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Effects of phase-locked acoustic stimulation during a nap on EEG spectra and declarative memory consolidation

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ABSTRACT

Objectives: Acoustic stimulation synchronized to slow waves (SWs) can enhance these sleep features and facilitate memory consolidation during nocturnal sleep. Here, we investigated whether a similar benefit could be accrued following stimulation during an afternoon nap. We also evaluated the event-related dynamics of associated EEG spectral changes and their correlation with memory performance.

Methods: Sixteen healthy young adults (mean age: 22 ± 1.4 years; nine males) were studied under two conditions: stimulation (STIM) and no stimulation (SHAM), in counter-balanced order. In the STIM condition, acoustic stimulation was delivered using blocks of five tones, each phase-locked to the SW up-state during a 90-min nap opportunity. In the SHAM condition, these time points were marked, but tones were not presented. Prior to the nap, participants learned 40 semantically related word pairs and immediate recall was tested. A delayed recall test was administered 45 min after awakening.

Results: Compared to the SHAM condition, acoustic stimulation increased SW amplitude, theta, and fast spindle activity and attenuated the forgetting of word pairs (p values < 0.05).

Conclusion: Phase-locked acoustic stimulation can promote sleep-dependent declarative memory during a daytime nap. This can be achieved by stimulation in Stage 2 and SWS without a requirement for high-amplitude slow wave detection.

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1. Introduction

There is compelling evidence that sleep facilitates consolidation of declarative memory [1–3], resulting in better memory performance after sleep than after a comparable duration of wakefulness. Sleep-dependent memory consolidation has been linked to neural oscillations, particularly those associated with slow-wave sleep (SWS) [4,5].

Low-frequency (0.5–4 Hz) brain oscillations constitute slow-wave activity (SWA), with slow oscillations greater than 75 μ V being the hallmark of SWS. External stimulation can enhance specific brain oscillations [6,7] leading to the reorganisation of memory traces and communication between cortical networks – processes that underlie systems consolidation of memory [8]. Slow (0.5–1 Hz) oscillations (SOs) generated within cortical and thalamic networks [9,10] appear to be particularly relevant for the consolidation

of declarative memories and are thought to act by coordinating the reactivation of memories during sleep [1,11]. SOs can be augmented by transcranial direct current stimulation (tDCS) [12–15], transcranial magnetic stimulation (TMS) [16], intracranial electrical stimulation [17], and acoustic stimulation [18,19]. This enhancement of SO amplitude can significantly improve overnight retention of learned word pairs in humans [12,13,15,19] and object-place recognition in rats [14]. In addition, activity in the delta frequency range (1–4 Hz) has also been suggested to be functionally similar albeit less potent than SOs [20,21]. These findings support a causal role of both SOs and delta waves (together termed SWA) in the offline processing of declarative memory [11,21].

Rhythmic acoustic stimulation presents a promising method of augmenting SWA with non-invasive, external stimuli as it (1) is non-invasive compared to its tDCS, TMS, and intracranial counterparts; (2) allows for the concurrent fine-grained analysis of EEG, and (3) offers more options for out-of-laboratory studies (eg, at home) due to low technical requirements. Such rhythmic acoustic stimulation during nocturnal sleep has been shown to entrain endogenous SWA and increase its amplitude. Both SWA augmentation and improvement in declarative memory appear to depend on the timing of auditory stimulation in relation to SWA phase [19].

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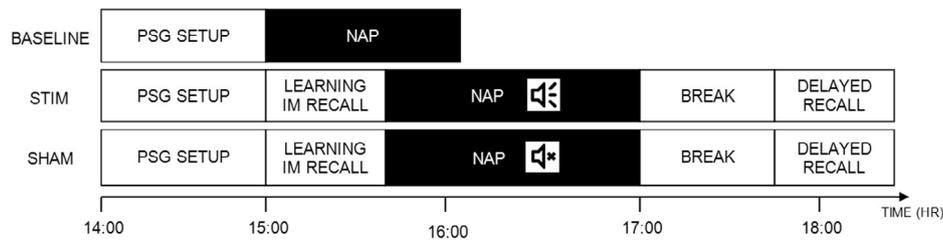


Fig. 1. Experimental procedure. In the BASELINE session, following preparation for polysomnography (PSG), participants napped for 1 h. Auditory tones were played in order to establish thresholds for use in the STIM session. In the STIM and SHAM sessions, PSG setup was followed by a learning period (LEARNING) where participants were required to remember 40 semantically related word pairs, and then an immediate recall phase (IM RECALL) where performance was tested. Afterwards, participants had a 90-min nap opportunity. In the STIM session, acoustic stimulation was presented throughout the nap period following certain criteria (see Methods), while none was administered in the SHAM session. The nap period was followed by a 45-min break to minimize any effect of sleep inertia, after which participants completed a delayed recall test (DELAYED RECALL).

We investigated a variation on these acoustic stimulation methods to determine whether similar improvements in declarative memory could be produced during a short interval of sleep in the afternoon. Prior work has demonstrated that, without any further intervention, a 60- to 90-min nap opportunity and nocturnal sleep can produce comparable benefits on memory [22,23]. We employed a novel, adaptive-detection algorithm to detect the slow wave (SW) up-phase (both SOs plus delta), which theoretically could accommodate the temporal jittering of naturally occurring SWs and improve in-phase presentation of acoustic stimuli. Furthermore, the procedure was adapted to increase the likelihood that oscillations that could benefit memory consolidation would be enhanced; rhythmic acoustic stimulation commenced once stable sleep stage 2 was detected, and the conventional SW amplitude threshold (75 μ V) was not required [24]. This protocol could thus accommodate SWs that arise under the lower sleep pressure [25] inherent in naps compared to nocturnal sleep.

2. Methods

2.1. Participants

Sixteen participants (mean \pm SD age: 22 \pm 1.4 years; nine males) took part in the study. They were selected from volunteers who reported that they: (1) were fluent English speakers, (2) had consistent and regular sleep habits, (3) had no history of sleep, psychiatric or neurological disorders, (4) consumed less than two caffeinated drinks per day, (5) were not on any long-term medications, (6) were non-smokers, (7) scored \leq 5 on the Pittsburgh Sleep Quality Index [26] (PSQI), and (8) scored \leq 10 on the Epworth Sleepiness Scale [27] (ESS).

Participants were required to follow a specific sleep schedule for at least one week prior to each sleep session. Each person was expected to maintain regular sleep and wake patterns (\pm 1 h) and to wake up no later than 08:30. To increase sleep propensity, participants were required to wake up before 06:00 on the morning of the experiment, after no more than 5 h of sleep. Adherence to the sleep schedule was ensured via wrist actigraphy (Actiwatch, Philips Respironics, USA). Participants were also required not to undertake any strenuous physical activity, consume alcoholic or caffeinated food or beverages in the 24 h preceding each sleep session, or to take any naps prior to the session. All participants provided written informed consent in accordance with this protocol that was approved by the National University of Singapore Institutional Review Board.

2.2. Study procedure

Participants completed three afternoon nap sessions: BASELINE, STIM (with acoustic stimulation), and SHAM (without acoustic

stimulation), each separated by a minimum duration of one week (Fig. 1). Baseline sessions were always conducted first. The order of STIM and SHAM sessions was counterbalanced across participants.

The BASELINE session was conducted to (1) allow the participant to become accustomed to sleeping under laboratory conditions, (2) verify that at least 5 min of Stage 2 or 3 sleep occurred in the allocated nap period in order to allow sufficient time for determining stimulation thresholds, and (3) establish the maximum volume of stimulation that would not wake up the participant. For this session, participants arrived at the laboratory at 14:00. After preparation for polysomnography (PSG), participants were given a 60-min nap opportunity at 15:00. Acoustic stimulation (50-ms bursts of pink noise) was presented in blocks of five tones (Fig. 2) through in-ear headphones once the participant entered stable stage 2 sleep (at least 5 min), according to predetermined thresholds (see *Adaptive detection of slow oscillations* for further details). During this session, a staircase titration of tone volume was performed to establish the maximum tone volume that did not awaken the participant. This volume was then used in the subsequent STIM session.

For STIM and SHAM sessions, participants arrived at the laboratory at 14:00, and underwent preparation for PSG recordings. At 15:00, participants self-rated their sleepiness levels using the Karolinska Sleepiness Scale [28] before performing a paired-associate task between 15:00 and 15:45 (see *Memory task* for further details). Participants were then given a 90-min nap opportunity. Acoustic stimulation was delivered throughout the entire nap period in the STIM condition. In the SHAM condition, in-ear phones were similarly used as in the STIM condition but stimulation volume was muted.

2.3. Memory task

In the paired-associate task, 40 semantically related word pairs (Supplementary Table S1) previously used by Payne and colleagues [29] (eg, animal – fox) were presented sequentially in a randomized order on a computer monitor for 5 s each with an inter-stimulus interval of 1 s. This was followed by an immediate recall test in which participants were shown the first word of each pair in a random order and were required to respond by typing the corresponding second word. Minor spelling errors were ignored (eg, omissions of letters that did not alter the meaning of the word-pair, such as ‘smoke’ and ‘smke’). There was no time limit. Immediately after each response, participants were informed whether they were “Correct” or “Incorrect,” but the correct word was not given as feedback would artificially increase performance by allowing re-encoding or learning to occur before the delayed recall phase [29]. Presentation of word pairs and immediate recall tests was repeated until participants reached an accuracy criterion of 60%.

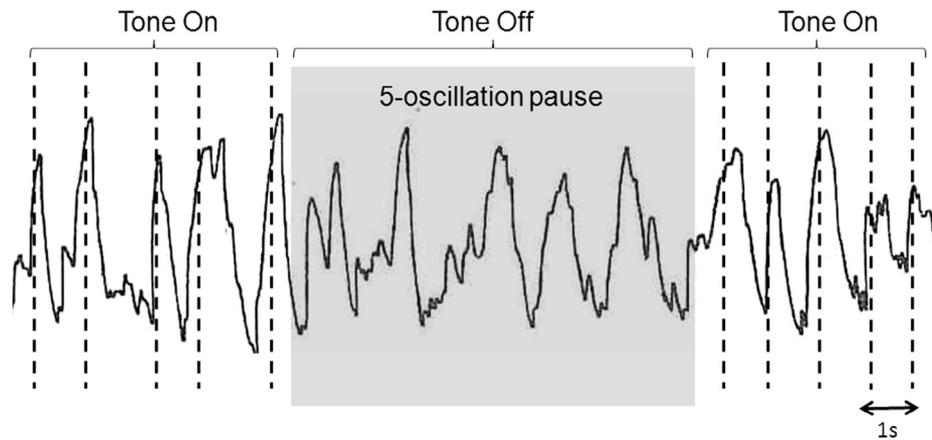


Fig. 2. Tone ON-OFF Blocks. Acoustic stimulation was delivered in blocks of five tones (vertical dashed lines), synchronized to the up phase of the detected slow waves (SWs). This was followed by a pause of 5 SW before continuation of phase-synchronized auditory stimuli. Dotted lines represent when a signal to commence tone stimulation was sent to the soundcard.

The mean number of whole-list presentations required to achieve this criterion was 1.5 (mean \pm SEM; STIM: 1.5 ± 0.13 , SHAM: 1.5 ± 0.16 , $t(15) < 0.01$, $p > 0.99$). A different word list was used for each experimental session, and the order of word lists was counter-balanced across participants and conditions.

During the delayed recall test after the nap, memory of the word pairs was tested in the same manner as in the immediate recall tests but without feedback. Delayed recall of word pairs commenced 45 min after waking, in order to minimize possible effects of sleep inertia. Memory change was indicated by the difference in the number of correctly recalled word pairs from the last immediate test to the delayed recall test.

2.4. EEG recordings

Continuous EEG was recorded using a BrainAmp MR amplifier (Brain Products GmbH, Munich, Germany) from eight channels (international 10–20 system, F3, F4, C3, C4, P3, P4, O1, and O2) referenced to linked mastoids (M1, M2). Electrooculography (EOG) and submental electromyogram (EMG) measures were also obtained. Ag-AgCl electrodes were used, and impedances were kept below 5 k Ω for EEG electrodes and below 10 k Ω for EOG and EMG electrodes. Signals were sampled at 500 Hz and filtered between 0.1 and 250 Hz.

2.4.1. Adaptive detection of slow oscillations and in-phase acoustic stimulation

EEG from the F3 electrode was used for real-time detection of SWs and synchronisation of the acoustic tones. Data were recorded in two ways: (1) via the Brain Vision Recorder acquisition software and (2) through a MATLAB Application Programming Interface (API) that communicated with the amplifier via a TCP/IP port. Every 20 ms a packet of 10 data points was sent to the MATLAB API. A custom-made MATLAB R2012a (MathWorks, Natick, MA) script (Santostasi et al., unpublished observation) processed the incoming EEG data for detection of SWs and application of acoustic stimulation in-phase with the SWs in real-time. EEG pre-processing consisted of applying a bandpass filter (Chebychev second order) with cut-off frequencies at 0.5 and 38 Hz. Data were down-sampled to 100 Hz to avoid aliasing. A phase-locked loop (PLL) was employed to dynamically track the phase of ongoing SWs in order to accurately deliver tones at the target phase. The PLL combines a voltage-controlled oscillator (VCO), a loop filter, and a phase detector in order to ensure that the output of the system is continuously at a constant phase angle (locked) with respect to an input, refer-

ence signal. A detailed discussion of PLL implementation has been previously published [30,31], as have examples of applications to analyse and classify EEG oscillations in real time [30,32]. Tones were delivered just before the up-phase of each SW (VCO centre frequency, $f_c = 0.85$ Hz, bandwidth of 3.7 Hz to adapt to the changes of the dominant SW frequency, 0.5–4 Hz). Specifically, each tone commenced about 30 degrees (corresponding to 70 ms in the case of a 0.85-Hz slow oscillation) before the projected peak of each SW up-phase in order to account for hardware delays (data packet delivery time of 20 ms + delay in soundcard activation of about 50 ms). Once the subject's EEG recording was visually determined to be in stable Stage 2 sleep, an SW detection algorithm was commenced. Upon criterion fulfilment ($SW_{\text{negativepeak_amplitude}} > 40 \mu\text{V}$ and time interval between 0.25 and 2 s between consecutive peaks), tones were applied in alternating 'ON-OFF' blocks of five SWs with in-phase acoustic stimulation followed by five SWs without acoustic stimulation (Fig. 2). Stimulation continued as long as at least 20% or 6 s of the last 30-s window contained SWs/K-complexes that fulfilled this criterion. As the algorithm dynamically adapted tone onsets to real-time EEG readings, block durations varied slightly. If arousals (defined by increases in both beta and alpha power above $4 \mu\text{V}^2$ and $28 \mu\text{V}^2$, respectively) were detected, stimulation was halted for at least 30 s. These parameters were selected following investigations from a series of pilot studies in order to maximize the likelihood that the acoustic tones would be delivered at the target phase (Santostasi et al., unpublished observation). In the SHAM condition, SW detection was similarly performed, but no tones were played. SW detection was applied continuously throughout each nap session.

2.5. Analyses of sleep measures

Sleep scoring analyses were performed using the FASST toolbox (<http://www.montefiore.ulg.ac.be/~phillips/FASST.html>). EEG signals were band-pass filtered between 0.1 and 25 Hz. Scoring was performed visually by two trained technicians following the criteria set by the AASM Manual for the Scoring of Sleep and Associated Events [24]. Total sleep time (TST) and time spent in different sleep stages were determined.

2.6. EEG preprocessing and analyses

Functions from the EEGLAB toolbox (<http://sccn.ucsd.edu/eeGLab>) along with custom scripts written in Matlab were used to pre-process and analyse EEG data. The performance of the adap-

tive SO detection algorithm was determined by measuring the instantaneous phase of each SO at tone onset. To do this, EEG recorded from the F3 channel was band-pass filtered from 0.7 to 1 Hz using a zero-phase shift, Hamming windowed sinc finite-impulse response (FIR) filter followed by a Hilbert transform to extract instantaneous phase.

To assess evoked activity arising from acoustic stimulation, the EEG signal for both STIM and SHAM conditions was bandpass filtered between 0.1 and 30 Hz and averaged across all ON blocks. Data were analysed for a 4-s window, time-locked to the first of the five acoustic stimuli within the ON block and baseline-corrected to a 1-s pre-stimulus interval. Epochs where arousals occurred were removed from further analyses. The numbers of ON blocks averaged in the STIM and SHAM conditions were 157.88 ± 29.1 and 161.88 ± 22.0 , respectively. EEG signals from the F3 electrode were analysed, given the prominence of slow oscillations in the frontal channels. Analyses from the central channels (used in prior work by Ngo and colleagues [19]) yielded similar results (Supplementary Fig. S1).

For spectral analyses, EEG was segmented into 4-s OFF and 4-s ON epochs for both STIM and SHAM conditions. For each epoch, power spectral density (PSD) estimates were computed for four event types – ‘STIM ON,’ ‘STIM OFF,’ ‘SHAM ON,’ and ‘SHAM OFF’ using a Hamming window over the entire segment (2000-point FFT). This resulted in a frequency resolution of 0.25 Hz. PSD estimates were averaged and subsequently smoothed using a three-point moving average. To account for inter- and within-individual variability, each average PSD was then divided by the mean cumulative power in the 0.25- to 30-Hz band across all ON and OFF blocks within each STIM/SHAM nap session.

Secondly, average percentage power increase in ‘STIM ON’ compared to ‘SHAM ON’ (normalized) in each of six specified frequency bands was computed using the formula: $(\text{STIM ON}_{\text{power}} - \text{SHAM ON}_{\text{power}}) * 100 / \text{SHAM ON}_{\text{power}}$. The six frequency bands were: SWA (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), slow spindle (12–14 Hz), fast spindle (14–16 Hz), and beta (16–20 Hz).

Thirdly, we characterized time-varying changes or perturbations in the spectral content of the data that might not have been captured in time-averaged signals. While *evoked*, phase-locked activity can be observed by averaging across multiple trials, *induced* (non-phase locked) activity is diminished or cancelled by signal averaging [33,34] due to phase cancellation. By computing event-related spectral perturbation (ERSP) plots that firstly estimate spectral changes over each single trial in a specified time-window, and then averaging these changes across trials normalized to a specified pre-stimulus baseline, identification of induced activity is made possible.

The EEGLAB function ‘newtimef.m’ was used to perform time-frequency analysis. This function employs a family of complex Morlet wavelets to decompose signals into time-frequency representations. The number of wavelet cycles used increased from 1 cycle at 0.8 Hz to 28 cycles at 25 Hz in 100 log-spaced frequencies corresponding to each frequency bin. Power estimates (in decibels) were normalized to the average baseline, defined as –200 to 0 ms before each ON block. To characterize the amount of phase-locking across trials, inter-trial coherence (ITC) was used to estimate phase consistency across trials at different frequencies. A significant increase

in ITC would indicate that EEG activity at a specified time and frequency was phase-locked with respect to stimulus onset.

Spindle detection was performed in stage 2 and stage 3 sleep based on an amplitude criterion similar to an algorithm by Mölle and colleagues [35] and implemented in the FASST toolbox. EEG was bandpass filtered between 12 and 16 Hz, and spindles were identified whenever the RMS value exceeded the 90th percentile for at least 500 ms for each recording.

2.7. Statistical analyses

The performance of the adaptive SO-detection algorithm was assessed using circular statistic functions from the CircStat toolbox [36]. For all other analyses, paired *t*-tests (two-tailed) using a significance level of 0.05 were used to determine differences in memory performance and EEG signals between the STIM and the SHAM conditions. In addition, Cohen’s *d* [37] was computed to indicate the size of the STIM effect on memory consolidation and SWA power. This measure expresses differences between two means divided by the pooled standard deviation.

3. Results

SW events marked did not significantly differ between STIM and SHAM conditions (Table 1). The average ISI between tones in the STIM condition was 1.112 ± 0.04 s (mean \pm SD).

3.1. Performance of adaptive SW-detection algorithm

The mean instantaneous phase when considering SW detections greater than 75 μ V in amplitude was 49.72 ± 26.69 degrees (STIM) and 49.62 ± 44.71 degrees (SHAM) (mean \pm standard deviation; Fig. 3a, b), and this difference was not significant (Watson Williams circular *t*-test, $p = 0.92$). When considering SWs between 40 and 75 μ V in amplitude, the mean phase was 54.07 ± 47.31 degrees (STIM) and 52.30 ± 50.29 degrees (SHAM) (Fig. 3c, d). This difference was also not significant (Watson Williams circular *t*-test, $p = 0.26$). When comparing the mean direction of these plots (Fig. 3a–d) to the target phase of 60 degrees, this difference was not significant (circular *v*-test, $p = 1$) indicating that the target phase was successfully detected by the algorithm.

3.2. Average auditory event-related potentials (ERPs)

Event-related potentials time-locked to the start of the first tone of each five-tone block were averaged for STIM and SHAM conditions (Fig. 4). Tones elicited a greater increase in the amplitude of slow oscillatory activity in the STIM condition, with this effect tapering off after the second oscillation.

3.3. Average power spectral density (PSD)

Spectral analyses revealed significant increases ($p < 0.05$) in normalized power between ON blocks in the STIM compared to the SHAM condition in specific frequency bins (Fig. 5a and b).

Table 1
Mean (\pm SEMs) for number and % of total tones played across sleep stages.

Sleep stage	SHAM (number)	STIM (number)	P	SHAM (% total)	STIM (% total)	<i>p</i>
All	914 \pm 100	872 \pm 119	0.76	–	–	–
S2	739 \pm 84	598 \pm 67	0.10	84.73 \pm 5.20	78.97 \pm 6.55	0.32
S3	170 \pm 63	254 \pm 117	0.41	14.73 \pm 5.18	17.83 \pm 6.70	0.61
Others	5 \pm 1	20 \pm 7	0.06	0.54 \pm 0.16	3.20 \pm 1.50	0.11

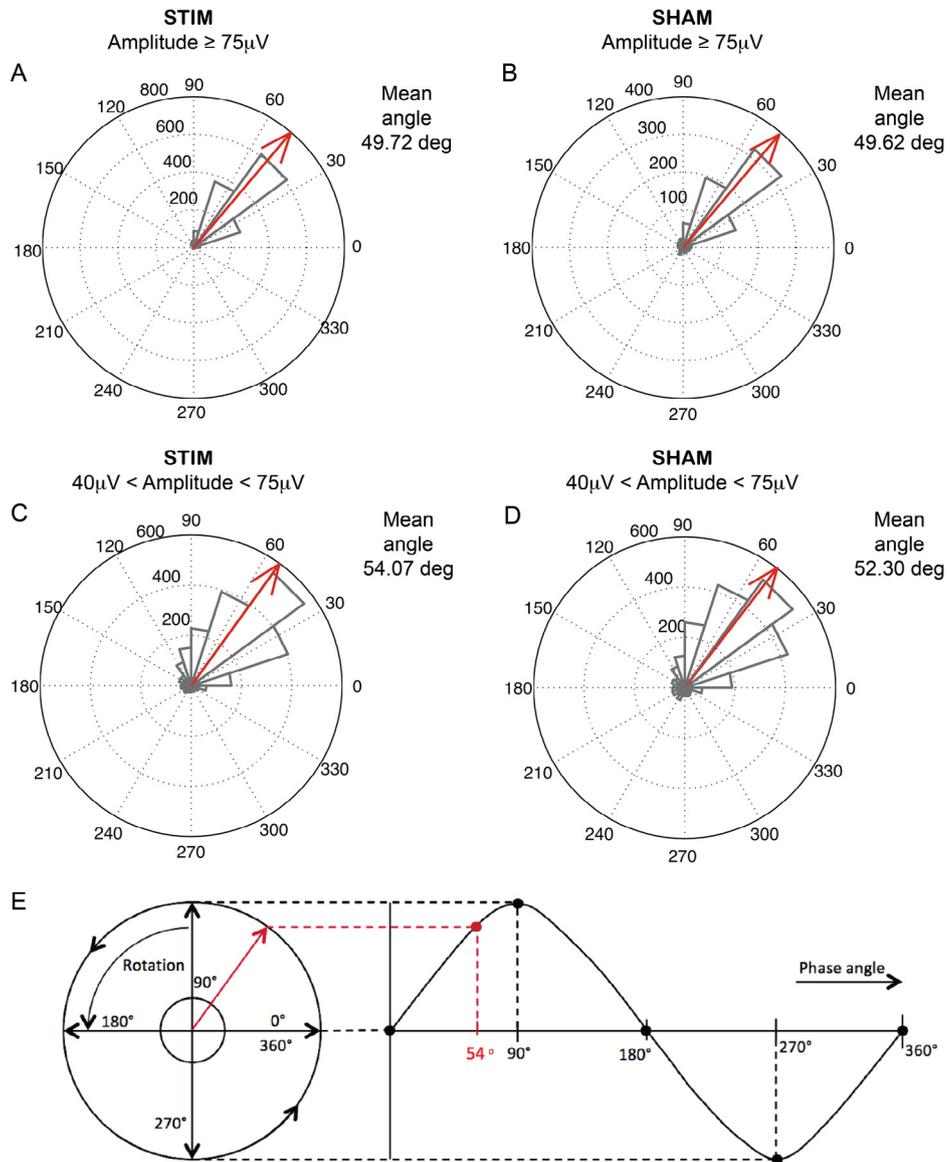


Fig. 3. Circular histograms for number of tones played during STIM and SHAM conditions collapsed across all participants, for slow wave (SW) amplitudes greater than 75 μV (A, B) and SW amplitudes between 40 μV and 75 μV (C, D). (E) Graphical depiction of phase angle.

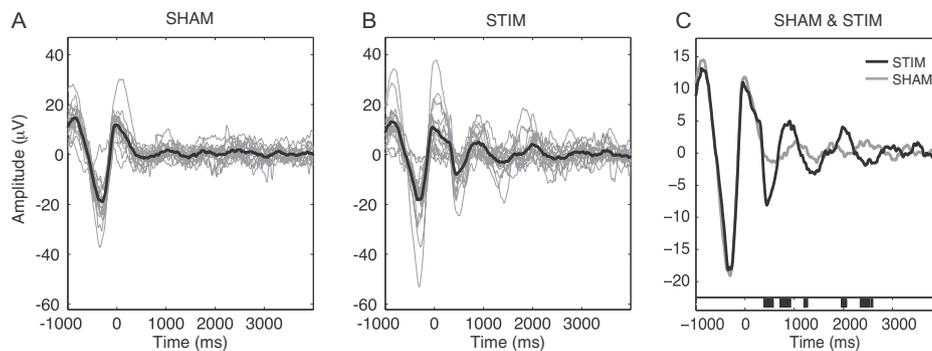


Fig. 4. Grand average event-related potentials (ERPs) for (A) SHAM and (B) STIM conditions, locked to the onset of the first tone of each five-tone block (time = 0) for F3. Light grey lines indicate individual subject averages while black lines indicate mean across all participants. (C) Grand average ERPs for SHAM and STIM. Black bars indicate time points when differences between the two conditions were significant ($p < 0.05$).

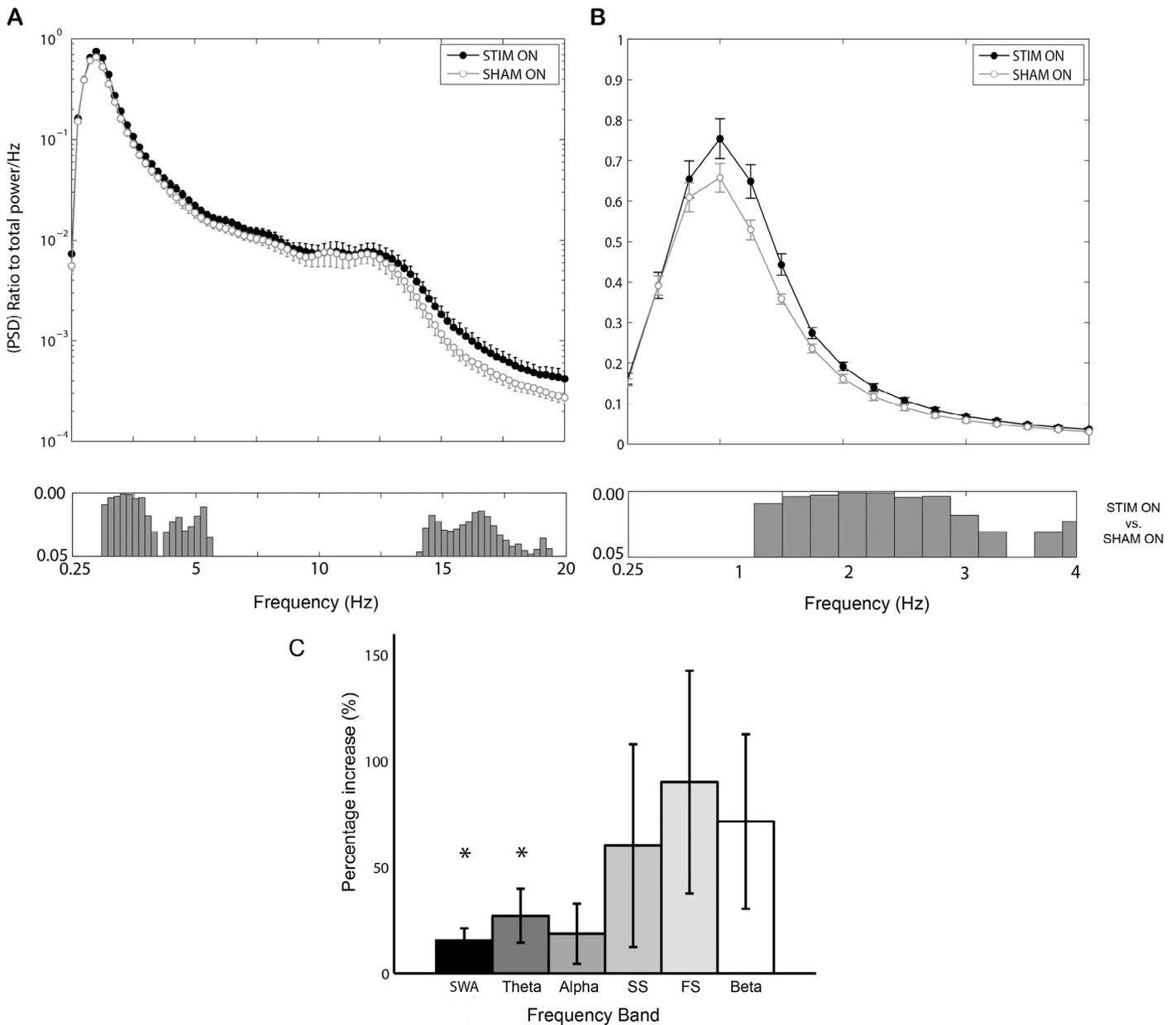


Fig. 5. Mean (\pm SEM) spectral power during 4-s STIM ON and SHAM ON blocks at F3, locked to the first of each five-tone block for (A) frequencies up to 20 Hz and (B) frequencies up to 4 Hz, normalized to the total power (0.25–30 Hz) across all ON blocks per condition. Below, *p*-value plots indicate frequencies where differences between conditions were significant (paired *t*-test, two-tailed). (C) Percentage increase in normalized power in the STIM ON compared to the SHAM ON conditions across six frequency bands: SWA (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), slow spindle (12–14 Hz), fast spindle (14–16 Hz), and beta (16–20 Hz). Paired *t*-tests revealed significant differences in the SWA and theta band ($p < 0.05$).

Analysis of percentage change in power across frequency bands in the STIM ON compared to the SHAM ON condition also revealed significant increases in the SWA and theta bands (SWA: $15.64 \pm 5.6\%$, theta: $27.15 \pm 12.7\%$, p values < 0.05 ; Fig. 5c).

3.4. Average auditory event-related spectral perturbations (ERSP) and inter-trial coherence (ITC)

ERSP plots (Fig. 6a and c) revealed that differential SWA and theta power for STIM versus SHAM increased across most of the epoch, whereas an increase in spindle activity (13–16 Hz) was temporally confined to subsequent SW up-state peaks, ie, at approximately 1000 and 2000 ms. ITC plots (Fig. 6b and d) revealed strong phase locking for STIM compared to SHAM only in the 0- to 1000-ms window post-stimulus onset in the SWA and theta range and not

in the fast spindle range. Together with grand average ERP plots, these show that: (a) increased SWA and theta activity in the first 1000 ms was successfully evoked as evidenced by increased ERP, ERSP and strong phase-locking across trials; (b) jittering of SWs occurred after 1000 ms as evidenced by ERP damping, high ERSP and low phase-locking across trials; and (c) fast spindle activity was induced as revealed by increased ERSP and low phase-locking across trials.

3.5. Effects on memory change

Participants recalled a similar number of word pairs at the immediate recall phase in the STIM and SHAM conditions ($t(15) = 0.26$, $p = 0.80$; Table 2).

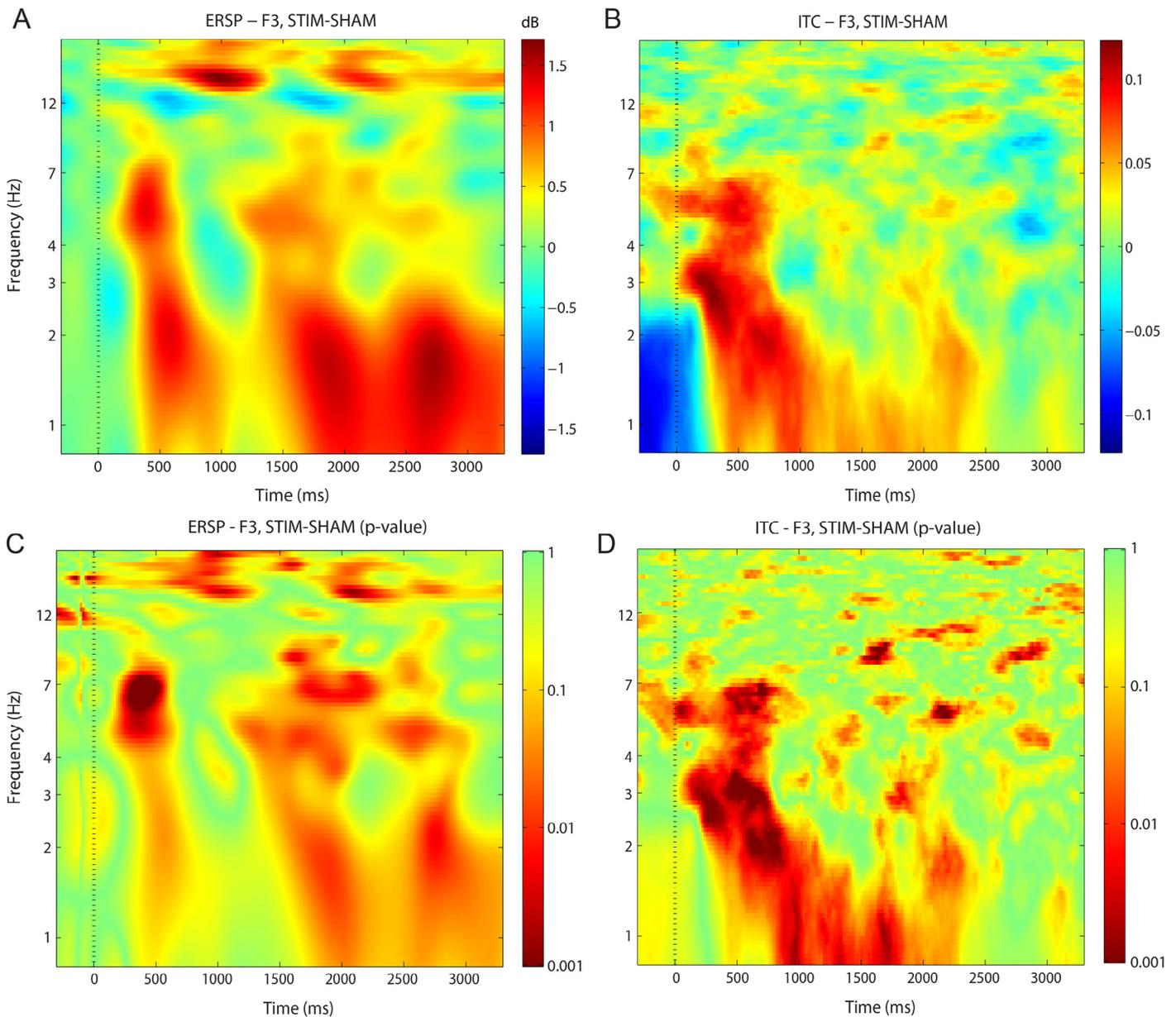


Fig. 6. (A) Event-related spectral perturbation (ERSP) and (B) inter-trial coherence (ITC) plots for STIM-SHAM at F3 locked to the onset of each five-tone block (time = 0). Increases in SWA and theta power were evoked (strong phase-locking across trials), whilst brief increases in fast spindle activity were induced (weak phase-locking across trials). Panels (C) and (D) show *p*-values for (A) and (B), respectively.

Forgetting of word pairs across the retention interval was significantly reduced in the STIM relative to the SHAM condition [$t(15) = 2.42$, $p = 0.03$; Fig. 7]. However, the effect of acoustic stimulation on declarative memory was modest (Cohen's $d = 0.43$).

3.6. Effects on nap macrostructure and microstructure

Sleep macrostructure as well as spindle density and spindle counts (Table 3) were similar between the two conditions, suggest-

ing stimulation-related changes were limited to SW and spindle power. No significant correlation between these sleep features and memory performance over the retention period was found (p values > 0.14, Table 4).

4. Discussion

Tones phase-locked to slow waves (SW) delivered during an afternoon nap enhanced SWA, theta, and fast spindle activity. Rhythmic acoustic stimulation was also associated with attenuated forgetting of words learned before the nap. Increases in SWA and theta were phase-locked across trials while fast spindle activity was not phase-locked, and could only be observed when individual trials were considered. These findings corroborate recent studies that have found acoustic tone delivery to be a promising, non-invasive method of enhancing overnight memory consolidation [19,38]. Additionally, we demonstrated the feasibility of delivering tones in both Stage

Table 2
Mean (\pm SEMs) for paired-associate performance.

	SHAM	STIM	<i>p</i>
Learning	83.75 \pm 2.62%	84.69 \pm 2.30%	0.80
Test	82.03 \pm 2.69%	84.69 \pm 2.67%	0.48
Change	-1.72 \pm 1.04%	0.00 \pm 0.94%	0.03

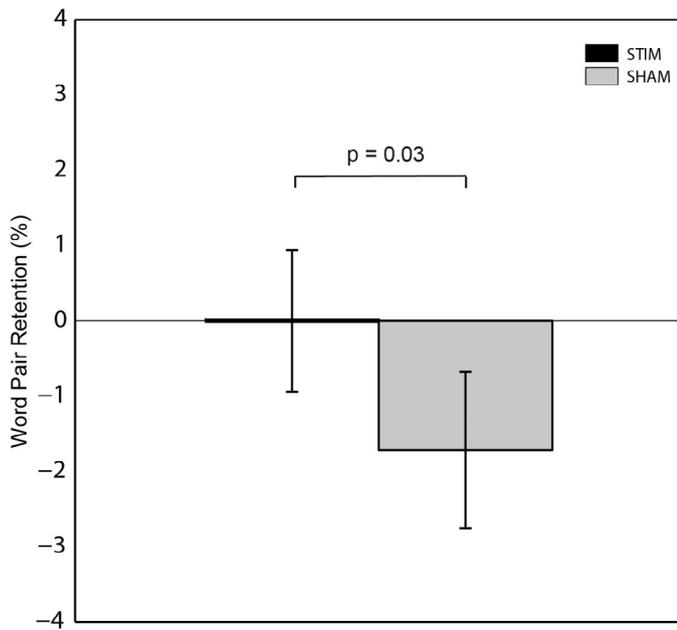


Fig. 7. Mean (\pm SEM) word pair retention (%) for STIM and SHAM conditions. Forgetting of semantically related word pairs was significantly reduced in the STIM condition (black bar) compared to the SHAM condition (grey bar).

2 and Stage 3 sleep, time-locked to the up-phase of slow waves lower in amplitude than the traditionally defined threshold (75 μ V) for determining slow wave sleep. This procedural advance could prove useful for memory augmentation during daytime naps and in middle-aged or elderly participants in whom SW amplitude is reduced [39,40].

Although SWA enhancement was specifically targeted, there was a concurrent increase in theta power observed. However, both delta and theta power have been linked to sleep homeostasis – increasing with duration of prior wakefulness and declining across the sleep episode. For example, delta and theta power have been shown to increase in recovery sleep following sleep deprivation [41] and across days of sleep restriction [42]. Together, these findings suggest that acoustic stimulation was able to induce an increase in sleep intensity.

While phase-locked acoustic stimulation elicited an increase in SWA (Cohen’s $d = 1.03$), there was only a modest reduction in forgetting (0.7 words on average; Cohen’s $d = 0.43$) when comparing retrieval performance between STIM and SHAM conditions. One reason could be the lower SWA content of a daytime nap on account of its short duration and length of prior wakefulness. In contrast, prior work examined the efficacy of acoustic tones over the first few

Table 3
Mean (\pm SEMs) of nap macrostructure (in minutes) and microstructure.

	SHAM	STIM	p
TST	82.00 \pm 2.49	79.34 \pm 2.33	0.32
Wake	16.31 \pm 2.32	17.13 \pm 2.27	0.78
Stage 1	7.44 \pm 1.25	8.69 \pm 1.42	0.59
Stage 2	57.88 \pm 2.62	51.75 \pm 4.07	0.12
SWS	6.66 \pm 2.45	10.06 \pm 4.41	0.38
REM	10.00 \pm 1.82	8.78 \pm 1.93	0.55
Stage 2 latency	13.66 \pm 2.01	12.06 \pm 0.75	0.41
REM latency	55.38 \pm 7.37	57.00 \pm 8.93	0.86
WASO	2.25 \pm 0.62	5.06 \pm 2.12	0.28
Spindle count	294.50 \pm 26.81	311.31 \pm 18.50	0.63
Spindle density*	4.63 \pm 0.30	4.83 \pm 0.24	0.60

TST, total sleep time; SWS, slow wave sleep; REM, rapid eye movement sleep; WASO, wake after sleep onset.

* Per minute time spent in Stage 2 and SWS.

Table 4

Pearson correlations of changes (STIM–SHAM) in nap macrostructure and microstructure with changes (STIM–SHAM) in word-pair performance across the retention interval.

	r_{15}	p
TST	-0.18	0.51
Stage 1	-0.25	0.35
Stage 2	-0.04	0.89
SWS	0.28	0.29
REM	-0.40	0.13
Sleep efficiency (%)	-0.02	0.94
Spindle count	0.13	0.63
Spindle density	0.01	0.97
SWA power	0.18	0.51
Theta power	-0.25	0.36
Fast spindle power	-0.22	0.41

r , Pearson’s r ; TST, total sleep time; SWS, slow wave sleep; REM, rapid eye movement sleep; SWA, slow wave activity.

hours of nocturnal sleep – an SWA-rich period [12,19]. Although more tones could have been played here (we used five-tone instead of two-tone blocks) which could have led to a greater increase in memory performance, Ngo and colleagues [38] have suggested that there may be no additional benefit of a five-tone block over a two-tone block owing to network refractoriness following initial stimulation [38,43–45]. This refractoriness could be attributed to induced spindle activity. Spindles have been shown to reduce sensory transmission during sleep [46,47] in order to protect sleeping individuals from disruptive environmental noise.

In contrast to previous work [19] feedback was not provided during the immediate recall phase in the present experiment. This prevented re-encoding prior to each nap session on recall test trials when participants answered incorrectly, and could potentially explain why we observed *reduced forgetting* [48,49] rather than enhancement of items retrieved at test.

The absence of an association between slow oscillations, spindles, and memory performance needs to be further examined in order to elucidate the neural mechanisms underlying memory improvement following acoustic stimulation. We speculate that there may be a complex temporal relationship between evoked SWs, induced spindles, and memory performance that has not yet been uncovered. For example, memory consolidation associated with spindles could be tied to their co-occurrence with SWs and hippocampal ripples in SWS [35,50–52]. SWS is the sleep stage when classic ‘replay’ of waking neural patterns are believed to occur [53]. Here, we did not separate spindles by sleep stage as seven participants had less than 5 min of SWS either in the STIM or SHAM condition. In addition, the range restriction in subject performance (change across retention interval from -1 to +3 words) limits the interpretation of the present results. There could also be significant inter-individual responses to these acoustic tones. Presumably, if SW generators possess an upper operational limit, it is likely that individuals with smaller endogenous SW amplitude or fewer SWs could stand to benefit more from this intervention.

The word pairs used here were semantically related. It is conceivable that there could be greater benefit to memory performance if unrelated word pairs were used. For example, Lo and colleagues found that a nap conferred greater benefits on memory of unrelated compared to related word pairs [23]. Finally, when comparing sleep and wake groups, Payne and colleagues found that after a 12-h retention interval there was pronounced deterioration in memory in the wake group only for unrelated but not related word pairs [29].

4.1. Limitations

Participants were partially sleep deprived in order to increase sleep propensity. Although the accompanying elevation in sleepi-

ness could in theory affect encoding performance, immediate recall prior to each STIM and SHAM nap session did not differ significantly. Additionally as sleep deprivation can elevate cortical excitability [54] and benefit synaptic plasticity, the benefit of acoustic tones on consolidation may not be comparable to that observed following a nap taken after a normal night of sleep.

When comparing spectral changes in the STIM ON vs SHAM ON conditions (Fig. 4), increases in beta power (14–20 Hz) were also detected (in addition to SWA and theta power) although epochs containing arousal events were removed from analyses. Although some of this may represent increased spindle activity, it is presently unclear why this occurs.

5. Conclusion

Acoustic stimulation is a viable, non-invasive method for enhancing SWA, theta, and fast spindle activity over a short afternoon nap. While SWA and theta activity were evoked, spindle activity was induced and not phase-locked across trials. Memory improvement in the form of reduced forgetting could be demonstrated. However, this behavioural benefit was not correlated with changes in SWA, theta or fast spindle activity, nap macrostructure or microstructure. Nevertheless, these results indicate that acoustic stimulation holds promise for applications where enhanced SWA might confer benefits to either patients or healthy individuals.

Conflict of interest

Drs Paller, Santostasi, and Zee have filed a provisional patent for the phase-locking technique used in this manuscript with the United States Patent and Trademark Office (U.S. Patent Application No. 62/038,700). The other authors have no conflicts of interest to disclose.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: <http://dx.doi.org/10.1016/j.sleep.2015.10.016>.

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Appendix: Supplementary material

Supplementary data to this article can be found online at [doi:10.1016/j.sleep.2015.10.016](https://doi.org/10.1016/j.sleep.2015.10.016).

References

- [1] Diekelmann S, Born J. The memory function of sleep. *Nat Rev Neurosci* 2010;11:114–26.
- [2] Rasch B, Born J. About sleep's role in memory. *Physiol Rev* 2013;93:681–766.
- [3] Walker MP. The role of slow wave sleep in memory processing. *J Clin Sleep Med* 2009;5:S20–6.
- [4] Stickgold R. Sleep-dependent memory consolidation. *Nature* 2005;437:1272–8.
- [5] Gais S, Born J. Declarative memory consolidation: mechanisms acting during human sleep. *Learn Mem* 2004;11:679–85.
- [6] Buzsáki G, Draguhn A. Neuronal oscillations in cortical networks. *Science* 2004;304:1926–9.
- [7] Varela F, Lachaux JP, Rodriguez E, et al. The brainweb: phase synchronization and large-scale integration. *Nat Rev Neurosci* 2001;2:229–39.
- [8] Paller KA, Voss JL. Memory reactivation and consolidation during sleep. *Learn Mem* 2004;11:664–70.
- [9] Steriade M. Grouping of brain rhythms in corticothalamic systems. *Neuroscience* 2006;137:1087–106.
- [10] Timofeev I, Chauvette S. Thalamocortical oscillations: local control of EEG slow waves. *Curr Top Med Chem* 2011;11:2457–71.
- [11] Marshall L, Born J. The contribution of sleep to hippocampus-dependent memory consolidation. *Trends Cogn Sci* 2007;11:442–50.
- [12] Marshall L, Mölle M, Hallschmid M, et al. Transcranial direct current stimulation during sleep improves declarative memory. *J Neurosci* 2004;24:9985–92.
- [13] Marshall L, Helgadottir H, Mölle M, et al. Boosting slow oscillations during sleep potentiates memory. *Nature* 2006;444:610–13.
- [14] Binder S, Berg K, Gasca F, et al. Transcranial slow oscillation stimulation during sleep enhances memory consolidation in rats. *Brain Stim* 2014;7:508–15.
- [15] Westerberg CE, Florczak SM, Weintraub S, et al. Memory improvement via low-frequency stimulation during sleep in older adults. *Neurobiol Aging* 2015;36:2577–86.
- [16] Massimini M, Ferrarelli F, Esser SK, et al. Triggering sleep slow waves by transcranial magnetic stimulation. *Proc Natl Acad Sci U S A* 2007;104:8496–501.
- [17] Vyazovskiy VV, Faraguna U, Cirelli C, et al. Triggering slow waves during NREM sleep in the rat by intracortical electrical stimulation: effects of sleep/wake history and background activity. *J Neurophysiol* 2009;101:1921–31.
- [18] Cox R, Korjoukov I, de Boer M, et al. Sound asleep: processing and retention of slow oscillation phase-targeted stimuli. *PLoS ONE* 2014;9:e101567.
- [19] Ngo HV, Martinetz T, Born J, et al. Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. *Neuron* 2013;78:545–53.
- [20] Buzsáki G. *Rhythms of the brain*. New York: Oxford University Press; 2006.
- [21] Ruch S, Marques O, Duss SB, et al. Sleep stage II contributes to the consolidation of declarative memories. *Neuropsychologia* 2012;50:2389–96.
- [22] Tucker MA, Hirota Y, Wamsley EJ, et al. A daytime nap containing solely non-REM sleep enhances declarative but not procedural memory. *Neurobiol Learn Mem* 2006;86:241–7.
- [23] Lo JC, Dijk DJ, Groeger JA. Comparing the effects of nocturnal sleep and daytime napping on declarative memory consolidation. *PLoS ONE* 2014;9:e108100.
- [24] Iber C, Ancoli-Israel S, Chesson A, et al. The AASM manual for the scoring of sleep and associated events: rules, terminology, and technical specification. Westchester, IL: American Academy of Sleep Medicine; 2007.
- [25] Riedner BA, Vyazovskiy VV, Huber R, et al. Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. *Sleep* 2007;30:1643–57.
- [26] Buysse DJ, Reynolds CF, Monk TH, et al. The Pittsburgh Sleep Quality Index (PSQI): a new instrument for psychiatric research and practice. *Psychiatr Res* 1989;28:193–213.
- [27] Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 1991;14:540–5.
- [28] Akerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. *Int J Neurosci* 1990;52:29–37.
- [29] Payne JD, Tucker MA, Ellenbogen JM, et al. Memory for semantically related and unrelated declarative information: the benefit of sleep, the cost of wake. *PLoS ONE* 2012;7:e33079.
- [30] Lo P-C, Lee Y-Y. Applicability of phase-locked loop to tracking the rhythmic activity in EEGs. *Circ Syst Signal PR* 2000;19:171–86.
- [31] Talbot DB. A review of PLL fundamentals. In: *Frequency acquisition techniques for phase locked loops*. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2012.
- [32] Hileman RE, Dick DE. Detection of phase characteristics of alpha waves in the electroencephalogram. *IEEE Trans Biomed Eng* 1971;18:379–82.
- [33] Makeig S. Auditory event-related dynamics of the EEG spectrum and effects of exposure to tones. *Electroen Clin Neuro* 1993;86:283–93.
- [34] Tallon-Baudry C, Bertrand O. Oscillatory gamma activity in humans and its role in object representation. *Trends Cogn Sci* 1999;3:151–62.
- [35] Mölle M, Marshall L, Gais S, et al. Grouping of spindle activity during slow oscillations in human non-rapid eye movement sleep. *J Neurosci* 2002;22:10941–7.
- [36] Berens P. A Matlab Toolbox for Circular Statistics. *J Stat Softw* 2009;31.
- [37] Cohen J. *Statistical power analysis for the behavioral sciences*. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.
- [38] Ngo HV, Miedema A, Faude I, et al. Driving sleep slow oscillations by auditory closed-loop stimulation—a self-limiting process. *J Neurosci* 2015;35:6630–8.
- [39] Landolt HP, Dijk DJ, Achermann P, et al. Effect of age on the sleep EEG: slow-wave activity and spindle frequency activity in young and middle-aged men. *Brain Res* 1996;738:205–12.
- [40] Gaudreau H, Carrier J, Montplaisir J. Age-related modifications of NREM sleep EEG: from childhood to middle age. *J Sleep Res* 2001;10:165–72.
- [41] Cajochen C, Foy R, Dijk DJ. Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. *Sleep Res Online* 1999;2:65–9.
- [42] Akerstedt T, Kecklund G, Ingre M, et al. Sleep homeostasis during repeated sleep restriction and recovery: support from EEG dynamics. *Sleep* 2009;32:217–22.
- [43] Firth H. Habituation during sleep. *Psychophysiology* 1973;10:43–51.
- [44] Bastien C, Campbell K. Effects of rate of tone-pip stimulation on the evoked K-Complex. *J Sleep Res* 1994;3:65–72.
- [45] Bellesi M, Riedner BA, Garcia-Molina GN, et al. Enhancement of sleep slow waves: underlying mechanisms and practical consequences. *Front Syst Neurosci* 2014;8:208.
- [46] Elton M, Winter O, Heslenfeld D, et al. Event-related potentials to tones in the absence and presence of sleep spindles. *J Sleep Res* 1997;6:78–83.

- [47] Schabus M, Dang-Vu TT, Heib DP, et al. The fate of incoming stimuli during NREM sleep is determined by spindles and the phase of the slow oscillation. *Front Neurol* 2012;3:40.
- [48] Jenkins JG, Dallenbach KM. Obliviscence during sleep and waking. *Am J Psychol* 1925;35:605–12.
- [49] Ebbinghaus H. *Über das Gedächtnis*. Leipzig: Duncker & Humblot; 1885.
- [50] Clemens Z, Molle M, Eross L, et al. Temporal coupling of parahippocampal ripples, sleep spindles and slow oscillations in humans. *Brain* 2007;130:2868–78.
- [51] Mölle M, Eschenko O, Gais S, et al. The influence of learning on sleep slow oscillations and associated spindles and ripples in humans and rats. *Eur J Neurosci* 2009;29:1071–81.
- [52] Cox R, Hofman WF, Talamini LM. Involvement of spindles in memory consolidation is slow wave sleep-specific. *Learn Memory* 2012;19:264–7.
- [53] Buzsaki G. The hippocampo-neocortical dialogue. *Cereb Cortex* 1996;6:81–92.
- [54] Huber R, Maki H, Rosanova M, et al. Human cortical excitability increases with time awake. *Cereb Cortex* 2013;23:332–8.