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

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## ABSTRACT

Sleep spindles are neural events unique to non-rapid 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 to 16 Hz) at about 1 s post stimulation, 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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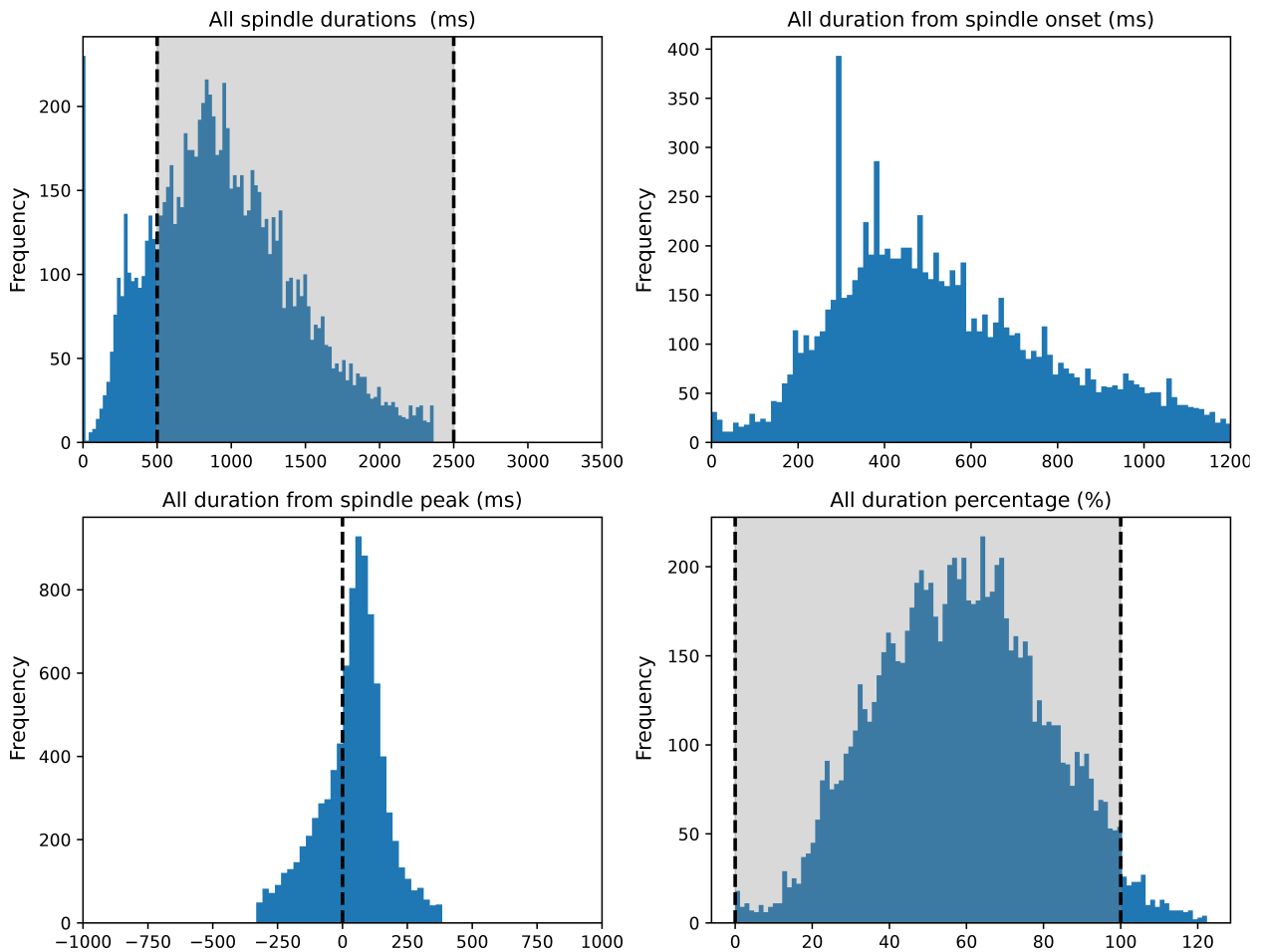
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## Effects of sleep spindle stimulation

	Number of epochs included in each analysis									
	#STIM all epochs	#SHAM all epochs	#STIM main	#SHAM main	#STIM before peak	#SHAM before peak	#STIM after peak	#SHAM after peak	#STIM evoked	#SHAM evoked
S01	605	879	571	852	90	91	291	480	34	27
S02	2085	2066	1881	1955	312	255	831	923	204	111
S03	460	362	383	333	52	47	162	161	77	29
S04	1079	682	969	637	150	96	489	318	110	45
S05	1533	416	1386	389	288	45	536	178	147	27
S06	1927	1245	1804	1202	307	194	859	542	123	43
S07	231	629	226	624	34	87	116	320	5	5
S08	170	927	157	890	20	119	78	434	13	37
S09	1280	1087	1219	1052	116	115	607	572	61	35
S10	1138	1097	986	985	149	103	443	437	152	112
Mean	1050.8	939	958.2	891.9	151.8	115.2	441.2	436.5	92.6	47.1
Std dev	677.1	492.5	621.6	467.2	112.9	64.3	279.62	220.7	65.7	35.7

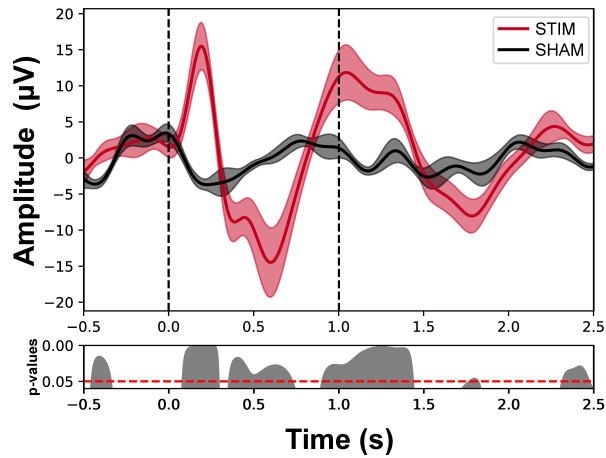
**Table S1**

Supplementary Table 1 lists the number of epochs included in the main analysis (left), in which only stimulations that were followed by a 2.5 s gap were included so as to observe the undisturbed evolution of the evoked response (i.e., represented in Figure 2). The middle columns indicate the number of epochs included in the analysis of the effect of stimulation timing within the spindle, and the right column indicate the number of spindles included in the analysis of the effects of stimulating evoked sleep spindles.

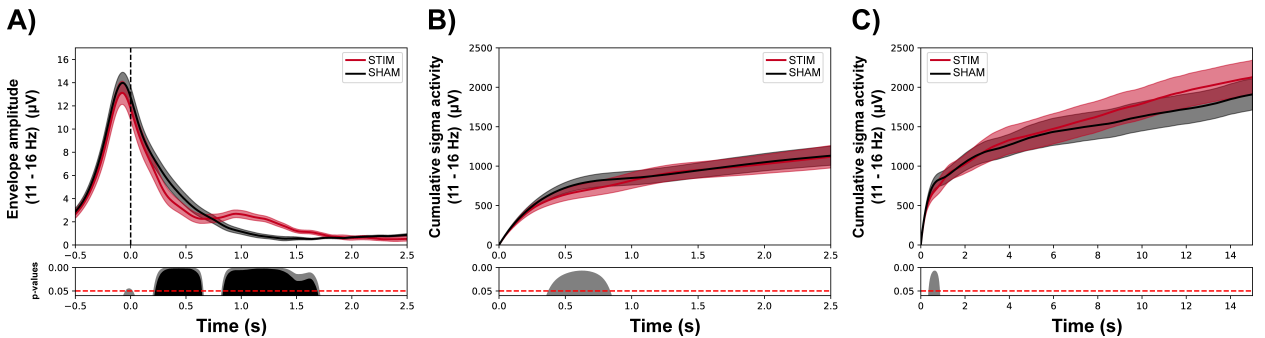


**Figure S1:** Post-hoc characterization of online sleep spindle detection. Spindle onset and offset were identified post-hoc based on deviations in sigma power. A) Spindle duration was calculated as the time difference between the offset and onset. Vertical dashed lines represent the American Academy of Sleep Medicine definition of spindle duration (i.e., 500 ms to 2.5 s). B) For each spindle, the time from spindle onset to stimulation was measured as the interval between spindle onset and the stimulation event. C) Similarly, the duration from the spindle peak was determined by calculating the interval between the stimulation event and the peak of the sigma envelope. The vertical dashed line represent the peak of the spindle envelope. D) The percentage of spindle duration at the time of stimulation provides insight into the phase of the spindle envelope when stimulation occurred. Vertical dashed lines represent the interval for which stimulation occurred within the spindle (97.6% of stimulations in our experimental design).

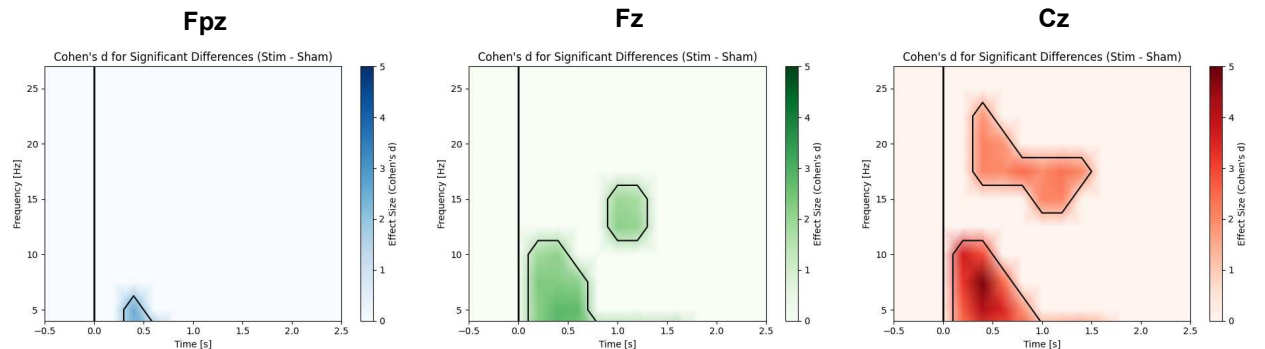
### Slow wave activity at Cz (0.1 - 4 Hz)



**Figure S2:** Effect of trains of spindle stimulation on slow wave activity (for comparison with the effect on neural responses to non-evoked spindle stimulation in Figure 2, and for comparison with sigma activity evoked by stimulating evoked responses in Figure 4 in the main paper). Dashed vertical lines represent timing of auditory stimulations. Grey areas represent uncorrected p-values and black areas represent corrected p-values.



**Figure S3:** Cumulative sigma power (main analysis, i.e., including epochs in which only one stimulation occurred, see also Figure 3 in the main paper, which shows the same information broken down by stimulations that occurred early vs. late in the spindle envelope’s evolution). Dashed vertical line represent timing of auditory stimulation. Grey areas represent uncorrected p-values and black areas represent corrected p-values.



**Figure S4:** Effect sizes for statistically significant clusters in response to closed-loop auditory stimulation of sleep spindles at Fpz, Fz and Cz (for visualization purposes only significant clusters bigger than a single time-frequency bin are represented)