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EFFECTS OF CLOSED LOOP TRANSCRANIAL ALTERNATING CURRENT STIMULATION TARGETING SLOW OSCILLATION SLEEP ON POST-SLEEP OBJECT LOCATION LEARNING

Bradley M. Robert
University of New Mexico

Bradley M. Robert

Candidate

Psychology

Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Vincent P. Clark, Ph.D. , Chairperson

James F. Cavenagh, Ph.D.

Jeremy Hogeveen, Ph.D.

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BY

BRADLEY M. ROBERT
B.S., Psychology, The University of New Mexico, 2017

THESIS

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ABSTRACT

Modulating sleep architecture to augment memory performance can provide valuable insight into how sleep contributes to memory. Slow wave activity has been identified as an important contributor to sleeps impact on memory. While the beneficial role of SWS in offline consolidation is well established research investigating SWS's role in post-sleep memory formation is limited. Here we investigated the relationship between SWS and post-sleep learning by modulating slow wave oscillations (SWO) with closed loop transcranial alternating current stimulation (tACS) with an aim toward enhancing learning capacity for object location associations. Thirty-six subjects were randomly assigned to either sham (21) or verum (15) tACS delivered to match the phase and frequency of the dominant slow-wave oscillation during sleep. Participants completed a novel object location memory task the following morning immediately after encoding as well as a delayed test the following evening. Results revealed a significant effect of stimulation condition where sham outperformed verum ($\eta^2 = 0.135$). Planned simple contrasts revealed that verum stimulation significantly reduced object location accuracy by 12.9% in the morning test with the delayed test trending in the same direction with a reduction of 17.8%. These data suggest a decrease in post sleep encoding capacity following a full night of SWS modulation. This result, along with reported benefits of stimulation during a 90-minute nap, suggest that limited augmentation of SWS

may accelerate sleep's beneficial effect on synaptic downscaling, but extended stimulation may drive these processes beyond the sleep systems homeostatic balance, resulting in a negative net effect on post-sleep learning.

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Introduction

While a consensus has not yet been reached regarding the function of sleep, many possibilities have been proposed. One such proposal is its role in sleep-dependent memory processes. Characterization of this dynamic role remains incomplete, due in part to the complexities of its neurobiology in its various stages, as well as the differentiated nature of memory (Walker & Stickgold, 2004). Modulating sleep and/or sleep architecture with non-invasive techniques to augment memory performance can provide valuable insight into how sleep and its different features contributes to memory, as well as elucidating potential new avenues for cognitive enhancement. An important aspect of sleep architecture – slow wave activity (SWA), is a high-amplitude neocortical neural phenomenon identifiable by electroencephalography (EEG) and characterized by synchronous slow-wave oscillations (SWOs) activity in the 0.5 – 4 Hz frequency range. These oscillations are the major architectural marker of slow wave sleep (SWS), an important contributor to sleep dependent memory processes (Diekelmann & Born, 2010). While the benefit of SWS to offline consolidation is well established (Rasch & Born, 2013), and numerous studies have investigated the role of SWS in consolidation of previously formed memories (Daurat et al., 2007; Fowler et al., 1973; Plihal & Born, 1999; Yaroush et al., 1971), research investigating the role of sleep in modulating memory formation is limited. Current theory posits that during waking periods neural networks involved in encoding new information become saturated from repeated potentiation decreasing the capacity of networks and diminishing efficacy of continued encoding processes (Tononi & Cirelli, 2006). SWS serves to promote global downscaling of these networks and desaturation of the synapses renewing the networks capacity for encoding new information in the post sleep period, a phenomenon known as the synaptic homeostasis hypothesis (Tononi & Cirelli, 2006).

Modulation of SWA pharmacologically (J. Walsh et al., 2006; J. K. Walsh et al., 2008), with acoustic stimulation (Ong et al., 2018), and through transcranial electrical stimulation (Antonenko et al., 2013; Ketz et al., 2018; Marshall et al., 2004) have shown promising results for altering sleep's impact on memory, albeit with limited reproducibility (Eggert et al., 2013). A potential contributor to this inconsistency is the open-loop nature of stimulation protocols being implemented, which do not account for the endogenous phase and frequency of ongoing neocortical oscillatory activity, as opposed to closed-loop stimulation, where stimulation can be implemented to coincide with endogenous phase and frequency. Closed-loop stimulation has been shown to be more effective than open-loop stimulation in a computational neural model of Parkinson's disease (Li et al., 2015) and has been argued to have the potential for optimized modulation of brain activations in a state dependent manner (Zrenner et al., 2016). In light of technological advancements allowing for application of closed-loop models of stimulation with concurrent EEG, and our group's successful implementation in previous studies using closed-loop transcranial alternating current stimulation (CL-tACS) to improve sleep-dependent memory consolidation by enhancing endogenous slow oscillations(Jones, Aaron P. et al., 2018; Ketz et al., 2018) and improve subjective sleep quality (Robinson et al., 2018), the current study investigated the effects of CL-tACS time-locked to up-states of ongoing SWOs and delivered at a matched frequency on hippocampally dependent post sleep object location encoding of a novel spatial location memory task.

Characterization of Memory

Memory is commonly differentiated into distinct types or categories and stages. While this is useful in characterizing the varied nature of memory, the multiple interrelated processes engaged throughout the nervous system that result in human memory do not operate as fully

independent processes and are reliant on different, interactive brain systems and networks. The most common classification scheme is based on the distinction between declarative and nondeclarative memory (Tulving, 1985). Declarative memory is memory that is fact-based and can be consciously recalled. Subcategories of declarative memory are semantic memory (memory for general knowledge) and episodic memory (contextual memory). Nondeclarative memory is regarded as nonconscious and is subcategorized into procedural memory (motor skills, how to do things), and implicit memory (automatic or unconscious memory). Additionally, memories are formed through unique stages of information processing over time where information is initially encoded, then stabilized into longer-term memory systems through consolidation for later retrieval (Walker & Stickgold, 2004). Encoding is the process beginning with perception of information uptake during learning where information initially registered in the cortex is transformed into a memory trace (Squire et al., 2015). The initial binding of the memory trace in the cortex is believed to be completed within seconds or minutes (Moscovitch, 1994). This manuscript will primarily focus on declarative memory encoding.

Hippocampal Learning and Long-Term Potentiation

Since the surgical removal of the well-known patient H.M.'s bilateral medial temporal lobe (MTL) resulted in severe memory impairment (Scoville & Milner, 1957), the MTL, and especially the hippocampal complex (HPC), has been recognized as playing a vital role in formation of declarative memory. The relationship between HC and declarative memory is supported by a robust literature (for a review see Eichenbaum, 2001) and prominent models of memory function provide consensus on HPC structures being involved in memory formation. The Standard Model of Memory Consolidation (SC; Alvarez & Squire, 1994) suggests memories are encoded by the MTL, including the HPC, which is made up of the hippocampus (HC),

parahippocampal (PHC), entorhinal (EC), and perirhinal (PC) cortices, and later migrated for longer-term storage to cortical association areas. Similarly, Multiple Trace Theory (MTT; Nadel & Moscovitch, 1997)) agrees with the role of the HPC in encoding but argues for its continued involvement in the retrieval and reconsolidation of memories.

One type of hippocampal dependent declarative memory is object location memory OLM); memory for locations of objects in the environment independent from routes (R. P. Kessels et al., 2001). Episodic OLM enables the explicit recall of specific details of objects and their locations within spatial and temporal context after a single exposure to an environment (Moscovitch et al., 2005). OLM has three processing components: object processing, spatial-location processing, and object to location binding (Postma et al., 2008). Spatial-location processing involves coordinate spatial codes for an object's exact position (Lansdale, 1998) and knowledge of global relative position (Kosslyn et al., 1992). Spatial memory deficits have been observed in hippocampally-lesioned rodents and primates (Redish & Touretzky, 1997), as well as humans with hippocampal damage(Nunn et al., 1998; Smith, 1987). (R. P. C. Kessels et al., 2004) compared spatial memory in epileptic patients with selective amygdalohippocampectomy to healthy controls. Results showed participants with neurosurgical lesions in the medial temporal lobe were impaired on a task assessing coordinate position information. Episodic memory models based on functional neuroimaging and lesion studies also indicate hippocampal involvement in remembering contextual details of encoded objects, including location information (Rugg & Vilberg, 2013; Slotnick, 2013).

Hippocampal dependent learning is considered to occur through plastic changes in synaptic efficiency that allow associations between neurons to be strengthened, or long-term potentiation (Bliss & Collingridge, 1993). More than one-hundred years ago, Cajal (1911)

hypothesized that changes in the strength of synaptic connections between neurons is what underlies information storage. This idea was extended by Hebb's (1949) proposal that if two neurons are active at the same time, the synaptic efficiency of the appropriate synapse will be strengthened. These ideas led to Hebbian Plasticity, and the axiom, "Neurons that fire together, wire together." A great deal of research and effort since that time has served to strengthen our understanding of LTP and Hebbian learning (Lynch, 2004); external events are represented in the brain as spatio-temporal patterns of neural activity which create synaptic changes in cells that are temporally proximal in activation. Throughout periods of wakefulness, as humans interact with the environment, learn, and form memories, plastic changes in the strength and number of connections between neurons take place, resulting in a net increase in synaptic weight impinging onto cortical neurons (Tononi & Cirelli, 2003). Anatomical work with animals exposed to enriched environments in which LTP is more likely induced exhibit a net increase in synaptic density (Klintsova & Greenough, 1999). Additionally, wakefulness is associated with diffuse induction of molecular changes usually associated with LTP as well as the induction of genetic activity associated with LTP (Cirelli et al., 2004; Cirelli & Tononi, 2000a), and can be increased further if animals are kept awake longer or engage in extensive exploration of their environment. During sleep, by contrast, LTP-related gene expression is muted or severely reduced (Cirelli & Tononi, 2000b), providing a molecular correlate for the observation that memories are established preferentially during waking and that sleep may be a time for their restoration.

Sleep

Sleep is a homeostatic process characterized primarily by distinct electrophysiological changes in brain activity accompanied by relative behavioral inactivity. Disruption of sleep affects daytime cognitive function in various realms, including attention, alertness, vigilance, and

divergent thinking (Killgore, 2010). Several hypotheses regarding the function of sleep have been proposed, including brain detoxification, tissue restoration, brain thermoregulation and energy conservation. More recently, prominent hypotheses have proposed that sleep periods are favorable for brain plasticity and learning and memory.

Sleep is characterized by two main stages: slow-wave sleep (SWS) and rapid eye-movement (REM) sleep. These stages are described in terms of time-frequency EEG recordings during the sleep episode, called polysomnography (PSG). Electrodes are placed over the scalp, as well as around the chin (to measure muscle tone in REM sleep), and around the eyes (to monitor vertical and horizontal eye movements, and blinks). During SWS, which is most prominent in the first half of the night, high-amplitude neocortical oscillations called slow-wave activity (SWA) which peak at 0.75 Hz (Marshall et al., 2004) are a prominent feature (Rasch & Born, 2013). SWA increases in proportion to the duration of prior wakefulness and decreases in density and distribution throughout the sleep period (Borbly, 2001). REM sleep is characterized by low amplitude, fast oscillatory brain activity resembling waking EEG, accompanied by loss of muscle tone. SWS replenishes the brain and body, whereas REM sleep modulates transition between SWS and waking (Vertes, 2004). Support for the causal role of sleep in hippocampal-dependent learning includes negative effects of sleep deprivation on hippocampal function during learning (McDermott et al., 2003) and decreased performance on tasks requiring hippocampal involvement (Guan et al., 2004; Yoo et al., 2007). These studies provide evidence that pre-learning sleep, and specifically SWA, play a critical role in preparing the brain for initial learning.

Studies in which participants were subjected to sleep deprivation prior to learning exhibited significant impairments in temporal memory (Harrison & Horne, 2000) – memory for

when events occur tested by requiring subjects to discriminate the recency of previously shown items - and a verbal learning task that altered between determining whether a list of nouns was in upper or lower case (control condition) and memorizing a list of nouns (experimental condition) (Drummond et al., 2000), while functional magnetic resonance imaging (fMRI) also showed a significant decrease in temporal lobe activation during encoding and compensatory activation in the parietal lobe and prefrontal cortex when compared to control. These findings confirm that sleep deprivation results in significant behavioral impairment and suggest that such deficits are mediated by overcompensation in other brain areas when MTL does not engage normally, evidence of sleep's role in hippocampal dependent learning.

Synaptic Homeostasis Hypothesis

One prominent hypothesis for explaining the function of sleep in learning and memory is the Synaptic Homeostasis Hypothesis (SHY; Tononi & Cirelli, 2003) which posits that SWA in brain areas involved in learning during waking periods would increase during the subsequent sleep period promoting a global down-scaling and desaturation of synapses potentiated from encoding, renewing the networks capacity for encoding in subsequent wakefulness. The slow rate of synchronized neuronal firing during SWA favors these processes of synaptic depression (Czarnecki et al., 2007; Vyazovskiy et al., 2008). Another factor promoting downscaling is the sequence of depolarization (up-phase) and hyperpolarization (down-phase) characterizing slow-oscillations. This proximal temporal pairing between generalized spiking at the end of the depolarization phase and generalized hyperpolarization at the beginning of the down-phase may communicate to synapses that presynaptic input was not effective in driving postsynaptic activity, a key requirement for depression (Kemp & Bashir, 2001). In one study, researchers selectively targeted and reduced SWA using acoustic feedback aimed at selective interference

with SWA. Performance on "simple" and "complex" vigilance tasks, a declarative learning task, and an implicit learning task were assessed after a night of sleep, with and without stimulation. fMRI imaging showed a reduction in HPC activation during encoding and reduced performance on a declarative learning task while not affecting reaction time on vigilance tasks or performance on implicit learning tasks (Van Der Werf et al., 2011). Additional support for SHY comes from Mander et al., (2011) where 44 participants performed two learning sessions on an episodic memory encoding task, each followed by immediate testing, and were then either kept awake or allowed to take a 100-minute nap. Those in the nap group improved performance on the post-nap learning session compared to the awake group, suggesting the sleeping period provided a means of recovery for learning resources. Results also showed a positive correlation between time in N2 sleep and learning ability, while no changes in performance were reported on a procedural memory task. Given the role of SWA in post-sleep learning and temporal coordination by which synaptic downscaling occurs, time dependent neurophysiology is a promising target for augmentation studies attempting to determine causal coordination between oscillatory activity during sleep and desaturation of synapses in the hippocampus involved in encoding in the subsequent waking period (Helfrich et al., 2014).

Transcranial Alternating Current Stimulation

Over nearly the past two-decades, an emergent tool for researchers, and increasingly clinicians, has been non-invasive brain stimulation (NIBS). NIBS provides an opportunity to expand on the correlational relationships in functional neuroimaging studies, which reveal alterations in neural activity temporally related to behavioral outcomes, by distinguishing epiphenomena from causal relevance by selectively modulating network function. While NIBS has been used in many forms to attempt to elucidate the relationships between neuronal function,

network activation, and behavioral outcomes, transcranial electrical stimulation (tES) during sleep has been shown to improve memory performance in various ways, including encoding (Kirov et al., 2009) and recall (Marshall et al., 2004, 2006). tES involves placing at least two electrodes, an anode and a cathode, on the scalp which are attached to a current generator that produces a weak electrical current. One form of tES, transcranial alternating current stimulation (tACS) provides an advantage in the study of oscillatory dynamics by enabling exogenous manipulation and entrainment of intrinsic oscillations through the injection of time-varying electrical fields by rhythmically reversing electron flow between electrodes (Antal & Paulus, 2013; Paulus, 2011). tACS has been shown to be an effective tool in entraining brain oscillations (Ali et al., 2013; Helfrich et al., 2014) by affecting neuronal firing rates in an oscillatory manner without affecting the mean overall firing rate, suggesting tACS as a candidate tool to modulate brain dynamics relevant to cognitive functions to determine their impact. SWA specifically has successfully been modulated using transcranial slow-oscillatory stimulation (tSOS) at 0.75 Hz resulting in an increase in the slow-oscillation band (0.5 – 1.0 Hz) (Marshall et al., 2006; Westerberg et al., 2015). While it is recognized that these studies do not employ pure tACS but rather oscillating components driven by a direct component, researchers commonly conclude that the oscillatory states induced similarly predict cognitive phenomena (Schutter & Wischnewski, 2016; Wang, 2010). While the majority of research involving augmentation of SWS using electrical stimulation has been directed toward memory consolidation, Antonenko et al., (2013) applied 0.25 mA or sham tSOS during two naps, one month apart, after a learning session including several declarative and procedural learning tasks. Results demonstrated tSOS not only increased SWS and SWA, as expected, but resulted in improved declarative learning of pictures, word pairs, and word lists, whereas procedural learning on a finger sequence tapping skill

remained unaffected. This finding, in corroboration with Van Der Werf et al., (2011) clearly point to a causative role of SWA in providing capacities for encoding new information in the HPC dependent learning system.

Open-Loop vs. Closed-Loop tACS

Although tACS can successfully entrain brain networks at the exogenous frequency, the phase of stimulation plays an important role in how quickly networks are entrained (Ali et al., 2013). In-phase stimulation can amplify backpropagation of an action potential, or result in LTP, while out-of-phase stimulation can selectively suppress oscillations in a network (Buzsáki & Draguhn, 2004). A study demonstrating the effects of phase-dependent tACS utilized 10 Hz tACS 1.0 mA for 20 minutes over parieto-occipital cortex, with simultaneous EEG recording presented visual oddball stimuli at four different phases of the tACS waveform. The results indicate significant interaction between stimulation condition and phase of stimulus presentation on target detection, demonstrating performance was modulated in a phase-dependent manner. Additionally, EEG alpha band (8 – 12 Hz) power was significantly increased during the stimulation period (Helfrich et al., 2014). This study illustrates that the interaction between endogenous phase and frequency of the targeted oscillatory activity and phase and frequency characteristics of exogenous stimulation must be considered to optimize tACS as a method for entraining networks. Closed-loop AC stimulation can selectively target candidate brain states for stimulation and match AC phase and frequency to provide stimulation within optimized parameters (Wilde et al., 2015) and is the optimal method to augment SWA during sleep to further characterize its role in learning post-sleep.

Recent work from our lab has successfully demonstrated a closed-loop transcranial alternating current stimulation (CL-tACS) paradigm to target and modulate SWOs to affect sleep

dependent memory consolidation. EEG slow-wave detection was used to deliver tACS matching the phase and frequency of the dominant oscillation in the frequency range of 0.5 – 1.2 Hz with an aim toward enhancing declarative memory consolidation on a target detection task. Results showed that CL-tACS targeting SWOs during sleep enhanced target detection accuracy for generalized images compared to sham and that this behavioral effect was significantly correlated with a post-stimulation increase and subsequent decrease in EEG spectral power in the slow-wave band (Jones et al., 2018; Ketz et al., 2018).

Objectives and Hypotheses

To briefly review, previous work has demonstrated LTP-like plastic changes in the HPC mediate encoding of declarative information, including coding for contextual information such as location of an object. These synaptic changes during hippocampal learning lead to saturation of synapses throughout the waking period. SWS plays a causal role in downscaling saturated synapses in HPC freeing synaptic resources for future learning by promoting high-amplitude slow-oscillations whose temporal dynamics promote large scale synaptic depotentiation. Modulation of SWS is a promising avenue by which causal relationships between oscillatory dynamics during sleep and large-scale synaptic downscaling can be investigated. SWS has been successfully augmented via NIBS in humans to increase SWA and has resulted in modulation of hippocampal dependent memory function but this research has focused on consolidation of previously learned memories during sleep leaving the relationship between SWS and post-sleep learning largely uncharacterized. Despite the depth of literature regarding sleep's relationship to learning and memory, only two studies to date have directly attempted to investigate a causal relationship between SWA and post-sleep encoding. In one such study, healthy participants receiving 0.75 Hz tSOS to induce SWA during a daytime nap were tested for encoding capacity

of declarative materials (pictures, word pairs, word lists) and procedural skills post-sleep (Antonenko et al., 2013). Compared with control, stimulation during the nap enhanced SWA and significantly improved encoding on all three declarative tasks, while conferring no benefit to procedural skills. Another group investigated the effect of slow oscillation acoustic stimulation during a daytime nap on post-sleep encoding of visual memories. A deep learning algorithm performed real-time sleep scoring and detected the up phase of ongoing SWA, selectively delivering acoustic stimulation (40 ms pink noise). Results showed enhanced low frequency (delta and theta) power and duration of SWS, compared to control. Participants with greater slow-oscillation evoked response performed better on the encoding task and exhibited greater anterior hippocampal activation at encoding (assessed via fMRI).

tACS has been shown to be an effective tool for entraining brain oscillations by introducing time-varying electrical fields in an oscillatory manner. Given the phase and frequency-dependent nature of neural oscillatory dynamics, stimulation protocols that consider individualized endogenous brain activity in application of tACS are likely to convey benefits in network entrainment. Following this lab's successful implementation of a CL-tACS paradigm to selectively augment SWOs, the current study investigated the effects of CL-tACS during nocturnal sleep time-locked to up-states of ongoing SWOs, and delivered at a matched frequency, on post sleep encoding of a declarative spatial location memory task. To the authors knowledge, this is the first study employing tACS in a closed loop fashion during a full night of nocturnal sleep to investigate a causal role of SWA in post-sleep declarative memory encoding. The present study also introduces a novel object location memory task paradigm to investigate hippocampally dependent object location encoding.

Based on the literature discussed, it is hypothesized that application of CL-tACS targeting SWA during sleep will improve post-sleep encoding of a declarative spatial location memory task. Specifically, it is hypothesized that (1) verum (1.5 mA/hemisphere tACS) stimulation during sleep will lead to increased recall accuracy compared to sham (0.0 mA/hemisphere tACS) stimulation during sleep at immediate (time point 1) test, (2) verum stimulation during sleep will lead to increased recall accuracy compared to sham stimulation during sleep at delayed (time point 2) test, and (3) verum stimulation during sleep will lead to less forgetting over time (between time points 1 and 2) than sham stimulation during sleep.

Methods

Inclusion/Exclusion Criteria

This data was collected during a single phase of a larger multi-phase project using tES to enhance memory consolidation. Participants were 18-40 years of age, used English as a first language, completed high school, and reported no history of head injury with loss of consciousness for longer than five minutes. They were right handed according to the Edinburgh Handedness Inventory, reported no history of neurological or psychiatric disorders, or alcohol or drug use disorders, were non-smoking, reported no excessive alcohol or caffeine consumption, were not currently taking any medication with significant effect on 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 excluded.

Participants

A total of forty-two participants were recruited using flyers placed around the University of New Mexico campus and surrounding community were assigned to either sham and verum conditions of the experiment and received monetary compensation for their time. Six of the original forty-two healthy participants were excluded from the analysis (4 from the verum and 2 from the sham group) due to technical difficulties with delivery of brain stimulation during sleep. The remaining thirty-six participants were included in the analysis (mean age = 20.67 years, SD = 2.93 years, 22 female). Of these 36 participants, 15 received verum stimulation and 21 received sham stimulation. 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.

Object Location Memory Task

A novel object location memory task using black and white drawings of objects, based on those used by (Eals & Silverman, 1994), was created to allow for testing hippocampally dependent spatial memory at multiple time points. The experiment was performed using Presentation® software (Version 16.5, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com). Participants were presented with an encoding image which consisted of a 5 x 5 matrix of black and white line drawn objects, randomly selected from a larger set of object stimuli, presented for 30 seconds. The stimuli were then removed, a mask was presented for 1000 ms consisting of a black background with a white centered crosshair. Eleven objects within the matrix were randomly chosen to switch locations making a new test matrix. The test matrix was saved and presented to participants for the immediate test and delayed test to test their accuracy in identifying which objects changed location, to be used as a measure of encoding.

Participants were instructed to select the objects that changed location from encoding image to test image. An example of presented stimuli can be found in Figure 2.

Waking EEG Acquisition

Stimulation and EEG recording were administered using a prototype Starstim64 system (Neuroelectrics, Barcelona, Spain). Electrodes were affixed to the scalp using a 64 channel neoprene cap including 29 Ag-AgCl EEG channels placed according to the international 10-20 system (P7, T7, CP5, FC5, F7, F3, C3, P3, FC1, CP1, Pz, PO4, 02, Oz, O1, CP2, Cz, FC2, Fz, Fp1, Fp2, P8, T8, CP6, FC6, F8, F4, C4, P4). EEG data were sampled at 500 Hz with CMS/DRL reference electrodes placed on the right mastoid. Three additional electrodes recorded both vertical and horizontal electrooculogram (EOG): one placed 1cm inferior and 1cm lateral to the left outer canthus and one placed 1cm superior and 1 cm lateral to the right outer canthus and electrocardiogram (ECG) placed 3 cm inferior to the left collarbone.

Sleep Polysomnographic (PSG) Acquisition

Polysomnographic (PSG) acquisition protocol during sleep was the same as waking EEG acquisition with a few exceptions. EMG electrodes (Oz, PO4) were placed to assist in sleep scoring: Oz placed superior to the chin, inferior to the lower lip and PO4 placed on the right jawline 3 cm (approx.) lateral to the chin, in accordance with PSG recording guidelines set by the American Academy for Sleep Medicine (Berry et al., 2015).

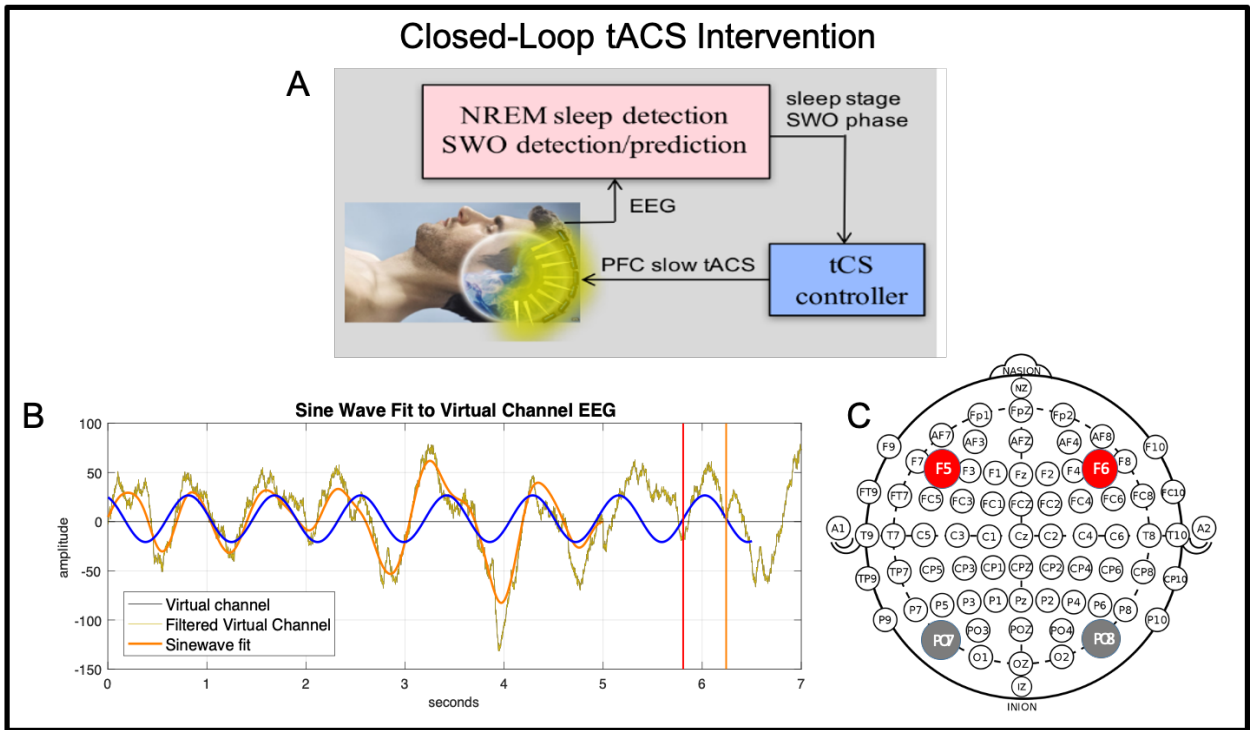
Closed-Loop Transcranial Alternating Current Stimulation (CL-tACS) During Slow-Wave Sleep

During sleep, EEG data were monitored, and the closed-loop stimulation algorithm was started when 4 minutes of continuous N2/N3 sleep was observed by research assistants trained in

identifying sleep stages based on PSG, then allowed to run for the remainder of the sleep period unless paused. Stimulation was paused if the participant showed signs of waking, then resumed after the participant returned to N2/N3 sleep. Both verum (1.5 mA/hemisphere) and sham (0 mA) closed loop transcranial alternating current stimulation (CL-tACS) was delivered over F5 and F6 in phase with ongoing SWOs and 180^{o} out of phase with electrodes at PO7 and PO8.

The CL-tACS intervention for modulation of SWOs was developed in MATLAB 2016a (The MathWorks, Inc.) using EEGLab (Delorme & Makeig, 2004), and is described in more detail in (Ketz et al., 2018). First, the closed loop algorithm detects the presence of SWOs (0.5–1.2 Hz) from data stored in a running 5-second buffer collected from 13 frontocentral channels (Cz, FC1, FC2, CP1, CP2, Fz, C4, Pz, C3, F3, F4, P3, and P4) that was continuously updated, and averaged together to create a virtual channel. The data then undergoes moving average subtraction within a 1-second window to mean center the signals, and noisy channels above 500 μV peak to peak amplitude across the buffer were rejected. Power within the slow-wave band in the virtual channel was compared to the total broadband power (1.5-120 Hz), and the center frequency was computed when the ratio of slow-wave power to higher frequency power was greater than 20%. Next, the algorithm attempted to match the stimulation frequency and phase with ongoing SWA, accounting for the dynamic latency using the system clock, so that the temporal dynamics of the endogenous SWOs were best matched by the sinusoidal waveform of the tACS by fitting a sine wave to the virtual channel using the dominant frequency in the slow wave band, with the offset and phase parameter values optimized. The sine wave was then projected into the future, identifying temporal targets that would synchronize brain stimulation to the predicted endogenous signal. 1.5 mA per hemisphere (verum) tACS was applied for 5 cycles at the detected SWO frequency, then the system idled for 3 seconds to avoid collection of

stimulation artifact in the data buffer. For the sham group, SWOs were detected and stimulation planned in the same manner, but 0 mA of current was delivered.



Note. Adapted from "Dose-Dependent Effects of Closed-Loop tACS Delivered During Slow-Wave Oscillations on Memory Consolidation", by Jones, A.P., 2018, Frontiers in Neuroscience, Vol. 12, https://doi.org/10.3389/fnins.2018.00867

Figure 1. Illustration of method of closed-loop alignment of tACS to SWOs. (**A**) During sleep, custom programmed closed-loop software delivered 1.5 mA tACS per hemisphere. The custom algorithm detects the presence of SWOs in the sleep EEG. Power in the slow wave band is calculated and a virtual channel is created by averaging 13 frontocentral EEG channels from a continuously updated buffer. tACS is applied at a matching frequency and phase to the predominant endogenous SWO for 5-cycles. (**B**) The virtual EEG channel (gold) in the buffer is bandpass filtered in the SWO frequency range (0.5 – 1.2 Hz; red). When stimulation criteria are met, a sine wave at the dominant SWO frequency (blue) is fit to the virtual channel and projected into the future to predict available up states. The dynamics of tACS and the predicted endogenous signal are aligned by matching the phase and frequency of tACS to this projected function. (**C**) Standard 10-10 electrode montage displaying Anode (red) and Cathode (Gray) locations used in CL-tACS protocol.

Experimental Procedure

The experiment was conducted as part of a larger, multi-night study, taking place on the first night (acclimation night). Participants were randomly assigned to one of two manipulations in a between subjects, sham controlled, single blind design: Verum and Sham stimulation. Participants were invited to provide informed consent at an orientation session prior to spending the night in the sleep lab and were given several questionnaires to assess various aspects of their sleep habits, personality traits, and to collect an IQ estimate. During the orientation participants were fitted for an EEG cap to be worn during sleep, and both testing sessions, then were given a tour of the sleep laboratories and an explanation of the EEG/stimulation equipment and experimental procedures.

Participants arrived at the sleep laboratory by 17:00 and were prepped and fitted with an EEG cap and were administered an adapted version of Raven's Progressive Matrices called Sandia Matrices (Matzen et al., 2010). Participants were then allowed to relax in the laboratory until approximately 21:00, when they were prepped for polysomnographic (PSG) recording during sleep. At roughly 22:00, participants were instructed to lie down in a supine position to perform bio-calibrations in order to identify and mitigate sources of noise in later EEG acquisition. Lights out for participants occurred between 22:00-23:00, and they were allowed to sleep uninterrupted for up to 8 hours before being awoken. Upon waking, participants were allowed to use the restroom and were offered snacks and water. They then completed a 1-back task for 21 minutes to arrest sleep inertia and assess alertness. Next, participants completed 2 practice trials of the spatial location memory task with feedback to ensure proper understanding of the task, followed by an encoding and test session. Participants were then disconnected and cleaned from the EEG equipment and released.

For the follow-up session, participants arrived approximately 10 hours after their morning departure (17:30), were prepped for waking EEG acquisition, and a test only session was administered to assess the effects of SWO augmentation on more long-term recall/recognition performance. At that time, participation in this experiment was concluded and participants continued on to perform tasks in the larger study using transcranial electrical stimulation (tES) to enhance memory consolidation.

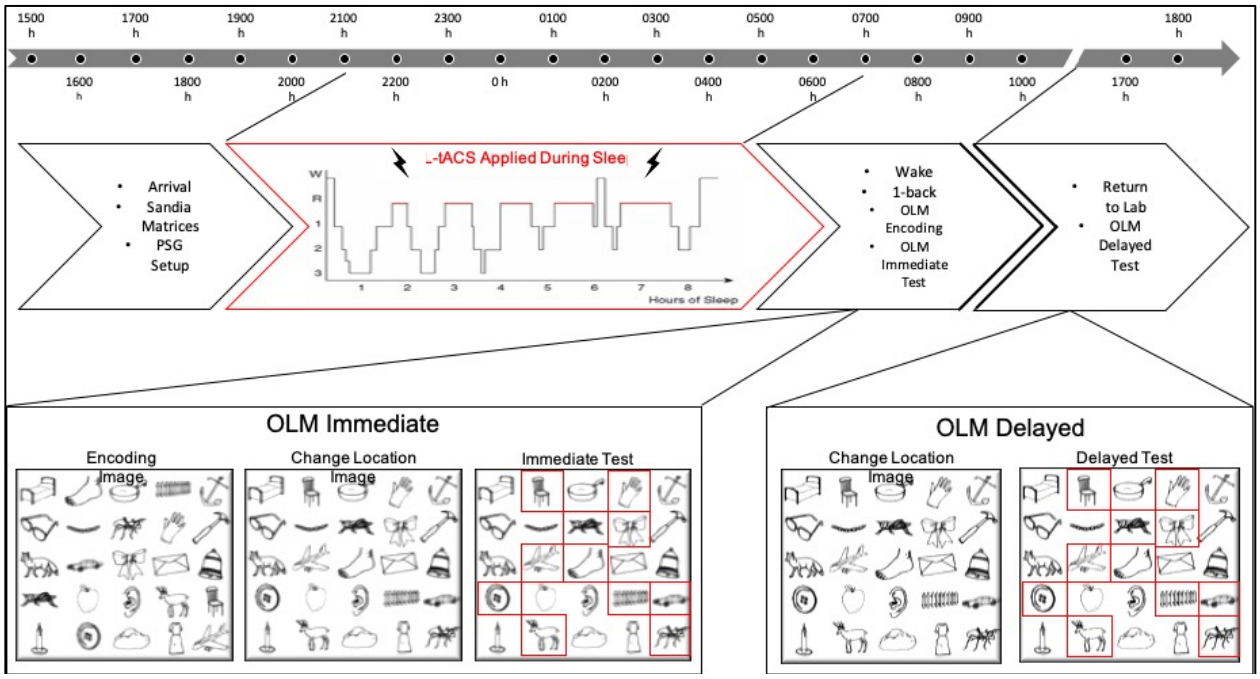


Figure 2. Experimental timeline and OLM task illustration. **Top:** Participants arrived at 1700 h, completed Sandia Progressive Matrices, and were set up for PSG recording. Lights out was between 2200 - 2300 and slept uninterrupted for up to 8-hours during which time the CL-tACS algorithm was run. After waking, participants completed a 1-back task for 21 minutes then completed the OLM immediate task. Participants returned approximately 10-hours after the immediate test for the delayed test. **Bottom:** Example images from OLM task. The OLM immediate test consisted of a 5 x 5 matrix of line-drawn objects (left) for encoding, displayed for 30-sec. After a delay, the change location image (middle) was presented for participants to identify objects that changed location. The right panel identifies objects that changed location in this example for illustration only. For the delayed test, the same change location image (left) presented during immediate testing was presented again for

participants to select the objects that changed location from the original encoding image (Objects that changed location are identified for illustration only).

Measures

Metrics to quantify sleep characteristics were calculated to test for statistical differences between conditions that may confound the results, including: total sleep time (TST) (sum of 30 second EEG sleep epochs scored as NREM/REM sleep by an expert sleep scorer/2) in minutes; sleep efficiency (computed as $\frac{total\ sleep\ time}{total\ time\ in\ bed}$ (Shrivastava et al., 2014)); and SWS ratio ($\frac{N3+\ N4\ epochs}{total\ sleep\ epochs}$), where N3 + N4 epochs are the count of 30 sec. epochs of EEG sleep data scored as N3 & N4 by an expert sleep scorer. Demographic and cognitive characteristics were also assessed between groups to check for confounds, including age, working memory (3-back), fluid intelligence (Sandia Progressive Matrices), and the abbreviated Shipley-2 IQ score (Shipley et al., 2009).

Data Analysis: A priori

Data were analyzed within a repeated measures ANOVA framework, comparing time (within subjects: immediate test, delayed test) and two stimulation conditions (between subjects: verum, sham). For analysis of encoding in the spatial location memory task, performance at the immediate and delayed tests were quantified as retrieval accuracy using F1 accuracy scores (Eq. 1). F1 scores are the harmonic mean average of precision and recall ((McSherry & Najork, 2008), where precision (Eq. 2) is the number of true positive responses divided by the number of all positive responses, and recall (Eq. 3) is the number of true positive responses divided by the sum of true positive responses and false negatives. Its range is from 0 to 1, where 1 is perfect precision and recall.

$$F_1 = 2 * \frac{precision * recall}{precision + recall} \quad \text{(Equation 1)}$$

$$Precision = \frac{true\ positives}{true\ positives + false\ positives} \quad \text{(Equation 2)}$$

$$Recall = \frac{true\ positives}{true\ positives + false\ negatives} \quad \text{(Equation 3)}$$

F1 accuracy scores for the immediate test and delayed test were used as the dependent variables. Since the hypotheses for this experiment include estimates of between group differences at each individual time point, planned simple contrasts between conditions at each time point were included, in addition to the test of interaction between conditions across time to test the difference in forgetting. Additionally, sleep metrics (sleep efficiency, SWS ratio, stimulation events), and demographic (age, gender) and cognitive (Shipley IQ, Sandia Matrices) characteristics were tested between conditions using independent samples *t*-tests investigating potential confounds associated with condition assignment.

Data Analysis: Post Hoc

In order to investigate the effect of SWS on encoding performance, four separate regression models were built to separate the unique effects of SWS ratio in each group at both the immediate and delayed tests. Data were split by treatment group then regression models built where SWS ratio was used as a predictor for F1 accuracy scores at both the immediate and delayed test individually.

Finally, the originally planned repeated measures ANOVA model was again run using SWS ratio as a covariate to investigate the effect of condition on task performance independent of the effect of SWS ratio.

Results

Results of independent samples *t*-tests for demographic (age, gender) and cognitive (Shipley IQ, Sandia Matrices; Table 1) characteristics indicated there were no statistically

significant differences between groups (all *p's* > 0.25), signifying the groups were matched on demographic variables. Measures of total sleep time, time spent awake after sleep onset, and sleep efficiency were not significantly different between verum and sham stimulation conditions suggesting length and quality of sleep were not adversely affected by the stimulation protocol (Table 2). Participants received an average of 544 stimulations (SD = 170) in the verum condition, compared with 500 marked SWO events (SD = 205) in the sham condition. While this difference was not significant ($p = 0.49$) the number of stimulations across participants in the verum group ranged from 294 – 804. The number of stimulation events was not a significant predictor of object location task performance ($F_{1,34} = 3.83$, $p = 0.540$). Additionally, independent samples *t*-tests for sleep stage 1 ($p = 0.33$), sleep stage 2 ($p = 0.70$), SWS ($p = 0.82$), and REM sleep ($p = 0.56$) reveal no significant effect of stimulation condition on sleep architecture.

Table 1. Demographic and Cognitive Characteristics

	Verum			Sham				
	N	Mean	SD	N	Mean	SD	*t*-test	*p*
Age	15	21.33	3.81	21	20.19	2.06	-1.056	0.30
Shipley IQ	15	104.07	12.93	21	100.67	10.35	-0.844	0.41
Sandia Matrices	15	0.45	0.14	21	0.45	0.15	0.054	0.96

Note. There are no significant differences in means between groups

Table 2. Sleep Architecture information

	Verum			Sham				
	N	Mean	SD	N	Mean	SD	*t*-test	*p*
Stimulation Events	15	544.00	170.35	20	499.95	205.6	-0.692	0.49
Total time in bed	15	478.06	22.57	21	479.71	30.17	0.187	0.85
Total sleep time	14	427.86	40.68	20	441.25	41.98	0.932	0.36

Awake	14	15.53	24.55	21	15.19	21.39	-0.44	0.97
Sleep stage 1	14	7.7	6.24	21	9.64	5.18	0.99	0.33
Sleep stage 2	14	262.23	82.62	21	273.19	81.59	0.394	0.70
Slow wave sleep	14	72.37	33.12	21	74.81	28.26	0.232	0.82
REM sleep	14	55.93	32.91	21	61.71	22.77	0.587	0.56
SWS Ratio	14	0.18	0.07	20	0.18	0.06	-0.132	0.90
Sleep efficiency	14	0.90	0.09	20	0.92	0.05	-0.692	0.49

Note. Time is indicated in minutes. Sleep efficiency represents the relative amount of sleep in relation to the total scored sleep time. There are no significant differences in means between groups.

Behavioral Measures

A 2x2 repeated measures ANOVA comparing group (verum SOS, sham SOS) by time (repeated measure - immediate test, delayed test) was run to investigate the effect of SOS on the ability to encode object locations. Repeated measures ANOVA assumptions for homogeneity of variance and sphericity were tested using Levene's test and Mauchly's test, respectively, both were non-significant, indicating the assumptions of homogeneity of variance and sphericity in this sample are reasonable. Shapiro-Wilk tests for normality supported this assumption, with the exception of the delayed test F1 scores for the verum condition being significant ($p = 0.046$). No correction was used for this analysis as the repeated measures ANOVA is robust to violations of the normality assumption. Results suggest a significant main effect of time ($F_{1,34} = 49.155$, $p < 0.001$; Figure 3a) where F1 accuracy at the immediate test was significantly higher ($M = 0.721$, $SE = 0.024$) than the delayed test ($M = 0.542$, $SE = 0.028$), indicating object location retrieval decreased as a function of time, as expected due to forgetting. The main effect of stimulation was also significant ($F_{1,34} = 5.310$, $p = 0.027$, $\eta^2 = 0.135$; Figure 3b), where sham ($M = .683$, $SE = 0.029$) outperformed verum ($M = .580$, $SE = 0.034$), with the η^2 effect size indicating

stimulation condition accounted for 13.5% of the overall variance between groups. The interaction effect was not significant, indicating forgetting between immediate test and delayed test was not statistically significantly different between stimulation conditions. Planned simple contrasts revealed that verum stimulation significantly reduced F1 scores on the immediate test by 12.9% ($p = 0.042$), while the reduction in F1 scores of 17.8% on the delayed test was not significant ($p = 0.064$).

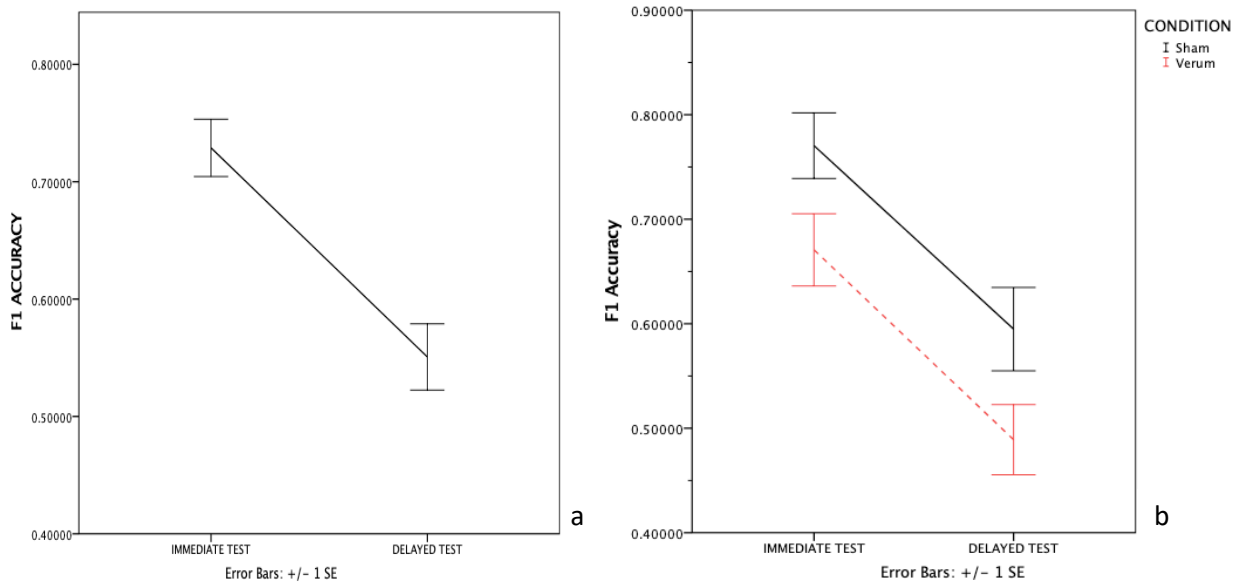
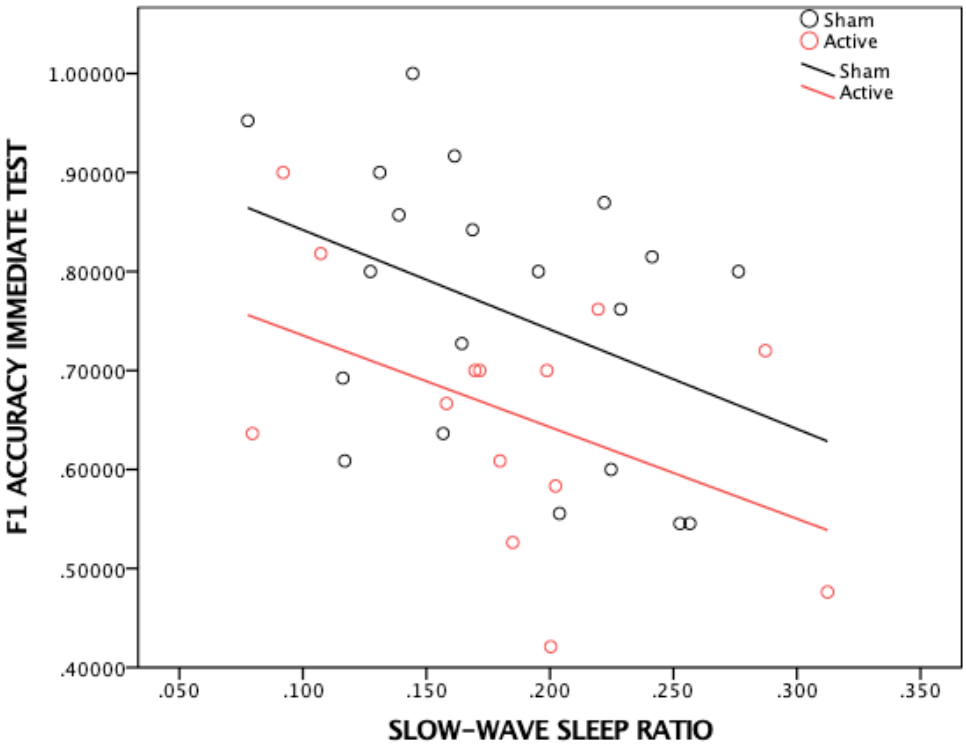


Figure 3. OLM F1 accuracy scores at immediate and delayed tests. 3a shows object location retrieval decreases as a function of time ($p < 0.001$). 3b shows the main effect of stimulation was significant ($p = 0.027$) where sham significantly outperformed verum at immediate test ($p = 0.042$), while the performance difference at delayed test was marginal ($p = 0.064$). The interaction effect was not significant, indicated forgetting across time was not significantly different between groups.

To investigate the effect of SWS on task performance a series of regression models were built using SWS ratio to predict F1 accuracy scores at both the immediate and delayed tests for the sham group and verum group separately. The models were split to test unique effects of SWS ratio in each group given the significant main effect of condition in the ANOVA model. Two subjects (1 verum, 1 sham) had to be excluded due to technical issues

during sleep EEG not allowing for scoring of sleep EEG data. Thus, a total of 34 participants (mean age = 20.71; SD = 2.97; 21 female) were included in the regression analyses. For the sham group, SWS ratio was a significant predictor at both immediate test (β = -0.446, $F_{1,18}$ = 4.466, p = 0.049) and delayed test (β = -0.495, $F_{1,18}$ = 5.845, p = 0.026). For the verum group, SWS ratio was a significant predictor of F1 accuracy at the delayed test (β = -0.624, $F_{1,12}$ = 7.670, p = 0.017), but not the immediate test (β = -0.421, $F_{1,12}$ = 2.588, p = 0.134).

Given that the ratio of SWS was a significant predictor of task performance, a 2x2 repeated measures ANCOVA (Figure 4) was run as indicated above with the addition of SWS ratio as a covariate. Results indicate there is no longer a main effect of time ($F_{1,31}$ = 1.121, p = 0.298) when controlling for SWS ratio. The covariate SWS ratio is a significant predictor of performance ($F_{1,31}$ = 14.107, p = 0.001). After controlling for SWS ratio the effect of stimulation condition remains significant ($F_{1,31}$ = 6.364, p = 0.017).



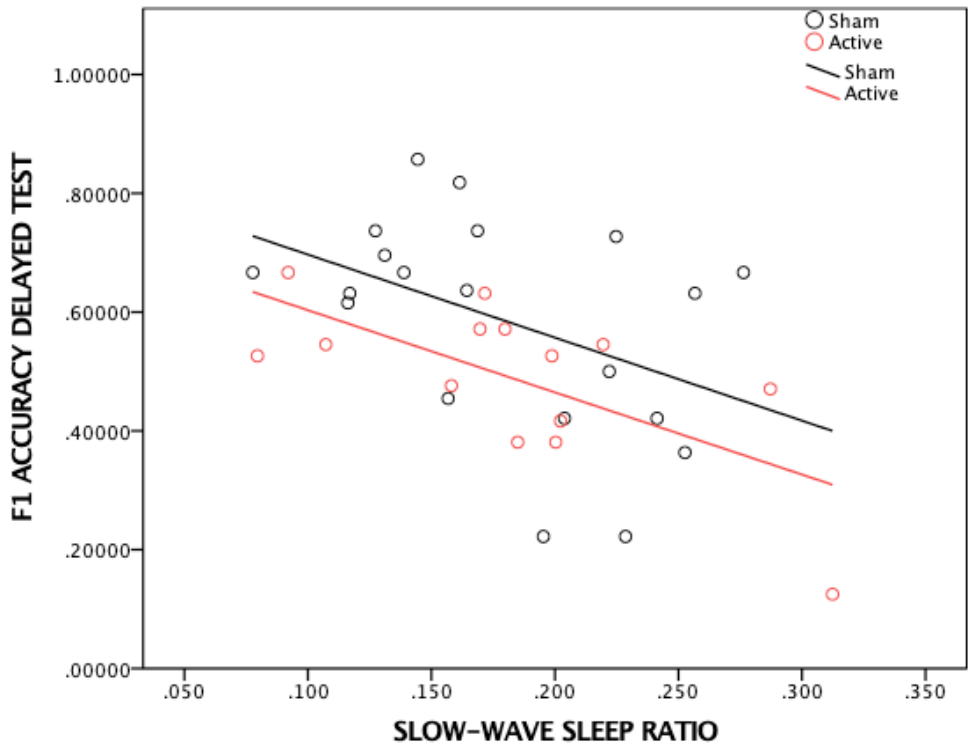


Figure 4. OLM F1 accuracy scores at immediate test (top) and delayed test (bottom) as a function of SWS ratio. Object location retrieval decreased as relative SWS increased ($p = 0.001$). The effect of CL-tACS condition remains significant ($p = 0.017$) when controlling for relative amount of SWS.

Discussion

To date, most research on how SWS affects learning and memory has focused on its role in the consolidation of declarative information. SHY suggests an additional role for SWS in preparing hippocampal resources for post-sleep encoding. According to this hypothesis synaptic downscaling during SWS reduces synaptic weighting saturating memory systems following sustained wakefulness, thus restoring encoding capacities. This theoretical framework is supported by a decrease in encoding capacity for new information after loss of sleep (Mander et al., 2011) and improved learning of new materials post-sleep as well as modulation studies

whereby quantity of SWA was artificially induced (Antonenko et al., 2013) resulting in increased encoding capacity, or selectively depressed (Van Der Werf et al., 2011) thereby reducing it. While this literature provides compelling evidence for SWS's role in post-sleep memory processing, the mechanisms by which SWS contributes to this relationship are not well understood.

This study employed SWS augmentation using tACS matched in phase and frequency to endogenous SOs in an online EEG-feedback controlled manner, which has previously been demonstrated to improve overnight consolidation, to further elucidate the relationship between SWS and subsequent encoding. Based on the results of previous studies employing forms of SO augmentation during a daytime nap, it was suggested that CL-tACS targeting SOs during nocturnal sleep would support synaptic downscaling of hippocampal resources used in encoding of declarative information, thus increasing capacity for hippocampally dependent learning in the subsequent waking period. Although a reduction in F1 accuracy over time was expected, we hypothesized less forgetting over time for the verum condition as compared to sham. This hypothesis was not supported by our findings as the absence of a significant interaction between time and stimulation condition suggests verum stimulation did not modulate the rate of forgetting.

We also hypothesized that verum CL-tACS would lead to increased F1 accuracy scores for changed object locations compared to sham at both the immediate and delayed test. Our results suggest the opposite of the hypothesized relationship where verum stimulation resulted in a 12.9% decrease in accuracy at the immediate test and 17.8% decrease in accuracy at the delayed test. These results clearly indicate that stimulation across the full sleeping period was detrimental to subsequent encoding for declarative object location information. Collapsed across

time, participants that received verum stimulation during sleep displayed a reduced ability to identify objects that changed location by 15%. Importantly, no effect of verum CL-tACS as compared to sham stimulation on measures of sleep efficiency, ratio of SWS to total sleep, or number of slow-wave events targeted by the closed-loop algorithm was found, suggesting that these changes are not explained by differences in sleep architecture as a possible result of disruption by the stimulation protocol. Further, the relationship between stimulation condition and encoding capacity remained significant when amount of SWS was controlled for.

These results, along with results from SO augmentation studies during daytime nap, demonstrate that the processes governing synaptic downscaling are sensitive to either the timing or dose of stimulation. While the present study is not the first to investigate augmentation of SWA on post-sleep encoding, it is the first study to investigate such effects after augmentation of SWS in a full nocturnal sleep period. The length of the sleep period, and duration of the targeted SWA augmentation may both be important factors in the present findings. Both previous studies targeting SWA augmentation during sleep occurred during controlled nap periods of up to 90 minutes (or 1-full sleep cycle). PSG analysis consistently shows that SWS is most prevalent early in the sleep period and diminishes in intensity and duration while SWA is decreased throughout the night as networks achieve homeostasis. SWA has been shown to be a regulated process that increases in proportion to the duration of prior wakefulness, progressively decreasing during sleep (Borbly, 2001). These time/quantity dependent decreases in SWA suggest the network level conditions driving SWA decrease as optimization of network conditions are achieved. One explanation for the natural decrease in SWA throughout the sleeping period is processes affected by SWA are sensitive to such a balance. Together, these results suggest the homoeostatic balance of SWS that benefits subsequent encoding is achieved

early in the sleep period such that further optimization via continued augmentation beyond the optimization achieved through natural sleep is not possible, or possibly detrimental. Limiting SWA enhancement to the first cycle of sleep, when network conditions driving SWA are strongest, may increase the efficiency with which SWA frees hippocampal resources for future learning, while continued augmentation of the duration and intensity of SWA beyond this natural balance may continue the downscaling process beyond the point of optimization, resulting in a non-optimized state and deficits in network function as well as synaptic plasticity. This information suggests augmentation of SWA is time and/or dose dependent whereby timing of SWA enhancement for subsequent encoding benefit must occur before nocturnal sleep has achieved network optimization and dosage of CL-tACS must be carefully designed to avoid downscaling beyond homeostasis. This notion supports the idea that sleep can be augmented such that it's beneficial effects on post-sleep learning can be achieved in a shorter period of time, such as a 90-minute nap, but possibly not enhanced beyond the sleep systems already optimized performance achieved during the nocturnal sleeping period. This is an important topic to address in the field of sleep research, as much recent attention is focused on using NIBS to augment consolidation during sleep, it is possible that other systems benefitting from sleeps restorative effects are sensitive to disruption of the homeostatic balance of sleep.

Limitations/Future Directions

The current study has several limitations which should be noted. The OLM task employed in the current study is novel to sleep research and while the task design has been used in memory research, it has not been employed in research specifically targeting hippocampally dependent declarative memory. Previous research of sleep and declarative memory have largely employed versions of the PAT. This difference in task paradigm is a caveat when drawing direct

comparisons to results of previous research of post-sleep encoding. Further, our conclusions regarding the effects of CL-tACS during nocturnal sleep on post-sleep encoding are limited to declarative spatial memory, as there was no measure of procedural learning. A lack of double-blinding of stimulation condition was another possible limitation, as research assistants were aware of the stimulation condition. Likewise, the current study employed a between-subjects design comparing a single verum stimulation condition to sham, limiting opportunities for further understanding of dose dependent effects of amplitude and duration of stimulation as well as timing. Employing a similar stimulation protocol in a within-subjects design would provide added insight into intra-individual variation resultant from the CL-tACS protocol. Given reports of dose dependent outcomes using tES in other cognitive domains, further investigation into the possible effects of CL-tACS at multiple amplitude conditions, as well as comparisons between stimulation duration such as limiting stimulation to the first quartile vs. the first half of the nocturnal sleep period, etc., in addition to varying when in the sleep cycle stimulation is initiated, such as second half of the night vs. first half are avenues for future research.

Arguments about the specificity of the sleep measures reported in the present research may also be raised. In comparison with EEG measures of SO amplitude, duration, power and phase coupling, the sleep measures reported here, namely the number of 30 second sleep epochs scored as SWS or the ratio of SWS to total sleep, are poor metrics for the acute effects of stimulation. Indeed, one notable limitation of the current study is the exclusion of sleeping EEG analysis. Despite the closed loop algorithms reported accuracy in previous research, validation of the accuracy with which the CL-tACS algorithm predicted SOs, as well as their phase and frequency, has not been performed on the current sample. While such analyses were beyond the scope of the current investigation, they remain a possibility for future research and may provide a

more complete understanding of the mechanisms resulting in the behavioral outcome reported here. This is a resounding caveat for the behavioral outcomes discussed, as stimulation that did not accurately identify and target SOs, or apply tACS in phase and frequency with endogenous SOs may decrease the efficacy with which SWA benefits learning, as has been demonstrated when SWS is selectively depressed resulting in decreased encoding capacity (Van Der Werf et al., 2011). Additional development of effective methods to resolve interference in the EEG signal during stimulation are necessary however, as this interference precludes reliable evaluation of such acute effects on individual SOs and their subsequent contribution to behavioral outcomes during the stimulation period. Closed loop augmentation of SWA employing auditory stimulation similar to Ong et al. (2018) implemented during nocturnal sleep is one possible avenue to circumvent EEG interference inherent to tES.

Conclusion

Previous work implementing NIBS targeting SWA during afternoon naps to modulate post-sleep encoding suggest that augmentation of SWA during sleep may improve hippocampally dependent declarative learning, providing support for SHY. In the first study employing CL-tACS targeting SOs during nocturnal sleep to investigate post-sleep encoding to date, we found that stimulation across the full sleeping period was detrimental to subsequent encoding of declarative object location information. While contrary to our hypotheses, these results support a causal role for SWS in hippocampal synaptic plasticity, while also introducing questions about the nuances of this relationship. The decrease in encoding capacity after augmentation of SO's during nocturnal sleep, along with the reported benefit of SOS during a 90-minute nap, suggests that augmentation of SWA may accelerate sleep's benefit on synaptic

downscaling, but continued stimulation may drive downscaling beyond the sleep systems homeostatic balance. Future research should test the effects of this protocol during both nap and partial sleeping periods. If targeted enhancement of SWA can confer the memory benefits of nocturnal sleep on truncated sleep, it may prove a beneficial treatment given our societies decreasing emphasis on getting a full night's sleep. Additionally, given the popularity of research on how SWS aids consolidation of memory, and the growing interest in NIBS to modulate such processes, additional consideration to account for down-stream effects on memory processes such as encoding in future studies employing modulation of sleep architecture are proposed.

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