PNAS Nexus, 2024, 3, pgae479
https://doi.org/10.1093/pnasnexus/pgae479
Advance access publication 1 November 2024
Research Report

Auditory processing up to cortex is maintained during sleep spindles

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Edited By Eric Klann

Abstract

Sleep spindles are transient 11–16 Hz brain oscillations generated by thalamocortical circuits. Their role in memory consolidation is well established, but how they play a role in sleep continuity and protection of memory consolidation against interference is unclear. One theory posits that spindles or a neural refractory period following their offset act as a gating mechanism, blocking sensory information en route to the cortex at the level of the thalamus. An alternative model posits that spindles do not participate in the suppression of neural responses to sound, although they can be produced in response to sound. We present evidence from three experiments using electroencephalography and magnetoencephalography in humans that examine different evoked responses in the presence of and following sleep spindles. The results provide convergent empirical evidence suggesting that auditory processing up to cortex is maintained during sleep spindles, and their refractory periods.

Keywords: sleep spindles, refractory period, auditory processing, thalamic gating, sleep

Significance Statement

Due to their thalamocortical origin, sleep spindles are thought to preserve the sleep state by repressing the flow of sensory information to cortex at the level of the thalamus. We present three lines of evidence that focus on responses to sound that are generated in cortex, using electroencephalography and magnetoencephalography. Frequency-following responses, evoked responses extracted from the auditory cortex, and effects of closed-loop auditory stimulation of slow oscillation up-states in the presence of sleep spindles were all unaffected by the presence of a spindle or its subsequent refractory period. These results update models of sleep spindles' roles, and critically, open the door to potential therapeutic interventions targeting spindles with brain stimulation.

Introduction

Sleep has important functions in removal of waste metabolites produced during wakefulness (1), synaptic rescaling (2), and in actively consolidating memory (3–6). These functions could not efficiently occur if sleep were constantly interrupted. Empirical support for the presence of a protective mechanism ensuring sleep continuity comes from studies demonstrating a reduced capacity to process external stimuli (e.g. (7–10)) and a reduced propensity for arousal by them during sleep (reviewed in (11)). The nature of such a protective mechanism and how the sleeping brain might maintain a balance between monitoring the surroundings and reducing sensory interference with other sleep-dependent processes remains unclear (11).

Neural events that occur only in sleep and whose presence correlates with reduced arousability are prime candidates as potential mechanistic explanations. Several such sleep-specific neural events occur during nonrapid eye movement (NREM) sleep (stages 2 and 3), among them sleep spindles and slow oscillations (SOs). Spindles are transient (<2.5 s) 11–16 Hz brain oscillations that are generated through thalamocortical interactions (12) (see Figure 1A), and SOs are low frequency (usually < 1.5 Hz), large-scale fluctuations in cortical and subcortical excitability (13–15). Spindles in particular are a credible candidates for interrupting sensory transmission through thalamus to cortex, because they are observed when neurons within the thalamic reticular nucleus shift their activity from a tonic to burst mode of firing (12). As tonic mode firing is present during normal wakeful sensory processing, it follows that quite a different firing pattern may not allow normal sensory transmission to cortex.

Competing Interest: The authors declare no competing interests.
Received: February 29, 2024. **Accepted:** October 13, 2024

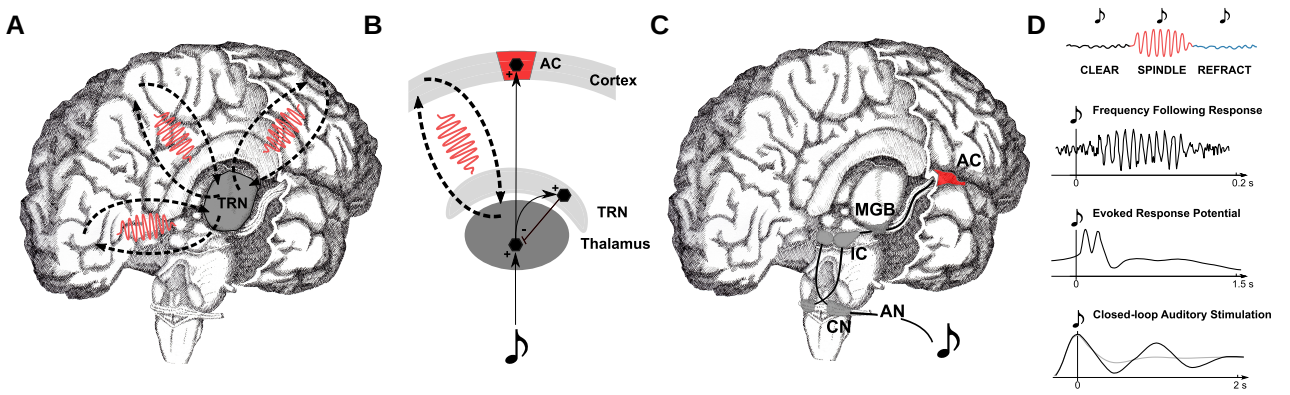

Fig. 1. Proposed mechanisms by which sleep spindles might gate access of auditory information to the cortex. A) Schematic representation of the thalamocortical loops involved in the generation of sleep spindles. The thalamic reticular nucleus (TRN), which wraps around the thalamus, is responsible for the initiation of sleep spindles (in NREM2 and NREM3 sleep stages). B) According to the thalamic gating hypothesis, the TRN is thought to modulate the flow of information through the thalamus to cortex (41), protecting the sleep state by disrupting transfer of sensory information through thalamus to cortex. C) Diagram of the ascending auditory pathway (right dorsolateral view), showing the auditory nerve (AN); cochlear nucleus (CN), and inferior colliculus (IC) in the brainstem; medial geniculate body (MGB) of the thalamus; and the auditory cortex (AC) in the temporal lobe. D) The thalamic gating hypothesis is tested in the current work by comparing the magnitude of three types of evoked auditory responses localized to or known to involve cortical brain regions (FFR, ERP, and effects of CLAS) during spindles as identified using EEG (labeled "Spindle"), during their refractory periods ("Refract"), and during stages 2 and 3 sleep when neither a spindle nor refractory period is occurring ("Clear"). FFR, frequency-following response; ERP, evoked response potential; CLAS, closed-loop auditory stimulation.

Further conceptual support for this idea comes from other known thalamic roles; in wakefulness, the thalamus acts as a selective filter and attentional controller for sensory inputs, regulating their transmission based on task relevance and attention (e.g. (16–18)). This concept has been proposed to extend to sleep spindles, where the thalamus continues its gating role but in a different context. During sleep, sleep spindles are believed to represent a mechanism by which the thalamus limits the influx of external sensory information to the cortex, dampening responsiveness to the environment (19–21). This selective gating mechanism is thought to help sustain a stable sleep state, protecting against full awakenings caused by external stimuli (see Figure 1B).

Evidence concerning this proposal is, however, equivocal, as some sensory-evoked brain responses appear to be unaffected by the presences of spindles (reviewed in (11, 12)). Much of the work suggesting a role of spindles in blocking sensory information is indirect. For example, sleep stage or overall spindle density might be correlated with brain responses (e.g. (22)), without considering specifically when a stimulus arrives relative to the appearance of a spindle. To date, there are few studies using time-resolved methods in healthy humans that directly investigate whether and how sleep spindles, or subsequent spindle refractory periods (23), influence gating of sensory information between thalamus and cortex (but see (20, 24) for evidence that hemodynamic responses to sound presented during spindles are reduced). If sensory information is gated by spindles (or their refractory periods (23)) at the thalamic level, the neural representation of the stimulus or reaction to it should be weaker or absent in the cortex when sleep spindles occur as compared with periods of time in which they do not. In sum, there are two competing explanations for the patterns of results reported in the previous literature: (i) that spindles (or their refractory periods) gate sensory access to the cortex to protect the sleep state, and (ii) that other mechanisms are responsible for diminished sensory processing and arousal observed during sleep.

The auditory sensory modality lends itself particularly well to investigating whether spindles block sensory information, as peripheral auditory structures remain accessible during the sleep state, and the origin and timing of specific neurological responses evoked by sound within the auditory pathway are quite well understood (see Figure 1C) (25, 26). Notably, recent developments in techniques to capture early responses to sound using magnetoencephalography (MEG) and electroencephalography (EEG) offer a means of measuring the effects of brain state on sensory process as sensory information ascends the central nervous system (7, 25–28).

An interesting property of the auditory system is that stimulation at specific times during neural events, notably during peaks in SOs, generates additional SOs and sleep spindles in the following seconds, and thereby enhances sleep-related memory consolidation processes. This technique is called closed-loop auditory stimulation, or CLAS (for recent reviews, see (29, 30)). Evidence suggests this phenomenon is likely to be mediated through the activation of nonlemniscal ascending auditory pathways, which project broadly to association areas including frontal regions (along with secondary auditory areas), likely also involving the ascending reticular activating system (31, 32). Measuring the ability of CLAS to generate additional SOs and spindles therefore offers the prospect of assessing how this pathway functions in the presence of sleep spindles, in addition to measuring auditory-modality-specific evoked responses.

In the present work, we conducted three experiments to assess the impact of sleep spindle occurrence and their refractory period on different measures of auditory cortical processing (see Figure 1C, right). First, we used combined MEG–EEG and distributed-source modeling techniques in a nap experimental design and measured frequency-following responses (FFR), a brain response used in auditory and cognitive research to measure the fidelity and precision of sound encoding (FFR; for reviews, see (33–35)). Critically, this signal is an early sound response that can be measured in the auditory cortex using MEG (25), and so allows us to investigate how periodicity encoding of auditory stimuli in the primary auditory (lemniscal) pathway is affected by the presence of sleep spindles. In a second experiment, overnight recordings allowed us to acquire sufficient trials (with the necessary inter-trial intervals) to measure slower cortical evoked response components extracted from right auditory cortex (namely P100, P200) in the presence of sleep spindles (36), and quantify their amplitude as a function of spindle presence. Finally, using a closed-loop auditory stimulation design based on EEG detection

of slow oscillations, we investigated the impact of auditory stimulation in the presence and absence of spindles which were coupled to SO up states. Coupling between SOs and spindles is reported to occur in less than 10% of SOs (37, 38); we therefore collected five whole-night recordings per participant to achieve high statistical sensitivity. In addition to clarifying one of the putative roles of sleep spindles, the results have practical use for optimizing the timing of brain stimulation delivery relative to endogenous neural events (39, 40).

Results

Experiment 1: evidence that the lemniscal auditory pathway operates during spindles

Fourteen subjects were included in a nap study [mean age: 24.8 (SD: 4.0; range: 21–36), 9 females].

Sleep scoring analysis confirmed that all subjects included in the analysis slept during the 2.5 hr nap opportunity. On average, subjects spend 70 minutes (SD: 35) in NREM2 and NREM3 sleep stages (combined), in which sleep spindles occur. On average 293 spindles (SD: 177) and 370 slow oscillations (SD: 348) were detected per subject using EEG (NREM2 and NREM3). See Table S1 for time spent in other sleep stages.

Brain responses to incoming sounds were cut into 300 ms epochs (from −50 ms prior to the sound to 250 ms post sound) and sorted according to their timing with respect to spindle onset and offset (i.e. "Spindle"), and a refractory period defined as a fixed window of 2.5 seconds post offset, as in previous work (i.e. "Refract" (23)). The "Clear" condition was defined as the absence of an SO, spindle, or its refractory period at the time of stimulation, only considering NREM2 and NREM3 sleep stages. After sorting the auditory stimulation events, the mean number of epochs in the Spindle condition was 1,540 (SD: 880), in the Refractory condition 2,430 (SD: 1,290), and in the Clear condition, 11,430 (SD: 7,480; see Table S1 for further details).

To confirm that the detection (at Cz) and sorting procedure was successful to separate time periods that included high and low spindle activity, we compared the electroencephalography absolute sigma band power (root mean square of the amplitude within 11–16 Hz; see Methods) during each of the three epoch types in 2 second windows centered on the sound presentation. Both absolute spectral power and relative spectral power (i.e. selected frequency band divided by broadband activity) are commonly used metrics, we favored the absolute power approach for its simplicity of interpretation and lack of dependency on power differences in other spectral bands, throughout the paper. We confirmed that the distributions (see Figure S1) were statically different using a repeated measures ANOVA $F(2, 24) = 144.64$, $P < 0.001$). Post hoc pairwise comparisons revealed that sigma band power was significantly greater in Spindle as compared with Clear (mean difference = 3.00e − 6, SE = 2.33e − 7, 95% CI = [2.36e − 63.65e − 6], $P < 0.001$), Refractory as compared with Clear (mean difference = 1.30e − 6, SE = 1.14e − 7, 95% CI = [9.86e − 71.62e − 6], $P < 0.001$), and Spindle as compared with Refractory conditions (mean difference = 1.71e − 6, SE = 1.65e − 7, 95% CI = [1.25e − 62.16e − 6], $P < 0.001$).

To test the main hypothesis that spindles (or their refractory periods) play a protective role in sleep by impeding the transmission of auditory information between thalamus and cortex, we investigated the strength of acoustic periodicity encoding in the right auditory cortex (i.e. the amplitude of the fundamental frequency in the FFR, which is associated with pitch information) using magnetoencephalography, across Spindle, Refractory, and Clear epochs. To test whether the cortical FFR was affected by the presence of a spindle, we focused on the right auditory cortex. Due to hemispheric specialization in the auditory system (42), the strongest phase-locked neural response to pitch information (FFR) is found in this region (25, 26, 43).

To confirm that the FFR was of sufficient quality and clarity to be used as a basis for investigating the main research questions, we statistically compared the amplitude of the FFR at the fundamental frequency (98 Hz) with the amplitude at the same frequency during the prestimulus period (50 ms), as in previous work (25). The mean signal-to-noise ratio (SNR) for the Clear condition was 11.75 (SD: 7.24), Spindle condition: 5.11 (SD: 6.28); Refractory: 10.39 (SD: 20.15); with a minimum SNR across all conditions of 1.28. We confirmed that the strength of the FFR during the stimulus was well above the baseline period in all three conditions (for each of Clear, Spindle, and Refract: $V = 91.00$, $P < 0.001$, rank-biserial correlation: $r_{rb} = 1.00$, 95% CI [1.00, $+\infty$]; Wilcoxon signed-rank tests were used due to a violated Shapiro–Wilk test of normality).

We then compared the magnitude of the fundamental frequency in the FFR between our conditions (Spindle, Clear, and Refractory). A nonparametric equivalent of a repeated-measures ANOVA was used (due to a violated normality assumption). A Friedman's Test did not show any significant difference between FFR magnitude in the rAC across Clear, Spindles, and Refractory conditions ($\chi^2(2) = 0.154$, $P = 0.926$). The FFR amplitude from right auditory cortex was therefore not significantly reduced during spindles, nor refractory periods, as compared with clear periods of NREM sleep (see Figure 2).

The frequentist statistical approach used thus far does not allow us to evaluate evidence in favor of a null hypothesis, here being that the strength of the cortical pitch representation is not systematically diminished by the presence of a spindle or its refractory period. Baysian statistical approaches allow for assessing evidence in favor of both an alternative and null hypothesis (44–48). The resulting metric, known as a Bayes factor (BF), is a likelihood ratio of the marginal likelihood of two competing hypotheses (e.g. the null hypothesis and an alternative). Bayes factors are expressed as a positive number on a continuous scale. For BF10 values, numbers greater than 1 are interpreted as evidence in favor of an alternative hypothesis, where bigger numbers indicate stronger evidence. On the other hand, small BF10 values, between zero and 1, indicate evidence in favor of the null hypothesis instead. BF10 and BF01 values operate symmetrically; thus BF01 values greater than 1 is another measure of support for the null hypothesis (i.e. small BF10 values). (44, 49).

We first ran a Bayesian version of a repeated measures ANOVA comparing our 3 conditions (Clear, Spindle, and Refractory). The analysis revealed substantial evidence in favor of the null hypothesis, with a BF01 value of 4.27, indicating strong support for the absence of an effect of conditions. We then ran post hoc tests comparing Spindle and Refract to Clear.

In agreement with the frequentist statistics, there was little evidence in favor of the hypothesis that spindle presence affects FFR amplitude [$BF_{SpindleEffect} = 0.313$, error = 0.009, 95% CI: (−0.621, 0.372)], nor of an effect of the Refractory period [$BF_{RefractoryEffect} = 0.299$, error = 0.009, 95% CI: (−0.397, 0.593)], on FFR strength. Conversely, there was moderate to strong evidence supporting the absence of a protective role of the presence of spindle [$BF_{NoSpindleEffect} = 3.200$, error = 0.009, 95% CI: (−0.621, 0.372)], and similarly, there was moderate to strong evidence in favor of there being *no* change in amplitude due to the refractory period [$BF_{NoRefractoryEffect} = 3.343$, error = 0.009, 95% CI: (−0.397, 0.593)].

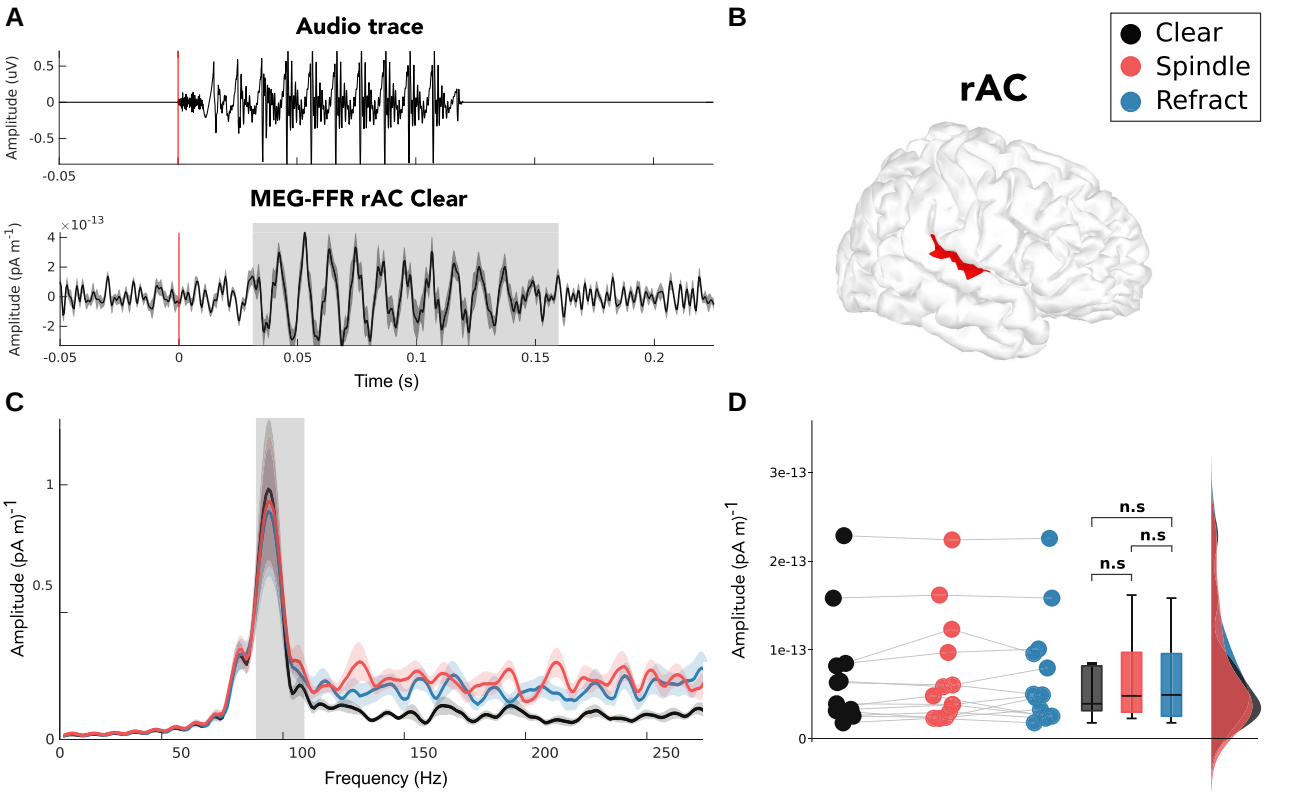

Fig. 2. The presence of neither a spindle nor its refractory period impedes propagation of fine auditory information through the lemniscal system from thalamus to cortex. A) Timeseries of the auditory stimulus (speech syllable /da/), and its neural phase-locked evoked response extracted from the right auditory cortex in NREM2 and 3 sleep (combined). B) Location of the right auditory cortex region of interest from which data are extracted. C) Frequency domain plot of FFR amplitude across conditions. D) FFR fundamental frequency (98 Hz) amplitude during Spindle, Clear, and Refractory periods. FFR, frequency-following response; Spindle, responses during a spindle; Refract, responses measured during the refractory period; Clear, responses measured during neither a spindle nor refractory period.

Experiment 2: evidence that sleep spindles do not greatly affect early cortical sound processing

The FFR examined in experiment 1 represents periodicity encoding in the right auditory cortex, which is isolated from other evoked responses based on its high-frequency range (>80 Hz). Filtering the same data in lower frequency ranges (i.e. 1.5 and 40 Hz (32, 50)) reveals "long latency" evoked responses (i.e. P100, P200) that are associated with other aspects of sound processing in the primary and secondary auditory cortex. The study design of experiment 1 is unsuitable for examining these slower evoked components, as a too-short inter-stimulus interval is used to maximize the number of trials (34); more time must be left between sound stimulations to observe the brain's extended response to sound, which is in the order of seconds rather than hundreds of milliseconds as for the FFR. Getting a sufficient number of stimulations while leaving an inter-stimulus interval long enough to observe these long latency components require a longer sleep opportunity than a nap timeframe. We therefore adopted an overnight study design with a limited number of subjects and conducted statistics within-subject, assessing the consistency of results by repeating the process independently on multiple subjects using linear mixed effect models (see Methods). Five neurologically healthy young adults without sleep conditions or taking medication were included in an overnight study. The mean age was 21.2 (SD: 1.33; range: 19–23), and three were female.

As in experiment 1, sleep scoring analysis confirmed that all subjects slept reasonably well despite the constraints of the scanner. Importantly for the present analysis due to the presence of spindles, subjects spent 179 minutes (SD: 59) in NREM2 and NREM3 sleep stages (combined; see Table S2 for further details on sleep duration). On average, 690 spindles (740) and 1,655 SOs (SD: 890) were detected per subject using EEG from NREM2 and NREM3 combined. During stages NREM2 and NREM3, an average of 224.8 stimulus presentations coincided with sleep spindles (SD: 81.8; range: 85–289) and 459.8 were presented during the refractory period (SD: 191.2; range: 149–614). An average of 2,284.2 epochs were sorted into the Clear condition, in which stimuli did not arrive concurrently with an SO, or within nor immediately after a spindle (SD: 650.5; range: 1,325–2,850).

To confirm that the detection (at Cz) and sorting procedure was successful to separate time periods that included high and low spindle activity, we compared the electroencephalography absolute sigma band power (root mean square of the amplitude within 11–16 Hz; see Methods) during each of the three epoch types in a 2 second windows centered on the sound presentation. We confirmed that the distributions were statically different using a repeated measures ANOVA $F(2, 8) = 25.543$, $P < 0.001$) (see Figure S2). Post hoc analyses were conducted to confirm that each conditions was statistically different from the comparison (Clear) condition. Post hoc pairwise comparisons revealed that sigma band power was significantly greater in Spindle as compared with Clear (mean difference = 3.37e – 6, SE = 5.18e – 7, 95% CI = [1.81e – 64.96e – 6], $P < 0.001$) and Spindle as compared with Refractory (mean difference = 3.00e – 6, SE = 5.18e – 7, 95%

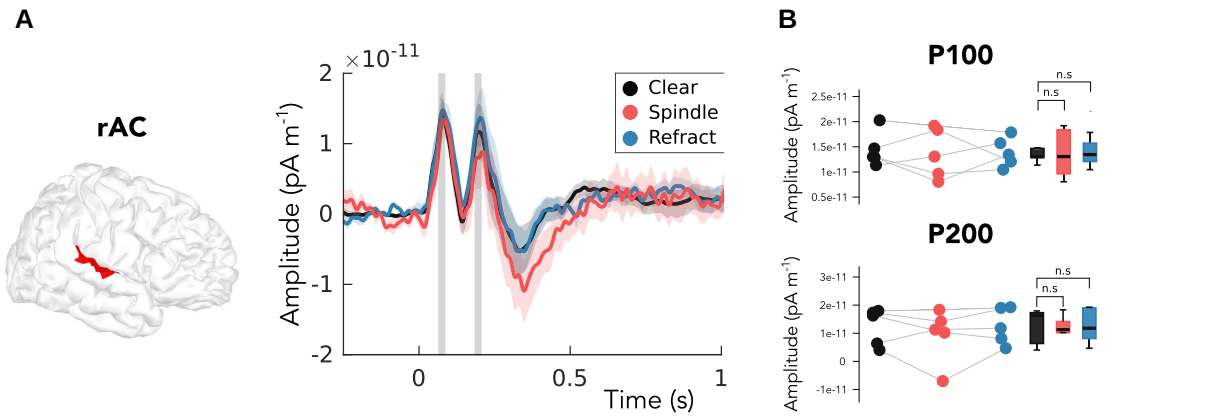

Fig. 3. The presence of neither a spindle nor its refractory period greatly affects the slow cortical evoked responses "P100" and "P200" (filter: 0.5–40 Hz). A) Timeseries of the auditory evoked responses in electroencephalography (Cz electrode), by condition. The right auditory cortex region of interest is shown at left. B) Within-subject means across all epochs are indicated by horizontal lines; amplitudes were similar across all three conditions.

CI = [1.44e – 64.56e – 6], $P < 0.001$). The Clear and Refractory conditions were not significantly different [mean difference = –3.71e – 7, SE = 5.18e – 7, 95% CI = (– 1.93e – 61.19e – 6), $P = 0.495$].

To visualize whether the presence of a spindle or its refractory period influences the low-frequency cortical response, we first produced subject average evoked responses from extracted signals from the rAC for each of the three conditions (Clear, Spindle, and Refractory period), within the 0.5–40 Hz frequency band; see Figure 3A. Following a similar logic to the FFR experiment, the unaltered appearance of cortical responses when sound onset coincides with an endogeneous sleep spindle would indicate that auditory information has passed through the thalamus and arrived at the cortex, unimpeded. The first components of evoked auditory responses (e.g. P100 and P200), i.e. those occurring within about 200 ms of sound onset, originate in auditory cortical areas (see Figure 3 in (26) for group-level whole-brain MEG topographies of the P1 and P2 components, see also (36, 51)) and are reliably produced by the right auditory cortex during sleep (see Figure 6 in (32)). For statistical analysis of the amplitude of early components, we extracted both P100 and P200 amplitudes for each epoch based on their peak timing as observed in the group averages (20 ms windows centered on 80 ms for P100 and 200 ms for P200, in agreement with previous work (52)). To confirm that P100 is observed in the absence of spindles and refractory periods (i.e. Clear condition), in the signal extracted from the right auditory cortex in each participant, we used simple nonparametric Wilcoxon signed-rank tests (one-tailed) to test the distribution of amplitude values across all single-trials epochs. For every subject, they were significantly higher than 0, meaning that a P100 component was clearly present (Sub 1: $V = 1.96e – 6$, $P < 0.001$; Sub 2: $V = 0.69e – 6$, $P < 0.001$; Sub 3: $V = 1.06e – 6$, $P < 0.001$; Sub 4:$V = 2.95e – 6$, $P < 0.001$; Sub 5: $V = 1.95e – 6$, $P < 0.001$). We conducted the same analysis for P200. For every subject, amplitudes were significantly higher than 0, meaning that a P200 component was clearly present (Sub 1: $V = 1.54e – 6$, $P < 0.001$; Sub 2: $V = 0.68e – 6$, $P < 0.001$; Sub 3: $V = 1.08e – 6$, $P < 0.001$; Sub 4: $V = 2.59e – 6$, $P < 0.001$; Sub 5: $V = 1.66e – 6$, $P < 0.001$). Note that the P200 component in Figure 3A appears to be of lower amplitude in the Spindle condition because a single subject had a lower number of detected spindles and less evident P200 component (see Figure 3B and Table S2).

To robustly evaluate potential differences in amplitude of the P100 component depending on the presence of a spindle, its refractory period or its absence, we conducted LME analyses at the single trial level, using subjects as a random effect. We removed outliers from each Condition (Clear, Spindle, and Refractory) by excluding values based on thresholds defined as 1.5 times the interquartile range (i.e. below Q1 and above Q3). The mean percentage of retained epochs across subjects was 93.7% (SD = 4.7). We compared a model with Condition as a fixed effect to a null intercept-only model and found that the addition of Condition did not significantly increase model fit ($\chi^2(2) = 1.62$, $P = 0.44$). Post-hoc analysis using estimated means did not show any statistically significant differences between conditions. (Clear-Refract difference, $M = 2.04e – 13\ pAm^{-1}$, SE = 8.68e – 13, $P = 0.97$) and (Clear-Spindle difference, $M = 1.52e – 12\ pAm^{-1}$, SE = 1.19e – 12, $P = 0.41$).

We conducted the exact same analysis for P200 amplitude and found similar results. The mean percentage of retained epochs across subjects was 93.2% (SD = 4.6). We compared a model with Condition as a fixed effect to a null intercept-only model and found that the addition of Condition did not significantly increase model fit ($\chi^2(2) = 5.95$, $P = 0.05$). Post-hoc analysis using estimated means did not show any statistically significant differences between conditions. (Clear-Refract difference, $M = –1.47e – 12$, SE = 1.06e – 12, $P = 0.35$) and (Clear-Spindle difference, $M = 2.63e – 12$, SE = 1.44e – 12, $P = 0.16$). For completeness, we also ran Bayesian statistics on each subject on epoch-level data, finding coherent results (see Table S3). These results indicate that the occurrence of spindles and their refractory period does not affect auditory cortical responses associated with early sound processing in the primary and secondary auditory cortex (P100, P200).

Later components (N550-P900) associated with widespread changes in brain activity and evoked slow oscillations (32), which are integral to sleep-dependent memory consolidation particularly when they are coupled to sleep spindles (i.e. co-occurring in a phase-amplitude relationship), have been shown to be dependent upon brain activity at the time of sound onset (14, 29, 30, 53, 54)). As auditory stimulation has been used to alter memory processes noninvasively in a technique known as closed-loop auditory stimulation (CLAS), it is of interest to further explore the influence of spindles on how well CLAS generates additional SOs and spindles in a reactive fashion.

Experiment 3: evidence that sleep spindles do not reduce the effect of closed-loop auditory stimulation

Because SO–spindle coupling, i.e. co-occurrence of the spindle and the up-state of the SO, is thought to be particularly important for

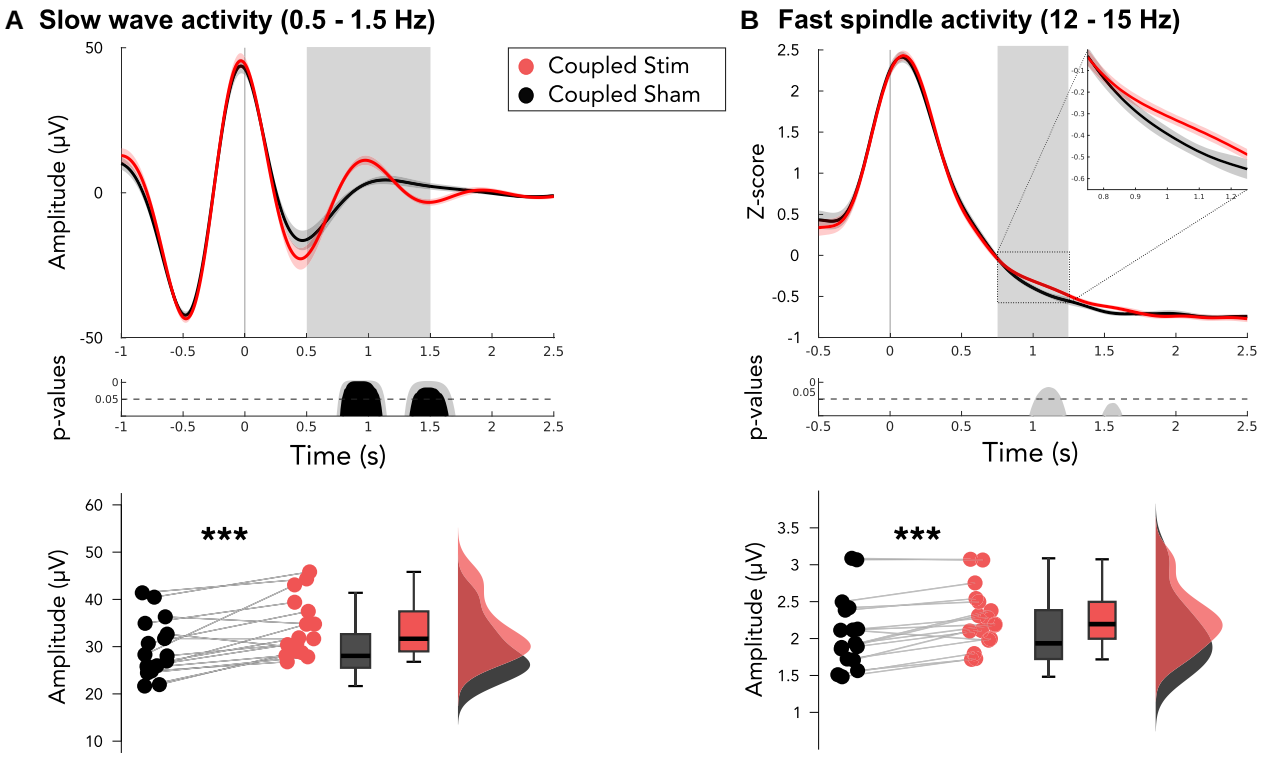

Fig. 4. The presence of a spindle coupled to an SO up-state does not impede closed-loop auditory stimulation effects on slow wave nor fast sigma activity at electrode Cz. A) Timeseries of low-frequency EEG time-locked to the detected SO up state in both Stim and Sham conditions only when the detected slow wave was coupled to a sleep spindle. Statistical significance of the difference between conditions is represented below. Gray shading represent uncorrected *P*-values and black shading represent these *P*-values after Benjamini–Hochberg correction. Amplitude (RMS) of the subsequent (generated) SO across conditions are reported for each subject, below. B) Timeseries of amplitude (z-score of root mean square) in the fast sigma band (12–15 Hz) for both stimulated and nonstimulated coupled SOs. Statistical significance of the difference between conditions is represented below the timeseries. Amplitude (RMS) of the subsequent (generated) fast sigma activity across condition are reported for each subject, below. Red denotes Stimulation; Black, Sham. Error bars = standard error. *** *P*-values < 0.001.

memory consolidation (55)), and because sound presented during up states provokes additional slow wave and spindle activity and boosts memory performance (closed-loop auditory stimulation) (14, 53, 54)), we investigated whether the co-occurrence of spindles during CLAS affects its effectiveness (i.e. ability to generate SOs and spindle activity). As <10% of SOs are coupled with spindles (37)), and each stimulation must be separated by several seconds to allow for the analysis of the slower evoked components (32), more data per participant are needed to address this research question. Experiment 3 used an at-home design so as to record five nights per subject.

Seventeen participants were included in this multiple-night experiment. The mean age was 27.56 (SD: 9.39; range 22–43), and 9 were female. On average, the mean number of usable recordings per subject for the remaining 17 subjects was 4.71 (SD: 0.77) (see Table S4). On average 7,300 spindles (SD: 2,590) and 12,150 SOs (SD: 4,720) were detected (at Fpz) per subject using EEG (NREM2 and NREM3). The mean number of total stimulations was 6,437.65 (SD: 2,463.19), and sham stimulations (in which an SO peak was detected but not stimulated, for comparison) was 5,716.41 (SD: 2,271.22). The mean number of spindles occurring during stimulated SOs was 361.76 (SD: 190.96), and spindles occurring during sham stimulations was 349.82 (SD: 177.67). In accordance with previous observations, the mean percent coupling across all detections was 6.50% (SD: 3.74).

To confirm that CLAS generates a subsequent SO, as has been observed in previous work (e.g. (14, 29, 30)), we first filtered the signal in the slow wave activity (SWA) range (0.5 to 1.5 Hz) and compared the amplitude of the generated SO between all Stimulation and Sham trials. A paired sample t test was conducted to assess the differences in mean amplitude in the SO range between 0.5 and 1.5 second post stimulation (14) in both Stimulation and Sham conditions. It revealed a significant difference in amplitude between the Stim and Sham conditions ($t(16) = 5.69$, $P < 0.001$, Cohen's $d = 1.38$). These results replicate previous findings (14) showing that the mean amplitude in the stimulation condition was significantly higher than in the sham condition (see Figure 4A).

Next, we split epochs according to the presence or the absence of spindles (Coupled SO and Uncoupled SO) to assess the impact of the presence of a sleep spindle at the time of stimulation. A paired sample t test revealed a significant difference in amplitude between the Coupled Stim and Coupled Sham conditions ($t(16) = 4.30$, $P < 0.001$, Cohen's $d = 1.04$). This result suggests that the presence of a sleep spindle nested in the SO up-state does not prevent the physiological effect induced by auditory stimulation.

To confirm that CLAS also increases fast spindle activity as has been observed (14), we first filtered the signal in the fast sigma range (12–15 Hz; (14)) and compared the route mean square within that band during the generated SO (0.75 to 1.25 s post SO upstate detection), between Stimulation and Sham across all trials. Spindle activity following stimulated SOs was significantly larger than that after unstimulated SOs ($t(16) = 4.14$, $P < 0.001$, Cohen's $d = 1.00$). We then split epochs according to the presence

or the absence of spindles to assess the impact of the effect of sleep spindle presence at the time of stimulation. A paired sample t test was conducted to assess the differences in both Coupled Stimulation and Coupled Sham conditions; it revealed a significant difference in amplitude between the Coupled Stim and Coupled Sham conditions ($t(16) = 2.28$, $P = 0.01$, Cohen's $d = 0.55$). This result suggests that the presence of a sleep spindle nested in the SO up-state does not prevent the physiological effect induced by auditory stimulation (see Figure 4B). In sum, sleep spindles do not impede the physiological enhancement of SOs and spindle activity by CLAS.

Discussion

Our results suggest that neither the co-occurrence of sleep spindles nor their refractory periods decrease time-resolved indices of auditory processing in healthy adults. In the first experiment, we showed that the amplitude of the frequency-following response extracted from the right auditory cortex was conserved across conditions. These data indicate that the lemniscal pathway that carries fine sound information from the auditory periphery to primary auditory cortex (via the auditory nerve, cochlear nucleus, and inferior colliculus in the brainstem, and medial geniculate body in the thalamus), operates independently from spindle dynamics (Figure 2).

In the second experiment, we measured the more commonly recorded long-latency evoked responses, P100 and P200. P100 in humans is thought to be generated in nonprimary regions of the superior temporal gyrus that are innervated by extra-lemniscal auditory input from nonspecific thalamic nuclei (e.g. medial pulvinar, nucleus limitans, and suprageniculate nuclei), whereas P200 is generated by downstream processing steps over an extended area of the auditory cortex (50, 56–58). As with the FFR, neither the P100 nor P200 evoked components were noticeably diminished by the presence or recent history of a spindle (Figure 3). This result indicates that a second branch of the auditory processing pathway, which is dependent upon different thalamic regions than the FFR, is also not strongly affected by spindle activity.

In the third experiment, we investigated how physiological responses to closed-loop auditory stimulation of cortical SOs are affected by the presence of spindles. The process of generating SOs and sleep spindles is thought to be mediated via the ascending reticular activating system sending inputs to ventral frontal regions that are strong generators of slow waves in sleep (31, 32). Under the hypothesis that sleep spindles serve in part to suppress sound and preserve the sleep state, their presence would be expected to reduce or eliminate the previously demonstrated effect of the auditory stimulation on slow wave activity and fast spindle activity. However, instead we find that stimulation during spindles generates additional SO and spindle-band activity, as has been noted in previous work that did not distinguish between SOs that were coupled with spindles and those that were not (e.g. (14)). This result suggests that a third means through which sound can influence cortical activity is also functional during spindles and their refractory periods (Figure 4).

The idea that sleep spindles play a protective role in sleep comes from several lines of research (summarized in (12)). In undisturbed NREM sleep, the density of spindles correlates with the duration of NREM2 sleep (59), and people who generally have more spindles exhibit higher tolerance for sleeping in noisy conditions (60). In rodents, optogenetic manipulations that increase spindle activity increase the duration and stability of NREM2 sleep (61, 62). A relationship between sleep spindles and sleep continuity is also observed in aging human populations, in whom sleep spindle amplitude and density are reduced (63, 64). Sleep spindle density is correlated with sleep efficiency and stability across the lifespan (65–67). Studies experimentally investigating arousability in humans and rodents also implicate sleep spindles in protecting the sleep state. Enhancing sleep spindles causally, either pharmacologically (using benzodiazepines) in humans (68), or through genetic manipulations in animal models (69), elevates arousal thresholds. Another line of work looks at how the brain's responses to sensory input changes over brain states. For example, Mai et al. noted that frequency-following response amplitude was lower during recordings in which more sleep spindles were present (22). As regards longer-latency auditory evoked responses, results are unclear. Elton et al. observed some difference in gross ERP morphology of responses to sounds occurring close to spindles vs. when they were absent, when sounds were presented to six participants (at 65 dB sound pressure level). P100 and P200 appeared to have higher amplitude during spindles (70). Cote et al. investigated P200 amplitude in response to different sound levels across sound levels of 60, 80, and 100 dB in eight participants. They did not observe differences between responses to sounds that were concurrent with spindles vs. those which were not (although they did find a difference at high sound intensities with a third condition, in which spindles occurred after sound presentation—possibly related to the CLAS effect) (71).

One reason for lack of clarity on the relationship between spindles and sensory information might be a matter of definition. Many studies invoking the thalamic gating hypothesis (according to which the thalamus filters sensory input during sleep spindles) do not differentiate between the potential roles of spindles in preventing sensory information from reaching cortex; or alternatively, in producing a subsequent, reactive response that could stabilize the sleep state itself (preventing an arousal from a subsequent sensory event). The terms "protective" and "reactive" were used to distinguish these ideas in a recent review (12); however, they may still be ambiguous. For example, "protective" might refer to shielding endogenous sleep-related cognitive processes (like memory consolidation) from external interference. Or, "protective" could mean maintaining the sleep state itself, which could include generating slow oscillations and spindles in the seconds following a response (which would also fit under the "reactive" term)—or even just not causing an arousal, as in studies that evaluate propensity to waking following sensory input. In the latter case, a mechanism is still lacking, returning us to the question of whether sleep spindles modulate the strength of sensory input to higher-level processes.

Importantly, auditory transmission is fast; information travels from ear to cortex in less than 15 ms (72). If the spindle were to gate sensory information to cortex as a protective mechanism, the spindle must already have started when sound information arrives at the thalamus for a blocking mechanism to make sense. For this reason, study designs that specifically separate brain responses according to their co-occurrence with spindles are critical to clarify the question of whether spindles play a protective role by impeding sound transmission.

To date, few studies have directly assessed responses to sound presented during a spindle. In addition to the two ERP studies presented above (70, 71), a notable exception is a pair of studies which used simultaneous EEG to mark the timing of sleep spindle and functional magnetic resonance imagining (fMRI) to investigate brain responses to tones during wakefulness and NREM sleep (20, 21). The authors showed that whereas elicited responses

were observed in the thalamus and the transverse gyrus during wakefulness as well as during NREM, responses were smaller and more variable when sounds were presented during a sleep spindle, suggesting that sound information is less prone to be faithfully transmitted to the cortex. They concluded that changes in sensory processing at the thalamic level during spindles allows for functional isolation of brain circuits from incoming stimuli, to promote and protect cellular interactions underpinning brain plasticity. However, other researchers have suggested that the higher response variability observed in these studies may also reflect a low number of trials recorded during sleep spindles (73).

A potential issue for the idea that spindles impede sensory transmission is that quite a lot of cortical sensory processing seems to take place during sleep, including spindle-rich sleep stages (NREM2 and NREM3; reviewed in (11)). In mice, presenting meaningful sounds (especially those previously associated with aversive tasks), can lead to disruptions in sleep-associated brain oscillations without necessarily causing full behavioral arousal (74). In-line with these findings and the observation that voice familiarity is processed in NREM (and also REM) sleep stages, Blume et al. proposed that the auditory system acts as a "sentinel system" by continuing to evaluate environmental stimuli and initiate awakenings when necessary to respond to potential threats (75). While some of these processes, particularly those involving fear conditioning, may be mediated by subcortical structures (e.g. thalamo-amydala circuitry (76)), evidence for higher-order (cortical) information processing in humans has also been reported during spindle-rich NREM sleep. For example, sleepers are able to selectively amplify informative vs. meaningless competing speech streams in NREM (and REM) sleep (77, 78). It is hard to reconcile a role for intermittent spindle activity in generally suppressing sensory transmission to cortex with a role for the auditory system to monitor the environment, particularly if higher-level cognitive processing such as recognizing and separating sound sources is needed.

To our knowledge, only one group has conducted time-resolved spindle analyses investigating auditory processing with direct recordings from the auditory cortex. In 2016, Sela et al. measured local field potentials and multiunit activity responses to auditory stimuli in rat primary auditory cortex. They reported that when sleep spindles (measured locally) co-occurred with the stimulus, neural responses were nearly identical in terms of local field potential morphology (latency and amplitude) and multiunit activity firing rate to those observed across NREM sleep (<6% difference). Even when narrowing their analysis to a subset of the highest amplitude sleep spindles so as to maximize the sensitivity of the analysis to amplitude modulations, they did not observe weakening of the responses, nor was there a correlation between strength of auditory response and sigma power (73). These results make a strong case against spindles impairing auditory thalamocortical transmission, although the authors acknowledge the restriction of the conclusions to auditory activity in primary auditory cortex (likely through the lemniscal pathway), leaving open the possibility that sleep spindles impair auditory processing downstream in other auditory (or nonauditory) regions (73). The view that spindles play an active role in blocking sound continues to be prevalent, particularly in human literature (12, 22, 79, 80). In the present work, we investigated this question in humans using MEG and EEG to assess auditory processing in the presence of sleep spindles through the main leminiscal pathway (see Figure 2) as well as other nonlemniscal auditory pathways (see Figures 3 and 4).

Our results using time-resolved and whole-brain techniques suggest that the presence of sleep spindles does not significantly impede auditory information from reaching the cortex through neither lemniscal nor two nonleminiscal auditory pathways. Previous work has shown that evoked responses to sound do however change considerably across NREM and REM sleep stages (32, 36). One explanation that might reconcile the discrepancy between our observations from EEG/MEG showing that evoked responses are preserved and those from earlier work showing that hemodynamic responses are reduced during spindles (20, 21) could be that it is not the thalamic relay itself that impedes sound transmission but rather the state of the cortex upon arrival of sensory information that determines its cognitive fate (the cortical gating hypothesis (11, 81)). Overall differences in levels of neurotransmitters within the system, which do vary considerably across sleep state and affect neural firing patterns (82), could perhaps affect the ability of auditory information to propagate within the cortex due to changes in tissue properties and/or functional connectivity between brain areas during sleep (83).

Recent work highlighted that sleep spindles are organized temporally according to an infraslow rhythm of around 0.02 Hz. This would correspond to sleep spindles being clustered within spindle-rich and relative spindle-free periods of 50 seconds alternating throughout the night. Interestingly, this pattern aligns with the alternating organization of NREM sleep into fragility and continuity periods, distinguished by acoustic arousability (84) and also observed in autonomic physiological fluctuations such as heart-rate rhythm in humans (84) but also pupil diameter (85) and brain temperature in rodents (86). This prevalent 0.02 Hz rhythm then might reflect a widespread brain–body rhythm impacting behavioral arousal, brain activity, and cortical cellular dynamics. In that case, the decrease in auditory cortex activity observed in previous neuroimaging studies might come from a decrease in general functional connectivity during deeper sleep and perhaps specific windows of time during which spindles happen to be more common, rather than due to thalamic gating during the spindle itself. We propose that spindles are one of many consequences of this sleep protective mechanism rather than its cause. Further work might consider assessing auditory processing comparing epochs not based on the presence or the absence of a spindle but rather on their timing relative to other physiological processes (e.g. infraslow rhythm, phase of SO, evolution of spindle envelope), and using complementary study designs which quantify sleep fragmentation as a function of sensory stimulation (e.g. with longer inter-stimulus intervals and varied stimulus intensity), using sensitive measures of arousal (87). Furthermore, the physiological and behavioral impact of targeting spindles with different properties (i.e. fast vs. slow (88)) and from different brain regions (89) remains to be explored.

Conclusion

Altogether, our data suggest that auditory information reaches the cortex through multiple pathways even during the presence of a sleep spindle (and its refractory period). These data therefore do not support a direct role of sleep spindles in protecting the sleep state by impeding its interruption by auditory input. This view is coherent with Sela et al.'s observation that cellular-level responses to sound in auditory cortex in rats are unchanged by sleep spindles (73), and with the idea that the roles of sleep spindles lie in other directions, for example in active memory consolidation as has been suggested in previous work (reviewed in (12)). More research is needed to clarify whether these results might extend to other sensory modalities (e.g. vision, touch), which seems likely considering that nonarousing somatosensory and visual

stimuli generally evoke similar brain responses to auditory stimuli in sleep (90, 91), and to evaluate the hypothesis that electrochemical changes in cortical tissue are responsible for observed differences in evoked responses across sleep stages. It also raises questions that can only be answered using more granular levels of investigation, i.e. concerning how distinct firing modes in thalamus during spindles can in fact still allow for transmission of sensory information. Finally, our results show that sounds presented during concurrent SOs and sleep spindles generate additional SOs and spindle activity, as has been shown when SO up-states are stimulated randomly with respect to spindle presence (14). As closed-loop auditory stimulation has generated a lot of interest for its potential to causally investigate the roles of neural oscillations and restore them in disease states (29, 30, 40, 92, 93), this result encourages further exploration into how and when sound can be used to modulate and improve sleep-dependent brain processes (94).

Methods and materials

See Supplementary Material for methods, including Participant recruitment and selection, Statistical approach, Study design and participant preparation, Stimulus Presentation, Data Acquisition and Processing, and Definition of Conditions. Subjects gave written informed consent, and were compensated for their time. All experimental protocols were approved by Concordia University's Human Research Ethics Committee, and concerning the MEG experiments, also by the McGill University Research Ethics Board.

Acknowledgments

The authors would like to acknowledge team members past and present who assisted in data collection: Arina Ujevco, Noam Thillou, Meredith Rowe, Keelin Greenlaw, Alix Noly-Gandon, and Raphaëlle Merlo. We thank Karine Lacourse for advice concerning spindle detection; David Levesque and Latifa Lazzouni for developing and testing the SO detection algorithm on the ecHT; and David Wang for technical assistance with the ecHT; Sylvain Baillet and Marc Lalancette for assistance with MEG access and technical matters.

Supplementary Material

Supplementary material is available at *PNAS Nexus* online.

Funding

H.R.J. was supported by a scholarship from the Quebec Research Funds (Fonds de Recherche de Quebec-Nature et technologies; FRQNT). E.B.J.C. is financially supported by grants from the FRQNT (2022-301806), Natural Sciences and Engineering Research Council of Canada (NSERC (2019-06976)), and a Concordia University Research Chair in Sleep and Sound.

Author Contributions

H.R.J.: Conceptualization, methodology, formal analysis, data curation, writing—original draft, writing—review and editing, visualization. E.B.J.C.: Conceptualization, methodology, formal analysis, data curation, writing—original draft, writing—review and editing, visualization, supervision, project administration, funding acquisition.

Data Availability

MEG and EEG data used in experiments 1 and 2 are available in the Open MEG Archive (OMEGA) (95). Extracted values used in this work have been deposited in the Open Science Foundation (https://osf.io/nawzq/?view_only=e4c4df7ac10342c199a808d4473781a1).

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