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Original Article

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Neurophysiological effects of targeting sleep spindles with closed-loop auditory stimulation

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Abstract

Sleep spindles are neural events unique to nonrapid eye movement sleep that play key roles in memory reactivation and consolidation. However, much of the evidence for their function remains correlational rather than causal. Closed-loop brain stimulation uses real-time monitoring of neural events (often via electroencephalography; EEG) to deliver precise auditory, magnetic, or electrical stimulation for research or therapeutic purposes. Automated online algorithms to detect and stimulate sleep spindles have recently been validated, but the time- and frequency-resolved physiological responses generated by them have not yet been documented. Building on the recent findings that sleep spindles do not block the transmission of sound to cortex, the present work investigates the neurophysiological responses to closed-loop auditory stimulation of sleep spindles. EEG data were collected from 10 healthy human adults (6 nights each), whilst sleep spindles were detected and in half the nights, targeted with auditory stimulation. Spindles were successfully stimulated before their offset in 97.6% of detections and did not disturb sleep. Comparing stimulation with sham, we observed that stimulation resulted in increased sigma activity (11–16 Hz) at about 1 second poststimulation but that stimulation occurring at the beginning of the spindle also resulted in early termination of the spindle. Finally, we observed that stimulating an evoked spindle did not elicit additional sigma activity. Our results validate the use of closed-loop auditory stimulation targeting sleep spindles, and document its neural effects, as a basis for future causal investigations concerning spindles' roles in memory consolidation.

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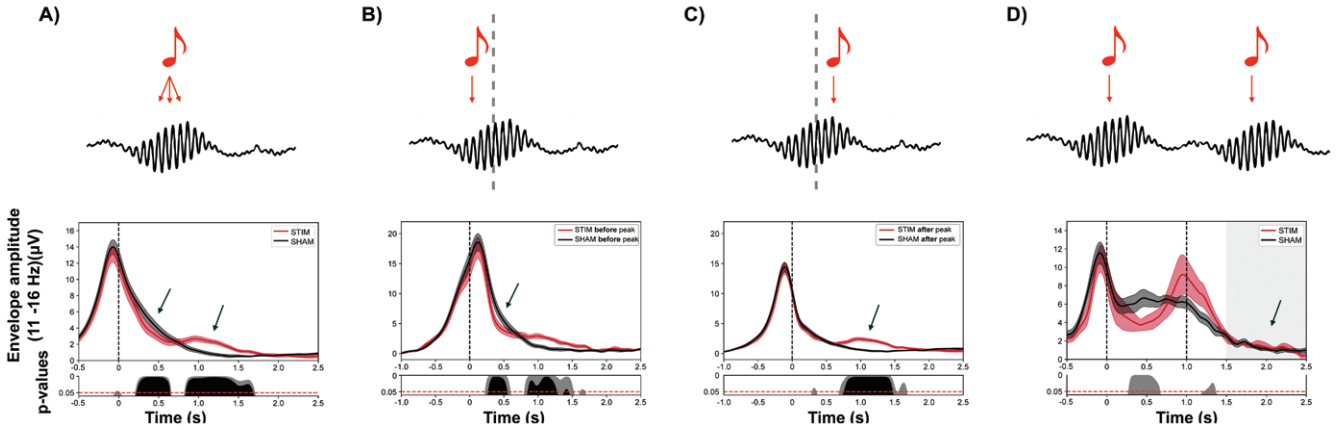
Graphical Abstract

The neurophysiological effects of closed-loop auditory stimulation targeting sleep spindles

Results:

1. Spindles were successfully stimulated before their offset in 97.6% of detections
2. Stimulation did not disturb sleep
3. Stimulating spindles terminated them early and evoked slow wave (0.1-4 Hz) and sigma (11-16 Hz) activity 1 s post stimulation (A)
4. Stimulating at the beginning (B) vs. end (C) of spindle yielded different effects
5. Stimulating an evoked spindle did not yield additional sigma activity (D)



Key words: sleep spindles; closed-loop auditory stimulation; slow oscillations; sleep; causal manipulation; brain stimulation; audition memory

Statement of Significance

In the present work, we document the neurophysiological effect of closed-loop auditory stimulation targeting sleep spindles across frequencies in the seconds following stimulation and further evaluate when and how stimulation affects spindle and slow oscillation activity, which have been most closely tied to memory consolidation processes in literature. The present work represents advances in demonstrating that stimulating sleep spindles with sound is technically and biologically possible. It supports the use of closed-loop auditory stimulation to causally manipulate sleep spindles, as a means of investigating their roles in learning and memory as well as in other cognitive processes.

Sleep spindles are transient (0.5–2.5 seconds) neural events with frequencies of 11–16 Hz, which are specific to nonrapid eye movement sleep. They are believed to be instrumental for sleep-dependent memory reactivation and consolidation (see [1] for a recent review of spindle function, and [2, 3] for current thinking about sleep's role in learning and memory). There has been much pioneering work describing sub-types of sleep spindles [4], how spindles differ between individuals [5], and how they are related to memory deficits in clinical populations (e.g. in schizophrenia [6–8]). However, the majority of evidence concerning their roles are either from invasive investigations using nonhuman animals [9] or from correlational studies in humans [10, 11].

Noninvasive brain stimulation techniques offer the possibility of precisely interacting directly with neural brain processes so as to educe evidence for their roles in complex, real-world learning which is relevant to human cognition [12–14]. Closed-loop brain stimulation is a technique in which neural events of interest for research or therapeutic purposes are measured (frequently using electroencephalography; EEG) and quickly identified in real-time, such that auditory, magnetic, or electrical stimulation can be used to interact with brain processes in a temporally precise fashion. This technique has been used successfully to enhance slow oscillations (SOs: 0.5–1.5 Hz), which, such as spindles, are involved in memory consolidation [15]. By stimulating SO up-states, when cortical neurons are partly depolarized and more excitable, Ngo et al. [16] enhanced the amplitude of SOs and reported an overnight improvement in memory performance. These results have now been replicated and extended (see [17–20] for reviews), demonstrating the effectiveness of precisely timed noninvasive auditory stimulation in modulating neural events—and the techniques' scientific value for investigating the neural substrates of memory processes. In previous work, Jourde et al. [21] investigated the neural mechanism by which sound influences slow oscillations [21] and found it likely to be related to a domain-general activation of the ascending reticular activating system, in accordance with prior hypotheses [22]. It has also been confirmed that sleep spindles do not significantly attenuate brain responses to acoustic information [23, 24], which would have precluded using auditory stimulation as a means of interacting with endogenous processes associated with sleep spindles.

Sleep spindles can be induced by brain stimulation in an open-loop fashion, either as part of an evoked response to an impulse (e.g. as seen in [25]) or in the form of entrainment to frequency-modulated stimulation [26]. Lustenberger et al. (2016) applied transcranial alternating current stimulation (tACS) within the spindle frequency range (12 Hz) and observed enhanced

cortical synchronization. The degree of stimulation-induced change in fast spindle activity was correlated with enhancement of motor (but not declarative) memory consolidation. Using a similar approach but in the auditory modality [27] explored the induction of spindles using auditory steady-state stimulation. Their design involved presenting white noise modulated at 12, 15, or 50 Hz, intermittently during NREM2 or NREM3; the intervention did increase spindle activity. In a follow-up study, the same team investigated how the timing of stimulation affected memory performance in a spatial location task. They concluded that stimulation delivered well after a spindle (i.e. 2.5 seconds after a spindle offset) resulted in better performance compared with stimulation sent immediately after the spindle (i.e., within 0.25 seconds of spindle offset [28]), suggesting a postspindle refractory period.

A series of nap experiments by Choi et al. [29–31] represent initial attempts at the challenging task of detecting spindles in real-time and stimulating them, with short bursts of pink noise (i.e. closed-loop auditory stimulation; CLAS). Due to detection latency of the equipment, however, the majority of auditory stimulations occurred after the spindles had already ended, with only 20%–23% of stimulations hitting the desired target sleep event. They showed that stimulating toward the end or after a spindle enhances both slow wave and sleep spindle activity [31], as occurs generally with stimulation during NREM sleep stages 2 and 3 [23, 32, 33]. Their results also suggested that stimulation at the end of the spindle rather than presented in a randomized fashion might reduce sleep fragmentation in a nap setting and potentially increase procedural memory consolidation. Because spindles have proven difficult to target, and because fine-grained analysis requires many stimulations and thus multiple nights of recordings, there remain few studies and many open questions on the neurophysiological effects of the timing of stimulation on evoked neural oscillations (and their cognitive and behavioral consequences). Furthermore, it is unknown whether spindles evoked by sound can in turn be stimulated to generate additional, trained spindles.

Automated online algorithms to detect and stimulate sleep spindles in real-time have recently been validated [34, 35]. In our previous work [35], we introduced the Portiloop, a deep learning-based, portable, and low-cost closed-loop stimulation system able to target specific brain oscillations, and validate its ability to detect spindles in a large database of sleep recordings in a simulated online context. However, work to date was conducted offline to document detection performance; the physiological response to single-pulse auditory stimulation successfully delivered selectively during spindles has not yet been documented. Confirming that sleep spindles can effectively be targeted under experimental conditions and measuring the neurophysiological effects of auditory stimulation is a necessary precursor to applying these techniques to questions about the roles of spindles in learning and memory.

In the present work, we collected EEG data from 10 healthy human adults (6 nights each), while sleep spindles were detected and, in half the nights, targeted with auditory stimulation. We document the neurophysiological effect of closed-loop auditory stimulation across frequencies in the seconds following stimulation and further evaluate when and how stimulation timing affects spindle and slow oscillation activity, which have been tied to processes of memory consolidation in literature. Our results validate the method of closed-loop auditory stimulation targeting sleep spindles and document the neural effects as a basis for future causal investigations concerning spindles' roles in memory consolidation.

Materials and Methods

Participants

Ten neurologically healthy adults were recruited from the local environment (6 female, $M = 28.9$ years, $SD = 7.4$, range = 23–45). Participants were screened via self-report for neurological, hearing, sleep problems, and sleep or wakefulness altering drug usage (which can affect spindle density [36]). Participation was on a voluntary basis. This research received approval from Concordia University's Research Ethics Board.

Study design

Participants were first screened for neurological conditions and briefed about the nature of their involvement. Following recruitment, participants received a kit containing the required equipment (i.e. Portiloop, electrode bundle, battery, skin cleaning supplies, electrode paste, tape, earphones, etc.) and detailed instructions as regards equipment operation, electrode placement, sound testing, and factors affecting signal quality. Each participant met with an experimenter to be trained on the handling of the material and data collection was closely monitored by the experimenter in the morning after each recording, ensuring proper electrodes placement and optimal data quality for an at-home multinight design. If the data quality was suboptimal, the experimenter contacted the participant to remind them of the procedure. Participants were asked to select 6 nights during which they expected to have a normal sleep schedule. We elected to collect multiple nights of sleep data on a small group of subjects to buffer against data loss due to electrode detachment or improper placement in the home environment, and to obtain a large number of epochs per subject. Subjects were asked to alternate between stimulation and sham nights (i.e. during which sleep spindles were detected and marked, but no sound was delivered).

Closed-loop auditory stimulation

Closed-loop auditory stimulation was accomplished using the Portiloop v2, a portable EEG system that is capable of stimulating sleep spindles with sound within about 300 ms of spindle onset as detected using offline algorithms [35]. In brief, the detection algorithm running on the Portiloop is a model based on a Convolutional Neural Networks followed by a Recurrent Neural Networks, trained on a gold-standard annotated dataset (Massive online data annotation [37]) to provide a real-time confidence score of the presence or absence of a sleep spindle. Based on performance evaluation and validation work described in Valenchon et al. [35], we set a confidence threshold of 0.75 for the current experiment. Portiloop is in active development, with recent additions including online sleep scoring subject-specific adaptation (Sobral et al., in review); the current work uses the architecture described in Valenchon et al. [35]. Portiloop plans are available to the community as an open science initiative to encourage further development and advance closed-loop neuroscience research.[1]

The electrode montage consisted of four midline positions (Fpz, Fz, Cz, and Pz), with a unilateral (left) reference placed on the left mastoid and a ground electrode placed on the left earlobe. A "right-leg-drive" circuit (i.e. the built-in bias drive amplifier in the Portiloop's EEG amplifier; Texas Instruments ADS1299) is used to ensure good common-mode rejection ratio performance, removing sources of environmental noise that are common

[1] https://github.com/Portiloop

across electrode sites [38]. In brief, the signal from the electrodes that are not used for detection (i.e. Fpz, Fz, and Pz) is averaged and reinjected at the left earlobe ground electrode. This configuration greatly reduces signal contamination from environmental sources such as 60 Hz power line noise and enhances signals that are relatively focal to Cz, notably fast spindles, allowing for their detection even in electrically unshielded environments. The midline configuration of the electrodes as selected in the current study also has the effect of slightly modulating the appearance of the evoked responses recorded using the same configuration as compared with that observed in a standard Cz-mastoid channel; specifically, neural patterns with a focal topography close to Cz (such as first components of the auditory evoked response) have higher relative amplitude to neural patterns with a more distributed topography such as evoked slow oscillations (note that amplitudes from different montages across studies are not directly comparable). The Cz electrode was used for spindle detection. Electrodes were secured with tape or gauze, according to hair coverage. Data were recorded locally on the Portiloop device at a sampling rate of 250 Hz and were transferred to the experimenter upon equipment return.

Sound stimulation consisted of 15 ms pink noise bursts (with 5 ms cosine ramps to avoid earphone clicks; normalized to −1 to +1 μV and sampled at 48 000 Hz), presented binaurally at 55 dB SPL, a sound level that generates robust evoked neural responses yet does not awaken participants [21].

The minimum interval between stimulations was set to 400 ms (selected based on pilot testing, to minimize the likelihood of a single spindle being detected and stimulated twice). Broadband (pink) noise was selected for similarity to the majority of CLAS work published to date, and because it generates a robust response that is likely invariant to minor differences in hearing sensitivity between subjects due to broad recruitment across the basilar membrane, noting that the underlying mechanism by which CLAS influences endogenous oscillations does not seem to depend on the type of noise used [21, 22, 39]. Sound was delivered through commercially available earbuds (Hiro: wired, Wicked Audio) which were secured in the ear using medical tape. This design choice was made to support our wider goals of making closed-loop research tools widely available and cost-effective [35]. All participants used the same earphones, with the same configuration.

EEG processing and analysis

We analyzed data from three out of the four electrodes (Fpz, Fz, and Cz) due to the high heterogeneity in data quality on the fourth electrode (Pz), which is located toward the back of the scalp and frequently becomes detached. All data were analyzed in Python using custom scripts based on freely accessible packages. NumPy was used for array manipulation, SciPy's signal module (scipy.signal) for filtering operations (including notch, band-pass, and band-specific filtering with butter and filtfilt), and Matplotlib for visualizing both the frequency response of the filters as well as the average brain responses. A fourth-order Butterworth band-pass filter (0.5–30 Hz) was applied to look at event-related potentials and to compute the event-related spectral perturbations. Epochs were extracted (−15 to 15 seconds following spindle detection), and baseline-corrected over the mean amplitude of the signal prior to the detection (from −1.5 to −1 seconds). This window was defined to avoid capturing the potential change in amplitude generated by slow oscillations occasionally coupled with the detected sleep spindle [40].

To control for data quality, a custom artifact rejection script was applied to each recording, which automatically removed poor sections of data by detecting both the absolute amplitude of the signal (for electrode-off detection) and sudden changes in amplitude (such as those caused by movements). Epochs with amplitudes exceeding ±200 μV were excluded. Due to the design choice of setting the minimum stimulation delay to 400 ms, note that it is not possible to definitively differentiate between detected sleep spindles that are endogenous and those that are evoked by stimulation. To ensure that each epoch contained only a single stimulation event, so as to characterize brain responses uninterrupted by additional stimulation, only stimulations spaced at least 2.5 seconds apart were included in the main analysis.

Event-related spectral perturbation plots were computed using the Python package scipy, with segments of 100 datapoints (~0.4 seconds) and a 50 % overlap. Statistical comparisons were computed across subjects for each time–frequency bin and corrected for False Discovery Rate using a Benjamini–Hochberg correction (alpha = 0.05). Concerning the analysis of the impact of stimulation in the slow-wave band, analysis was similar except the use of a different fourth-order Butterworth band-pass filter (0.1–4 Hz), which was applied to the raw data. Similarly, analysis of the spindle band activity followed a similar procedure with a third filter (same parameters with a 11–16 Hz frequency band). For each analysis, the filters were applied before defining epochs to avoid border effects. Because phases of both the detected and evoked sleep spindles can vary, we used the envelope of the signal as our metric to estimate instantaneous spindle power.

To quantify the distribution of stimulation delays achieved by the online detection algorithm, we implemented an offline detection method based on sigma power deviation, as defined in the work by Choi et al. [31] (see Supplementary Figure S1 for a detailed characterization of our online sleep spindle detection).

To investigate the long-term effects of stimulation and ensure the maintenance of sleep continuity, we calculated the cumulative sigma power for both STIM and SHAM conditions by summing the amplitude of the envelope signal during the 15 seconds following stimulation. For this and subsequent analyses, we focused on Cz, which showed clear evoked responses in both frequency ranges of interest. Temporal statistical comparison was computed using subjects averages and corrected similarly using the Benjamini–Hochberg procedure (both uncorrected and corrected p-values are represented below the timeseries figures).

We also investigated the interaction between stimulation timing and electrophysiological outcomes by sorting epochs based on the relative position of the stimulation to the peak of sigma activity for each detected spindle (as measured by the peak of the envelope signal). If stimulation occurred more than 50 ms prior to the peak, it was sorted into the first category (referred to as "before the peak"). If it occurred more than 50 ms after the peak, it was sorted into the second category: "after the peak." In addition to analyzing the effects of spindle timing on evoked sigma power as in the main analysis, we explored differences between the conditions in cumulative sigma power over the subsequent 15 seconds, as a means of observing overall differences in the amount of evoked sigma activity.

To explore the effect of stimulating the *evoked* sleep spindles, we isolated epochs in which two detections occurred consecutively (within −1.5 to −0.5 seconds; see Supplementary Table S1

for the number of epochs included per subject). The second of the two spindles is likely to have been evoked, (1) on the basis of our average results Figure 2D and (2) because the rhythms of endogenously produced spindles follow a longer (~0.02 Hz) cycle [41]. The strength of evoked sigma activity following the second stimulation was evaluated by statistically comparing the mean sigma envelope amplitude 1.5–2.5 after the onset of the first spindle.

Finally, to investigate the potential disruptive (or stabilizing [31]) effects of CLAS to sleep spindles, we statistically compared the number of overall detections in the STIM and SHAM conditions and computed cumulative sigma power over the 15 seconds epochs, which as they are overlapping collectively cover the duration of NREM2 and 3 sleep (noting that a validated sleep scoring procedure for Portiloop's unique montage has not yet been validated).

Results

Spindle detection

We first confirmed the effectiveness of spindle detection. As anticipated, improper electrode placement or loss in the in-home recordings resulted in poor signal quality for some time intervals. However, a large amount of data was usable; on average, 2.6 (*SD*: 0.5) stimulation and 2.2 (*SD*: 0.6) sham nights per subject were retained in the analysis (i.e. 20% data loss). Across all nights, the mean number of epochs included in the main analyses in the STIM condition which met the criteria for inclusion in the main analysis (i.e. no additional stimulation in the subsequent 2.5 seconds) was 958.2 (*SD*: 621.6), and in the SHAM condition, it was 891.9 (*SD*: 467.2; please see Supplementary Table S1 for per-subject values). A Wilcoxon Signed Rank Test confirmed that the number of spindles detected during sham and stimulation nights did not differ systematically ($W = 31$, $z = 0.36$, $p = .770$). The effect size ($r = .127$) was measured using Rank-Biserial Correlation.

As observed in Figure 1, the in-home Portiloop approach was successful in detecting spindles, and in stimulating them before they ended, with 97.6% of stimulations arriving before the end of the spindle as determined using an offline detector (see Supplementary Figure S1D). Spindle power at the time of detection and at baseline level (Baseline STIM: Mean: 0.21, *SD*: 0.14; Baseline SHAM: Mean: 0.23, *SD*: 0.16; Detection STIM: Mean: 11.76, *SD*: 3.20; Detection SHAM: Mean: 12.75, *SD*: 2.89) did not differ significantly between stimulation and sham conditions (Baseline STIM vs SHAM: $t(18) = -0.30$, $p = .77$, Cohen's $d = -0.13$; Detection STIM vs SHAM: $t(18) = -0.69$, $p = .75$, Cohen's $d = -0.31$) but is significantly higher than one second prior to detection (Baseline vs Detection: $t(38) = -16.97$, $p < .001$, Cohen's $d = -5.36$). The mean spindle duration as computed by the offline algorithm (see Materials and Methods section) was 958.7 ms with the bulk of stimulations falling between about 300–500 ms, in accordance with expectations from offline estimates [35]. Supplementary Figure S1B illustrates the distribution of delays between (offline-detected) spindle onset and stimulation. These results confirm that the Portiloop device successfully detected spindles and did so similarly for the stimulation and sham conditions, such that differences in postdetection neurophysiology may be meaningfully be compared.

Effects of stimulation vs sham

To document the effects of auditory stimulation to sleep spindles without bias as to frequency band or time window, we used an event-related spectral perturbation approach to assess the strength of changes in oscillatory activity locked to the stimulation, between stimulation and sham conditions for each of the three electrodes of interest (averaged broadband evoked response potentials for the stimulation condition are overlaid, see Figure 2B). Statistical analyses confirm an increase in high theta immediately following stimulation (from around 100 to 500 ms) and an increase in spindle power about 1 second poststimulation. This pattern was most prominent at the Fz and Cz electrode sites and did not reach significance at the most frontal site (Fpz). Effect sizes are reported in Supplementary Figure S4.

Next, we evaluated the effects of stimulation specifically on slow wave activity (0.1–4 Hz). A clear evoked auditory response and subsequent slow oscillation is observed when sound is delivered during spindles, with statistically significant differences from sham for the majority of the duration between stimulation

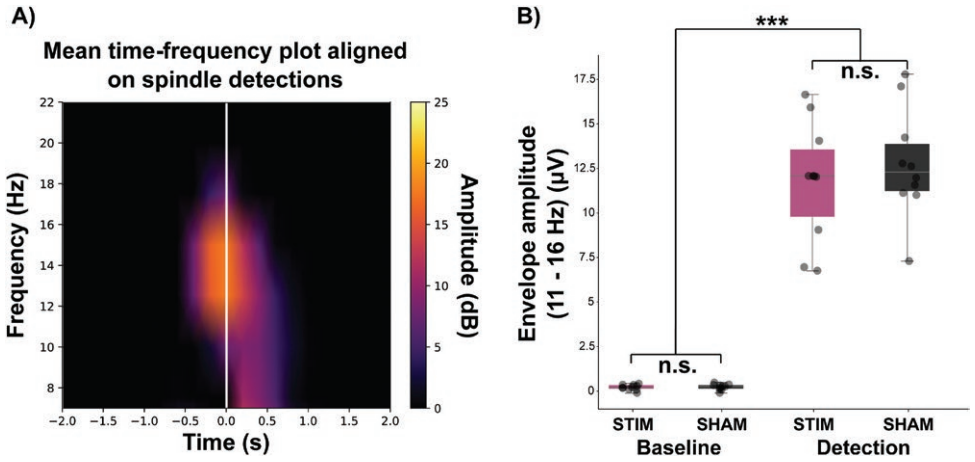


Figure 1. Successful real-time detection of sleep spindles. (A) Mean time–frequency plot shows a clear burst of sigma activity at the time of detection. (B) Comparison of the envelope of the sigma activity (11–16 Hz) 1 second prior to the detection (Baseline) and at the time of the detection suggests the presence of a spindle. "***" indicates significance at $p < .001$, FDR-corrected.

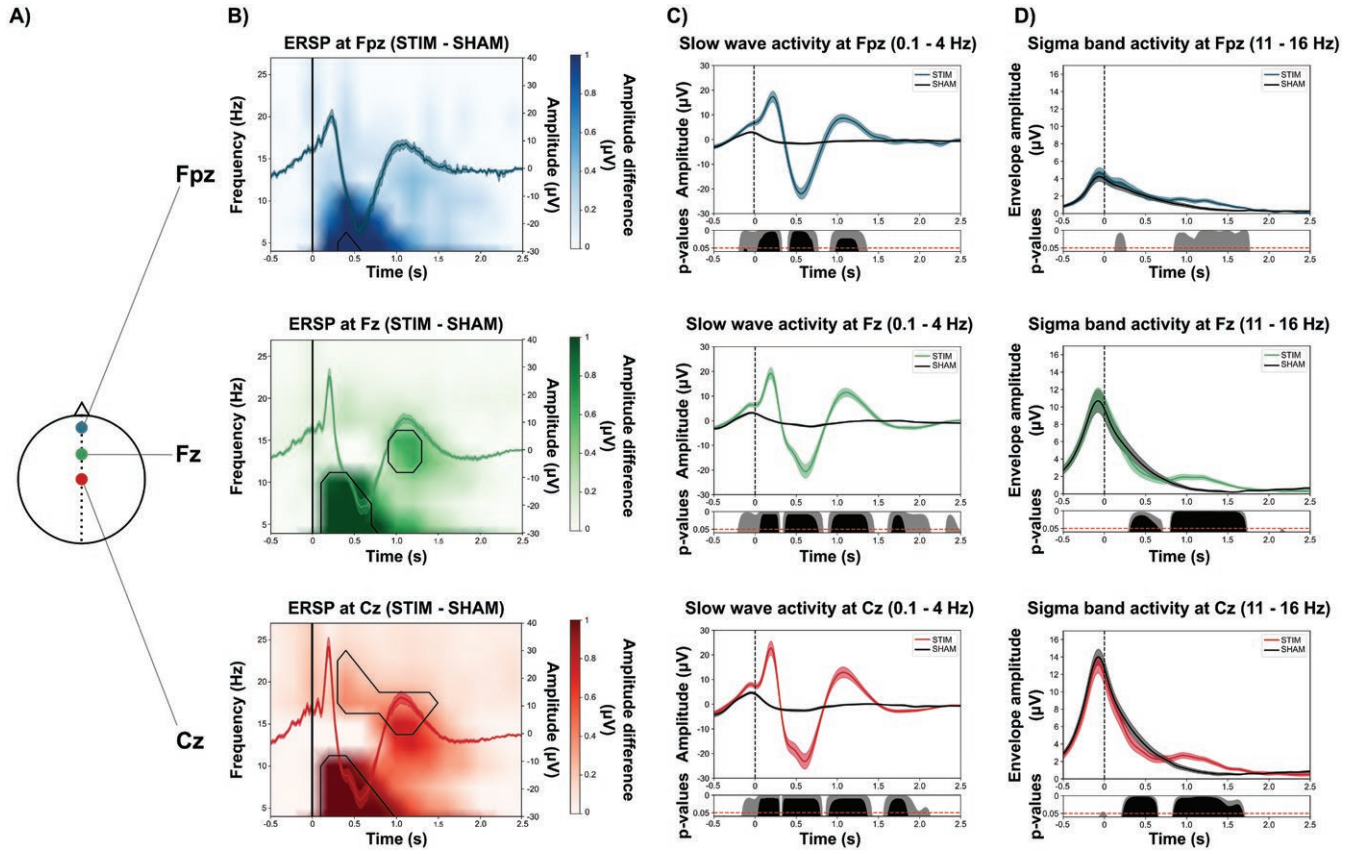


Figure 2. Effects of closed-loop auditory simulations of sleep spindles (detected at Cz). (A) Position of the three electrode sites. (B) Event-related spectral perturbation following closed-loop auditory stimulation of sleep spindles as compared with sham. Black contours highlight the significant differences between conditions (for visualization purposes, only significant clusters bigger than a single time–frequency bin are represented). The overlaid line represents the broadband (0.5–30 Hz) mean evoked response potential (shaded area: standard error of the mean). (C) Slow wave-filtered evoked responses. (D) Spindle-band activity. In C and D, solid lines indicate that group mean and shaded lines represent standard error of the mean. Statistical differences (STIM vs SHAM) are represented in the bottom panels. Gray areas represent uncorrected *p*-values and black areas represent corrected *p*-values.

and 2 seconds at Fz and Cz (Figure 2C). The most frontal site (Fpz) also showed a significant evoked response, though with fewer clear differences from the sham condition.

In the spindle range (11–16 Hz), we observe a significant decrease in power toward the end of the detected spindle (around 500 ms), possibly indicating early termination [24], followed by a strong increase in activity between 750 ms and 1.75 seconds poststimulation (see Figure 2D). As in the previous analysis, the pattern was more pronounced at the central electrodes than the frontal electrode.

Effects of stimulating the beginning vs the end of a sleep spindle

To investigate the interaction between stimulation timing and evoked brain activity in the sigma range, we compared the difference between STIM and SHAM trials for epochs in which stimulation occurred before or after the spindle peak (as defined in Materials and Methods section). As observed in Figure 2D in which all epochs were considered, stimulation occurring before the peak of sigma activity also resulted in an early termination of the current spindle (<0.5 seconds poststimulation) and a subsequent increase in evoked activity around 1 second after stimulation. In contrast, when stimulation occurred after the peak of the sigma activity, there was no early termination of the current spindle, but clear evoked activity was still generated ~1 second poststimulation (see Figure 3). Interestingly, when investigating the long-lasting effects through cumulative sigma analysis, we observe that while targeting stimulation before the peak did not modify overall sigma power, stimulating the end of the sleep spindle enhanced sigma activity as observed in the 15 seconds poststimulation. This difference was statistically significant at uncorrected alpha values from 4 seconds onward but did not survive the Benjamini–Hochberg procedure.

Effects of stimulating evoked sleep spindles

Due to the design of our experiment in which the minimum interstimulus interval was set to 400 ms, some evoked spindles occurring 1 second after stimulation were also detected and subsequently stimulated. By filtering for these specific epochs, we can investigate the effect of auditory stimulation on spindles that are most likely *evoked* (see Figure 4). Interestingly, we observed an absence of evoked activity following the *second* stimulation. This result was statistically confirmed by comparing the extracted values between STIM and SHAM conditions from 1.5 to 2.5 seconds post the original stimulation ($t(9) = -0.593$, $p = .58$, Cohen's $d = -0.19$).

Effects of whole-night spindle stimulation on sleep microarchitecture

To investigate the potential disruptive or stabilizing consequences of whole-night spindle stimulation, we computed

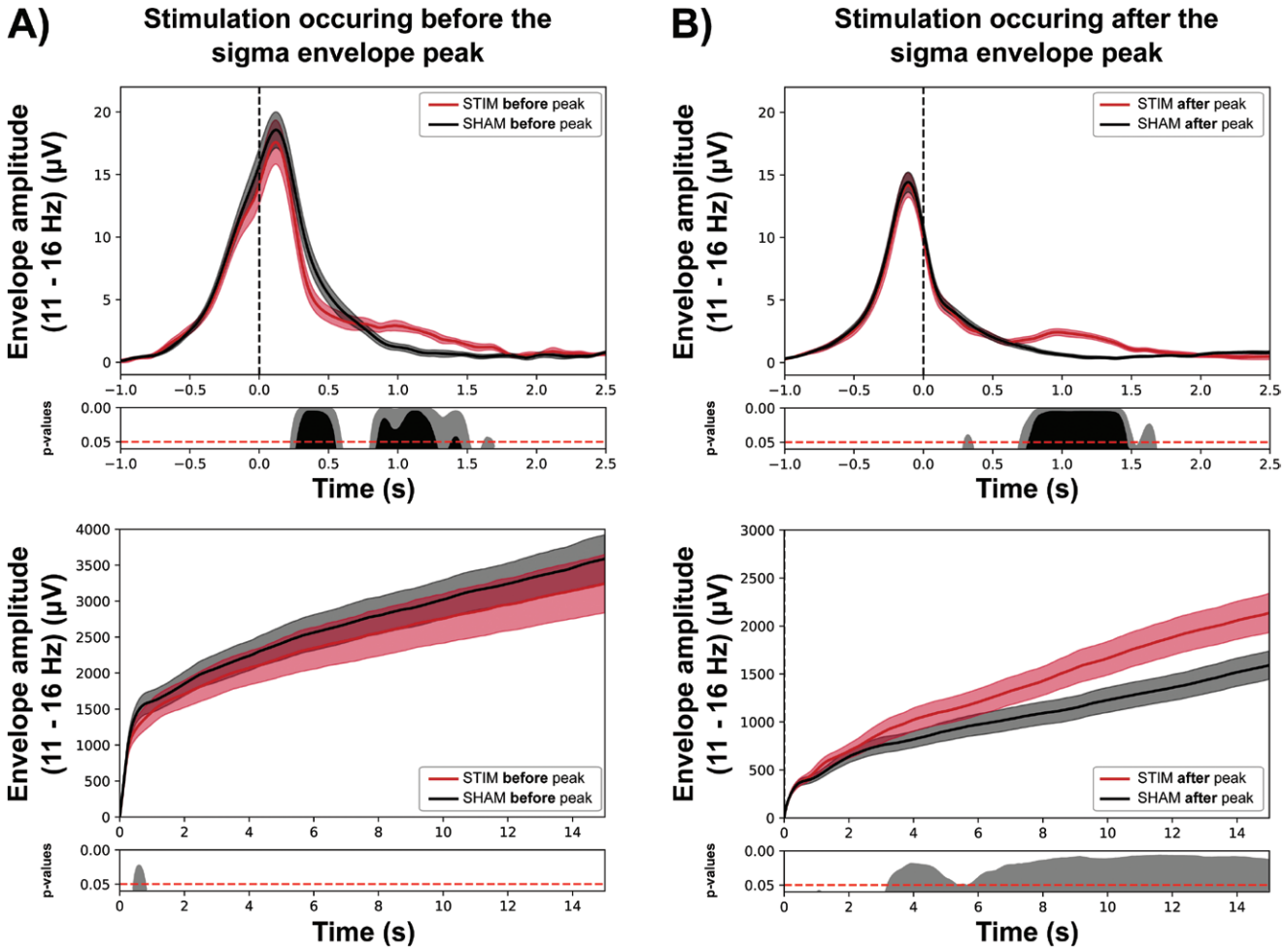


Figure 3. Timing relative to the ongoing sigma activity impacts the neurophysiological response to stimulation. (A) Top: Comparison of the envelope of the sigma activity (11–16 Hz) for both STIM and SHAM conditions when stimulation occurred before the peak of the detected spindle. Bottom: Cumulative sigma power in the 15 seconds following stimulation. (B) Top: Comparison of the envelope of the sigma activity for both STIM and SHAM conditions when stimulation occurred after the peak of the detected spindle. Bottom: Cumulative sigma power in the 15 seconds following stimulation. Dashed vertical line represent timing of auditory stimulation. Gray areas represent uncorrected *p*-values and black areas represent corrected *p*-values.

average difference time–frequency plots extended to 10 seconds and found no evidence of increased high-frequency activity that might indicate arousals as would be expected if the sound stimulation disturbed sleep. Next, we statistically evaluated the overall number of spindle detections between conditions. Across all nights, the mean number of spindles detected in the STIM condition (comprising those before and after 2.5 seconds, to allow for a total detection count including evoked responses) was 1050.8 (*SD*: 677.1), and in the SHAM condition, it was 939.0 (*SD*: 492.5) (see Supplementary Table S1 for details). A Wilcoxon Signed Rank Test confirmed that the number of spindles detected during sham and stimulation nights did not differ systematically ($W = 34$, $z = 0.663$, $p = .557$). The effect size ($r = 0.236$) was measured using Rank–Biserial Correlation. Finally, we computed the overall cumulative sigma power across a 15 seconds window following each stimulation onset (see Supplementary Figure S3). Results showed no significant difference after correction for multiple comparisons and showed only a short window (400–800 ms) of decreased sigma activity in the stimulation condition, corresponding to the spindle termination described above (Figure 2D), at uncorrected statistical levels.

Discussion

The aim of this work was to document the neurophysiological effects of auditory spindle stimulation on neural activity. We first confirmed that our method of stimulating spindles successfully detected spindle-band activity (i.e. using the Portiloop [35]; Figure 1) and that it was able to stimulate spindles prior to their termination (with almost all stimulations hitting spindles). Comparing stimulation with sham conditions, we found that the earliest difference between stimulation and sham is an increase in lower frequency activity (i.e. up to about 11 Hz), between 100 and 500 ms postdetection (Figure 2B). While some results suggests that this activity is associated with memory functions [42, 43], its function has not been causally explored.

In the slow oscillation band, we observe a strong sleep-specific evoked response resembling that reported in open-loop studies [21, 44, 45], closed-loop auditory stimulation studies that target SO up-states [16], and in studies that stimulated the end or following the offset of spindles [30, 31]. In brief, the auditory evoked response components observed in wakefulness within about 300 ms following stimulus presentation are followed in the sleep state by a pronounced negativity from about 300–600 ms (sometimes referred to as components N350 and N550) and then a

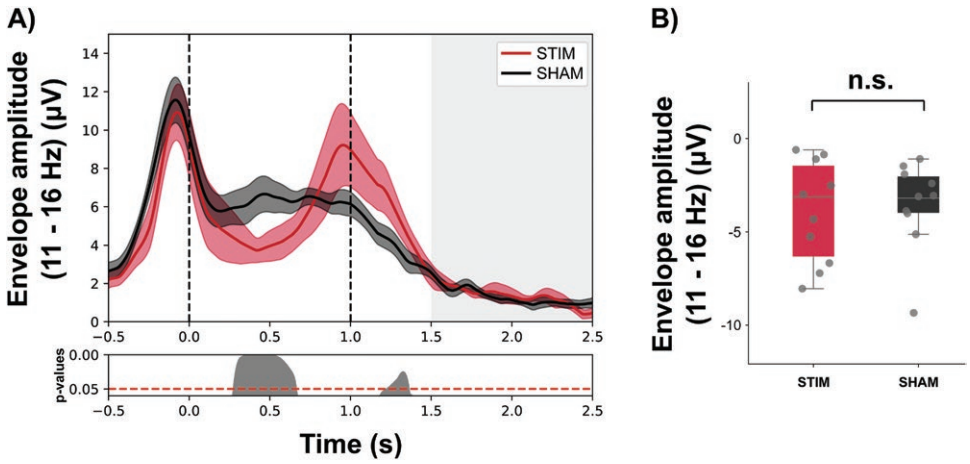


Figure 4. Effect of trains of spindle stimulation showing that stimulating evoked spindles (i.e. two stimulations in a row) does not appear to elicit additional sigma activity (A) as observed in the envelope amplitude timecourse and (B) confirmed statistically within a window 1.5–2.5 seconds (gray shading in A) following the first sound onset. Dashed vertical lines represent timing of auditory stimulations. Gray areas represent uncorrected *p*-values and black areas represent corrected *p*-values.

positive component after 900 ms (P900). As spindles were present during stimulation in this experiment, the results support prior observations that their presence does not impede the CLAS effect [23].

Most importantly given our focus on manipulating sleep spindles experimentally, we observed two clear changes in the spindle frequency band. First, stimulated spindle is truncated around 500 ms poststimulation. This observation is consistent with work showing that stimulating the locus coeruleus, a brainstem nucleus that produces the neuromodulator noradrenaline and projects broadly to frontal brain regions [46, 47], suppressing sleep spindle activity [48]. As the locus coeruleus is connected to the ascending auditory pathway and is thought to be critical for generating the evoked N550-P900 complexes, it has been implicated in a proposed mechanism by which CLAS to slow oscillations induces more slow oscillations, sleep spindles, and consequently, memory benefits [21, 22]. Spindle activity power increased between 750 ms and 1.75 seconds poststimulation, which coincided with the induced SO upstate. As the degree of temporal coupling between spindles and slow oscillations is predictive of successful memory consolidation [49], this result suggests that it will be possible to manipulate and perhaps enhance memory processes.

Our results also demonstrate that the specific timing of stimulation may have different physiological effects. If stimulation hits the early portion of the spindle, the spindle is most likely to terminate early (Figure 3A). Early termination of spindles as a result of sound stimulation in sleep has been previously observed in the rat auditory cortex [24]. Sela et al. noted that termination occurred within about 150–200 ms poststimulation and was more likely for louder sounds. In the present work, early termination was followed by an increase in sigma activity relative to the sham condition (between 900 and 1500 ms) yet did not lead to overall changes in cumulative sigma activity (nor awaken the participants). If stimulation hits the later, waning part of the spindle, there is no appreciable influence on the present, detected spindle, but a clear increase in sigma band activity is elicited, also from about 800–1500 ms (Figure 3B), similar to observations by Choi et al. [30]. The overall cumulative sigma band activity appears greater in the stimulation versus sham conditions over the subsequent sleep period (though at uncorrected significance levels).

An intriguing result emerged when we separated cases in which an evoked spindle was strong and clear enough to be detected by the detection algorithm, and itself stimulated—although the second consecutive stimulation did evoke a response in the slow frequency band (see Supplementary Figure S2), no concurrent evoked sigma activity was observed (Figure 4B). This finding suggests a limit to the temporal frequency with which spindles can be successfully evoked, in line with the concept of a postspindle refractory period [1, 50], and hints that constraints on externally driving spindles and slow oscillations may be set by different underlying mechanisms [51]. These results also corroborate the observation made by Ngo et al. [51], which stated that the phase-locked increase in spindle activity was restricted to the first stimulus presentation.

An open question is whether sound stimulation alters the architecture of sleep. Noise has been investigated both for its disruptive properties in the context of unwelcome environmental noise (e.g. in hospital settings or in neighborhoods adjacent to heavy transportation routes), and as a means of intentionally stabilizing sleep (e.g. by playing white or colored noise, music, or sound bursts). While recent reviews have emphasized in both cases that heterogeneity of study design make general conclusions difficult to draw (see [52, 53]), the effects may depend on sound intensity, predictability, meaning, sleep depth, and individual factors such as hearing ability. In the current work, we concluded that presenting short bursts of pink noise devoid of semantic content at low intensity has little effect on sleep continuity or overall depth (as measured by the number of spindles detected and lack of observed arousals). However, it does appear to alter the temporal organization of sigma activity (Figure 3).

It is noteworthy that while random sound stimulation and CLAS targeting different sleep features during NREM sleep have broadly similar physiological effects (i.e. evoked SO and sigma activity), subtle differences in neurophysiological responses arise due to timing variations with respect to the phase of endogenous activity. These differences, however, may have distinct consequences for memory processes [16].

Comparing the outcomes of stimulating different neural oscillations and examining phase and timing-specific effects would help determine whether CLAS mechanisms are fundamentally the same but vary in intensity, or if they differ qualitatively, by enhancing or

disrupting specific processes. In any case, it will be necessary to establish the optimal stimulation paradigms and their behavioral effects through direct empirical comparison (i.e. using the same equipment and methods). Future work could also personalize detection parameters and explore the optimal stimulation features (i.e. sound content, level, and duration). Developments such as online sleep scoring will enable the detector to activate only during the targeted sleep stages for better precision. The present work supports the value of research tools that can detect and stimulate neural events quickly and flexibly along their time courses. Practically speaking as regards spindles, our results suggest that they might be experimentally repressed by targeting them early after onset, possibly using pairs of stimulations to also disrupt the evoked sigma activity at ~1 second. Sigma activity might instead be enhanced by targeting spindles' waning phase, leaving several seconds between stimulations so as to avoid the refractory period and benefit from the evoked sigma activity.

Although much work lies ahead, the present results represent advances in demonstrating that stimulating sleep spindles with sound is technically and biologically possible, with distinct effects depending on stimulation timing. It supports the use of closed-loop auditory stimulation to causally manipulate sleep spindles, as a means of investigating their roles in learning and memory, health and disease, and potentially as a means of restoring processes degraded by disease state [6, 8] or aging [54, 55]. We hope that the closed-loop auditory stimulation technique will facilitate the expansion of much previous research by Professor Robert Stickgold on sleep spindles' functions [4, 10, 56–60] to the noninvasive and nonpharmacological causal domain and thereby help deepen our understanding of sleep's role in memory.

Supplementary material

Supplementary material is available at *SLEEP Advances* online.

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Author contributions

Hugo R. Jourde (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Methodology [equal], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal]), Milo Sobral (Data curation [equal], Methodology [equal], Writing—review & editing [equal]), Giovanni Beltrame (Funding acquisition [equal], Methodology [equal], Resources [equal], Writing—review & editing [equal]), and Emily B. J. Coffey (Conceptualization [equal], Funding acquisition [equal], Methodology [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal])

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Data availability

The dataset generated and/or analyzed during the current study are not publicly available, but they may be made available on reasonable request by contacting the corresponding authors to establish a data sharing agreement.

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