vmPFC codes for global or general relationships between items (Preston & Eichenbaum, 2013). Delayed increase in synaptic transmission was found in the hippocampal-prefrontal cortex pathway during training in an associative learning task. Further, efficacy of potentiation was different in the EC input to the HC and the output to frontal cortex. In the inputs, potentiation was evident early in learning, but potentiation from HC to frontal cortex was delayed until later when learning was greater, suggesting that this pathway is involved in late consolidation which could help stabilize the cortical representation of learned events (Laroche, Davis, & Jay, 2000).

Forgetting is modulated by separate processes in different memory systems. The neocortex is thought to be involved in content representations, whereas the hippocampus is involved in spatial-contextual representations in addition to linking and indexing cortical representations. The hippocampus is excellent at pattern separation; thus, interference is not likely to impair memories there, but rather forgetting will be produced by decay. The opposite is true of the cortex, where overlapping traces can interfere with encoding or retrieval, a process called catastrophic interference (Hardt, Nader, & Nadel, 2013).
SC cannot explain data showing similar activation of hippocampal areas during remote and recent retrieval (Nadel & Moscovitch, 2001). However, Haist, Gore, & Mao (2001) show that in older adults taking a famous faces remote memory task, the HC may only participate in processes of consolidation lasting a few years, as recollection of recent faces produced activation in right anterior HC and amygdala, whereas the right hemisphere EC may play a role in temporally graded changes for up to 20 years. Data also suggest that increased functional connectivity between HPC and lateral occipital cortex was positively related to memory performance in an associative encoding task, and that memory information becomes distributed across cortico-hippocampal circuits early in the process of consolidation.

Evidence from fMRI studies indicate that the HPC is involved in retrieval of information, at least autobiographical, regardless of age, and from electroencephalography (EEG), suggesting that this is coordinated via neuronal oscillations in the theta and gamma frequencies. Gamma synchronization during encoding helps bind sensory features into objects in posterior cortices, and theta-gamma coupling is responsible for integrating objects into events in the HPC. During retrieval, access to a fraction of the HPC representation activates the theta cycle, which then triggers the nested gamma activity, representative of the neocortical representation of memory (Moscovitch, Cabeza, Winocur, & Nadel, 2016).

Poldrack et al. (2001) used fMRI in a classification learning task, where either declarative (associative), or non-declarative (probabilistic) aspects of the task were emphasized. MTL structures were engaged for the declarative portion, and the striatum was engaged for the non-declarative portion. Further, activity in these areas was negatively
correlated, suggesting competition between two memory systems during the same task depending on the type of information to be processed.

Whether memory impairment reflects imprecise or insufficient encoding, storage, or retrieval is debatable (Nadel & Moscovitch, 2001). Preston & Eichenbaum (2013) provide a review of prefrontal cortex damage in monkeys and humans, suggesting that patients with prefrontal damage are severely impaired in learning novel associations between stimuli once an association is established (the A-B, A-C problem). Monkeys with medial prefrontal cortex (mPFC) damage are unable to switch between remembering place versus response in a spatial memory task. The mPFC may be involved in the retrieval of remote memories, but recent evidence suggests that it may also be involved in development of schemas, which are generalized categories of information that make assimilation of related information easier and can lead to very rapid learning once formed (Dudai, Karni, & Born, 2015). Mumby (2001) suggests that lesioning the hippocampus does not in general impair rats on an object-recognition task, the delayed-non-matching-to-sample (DNMS) task, contrary to previous reports. Lesions of the HC lead to deficits in contextual or spatial aspects of an object-place association task, but not to object recognition (Mumby, Astur, Weisend, & Sutherland, 1999b; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002). Rats with parahippocampal lesions could not learn to distinguish paired associates from mispairings (Eichenbaum & Bunsey, 1995).

Comparison between animal and human lesion models is often problematic because animal lesions can be more tightly controlled, whereas human lesions are often the result of disease or injury, and thus are less precise (Gluck & Myers, 1995). There is an extraordinarily rich literature of both human and non-human animal studies of brain damage
and relationships to various forms of memory. Though beyond the current scope of this manuscript, readers are referred to Mumby (1999a; 2001) for reviews.

1.4 Neural Network Models of Memory Consolidation

Complementary Learning Systems Theory

Complementary learning systems (CLS) is a theory to overcome catastrophic interference, where new learning completely overwrites existing information. In this model, the HC is responsible for conjoining information from disparate brain regions to form a representation of an event via LTP (McClelland, McNaughton, & O’Reilly, 1995), and has been used to model several memory domains, including recognition memory and fear conditioning (O’Reilly & Norman, 2002). Memory retrieval occurs when a cue triggers completion of the original CA3 pattern, which then drives CA1 and reactivates the patterns throughout cortex. This replay is crucial to allow consolidation of information in the slow learning cortex (McClelland, McNaughton, & O’Reilly, 1995; O’Reilly, Bhattacharyya, Howard, & Ketz, 2011). A basic premise of this theory is that long term memory systems containing distributed representations cannot afford to undergo rapid changes in synaptic weights, as these rapid changes would destabilize existing memory traces, causing catastrophic interference. Thus, two complementary systems, one that learns quickly, is excellent at pattern separation, and is robust against interference, can work with another, incremental learning system, to build long-term memory traces over time, where the HC is the fast learner and the prefrontal cortex (PFC) is the slow learner (Nadel, 1992; O’Reilly, Bhattacharyya, Howard, & Ketz, 2011).

Norman, Newman, & Perotte (2005) suggest a neural network approach that can reduce interference in the CLS model, consisting of oscillating inhibition and autonomous
memory rehearsal. An oscillating inhibition algorithm provides a means to strengthen parts of weak memories, while punishing strong competitors, and autonomous memory rehearsal combined with this algorithm (presumably during REM sleep) reduces the rate of forgetting of input patterns no longer present in the system, as well as prevents catastrophic interference better than other models, such as basic Hebbian learning.

**Hierarchical Relational Binding**

Another framework called hierarchical relational binding theory (Shimamura, 2010) proposes that the MTL functions as a unit geared toward binding features of an event in a hierarchical manner, such that bindings in lower levels are nested within higher level bindings. Memory strength is increased in a superadditive way in the hierarchy. The hippocampus in this model is the final zone in the MTL and has the capacity to relate and reinstate ensembles from disparate regions of cortex. During retrieval, activation through MTL levels assists in the reinstatement of event features associated with specific episodic memories. These ideas have been validated through computer modeling of signal detection curves in source item memory (Shimamura & Wickens, 2009).

Lorincz & Buzsaki (2000) describe a neural model of hippocampal-entorhinal function for long-term memory storage. In their model, the major function of this system is to alter synaptic connections in the neocortex. This is accomplished by the EC comparing the error between neocortical representations and feedback from the HC. This error detection initiates plastic changes in the hippocampus. The model then requires that the reconstructed input from the hippocampus and the information from cortex are there simultaneously, which is accomplished by predictive structures, namely the CA3 recurrent network and EC-CA1 connections. This leads to the hippocampus generating new independent components as
output, which are then used to train long-term memory trace in the EC for transfer to the cortex.

1.5 EEG Correlates of Information Encoding and Retrieval

Brain oscillations across the spectrum have been implicated in successful encoding and recall/retrieval of information. Backus et al. (2016) suggests a role for hippocampal/medial prefrontal cortex (mPFC) theta in integrating new memories into existing frameworks. Kahana (2006) Suggests that theta and gamma are involved in memory processes, including spatial working memory (WM)/navigation, and word/verbal working memory/encoding. In a review, Klimesch (1996) suggests that alpha desynchronizes during mental effort. Further, alpha shows considerable interindividual variability which should be accounted for. For example, the author suggests two alpha bands: lower (Alpha I; 7-11 Hz), and upper (Alpha II; 10-13Hz). Upper band desynchronization (reduction in power) is related to semantic processing, while lower band activity is related to attentional processes. The author suggests that the strongest desynchronization occurs ~500ms post-stimulus in a semantic feature word task. He also suggests that lower alpha responds to task difficulty by modulating attention, and theta synchronization is associated with episodic demands. For example, during a word recognition memory task, increased theta was related to correct recall, as was reduced alpha. Klimesch (1999) points out that alpha also decreases at night during sleep, especially during REM sleep. Tonic theta decreases but phasically increases during increasing waking cognitive performance, whereas alpha tonically increases and phasically decreases. Desynchronization in lower alpha reflects attentional processes, while desynchronization in upper alpha reflects semantic memory processing.
Based on Hebbian learning, it may be assumed that synchronization of neurons is required for memory formation (i.e., fire together, wire together). But mathematical information theory postulates that synchronization at low frequencies could reduce information encoding. Hippocampal synchronization leads to information binding in those structures, but desynchronization of lower frequencies (specifically theta and alpha, but also beta) in the neocortex mediates the representation of information in those structures. Cortical desynchronization in alpha/beta at left inferior prefrontal cortex is associated with semantic processing, and memory formation for visual material is associated with a power decrease in alpha over parietal/occipital regions. Importantly, these processes appear to be similar for encoding and retrieval processes (Hanslmayr et al., 2016). Using information theory, models suggest an increased amount of information encoded as desynchronization is increased (using Shannon's entropy), where the most information is encoded in the most desynchronous state. For episodic memory, perhaps the desynchronous nature of neurons allows for unique codes for each memory trace, since there can be an infinite combinations of firing patterns, even in a small number of neurons. Perfect synchrony (like in a seizure) is deleterious for information processing because there is not variability in the firing pattern to code pieces of information. Similarity, in a completely chaotic state or complete desynchrony, the system would be too noisy to code anything, and information would be lost (Hanslmayr et al., 2012).

Hanslmayr et al. (2012) also suggests most memory research focuses on synchronization of frequencies, specifically theta and gamma for memory encoding and retrieval. However, other processes, including desynchronization of alpha and beta for encoding and retrieval are important to consider as well. These phenomena are often correlated, suggesting a similar underlying mechanism or process. Beta power decreases
specifically reflect semantic processing and is often localized to left IFG. For episodic memory retrieval, decreased alpha/beta was associated with improved performance and showed a parietal topography. These processes may reflect the reactivation of the sensory features of a memory trace. Retrieval power decreases are seen in multiple regions, including frontal, parietal, and temporal areas.

Klimesch et al. (1994) suggests that theta activity is assumed to reflect longitudinal pathways from hippocampus to cortex. The encoding of semantic information relies on episodic memory processes because context is omnipresent when learning. In ERP research, the P300 reflects episodic memory processes as evidenced by larger P300 for words later recalled compared to non-recalled words. Participants performing only episodic encoding, but not semantic, showed this P300 effect, which is thought to reflect in part, theta activity. Word-features were presented to participants who then had to describe them as “congruent" in the semantic task (like bird-swallow), or "presented during the semantic task" for the episodic task. Event related synchronization (ERS; reflecting an increase in power)/event related desynchronization (ERD; reflecting a decrease in power) was computed based on scalp EEG, and only correct trials were used for analysis. EEG bandwidths were customized for each participant, which is thought to reduce error variance and provide a more accurate representation of neural dynamics, which can be variable among subjects, and be influenced by things like age, circadian rhythm, etc. Results suggested a decrease in alpha power, which is expected because ERD of alpha is associated with attentional processes. Theta ERD was suppressed, suggesting that increased theta was associated with performance in the semantic and episodic tasks. Specifically, theta showed ERS when episodic memory demands are required. Upper alpha band activity showed ERD during the semantic task only. The author
interprets these finding as theta being responsible for episodic encoding, and upper alpha being responsible for semantic encoding.

Weiss & Rappelsberger (2000) studied either auditorily presented concrete nouns or visually presented concrete nouns. EEG was recorded during encoding and for interspersed one-minute rest periods. Encoding EEG from correctly and incorrectly recalled words were used for analysis and EEG coherence analysis was conducted. For visually studied words, an increase in delta and theta band activity in correctly recalled words compared to incorrectly recalled words was observed. Further, there was a reduction in alpha for correctly vs incorrectly recalled words. For auditorily studied words, an increase in delta and theta activity (measured as root square power) was observed in correct compared to incorrectly recalled words. Higher synchronization overall was observed for correctly compared to incorrectly recalled nouns. The authors conclude that theta synchronization reflects successful encoding and storage of episodic information. Specifically, higher coherence in the alpha-2 and beta-1 bands reflect semantic processing of the words.

Pastötter & Bäuml (2014) suggest a role for theta in memory encoding and retrieval. Participants performed a word learning task where words were presented visually, and then a distractor task (number counting). Participants then performed a cued recall (the cue was based on first letter of the word - all first letters were unique in each list). EEG was recorded during the task and time frequency metrics of synchronization (power) were calculated. These measures included slow (2-4 Hz) and fast (5-7.5) theta. Synchronization of slow theta was positively related to retrieval success. Increases in fast theta power was negatively related to retrieval success. Slow theta localized over fronto-central and right parietal electrodes. Fast theta localized over mid-frontal and left-temporal sites. The authors suggest a
dissociation between fast and slow theta is related differently to memory processes, where slow theta reflects recollection and conscious awareness, and fast theta reflects interference and interference resolution.

Griffiths et al. (2016) investigated learning in a real-world memory paradigm using mobile EEG. A subsequent memory effect paradigm was utilized, which measures EEG during encoding, then based on behavioral performance, only the EEG from later recalled or non-recalled words are used for analysis. While they report successful recall is marked by decreased power in alpha and beta bands, theta remains a mystery, where some groups report increases, and others report decreases being associated with memory performance. Participants navigated around campus while EEG data were collected. Words were learned at specific locations, and a free recall test was given later. Then the participants led the experimenter to the locations where the correctly recalled words were learned. Results suggest low-frequency power decreases in successful memory formation. Reduction in beta power was localized to left frontal/temporal pole. Theta power decreases were also observed, particularly for items that demonstrated strong spatial coupling at recall. Theta was localized to temporal poles, posterior parietal cortex (PPC), and MTL regions. The authors suggest that beta power reflects verbal information processing and theta reflects activation of and communication between regions responsible for processing semantics and spatial location.

Werkle-Bergner et al. (2006) also suggest that theta is important for memory encoding. LTP processes are enhanced through stimulation in the theta frequency range. ERD in the upper alpha band was observed during successful encoding, while fronto-posterior ERS theta activity was observed during successful word encoding. This theta activity was localized over left frontal areas and increases in long-range coherence was
observed within the left hemisphere. Theta-gamma coupling may also underly memory formation. Individual memory components are refreshed at the gamma frequency, and the entire memory episode is refreshed at the theta frequency. Older adults showed less theta ERS during encoding and retrieval in a Sternberg memory task with words. They also showed greater alpha ERS during encoding and smaller alpha and beta ERD during retrieval. When children are compared to adults in an auditory Sternberg memory task with verbal stimuli, they showed delayed and smaller amplitude theta ERS and alpha ERD, particularly during retrieval, suggesting that memory systems at 12 years old are not fully developed, especially the retrieval systems.

Berka et al. (2007) administered a cognitive battery, including a paired associates test, and a B-Alert mobile EEG system was used for time frequency analysis. Classifiers for task engagement and workload metrics were derived from the EEG data. Engagement was calculated from fully rested and sleep deprived individuals, and workload was calculated from combinations of low and high difficulty cognitive tasks (arithmetic, digit span, grid location). The classifier results suggested engagement was comprised mostly of alpha and gamma band activity, whereas workload was comprised of theta, beta, and gamma. Among other results, for the memory tests, engagement and workload were higher during the encoding phase than recognition and increased as a function of task difficulty. While interesting, no thought was given to the existing literature regarding how correct encoding/retrieving leads to a different EEG profile compared to incorrect recall.

Jacobs et al. (2006) administered 576 trials distributed across two sessions in two days where participants viewed a list of 2, 4, or 6 consonants followed by a test probe and were required to press a button to indicate a target or lure item. Results suggest increases in
left parietal theta was related to memory recognition, and decreased theta power was associated with increased working memory load. Though similar effects have been observed after viewing open class words (nouns) compared to closed class words (conjunctions), this task may have tapped more into working memory than long-term memory processes.

Long et al. (2014) had participants learn 16 lists of 16 words each and then had them perform an immediate free recall test. Participants were part of an intracranial EEG (iEEG) epilepsy study and were compared to healthy controls (with scalp EEG). Results suggested a fronto-medial theta increase in accurately encoded words. Also, decreases in hippocampal theta in both the iEEG and scalp EEG groups was related to correct encoding. An increase in gamma was also observed, which was related to encoding performance. They also report evident alpha and beta decreases related to encoding performance across ROIs, including dorsolateral prefrontal cortex (DLPFC), inferior frontal cortex (IFC), MTL, and others.

Alekseichuk et al. (2020) suggests that parietal theta is necessary as a "glue" for associative memories. This oscillation has been observed during both recognition and encoding. In this study, 918 color photos of human faces were used as stimuli. Two tasks were performed, separated by a 20-minute delay period. The first task was a short-term memory task, where participants viewed 4, 5, or 6 face-monetary value pairs and had to choose the correct face-value pair in a two-alternative forced choice. Following 20 minutes, participants encoded 180 faces (half of which were displayed during the encoding task). They indicated on a 6-point Likert scale how familiar the face was, and how confident they were in their decision. In addition, theta tACS was delivered in a double-blind, placebo-controlled, randomized, counterbalanced crossover design with three conditions: target stimulation, active control, and placebo control. All sessions were spaced by at least 72 hours to avoid
carry over effects, and EEG was also recorded. Results suggest that right posterior parietal cortex is crucial for long term memory encoding. Low theta (4 Hz) reflects encoding processes, and tACS during encoding improves memory familiarity but not recollection. Thus, theta appears to work as a binding agent that leads to a higher degree of familiarity during test.

Küssner et al. (2016) had participants perform a vocabulary learning task. Introverts and extroverts were recruited, and participants were subjected to either baroque music or silence while studying. Participants with higher beta power performed better than participants with lower beta power. Further, increased beta power was associated with improved recall at test.

Marko et al. (2019) had participants perform an associative chain test with three variations. The first was associative, where participants had to generate words that were associated (doctor, nurse, etc.). The second was dissociative, where participants had to generate words that were not related (kitchen, car, dog, etc.). The third was associative-dissociative, where participants alternated between the two. Left DLPFC-temporal parietal cortex tACS at 6 Hz was delivered during three experimental sessions with three different stimulation conditions: in phase, anti-phase, sham. Associative retrieval was inhibited by anti-phase vs sham tACS. Dissociative retrieval was enhanced by anti-phase tACS but inhibited by in-phase tACS. The authors suggest that entrainment of tACS in phase strengthens the stability of prepotent semantic connections, while anti-phase stimulation decreases binding of these connections, making the dissociative task easier because the prepotent responses do not have a chance to interfere. No EEG was recorded during this experiment, which could have elucidated the functional correlates of these processes.
Mathewson et al. (2012) had 39 participants perform a task, a game called the Space Fortress task. The game entails maneuvering around objects and avoiding enemy missiles, keeping the ship within a predefined area and under a certain velocity, and destroying the fortress with a specific combination of attacks. Secondary tasks included an auditory oddball and a cognitive task battery testing attention, cognitive control, and working memory. EEG was time locked to four events of interest (mine onsets, fortress hits, and rare tones in and outside of the game). Results suggest that a laterализed increase in alpha power was associated with learning the task during training, and participants whose alpha power was highest at baseline (before training) performed the best. Delta power was also positively related to learning rate. The only event of interest that predicted learning rate was a fortress hit. They suggest that frontal alpha is related to cognitive control and attention to salient learning events in the game, specifically fortress hits. This makes some sense because that is mainly the point of the game, so participants paid attention to this piece during training, as it was the most salient event/feature.

Bays et al. (2015) suggests that alpha activity is generated mainly in thalamo-cortical circuits, and evidence exists for intracortical sources as well. Alpha power is reduced with greater task related effort and increased attention. Participants with lower overall alpha power better discriminated brief visual stimuli. Alpha can reflect top-down control processes, where increased alpha is associated with inhibition and decreased alpha is associated with task processes. They had eight participants perform a visual search perceptual learning task. Training took place over eight days (one hour/day). The task involved finding a target line in a sea of distractor lines. Participants reported with a keypress what color the target line was (white or black). EEG was collected at a pretest (before the eight days of training), and at
posttest following training. Pre-test versus post-test alpha power was the main dependent variable. Results suggest that alpha power was increased in the pre-stimulus period at post-test compared to pre-test. Alpha was reduced once the stimulus appeared (as evidenced by alpha ERD). The amount of ERD was greater at post-test than at pre-test. Interestingly, no EEG measures correlated with behavioral performance. They conclude that participants are allocating their attentional resources better following training, as evidenced by the ERD observed in alpha. They say that this processing efficiency could possibly be due to automaticity effects of learning the task. The lack of correlation with behavior could be due to numerous factors, including difference in wakefulness, or that changes in alpha may not be linearly related to behavioral performance change or improvement. Participants may consciously or subconsciously regulate alpha activity during the task, then changes in alpha power after training would be related to the amount of alpha at test, meaning accuracy could fluctuate but alpha remains constant, leading to no correlation.

Laera et al. (2021) investigated prospective and retrospective memory using EEG. Prospective memory is remembering to execute an intention at the appropriate moment (like remembering a friend's birthday). Retrospective memory is retrieving the intended action (call your friend whose birthday it is to wish them a happy one). A modified version of the encoding/retrieval prospective memory paradigm was utilized. There were three phases to the experiment - an encoding phase, a maintenance phase, and a retrieval phase. The ongoing task was a semantic categorization task using German words. In the encoding phase, participants had to memorize the association between letters and colors. At retrieval, participants had to press a key whenever they saw word pairs in the correct color. The prospective aspect was manipulated by varying the number of colors participants had to
memorize during encoding. In high distinctiveness, there were only two colors, in low distinctiveness there were four colors. The retrospective aspect was manipulated by varying the time available at encoding and by increasing the number of letter-color associations. Results suggest that older adults showed a decrease in parietal alpha in the detection of distinct prospective memory cues, and a decrease in parietal beta when detecting less distinct cues. Older adults showed less beta activity compared to younger adults across the experiment. No age-related differences were evident in the retrospective component. Older adults also showed reduced theta activity for the highly distinctive prospective memory cues. The change in oscillatory dynamics as a function of age could be related to the reallocation of attentional resources needed to perform the task. Beta could be involved in trying to enhance task coordination processes, suggesting perhaps the older brain does not work as efficiently and must compensate.

Brokaw et al. (2016) investigated whether a quiet rest (non-sleeping) period would produce similar effects as a nap, as shorter sleep periods such as a nap can boost memory consolidation much like a full night’s sleep can. Participants performed a recall task where they listened to a short story, then either quietly rested with eyes closed for 15 minutes or performed a distractor task for 15 minutes. Next, a recall test was administered. EEG was recorded to investigate the neural dynamics involved. The computer game "Snood" was used as the distractor task. Participants completed both conditions in a counterbalanced design with 10 minutes in between tasks. Results suggested that participants in the quiet rest condition performed better on the recall task than did participants in the distractor task condition. Participants in the quiet rest condition showed an increase in slow wave activity and alpha power. Memory recall scores were positively associated with slow-wave power,
but negatively associated with alpha power. They conclude that quiet rest can facilitate memory consolidation and improve performance much like a nap or sleep can do, and likely by similar mechanisms. Specifically, reactivations are thought to occur during times of quiet rest, thus facilitating the strengthening of memory traces via reconsolidation mechanisms.

In Hanouneh et al. (2018), EEG was measured during a recall task and compared against a resting-state baseline that was acquired before the encoding portion of the task. Participants watched educational videos (10 minutes long) dealing with science concepts of human anatomy and physiology. They completed a baseline resting state EEG period with eyes open, then watched the video three times, followed by a 30-minute retention interval where participants engaged in casual conversation. They were instructed not to sleep or rehearse the information in the videos that they encoded. EEG was acquired during the recall task, where 20 multiple choice questions relating to the video were given. Results suggest that delta and theta band power were negatively correlated with task performance at frontal regions. Alpha band power was positively associated with performance in frontal regions but negatively associated in parietal and temporal regions. High beta was positively related to performance in frontal lobe sites. EEG coherence was negatively associated with performance in the delta band at fronto-parietal and fronto-central sites. Gamma coherence was positively associated with performance all over the scalp. EEG phase delay (representing synchronization) showed high connectivity in delta and alpha bands in frontal regions, and was negatively correlated with memory scores, reflecting increased synchronization. They conclude that decreased theta represents increased mental effort during recall. Decreased alpha in general has been shown to be related to successful retrieval, and specifically decreases in parietal alpha have been shown to be associated with memory performance.
Stokes et al. (2012) had participants perform a memory-guided visual attention task, where they searched for a target stimulus in a naturalistic scene. Previously learned locations were 100% accurate in predicting where the target stimulus would be (if there was a target present). Results suggested that posterior alpha power was reduced during the presentation of a valid memory cue with a latency of 650-750ms post-cue. They conclude that alpha desynchronization reflects attentional processes in naturalistic visual search.

Yaffe et al. (2014) suggests that reinstatement/reactivation of neural activity is related to the ability to recover a representation of a previous memory, essentially recovering a context in which an episodic memory is embedded for retrieval. iEEG was recorded from participants during a verbal paired associates task, and iEEG recordings from correct and incorrect trials were analyzed. Results suggested theta and high gamma were associated with reinstatement in correctly recalled paired associates, with an ROIs in the inferior temporal lobe for theta and gamma, and right ventrolateral prefrontal cortex (VLPFC) for theta and bilateral VLPFC for high gamma.

Fukuda & Woodman (2017) had participants perform a visual working memory/long term memory task, where they learned to memorize arrays of different sizes with objects in them. Results suggested that suppression of parieto-occipital alpha was present during both encoding and retrieval of information. They suggest that this is thought to reflect the processes in working memory, both during encoding and retrieval, where information must be called up back to working memory to be acted upon (in this case, indicating recall of the image).

Mölle et al. (2002) had participants perform a word and face paired associates task, where they experienced both conditions. Resting state EEG was recorded after the first task,
then again after both tasks had been completed. The order was counterbalanced across participants. Results suggested that alpha II ERD and theta ERS was observed in correctly encoded trials compared to incorrectly encoded trials. Combined measures of alpha ERD and theta ERS in efficient learning trials was localized over left frontal and temporal regions for words and over right parietal cortical regions for faces. Higher power alpha I and beta activity were observed in words compared to faces. They conclude that these dynamics reflect intentional encoding of information.

1.6 Augmentation of Memory Consolidation in Humans

There are numerous theories and models of human memory, but they all seem to agree broadly on the structures involved in consolidating and storing information, even if the details are contended. There are myriad ways to augment consolidation in humans, including pharmacologically (Soetens, Hueting, Casaer, & Hodge, 1995), by utilizing the testing effect (Hogan & Kintsch, 1971; Karpicke & Roediger, 2007; Thompson, Wenger, & Bartling, 1978; Wheeler, Ewers, & Buonanno, 2003), distributed learning (Litman & Davachi, 2008), dreaming (Wamsley, Tucker, Payne, Benavides, & Stickgold, 2010), and manipulation of physiological states (Hoscheidt, LaBar, Ryan, Jacobs, & Nadel, 2014) among others. Recently, transcranial current stimulation (tCS) has become a popular method to communicate with the brain in its own language, electricity (Buzsaki & Draguhn, 2004), as well as augment cognition (Coffman, Clark, & Parasuraman, 2014).

Transcranial Current Stimulation

Utilizing tCS to enhance cognitive functioning is becoming increasingly popular (Sarmiento, San-Juan, & Prasath, 2016). tCS may be involved in neural plasticity (Mohammadi, 2016), and several review papers and meta-analyses have shown the efficacy
of tCS in enhancing cognitive performance in both healthy and clinical populations (Kuo & Nitsche, 2012; Coffman et al., 2013; Fröhlich, 2014; Hsu et al., 2015). Coffman et al. (2014) provides a comprehensive review of the cognitive effects of transcranial direct current stimulation (tDCS) in healthy samples. Though meta-analytic techniques were not utilized, positive effect sizes of active stimulation were reported in the domains of learning, memory, perception, and attention. Using meta-analysis to investigate effect sizes in both healthy and Alzheimer’s samples, Hsu et al. (2015) report a modest effect size (standardized mean difference; SMD) of 0.42 in healthy samples, but a large effect size of 1.35 in Alzheimer’s samples. Though transcranial magnetic stimulation (TMS) was also included in these calculations, results suggest that targeting clinical populations, with brains that are functionally sub-optimal may be a better application of neuromodulation with tCS. However, another meta-analysis suggests that single-session tDCS has no substantial effect on improving cognition. Horvath, Forte, & Carter (2015) conducted 59 separate analyses investigating attention, working memory, and executive functioning, and found no evidence that active tDCS leads to an improvement in performance compared to a control group. However, they only focused on healthy populations and studies where cross-laboratory replication had taken place.

Verbal memory has been augmented with tDCS as well. Elmer et al., 2009 used anodal, cathodal, and sham tDCS over either the right or left DLPFC to identify hemispheric differences of stimulation effects. Participants did an episodic verbal memory task during stimulation and were asked to recall words that were spoken to them. Each participant received anodal, cathodal, and sham stimulation in a randomized order. Electrodes were placed over F3 and F4 using the 10/20 electrode placement system, which is the right and left
DLPFC. Anodal and cathodal stimulation were applied at a constant current of 1.5 mA for a duration of 5 minutes. Participants in the sham condition received no stimulation. They found that cathodal tDCS over the left DLPFC impaired short-term memory in the auditory verbal memory task compared to the sham condition.

**Transcranial Alternating Current Stimulation (tACS)**

There is considerable evidence that transcranial alternating current stimulation (tACS) is effective in entraining brain oscillations (Ali, Sellers, & Fröhlich, 2013; Merlet et al., 2013; Herrmann, Rach, Neuling, & Strüber, 2013; Helfrich et al., 2014). Whereas tDCS affects firing rate, tACS regulates the firing rate in an oscillatory manner without affecting the average overall firing rate, and even low amplitude AC stimulation results in increased coherence between neuronal spikes and the frequency of stimulation (Herrmann, Rach, Neuling, & Strüber, 2013). Helfrich et al. (2014) suggest that tACS is a promising tool to modulate brain areas relevant to distinct cognitive functions to determine their functional impact.

The phase at which tACS is delivered to the cortex is critical to drive entrainment. In-phase stimulation can amplify the backpropagation of an action potential, or result in LTP, but out-of-phase stimulation can selectively suppress oscillations in a network (Buzsaki & Draguhn, 2004). Helfrich, Schneider, Rach, Trautmann-Lengsfeld, Engel, & Herrmann, (2014) applied 1.0 milliamp (mA) tACS at 10 Hz for 20 minutes over parieto-occipital cortex, while simultaneously recording EEG during a visual oddball task. To investigate phase-dependent effects, the visual stimulus was presented at four different phases of the tACS waveform. The data demonstrate a significant interaction between stimulation condition and phase of stimulus presentation on target detection, suggesting that tACS is
modulating performance in a phase-dependent manner. Further results revealed that alpha band power was significantly enhanced during active stimulation. Thus far, a fine-grained analysis of EEG data during and immediately following tACS stimulation has not been possible, however an algorithm was developed for this study to remove the tACS artifact, which opens the possibility of investigating the neural effects during and shortly bordering the application of stimulation, though empirical validation of this algorithm is currently lacking.

Computer modeling studies have shown that tACS can modulate large-scale networks. Using tACS to induce weak perturbations in oscillatory rhythms to investigate the effects on large-scale cortical networks in computer simulations, Ali, Sellers, & Fröhlich, (2013) report that short-term depression leads to large effects on cortical oscillations. A simulated neural network of 200,000 neurons (160,000 pyramidal cells and 40,000 interneurons) was subjected to a direct current waveform and an alternating current waveform at 3Hz (the endogenous frequency of the network). The results show that tACS produced larger average relative power at 3Hz compared to tDCS. Although tACS could successfully entrain the network (90% of peak steady-state power at the stimulation frequency) regardless of onset phase, phase played an important role in the speed at which networks were entrained. Stimulation started near the hyperpolarizing down-state of the oscillation produced a quieting effect, allowing for a larger gain in pyramidal-pyramidal interaction during the subsequent up state. This suggests that tACS is a better candidate waveform to entrain neurons, and the phase at which tACS is applied is crucial.

Closed-loop AC stimulation provides the most optimized method for delivering stimulation and entraining neurons in the human brain, is technically complex, and has only
recently been demonstrated (Wilde, Bruder, Binder, Marshall, & Schweikard, 2015). Berényi, Belluscio, Mao, & Buzsáki (2012) utilized closed loop tACS at 1Hz during seizure-triggered spike and wave (SW) episodes in a rodent model of generalized epilepsy. This paradigm led to a 60% reduction in the duration of SW events and the fraction of time spent in this state. Mean duration of SW was significantly shorter in all tested rats, and time spent in SW episodes was reduced in 7 of 9 rats. Being able to detect on-line changes to endogenous brain rhythms and apply targeted stimulation is the optimal method by which to invoke change in brain function and modulate behavior.

However, other theories and techniques have been proposed. For example, Bergmann, 2018 explains that brain state-dependent brain stimulation (BSDBS) is a different type of stimulation, distinct from closed-loop stimulation. BSDBS involves a continuous cycle of EEG monitoring, triggering stimulation, which modifies the brain state, leading to new EEG signals and so on. He describes traditional closed-loop stimulation as open-loop brain state-dependent brain stimulation because there is a missing part of the loop whereby the stimulation does not change the brain state.

1.6 Sleep

Humans spend roughly one-third of their lives asleep (Sejnowski & Destexhe, 2000). Sleep is a universal phenomenon and has been identified in every animal studied (Tononi, Riedner, Hulse, Ferrarelli, & Sarasso, 2010; Abel, Havekes, Saletin, & Walker, 2013). It is a homeostatic process, governed by a circadian system (Pace-Schott & Hobson, 2002), disruption of which leads to cognitive inefficiency (Borbely, Daan, Wirz-Justice, & Deboer, 2016). It has been shown to accelerate the process by which ribonucleic acid (RNA) is synthesized and to elevate cortical messenger RNA levels of genes critical for building and
maintaining synapses (Abel, Havekes, Saletin, & Walker, 2013). Sleep deprivation leads to a host of deficits in various cognitive realms, including attention, alertness, vigilance, and creative, innovative, and divergent thinking (Killgore, 2010; McCoy & Strecker, 2011).

Sleep is crucial for consolidation of memory, though the contribution of different sleep stages to domain-specific memory consolidation (Walker & Stickgold, 2006; Diekelmann & Born, 2010; Rasch & Born, 2013), and how memories change from waking to sleep (Paller & Voss, 2004) is unclear. This process is thought to occur via neuroplastic mechanisms (Walker & Stickgold, 2003) driven by long-range communication via neuronal oscillations (Piantoni, Van Der Werf, Jensen, & Van Someren, 2015). Slow wave sleep (SWS) appears to be involved in declarative memory consolidation (Plihal & Born, 1997; Marshall & Born, 2007; Diekelmann & Born, 2010; Born & Rasch, 2013) where large-scale neuronal up-states, resulting in depolarization across cortical and sub-cortical regions ideal for synchronization across disparate brain regions, allow information transfer from hippocampal to frontal areas. Nested within these events are sleep spindles and ripples, which are thought to coordinate the transfer of information (Oudiette, Santostassi, & Paller, 2013). Theta oscillatory coupling between CA1 regions and mPFC is suggested as a potential mechanism by which connectivity between these areas can facilitate learning (Igarashi, 2015). Large negative potential DC shifts have been recorded at the scalp during transitions from wake to SWS, reflecting cortical excitability (Marshall, Molle, & Born, 2003). REM sleep is thought to be involved in procedural memory consolidation (Diekelmann & Born, 2010; though see Crick & Mitchison, 1983 for an interesting theory of “parasitic” states and REM sleep forgetting). Memories that are part of a formed schema may also selectively benefit from REM sleep, as the route of consolidation may be dominated by the medial prefrontal cortex (Durrant,
Cairney, McDermott, & Lewis., 2015) Schemas, though thought to reflect cellular consolidation processes, may in fact be explained by systems consolidation (Rudy & Sutherland, 2008). The SWS-dependent declarative, REM-dependent procedural consolidation dichotomy represents the “dual-process hypothesis” (Diekelmann & Born, 2010) and has experimental support using “half-sleep” paradigms, where subjects are selectively trained and tested before and after specific sleep stages (Rasch & Born, 2013). On the contrary, the “sequential hypothesis” states that the succession of SWS by REM sleep facilitates consolidation of both declarative and procedural memories (Ambrosini & Giuditta, 2001; Diekelmann & Born, 2010; Rasch & Born, 2013).

Plihal & Born (1997) showed that selectively learning and testing when waking from either SWS or REM sleep produced a variable effect on memory performance. Early sleep rich in SWS was required for declarative memory consolidation, whereas REM sleep was crucial for procedural memory consolidation, evidence for the dual process theory. Walker & Stickgold, (2003) used a motor sequence tapping task in participants who either learned the task then slept or stayed awake, which led to a sleep dependent performance improvement. Another group learned the first task, then trained on a second motor sequence prior to sleeping. Interestingly, an interference effect was observed, where participants in the second group failed to show the sleep dependent improvement in the first task but did improve significantly on the second sequence task. Stickgold et al. (2000) show that performance on a complicated visual discrimination task exhibited a unique sleep dependent profile, where subjects had to sleep at least 6 hours after performing the task for improvement to be observed on subsequent testing. The increase in performance appeared to be related to the number of hours above six that they slept. Interestingly, performance gains on the task were
related to the amount of time subjects stayed in early (first quarter of the night) SWS and late (last quarter of the night) REM sleep, providing evidence for the sequential hypothesis. Awakenings during either SWS or REM sleep did not lead to a decrement in performance in a declarative word-pair associates test (Genzel, Dresler, Wehrle, Grozinger, & Steiger, 2009). Stickgold (2005) suggests that sleep improves performance in a visual texture discrimination task, even after finger movement was controlled for by having participants wear mittens during waking periods. Further, performance enhancement on a motor adaptation task shows a sleep-dependent effect, which is correlated with slow-wave EEG activity in task-related cortical regions.

**Sleep Architecture**

In mammals, sleep is characterized by two main stages: SWS and REM sleep, mirrored by complementary stages during wake – theta band activity during active exploration (like the hippocampus during REM sleep), and high-frequency ripples during quiet rest (like the hippocampus during SWS; Sejnowski & Destexhe, 2000; Rasch & Born, 2013). These stages are discernable by collecting EEG data during the sleep episode, a method called polysomnography. Electrodes are placed over the scalp, as well as around the eyes (to monitor eye movements and blinks), and the chin and/or leg (to measure muscle atonia in REM sleep). SWS is rich in the first half of the night, decreasing in distribution and density throughout the night, and is marked by slow, high-amplitude oscillations, called slow-wave activity (SWA), which peaks at around 0.75Hz (Marshall et al., 2004). Monoamines (serotonin and norepinephrine) and acetylcholine serve as a switch between brain modes from encoding during wake to consolidation during SWS, as well as between
sleep stages (Payne & Nadel, 2004), while glucocorticoids are at a minimum during SWS (Diekelmann & Born, 2010).

REM sleep, in contrast, is characterized by low amplitude, fast oscillatory brain activity that resembles waking EEG, and muscle atonia. Noradrenergic and serotonergic tone reaches a minimum during REM sleep, while cholinergic activity is highest (Diekelmann & Born, 2010). SWS is restorative for the brain and body, whereas REM sleep modulates transitions between SWS and waking (Vertes, 2004). Approximately half of sleep is spent in lighter, stage 2 (N2) sleep, characterized by sleep spindles and K-complexes, and minor SWA. Stage N2 is not distinguished from SWS in rodents, and REM sleep is primarily associated with memory consolidation in the rat, whereas SWS in humans is most often associated with hippocampal-dependent memory consolidation, though this discrepancy may be because studies with rats always involve reward, whereas human studies do not always do (Abel, Havekes, Saletin, & Walker, 2013). These concerns make translational suggestions between rats and humans difficult.

**Hippocampal Sharp Wave Ripples (SWR)**

Hippocampal sharp waves are fast depolarizing events that are generated in the CA3 region of the HC, on which fast (100-300 Hz) activity originating from interneurons and pyramidal cells in CA1 regions of the HC are superimposed. SWR occur during the positive half-wave of SWA (Molle, Yeshenki, Marshall, Sara, & Born, 2006), but also during waking (Oudiette, Santostassi, & Paller, 2013), and accompany the reactivation of neurons that were active during a waking experience. SWR events nest in spindle troughs, a finding verified using intracranial recording in patients with epilepsy (Staresina et al., 2015) thereby facilitating information transfer from HPC to cortex by feeding reactivations directly into the
excitatory phase of the spindle (Marshall & Born, 2007; Diekelmann & Born, 2010). Multiunit activity in the PFC precedes SWR by approximately 30 ms, which is thought to participate in synchronizing these areas for information transfer (Molle, Yeshenki, Marshall, Sara, & Born, 2006). Reactivations of neuron traces during SWS in the rat occur mostly during ripple events (Kudrimoti, Barnes, & McNaughton, 1999). Neocortical delta wave and spindle events may select which hippocampal neurons will participate in the ripple events (Sirota, Csicsvari, Buhl, & Buzsaki, 2003). These inputs can select initiators of the SWR, and in turn, provide a synchronous output preferably to those neocortical cell assemblies that continue to participate in the spindle event (Payne & Nadel, 2004). Ripple events closely co-occur with spindles, where ripples precede spindle-ripple events in the rat (Siapas & Wilson, 1998), suggesting these ripple events may bias which neocortical cells participate in the spindle event, providing guided plastic modification and thus consolidation. Sharp waves arise in the CA3 region and are propagated to CA1 and to the output layers of EC, from which they are propagated to the cortex. Patterns in the hippocampus could be completed during sharp waves, allowing for reinstatement in neocortical areas (McClelland, McNaughton, & O’Reilly, 1995).

Sirota, Csicsvari, Buhl, & Buzsaki (2003) recorded from hippocampal and cortical sites in freely exploring rodents during wake and sleep. Hippocampal ripple events were associated with an increase in delta and spindle bands. The cooccurrence of neocortical and hippocampal events was modulated by slow and ultraslow oscillations observed previously in these areas. Power in ripple events was correlated with sleep spindle and delta waves at the 1-2 second timescale in rats and mice. Pyramidal cell discharge is locked to the troughs of ripple waves and troughs of sleep spindles and delta waves in deeper cortical areas are
associated with the maximum discharge of neocortical cells, suggesting a discharge of the entire network. The maximum probability of ripple discharge occurred 50 ms after the trough of delta waves and 50 ms before the trough of sleep spindles. These findings suggest a temporal framework for coordinated information transfer between the hippocampus and neocortex during SWS.

Replay and Hippocampal-Cortical Communication

Evidence that the hippocampus replays events during wake (Carr, Jadhav, & Frank, 2011) and sleep, suggested by data showing that cell ensembles that were active during a waking experience show repeated similar activation during sleep episodes (Pavlides & Winson, 1989; Wilson & McNaughton, 1994; Sirota, Csicsvari, Buhl, & Buzsaki, 2003; Ribeiro et al., 2004) has been established, and are thought to arise from excitatory CA3 pyramidal cells acting as an autoassociative pattern completion network that, through reverberating activity, leads to a settling into an attractor state corresponding to a previously stored memory that is then reinstated in CA1 through feed-forward excitation (Carr, Jadhav, & Frank, 2011), however other areas appear to replay events as well (Ribeiro et al., 2004). The noradrenergic system may be critical in these processes (Sara, 2010). Insights from CLS theory suggest that REM replay may also play a role by reducing forgetting through oscillatory learning triggered by the theta rhythm which strengthens weaker memories and weakens conflicting ones through a process of oscillatory inhibition. During one phase, lower inhibition leads to the activation of potentially conflicting memory traces, then in another, higher inhibition increases synaptic weights of neurons, effectively re-encoding memories to reduce interference from overlap (O’Reilly, Bhattacharyya, Howard, & Ketz, 2011). Plasticity plays a crucial role in replay and blocking NMDA receptors impairs learning-
enhanced sleep reactivation (Atherton, Dupret, & Mellor, 2015). In the rat, Ribeiro et al. (2004) used neuronal ensemble correlation to show that novelty-induced neuronal firing in several forebrain areas were still present 24 and 48 hours after a pre-novelty baseline recording in the HC, thalamus, putamen, and cerebral cortex. Further, this activity was greater during SWS compared to both wake and REM sleep. This suggests that, in the rat, replay occurs in multiple cortical and subcortical areas. Replay may also be involved in building cognitive schemata by selectively strengthening shared elements through CLS-like processes and the synaptic homeostasis hypothesis, a mechanism called “information overlap to abstract”, or iOta (Lewis & Durrant, 2011).

In rodent models, Wilson & McNaughton (1994) showed that hippocampal place cells that were activated during exploration were selectively reactivated during sleep, and this activation was observed during non-REM (NREM) sleep, not REM sleep (Stickgold et al., 2000). A comparable pattern of reactivation is observed in the Zebra finch during song rehearsal, suggesting that the song is undergoing replay while the bird is sleeping.

Sleep Spindles

Sleep spindles are waxing and waning 10-15 Hz activity, generated in the thalamus from an interaction between GABAergic neurons in the nucleus reticularis and glutamatergic thalamo-cortical projections that propagate to cortical regions (Diekelmann & Born, 2010; Andrillon et al., 2011). Rhythmic inhibitory post-synaptic potentials (IPSPs) caused by repetitive spike bursts in GABAergic thalamic reticular neurons lead to rebound in thalamocortical glutamatergic neurons, which generate excitatory post-synaptic potentials (EPSPs) in cortical cells (De Gennaro & Ferrara, 2003), thus making spindles a prime target for cortical LTP (Fogel, Nader, Cote, & Smith, 2007a). Sleep spindles are modulated by
circadian factors and are strongest during the initial part of a sleep episode, occurring with K-Complexes, which reflect information processing during sleep (Fogel & Smith, 2011), and exert excitatory and inhibitory actions on cortical neurons, synchronizing spindle and delta waves from the thalamus (De Gennaro & Ferrara, 2003). They are split into functionally distinct bands, slow (9-12 Hz), occurring 200 milliseconds prior to (fast 13-15Hz) spindles, generated over frontal and centro-parietal cortices, respectively (Andrillon et al., 2011), though others argue that there is only one type of spindle (fast), with an anterior alpha peak (De Gennaro & Ferrara, 2003). Hippocampal-neocortical connectivity is greatest in N2 sleep (Andrade et al., 2011), and spindle density increases over the sleep cycle (De Gennaro et al., 2000). Spindles appear to reduce environmental influences on neocortical activity, shifting sleep into deeper stages (Buzsaki & Draguhn, 2004). They are thought to be related, in general, to memory consolidation (Fogel & Smith, 2011). Low-frequency spindle activity and density over left frontal cortex was observed during a daytime nap after learning a difficult word-pair list compared to an easier encoding condition, and the changes during sleep were correlated with improved memory during a subsequent waking test (Schmidt et al., 2006). Fronto-central spindle count is associated with verbal memory performance in a face-name association task (Clemens, Fabo, & Halasz, 2005), and a declarative memory task (Holz et al., 2012). Density of spindles both before and after sleep are related to improvement in a paired associates learning task (PAT; Gais, Molle, Helms, & Born, 2002). Spindles may be abnormal in sleep disorders, such as obstructive sleep apnea, leading to memory impairment (Barner, Ngo, Diekelmann, Weess, & Schlarb, 2016). They are highly stable within, but highly variable between individuals, and have been suggested as a marker for intelligence (Fogel, Nader, Cote, & Smith 2007a; Fogel & Smith, 2011; Lustenberger, Marie,
Durr, Achermann, & Huber, 2012), as well as predict susceptibility to sleep disturbance due to external noise, as participants with higher spindle count on an acclimation night showed more stable sleep in the presence of noise (Dang-Vu, McKinney, Buxton, Solet, & Ellenbogen, 2010).

*Slow-Wave Oscillations/Activity*

Slow wave oscillations/activity (SWO/A) are generated in cortical and thalamic networks and reflect a global depolarizing up-state phase and a complimentary down-state hyperpolarizing phase, which spreads across the cortex, as well as through subcortical structures like the HC (Ngo et al., 2013a). SWA is defined by the 0.5-4.0 Hz frequency band and is composed of the delta rhythm (1-4 Hz), and the SWO (<1 Hz, 0.8 Hz peak frequency; Rasch and Born, 2013). Delta rhythm is suppressed by the thalamus during the presence of spindles, when the membrane potential is more depolarized (Nunez, Dossi, Contreras, & Steriade, 1992). SWOs are thought to both be involved in synaptic downscaling (Tononi & Cirelli, 2003; 2006) as well as facilitate communication between the HC and neocortex (Diekelmann & Born, 2010; Rasch & Born, 2013), and are related to declarative learning (Molle, Marshall, Gais, & Born, 2004). SWA is highest at the beginning of the sleep episode and declines exponentially over the first four NREM cycles before eventually stabilizing for the remainder of the interval (Aeschbach & Borbely, 1993; Riedner et al., 2007).

Using high-density EEG, Murphy et al. (2009) showed that SWOs preferentially arise in the insula or cingulate gyrus, propagate via the cingulate, and show hot spots of activation in several brain regions, including anterior and posterior cingulate (PCC), precuneus, left inferior frontal gyrus, and insula, a finding largely replicated by Dang-Vu et al (2008). These areas show considerable overlap between regions involved in the default mode network.
(DMN), discovered in fMRI. DMN activity was apparent throughout all stages of sleep, but as sleep depth increased, PCC, retrosplenial cortex, parahippocampal gyrus and mPFC contributions to DMN activity decreased (Samann et al., 2011). Amount of time spent in NREM is correlated with facial recognition score improvement (Clemens, Fabo, & Halasz, 2005), and declarative memory enhancement (Holz et al., 2012). Though assumed to decline over the course of a sleep event, as sleep pressure relaxes, and synaptic strength is reduced, SWA may in fact be more related to circadian phase than previously realized. Lazar, Lazar, & Dijk (2015) recorded EEG from 34 participants across 231 separate sleep episodes in a forced desynchrony protocol. They showed that circadian factors influenced the count, frequency, amplitude, and slope of SWA, mainly over frontal brain regions. A decline in early, nocturnal SWS because of ageing has a detrimental effect on declarative memory consolidation compared to young participants (Backhaus et al., 2007).

**Ponto-Geniculo-Occipital (PGO) Waves**

PGO waves originate from bursts of synchronized activity from the pontine brainstem to lateral geniculate nucleus (LGN) and visual cortex. They have not been reliably defined in humans but are prominent during REM sleep in both the cat and rat (Diekelmann & Born, 2010). Using a novel method of REM sleep deprivation, Datta, Mavanji, Ulloor, & Patterson, (2003) showed that activating the pontine wave generator (a specific group of neurons in the pons) using carbochol in selectively REM-deprived rats led to no impairment in a two-way avoidance learning task compared to a REM-deprived saline-vehicle group. The REM-deprived carbochol group performed as well as a saline-vehicle control group that could sleep normally. The improvement of learning was proportional to the level of pontine wave
activity, suggesting a causal role for pontine waves in REM memory consolidation in rodents.

**Theta Oscillations**

Theta oscillations (4-8 Hz) predominate in the HC during tonic REM sleep in rats, but are less well characterized during human sleep, because the HC is not easily measured with EEG. They are one of the most well-known electrophysiological features of the HC, and place cell spike firing advances from the peak to trough of theta as a rat enters and travels toward the center of the field. Consequently, future positions of place fields can be predicted from the phase sequence, known as theta phase procession (Buzsaki, 2002; Buzsaki & Draguhn, 2004). Place cells that were activated during exploratory behavior during waking are selectively reactivated during slow-wave sleep (Wilson & McNaughton, 1994; Sutherland & McNaughton, 2000).

One study of theta waves in human sleep was conducted by Cantero et al. (2003). They recorded directly from the HC and frontal cortex in three of nine patients suffering from epilepsy. Theta oscillations were evident only during REM sleep, but not in SWS (<4% of total bursts occurred in SWS). However, in the transition from sleep to waking, theta activity was observed both in the HC and in the neocortex. Gamma activity was also recorded during hippocampal theta events but did not show any phase modulation by hippocampal theta, a finding that is in stark contrast to rodent research. However, theta activity in humans during sleep may represent “off-line” reactivation of memories for replay to the neocortex for long-term storage.
1.7 Sleep and Memory

The memory function of sleep may be the only way in which to explain the fact that unconsciousness during sleep leaves an organism incredibly vulnerable (Born, Rasch, & Gais, 2006). Sleep benefits consolidation in children as young as 6.5 months (Simon et al., 2016), but whether sleep provides protection against forgetting or is actively involved in the process of consolation remains debatable (Diekelmann & Born, 2010). New information can be learned during sleep (Arzi et al., 2012). Brown & Robertson (2007) found that both procedural and declarative learning can be blocked by learning the opposing task over a period of wake, but not sleep, suggesting that overlapping systems are involved in the consolidation of both types of memory, and that sleep may confer a benefit in reducing the interfering effects of the second task on performing the first. These processes may overlap, and work based on a pattern of reduced interference during SWS where the brain can then opportunistically consolidate memories since the hippocampus is not occupied with new memory consolidation (Mednick, Cai, Shuman, Anagnostaras, & Wixted, 2011).

SWS/SO coordinates or orchestrates activity across the brain, SWR, delta waves, and spindles. Slow oscillations originate from the transition between dorsolateral and orbitofrontal cortices and sleep spindles are generated in the thalamus. SWOs are a global phenomenon that may be modulated locally, depending on previous waking activity (synaptic homeostasis hypothesis) which is why there is more SO activity in brain regions involved in learning a task before sleep. SWS/SWA is positively correlated with the amount of time spent awake. SWS decreases with age, possibly due to gray matter decline or glial function. Buzsaki proposed reactivation (the so-called Reactivation Hypothesis; Ghorbani & Marshall, 2020; Born, 2010) as a mechanism for system consolidation, with theta oscillations relevant
for during encoding, and SO/SWR/Spindle oscillations relevant for sleep-dependent consolidation. Physiological functions of Slow-Wave Sleep could include energy saving as SWS has active role in the reuptake of ATP, hormone release and regulation, as HPA and SNS axes are down regulated during sleep, while HGH, prolactin, and melatonin is upregulated, immune system support, or for cleaning of metabolites (like B-amyloid proteins). SWS is disturbed in many clinical populations and parasomnias, including somnambulism (sleepwalking), insomnia, sleep apnea, restless leg syndrome, dementia (poor sleep precedes dementia by years), Fibromyalgia, ADHD, and other psychiatric disorders (Léger et al., 2018).

Sleeping following a learning episode changes the functional organization of the brain. Walker et al., (2005) provides evidence that sleeping after learning a motor sequence task results in unique alterations in functional brain activity compared to an awake group. Participants learned a motor skill task, then could sleep, or had to stay awake, before being tested after a 12-hour delay. Subjects who could sleep showed increased activation in right primary motor cortex, mPFC, HC, and left cerebellum, while reductions were observed in parietal cortices, left insula, temporal pole, and fronto-polar regions, suggesting less of an emotional task burden and more automatic processing.

Few studies have looked at the oscillatory activity in EEG during and following learning, and how these dynamics are associated with learning gains relating to sleep consolidation. Mölle, Marshall, Gais, & Born (2004) found a large increase in learning-dependent coherence when data were time locked to the occurrence of SWOs. Specifically, coherence was observed after learning in the slow-oscillatory, delta, slow-spindle, and gamma bands.
Napping may provide as large a benefit as an entire night of sleep in a texture discrimination task (Mednick, Nakayama, & Stickgold, 2003). Quiet restfulness also confers a benefit on memory, and this benefit is related to power in SO-band and a reduction in alpha band activity (Brokaw et al., 2016). Mentation of task related information during a nap is associated with improved performance on a virtual navigation task in humans (Wamsley et al., 2010). Few labs suggest that sleep is not involved in memory consolidation (Vertes, 2004).

The mechanism by which consolidation is thought to occur during sleep appears to be mainly in the form of synaptic plastic changes because of sleep (Maquet, 2001; Walker & Stickgold, 2006), and is dependent on neurotransmitter levels, specifically acetylcholine, where lower levels of the neurotransmitter was critical for consolidation of declarative information (Gais & Born, 2004).

A potential mechanism for memory consolidation during sleep is proposed by Sejnowski & Destexhe (2000). They hypothesize that SWS could be part of ‘recall’ and ‘store’ sequences, where fast oscillations reflect recalled events, which are then stored through SWS processes. Once asleep, thalamic bursts generate spindle activity, thereby driving strong excitatory or inhibitory inputs in cortical neurons. This triggers calcium entry and may facilitate gene expression for long-term synaptic modification for the consolidated information. Synapses tagged by short-term potentiation during fast oscillations are then consolidated by calcium-mediated processes in SWS, where the synchronized firing pattern selects the tagged cells.

*Synaptic Homeostasis Theory*
The synaptic homeostasis model, proposed by Tononi & Cirelli (2003) suggest that wakefulness is associated with synaptic potentiation in several cortical circuits, potentiation is tied to the homeostatic regulation of SWA, SWA is associated with synaptic downscaling, and downscaling is tied to the beneficial effects of sleep on performance. The theory predicts SWA activity in areas that were involved in learning a task would increase after learning during sleep, to downscale the synaptic weights of active areas to baseline levels. REM sleep may provide a polishing of synapses via insertion of AMPA receptors in those synapses that survive downscaling, which could explain the regular alteration of these states. Support for the synaptic homeostasis model comes from Mander, Santhanam, Saletin, & Walker, (2011) where 44 participants learned face-name pairs in two learning sessions, immediately followed by test sessions prior to either taking a 100-minute nap or staying awake. Those in the nap group performed better on the post-nap learning session than the group that stayed awake, suggesting that the nap provided a means of recovery for learning. Further, learning ability was correlated with time in N2 sleep, as well as fast spindle activity over bilateral PFC. There were no changes in performance on a procedural task, however.

Active Systems Consolidation (ACS) Theory

In a review, Navarrete et al. (2020) explains the Active Systems Consolidation (ASC) hypothesis of memory. They suggest that information is consolidated by the interaction between hippocampal and cortical structures. The coordination of ripples (150–250 Hz), thalamocortical spindles (11–16 Hz) and cortical slow oscillations (SO, 0.5–3 Hz) during SWS enable reactivation, plasticity, and stabilization of recently encoded information, while information processed when awake can be encoded both in the hippocampus and in cortex, with temporal and spatial information being stored in the hippocampus and other information
in the cortex. Cortico-hippocampal sharing of information during SWS then helps to reorganize and stabilize recent memory traces. They propose that, “hippocampal replay may be triggered by such oscillatory interactions in cortical reactivation around SOs, and that these mirror cortical activity during learning, initiating the TOP-DOWN link. Notably, cortical reprocessing associated with the BOTTOM-UP process might rely on a different process from that used in this TOP-DOWN link. During SOs, cortical downstates (synchronized neural hyperpolarizations) isolate neural inputs to the prefrontal, entorhinal and hippocampal areas, coordinating the temporal patterns of neural firing that establish connectivity between these regions.”

_Sleep-Dependent Memory Consolidation_

Tucker et al. (2006) had participants who either took naps with NREM sleep but not REM sleep or stayed awake. Results suggest that the NREM nap group participants improved significantly on paired associates but not mirror tracing performance compared to the awake group. Further, SWS correlated with improvement in the declarative (paired associates) task. They conclude that SWS is important for declarative memory consolidation, but not for procedural memory consolidation.

Wilhelm et al. (2011) had 142 adults perform several tasks, including paired associates, object location, and finger tapping tasks. Participants then either slept or stayed awake after learning and were either told that there was going to be a retention test following learning or had the information regarding the retention test withheld. Results suggested that sleep conferred a benefit in paired associates learning, but only in the group who were told that there would be a retrieval test. Waking groups did not differ with regards to paired associates as a function of expectancy. There was an increase in SO and spindle activity in
the expectant group, and that activity correlated with declarative memory performance only in the expectant group. The authors conclude that sleep selectively or preferentially benefits consolidation of information that is relevant for the future in some aspect, likely through SWS and reactivation.

Cordi & Rasch (2021) suggests that the relationships between sleep, specifically SWS, and memory are not clear cut. Often relationships are found in a subset of participants, under varying circumstances, and sometimes the relationships are in the opposite (negative) direction. To address this, they recruited a large sample size (159 participants; 149 female) and had them take two separate 90-minute naps in the laboratory. This within subjects design was used to achieve their goal of investigating intra-individual factors (specifically amount of SWS) associated with sleep dependent memory consolidation. They had participants perform a paired associates task, which was used to measure declarative memory. An additional manipulation, which consisted of either a control text (about mineral deposits) or an intervention (e.g., relaxing music) was played for subjects for approximately 15 minutes before sleep. Results suggested that the percent of SWS achieved in the nap and memory performance was not significantly associated. Also, minutes spent in SWS not a significant predictor of memory either. The authors suggest that while it is possible that a longer sleep episode (an entire night) is important for memory consolidation, intra-individual factors are an important aspect of the sleep-dependent memory consolidation literature.

Gais (2006) had participants learn German-English nouns. Learning occurred either at 8:00 am or 8:00 pm and was either followed by a sleep session or an active day awake, and all conditions were then followed by a second sleep session, then a recall test occurred at either 8:00 am or 8:00 pm. All derivations of learning and recall testing were investigated to
test the effect of time between learning and recall with relation to sleep. The results suggested that recall was better only if sleep immediately or shortly followed learning compared to the other conditions, where learning was followed by an awake period. A second experiment confirmed this, where two groups were tested, where both groups learned at 8:00 pm, then one group could go to sleep immediately while the other group was sleep deprived after learning, but still allowed to sleep after the deprivation period. Both groups were tested 48 hours after initial learning. The deprivation group performed significantly worse than the sleep group, suggesting that sleeping immediately after learning provides the best benefit for memory consolidation.

Genzel et al. (2009) tested participants on declarative (verbal paired associates) and procedural (finger tapping) memory tasks. Participants were then either experienced slow-wave disturbed, REM disturbed, or undisturbed sleep, in a within subjects design. Results suggested that sleep deprivation led to reduction in the relevant sleep stages, but memory performance remained unchanged. An association was found with declarative task performance and sleep spindle activity only in the undisturbed sleep condition. The authors concluded that it is fact spindles, and not SWS, that are important for declarative memory consolidation. This effect is especially related to spindles in the first 1/3 of the night.

Forcato et al. (2020) had participants perform a sound-word association paradigm, then did a targeted memory reactivation protocol during wake or sleep, where either incomplete (only sound with first word syllable) or complete (sound word pairs) were presented to participants. Results suggested that both types of cues labilize memories during wake and both types were good at stabilizing memories over a 40-minute nap at short term testing. However, if the retention interval was extended to 7 hours after sleep only
incomplete reminder cues stabilize memories in the long term. They conclude that incomplete reminders lead to better memory labilization via the mismatch hypothesis. This states that a prediction error (in this case a mismatch between the sound and the prediction of the word for which only the first syllable was presented (incomplete reminder) leads to labilization of memory and stronger reconsolidation.

Chatburn et al. (2021) suggests also that individual alpha frequency (IAF) could be an important factor in memory processes, specifically the generalization of learned rules or information. They had participants learn Chinese-English Paired Associates, and generalization was accomplished via rule-congruence built into the task. A within subjects (early/late sleep and wake control) design was utilized to control for individual differences in a three-night (acclimation, early/late sleep, opposite of night 2 on night 3) sleep and memory study. Results suggest that sleep spindles were associated with memory consolidation, generalization, and transfer from implicit to explicit knowledge. Recognition was associated with an interaction of initial encoding level and sleep spindle density. Generalization was related to the interaction between IAF and spindle density. They conclude that better encoding pre-sleep led to a negative association with spindle density (i.e., more spindles were detrimental). Spindles may be important for generalization, which could explain the effect that well encoded information was less likely to be recalled correctly when many spindles were observed overnight, because the information was schematized. Spindles and IAF interact to produce generalization and higher IAF could be related to increased trace replay.

Jenkins & Dallenbach (1924) had participants learn nonsense words with a sleep session or waking session following. Results suggested that more information was correctly recalled after sleep than wake. They suggest that forgetting is, "not so much a matter of
decay of old impressions and associations as it is a matter of the interference, inhibition, or obliteration of the old by the new."

Tucker & Fishbein (2009) had participants perform paired associates, motor sequence, and digit span tasks in a sleep paradigm where they either slept a half night or full night. The results suggested no difference between half night and full night sleep in paired associates task performance. They conclude that since SWS dominates in the first half of sleep, the results seen suggest that SWS is important for declarative memory consolidation, at least for paired associates learning. In fact, a nap may be as good as a night's sleep.

Levy et al. (1972) attempted to teach Russian-English paired associates while participants slept, and results suggested that participants can learn information while asleep.

Fowler et al. (1973) compared first half night sleep (SWS) with second half night sleep (REM) with a waking control group on verbal and shapes paired associates tasks. Participants in the sleep groups were awoken in Stage 2 for learning in the SWS group, or four hours after Stage 2 sleep for learning in the REM group. The results suggested that first half night (SWS) was essential for verbal but not shape paired associates, while the second half night showed better memory performance than the awake group. These results cannot be interpreted as sleep merely sheltering information from interference, as subjects slept the same amount of time in the two conditions, just one experienced more SWS than REM and vice versa for the other. Both sleeping conditions were better than waking control.

Castaldo et al. (1974) had participants learn consonant-consonant-consonant trigrams and verbal paired associates before sleep, then were tested upon waking. They found no effect of sleep variables, including REM or Delta sleep (SWS) on memory. In a separate experiment, no effect of a pharmacological intervention (imipramine hydrochloride, a
Fenn & Hambrick (2015) gave a cognitive battery including a verbal paired associates task to participants, where the criteria for learning was set at 60%. There was a waking group trained in the morning and tested in the evening, and a sleep group that was trained in the evening, allowed to sleep at home, then tested in the morning after sleep. Results suggested that the wake group showed memory loss compared to the sleep group for paired associates. Further, intelligence correlated with performance in the sleep group but not in the wake group. The authors then suggest that since intelligence is correlated with sleep variables, such as spindles, this may be the moderating relationship observed in the sleep group. However, they did not control for long-term memory performance, so that could be driving the effect (i.e., it may not be intelligence per se, but rather something else).

Tucker & Fishbein (2008) gave participants several tests including a non-associative paired associates task, a maze learning task, and the Rey-Osterrieth complex Figure training (with or without test) in a nap paradigm. Results suggested that compared to subjects who stayed awake during the training-retest interval, subjects who took a NREM nap demonstrated enhanced performance for word pairs that were tested during training, but not for untested word pairs. For all tasks, participants who most strongly acquired the information during learning showed the biggest sleep-dependent performance enhancement. They suggest then that NREM sleep benefits declarative memory performance, but that this relationship depends on strength of initial task acquisition during learning. However, they tested the subjects in the nap group two hours after waking so there was potential for interference to occur, which may have influenced these results.
Potkin & Bunney (2012) had 10-14-year-old participants perform paired associates and working memory tasks. Sleep benefited paired associates learning by 20.6% but had no effect on working memory task performance. They conclude by suggesting that your mother was in fact right, when she told you to make sure to get a good night’s rest.

Sleep and Memory Interference

In a review, Ellenbogen et al. (2006a) sought to answer sleep-dependent memory consolidation questions, like does sleep provide passive protection for memories, permissive consolidation, active consolidation, or is it not related to memory consolidation at all? They point out that there is little support from the literature that sleep does nothing regarding memory consolidation. Passive protection suggests that sleep merely shelters information from being modified by the process of being unconscious, but several studies have shown an increased protection from interference even while awake, following sleep. Sleep therefore does not seem to passively protect information. The permissive consolidation hypothesis states that sleep passively allows innate consolidation processes to occur unabated, or without interfering information, much like becoming intoxicated after learning will help with consolidation. The active hypothesis seems to have the most support, coming from animal literature (replay), spindle activity being correlated with memory performance, and split night studies, among other literature.

Norman (2006) suggests that sleep may play a role in reinforcing and protecting knowledge. Sleep may also be able to repair or reorganize broken or disorganized memories into coherent stable ones. It could be the case that by sleeping, memories are strengthened, (or at least not weakened) by interfering information due to simple the lack of input to the brain during sleep. However, this does not appear to be the case. Sleep tunes memory traces
so they are more coherent and likely to be retrieved later. Sleep could also clear out contextual information from the memory, allowing it to be reactivated in different contexts, thus strengthening the trace.

Interference paradigms are distinguished by whether the words in each list are unique. In the so-called AB-AC paradigm, also known as associative interference, the “A” word in list AB is re-paired with “C” words in the AC list. In the so-called AB-CD paradigm, also known as non-associative interference, each word-pair list is unique. Proactive and retroactive interference effects are present for both kinds of interference paradigms (Sheth et al., 2012). Learning new information interferes with previously learned information, and previously learned information interferes with learning new information (Unsworth et al., 2013).

In Sheth et al. (2012), participants performed a paired associates task in a series of experiments utilizing either associative or non-associative interference paradigms after participants either slept or stayed awake after learning. The results suggested that sleep following learning protects from both associative and non-associative types of interference, but no difference was found between associative and non-associative interference in terms of sleep protection of one over the other. Finally, sleep specifically protected weakly encoded memories from retroactive interference. The authors suggest that perhaps weakly encoded memories are preferentially reactivated during sleep somehow, which explains the effect of sleep on retroactive interference only for weakly encoded memories. They go on to suggest a more passive mechanism where the protection seems to be more general and less trace specific because of the non-associative interference protection observed. The authors pit active (reactivation systems consolidation) against passive (memory isolation) consolidation
frameworks, where memory isolation is the idea that sleep renders the encoded information inaccessible for reactivation unless specific contextual cues are provided.

Ellenbogen et al. (2006b) had participants perform a verbal paired associates test in an interference paradigm. They first learned list AB, followed by 12 hours of sleep or wakefulness. Each group was then split into interference groups, where one group learned a new list (AC), in an associative interference AB-AC paradigm, while the other group did not. They were then tested on list A-B. Learning criteria for both lists was set at 100%. The results suggested that the sleep group overall had slightly better memory performance compared to the wake group in the no interference condition. However, in the interference group, a highly significant difference was found between the sleep and wake groups, where the sleep group had much better memory performance. The authors conclude that sleep protects from associative retroactive interference.

Alger et al. (2012) had participants perform a bimodal paired associates task (word pairs paired with sounds) in an interference paradigm. The learning criteria was set at 75% for the test list and 90% for the interference list. Participants first learned the test list, comprised of 30-word pairs, then either took a nap or stayed awake watching nature videos. There were three total groups: a wake group, a 10-minute nap group, and a 60-minute nap group. They were then tested on the first 10-word pairs from the 30, then learned the interference task, and were tested on the second 10 (and the interfering words), then came back a week later for the final 10 pair test. The results suggested that at the immediate test before interference, the wake group performed the worst, the 10-minute nap group performed at an intermediate level, and the 60-minute nap group performed the best. In the interference test, the profile was similar, but a large effect was observed for the 60-minute nap group
compared to the other groups. In the long-term test, there was no difference between wake and 10-minute nap groups, but the 60-minute nap group performed significantly better than the other two groups. EEG measures did not correlate with any behavior (total sleep time, SWS or REM, etc.). The authors concluded that SWS is important for the protective benefit of sleep on retroactive interference and on long term memory formation, as little or no SWS was evident in the 10-minute nap group but was in the 60-minute group.

Ekstrand (1967) had participants perform a verbal paired associates task in a 3x2 (no interference, proactive interference, retroactive interference) x (sleep, wake) design. Results suggested that sleep conferred a benefit for the no interference condition, and protected from both proactive and retroactive interference, but even more so for retroactive. The author concludes that sleep produced spontaneous recovery of extinguished 1st list responses.

McDevitt et al. (2015) used a visual texture discrimination task with two sets of stimuli (A-B and C-D) in a non-associative interference paradigm (high interference, moderate interference, low interference) in four participant groups (awake, 75-minute quiet rest, 60-minute nap, 90-minute nap). EEG/PSG measures were also recorded. Results suggested that the low interference group showed the greatest amount of learning. Some learning was obtained in proactive interference and no learning was obtained with retroactive interference. Overall, REM naps led to less interference. While NREM sleep rescued from moderate proactive interference, REM sleep rescued information from high retroactive interference, which could be related to cholinergic transmission during REM sleep. They posit that high information input and high plasticity during low interference leads to increased signal in a smaller network of neurons, improving memory, while in moderate interference, reduced information input and low plasticity in NREM are sufficient to resolve
moderately damaged memories, but the unique low information/high plasticity environment of REM sleep is necessary to rescue severely damaged memories to reconstruct target templates.

Schönauer et al. (2014) administered a cognitive battery, including a city map, vocabulary, remembering objects, phone numbers, details of a story, and signs to participants. Verbal/non-verbal, single/associates, recall/recognition, free recall/cued recall were all manipulated in a within subjects design where participants either napped, stayed awake, or did a mindfulness meditation (which was supposed to emulate a reduced interference condition that was not due to sleep). Results suggested that all types of memory were enhanced by sleep, however EEG measures was not associated with memory enhancement in the sleep group. There was no difference between the mindfulness (low waking interference) condition and active wakefulness (high interference condition), suggesting that sleep related consolidation processes are not merely a function of reduced interference, as the sleep group showed superior memory compared to waking groups. Lastly, there was no effect of preferential consolidation on any of the cognitive battery subscales (which were presented in the same order across participants), suggesting that sleep protected from retroactive interference.

Deliens et al. (2013a) used a modified AB-AC interference paired associates task, where half of the list AB words were paired with items in the AC list, while the rest were unique. Participants learned list AB, and then either took a nap or stayed awake, then learned list AC, and finally were tested on list AB in a within subjects design separated by one month. Results suggest that sleep, specifically naps with SWS, protected from retroactive
interference. They conclude that SWS, but not REM, is important for protection from post-sleep retroactive interference.

Drosopoulous et al. (2007) had participants perform a verbal paired associates task with interference in four groups: a sleep group, a wake group, an immediate recall group, and a delayed recall group. First, 20-word pairs were learned to 90% criterion. Participants then played a distractor game for 15 minutes, then the interference list was learned in an AB-AC associative interference paradigm. In a second experiment the strength of encoding was manipulated in learning the lists, in a non-associative AB-CD interference paradigm. In the intense encoding condition, feedback was displayed for longer, and participants had to reach 90% performance. In the weak encoding condition, feedback was shorter, and only 60% performance was required. Results from the first experiment suggested that after sleep list AB performance was better than in the waking condition. However, list AC showed no effect of sleep. Further, there was no effect of immediate or delayed recall. Experiment 2 results suggested that strongly encoded word pairs were better remembered, however only in the sleep group was there an effect on weakly encoded information, suggesting that sleep preferentially benefits weakly encoded information. It is important to note that in all conditions, participants recalled fewer words at test than encoding. The authors suggest then that sleep is associated with declarative memory consolidation and may preferentially benefit weakly encoded information, regardless of why the information was weakly encoded (interference vs encoding strength).

Further evidence of encoding strength effects comes from Pöhlchen et al. (2021), where participants performed a paired associates task in an AB-AC interference paradigm. Participants either slept or stayed awake following AB list learning. A second experiment
had participants take a nap instead of sleeping a full night. Participants were trained to 100% correct recall on list AB and AC. Results suggested no protective effect of sleep (either nap or full night) on interference. In fact, retroactive interference was only seen after sleep but not after wake. They suggest that subjects rapidly encoded words into the neocortex (criterion set to 100%), and thus sleep consolidation was not necessary. They suggest that only weakly encoded information would benefit from sleep dependent memory consolidation processes.

Deliens et al. (2013b) had participants perform an associative AB-AC paired associates interference paradigm, where half of list AB was interfered with by AC. The design was within subjects with a two-week interval - one post-training sleep, one post-training sleep deprivation. All participants could sleep normally for two additional nights before testing. Participants were split into evening and morning groups to account for time-of-day learning effects. Results suggested no evidence for protection from retroactive interference after sleep. There was an interference effect observed in the sleep condition but not in the sleep deprived condition. They conclude that reactivation makes information labile again, and the AC associations interfered with AB associations learned before, and consolidated during, sleep. They say in fact that sleep may set memory traces in a state sensitive to updating through reconsolidation processes. There were however several methodological differences in this work that could account for the observed effect, which is incongruent with most of the literature. A within subjects design was used, which controls for intra-individual differences. Participants were all allowed two recovery nights of sleep, so perhaps that allowed for other consolidation processes to occur, leading to the observed
results. Finally, this study also lacked a no interference condition for comparison with the other groups. Further research is needed to understand the temporal dynamics of interference.

Abel & Bäuml, (2014) had participants perform a paired associates task with an AB-AC interference design with four groups: AM control, PM control, 12-hour wake, 12-hour sleep. Control groups experienced both proactive and retroactive interference. Results suggested that overall, sleep led to better memory than wake, and sleep showed no retroactive interference effect like was shown in the waking condition. Proactive interference was observed in both 12-hour conditions (sleep and wake), but sleep showed less interference than wake. The authors conclude that sleep reactivates memories and stabilizes them overnight, making them less likely to be interfered with, either proactively or retroactively.

1.8 Augmentation of Memory Consolidation During Sleep

Augmentation of memory consolidation during sleep has previously been explored using pharmacological methods (Feld et al., 2013; Gais & Born, 2004; Mednick et al., 2013; Schredl, Weber, Leins, & Heuser, 2000) and auditory stimulation (Cox et al., 2014; Marshall, Helgadóttir, Mölle, & Born, 2006; Lafon et al., 2017; Leminen et al., 2017; Ngo et al., 2013b; Ngo et al., 2019; Ong et al., 2016; Papalambros et al., 2017; Papalambros et al., 2019; Weigenand, Molle, Werner, Martinez, & Marshall, 2016; Zhou et al., 2012).

Pharmacological

GABAergic activity contributes to the generation of SWS, and GABA agonists typically enhance SWS and SWA, though also reduce spindle activity. Feld et al. (2013) investigated the effect of the GABA reuptake inhibitor tiagabine on sleep parameters and memory consolidation in 14 healthy male participants during two overnight experimental sessions (one placebo, one tiagabine) separated by at least 14 days, while EEG data were
collected. In both conditions, participants performed an emotional picture task, a PAT to measure declarative memory, and a finger tapping task to measure procedural memory. Performance did not differ in the emotional picture task, nor in the PAT before and after sleep for the experimental versus control conditions, however, the procedural task showed a decrement in performance after the experimental night even though participants in this condition spent more time in SWS and REM sleep compared to the control condition. Further, there were no differences in fast or slow spindle overall power between groups. Fast spindle activity was reduced following the negative half-wave peak at electrode Cz. This suggests that modulation of spindle parameters is what could be driving the behavioral effects seen in other studies, as data suggest that the synchronization of fast spindle activity to the up state of the SO is necessary for consolidation.

Mednick et al. (2013) manipulated both sleep spindles and REM sleep with various doses (10mg, 5mg) of the GABAa agonist zolpidem, which has been shown to modulate sleep, specifically spindles and SWR, (3g, 2.5g) of the GABAb agonist sodium oxybate, which does not pharmacologically alter sleep, or placebo in 49 healthy young participants over the course of two experimental nights separated over 5-10 days in a within-subject crossover design. Subjects went to sleep at 23:00h, and were awoken at 05:00h, and either allowed to watch TV or were given cognitive tests. Next, the drug or placebo was administered, and they could sleep for 90 additional min. The hypothesis was that REM sleep would be heavier, due to circadian influences, and that the drug would lead to increased SWS and spindle activity, and reduced REM. Cognitive tasks included PAT, a texture discrimination task, and a motor sequence task. Results suggest that zolpidem increased both fast and slow spindle count and density over fronto-central regions, as well as improved
declarative memory recall, reduced visual texture discrimination scores, and produced no change in motor sequence learning, compared to sodium oxybate and placebo. Further, spindle density was correlated with improvement in paired associates recall.

Another study, done in 10 healthy elderly adults, suggests that augmentation of REM sleep is associated with improved performance on a declarative word-list learning task, not just procedural memory tasks, as is suggested by others. Donepezil was administered to older, healthy adults prior to sleep over several nights. Donepezil is an acetylcholinesterase inhibitor, thought to enhance REM sleep. Following the administration of the drug, participants spent more time in REM sleep, and performance on the word-list learning task was correlated with amount of time spent in REM sleep during the nights where the drug was administered. (Schredl, Weber, Leins, & Heuser, 2000).

Gais & Born (2004) showed that acetylcholine level during SWS is crucial in memory consolidation. Twenty-nine participants learned a PAT, then were either allowed to sleep for 3 hours or remained awake. Physostigmine, a cholinesterase inhibitor was administered, and after waking (or 3 hours for the wake group) a recall test was performed. Drug infusion led to an impairment only in sleep subjects, leaving waking subjects’ performance unaffected, whereas a placebo group showed an improvement in declarative memory after the sleep interval. This suggests a direct role for acetylcholine in hippocampal-dependent memory consolidation during sleep.

Targeted Memory Reactivation

Targeted memory reactivation (TMR) involves the pairing of external stimuli (sound tones, smells, etc.) with target information during learning, then presenting the stimuli back during sleep and is thought to facilitate reactivation of the memory trace, leading to improved
consolidation and recall following sleep (Rudoy, Voss, Westerberg, & Paller, 2009). It could potentially be used to facilitate erasure of painful or maladaptive memories, in post-traumatic stress disorder (PTSD), for example (Oudiette & Paller, 2013). The degree to which TMR is effective depends on the phase of SWO when the cue is replayed during sleep (Batterink, Creery, & Paller, 2016). Diekelmann, Buchel, Born, & Rasch (2011) showed that reactivation of memory during SWS stabilized memories, using an interference task, compared to waking reactivation. TMR benefits visuo-spatial learning in a short (40 minute) nap, like performance after a non-cued longer (90 minute) nap (Diekelmann, Biggel, Rasch, & Born, 2012). Targeted sounds result in activation of right parahippocampal cortex, and post-sleep memory accuracy was associated with increased activation of thalamus, bilateral MTL, and cerebellum. Improvement in memory due to TMR was associated with increased functional connectivity between parahippocampal-medial prefrontal areas (Dongen et al., 2012).

Batterink & Paller (2015) investigated effects of TMR on linguistic rule learning. Forty-four participants were presented with nonsense monosyllabic words arranged in a hierarchical artificial structure, as well as passive exposure to a probabilistic tone sequence. Subjects then took a 90-minute nap, during which grammar cues or tone sequence cues were presented in SWS. Results suggest that subjects who were cued with the grammar information during SWS were more accurate in the test phase post-nap compared to subject who were cued with the tone. However, they did not include a waking control condition or a no-cue condition, which would have allowed for a more precise estimate of the effect. These results suggest that it is possible to enhance the extraction of abstract rules more efficiently by using TMR during sleep. Similar results have been found using button presses (Cousins et
al., 2014), musical melodies (Antony et al., 2012), and tones in spatial navigation in rats (Bendor & Wilson, 2012). Interestingly, Bendor & Wilson (2012) found that presentation of the auditory cue during SWS caused place cells in the hippocampus to fire more frequently to a cued direction compared to an uncued direction. Further, the number of replay events did not differ between the control and cue conditions, indicating that the content of the replay was the only thing affected by the cue, not the frequency of replay events.

Rasch, Buchel, Gais, & Born (2007) presented a rose odor to 18 subjects while they learned the spatial locations of 15 item pairs. They then presented the odor or a vehicle control again during the first two periods of SWS, which led to improved consolidation and recall following sleep. This effect was associated with increased activity of the left anterior and posterior hippocampus, measured with fMRI. Presenting the odor alone during SWS did not influence retention. Using TMR during a waking condition did not have an effect and delivering the odor during REM sleep did not affect performance on a procedural memory task. However, subjects were not exposed during N2 sleep, when sleep spindles are most robust and have shown to be correlated with consolidation. Doing so would have proven to be a compelling dissociation of consolidation processes during N2 sleep and SWS.

TMR depends on several factors, including emotional salience, motivation to remember, amount of prior learning, pre-sleep memory strength, and mental state, like being fatigued or well-rested (Nadel, Hupbach, Gomez, & Newman-Smith, 2012). Using TMR during SWS, Creery, Oudiette, Antony, & Paller, (2015) investigated performance in an object location paradigm (Bridge & Paller, 2012) with 20 participants while EEG was recorded. Associated sound cues were played during SWS for half (25) of the studied object, and object locations were tested after waking. Results suggest that cueing overall had no
benefit in terms of accuracy in the post-sleep test. However, for those items that were more accurately recalled in the immediate test prior to sleep, TMR provided a benefit. Cueing provided a benefit only for those items that were neither very poorly recalled, nor perfectly recalled, suggesting that once a certain level of learning is achieved, reactivation occurs spontaneously, and cueing no longer provides an enhancing benefit. The cueing benefit was correlated with relative delta band power, and more efficient learners had more delta band power than less efficient learners. Although correlational in nature, this result suggests the possibility to investigate other manipulations during encoding and wake that have a synergistic effect with augmentation of sleep. These could include manipulating the amount of learning, by invoking the principles of the testing effect, manipulating the emotional salience of information, pairing neutral information with emotionally salient stimuli, changing reward value of remembering, or applying tCS. Perhaps this effect is why not all sleep augmentation experiments show effects on memory consolidation.

Determining what information is tagged and replayed during sleep, the subsequent effect of this on lasting memories, and how targeted memory reactivation could modulate these processes are questions that have not yet been answered. Oudiette, Antony, Creery, & Paller (2013) provided some evidence to suggest that TMR may interact with waking assumptions about information to lead to enhanced consolidation. Sixty young adults participated in a study, where object locations for 72 items were presented. Each item was assigned a value, corresponding to the future payoff of remembering the item; some were low value, others high value. Each item was always associated with a sound during the learning phase of the study. Subjects then either took a nap or stayed awake for 90 minutes. During the nap, associated sounds were delivered for half of the low value items. In two
additional experiments, the learning phase was the same, but subjects stayed awake during the subsequent 90 minutes and either watched a documentary movie, while sounds were played during learning and testing only, or a difficult working memory task, where sounds were played during learning and while performing the working memory task. As expected, high value items were better remembered than low value items across experiments. TMR during SWS saved the cued low-value items from being forgotten compared to the non-cued items.

Rasch et al., 2007 found that visual memories can be improved through targeted memory reactivation, and Schreiner and Rasch, 2014 found that verbal memories can also be strengthened through TMR. Further, it can enhance motor skills (Antony et al., 2012), bolster fear extinction (Hauner et al., 2013), and modify social biases (Hu et al., 2015).

Lerner et al., 2019 used tDCS and tACS in a unique pattern as a targeted memory reactivation method to improve recall of sequential events. They used tDCS Spatio-Temporal Amplitude-Modulated Patterns (STAMPs, see Pilly et al., 2020) during training of a 3-D virtual environment search task, and alternated between closed-loop tACS (1.5 mA/hemisphere) and 2.5 mA tDCS STAMPs during slow wave oscillation up states. They found that the number of stimulation events during SWOs was related to performance change on items involving insight ($F(1,58.19) = 5.01$, $p = 0.029$), and stimulation was not related to an increase in memory consolidation of rules in the task.

Pilly et al. (2020) used STAMPs to tag individual memories during encoding in a VR episodic memory task. Participants were shown short videos of scenarios (e.g., a fire breaking out in a building after a man with blue pants ran in and out). Individual scenarios were tagged, then the same STAMPs applied during learning were also applied during up
states of the SO (“cueing” the memory for reactivation and consolidation during sleep).

Participants were then tested several times at various intervals on the information encoded from the scenarios (in the above example, an image of several characters would be presented, one with blue pants, and the participants would be asked which character ran out of the building prior to the fire starting). Results suggest that scenarios for which tags and cues were applied showed better metamemory by participants 48 hours after learning.

**Augmentation of SWS with Exteroception**

Simply rocking a participant while asleep can facilitate the enhancement of power in the SO frequency band. Bayer et al. (2011) placed 12 male participants either in a stationary or slowly rocking bed (.25Hz) during dual 45-minute afternoon naps in a within-subjects design. Slowly rocking participants facilitated the transition from waking to sleep, led to an increased duration of N2 sleep, and boosted slow oscillations and sleep spindles compared to the stationary bed condition.

The phase at which external stimuli are presented has a marked effect on how stimulation during SWS may lead to a memory performance improvement. Weigenand, Molle, Werner, Martinez, & Marshall, (2016) delivered quasi-phase-dependent open-loop auditory stimulation to 26 healthy participants during SWS. SWA increased because of the stimulation, whereas both fast and slow spindle power decreased, however memory performance on a PAT failed to show a benefit of SWA enhancement.

Ngo et al. (2013b) used closed-loop feedback auditory stimulation to induce slow-wave trains and enhance power in the SWO frequency band. Eleven participants spent two experimental nights in the laboratory while receiving either phase-locked auditory stimulation during non-REM sleep, or no stimulation. Two auditory stimuli, consisting of 50
ms of pink noise, was delivered during the predicted up-state of the SWO. Compared to sham, the auditory stimulation resulted in enhanced slow oscillatory activity, as well as in propagation of the SO. Stimulation resulted in increased power in the SO band (0.5-1.0Hz), but a reduction in overall delta band (0.5-4.0Hz) power. In addition, participants performed a PAT (Marshall, Helgadóttir, Mölle, & Born, 2006), to assess the effect of stimulation on declarative memory consolidation. Percentage of time spent in SWS was significantly correlated with retention of word pairs only in the active group, suggesting that entrainment of SO during SWS is critical for improved performance. Stimulation enhanced spindle activity in both slow and fast spindle frequency bands, a phenomenon also found in Ngo et al., (2019). Stimulation led to an enhancement of the SO slope, with a concomitant decrease that was greater than in the sham group. Though the authors failed to speculate on this finding, this may suggest spindle efficiency, given the relationship between spindle activity and memory performance observed. No differences in sleep architecture were evident between conditions, however, only two electrode channels C3 and C4, were used for sleep scoring, which is a vast departure from the sleep literature, where typically 10 channels are used.

In Ong et al. (2016), participants learned 40 semantically related word pairs prior to taking a 90-minute nap. During this nap, oscillatory activity was enhanced by presenting blocks of 5 tones, which were phase-locked to the up-state of the SWO. Upon waking, an immediate test of the word pairs was given, followed by a delayed test 45 minutes later. Results suggest that the auditory augmentation changed SWO spectral frequency characteristics, by increasing amplitude, theta band activity, and fast spindle activity.
Additionally, the experimental group showed an attenuation of forgetting of word pairs compared to the control group.

A closed-loop auditory stimulation study was conducted in healthy older adults by Papalambros et al. (2017). Participants wore headphones designed for sleep that either delivered trains of 5, 50 ms pulses of pink noise during the up-state of the SWO during SWS or no sounds during sleep in a within-subject design. They were tested on a PAT before and after sleep. Recall was superior in the stimulation condition compared to sham. SWA and spindle activity was increased in the stimulation group. Interestingly, the degree to which participants benefitted from the stimulation was related to the phase at which the stimuli were delivered. Participants were divided into performance groups, and the group that benefitted the most had, on average, more tones presented to them closer to the up state of the SO, defined as 360º (352.1º) compared to the lower performing group (342.3º) providing compelling evidence that the phase at which stimulation is delivered is a crucial aspect of enhancement.

Cox et al. (2014) presented realistic sounds (dog barking, traffic noise, etc.) to participants during SWS using a closed-loop prediction algorithm to identify SO phase. Twelve subjects slept from 2-2.5 hours, during which time white noise was presented at a constant rate. The prediction algorithm identified the phase of the SWO, and sounds were played either during up or down states. Sounds from the up and down states were paired, and participants were then tested after the nap in a forced-choice manner. They were asked which sound of the pair was familiar to them. Results suggest an increase in spindle power for up-targeted stimuli compared to down-targets across most of the scalp, roughly 70% of channels. There were differences in processing the stimuli, as evidenced by unique time-
frequency and event-related potentials for the up and down-targeted stimuli. However, these electrophysiological differences did not manifest in behavioral outcomes, that is, sounds presented in the up-state were not reported as being more familiar than those in the down-state, either compared to each other, or to novel sounds only presented during waking.

Debellemaniere et al., 2018 aimed to enhance SO during N3 sleep slow oscillations using an in-home wireless dry-electrode EEG device for auditory closed-loop stimulation. They found that this pink noise stimulation was associated with an increase of 43.9% of delta power within 4 seconds following the first stimulation event, and continued through the last stimulation event, where there was an average increase of 11.8% of delta power. Additionally, stimulation effects were consistent when delivered across 10 consecutive days with no adaptation effect detected.

Shimizu et al., 2018 used closed-loop auditory stimulation during sleep to enhance learning in a virtual reality navigation task. They targeted the transition between down-states and up states of SOs during N2 and N3 as well as during the task. This stimulation was related to a decreased testing time following the nap and improved performance on their overall testing scores compared to the control group.

Schneider et al. (2020) had participants (older adults versus younger adults) perform a paired associates task, a finger tapping task, and a picture encoding task in a within subjects verum stimulation and sham closed-loop auditory paradigm during sleep. Acoustic trains were delivered at the SO up-state. Whereas performance improvements were seen in younger adults, this effect was not observed in older adults. In fact, declarative memory was impaired with verum stimulation in older adults. Amplitude of the SO was not modulated by verum stimulation in old adults like was seen in younger adults. Finally, the profile of spindles and
SOs were different in older adults compared to younger adults. They suggest that the ageing brain is not susceptible to modification like the young brain, maybe due to smaller engaged cortical populations or reduced thalamo-cortical connectivity due to prolonged cell refractoriness. Or perhaps the timing or other parameters of the stimulation procedure need to be adjusted for the older brain.

1.9 Electrical Stimulation During Sleep

The use of tDCS or tACS during sleep has been shown to improve memory performance in various ways, including recall (Marshall et al., 2004, 2006), and encoding (Kirov, Weiss, Siebner, Born, & Marshall, 2009). It could be a rehabilitation tool for those afflicted with sleep disorders, such as chronic insomnia (Saebipour et al., 2015), or epilepsy (Berenyi et al., 2012; Del Felice et al., 2015a,b), or for children with attention deficit hyperactivity disorder (ADHD, Prehn-Kristensen et al., 2014). In a recent meta-analysis, Barham et al. (2016) provided evidence that electrical stimulation during sleep improves declarative memory, while leaving procedural memory unchanged, with an average effect size (SMD) of .447 for declarative memory enhancement, and -.476 for disruption, both of which are significant. The enhancement of SWS appears to be particularly important for the effects, where these procedures drive the brain from electrodes placed on the scalp, mainly over bilateral dorsolateral prefrontal cortex (DLPFC; Marshall et al., 2004, 2006), or using transcranial magnetic stimulation (TMS; Massimini et al., 2007), leading to an increase in nearly global SWA, whereas median nerve stimulation during SWS led to an increase in SWA that was restricted to somatosensory and motor cortices (Tononi, Riedner, Hulse, Ferrarelli, & Sarasso, 2010), suggesting targeting of specific brain regions is possible if necessary.
Encoding Before Sleep

Ambrus et al. (2015) had participants perform a verbal paired associates task while 140Hz (ripple frequency) Bilateral DLPFC tACS was administered during encoding. The study was a counterbalanced, fully crossed within subjects design, so participants received both verum and sham tACS. Performance was tested 10 minutes after encoding, then again in the morning after participants went home to sleep. Results suggested that participants in the verum condition did not forget overnight, whereas in the sham condition, there was a significant amount of forgetting overnight. An additional analysis included only who were >60% performers, and these results showed that verum stimulation led to enhancement of performance overnight (~1 word extra remembered), whereas the sham group forgot about the same amount (~1 word on average). The authors conclude that stimulating at the ripple frequency could help with hippocampal reactivations and strengthen encoding. They say that this could be due to reduced inhibition of stimulated areas which led to an enhanced exchange of information between subcortical structures at night, resulting in improved consolidation. Or perhaps led to increased synchronization between hippocampus and cortex, which enhanced consolidation.

Sleep Spindles

Lustenberger et al. (2016) demonstrated a functional role for fast sleep spindles in motor, but not declarative, memory consolidation using feedback controlled tACS (FB-tACS) during detected spindle (11-16Hz) events throughout sleep. 16 male participants underwent the procedure: a crossover, counterbalanced, within-subjects design, where they either received active tACS (1.0 mA), or sham (no current) stimulation over the course of two nights. Spindle activity was significantly enhanced after the active night of stimulation,
but only in N2, while delta and theta power in N2 and SWS were reduced. Motor memory, assessed by a motor sequence tapping test, was improved after stimulation compared to sham, however, no improvement was observed in a PAT after spindle-enhanced sleep. Fast spindle activity in both groups was negatively correlated with response time, suggesting a functional role for fast spindles in motor memory consolidation.

Sleep spindles have also been targeted with stimulation in clinical populations, including temporal lobe epilepsy (TLE). Del Felice et al. (2015a, b) applied SO-tDCS (frequency: 0.75 Hz, amplitude: 0.0 – 250 µA) in a fronto-temporal setup (electrode locations F8-T8 or F7-T7) over the discharging hemisphere for a total of 30 minutes (6, 5-min intervals) during N2 sleep. One group received active stimulation during wake and sham during sleep, while the other group received the opposite in a randomized, cross-over design. Declarative memory was assessed with the Rey Auditory Verbal Learning Test (RAVLT), and visuospatial memory with the Rey-Osterrieth Complex Figure Test. Results suggest that total sleep time was increased in the active condition, and current density of slow spindle generators was increased, as was declarative memory performance, compared to sham. They suggest that electrical stimulation could be a potential memory rehabilitation tool for those afflicted with seizure disorder.

**Slow-Wave Sleep**

Marshall, Molle, Hallschmid, & Born (2004) administered 0.26mA/cm² anodal tDCS over bilateral fronto-cortical electrode sites during wake (n =12) and separately during SWS-rich sleep stages (n = 18) repeatedly for 30 minutes in 15-second on/off cycles. Results suggest that declarative memory retention was superior in the active group only when stimulation was delivered during sleep compared to a placebo stimulation group; there was
no effect for the waking cohort. Learning in the active sleep group was marginally better than
the wake group. Procedural memories, assessed by a mirror-tracing task, were not affected by
SWS augmentation with tDCS. They also found that participants’ moods were improved both
after tDCS during sleep as well as in waking periods. Finally, they found that slow wave
activity (< 3Hz) was enhanced, and higher frequency activity, including theta, alpha, and beta
band activity, was reduced.

Marshall, Helgadottir, Molle, & Born (2006) used open-loop tACS on medical
students during SWS. A 0.75 Hz (maximum current density: 0.517mA/cm²) sine wave was
applied over bilateral frontal cortex in 5, 5-minute intervals after subjects had entered SWS.
Cognitive tasks included PATs, and tests of mirror tracing and finger tapping. The
enhancement of SWS with tACS led only to an improvement in the verbal PAT, however no
benefit was observed for the non-verbal PAT, suggesting that this intervention is specific to
verbal memory. No sleep-related improvement in performance was observed for the
procedural memory tasks. More time was spent in SWS after tACS compared to sham,
whereas the rest of sleep architecture did not differ between groups. Stimulation enhanced
EEG power within the slow oscillation (0.5–1.0 Hz) and slow spindle frequency (8-12 Hz)
bands at electrode Fz. To dissociate the effect of slow oscillation enhancement on learning, 5
Hz theta stimulation was applied during SWS. Compared with sham stimulation, theta
stimulation reduced slow oscillation power and frontal slow-spindle power and did not
benefit word-pair learning.

In a follow-up study investigating the effect of theta-band stimulation over DLPFC
during either stable NREM sleep or during REM sleep, Marshall, Kirov, Brade, Molle, &
Born (2011) found that stimulation during NREM in this frequency range disrupted the
amount of time spent in SWS as well as increased time spent in lighter, N2 sleep, reduced power in SWA and spindle bands, and produced an impairment in declarative memory consolidation, assessed with a PAT, while leaving performance on procedural tasks unchanged. Theta-tDCS during REM sleep did not affect sleep architecture, increased global gamma power, and affected beta band power.

This effect is apparent even after a 90-min nap. Antonenko, Diekelmann, Olsen, Born, & Molle (2013) delivered 0.25 mA or sham transcranial slow oscillation stimulation (tSOS) during two naps in 15 participants, separated by a month, after a learning phase in several cognitive tasks, including a PAT. Time spent in SWS, fast and slow spindle power, and an improvement in recall in the PAT were observed in the active stimulation condition compared to sham, where no improvement was observed in a procedural memory task.

Westerberg et al. (2015) delivered tACS on older adults during SWS in a 90-minute nap. Bilateral 0.75 Hz tACS (0.0 – 260 µA) was administered over lateral frontal cortices for the active group, and no stimulation was administered for the sham group. Results suggest that SWO amplitude was higher in the experimental group, and this group showed a larger improvement in word-pair recall compared to the sham group. Results in this sample are inconsistent (Ladenbauer et al., 2016), but incongruent experimental procedures may be responsible.

This effect has also been demonstrated in rats. Using analogous methods to studies in humans, Binder et al. (2014) trained rats in an object place recognition task, a hippocampal-dependent one trial learning task and applied trapezoidal SO-tDCS during SWS (frequency: 1.33-1.5Hz, amplitude: 0.0 - 9.0 µA). Results suggest that preference for the displaced object
was only above chance level for the stimulation group. This effect was apparent in 9 out of 12 animals tested.

There is also evidence that stimulation during SWS can accelerate sleep homeostasis. Synaptic downscaling observed during SWS has been posited as a mechanism by which the brain maintains computational efficiency by increasing the signal to noise ratio. Reato et al. (2013) stimulated subjects during SWS bilaterally for 25 minutes over DLPFC with a 0.75 Hz trapezoidal DC waveform (0.0 – .26 mA), which led to a slowing of the decay of SWO, suggesting a putative mechanism for the cognitive enhancement of SWS augmentation with electrical stimulation.

The findings discussed above are not without controversy, and have not always been replicated (Sahlem et al., 2015). For example, Eggert et al. (2013) failed to show a benefit of slow-oscillatory tDCS (SO-tDCS) on declarative memory, assessed via a PAT, in 26 elderly adults. Subjects spent less time in SWS and more overall time awake in the stimulation condition compared to sham. There were methodological differences between this and other studies, including a reduced current density (0.331 mA/cm² versus 0.571 mA/cm² in Marshall et al., 2006), and a different applied waveform (trapezoidal versus sinusoidal) that could have accounted for the failure to find an effect (a similar waveform and concomitant failure to find a result in a younger healthy sample was also apparent in Sahlem et al. 2015). Another study, Frase et al. (2017) also failed to find an effect in an older, healthy sample, however, the position of the electrodes was different than previous studies (electrode locations Fp1/Fp2 and P3/P4). Using a trapezoidal waveform may interfere with endogenous brain rhythms, rendering SW enhancement ineffective. Paßmann et al. (2016) also showed that whereas SWA and spindle activity was enhanced with SWS stimulation in 21 healthy older adults, it
failed to produce a benefit in a PAT, and visuo-spatial memory was significantly impaired compared to sham. This effect is still very much of interest in the scientific community and is a continuing area of research (Bueno-Lopez, Eggert, Dorn, & Danker-Hopfe, 2017).

Memory consolidation can be disrupted via tACS. Garside et al. (2015) applied cross-hemispheric tACS during SWS in an afternoon nap, with methods like those outlined in Marshall et al. (2006), disrupting both slow wave activity and memory consolidation. Eight subjects participated in a cross-over within subject design, where two naps were taken, during which either active or sham stimulation was applied during SWS. Declarative memory was assessed with a PAT. Though non-significant, this stimulation procedure produced a decrement in recall following the nap, and this decrement was associated with a significant reduction in slow oscillations compared with sham stimulation. Interestingly, SWA showed a rebound, reflecting homeostatic sleep pressure, following the final stimulation interval compared to sham.

Impairing memory consolidation is an important potential aspect of understanding the underlying mechanisms of memory in sleep. The ability to selectively activate traumatic memories, for example, then disrupt the reconsolidation procedure could be a valuable treatment protocol for phobias, anxiety disorders, PTSD, or addiction. Oudiette, Antony, & Paller (2014) review two studies, one in mice, and one in humans, showing that manipulating sleep can lead to the forgetting of fearful associations. In the first study, mice were conditioned to fear an odor that was paired with a foot-shock. The odor was then presented during SWS, and an injection of an amygdala protein-synthesis inhibitor was delivered. This intervention led to a diminution of fear expression when tested 24 hours later. Interestingly in humans, a conditioned fear response to faces was diminished after odors that were associated
with the conditioned fear were presented during SWS, without the need of a protein-synthesis inhibitor. The conditioned fear response decreased throughout the sleep period, indicating that TMR during sleep was reorganizing the memory from a fearful to a safe one. These changes were also associated with decreased hippocampal activation and the reorganization of ensemble pattern activity in the amygdala, as revealed with neuroimaging.

Cellini et al., 2019 tested short duration repetitive tES (SDR-tES) over the DLPFC as an enhancement method for declarative memory of facts during a daytime nap. They found that it was associated with an increase in memory retention compared to the sham condition immediately after sleep as well as 48 hours later. Additionally, there was an increase in the amount of time spent in N3 sleep, and there was an increase in the rate of slow oscillations during N2 and N3 sleep.

Jones et al., 2018 and Ketz et al., 2018 aimed to use tES to strengthen endogenous SWOs and enhance memory consolidation. Their target detection task involved learning to identify threats present in an image through a discovery learning paradigm, and the test involved identifying these threats in repeated and generalized images. The repeated images consisted of images that had been presented during training, and the generalized images were similar in content to the images they had seen during training but were presented from a novel perspective. They applied 1.0 mA of tDCS during training, and 1.5 mA per hemisphere of closed-loop tACS alternating over the right and left DLPFC to active subjects during slow wave up states, phase and frequency matched to the endogenous oscillations. Sham subjects received 0.1 mA tDCS over the right DLPFC during training and no stimulation during sleep. Ketz et al., 2018 found that active closed-loop tACS was associated with an increase in slow wave power to spindle coupling as well as an improved accuracy for identifying generalized
images was improved with stimulation. Jones et al., 2018 found that there was a stimulation
dose-dependent enhancement of memory for the generalized images, such that memory was
boosted the most for the subjects who received a number of stimulation events during the
night that was closest to the median.

Ladenbauer et al. (2017) tested whether slow oscillations, fast spindles, and the
coupling between them could be enhanced by SO-tDCS during a nap in patients with mild
cognitive impairment (MCI). Nine males and seven females participated in a balanced
crossover design, receiving verum stimulation and sham stimulation separated by at least two
weeks. They did a verbal paired associate learning task, a visuospatial learning, and a finger
sequence tapping task followed by an afternoon nap and finally, memory tests 30 minutes
after awakening. SO-tDCS was administered beginning 4 minutes after sleep stage 2 began
(frequency: 0.75 Hz, amplitude: between 0 and 262.5 µA) across frontal locations F3 and F4
for 5 minutes at a time, separated by 1 minute stimulation-free intervals. They found that SO-
tDCS significantly increased slow oscillations and spindle power. Specifically, the spindle
power was enhanced during slow oscillatory up-states and the synchronization between slow
oscillations and the fast spindle power was strengthened. This method also led to an
improvement in visual memory performance.

Finally, augmenting SOs with CL-tACS has been found to enhance subjective sleep
quality. Robinson et al., 2018 stimulated the right DLPFC with 1.0 mA of anodal tDCS
during training of a visual search paradigm, followed by closed-loop tACS in phase with
endogenous SWOs during sleep, alternating between the right and left DLPFC. They found
that subjective sleep quality was enhanced in participants who received active stimulation as
compared to the first adaptation night spent in the lab ($p = 0.032$) and compared to
participants who received sham stimulation ($p = 0.029$). Additionally, sleep efficiency for subjects who received active stimulation was higher compared to all subjects on the first adaptation night in the lab ($p = 0.025$).

However, tACS during sleep may not always show a neural entrainment effect. For example, Lafon et al (2017) found no entrainment effect of low amplitude tACS (2.5 mA) on slow oscillations during SWS using implanted recording electrodes in epileptic patients.

**1.10 Prior Threat Detection Work from Our Laboratory**

In work from our lab (Clark et al. 2012), we have investigated the effects of tDCS in a complex visual object detection paradigm. Stimuli were adapted from the “DARWARS Ambush!” program (Macmillan et al., 2005; Raybourn, 2009), which was used to train soldiers being deployed into combat in the Middle East. Participants were instructed to view a series of images and decide whether an object (sniper rifle, shadow indicating an improvised explosive device, etc.) was present in each image (a detailed description of the task can be found in the methods section below). Functional magnetic resonance imaging (fMRI) was used to determine neural activity elicited by the task as participants progressed from novice to intermediate and then to expert states of performance.

Before any training, novice subjects were scanned while performing the object detection task without receiving feedback regarding their decisions. When comparing images with a target object to those without, results suggested that many brain areas were more metabolically active when target objects were present and accurately detected in the images, including bilateral anterior caudate and putamen, anterior and posterior insulae, parahippocampal gyri, cingulate gyri, superior temporal gyri, and inferior and superior parietal lobules. Subjects were then trained outside of the scanner until they reached an
intermediate (>78% accuracy), or for a subset of seven subjects, expert (>95% accuracy) level of performance. Once trained, they were scanned again and results suggested that more anterior brain areas were active, including right middle and inferior frontal cortex and left inferior frontal cortex in the intermediate group, and bilateral inferior frontal cortex, right cingulate, and right inferior parietal lobule in the expert group.

Learning this task (contrasting the difference between novice and intermediate groups) suggested that several brain areas including right middle and medial frontal cortex, right parahippocampal cortex, right cingulate cortex, right middle temporal/inferior parietal lobule and left superior temporal cortex were involved. Dynamic Bayesian Network (DBN) analyses suggested several networks involved in learning the task as well, including one comprised of right fusiform and right inferior parietal cortex.

Based on these fMRI results, two electrode placements were identified: right inferior frontal cortex (site F-10 in the standard EEG 10-10 system, above Brodmann’s area 44), which was the most significant using GLM methods, and right inferior parietal cortex (10-10 site P4 above Brodmann’s area 39), which was the most significant using DBN analyses. When right inferior frontal cortex location F10 was stimulated for 30 minutes (2.0 mA, with the cathode on the left arm) the results were astonishing. Participants receiving the active dose of stimulation exhibited nearly a doubling in their ability to identify concealed target objects compared to the sham stimulation (0.1mA) group after training combined with tDCS (26.6% for the active group, 14.2% for the sham group). Furthermore, this effect was also observed in a delayed testing condition (21.4% increase in learning for the active group, 10.5% for the sham group), which occurred 1 hour after stimulation, suggesting that the effects lasted at least 90 minutes after the end of tDCS administration (Clark et al., 2012).
Anodal stimulation over site P4 also showed an increase in learning, which was slightly less than the magnitude seen for the F10 placement (22.5% for the active group). The effect of F10 stimulation on learning this task has been replicated twice (Coffman et al., 2012a; Falcone et al., 2012) and has been shown to persist for at least 24 hours after stimulation ends (Falcone et al., 2012).

In a separate study, based on the finding of reduced blood-oxygen level dependent (BOLD) fMRI activity in left occipital-temporal regions when objects were present versus when they are absent (Clark et al., 2012), cathodal stimulation over area this area (10-20 site T5 above Brodmann’s area 37) was investigated for its effects on learning. The hypothesis was that reducing activity with cathodal tDCS in areas showing reduced BOLD fMRI activity which are associated with exogenous attentional processes that are easily confounded by camouflage, would reduce distraction by camouflage present in the images. The results suggested learning effects on par with the F10 anode placement (25.4% for the T5 active group, 13.35% for the sham group; Clark, Coffman, Trumbo & Wegele, 2014).

Using the same paradigm described in Clark et al. (2012), Coffman et al. (2012a) investigated the differential effects of tDCS on stimulus type. In the original study, 82% of the testing stimuli were novel (i.e. – had not been presented during training), and only 18% were repeated images. To investigate specifically the impact of tDCS on object detection in repeated versus novel images an experiment was conducted investigating signal detection metrics using the same types of images and procedures, but which was modified to include 50% repeated and 50% novel images.

In signal detection theory, perceptual sensitivity (d’) and response bias (β) are used to determine response characteristics in participants. Perceptual sensitivity is a measure
indicating how well one discriminates signal from noise (or in terms of this study, objects present, and objects absent), and is calculated as the normalized (z-scored) hit rate minus the normalized false alarm rate. Response bias is a measure of how likely one is to respond one way or the other, where values greater than one suggest a bias toward responding “object absent” and values less than one indicating a bias toward responding “object present” and is calculated by raising e to the power of ½ the difference between the squared normalized hit rate and the squared normalized false alarm rate. Ideally, when trying to improve performance on signal detection metrics, one would want to increase a participant’s d’ while not changing their bias to respond in a certain way.

Data from nine active (2.0mA) and ten sham (0.1mA) participants were included in Coffman et al. (2012a). Results suggest that active tDCS enhanced d’ compared to sham without changing the way in which the groups responded to stimuli, evinced by no change in β across groups. Further, this effect was enhanced when comparing repeated images to novel images.

Active tDCS also showed an improvement on both hit rate (choosing correctly that an object was present in the image) as well as correct rejections (choosing correctly that no object was present in the image) across both stimulus types. There was an interaction observed between stimulus type (object present vs. object absent) and repeated versus novel images, where the active tDCS group showed a greater improvement for repeated images only when objects were present in the image (hits), but not when objects were not present (correct rejections).

Investigation of whether physical sensation during administration of tDCS (itching, tingling, burning) as well as self-reported mood had any effect on performance was
conducted as well. Results suggest that neither sensation nor mood had any effect on performance.

Using the same paradigm as Coffman et al. (2012a), a study was conducted by colleagues at George Mason University (Falcone, Coffman, Clark, & Parasuraman, 2012) to examine the validity of the findings obtained in our lab as well as investigate the rate of retention in performance observed after training and tDCS.

Thirty-seven participants took part in the study. In addition to the object detection paradigm procedures outlined in other studies (Clark et. al., 2012; Coffman et al., 2012a,b), participants were asked to return to the laboratory after a 24-hour delay, so retention of performance observed after F10 anodal stimulation could be assessed.

Data suggest that active tDCS over F10 resulted in an improvement of hit rate over sham stimulation (active = 76% peak hit rate; sham = 61% peak hit rate). In addition, false alarm rate was reduced in active participants as well compared to sham (active = 18% false alarm rate, sham = 35% false alarm rate). Perceptual sensitivity scores were also significantly higher in the active group active by the end of training (active = 1.86; sham = 0.73). There were no group differences in response bias, suggesting that tDCS was modulating sensitivity to discriminate images with and without hidden objects, without changing the way in which participants tended to respond. Interestingly, the improvement of the active group over the sham group persisted after a 24-hour retention interval, with only a slight reduction of d’ scores, which were reduced in the active group by approximately 8% from the immediate retention condition to the 24-hour retention condition, suggesting that the effect of tDCS on learning lasts at least 24 hours.
2.0 STUDY DESIGN

2.1 Rationale

There are many unanswered questions regarding how sleep affects memory consolidation. We currently do not have a complete picture of what types of memories are consolidated during sleep and when, and the processes by which this happens. For example, Stickgold (2005) suggests that some, but not all, procedural memories are consolidated during sleep. What allows the failure of consolidation of some information, while sparing other types?

Thus far, studies investigating the effects of electrical stimulation during sleep on memory have focused on a very narrow range of tasks, namely a verbal paired associates task as a proxy for declarative learning, and finger tapping or mirror tracing for procedural learning. This provides a myopic view of information that can benefit from sleep consolidation or augmentation. There have been positive effects on an artificial grammar rule learning task (Paller, 2015), but how this translates to actual grammar acquisition is unknown. Would this effect be apparent in children learning grammatical structure? What about in illiterate adults or children in special education? Would the same effect be seen in other tasks of abstract rule learning, or in other learning and memory paradigms? Translating lab effects into ecologically valid scenarios (far transfer) would allow for the application of tES technologies to actually help individuals in their lives.

Failure to replicate results on verbal paired associates task performance after an augmented night of sleep is perplexing. Not all studies used phase locked, closed-loop stimulation during sleep, which is important in entraining oscillations and boosting power in target frequency bands (Ali, Sellers, & Fröhlich, 2013). Using phase and frequency locked
AC stimulation during sleep for the PAT could provide evidence that endogenous rhythms are sometimes rendered suboptimal when open-loop systems are used. There is evidence that brain stimulation during waking improves cognitive performance across several domains (Coffman, Clark, & Parasuraman, 2014); perhaps combining waking stimulation with overnight augmentation of sleep could produce synergistic effects.

Being able to successfully remove the stimulation artifact from ongoing EEG during sleep will be critical to investigate how stimulation is altering architecture in a fine-grained way. Generating an artificial waveform for tACS that is close enough to endogenous activity to influence it, but different enough to be filtered out for analysis of ongoing activity during stimulation would be extremely valuable.

In rodents, implanted electrodes in the thalamus, hippocampus and PFC would allow the investigation of the contribution of each area to consolidation by knocking out selected areas or concurrently stimulating other regions. Targeting one component in the system, either during SWS or REM sleep, could provide useful information in terms of how areas communicate and consolidate information. The entrainment of endogenous networks, by stimulating in theta band in the HC and slow-wave band in the frontal cortex and investigating the effects on behavior is important future research. Applying stimulation out of phase to disrupt communication in rodent models would be interesting as well.

Whether sleep augmentation leads to an improvement in passive memory consolidation is unclear. Studies have not looked at passive information consolidation while subjects watched a documentary, for example, then had tACS-augmented sleep, and were subsequently tested upon waking. This could answer if normal, passive memory
consolidation benefits from the same sort of intervention as will-to-be-remembered information.

Another, surprisingly lacking, corpus of data involves the analysis of the neural signatures of encoding before and recall after sleep, and how these processes interact with enhancement or impairment of consolidation during sleep with brain stimulation. There are few studies investigating this important question (Ong et al., 2016; Marshall, Molle, Hallschmid, & Born, 2004; Molle, Marshall, Gais, & Born, 2004). Time frequency analysis techniques using EEG have become increasingly popular in cognitive neuroscience research and oscillatory activity has been linked to perception, working memory, and cognitive control, among other functions. However, oscillatory activity as it relates to the augmentation of sleep cycles and subsequent effects on cognition has largely gone uninvestigated.

Questions regarding what consequences external augmentation of sleep architecture may have on a person’s quality of life, including sleepiness, the ability to focus or pay attention at work or school, thinking critically or creatively, as well as effect dreaming, and further cognitive functioning remain. We do not yet understand how augmentation of sleep with tACS could functionally alter macro-level sleep architecture, as few studies have investigated this question.

The synaptic homeostasis hypothesis would predict that if brain stimulation was driving neurons to a homeostatic baseline, then perhaps stimulation over a shortened night of sleep would be enough to “reset” the brain for improved learning or performance upon waking, thus providing an optimization method for individuals whose profession or illness prevents them from acquiring the requisite amount of natural sleep.
Is there a way to augment only one memory trace from two related traces using reactivation and reconsolidation? There is an argument about whether reactivation leads to a new trace or a modified trace, and perhaps stimulation could help shed light on that debate.

To date, no one has investigated whether electrical TMR leads to memory enhancement, like olfactory or auditory reactivation does. Does electrical TMR lead to increased theta bursting, or theta coherence in the HPC (Cantero et al., 2003), and how may this relate to behavioral improvement?

Technologies with precise temporal and spatial resolution, like magnetoencephalography (MEG), may allow investigation into the early processes of HPC and PFC integration during consolidation, replay, reactivation, reconsolidation, and recall, during both wake and sleep, which would be interesting for future research, as this work has only been done using fMRI. This could help answer the question of for how long the hippocampus is involved in memory retrieval, lending support to either SCT or MTT.

Effects of repeated exposure to stimuli on sleep correlates have not been investigated. How do reactivated memory traces look different than new ones in terms of spectral characteristics? What role does the pontine wave have for human memory consolidation? No studies have investigated this phenomenon, as it is incredibly invasive. Or is there a human analog of the rat p-wave? Is more frequent replay during sleep the mechanism by which information with emotional salience or import are more likely to be remembered? There are seemingly limitless questions still unanswered in the sleep-dependent consolidation field, which leads to the specific aims of the current project, which endeavored to answer three of these outstanding questions: does closed-loop stimulation lead to improved PAT performance compared to open-loop methods, can stimulation mitigate the effects of retroactive
interference, and can stimulation-augmented sleep improve learning in other domains outside of paired associates learning?

2.2 Specific Aims and Hypotheses

Sleep is universal and understanding it more fully could lead to a host of benefits, from improved cognitive performance to quality of life. Memory consolidation during wake and sleep is a complicated process, and there are many unanswered questions. tCS is a tool that can be used to both help us to understand, as well as augment, sleep-dependent memory consolidation processes, as well as the quality of sleep in general. If realized, targeted memory consolidation has tremendous promise for both healthy and clinical populations by selectively reactivating memories, and using tCS to either facilitate their strengthened reconsolidation, in the case of desirable memories, or disrupt reconsolidation processes, in the case of undesirable ones, which could have educational, occupational, athletic, and psychiatric benefits.

The current study was designed to expand the understanding of what kinds of information consolidation can be augmented with tACS and considering conflicting findings regarding electrical brain stimulation delivered during SWS in an open-loop method. Specifically, this series of experiments aimed to evaluate a novel closed-loop tACS method to deliver brain stimulation timed to predicted slow-wave up states, and at the endogenous oscillation of an individual’s slow-wave pattern, both throughout a sleep session as well as between sleep sessions. This project had three specific aims:

1. **To examine the behavioral effects of Closed-Loop tACS delivered during predicted SWOs on three learning tasks that each focus on a different aspect of**
learning, including the Declarative Paired Associates Task, the Paired Associates Interference Task, and on an Object Detection/Category Learning task.

2. To examine the relationships between EEG characteristics and/or CL-tACS stimulation parameters on performance during these tasks.

3. To examine the relationships between stimulus characteristics (namely repeated information versus generalized information) and effects of CL-tACS (e.g., what stimulus responses and/or memory and recall or recognition effects for specific types of stimuli are affected by CL-tACS).

In Experiment 1, the aim was to begin to expand the investigation of sleep dependent memory consolidation augmentation with tES to include other types of memories, beyond basic declarative word-pair learning. Participants completed a novel variant of the category learning paradigm shown previously to be sensitive to tES (Clark et al., 2012; Coffman et al., 2012, Falcone et al., 2012).

The experimental questions for the behavioral data were:

1. Does verum CL-tACS improve learning in the object detection task compared to sham?

2. Is there an interaction between stimulus characteristics (repeated images vs. generalized images) and stimulation in the object detection task?

The experimental questions for the EEG and Stimulation data were:

1. Do higher stimulation counts predict enhanced learning?

2. Are specific EEG frequencies (Biomarkers) associated with good/poor encoding during the immediate test following training, and recall following sleep, and how does stimulation influence these dynamics?
Hypotheses for this experiment were:

1. *Verum tDCS stimulation during wake will lead to improved immediate performance on the task compared to sham.*

2. *Verum CL-tACS stimulation during sleep will lead to improved delayed performance on the task (at morning and afternoon tests) compared to sham.*

3. *Memory for repeated stimuli will be enhanced with wake and sleep stimulation compared to generalized images.*

4. *EEG dynamics will change as a function of performance and stimulation condition.*

In Experiment 2, the aim was to investigate if a novel closed-loop SWO augmentation algorithm which delivered tACS during predicted up-states can benefit sleep dependent memory consolidation like the open-loop methods previously accomplished using a standard paired-associates task (Marshall, 2004, 2006).

The experimental questions for the behavioral data were:

1. *Does verum CL-tACS lead to improved recall of List A-B compared to sham?*

2. *Does initial encoding strength of List A-B predict memory following sleep, and does stimulation interact to influence performance?*

The experimental questions for the EEG and Stimulation data were:

1. *Do higher stimulation counts predict improved behavioral performance?*

2. *Are specific EEG frequencies (biomarkers) associated with strength of List A-B encoding and/or recall following sleep, and how does stimulation influence these dynamics?*

Hypotheses for this experiment were:

1. *Verum stimulation will lead to improved performance on the PAT compared to sham.*
2. Low encoders will show a larger benefit of stimulation compared to high encoders.

3. EEG dynamics will change as a function of performance and stimulation condition.

In Experiment 3, the aim was to investigate if a novel closed-loop SWO augmentation algorithm which delivers tACS during predicted up-states protects from retroactive interference in a novel interference paired associates task, modeled after Marshall (2004; 2006).

The experimental questions for the behavioral data were:

1. Does active CL-tACS protect from retroactive interference of List C-D on List A-B recall?

2. Does higher initial encoding strength of List A-B and stimulation interact to influence performance?

The experimental questions for the EEG and Stimulation data were:

1. Do higher stimulation counts predict improved behavioral performance on list A-B recall?

2. Are specific EEG frequencies (Biomarkers) associated with encoding strength of List A-B, and recall following sleep, and how does stimulation influence these dynamics?

Hypotheses for this experiment were:

1. Verum stimulation will lead to less interference of List C-D encoding on List A-B test performance.

2. Low encoders on List A-B will show a larger benefit of stimulation compared to high encoders.

3. EEG dynamics will change as a function of performance and stimulation condition.
Overall, the goals of the project were to investigate whether CL-tACS provides a larger memory benefit than previously conducted open-loop studies, study the effects of CL-tACS on a novel memory task, and explore whether sleep stimulation protects from retroactive interference. In addition, we studied stimulus characteristics in the novel memory task (namely veridical recall versus generalization), and whether encoding ability at baseline interacted with stimulation in the PAT. Finally, we wanted to investigate the effects of our intervention on waking EEG activity during testing before and after sleep.

2.1 DARPA Restoring Active Memory (RAM) Replay Project

The experiments outlined in this manuscript were part of a larger, multisite study funded by the Defense Advanced Research Projects Agency (DARPA) to investigate the possibility of cueing active memory during sleep using non-invasive exteroceptive interventions to improve sleep dependent memory consolidation. Our group consisted of researchers from HRL Laboratories in Malibu, CA, Rutgers in New Brunswick, NJ, USC in Los Angeles, CA, and Cardiff University in Wales, UK. The program consisted of five separate experiments, using four different tasks, three of which were performed in virtual reality (VR), and three different kinds of brain stimulation during both awake learning as well as during SWS. The first two experiments were pilots using the DARWARS Target Detection Task. The first pilot (described in Experiment 1 below) used waking tDCS and closed-loop tACS during SWS to accelerate learning (Jones et al., 2018, Ketz et al., 2018). The second pilot used the same task but employed a novel method of neurostimulation called Spatiotemporal Amplitude-Modulated Patterns (STAMPs) to “tag” information during learning to be “cued” during sleep to facilitate reconsolidation. The third experiment aimed to improve sequence learning in VR (called the Situational Awareness (SA) task) using both
The fourth experiment (part of which is described in Experiment 2) aimed to improve episodic memory learned in VR (called the Operational Event Reporting (OER) task) using STAMPs (Pilly et al., 2020). In addition, during the acclimation night, a Paired-Associates Test paradigm was administered, and participants got either verum or sham CL-tACS during SWS. The fifth experiment (part of which is described in Experiment 3) looked to improve difficult rule learning during a VR spatial navigation task (called Operational Rule Understanding (ORU) task) with STAMPs. In addition, an interference version of a Paired Associates Test paradigm was administered, and again participants either received verum or sham CL-tACS during SWS.

Contributions by Study Personnel

I worked with Dr. Mike Trumbo to develop the modified version of the DARWARS target detection task by splitting the stimulus set into “people” threats and “object” threat. Also, we recoded the Presentation script to work with the Neuroelectrics EEG system. I wrote a task script in MATLAB to present stimuli, send EEG markers to the Neuroelectrics control software, and capture and score responses. I designed the interference paired associates task and wrote a task script in MATLAB to present the stimuli, send EEG markers to Neuroelectrics, and capture and score responses. I performed all the behavioral analyses for the effects of stimulation for the DARWARS target detection task (Experiment 1), and all waking EEG analyses. I performed all analyses of behavioral analyses for the paired associates task (Experiment 2), and all waking EEG analyses. I performed all analyses of behavioral analyses for the interference paired associates task (Experiment 3), and all waking EEG analyses. In addition to that, I also assisted with recruitment, screening, and scheduling of participants. I ran participants through the experiments and collected data from all the
experiments, both for the testing phases, as well as during sleep. I became an expert in identifying sleep stages from ongoing EEG activity to determine when to run (or stop) the closed-loop stimulation code, depending on what sleep stage the participant was in (for the former), or if they awoke unexpectedly (for the latter). I did not write the closed-loop code, that honor goes mainly to Praveen Pilly and Jaehoon Choe at HRL (see Ketz et. Al., 2018), but I did work closely with them to fine tune the parameters within the code to optimize stimulation so we could get as many stimulation events throughout the night during SWOs as possible. I was also the lead troubleshooter on the team and was responsible for making sure the equipment and code worked as best I could. In doing so, I frequently was in contact with the Neuroelectrics engineers and our collaborators at HRL with whom I worked closely to keep things running as smoothly as possible. The devices we used were custom-built, beta devices, so they required a good deal of technical troubleshooting to keep them working correctly. Finally, I managed an army of undergraduate and graduate RAs. This also included training them on experimental procedures and teaching them in general about sleep and sleep-dependent memory consolidation.

2.2 Experiment 1 – DARWARS Target Detection/Category Learning Task

Method

Participants

Participants were men and women who were between 18–40 years of age (mean age = 20.41, SD = 1.701), used English as a first language, completed high school, and had no history of head injury with loss of consciousness for longer than five minutes. They were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971), had no history of neurological or psychiatric disorder, had no history of alcohol or drug abuse, were
non-smoking, had no excessive alcohol or caffeine consumption, were not currently taking any medication significantly affecting the central nervous system, had no implanted metal, had no sensitivity or allergy to latex, had good or corrected hearing and vision, and reported no sleep disturbances. Women who were pregnant, or thought they may be, were also excluded. A total of 29 participants (12 female) were recruited using flyers placed around the campus of the University of New Mexico and surrounding community to complete both verum and sham stimulation conditions of this experiment and received monetary compensation. For the behavioral data analyses, A total of 21 participants (mean age = 20.1 years, SD = 1.67 years, 8 female), including data from 5 additional subjects who could not be included for the biomarker analyses in Ketz et al. (2018) due to insufficient sleep EEG data, were included for the behavioral analysis. For the EEG analysis, a subset of 19 participants with acceptable EEG data were included in the analysis. Since this was a multiple night study, eight participants did not finish the entire study, and thus were excluded from these analyses. All participants provided signed informed consent to participate in the study in accordance with the Declaration of Helsinki, which was approved by the Chesapeake Institutional Review Board.

**Tasks/Materials**

A modified version of the original task, described in Clark et al. (2012), was created to allow for the within-subjects design of the current study. Participants were trained to discover the presence of targets hidden in complex static images, and changes in performance were tracked over time. Though not a traditional memory assay, this task was chosen in part because it could be used to examine different forms of memory encoding simultaneously, including both veridical memory and consolidated or generalized memory. As noted above,
the aim of the current study was to produce as large a benefit for memory performance as possible, and thus the waking tDCS was leveraged in combination with sleep tACS to produce a synergistic, additive effect. Targets that were hidden in these images included explosive devices concealed by or disguised as dead animals (e.g., camels), roadside trash, fruit, flora, rocks, sand, or building structures; and enemies in the form of snipers, suicide bombers, tank drivers, or stone-throwers. The stimulus set was divided into two target categories: people target images (e.g., enemy snipers, friendly fire), and object target images (e.g., improvised explosive devices, trip wires). Half of the images presented to participants during tests following training were identical to those seen in training (repeated images, used to test veridical memory), and half were related, but with varying spatial perspective from the same corresponding scenes (generalized images, used to test for consolidated memory). Therefore, this design allowed for the investigation of effects of the sleep intervention on both veridical recall and generalization performances. Examples of images presented to participants can be found in Figure 1. Participants were instructed that they could stop the task at any time if the stimuli were too uncomfortable or made them anxious. No participants elected to stop for such a reason.
Figure 1. Example images presented to participants. The top row contains example threat absent images, and the bottom row contains analogous threat present images. The cut-out boxes are used here for display purposes only and were not present in the actual task. The first two columns display object threat images (roadside IED, and remote-control car bomb) and columns 3 and 4 display people threat images (snipers).

Procedure

Experimental Timeline

The experiment was conducted over the course of six days that included three nights spent in our sleep laboratory referred to here as “Acclimation,” “Night 1,” and “Night 2,” as well as two afternoon follow-up test sessions (“Day 2 Follow-Up,” “Day 3 Follow-Up”), as well as an initial orientation session. Participants were randomly assigned to one of four manipulations in a within-subjects, counterbalanced, single-blind design: Object Target/Sham Stimulation Day 2, People Target/Verum Stimulation Day 3 (SO/AP), Object Target/Verum Stimulation Day 2, People Target/Sham Stimulation Day 3 (AO/SP), People Target/Sham Stimulation Day 2, Object Target/Verum Stimulation Day 3 (SP/AO), People Target/Verum Stimulation Day 2, Object Target/Sham Stimulation Day 3 (AP/SO). The abbreviations
indicate the condition on (day2/day3), where S = Sham, A = Verum, O = Object targets, and P = People targets (see Table 1).

Table 1
*Experiment 1 Condition Assignments*

<table>
<thead>
<tr>
<th>Target Type</th>
<th>Stimulation Condition</th>
<th>Stimulation Condition</th>
</tr>
</thead>
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<td>Object Day 2/People Day 3</td>
<td>SO/AP</td>
<td>AO/SP</td>
</tr>
<tr>
<td>People Day 2/Object Day 3</td>
<td>SP/AO</td>
<td>AP/SO</td>
</tr>
</tbody>
</table>

Stimulation conditions applied to both waking and sleep interventions (e.g., a “Verum” assignment meant that a full dose of both tDCS during wake and tACS during sleep were administered). This combined protocol was implemented to achieve as large a long-term effect on memory performance as possible, given that waking tDCS was previously shown to improve the immediate performance on the task that persisted over at least 24 hours without any overnight changes (Falcone et al., 2012). At the orientation session, participants were invited to provide informed consent, and were given several questionnaires to assess various aspects of their personality and sleep habits, including the Big Five Inventory (John & Srivastava, 1999), which measures personality along five major dimensions, the BIS/BAS Scale (Carver & White, 1994), which measures behavioral activation/inhibition, the Barrett Impulsiveness Scale (BIS-11; Patton et al., 1995), which measures impulsiveness, the Pittsburgh Sleep Quality Index PSQI; Buysse et al., 1989), which is a trait sleep quality measurement, the Morningness-Eveningness Questionnaire (known as the “Owl/Lark”; Horne & Östberg, 1976), which describes the natural circadian rhythms of respondents, as well as the Shipley Institute for Living Scale (Shipley & Burlingame, 1941), to gather an IQ estimate consisting of a crystallized intelligence score and an abstract intelligence score, as
well as a composite IQ score. Following the questionnaires, head measurements were made (circumference, nasion to inion, and pre-auricular to pre-auricular) to fit an EEG cap. Participants were next given a tour of the sleep laboratories and an explanation of the EEG/Stimulation equipment and experimental procedures. Finally, each participant was issued a Fitbit wrist-worn biometric sensor (Dickinson et al., 2016) with instructions on how to correctly operate it to track sleep prior to their lab visits.

For the acclimation night, participants arrived at the sleep laboratory by 17:00, and were prepped and fitted with an EEG cap (see Waking Electroencephalographic (EEG) Data Collection section), and an adapted version of Raven’s Progressive Matrices called Sandia Matrices was administered (Matzen et al., 2010). Next, data was collected to calibrate biometrics (Patel et al., 2018) for use in a predictive computational model, including a breath count task to measure attentional lapses (Braboszcz and Delorme, 2011) that lasted 30 min, as well as a 3-back task to generate cognitive stress and mental fatigue (Hopstaken et al., 2015) that lasted 21 min was gathered. Participants could then relax in the laboratory (with the option of eating dinner) until roughly 21:00, when they were prepped for PSG recording during sleep (see Polysomnographic Data Collection section). EEG electrode locations were digitized using Polhemus FASTRAK System (Polhemus, Inc.) for data analysis purposes as well as to measure how much the cap may have shifted during the subsequent sleep episode. Participants were instructed to lie down in a supine position at approximately 22:00, when biocalibrations were performed to help identify sources of noise in later EEG acquisition. This included EEG data collection of eyes open for one minute, closed for one minute, looking up, down, right, and left, blinking slowly five times, clenching the jaw, and finally moving into a comfortable sleeping position. Lights out for the participants occurred between
22:00–23:00, and they slept for up to eight uninterrupted hours before being awoken. During sleep, EEG data were monitored, and the closed-loop prediction algorithm was started when four minutes of continuous N2/N3 sleep was observed by research assistants trained by a sleep research expert in identifying sleep stages based on PSG. During the acclimation sleep, no stimulation was applied, but the information gathered from the closed-loop prediction algorithm was used to verify the SWO relative power threshold of 20%, and reduced if needed, for subsequent experimental nights for each participant. Upon waking, participants could use the restroom and were offered water and snacks. They filled out the Karolinska Sleep Diary (KSD; Åkerstedt et al., 1994) to assess subjective sleep quality. Next, they completed a 1-back task for 21 minutes to assess alertness and were then disconnected and cleaned from the EEG hardware and released.

For Night 1, participants arrived at the laboratory at approximately 17:30, and were immediately set up for EEG data collection and tDCS. Participants were seated in front of the computer and instructed on how to respond to the stimuli but were not given specific information about the nature of the hidden targets or any strategies with which to find them. First, participants performed two baseline runs (test blocks one and two), consisting of 60 novel images per run. Each image was presented for 2 s, during which a binary response (target present/target absent) was made using the keyboard, and with an inter-image interval that varied from 4 to 8 s. Each baseline run lasted approximately eight minutes, and no feedback was given regarding performance.

Participants then took a brief baseline mood questionnaire to help assess effects of tDCS on subjective mood. The mood questionnaire consisted of nine questions on a 0–5 Likert scale. Items included feelings of nervousness or excitement, tiredness, confusion,
sadness, frustration, dizziness, nausea, physical pain or discomfort, and ability to pay attention. After all questions were answered, the training portion of the target detection task was administered.

Participants completed three training runs, the first two of which (training blocks one and two) were under 30 minutes of either verum (1.0 mA) or sham (0.1 mA) tDCS, followed by one more run (training block three) immediately following the administration of tDCS. The training blocks differed from the test blocks in that 1.5s following each image presentation, the participants were given audiovisual feedback using a short clip (5 s) regarding the consequence of their decision. If the participant indicated “target present” and was correct, a short video depicting the mission progressing as planned was shown, with a voiceover praising the participant for choosing correctly. If the participant indicated that a target was present when there was not, a voiceover chastised them for delaying the mission, or insulted them by indicating they were acting cowardly. If the participant correctly indicated that there was no target present, feedback was given that the mission was progressing as planned. If they indicated that there was no target present when in fact there was one, a video showing the consequence of missing the target was shown. For example, another member of the participant’s platoon was shot by a sniper, or a Humvee was destroyed by an IED. Further, a voiceover scolded the participant for missing the target and told them that members of their team had been killed. Each of the three training blocks consisted of 60 novel images and lasted approximately 16 minutes each. The audiovisual feedback did not provide specific details of the shape or location of the target, but enough information was available from the test image and feedback movie that the participant could infer its type and general position in the image. Following the three training runs,
two more test runs (test blocks three and four; “immediate test”) were administered to
gauge the immediate effect of tDCS on learning before sleep. Half of the stimuli used in the
immediate test had been presented during training (repeated images), while the remaining
stimuli were similar in content with the same targets seen before but had not been
presented during training (generalized images). Each test block presented 60 images (30
repeated, 30 generalized). Thus, memory for trained images and the generalization of the
training to novel images could be examined separately. Following the final test block,
participants were administered an exit mood questionnaire consisting of the same nine
questions in the initial mood assessment, as well as a questionnaire probing the strategy the
participants used to complete the task. Next, a new set of Sandia Matrices was
administered, as was a Language History Questionnaire (LHQ). Then participants could
relax in the laboratory until roughly 21:00, when they were prepped for PSG recording during
sleep (see Polysomnographic Data Collection section). EEG electrode locations were
digitized, and biocalibrations were performed. Lights out for the participants occurred
between 22:00–23:00, and they were allowed again to sleep for eight uninterrupted hours
before being awoken. During sleep, EEG data were monitored, and the closed-loop
stimulation intervention was started when four minutes of continuous N2/N3 sleep was
observed and allowed to run through the remainder of the night. If the participant showed
signs of waking, or needed to use the restroom, the stimulation was paused, and resumed
after the participant was again in N2/N3 sleep. Upon waking, participants could use the
restroom, and were offered water and snacks. They filled out the KSD to assess
subjective sleep quality. Next, they completed two more test blocks of the target detection
task (“morning test”) to assess the effect of SWO augmentation on performance, filled out
the strategy questionnaire, and then were disconnected from the EEG hardware, and released. Like the immediate test, each block in the morning test presented 60 images (30 repeated, 30 generalized). The repeated images used in morning test were different from those used in the immediate test.

For the Day 2 follow-up, participants arrived approximately 24 h after their day 2 arrival (17:30), were prepped for EEG data collection, and were administered two more test blocks (“afternoon test”) to assess the effects of SWO augmentation on more long-term retention and performance. Note that each block of the immediate, morning, and afternoon tests presented 30 repeated and 30 generalized images, and there was no overlap in stimuli across these testing runs.

Approximately 5 days after completing the Day 2 follow-up, participants came back to the laboratory for their Night 2 and Day 3 follow-up. The timeline and procedures were identical to Night 1 and Day 2 follow-up, the only differences being the target category (object targets/people targets) and stimulation condition (verum/sham) were opposite of their Day 2 assignments. Upon completion of the Day 3 follow-up, a final exit questionnaire was administered to gather subjective ratings from participants in terms of how they felt the intervention impacted their memory functioning generally. And they were debriefed, during which time they could ask questions about the nature of the experiment. See Figure 2 for a graphical description of the experimental procedures and Figure 3 for the target detection experimental procedure.
**Figure 2.** Experimental timeline for Experiment 1 (top), and an artificial hypnogram (bottom). It is worth noting that RECALL before sleep did not occur immediately before sleep, but rather several hours before. Likewise, participants did not complete the RECALL in the morning after sleep until roughly 30 minutes after waking, and again after approximately 8 hours away from the laboratory. Although the hypnogram shows only the first 200 minutes of sleep, the closed-loop algorithm could be triggered at any point in the night if the appropriate slow-wave conditions were met.
Figure 3. Experiment 1 training (top) vs. testing (bottom) trial timeline. On training trials, a short feedback movie was shown following a participant response, describing the consequence of the decision. No such feedback was given during testing trials.

**Waking Electroencephalographic (EEG) Data Collection**

A commercial StarStim R32 simultaneous EEG/Stimulation device (Neuroelectrics, Inc.) was used. Participants were prepped and fitted with a neoprene EEG cap that incorporated 32 Ag-AgCl electrodes (solid gelrodes: NE028, Neuroelectrics, Inc.), placed according to the extended 10–20EEGsystem (P7, T7, CP5, FC5, F7, F3, C3, P3, FC1, CP1, Pz, PO4, O2, Oz, O1, PO3, CP2, Cz, FC2, Fz, AF3, Fp1, Fp2, AF4, P8, T8, CP6, FC6, F8, F4, C4, P4). As some extracephalic electrodes were used, some of the channels labeled with cephalic 10-20 sites were placed elsewhere. These are mentioned here to clarify how the original raw data were organized. Three of the channels were utilized for electrocardiogram (ECG) and electrooculogram (EOG) recordings: The channel labeled “PO3” was placed
under the left collarbone for ECG, and channels labeled “AF3” and “AF4” were placed superior and lateral to the right outer canthus, and inferior and lateral to the left outer canthus, for vertical and horizontal EOG, respectively. Common Mode Signal (CMS) and Driving Right Leg (DRL) reference electrodes (stricktrodes: NE025, Neuroelectrics, Inc.) were placed on the piauricular, as stimulation was applied to the mastoids. Data were sampled at 500 Hz.

Polysomnographic (PSG) Data Collection

For polysomnographic (PSG) data collection during sleep, the setup was nearly identical to wake, with a few exceptions. First, two EMG electrodes were placed on (channel labeled “Oz”) and under (channel labeled “PO4”) the chin in accordance with PSG recording guidelines set forth by the American Academy for Sleep Medicine (Berry et al., 2012) to help with sleep scoring. Second, EEG data were collected from 25 electrodes. For closed-loop SWO augmentation, four channels were dedicated for stimulation; namely, F3, F4, and bilateral mastoids (where electrodes labeled “T7” and “T8” were placed). Finally, as these channels were used for stimulation, they were omitted from EEG data collection. Of the remaining electrodes, Fp1 and Fp2 along with C3, C4, O1, and O2 were used for sleep staging.

Waking Transcranial Direct Current Stimulation

Thirty minutes of continuous transcranial direct current stimulation (tDCS) was delivered during 48 minutes of training. A custom tDCS template for use during awake training was defined in the Neuroelectrics control software, CoreGUI. The anode electrode was set to +1000µA, and the cathode was set to -1000 µA, for a total dose of 1000 µA (1.0 mA) for verum stimulation; for sham stimulation, the current values were 100 µA and -100
µA, for a total dose of 100 µA (0.1 mA). Two SPONSTIM 25 electrodes with saline soaked sponges (25 cm²) were affixed to the participants. For verum stimulation, the anode electrode was centered over the right sphenoid bone (electrode site F10), and the cathode electrode was placed on the upper contralateral arm. For sham stimulation, the placement, polarities, and duration were identical to those for verum stimulation, but the current was set to 0.1 mA instead of 1.0 mA. Physical sensation ratings were solicited three times during tDCS administration: once after current ramp-up (approximately one minute), four minutes following ramp-up before the first training run began (approximately five minutes after stimulation had begun), and immediately following the first training run (approximately 21 minutes after stimulation had begun). Participants were asked to rate three different types of sensations (itching, heating, and tingling) on a 0–10 Likert scale, where 0 indicated no feeling of sensation at all and 10 indicated the worst possible feeling of sensation. Any report of a seven or above resulted in immediate cessation of stimulation and termination of the experiment, without penalty to the participant. No participants opted out due to waking tDCS.

Closed-Loop Transcranial Alternating Current Stimulation (CL-tACS) During Slow-Wave Oscillations

The closed-loop algorithm for electrical augmentation of memory consolidation during sleep first detected the presence of SWOs, which consist of slow synchronized positive and negative deflections of EEG that are associated with memory consolidation. The algorithm next attempted to match the stimulation frequency and phase with ongoing slow-wave activity such that maximal positive stimulation occurred at the up states (positive half waves) of the endogenous SWOs, as prior work suggests that these are the periods during which coordinated memory replays between hippocampus and neocortex occur to facilitate
long-term memory consolidation (Ji and Wilson, 2007). For robust SWO detection, a virtual channel was computed by averaging 13 fronto-central EEG channels (Cz, FC1, FC2, CP1, CP2, Fz, C4, Pz, C3, F3, F4, P3, P4 in the extended 10–20 system) to determine the overall synchronous activity of EEG recorded during sleep. The virtual channel allowed the observation of moments of relatively high slow-wave power, referred to as slow-wave events, while averaging out lesser magnitude activity on individual channels unrelated to the pattern of SWOs. The included channels were stored in a running 5-s buffer. They underwent moving average subtraction with a 1 s window (to center the mean of the signals at 0 µV). Noisy channels exceeding 500 µV min-to-max amplitude across the 5 s were rejected before the virtual channel was computed and not included in the average. Each discrete data fetch operation updated the buffer with a random transmission delay, which needed to be accounted for to plan and precisely time the next brain stimulation intervention.

The virtual channel data in the buffer was further processed to detect the presence of SWOs and predict the next up state (see Figure 4). The power spectrum, computed by Fast Fourier Transform (FFT), was used to plan stimulation when the ratio of the cumulative power in the slow-wave band (0.5–1.2 Hz) was more than 20% of the total cumulative power from 0.1 to 250 Hz. If this SWO relative power threshold of 20% was crossed, the algorithm then filtered the data in the slow-wave band with a second-order zero-lag Butterworth filter. Next, a sine wave was fit to the filtered virtual channel using the identified dominant slow-wave frequency and by optimizing the amplitude, offset, and phase parameter values. The sine wave was then projected into the future, identifying the temporal targets that would synchronize brain stimulation to the predicted endogenous signal. Throughout this process, the dynamic latency associated with data processing was timed using the system clock.
Together with distributions of calibrated latencies for data fetch and stimulation commands (mean = 5 ms, SD = 2 ms), which were measured offline, the algorithm determined the correct time point to communicate with the hardware to initiate the stimulation. For instance, suppose at a given moment the algorithm initiates data fetch to populate the buffer with the last 5 s of EEG data, the data becomes available for processing a few ms (say, 6 ms) into the future based on sampling from the distribution for data fetch latency. Then, say it takes 100 ms for data processing to predict the next up state, which happens to be 600 ms into the future from the starting time point. If it takes a few ms (say, 7 ms) to physically initiate stimulation based on sampling from the distribution for stimulation command latency, the algorithm would wait 487 ms after the EEG processing step to send the stimulation command to the device. tACS was applied for 5 cycles at the detected SWO frequency. Should the next possible stimulation start time be later than the start of the next predicted up state, yet at least 300 ms before its end, then synchronous stimulation was initiated and continued until 4 full cycles are completed (where a cycle is defined as the progression from 0° phase to 360° phase). If at least 300 ms of up state stimulation was not possible, then the algorithm planned the stimulation to start at the next upcoming up state based on the continued sine wave projections from the buffer. Following the offset of tACS delivery, the system idled for 3 s to avoid the collection of stimulation artifacts in the data buffer, then resumed the cycle of data update in the buffer, data processing and predictions, and stimulation planning as the criteria specified above are met. Thus, our closed-loop system adapted and adjusted stimulation parameters online to ensure the proper administration of stimulation at the correct temporal targets to match the predicted transient brain states of interest. It is able to minimize the pitfalls of temporal inaccuracies that arise because of
variable delays intrinsic to any recording/stimulation/processing hardware. On sham nights, up states were similarly predicted but no stimulation (i.e., 0.0 mA) was applied. For stimulation on verum nights, 1.5 mA sinusoidal currents (peak-to-peak = 3.0 mA) were applied at F3, F4, T7, and T8 with pistim electrodes (NE024, Neuroelectrics, Inc.) using the method stated above with F3 and F4 in phase with each other and with ongoing SWOs, and 180° out of phase with T7 and T8. Note T7 and T8 electrodes were placed on bilateral mastoids. See Figure 4 for a graphical representation of the CL-tACS intervention and stimulation montages for waking tDCS (Experiment 1) and overnight stimulation (All Experiments).

**Figure 4.** CL-tACS intervention (top) and Stimulation montages for waking tDCS (Experiment 1, bottom left), and overnight stimulation (All Experiments, bottom right). The virtual EEG channel (gold) in the 5 s buffer is bandpass filtered in the SWO frequency range (0.5–1.2 Hz). If the relative power in the SWO band is >20% of the broadband power across 0.1–250 Hz, a sine wave at the dominant SWO frequency is fit to the filtered virtual channel and projected into the future to predict the time points of the next available UP states. By matching the phase of the tACS to this projected function, the dynamics of tACS and the predicted endogenous signal are aligned. For verum stimulation, 5 cycles of closed-loop tACS was applied in response to observed SWO events through the sleep.
2.2 Experiment 2 – Paired Associates Task

Method

During the fourth RAM Replay experiment (OER), a paired associates task paradigm was developed and run during the acclimation night. Overnight, participants were randomized to receive either sham stimulation or closed-loop tACS in the same manner as in Experiment 1.

Participants

Thirty-Five participants were recruited in the same way as Experiment 1 and signed informed consent. Six participants did not complete the study due to noncompliance, leaving twenty-nine participants (mean age = 23.38, SD = 4.59, 16 female). One participant dropped due to scalp irritation (not from the tACS intervention, but rather from the STAMP intervention on a subsequent night), one never showed up for the study after orientation, one opted out after night 2 because of a family issue, two opted out due to scheduling conflicts, and the last was moved to the final (ORU) study. All exclusion criteria were the same as Experiment 1.

Task/Materials

Participants completed a verbal paired associates task on a computer using a custom-built task in Matlab. The design was modeled after Marshall (2004, 2006), where 54-word pairs were presented for participants to learn (List A-B). The task had three phases: an encoding phase where participants were given five seconds to study each word pair, an evening testing phase, where participants were given one of the words for each pair, and they had to produce the corresponding word. and a final test was administered phase in the morning, like the evening test. Participants responded by typing words into a text box. There
was no time limit imposed on participants to produce a response. Please see Figure 5 for the word list used.

Word-Pair List (A-B) From Marshall et al., 2006

<table>
<thead>
<tr>
<th>INSECT</th>
<th>WORM</th>
<th>AIRPLANE</th>
<th>KETCHUP</th>
<th>GIRL</th>
<th>DATE</th>
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<tr>
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<td>FIGHT</td>
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</table>

**Figure 5.** Word pair list A-B from Marshall (2006).

**Procedure**

*Experimental Timeline*

The experimental timeline was like Experiment 1 with a few notable exceptions. This study design was between subjects, and only occurred during the acclimation night/morning. Participants were seated in front of the testing computer approximately 24 inches from the screen. After studying the word pairs in the encoding phase, participants were administered the first evening test. If they did not accurately recall at least 60% of the word pairs, another encoding round was administered, followed by another evening test. This sequence was repeated up to three times or stopped once participants reached at least 60% accuracy. They were not given feedback regarding the word pairs that were correct or incorrect, but rather had to keep in mind which word pairs they may have been inaccurate on and adjust their
responses in the testing phase. They were then allowed to go to sleep, following the same timeline and stimulation procedures as Experiment 1. Following the sleep session, where they received verum or sham CL-tACS, they could use the restroom and were seated in front of the computer for a final test. This test was administered only one time and was used to measure the amount of retention overnight. Please see Figure 6 for a graphical representation of the experimental timeline.

![Diagram](image)

**Figure 6.** Experimental timeline for Experiment 2.

### 2.3 Experiment 3 – Interference Paired Associates Task

During the fifth RAM Replay experiment (ORU), a novel interference paired associates task paradigm was developed and run during the acclimation night.

**Participants**

Forty-seven participants were recruited in the same way as Experiment 1 and forty-five signed informed consent. Four dropped out due to scheduling issues, and four dropped
prematurely, two because they could not get comfortable enough to fall asleep in the laboratory, and two because they could not tolerate the STAMP stimulation. For the behavioral analysis, 37 participants (mean age = 21.34, SD = 3.47, 20 female) had data and were included in the analysis. For the EEG analysis, 21 participants had adequate data from the evening and morning tests to be included. All exclusion criteria were the same as Experiment 1.

Tasks/Materials

The task and materials were identical to Experiment 2 with a notable exception. In addition to learning list A-B (Figure 5), participants were asked to learn a new list (list C-D) in the morning upon waking, again to at least 60%, before being tested again on list A-B.

Please see Figure 7 for word list C-D.

**Word-Pair List (C-D) From Marshall et al., 2006**

<table>
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<td>ROAD</td>
<td>TAR</td>
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Figure 7. Word pair list C-D from Marshall (2006).

Procedure

Experimental Timeline
The experimental design was like that used in Experiment 2, with a notable exception. For this experiment, participants were required to learn a new list (C-D) to at least 60% accuracy in the morning prior to taking the final test for list A-B. All other procedures and parameters were the same as Experiments 1 (timeline, stimulation procedures) and 2 (task structure). Please see Figure 8 for the timeline for Experiment 3.

**Figure 8.** Experimental timeline for Experiment 3.

### 3.0 DATA ANALYSIS AND RESULTS

#### 3.1 Data Analysis

**Behavioral Analysis – Experiment 1**

Data were analyzed within a repeated measures ANOVA framework, comparing “time” (within subjects: baseline, training, immediate, morning, afternoon), two image “types” when appropriate (within subjects: repeated images, generalized images), and two “stimulation” conditions (within subjects: verum, sham). Number of stimulation events for the verum night was entered into the model as a covariate for all analyses except the models
that include pre-sleep immediate test performance. The number of verum night and sham night slow-wave events were highly correlated (Pearson’s $r = 0.643, p = 0.005$), meaning that participants with more SW events in the verum night also tended to have more events in the sham night. Therefore, only verum event count was used as a covariate due to multicollinearity concerns, as well as the fact that it was the electrical augmentation that was of interest, not slow-wave events per se. Two participants were missing one time point of data each, and for these data points a Non-linear Iterative Partial Least Squares (NIPALS) algorithm (Wold, 1974) was run for data imputation in XLSTAT. For analysis of learning in the target detection task, two main outcome metrics were calculated: correct rate (hits plus correct rejections), and F1 score, which is the harmonic mean average of precision and recall (McSherry and Najork, 2008). Its range is from 0 to 1, where 1 is perfect precision and recall. F1 score is often used in the machine learning literature but can be applied to human cognition as well. Precision is calculated as the proportion of correct responses to all affirmative responses made (hits/(hits + false alarms)), and recall is calculated as the proportion of correct responses to all targets present (hits/(hits + misses)). With these metrics, dependent variables were calculated using raw performance scores from baseline to immediate test (after waking tDCS), as well as morning and afternoon tests (after sleep intervention), and overnight performance changes from immediate to morning and afternoon tests for repeated and generalized images. Finally, contrast scores were calculated by subtracting repeated from generalized overnight performance changes for the morning and afternoon tests separately. Greenhouse-Geisser correction was used for interpretation of within subjects effects in the case of violated sphericity, and pairwise comparisons were adjusted using a Bonferroni correction. Curve fitting analyses were performed, fitting both
linear and quadratic effects, to investigate dose-dependent effects of our intervention on post-sleep raw performance. For these analyses, two observations per subject were used (namely, verum event count with verum performance, and sham event count with sham performance). Since we are primarily interested in the effects of verum tACS on performance, and not necessarily the relationship between performance and slow-wave events per se, stimulation event counts for the sham night were set to 0 because no stimulation was applied. Data were analyzed with SPSS version 24 (Armonk, NY, United States: IBM Corp, 2017). There were no significant differences between verum and sham nights in terms of the number of awakenings as determined by an experienced rater, suggesting that our CL-tACS intervention did not disturb sleep.

**EEG Analysis – Experiment 1**

*Preprocessing*

All preprocessing was accomplished in EEGLAB v14.1.2 (Delorme & Makeig, 2004) utilizing custom scripts developed in MATLAB R2017b (Mathworks, Natick, MA). Channel locations (Neuroelectrics 32 channel template file) were loaded. Raw EEG data were first high-pass filtered at 0.1 Hz. Next Artifact Subspace Reconstruction (ASR) was run to remove any non-stationary noise from the raw data. ASR works by first determining a clean portion of EEG data based on the signal variance. It accomplishes this by taking the channel-wise root-mean-square (RMS) with a 1-second window, and concatenates clean windows based on a z-score threshold of the data to obtain calibration data. Next, it uses principal components (PC) to determine cutoff values based on a user-defined measure of standard deviation (k) and mean/SD of RMS across all 0.5-second windows (0.25-second step). The variable k was assigned a value of 20 for the current analysis. Finally, ASR reconstructs the
clean window. It has been shown that values ranging from 10-100 are ideal for removing artifact without compromising brain signal (Chang et al., 2018). Next, data were epoched from -1000:2000 ms post-stimulus, and a 200 ms prestimulus baseline was subtracted from the data. Epochs were stimulus onset locked and were calculated separately for repeated and generalized images for which there was a correct response. Incorrect responses were not analyzed due to low trial numbers because task accuracy neared ceiling performance. Next Automatic Pipeline for Processing APPLE code (Singh et al., 2018) was run. APPLE cleans data using a conjunction of the FASTER algorithm (Nolan et al., 2010) and pop_rejchan from EEGLAB (Delorme & Makeig, 2004). Independent component analysis (ICA) was utilized for artifact rejection. Components were plotted and visually inspected for artifact, including blinks, lateral eye movements, ECG signal, and other movement and noise artifacts. Components were selected manually for removal. The data were then reconstructed and band-pass filtered from 1-40 Hz for time frequency decomposition.

**Time-Frequency Analysis (Channel)**

Individual set files were loaded into the STUDY framework in EEGLAB (Delorme & Makeig, 2004). Data were convolved with a 3 cycle Morlet wavelet with a 0.8 Hanning-tapered window applied. A total of 100 log-spaced frequencies were calculated from 1 to 40 Hz, and a 100 ms baseline was subtracted from the data. 200 equally spaced timepoints were calculated, with a sampling rate of 500 Hz. Event-related spectral perturbation (ERSP) values were calculated to perform comparisons across time and group in 4 separate frequency bins (theta, 3-7 Hz, alpha 8-12 Hz, beta 13-30 Hz, and gamma 30-40 Hz). Averaged ERSP values for each frequency range for each 100 ms time bin from 0-1400 ms post-stimulus were calculated for correlation analysis. Averaged ERSP data from significant results in the EEG
data were subjected to correlation analyses to investigate if the EEG effects could be considered as biomarkers for behavioral improvement. Gamma activity was not analyzed due to the applied 40 Hz lowpass filter. Because no analyses were performed, gamma activity was not visualized in the ERSP plots.

*Time-Frequency Analysis (Statistics)*

Data were analyzed in the STUDY framework (Delorme & Makeig, 2004). Statistical tests were run using bootstrap statistics based on a null distribution estimated from n = 2000 runs and a statistical threshold of $\alpha = 0.01$. Others have used a similar procedure to produce reliable results (Long et al., 2016; Kamiński, Brzezicka, Mamelak, & Rutishauser, 2020). Bootstrapping is a surrogate test method that does not make any assumptions on data distribution. This non-parametric method repeatedly draws samples from all the data (creating samples for each group with the same number of datapoints as the original groups) with replacement to create a distribution of difference. The original effect is then compared to the distribution of difference, and significance is indicated if the original effect is in the tail of the difference distribution (https://eeglab.org/tutorials/ConceptsGuide/statistics_theory.html#non-parametric-statistics). Bootstrapping has been shown to adequately control for Family-Wise Error Rate (Pernet et al., 2015). Variables included time (within subjects, 2 levels – pre-sleep and post-sleep), and stimulation condition (between subjects, 2 levels – Verum and Sham). For the correlation analyses for both channel and cluster values, behavioral data were correlated with channel or cluster ERSP values in the theta, alpha, and beta bands across time. A non-parametric correlation approach (Spearman’s Rho) was utilized due to non-normality in the data.


**Behavioral Analysis – Experiment 2.**

Two learning variables were calculated. The first was the difference between performance at the morning test and criteria performance from the last evening test (remember that participants had to achieve at least 60% correct in the evening). This variable was calculated as the difference in number of correct words recalled in the morning versus the evening (Absolute Performance). Words had to be exactly recalled to be counted as correct. The second metric was a relative performance score, calculated as the difference in number of words correct in the morning and evening, divided by evening performance (Penny Score). Stimulation count was used as a covariate and was calculated the same way as in Experiment 1. Data were analyzed in a Univariate Analysis of Covariance (ANCOVA), with Absolute Performance or Penny Score as the dependent variable, and stimulation count as a covariate. Stimulation condition (Verum/Sham) was entered as a between subjects variable. For the high vs. low encoder analysis, a median split was performed on participant performance to the first recall test following encoding. Participants who scored above the median were classified as high encoders, and participants who scored below the median were classified as low encoders. All alpha values were Bonferroni corrected for multiple comparisons. Data were analyzed with SPSS version 24 (Armonk, NY, United States: IBM Corp, 2017). There were no significant differences between verum and sham nights in terms of the number of awakenings as determined by an experienced rater, suggesting that our CL-tACS intervention did not disturb sleep.

**EEG Analysis – Experiment 2**

All EEG procedures were identical to Experiment 1.

**Behavioral Analysis – Experiment 3**
Variables and analyses were identical to that described in Experiment 2.

**EEG Analysis – Experiment 3**

All EEG procedures were identical to Experiment 1.

**3.2 Results**

**3.2.1 Experiment 1: DARWARS Target Detection Task**

*Overall Raw Scores*

A 5 × 2 (time*stimulation) RMANOVA on raw overall correct scores revealed a main effect of time ($F_{(4,80)} = 79.815, p < 0.000001$), but no significant effect of stimulation or a significant interaction between time and stimulation (see Figure 9A). A 5 × 2 (time*stimulation) RMANOVA on raw F1 scores also revealed a main effect of time ($F_{(4,80)} = 71.868, p < 0.000001$), but no significant effect of stimulation or a significant interaction between time and stimulation (see Figure 9B).
Figure 9. Raw performance scores through the experiment. (A) Overall correct rate through the experiment. (B) Overall F1 score through the experiment. There were no significant differences at any time point between verum and sham stimulation conditions. Error bars represent +/- 1 SEM.

Raw Scores (Immediate and Morning Tests)

To characterize the overnight performance changes and separate the effects of waking tDCS from sleep tACS, a 2x2x2 (time*type*stimulation) RMANOVA was run on raw correct rate and F1 scores, comparing performance at the immediate (after tDCS) and morning (after tACS) tests. Results for correct rate showed a three-way interaction of
time*type*stimulation \((F_{1,20} = 7.631, p = 0.0120)\), as well as a main effect of image type \((F_{1,20} = 42.429, p = 0.000002)\), where performance for repeated images was greater than for generalized images by a mean marginal difference of 5.0% (6.4% of the mean), collapsed across time and stimulation condition. Investigation of the interaction showed a simple effect of image type within levels of time and stimulation at the immediate test for both verum \((F_{1,20} = 45.249, p = 0.000002)\) and sham \((F_{1,20} = 5.285, p = 0.324)\) stimulation, as well as at the morning test for both verum \((F_{1,20} = 4.670, p = 0.0429)\) and sham \((F_{1,20} = 9.072, p = 0.0068)\) stimulation. Performance on repeated images was greater than for generalized images for both verum and sham stimulation conditions at both the immediate (7.9 and 4.1%) and morning tests (2.9 and 5.2%), respectively; Figure 10A. Note there were no significant differences in pre-sleep correct rate for either image type between verum and sham stimulation conditions.

Results for F1 score also showed a three-way interaction of time*type*stimulation \((F_{1,20} = 12.761, p = 0.0019)\), as well as a main effect of image type \((F_{1,20} = 26.481, p = 0.000049)\), where performance for repeated images was greater than for generalized images by a mean marginal difference of 4.8%, collapsed across time and stimulation condition. Investigation of the interaction showed a simple effect of image type within levels of time and stimulation at the immediate test for verum stimulation \((F_{1,20} = 14.083, p = 0.0012)\), as well as at the morning test for both verum \((F_{1,20} = 5.194, p = 0.034)\) and sham \((F_{1,20} = 18.168, p = 0.00038)\) stimulation. Generalized image performance was greater for verum stimulation compared to sham stimulation by 5.0% at the morning test \((p = 0.010)\). Performance on repeated images was greater that generalized images for both stimulation conditions at both the immediate and morning tests (6.8, 2.9, and 6.6%, respectively, for
immediate verum and morning verum/sham; Figure 10B). For F1 score metric as well, there were no significant pre-sleep differences for either image type between verum and sham stimulation conditions. In other words, waking tDCS did not have any learning effects in pre-sleep performance.

**Figure 10.** Raw performance scores from immediate (pre-sleep) and morning (post-sleep) tests broken down for repeated and generalized images in the verum and sham stimulation conditions. (A) Raw correct scores. Performance on repeated images was significantly higher
than on generalized images for verum and sham stimulation at both the immediate and morning tests. (B) Raw F1 scores. There was a significant effect of image type for verum stimulation at the immediate test, with performance on repeated images better on generalized images. There was a trend for sham stimulation performing better than verum stimulation at the immediate test on generalized images; however, performance for sham stimulation on generalized images declined after sleep. Following sleep, the significant effect of image type remained for verum stimulation and was additionally seen for sham stimulation. There was a significant effect of stimulation condition at the morning test for generalized images, where verum stimulation led to improved performance compared to sham stimulation. Note that no significant effect was observed at the immediate test for tDCS applied during training for either image type. Error bars represent +/- 1 SEM. Some of the immediate test data in (B) is also presented in Ketz et al. (2018).

Repeated/Generalized Overnight Changes

A 2x2x2 (time*stimulation*type) RM ANCOVA with tACS count as a covariate on correct rate overnight changes from immediate to morning and afternoon tests revealed a marginal effect of the covariate (namely, verum stimulation count) on performance ($F(1,19) = 3.866, p = 0.064$), and a main effect of image type ($F(1,19) = 11.132, p = 0.0034$), where performance for generalized images was greater than for repeated images by a mean marginal difference of 3.3%. Investigation of simple effects of image type within levels of time and stimulation condition revealed significant effects for verum stimulation, where overnight performance change on generalized images was greater than that on repeated images at both the morning ($F(1,19) = 7.504, p = 0.0099$; Cohen’s $d = 1.257$) and afternoon ($F(1,19) = 9.702, p = 0.018$; Cohen’s $d = 1.431$) tests by 5 and 6.6%, respectively. No significant effects were found for sham stimulation. See Figure 11A for these results.

A 2x2x2 (time*stimulation*type) RM ANCOVA on F1 score overnight changes from immediate to morning and afternoon tests revealed a main effect of image type ($F(1,19) = 5.7679, p = 0.0265$), where performance for generalized images was greater than for repeated images by a mean marginal difference of 1.5%, and a three-way interaction of
time*stimulation*type ($F_{(1,19)} = 4.468, \ p = 0.0475$). Investigation of the three-way interaction revealed a simple effect of stimulation condition at the morning test for generalized images ($F_{(1,19)} = 10.441, \ p = 0.0043; \text{Cohen’s} \ d = 1.482$), where verum stimulation led to a higher overnight change in F1 score than sham stimulation by 6.1%. No significant effects were found at the afternoon test, or at either time point for repeated images, suggesting that the effect of improved performance on generalized images did not come at the expense of impaired performance on repeated images. Investigation of simple effects of image type within levels of time and stimulation condition revealed marginal effects for verum stimulation at both morning ($F_{(1,19)} = 3.625, \ p = 0.0721$) and afternoon ($F_{(1,19)} = 3.960, \ p = 0.061$) tests, where overnight performance change for generalized images improved by 4.0 and 5.1%, respectively, than for repeated images. A simple effect was found at the morning test for sham stimulation ($F_{(1,19)} = 6.188, \ p = 0.0229$), where overnight performance change for repeated images was greater than for generalized images by 3.7%. See Figure 11B for these results.
Figure 11. Overnight performance changes from immediate to morning and afternoon tests broken down for repeated and generalized images in the verum and sham stimulation conditions. (A) Overnight correct rate changes. The overnight correct rate change on generalized images was greater than on repeated images for verum stimulation at both morning and afternoon tests. Further, the overnight correct rate change on generalized images at the morning test for verum stimulation was greater than that for sham stimulation at a trend level. (B) Overnight F1 score changes. The overnight F1 score change for generalized images was significantly greater for verum stimulation compared to sham stimulation at the morning test. Further, the overnight F1 score change on generalized images for verum stimulation was greater than on repeated images at both morning and afternoon tests at a trend level. However, the overnight F1 score change on repeated images for sham stimulation was significantly greater than on repeated images at the morning test. Some of the data in (B) is also presented in Ketz et al. (2018) and is further broken down by morning and afternoon tests. * - p < 0.05, ** - p < 0.01, error bars represent +/- 1 SEM.
Contrast in Generalized vs. Repeated Overnight Changes

Given the benefit of tACS on overnight performance change for generalized images compared to repeated images, we analyzed contrast scores for both correct rate and F1 score, which were obtained by subtracting repeated from generalized overnight performance changes for the morning and afternoon tests, using a 2x2 (time ∗ stimulation) RM ANCOVA with tACS count as a covariate. Results for correct rate showed a marginal effect of verum stimulation count on performance ($F_{(1,19)} = 3.927, p = 0.0621$) and an effect of stimulation condition ($F_{(1,19)} = 5.946, p = 0.0147$), where verum stimulation performance was greater than sham by a mean marginal difference of 5.1%, which was driven by a simple effect of stimulation condition at the morning test ($F_{(1,19)} = 7.261, p = 0.0143$; Cohen’s $d = 1.238$), where verum stimulation performance was greater than sham by 6% (see Figure 12A).

Results for F1 score revealed a main effect of stimulation condition ($F_{(1,19)} = 7.195, p = 0.0247$), an interaction of time ∗ stimulation ($F_{(1,19)} = 4.486, p = 0.0475$), and a main effect of verum stimulation count ($F_{(1,19)} = 4.591, p = 0.0453$). Investigation of the interaction showed a simple effect of stimulation condition at the morning test ($F_{(1,19)} = 12.196, p = 0.0024$; Cohen’s $d = 1.604$), where the generalized vs. repeated contrast in overnight F1 score
change for verum stimulation was greater than for sham stimulation by a mean marginal difference of 7.7%. No significant effect was found at the afternoon test (see Figure 12B).

**Figure 12.** Contrast in overnight performance changes between generalized and repeated images from immediate to morning and afternoon tests in the verum and sham stimulation conditions. (A) Generalized vs. repeated image contrast in overnight correct rate changes. The overnight correct rate change for generalized images over that for repeated images was significantly greater for verum stimulation compared to sham stimulation at the morning test. This difference was at a trend level at the afternoon test. (B) Generalized vs. repeated image contrast in overnight F1 score changes. The overnight F1 score change for generalized images over that for repeated images was significantly greater for verum stimulation.
compared to sham stimulation at the morning test. This difference was at a trend level at the afternoon test. * - $p < 0.05$, ** - $p < 0.01$, error bars represent +/- 1 SEM.

**tACS Dose Effects**

To better understand the contribution of closed-loop tACS to sleep-dependent memory generalization, we analyzed dose effects of tACS event count through the night on raw performance in correct rate and F1 score at morning and afternoon tests for both generalized and repeated images separately, as well as the contrast in generalized vs. repeated overnight performance changes, across participants and between the two experimental nights. One subject was an outlier (>2 standard deviations from the mean) for verum stimulation count, and one subject was an outlier for behavioral performance. They were thus excluded from the curve fitting analyses. Linear and quadratic trends were fit for each outcome variable. Quadratic effects (in the shape of an inverted U) were observed for correct rate measures at both morning ($F_{(2,39)} = 4.472, p = 0.0182$; quadratic $t = −2.984, p = 0.005, R^2 = 0.195$; Figure 13A) and afternoon ($F_{(2,39)} = 4.187, p = 0.0229$; quadratic $t = −2.820, p = 0.0076, R^2 = 0.185$; Figure 13B) tests for generalized images. No linear effect at the morning ($F_{(1,39)} = 0.034, p = 0.855$; linear $t = −0.184, p = 0.855, R^2 = 0.001$) or afternoon ($F_{(1,39)} = 0.356, p = 0.554$; linear $t = −0.597, p = 0.554, R^2 = 0.009$). No significant linear or quadratic effects were obtained for repeated images or the contrast scores.
**Figure 13.** Inverted U-shaped dose effects for correct rate metric, combining across verum and sham groups. Linear and quadratic fits to raw correct rate performance on generalized images as a function of number of tACS events during either experimental night are shown for the (A) morning and (B) afternoon tests separately. The legend is in (B). The quadratic fits in each panel were significant.

For F1 scores, significant quadratic effects were also observed at both morning ($F(2, 39) = 5.191, p = 0.0102$; quadratic $t = -3.042, p = 0.0043, R^2 = 0.195$; Figure 14A) and afternoon ($F(2, 39) = 4.625, p = 0.0161$; quadratic $t = 2.901, p = 0.0062, R^2 = 0.200$; Figure 14C) tests for generalized images. Furthermore, significant quadratic effects were obtained for repeated images at the afternoon test ($F(2, 39) = 5.191, p = 0.0102$; quadratic $t = 3.042, p = 0.0043, R^2 = 0.219$; Figure 14D) and for the contrast score (generalized – repeated overnight change) at the morning test ($F(2, 39) = 7.563, p = 0.0017$; quadratic $t = 3.7399, p = 0.00062, R^2 = 0.290$; Figure 14B). No linear effects were observed at the morning ($F(1, 39) = 0.034, p = 0.855$; linear $t = -0.184, p = 0.855, R^2 = 0.001$), or afternoon ($F(1, 39) = 0.699, p = 0.408$; linear $t = -0.836, p = 0.408, R^2 = 0.018$) tests for generalized images, nor for repeated images at the afternoon test.
\( (F_{1,39} = 0.930, p = 0.341; \text{linear } t = -0.964, p = 0.341, R^2 = 0.024) \) or the contrast score \\
\( (F_{1,39} = 0.854, p = 0.361; \text{linear } t = 0.924, p = 0.361, R^2 = 0.022) \).

**Figure 14.** Inverted U-shaped dose effects for F1 score metric, combining across verum and sham groups. Linear and quadratic fits to raw F1 score performance on generalized images as a function of number of tACS events during either experimental night are shown for the (A) morning and (C) afternoon tests separately. The legend is in (B). Further, the fits to raw F1 score on repeated images for the afternoon test are shown in (D). And the fits to the contrast in overnight F1 score changes for generalized vs. repeated images at the morning test are shown in (B). The quadratic fits in each panel were significant.
**Interim Summary**

Participants were able to learn the task, as evidenced by improving performance over time. There were no effects of tDCS delivered during training on performance for either generalized or repeated images. Overall, performance for repeated images was greater than for generalized. However, the change in overnight performance for generalized images was significantly better in the verum condition compared to sham. A dose-response effect was observed for morning and afternoon tests, as well as in an overnight change score. Inverted U-shaped quadratic effects predicted behavioral performance, where too little or too many unique stimulation events predicted worse performance than a middle range.

**EEG Effects**

**Channel ERSP**

**Morning Test**

An alpha effect was observed at Channel T8, both within subject (overnight) and between subjects at the morning test. A significant reduction ($p < 0.01$) in upper alpha/lower beta band power was observed in the sham condition overnight, whereas a non-significant increase in alpha and beta power was observed in the verum condition overnight. A weak effect between conditions was observed in late alpha, with the verum condition showing an increase compared to the sham condition. The 2X2 (time x condition) ANOVA interaction was also significant at several timepoints, suggesting that stimulation resulted in increased alpha band power overnight in the verum condition compared to sham (See Figure 15). Interestingly, the late alpha component was negatively associated with overnight change in behavioral performance (Spearman’s Rho = -0.7619, $p = 0.0280$) in the verum condition (see Figure 16). No such association was observed in the sham condition (see Figure 17).
Figure 15. Time frequency plots for Electrode T8. A significant decrease in alpha/beta activity was observed overnight for the sham condition, while a non-significant increase was observed in the verum condition. A small between group effect was observed at the morning test, where the verum condition showed increased alpha/beta activity compared to sham. There was also a significant 2x2 interaction at multiple timepoints. For reference, the top row of the figure is the sham condition, and the middle row is the verum condition. The first two columns in the third row are the statistics comparing across groups at the immediate and morning test. The final column in the third row is the 2x2 (time x condition) ANOVA statistics. The first column is the immediate test, and the second column is the morning test. The first two rows in the third column are the statistics comparing time within each condition. All subsequent time frequency plots are organized in the same way. The scale is -3 to 3 dB.
Figure 16. Relationship between overnight change in performance and overnight change in alpha band power at electrode T8 at 1400ms post-stimulus in the verum condition ($p = 0.0280$).

![Graph showing relationship between performance change and alpha band power change](image)

Figure 17. No relationship between overnight change in performance and overnight change in alpha band power at electrode T8 at 1400ms post-stimulus in the sham condition.

A theta effect was observed at Channel P3, both within subject (overnight) for the verum condition, and between subjects at the morning test. A significant reduction ($p < 0.01$) in theta band power was observed in the verum condition overnight, whereas no effect was observed in the sham condition. A significant effect between conditions was observed, clustered around 500ms post stimulus, with the verum condition showing a decrease compared to the sham condition. The 2X2 (time x condition) ANOVA interaction was also significant at 500ms, suggesting that stimulation resulted in decreased theta band power overnight in the verum condition, and no change in sham (See Figure 18). The reduction in theta power was negatively associated with behavioral performance improvement overnight.
only in the verum condition (Spearman’s Rho = -0.85714, \( p = 0.00653 \); see Figure 19), but not in the sham condition (see Figure 20).

Figure 18. Time frequency plots for electrode P3. An effect in the theta band was observed both within subject (overnight) for the verum condition, and between subjects at the morning test. Reduction in theta band power was observed in the verum condition overnight, whereas no effect was observed in the sham condition. A significant effect between conditions was observed, clustered around 500ms post stimulus, with the verum condition showing a decrease compared to the sham condition. The 2X2 (time x condition) ANOVA interaction was also significant at 500ms.

Figure 19. Relationship between overnight performance change and overnight change in P3 ERSP theta power 500ms post-stimulus in the verum condition (\( p = 0.00653 \)).
A theta effect was observed at Channel F3, where the 2X2 (time x condition) ANOVA interaction was significant approximately 250-350ms post stimulus. The interaction suggests that theta activity is increased overnight in the verum condition and decreased in sham. Further there is a trend effect of group at the morning test, where theta in the verum condition appears to be increased compared to sham (see Figure 21). Theta power in this time window is negatively associated with behavioral performance change overnight in the verum condition (Spearman’s Rho = -0.7381, \( p = 0.03655 \); Figure 22), but no such effect was observed in the sham condition (see Figure 23).
Figure 21. Time frequency plots for electrode F3. A theta effect was observed where the 2X2 (time x condition) ANOVA interaction was significant approximately 250-350ms post stimulus. The interaction suggests that theta activity is increased overnight in the verum condition, but not in sham.

Figure 22. Relationship between overnight performance change and overnight change in F3 ERSP theta power 200-300ms post-stimulus in the verum condition ($p = 0.03655$).
Figure 23. No relationship between overnight performance change and overnight change in F3 ERSP theta power 200-300ms post-stimulus in the sham condition.

**Afternoon Test.**

Theta and alpha effects were observed at Channel C4, both within subject for theta (overnight) and between subjects for theta and alpha at the afternoon test. A significant reduction ($p < 0.01$) in alpha band power at 500ms was observed in the sham condition overnight, whereas a significant increase in theta power, and decrease in beta power was observed in the verum condition overnight. An effect between conditions was observed early in theta, then overlapping and continuing in alpha, from approximately 250-500ms post stimulus, with the verum condition showing an increase compared to sham. The 2X2 (time x condition) ANOVA interaction was also significant at timepoints like the between subjects effects, suggesting that stimulation resulted in increased theta and alpha band power overnight in the verum condition compared to sham (see Figure 24). Increase in alpha power
was positively associated with performance only in the verum condition (Spearman’s Rho = 0.5321, $p = 0.04115$; Figure 25), but not in the sham condition (see Figure 26).

**Figure 24.** Time frequency plot for electrode site C4. Theta and alpha effects were observed, both within subject for theta and between subjects for theta and alpha at the afternoon test. A significant reduction in alpha band power at 500ms was observed in the sham condition overnight, whereas a significant increase in theta power was observed in the verum condition overnight. An effect between conditions was observed early in theta, then overlapping and continuing in alpha, from approximately 250-500ms post stimulus, with the verum condition showing an increase compared to sham. The interaction was also significant at timepoints like the between subjects effects.
Within subjects theta and alpha effects were observed at Channel C3. A significant reduction ($p < 0.01$) in alpha and beta power at several timepoints was observed in both the sham and verum conditions overnight, whereas a significant increase in theta power at 200ms was observed only in the verum condition overnight. There were no between session effects nor was the interaction significant (see Figure 27). Increase in theta power was positively related to performance only in the verum condition (Spearman’s Rho = 0.5251, $p = 0.04448$; Figure 28), but not in the sham condition (see Figure 29). Alpha power was not significantly related to performance in either condition.
Figure 27. Time frequency plot for electrode C3. Within subjects theta and alpha effects were observed, with a significant reduction in alpha band power at 500ms in both the sham and verum conditions overnight, whereas a significant increase in theta power at 200ms was observed only in the verum condition overnight.

Figure 28. Relationship between overnight performance change and overnight change in C3 ERSP theta power 200ms post-stimulus in the verum condition (p = 0.04448).
Figure 29. No relationship between overnight performance change and overnight change in C3 ERSP theta power 200ms post-stimulus in the sham condition.

Theta and alpha/beta effects were observed at Channel CP5, both within subject for alpha/beta (overnight) in the sham condition and theta for the verum condition, and between subjects for theta at 100-250ms in the afternoon test. A significant reduction ($p < 0.01$) in alpha band power at 250ms was observed in the sham condition overnight, whereas a significant increase in theta power was observed in the verum condition overnight in the same time window. An effect between conditions was observed early in theta, from approximately 100-250ms post stimulus, with the verum condition showing an increase compared to sham. The 2X2 (time x condition) ANOVA interaction was also significant at timepoints like the between subjects effects, suggesting that stimulation resulted in increased theta and alpha band power overnight in the verum condition compared to sham (See Figure 30). Increase in theta power was positively related to performance only in the verum
condition at 200ms (Spearman’s Rho = 0.77582, \( p = 0.00111 \); Figure 31), and 300ms (Spearman’s Rho = 0.61318, \( p = 0.0197 \); Figure 32), but was not in the sham condition (see Figures 33, 34; one outlier [>3SD from the mean for theta power] from the verum condition was removed).

Figure 30. Time frequency plot for electrode CP5. Theta and alpha effects were observed both within subject for alpha (overnight) in the sham condition and theta for the verum condition, and between subjects for theta at 100-250ms in the afternoon test. A significant reduction in alpha band power at 250ms was observed in the sham condition overnight, whereas a significant increase in theta power was observed in the verum condition overnight in the same time window. An effect between conditions was observed early in theta, from approximately 100-250ms post stimulus, with the verum condition showing an increase compared to sham. The interaction was also significant at timepoints like the between subjects effects.
Figure 31. Relationship between overnight performance change and overnight change in CP5 ERSP theta power 200ms post-stimulus in the verum condition ($p = 0.00111$).

Figure 32. Relationship between overnight performance change and overnight change in CP5 ERSP theta power 300ms post-stimulus in the verum condition ($p = 0.0197$).
Figure 33. No relationship between overnight performance overnight change in change and overnight change in CP5 ERSP theta power 200ms post-stimulus in the sham condition.

Figure 34. No relationship between overnight performance change and overnight change in CP5 ERSP theta power 300ms post-stimulus in the sham condition.
Theta and alpha effects were observed at Channel T7, both within subject for alpha (overnight) in the sham condition and theta for the verum condition, and between subjects for alpha at 500ms in the afternoon test. A significant reduction ($p < 0.01$) in alpha band power at 250ms was observed in the sham condition overnight, whereas a significant increase in theta power was observed in the verum condition overnight in the same time window. An effect between conditions was observed in alpha, at approximately 500ms post-stimulus, with the verum condition showing an increase compared to sham. The 2X2 (time x condition) ANOVA interaction was not significant (see Figure 35). Increase in theta power was positively related to performance only in the verum condition at 200ms (Spearman’s Rho = 0.66786, $p = 0.00651$; Figure 36), but not in the sham condition (see Figure 37).

**Figure 35.** Time frequency plot for electrode T7. Theta and alpha effects were observed, both within subject for alpha (overnight) in the sham condition and theta for the verum condition, and between subjects for alpha at 500ms in the afternoon test. A significant reduction in alpha band power at 250ms was observed in the sham condition overnight, whereas a significant increase in theta power was observed in the verum condition overnight in the same time window. An effect between conditions was observed in alpha, at approximately 500ms post stimulus, with the verum condition showing an increase compared to sham.
Figure 36. Relationship between overnight performance change and overnight change in T7 ERSP theta power 200ms post-stimulus in the verum condition ($p = 0.00651$).

Figure 37. No relationship between overnight performance change and overnight change in T7 ERSP theta power 200ms post-stimulus in the sham condition.
Interim Summary

Channel ERSP data at several locations showed changes overnight in both verum and sham, and condition differences were observed at the morning test at several sites as well. Further, these EEG effects were associated with behavioral performance change overnight only in the verum condition, not in sham. For the morning test effects in the theta band were observed at electrodes F3 and P3, and these effects were negatively associated with performance change overnight. An effect in the alpha band was observed at electrode T8, which was also negatively associated with behavioral performance.

For the afternoon test, theta effects were observed at electrodes C3, T7, and CP5, where verum exhibited more theta activity compared to sham. These EEG effects were positively associated with behavioral performance in the verum condition but not in sham. An effect at electrode C4 suggests an increase in theta in the verum condition compared to sham, but that activity was not associated with behavioral performance.

Discussion – Experiment 1

In this experiment, we demonstrate a selective dose-dependent enhancement in memory performance for generalized images compared to repeated images in a target detection task whose learning is parahippocampally-mediated (Clark et al., 2012). We show an inverted U-shaped dose effect of closed-loop tACS on post-sleep generalization performance in the target detection task, which is interpreted in terms of tACS-induced facilitatory and subsequent refractory dynamics of self-regulating SWOs in scalp EEG. Our intervention could be enhancing the brain’s natural processes during sleep that integrate information into long-term memory for improved generalization after sleep. We show that the verum condition led to an increase of between 5-6.6% on generalized image performance
compared to sham, meaning that under verum conditions, participants were able to recall between 6-7.92 more generalized images correctly compared to sham. Contrast this with work from Marshall et al. (2006), where participants in the verum condition recalled 2.69 more words on average compared to sham (a 5.8% improvement). Obviously, a direct comparison between these two experiments is impossible, as the tasks are completely different, but results from the current experiment do show a greater improvement overall with verum CL-tACS.

We also measured scalp EEG while participants took an evening test before sleep after training, and again at a final test in the morning after our novel tES intervention. To our knowledge, this is the first attempt to do so to understand the neural dynamics associated with stimulation-induced memory enhancement during sleep. EEG data were investigated at the channel and cluster level, and time frequency analyses were performed. We show here that our stimulation intervention during sleep leads to significant changes in neural dynamics, both across time (within a condition), and between conditions. Further, many of these effects are associated with performance change from the evening to morning tests. These therefore could be biomarkers of stimulation-induced performance enhancements in this task.

3.2.2 Experiment 2: Verbal Paired-Associates Task

Effects of CL-tACS on Learning

Unfortunately, technical limitations led to a dataset without enough participants (six left in the verum condition) to adequately perform a meaningful analysis, so results for this experiment will not be reported.
Effects of CL-tACS on EEG Power

Unfortunately, technical limitations led to a dataset without enough participants to adequately perform a meaningful analysis, so statistical results for this experiment will not be reported.

3.2.3 Experiment 3: Verbal Interference Paired-Associates Task

Effects of CL-tACS on Learning

*Overnight Change Scores*

Three participants were excluded (two low and one high encoder) from the analysis upon inspection of the dependent variable (< 3SD from the mean), leaving a total of 31 participants (17 sham, 14 verum; 16 low encoders, 15 high encoders). At the first evening test (used to determine encoding performance), the high encoders on average recalled 14 more words than did the low encoders (out of a total 54 possible). There was no significant difference in Shipley IQ ($t_{(25)} = -1.946, p = 0.062974$), nor Shipley Vocabulary Subscale ($t_{(27)} = -1.588, p = 0.123930$) between encoding groups. The difference in number of words recalled between the morning test and the last test before sleep was entered into a univariate ANCOVA. Stimulation count was entered as a covariate, as in Experiment 1. Condition (2 levels – verum, sham), and encoding strength (2 levels – low, high) were entered as between subjects variables. Results suggest no effect of stimulation count on performance ($F_{(1,23)} = 0.001, p = 0.974807$), and thus was taken out of the model. An independent samples t-test revealed no difference in predicted up states between verum and sham stimulation groups ($t_{(25)} = -0.832, p = 0.413443$). A univariate ANOVA was then run with an identical design without stimulation count as a covariate. Results suggest an overall trend-level effect of stimulation condition ($F_{(1,27)} = 3.630, p = 0.067433$; Cohen’s $d = 0.734$; see Figure 38), and a
trend-level interaction of encoding strength and stimulation condition \( (F(1,27) = 3.280, p = 0.081261) \). Planned simple effects tests of stimulation condition within levels of encoding strength revealed a significant effect of stimulation for low \( (F(1,27) = 6.750, p = 0.014998); \) Cohen’s \( d = 1.001 \), but not high \( (F(1,27) = 0.005, p = 0.946743) \) encoders. Within low encoders, verum stimulation led to an increase in 4.382 words recalled on average compared to sham. Within high encoders, only a negligible difference (0.111 average word difference) was observed. Please see Figure 39.

**Figure 38.** Trend level behavioral effect \( (p = 0.067433) \) for verum tACS overall. Error bars = +/- 1 SEM.
Figure 39. Interaction of encoding strength with stimulation condition. In low encoders, verum tACS led to more words being recalled than sham. Error bars = +/- 1 SEM.

**Penny Score**

The difference in number of words recalled between the morning test and the last test before sleep, divided by the pre-sleep test score, was entered into a univariate ANCOVA. Stimulation count was entered as a covariate, as in Experiment 1. Condition (2 levels – verum, sham), and encoding strength (2 levels – low, high) were entered as between subjects variables. Results suggest no effect of stimulation count on performance ($F(1,23) = 0.0001, p = 0.990913$), and thus was taken out of the model. Recall there was no difference in predicted up states between verum and sham stimulation groups. A univariate ANOVA was then run with an identical design without stimulation count as a covariate. Results suggest an overall trend-level effect of stimulation condition ($F(1,27) = 3.820, p = 0.061068$; see Figure 40), but no interaction of encoding strength and stimulation condition ($F(1,27) = 2.847, p = 0.103083$). Planned simple effects tests of stimulation condition within levels of encoding strength
revealed a significant effect of stimulation for low \( F_{(1,27)} = 6.481, p = 0.016921 \), but not high \( F_{(1,27)} = 0.037, p = 0.849777 \) encoders. Within low encoders, verum stimulation led to an increase of 0.129 compared to sham. Within high encoders, only a negligible difference (0.009 average difference) was observed. Please see Figure 41.

**Figure 40.** Trend level behavioral effect \( p = 0.061068 \) for verum tACS overall. Error bars = +/- 1 SEM.
Figure 41. Interaction of encoding strength with stimulation condition. In low encoders, verum tACS led to more words being recalled than sham. Error bars = +/- 1 SEM.

Effects of CL-tACS on Interference

Overnight Change Scores

To test the hypothesis that verum CL-tACS protects from retroactive interference, difference in number of words recalled between the morning test and the last test before sleep was entered into a univariate ANOVA. Since the experimental design did not have a condition where participants did not experience an interference list in the morning prior to final testing, the sham participants from Experiment 2 were used as a control group. Given that the design of Experiment 2 did not have an interference component, the sham group from that experiment is a reasonable choice to answer the current hypothesis. Condition (3 levels – verum, sham, Experiment 2 sham), was entered as a between subjects variable. One outlier (>SD from the mean) participant from the Experiment 2 sham group was removed
prior to analysis. Results suggest a main effect of condition ($F_{(1,43)} = 8.378$, $p = 0.000846$).

Simple effects testing of condition revealed significant differences between the sham and Experiment 2 sham groups (mean difference = 4.027, $p = 0.000613$), where the Experiment 2 sham group recalled significantly more words than did the interference experiment sham group. Interestingly, there was no significant difference between the verum and Experiment 2 sham groups (mean difference = 1.662, $p = 0.353067$), which suggests CL-tACS does provide some protection from retroactive interference. Though not significant, the numerical difference between the verum group and the Experiment 2 sham group also suggests that there was an overall proactive interference effect in the current study. Please see Figure 42.

![Figure 42](image)

**Figure 42.** Interference is reduced with CL-tACS. A significant difference between the sham and Experiment 2 sham groups was observed, where the Experiment 2 sham group recalled significantly more words than did the interference experiment sham group. There was no significant difference between the verum and Experiment 2 sham groups which suggests CL-tACS does provide some protection from retroactive interference. Error bars = +/- 1 SEM.
Penny Score

The difference in number of words recalled between the morning test and the last test before sleep, divided by the pre-sleep test score, was entered into a univariate ANOVA. Condition (3 levels – verum, sham, Experiment 2 sham), was entered as a between subjects variable. One outlier (>SD from the mean) participant from the Experiment 2 sham group was removed prior to analysis. Results suggest a main effect of condition ($F_{(1,43)} = 7.661, p = 0.001427$). Simple effects testing of condition revealed significant differences between the sham and Experiment 2 sham groups (mean difference = 0.111, $p = 0.001292$), where the Experiment 2 sham group recalled significantly more words than did the interference experiment sham group. Interestingly, there was no significant difference between the verum and Experiment 2 sham groups (mean difference = 0.036, $p = 0.737369$), which suggests CL-tACS does provide some protection from retroactive interference. Though not significant, the numerical difference between the verum group and the Experiment 2 sham group also suggests that there was an overall proactive interference effect in the current study. Please see Figure 43.
Figure 43. Interference was reduced with CL-tACS. A significant difference between the sham and Experiment 2 sham groups was observed, where the Experiment 2 sham group recalled significantly more words than did the interference experiment sham group. Interestingly, there was no significant difference between the verum and Experiment 2 sham groups, which suggests CL-tACS does provide some protection from retroactive interference. Error bars = +/- 1 SEM.

Interim Summary

Verum stimulation resulted in improved performance overnight compared to sham. Though overall, participants in both conditions had some degree of forgetting overnight, participants in the verum condition forgot less than in the sham condition. This effect is especially large for participants classified as “low-encoders”. The sham data from Experiment 2 (no interference condition) were used to evaluate the relative impact of CL-tACS on interference mitigation. The sham condition from the no interference experiment
forgot the least amount overnight. The verum condition from Experiment 3 had intermediate (though not significantly different from the no interference sham group) performance, and the sham group from Experiment 3 forgot the largest amount. These results suggest that interference was accomplished in the experiment, and that CL-tACS rescues or protects from retroactive interference, especially in participants classified as low encoders.

Effects of CL-tACS on EEG Power and Relationship with Learning

Channel ERSP and Correlations with Behavior

An alpha effect was observed at Channel Fz, between subjects at the morning test. A significant reduction \((p < 0.01)\) in alpha/low beta band power was observed in the verum condition compared to the sham condition from 200-400ms post-stimulus. However, this effect did not predict overnight behavioral performance change in either group. See Figure 44.

**Figure 44.** Time frequency plot for electrode Fz. An alpha effect was observed between subjects at the morning test. A significant reduction in alpha band power was observed in the verum condition compared to the sham condition from 200-400ms post-stimulus.
Theta and alpha effects were observed at Channel Cz, both overnight in the verum condition and between conditions at the morning test. A significant reduction in theta was observed overnight in the verum condition, and a significant reduction ($p < 0.01$) in theta and alpha was observed in the verum condition compared to the sham condition at the morning test. However, this effect did not predict overnight behavioral performance change in either group. See Figure 45.

**Figure 45.** Time frequency plot for electrode Cz. Theta and alpha effects were observed, both overnight in the verum condition and between conditions at the morning test. A significant reduction in theta was observed overnight in the verum condition, and a significant reduction in theta and alpha was observed in the verum condition compared to the sham condition at the morning test.

A significant reduction in theta overnight was observed at Channel CP2 only in the verum condition. No effects were observed in sham, or between conditions (see Figure 46 for ERSP plots). No linear relationships were observed between overnight change in theta and behavioral performance. However, overnight performance change and overnight change in
alpha band power was evident only in the verum condition at 400ms (Spearman’s Rho = 0.80719, \( p = 0.0046 \)), and 500ms (Spearman’s Rho = 0.75453, \( p = 0.0117 \); Figures 47, 48). No relationships were found in the sham condition (see Figures 49 and 50).

**Figure 46.** Time frequency plot for electrode CP2. A significant reduction in theta overnight was observed only in the verum condition. No effects were observed in sham, or between conditions.

**Figure 47.** A significant positive relationship was observed between overnight change in alpha amplitude measured at CP2 at 400-500ms and overnight change in behavioral
performance in the verum condition ($p = 0.0046$). No effects were observed in the theta band, where the ERSP effect was observed.

**Figure 48.** A significant positive relationship was observed between overnight change in alpha amplitude measured at CP2 at 500-600ms and overnight change in behavioral performance in the verum condition ($p = 0.0117$). No effects were observed in the theta band, where the ERSP effect was observed.
Figure 49. No significant relationship was observed for overnight change in CP2 alpha 400ms post-stimulus and overnight change in behavioral performance in the sham condition. No effects were observed in the theta band, where the ERSP effect was observed.

Figure 50. No significant relationship was observed for overnight change in CP2 alpha 500ms post-stimulus and overnight change in behavioral performance in the sham condition. No effects were observed in the theta band, where the ERSP effect was observed.

An effect was observed at Channel P3, both overnight and between conditions. Overnight in the sham condition, a significant increase in theta and alpha power was observed, while in the verum condition, a significant decrease in theta power was observed overnight. At the morning test, a significant reduction in high theta and alpha was observed in the verum condition compared to sham. However, these effects did not predict behavioral performance in either condition. See Figure 51.
Figure 51. Time frequency plot for electrode P3. Overnight in the sham condition, a significant increase in theta and alpha power was observed, while in the verum condition, a significant decrease in theta power was observed overnight. At the morning test, a significant reduction in high theta and alpha was observed in the verum condition compared to sham.

An effect was observed at Channel F3 overnight in the verum condition and between conditions at the morning test. In the verum condition, a small effect in alpha was observed overnight at 400ms showing reduced power in the morning. Reductions in alpha and alpha power were observed in the verum condition compared to sham at 200-400ms and 1000-1400ms post stimulus (see Figure 52).
Figure 52. Time frequency plot for electrode F3. An overnight effect in the verum condition as well as a between conditions effect at the morning test was observed. In the verum condition, a small effect in alpha was observed overnight at 400ms showing reduced power in the morning. Reductions in alpha power were observed in the verum condition compared to sham at 200-400ms and 1000-1400ms post stimulus.

Interim Summary

Effects mainly in alpha and beta were observed between conditions at the morning test, where activity was reduced in the verum condition compared to sham. Effects were observed at electrodes Fz, P3, Cz, F3, and CP2. Most of the EEG effects were not predictive of behavioral performance, except for alpha activity being positively associated with performance only in the verum condition at electrode CP2.

Discussion – Experiment 3

In this experiment, we aimed to investigate whether CL-tACS can enhance the protection from interference that sleep provides. A non-associative paired associates AB-CD interference paradigm was utilized. We first demonstrate that overall, participants in the verum condition forgot on average 2.40 fewer words than sham, an effect on par with previous studies. For example, Marshall et al. (2006) demonstrated a 2.69-word difference, with verum stimulation outperforming sham. Their study was conducted with a within subjects design, however. When looking within low encoders, we show a 4.382-word difference when comparing verum to sham, an effect twice the size of Marshall et al. (2006), although participants were not split into such categories in that study. We demonstrate an effect where participants in the verum stimulation condition exhibited less of a retroactive interference effect compared to the sham condition. The sham group from Experiment 2 was used to compare to the current groups. The Experiment 2 sham group (no interference group)
forgot the least amount overnight, the verum group from the current experiment had intermediate performance, not significantly different from the Experiment 2 sham group, and the current experiment sham group forgot significantly more words than did the verum group in Experiment 3 and the sham group from Experiment 2. This effect was largest for those participants who were classified as poor encoders. There is evidence from the literature arguing both that sleep selectively benefits strongly encoded information, but also that sleep selectively benefits weakly encoded information. So perhaps these results show that our CL-tACS intervention is helping poor encoders compensate. In future analyses, an item-by-item analysis could be conducted to look at this effect in a more fine-grained way.

We also measured scalp EEG while participants took an evening test before sleep after learning, and again at a final test in the morning after our novel tES intervention. To our knowledge, this is the first attempt to do so to understand the neural dynamics associated with stimulation-induced memory enhancement during sleep in an interference and/or paired associates task, which most of the sleep and memory literature is based upon. EEG data were investigated at the channel level, and time frequency analyses were performed. We show here that our stimulation intervention during sleep leads to significant changes in neural dynamics, both across time (within a condition), and between conditions, however these effects do not appear to be as robust as those in Experiment 1. Further, many of these effects are not associated with performance change from the evening to morning tests. There is some support in the literature that EEG measures do not correlate with behavioral improvement because, for example, the relationships could be non-linear, and thus would not be captured by standard linear model (GLM) techniques. The experimental design could be an issue with the lack of effects found here. Participants were allowed as much time as they needed to type in a response.
In future iterations of this experiment, a button press should be required when the participant had decided upon an answer, and then that event could be used to time lock for EEG analysis. However, these results still could be biomarkers of stimulation-induced performance enhancements an interference paired associates task, but more work is needed to verify this claim.

### 4.0 DISCUSSION

*Summary – Experiment 1*

In summary, we found that closed-loop tACS resulted in improvements both for both a category learning task and for an interference paired associates task. We found in both tasks that these behavioral benefits were associated with decreases in beta and high alpha, and increased theta power. These effects were observed after verum stimulation, but the same relationships with behavior were not often seen after sham. This suggests that closed-loop tACS may induce some long-term changes in brain function and its relationship with behavior.

In this series of experiments, we sought to test the effects of closed-loop tACS delivered during SWOs while participants slept in the laboratory on sleep-dependent memory consolidation processes. Our algorithm predicted up states of SWOs and delivered transient tACS at the same frequency and in phase with these endogenous oscillations, in up to hundreds of individual 5-cycle stimulation events. Ketz et al. (2018) showed that our closed-loop tACS was primarily applied during NREM stages 2 and 3 compared to the other sleep stages (see their Figure 3), and also validated the starting phase of predicted up states with respect to ongoing SWOs using a V-test for circular uniformity based on artifact-free data from the sham nights (see their Figure 2). The approach is flexible enough to deliver
stimulation in concert with other neural oscillations (such as spindles and ripples) during both sleep and wake.

This work is novel in terms of employing closed-loop non-invasive electrical stimulation to boost hippocampally-dependent memory processes occurring during sleep, as all previous studies of electrical stimulation targeting SWOs have only used open-loop methods to deliver stimulation. The phase and frequency of the applied stimulation is extracted from ongoing brain activity, providing the potential for entraining brain oscillations as well as enhancing their synchrony across brain areas, giving our intervention an advantage over previous work. A recent feedback-controlled method (Lustenberger et al., 2016), applying transient 12 Hz tACS in response to spindle activity during sleep, modulated overnight change in performance on a procedural memory task (namely, sequence tapping) but not on a declarative memory task (namely, word pairs). We are the first to not only adapt both phase and frequency of tACS in closed loop during sleep, but also enhance memory generalization performance in a discovery learning paradigm, and to show a protective benefit against retroactive interference in a paired associates task.

Previous work has shown that 30 min of 2.0 mA tDCS applied over right inferior frontal gyrus during training leads to a doubling in performance improvement, measured as the change in performance from baseline to immediate tests, for the target detection task used in the current study (Clark et al., 2012). This effect is one of the few cognitive enhancement studies using tDCS to be replicated independently (Falcone et al., 2012). Coffman et al. (2012) investigated the interactions of tDCS and stimulus characteristics and found that verum tDCS led to a greater improvement in change from baseline to immediate tests for repeated compared to generalized images prior to sleep, likely enhancing veridical recall. By contrast, the current results showed
the opposite pattern, with a larger effect of closed-loop sleep tACS on generalized compared to repeated images, which is congruent with theories of sleep consolidation, and has been shown with TMR (Witkowski, et al., 2021). Thus, there appears to be a differing effect when attempting to optimize performance on the target detection task by combining waking tDCS and closed-loop sleep tACS, which likely depends on when and how the brain stimulation is applied. Further, there was no significant effect of waking tDCS on any metric in the target detection task prior to sleep, likely because 1.0 mA of current was delivered here, which was chosen in part to reduce the amount of stimulation (current duration) for each individual participant over the course of the experiment, as opposed to 2.0 mA of current used in our previous tDCS-only awake studies. We also used a different device to apply tDCS (the Neuroelectrics StarStim) rather than the Activatek Activadose used in our prior studies. While both systems provide controlled DC current, subtle differences in how that current is maintained, and the stability of the current over time, may be important for the tDCS effect.

Note that the performance improvements on generalized images for verum stimulation did not come at the expense of repeated image performance. In fact, raw performance on repeated images compared to generalized images was greater for both stimulation conditions at the pre-sleep immediate test as well as at the morning test. However, after sleep, generalized image performance was increased for verum CL-tACS, but not for sham stimulation. In addition, this is the first attempt to measure waking EEG during learning and testing to understand how sleep stimulation changes oscillatory dynamics, and how these may relate to the improvement in behavioral performance observed. We show here that verum tACS leads to changes in theta, alpha and beta bands, and these changes are associated with performance change. We mostly observed increases in power of higher frequency activity and increases in
lower frequency power when comparing verum to sham stimulation, the associations with performance suggest some positive and some negative relationships. This is a paradoxical finding compared to most, but not all, of the literature (see Griffiths et al., 2016; Mathewson et al., 2012; Hanouneh et al., 2018). We show that at the morning test an increase in alpha in verum, but a negative association between alpha power and behavioral performance, as well as both a decrease and increase in theta at different electrode sites, both having negative associations with performance in verum. At the afternoon test, we show an increase in theta in verum, with positive associations with performance at multiple electrode sites. The afternoon test was a better assay, and the data suggest that CL-tACS modulates memory performance through theta activity, thought to be responsible for “binding” associative memory, and increasing familiarity during recall (Alekseichuk et al., 2020). This could be a mechanism by which participants in the verum condition performed better on generalized images. If the images are more familiar to the participant during testing, it is more likely that they would be able to extrapolate to a different visual angle within the scene and accurately determine whether a target was present. For Experiment 1 overall, it appears that stimulation is modulating mainly theta activity, and this activity is positively associated with performance improvement overnight in the verum condition, specifically at the afternoon test. It could be the case that stimulation is affecting neural dynamics and their relationships with behavior in novel ways, or that the relationships with behavior are non-linear, and should be further investigated and subjected to non-linear statistical techniques. In any case, the current results should be replicated, since this is the first attempt to characterize consolidation processes in these tasks during sleep using stimulation.
We propose that the 5-cycle bursts of our closed-loop tACS transiently enhance the power of SWOs through the night, which greatly boosts the transfer and consolidation of recently acquired task information from short-term storage in hippocampus to long-term storage in neocortical areas. In support of this possibility, Ketz et al. (2018) found significant tACS-induced changes in EEG spectral power for SWO band within the observable post-stimulation period (3–10 s from tACS offset) using a spatiotemporal clustering algorithm. Note the changes during the actual tACS application (5 cycles: 4.17–10 s) and the immediate 3 s following tACS could not be analyzed because of stimulation artifacts. Compared to sham stimulation, our closed-loop tACS was shown to increase (from 3.02 to 4.24 s following tACS offset) as well as subsequently decrease (from 4.28 s to at least 10 s following tACS offset) SWO power, while increasing SWO-spindle coupling. Furthermore, both positive and negative modulations in SWO power were significantly correlated with improved overnight F1 score change for generalized images, as well as the contrast in generalized vs. repeated overnight F1 score change, across subsets of clustered EEG channels in certain post-stimulation epochs.

Inverted U-shaped dose effects for tACS, seen only for the target detection task, point to a possible optimal number of stimulation events throughout the night to produce a benefit in performance of this task. If too few stimulations are applied, performance does not appear to be improved; likewise, beyond a certain dose, continued application of closed-loop tACS may perpetuate and even extend the compensatory refraction in response to significant enhancements in SWO power because of initial applications earlier in the night. Prolonged suppressions of SWOs may have a negative impact on sleep consolidation that would have occurred earlier, such that the influence of dose is an inverted U curve. The optimal dose for a given individual can be tracked online during the night by monitoring for the occurrences of
stimulation-induced suppression in SWO power following each tACS event. However, these relationships were not seen in the paired associates task sample, so perhaps this dose-dependent effect is specific to certain types of information. Further research will be needed to elucidate this finding.

Though we used a task that was shown previously to be sensitive to tDCS (Clark et al., 2012), the within subjects counterbalanced experimental design required that the task be modified such that it could be delivered over the course of two experimental days. The choice to split the stimulus set based on target characteristics, creating an object set and a people set of stimuli, may have rendered the task too easy to learn, as participants could extrapolate from one stimulus set to the other when learning for night 2. Though the manipulation order was counterbalanced across the participants reported in this study, this carryover effect makes it harder to find a behavioral effect of our closed-loop tACS intervention. Despite this, it is notable that we found a significant improvement in overnight performance change for generalized compared to repeated images between the verum and sham stimulation conditions, with an effect size comparable to those reported previously in the literature. In particular, Marshall et al. (2006) demonstrated a 3.85% improvement in post-sleep performance of word recall with verum stimulation compared to sham based on 46 word-pairs, which is comparable to the current study where verum stimulation outperformed sham by 5% for F1 score on generalized images at the morning test.

Summary – Experiment 3

For the interference paired associates task, we demonstrate for the first time that stimulation during sleep can enhance the protective benefits on retroactive interference. This effect appears to be most strong for those participants who are not efficient encoders. Though
there was an overall trend level \((p = 0.06)\) effect overall, a significant effect was found only in the low encoder group. This effect was compared against the sham group for Experiment 2, where no interfering information was presented to participants in the morning prior to the final test, and the results suggested that the no interference sham group forgot the least, and the interference sham group forgot the most. However, the verum interference group forgot an intermediate amount, suggesting that our intervention provided further protection against retroactive interference. We showed several EEG effects, suggesting that verum stimulation led to reduced higher frequency power, including alpha and beta in the morning test compared to sham (which is consistent with the memory literature that used paired associates learning), but this effect was not linearly related to behavioral performance, except for one instance in the verum group (though the stimulation effect in that channel was in the theta band). Increased alpha activity has been associated with semantic processing (Weiss & Rappelsberger, 2000), and decreases in power in alpha and beta are thought to reflect semantic processing and efficient memory recall (Klimesch, 1996). We did not have enough acceptable datasets to perform a low vs. high encoder analysis, which could have elucidated this effect further. We also showed reduced theta in the verum group compared to sham (which is inconsistent with the literature, though see Griffiths et al., 2016; Pastötter & Bäuml, 2014), however this effect was not related to performance. The EEG results from the target detection task suggest that overall, our intervention mostly enhanced theta activity, which was positively associated with memory improvement, while in the interference task, alpha and beta band activity was reduced in the verum condition compared to sham but was not linearly related to memory performance. The results from both experiments do however provide further evidence for a relationship between SWOs and memory consolidation.
Mechanistic Considerations

To further understand and validate the mechanisms of the phenomenon under consideration, it would be necessary to accomplish both enhancement and impairment of memory consolidation for different suboptimal and optimal doses. Closed-loop tACS has the capability to both enhance endogenous brain rhythms (by stimulating in phase and frequency) and disrupt them (by stimulating out of phase and frequency), so theoretically it would be possible to improve or impair consolidation. Future studies should include this manipulation by either stimulating out of phase or at a different frequency to impair memory consolidation. tDCS has been shown to modulate cortisol, among other compounds (Morgan, Davis, & Bracewell, 2014), but no studies have investigated if tACS acts in a similar fashion. Given the interaction of cortisol and memory consolidation (Payne & Nadel, 2004), investigation of the effects of cortisol levels throughout the night while undergoing tACS sleep augmentation could be a potential mechanistic explanation.

Expand the Scope of Sleep-Dependent Memory Consolidation

This study has focused on declarative memory consolidation, in the context of improving the skill of target detection in static images and verbal paired associates learning. Future studies utilizing closed-loop tACS could be conducted with a more traditional assay of declarative memory consolidation. We used here a paired associates task, as it is a foundational task used in most of the previous electrically augmented sleep-dependent memory consolidation studies, which have delivered stimulation in an open-loop manner only. Though our attempt at a standard paired associates test did not work for technical reasons, running such an experiment in the future would allow us to compare the effects of closed-loop tACS to prior open-loop approaches (Marshall et al., 2006, 2011; Sahlem et al., 2015). Regarding the paired
associates task, we set our learning criterion to 60%, as most others do. However, some studies have participants learn the material to 100%. Manipulating encoding strength using the current stimulation paradigm could be an interesting way to elucidate the effect we observed on low vs. high encoders. This work is the first to attempt to characterize the EEG dynamics of memory consolidation during sleep in a long-term way, including testing participants roughly 24 hours after initial learning in Experiment 1. Most of the literature focuses on short-term investigations of EEG-related memory consolidation, taking measurements minutes between the encoding/recall sequence, whereas these experiments measured EEG 8-24 hours after learning, and with an intervening sleep/stimulation period. The findings in the current series of experiments are mostly in line with those findings, but it could be the case that these dynamics shift with longer-term memory processes, especially those associated with sleep. Future studies will be needed to replicate and further elucidate the current findings.

Our CL-tACS intervention can be optimized by personalizing the most critical parameter of the SWO relative power threshold using prior sleep data from a given participant. The participant’s whole-night polysomnographic recordings can be staged by an expert rater, in order to extract the distribution of relative power of the SWO band in the identified NREM sleep stage 3 (N3), when SWOs are most likely to occur (Rasch and Born, 2013). SWO relative power threshold for triggering CL-tACS can then be set to the median of this distribution. Further, the ongoing SWOs during sleep can be highly variable and drift in frequency and amplitude over time. As a result, up state predictions derived using data collected further in the past could be less reliable. For this reason, the buffer length could be shortened to be just long enough to contain one or two cycles of SWOs at the lower bound frequency of 0.5 Hz (2 or 4 s). Increasing or personalizing the SWO relative power threshold
and shortening the buffer length will certainly minimize any false predictions of up states, such as those triggered in sleep stages other than N2 and N3 as well as those not coinciding with actual up states, to trigger the CL-tACS intervention. The number of cycles and the number of applications of CL-tACS can be adjusted dynamically through the night based on tracking the EEG biomarkers of memory consolidation in the post-stimulation periods (Ketz et al., 2018). In other words, CL-tACS can be adapted gradually to maximize the post-stimulation biomarkers. It could also be the case that the optimal time for enhancing memory consolidation and generalization is during the first two sleep cycles. Several studies show an improvement in learning with an intervention over the course of a nap (e.g., Rudoy et al., 2009; Lewis and Durrant, 2011; Ladenenbauer et al., 2016), and perhaps our intervention would be more effective if we restricted its delivery to the first 3h of sleep, when SWOs are the richest.

4.1 Limitations/Future Directions

Limitations

Sleep research presents a host of unique problems with data collection, including participant comfort and attrition (in the case of multi-night studies like the current design). Our intervention, while effective in modulating memory consolidation, was the first of its kind, which necessitated finding solutions to novel problems. The StarStim R32 EEG/stimulation device is relatively new and was not specifically designed for sleep research. Sleep EEG systems are designed to be comfortable enough to allow hours of sleeping on a pillow, and robust enough for reliable operation when connected to a tossing participant. We were the first group to attempt to use the R32 for closed-loop stimulation during wake as well as during sleep, which produced a host of technical hurdles that had to be overcome, in terms of being able to fetch EEG data in real time, keeping the electrode cap affixed to the participant.
throughout the night, and ensuring the continual operation of the device. One limitation of electrical stimulation during sleep, as in wake, is that a small subset of participants cannot tolerate the physical sensations associated with brain stimulation. Thus, it could be argued that electrical stimulation is suboptimal to auditory stimulation, however, the incidence of intolerability for electrical stimulation is exceptionally low on average, and electrical stimulation is robust against interference from ambient sensory stimuli in the environment owing to its non-sensory nature. The latter is likely a potential problem for auditory interventions for memory enhancement applications in less-than-controlled, real-world settings.

The EEG data that was collected and used in the current series of experiments was particularly degraded in the morning. Given the aims of investigating neural dynamics during wake, the EEG cap from sleep should have been removed and reapplied in the morning before any testing occurred. Although the RAs did what they could to ensure the best possible data quality, the time constraints and scope of these experiments within the larger RAM REPLAY project did not allow for such measures. The quality of the EEG data, particularly for the morning tests did suffer, and future studies should account for this and make sure a fresh EEG application is used at all testing times.

Correcting for multiple comparisons in neuroimaging data is always a consideration. The EEG predictions in the current series of studies were largely non-theoretical, and the analyses were exploratory because this was the first attempt to characterize waking EEG changes due to sleep and stimulation. As such, an attempt was made to control for Type-I error rate by employing a bootstrap statistical method for inferential statistics. Bootstrapping involves randomly sampling the data with replacement to build surrogate distributions for
each group. The difference between groups is then calculated and compared against the original group difference. If the original difference is observed in the tails of the bootstrapped difference distribution, then significance can be assumed, and Ho can be rejected. Though not a multiple comparison correction per se, bootstrapping has been shown to control for Family-Wise Error Rate in EEG data analysis (Pernet et al., 2015). To further restrict the probability of a Type-I error, a significance threshold of $\alpha = 0.01$ was applied. Since there were a large number of tests, it is likely that some false positives were evident, and future replication using more strict multiple comparison procedures (Bonferroni, FDR, etc.) will be required to more accurately characterize significant EEG effects like those reported in the current manuscript.

Given the findings in the literature, including this work, a careful study should be undertaken that investigates just these tasks with our closed-loop tACS intervention. These experiments were part of a larger study, and there were likely confounds in the form of interference, sleepiness, etc. that could have influenced the results reported here. For example, experiments 2 and 3 were conducted without an adaptation night, which is standard practice in sleep studies. There is a known “first-night effect”, where participants’ sleep is disrupted the first night sleeping in the laboratory (Toussaint et al., 1995). The results here are encouraging and should be validated with a carefully controlled replication study. A within-subjects design would be desirable to control for individual differences in encoding strength, sleep architecture, memory capacity, EEG dynamics, etc.

A limitation of Experiment 1 is the confounding factor of waking tDCS for the verum stimulation condition in accounting for the significant overnight performance changes for generalized images. In other words, we have not directly dissociated the individual
contributions of waking tDCS and sleep tACS to overnight performance changes. However, previous work did not find any overnight improvements in target detection following waking tDCS alone over 24 h (Falcone et al., 2012). Further, as noted above, a larger, more effective dose (2 mA) of tDCS during training had been shown to improve performance for repeated images significantly more than for generalized images before sleep (Coffman et al., 2012). Data collected before waking tDCS were not analyzed for Experiment 1. Though there were very few significant differences between conditions at the immediate test (following tDCS), suggesting that the overnight EEG effects reported were the result of the CL-tACS intervention, baseline data should be investigated as that would allow for a more complete disambiguation of tDCS and tACS effects. It could be the case that the EEG changes reported are the result of a recovery from tDCS, rather than a direct modulation of tACS. However, the current study shows significant inverted U-shaped dose effects of sleep tACS for post-sleep performances on generalized images in the context of a fixed waking tDCS dose of 1 mA over 30 min. These observations provide indirect evidence for the unique contribution of the closed-loop tACS in boosting long-term memory generalization for the target detection task.

Technical problems with the equipment and algorithms limited data collection for Experiment 2. That, coupled with the fact that we were using a new, prototype StarStim64 system (which, based in part on the problems we had with it, will not become commercially available) resulted in many technical problems that ultimately led to data loss. Future studies should use the commercially available StarStim32 system that is better understood and has more technical support from the manufacturer.

The interference paired associates task was not ideally set up for EEG analysis. Participants had an unlimited amount of time to respond to the cue word and entered their
response manually via keyboard. Normally, paired associates tests have participants verbalize their responses, which would be difficult to time in EEG, but future iterations of this experiment will include a button press that participants can make when they decide on a response, thus time locking that event in the EEG for subsequent analysis. These many sources of variability reduced the quality of the EEG data and resulting analyses.

**Future Directions**

Future work will include an item-by-item analysis for the interference paired associates task. In the current results, weak encoders showed more improvement, which could be because weakly encoded memories are preferentially reactivated, or because the memory isolation hypothesis shelters them from being activated/interfered with, but the best way to test that would be to either copy a previous design (where words are studied multiple times or up to a specific criteria) then tested, or by doing an item (correctly encoded/not) by item analysis of these data.

To talk about a phenomenon mechanistically, it is necessary that selective enhancement or impairment be accomplished. Closed-loop tACS has the capability to both enhance (by stimulating in phase) and disrupt (by stimulating out of phase; see Ngo et al., 2013b) endogenous brain rhythms, so theoretically it would be possible to improve consolidation of some information while impairing consolidation of other information, by stimulating in-phase one night for enhancement and out-of-phase another night for impairment for example. More studies should be conducted that selectively impair consolidation, including in the tasks presented here. The idea of targeted memory consolidation (TMC) could allow for the possibility of selectively augmenting memories and deciding externally which ones endure and which are driven to perish. This could be
beneficial to students, those stricken with phobias, post-traumatic stress disorder (PTSD), or addiction, by selectively targeting painful or maladaptive memories for reactivation and subsequent disruption of reconsolidation.

5.0 CONCLUSION

In conclusion, this series of experiments sought to investigate the effects of closed-loop tACS on memory consolidation in canonical tasks (paired associates), but also by expanding the scope of tasks (target detection) and theoretical constructs (retroactive interference) to further elucidate the possibilities of stimulation during sleep to improve memory consolidation. Further, EEG dynamics were investigated before and after sleep and stimulation, during waking learning and testing to investigate biomarkers associated with stimulation-induced performance enhancements. We show here that closed-loop tACS improves memory consolidation in a novel visual target detection discovery learning task, as well as protects from retroactive interference in a paired associates task, and that these behavioral improvements are related to EEG oscillatory dynamics. These results are encouraging, but future work is needed to better understand the underlying mechanisms of stimulation-induced sleep dependent memory consolidation enhancement.
6.0 REFERENCES


https://doi.org/10.1016/j.neuroscience.2005.01.011


https://doi.org/10.1016/j.neuroimage.2013.07.083


https://doi.org/10.1016/j.smrv.2012.04.001

Cordi, M. J., & Rasch, B. (2021). No evidence for intra-individual correlations between sleep-mediated declarative memory consolidation and slow-wave sleep. *Sleep, zsab034*.

https://doi.org/10.1093/sleep/zsab034


https://doi.org/10.1146/annurev.psych.55.090902.142050


https://doi.org/10.1016/j.brs.2015.01.400


https://doi.org/10.1016/j.nlm.2013.09.008


https://doi.org/10.1016/j.neurobiolaging.2015.04.016


https://doi.org/10.1126/science.aaa3841


https://doi.org/10.1038/31371


doi:10.1016/j.jml.2006.09.004


https://doi.org/10.1126/science.1095760


https://doi.org/10.1016/j.smrv.2018.01.008


https://doi.org/10.1093/sleep/zsx003


https://doi.org/10.1080/00049537208255807


https://doi.org/10.1016/j.tics.2011.06.004


https://doi.org/10.1016/j.nlm.2014.11.015


http://doi.org/10.1016/j.neurobiolaging.2015.05.014


https://doi.org/10.1017/S1355617711000683

