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Feedback-Controlled Transcranial Alternating Current Stimulation Reveals a Functional Role of Sleep Spindles in Motor Memory Consolidation

Highlights

- Feedback-controlled tACS (FB-tACS, 12 Hz) boosted subsequent sleep spindle activity
- FB-tACS enhanced sleep-dependent motor, but not declarative memory, consolidation
- Stimulation-induced fast spindle activity changes predicted motor memory benefits
- The correlation of spindles and motor memory in sham session agrees with FB-tACS results

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In Brief

Lustenberger et al. engineered a novel feedback-controlled spindle stimulation approach that selectively targeted and modulated sleep spindles in real time. This approach revealed, for the first time, that fast sleep spindles play a functional role in motor memory consolidation.

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Feedback-Controlled Transcranial Alternating Current Stimulation Reveals a Functional Role of Sleep Spindles in Motor Memory Consolidation

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SUMMARY

Transient episodes of brain oscillations are a common feature of both the waking and the sleeping brain. Sleep spindles represent a prominent example of a poorly understood transient brain oscillation that is impaired in disorders such as Alzheimer's disease and schizophrenia. However, the causal role of these bouts of thalamo-cortical oscillations remains unknown. Demonstrating a functional role of sleep spindles in cognitive processes has, so far, been hindered by the lack of a tool to target transient brain oscillations in real time. Here, we show, for the first time, selective enhancement of sleep spindles with non-invasive brain stimulation in humans. We developed a system that detects sleep spindles in real time and applies oscillatory stimulation. Our stimulation selectively enhanced spindle activity as determined by increased sigma activity after transcranial alternating current stimulation (tACS) application. This targeted modulation caused significant enhancement of motor memory consolidation that correlated with the stimulation-induced change in fast spindle activity. Strikingly, we found a similar correlation between motor memory and spindle characteristics during the sham night for the same spindle frequencies and electrode locations. Therefore, our results directly demonstrate a functional relationship between oscillatory spindle activity and cognition.

INTRODUCTION

Oscillatory patterns are fundamental to the organization of thalamo-cortical activity and are conserved across species [1, 2]. The presence of oscillations at different frequencies is dynamically regulated as a function of overall behavioral state and moment-to-moment fluctuations in cognitive demands [1–4]. The transient occurrence of pronounced rhythmic activity is commonly observed in recordings of cortical network dynamics. However, the causal role of the dynamic occurrence of brain oscillations remains poorly understood. Most prominently, sleep spindles are transient electroencephalogram (EEG) oscillations between 11 and 16 Hz [5, 6]. The functional role of sleep spindles in cognitive processes has been hypothesized, but not yet directly demonstrated [7, 8]. Besides the issue that the majority of previous studies on the role of sleep spindles are based on correlations between sleep spindles and memory consolidation, the few studies that manipulated sleep using tones, electrical stimulation, or pharmacology increased sleep spindles indirectly by enhancing slow oscillations/slow-wave sleep [9–13]. This fundamental gap in our understanding of these thalamo-cortical oscillations is the result of the lack of a tool to monitor and selectively enhance transient epochs of oscillatory activity in real time in humans. Transcranial alternating current stimulation (tACS) applies a weak electrical current to the scalp, and recent evidence demonstrates that tACS is capable of inducing frequency-specific effects on brain dynamics [14–19] and can be used to identify the functional role of brain oscillations in cognition [19–22]. However, no approach to selectively target transient oscillations has been described. Animal studies and computational models showed that the effectiveness of transcranial electrical stimulation (tES) relies on the internal network dynamics; therefore, stimulation paradigms that resemble the temporal structure of endogenous activity patterns are the most effective paradigms [16, 23–27]. Based on these findings, we hypothesized that real-time detection of transient oscillations that trigger short epochs of tACS resembling the targeted endogenous oscillation provides a means to boost transient oscillations. Sleep spindles represent the ideal target oscillation to apply this approach for several reasons: (1) Sleep spindles are clearly defined and distinct oscillations during non-rapid eye movement (NREM) sleep that can be targeted in real time. (2) So far, no approach has been described that enhanced sleep spindle activity without increasing other sleep oscillations or the time spent in specific sleep stages [10–12]. (3) Their proposed role in cognitive processes such as memory consolidation still needs to be demonstrated. (4) Several psychiatric and neurologic disorders are hallmarked by sleep spindle deficits,

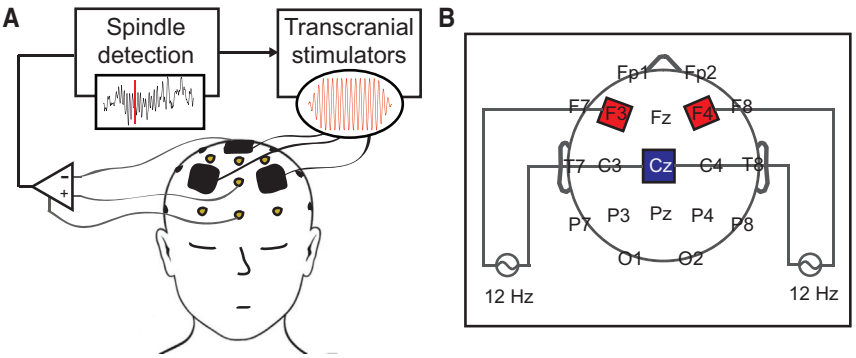

Figure 1. Feedback-Controlled Spindle tACS
(A) Graphical representation of real-time spindle detection and feedback-controlled transcranial current stimulation.
(B) Schematic of tACS current source and stimulation electrode configuration; stimulation electrode placement according to International 10-20 locations.
See also Figure S1.

such as Alzheimer's disease [28], autism [29], and schizophrenia [30–34]. We used an EEG-feedback-controlled approach that restricts the application of tACS (FB-tACS) in the spindle frequency range to when a sleep spindle during NREM sleep is detected and, therefore, only targets network dynamics when spindle activity is prevailing. We further performed two learning paradigms (a declarative word pair and a procedural motor sequence tapping task) that have typically been used to demonstrate sleep-dependent memory consolidation [8, 10]. This approach enabled us to ask whether sleep spindles play a causal role in memory consolidation. This is a question of significant translational relevance, given the number of neurological and psychiatric conditions associated with memory impairment [33, 34]. We found that spindle FB-tACS caused an enhancement of cortical synchronization in the spindle frequency range that intensified the spindling process and improved memory consolidation.

RESULTS

16 male participants underwent a screening night and, thereafter, completed two study nights (randomized, counterbalanced crossover design), one with spindle FB-tACS (verum) and one without stimulation (sham). During both study nights, participants performed an associative word pair (declarative) and motor sequence tapping task (procedural) in the evening and were retested in the morning to assess sleep-dependent memory consolidation. All-night polysomnographic recordings (8 hr EEG, EOG [electrooculogram], and EMG [electromyogram]) were collected. Participant-adapted thresholds based on spectral power values and spindle characteristics obtained during the screening-night EEG (Fz-CPz) were used to simultaneously evaluate in real time whether (1) the participant was in NREM sleep and (2) spindle activity reached an individually defined threshold (Figure 1A; Figure S1; Supplemental Experimental Procedures) during the study nights. If (1) and (2) were met, short epochs of alternating currents with a spindle-like waveform were applied bi-frontally during the verum condition (1 mA, 12 Hz sine wave, 1 s duration at maximum amplitude, 0.25 s linear ramp up, and 0.25 s linear ramp down; Figure 1B). Each stimulation was followed by a 6.5 s timeout (no stimulation even if spindles are present). Our electrode montage resulted in a stimulation that encompassed broadly frontal and centro-parietal regions (Figure 2). Participants were successfully blinded to the stimulation condition, as the two participants who reported sensation of electrical stimulation did so during the sham night. One subject was excluded from stimulation-related EEG analysis due to bad signal quality (see the Supplemental Experimental Procedures).

tACS Was Restricted to NREM Episodes with Prevailing Sleep Spindle Activity

Our spindle detection algorithm led to tACS application solely when sleep spindle activity was prevailing, as illustrated in Figure 3. In all participants, spindle activity was significantly higher at and around the algorithm spindle detection time point ("stimulation onset") compared to the rest of the epoch as verified by the Hilbert amplitude between 11 and 16 Hz during the sham nights (Figure 3C). Furthermore, combining the NREM and sigma threshold detection allowed for a successful identification of prevailing spindle activity during NREM sleep, with a negligibly low number of stimulations during REM or wakefulness (Figure S1E; Table S1).

Spindle FB-tACS Improved Motor Memory Consolidation

We found superior motor memory consolidation (absolute overnight difference; Figure 4A) assessed by speed for correct trials (reduction in response time, a measure for the tapping time between key presses) after spindle FB-tACS (−21.01 ± 5.72 ms) compared to sham (−10.97 ± 7.69 ms; robust linear mixed-model factor condition: $F(1, 11.8) = 5.7$, $p = 0.035$). 12 of 16 participants (responders) showed this beneficial effect of spindle FB-tACS on motor memory consolidation (Figure 4B). This effect was not driven by baseline performance differences, since the response time in the evening was not different between sham and verum conditions (factor condition: $F(1, 11.8) = 0.0$, $p = 0.97$). Furthermore, the reported motor sequence speed gains cannot simply be explained by an improvement in attentional reaction time, as performance in a psychomotor vigilance task was not significantly affected by stimulation (Supplemental Experimental Procedures; Table S2). Number of errors and number of correctly tapped sequences were not affected by stimulation (Figure S2; all p values for factor condition were >0.1). Number of correctly tapped sequences has previously been used as a measure for speed [10, 35, 36]. However, this measure likely assesses both accuracy and speed, because it is dependent on number of errors (accuracy) and response time (speed). Indeed, we found that overnight changes in correctly tapped sequences was negatively correlated with number of errors (pooled data for both conditions, $r(30) = -0.59$, $p < 0.001$). In addition, we found that decreased response time (increase in speed) across the

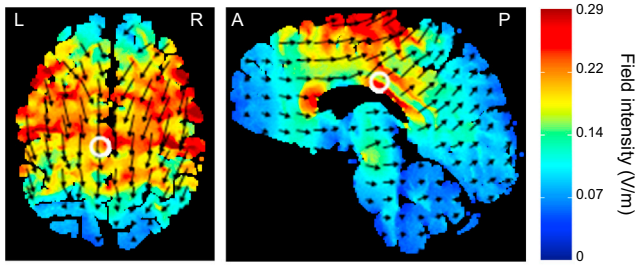

Figure 2. Electric Field Modeling of Used Electrode Montage
Electrodes were mounted over F3, F4, and Cz (return electrode). Field modeling of 1 mA tACS was performed using HDExplore v2.3 (Soterix Medical). This electrode montage led to a broad field distribution over frontal to parietal regions with greatest magnitude of the electric field localized to areas underneath and between the electrodes. Left, axial view (MNI [Montreal Neurological Institute] position of white circle {−6, −3, 50}); right, sagittal view (MNI position of white circle {−7, −10, 32}). L, left; R, right; A, anterior; P, posterior.

sleep period was related to an increase in the number of correctly tapped sequences; pooled data for both conditions, $r(30) = -0.52$, $p < 0.005$. Of note, speed and accuracy were not significantly correlated and, therefore, represent two independent components (pooled data for both conditions; $r(30) = -0.25$, $p > 0.1$). Hence, it is important to separate those two components of motor learning, because they might be differentially affected by stimulation. Thus, stimulation effects might be masked if combination measures (e.g., number of correct sequences) are used. To confirm that the speed aspect of the number of correctly tapped sequences was also significantly affected by stimulation condition, we further controlled the robust linear mixed model (dependent variable: number of correctly tapped sequences) for accuracy by including number of errors as a covariate. Indeed, this corrected model revealed a significant effect of stimulation condition on number of correctly tapped sequences, $F(1, 10.9) = 5.17$, $p = 0.04$, further confirming that, specifically, speed was significantly modulated by FB-tACS.

Spindle FB-tACS had no effect on declarative memory (difference in number of recalled word pairs: sham, 8.00 ± 1.23 words; verum, 7.94 ± 1.07 words; $F(1, 11.8) = 0.00$, $p = 0.97$). Collectively, spindle FB-tACS improved sleep-related gains in motor sequence tapping speed but had no influence on motor sequence accuracy or declarative memory.

Spindle FB-tACS Had No Effect on Sleep Architecture but Increased Post-stimulation Spindle Activity

Given this beneficial effect of FB-tACS on motor memory consolidation, we next investigated whether FB-tACS enhanced sleep spindle activity. We hypothesized that a selective enhancement of spindle activity by stimulation was the underlying mechanism of this memory improvement. First, we excluded the possibility that overall effects on the macroscopic structure of sleep could account for the effect on memory. None of the time spent in individual sleep stages or total sleep time was significantly different between the sham and verum conditions (Table 1; all p values of factor condition were >0.1). Furthermore, no significant effect of condition on sleep architecture was found if only motor memory responders (n = 12) were included (all p values of factor condition were >0.1). Due to the pronounced stimulation artifact (within ~2 s around the tACS start and around 4.3–6 s, caused by internal source switching in the stimulator; Figure S3; see the Supplemental Experimental Procedures for details), analysis was only possible in a stimulation-free interval. Thus, we then examined how short epochs of 12-Hz tACS affected the NREM sleep EEG in a short stimulation-free interval after the tACS artifact (2–4.3 s after stimulation onset; see the Supplemental Experimental Procedures for details) compared to sham condition (only spindle detection trigger, no tACS applied). For this analysis, 15 out of 16 participants were included due to an unusable EEG for one participant (see the Supplemental Experimental Procedures). We performed the analysis separately for NREM sleep stages 2 and 3 (N2 and N3, respectively) to account for number of included trials, light sleep (N2) and deep sleep (N3), and different thalamic hyperpolarization levels (see the Supplemental Experimental Procedures for details). Spindle FB-tACS led to a broad increase in spindle activity around 11–16 Hz only in N2 averaged over all electrodes, with motor memory responders (n = 11) showing an increase in very fast spindle frequencies (15–16 Hz) compared to non-responders (n = 4, show decrease; Figure 5). Besides a selective increase in spindle activity, our stimulation also significantly reduced power in the delta and theta ranges in N2 (Figure 5) and N3 (Figure S4). Since sigma activity overlaps with alpha activity during wakefulness, one might argue that the stimulation leads to arousal that could explain an increase in sigma activity. However, our results clearly show that this is not the case: (1) wakefulness alpha is between 8 and 12 Hz, whereas our increase in spindle activity is between 12 and 16 Hz (Figure S5); (2) the spectrogram after the stimulation has a similar profile for sham and verum epochs, looking clearly different from a typical wakefulness (eyes-closed) period; and (3) the number of wakefulness periods and perceived sleep depth were not significantly different between conditions (Table 1; Table S2).

FB-tACS-Induced Enhancement of Spindle Activity Predicted Improvement in Motor Memory Consolidation

In order for sleep spindle activity to promote motor memory speed gains, the stimulation-induced increase in spindle activity should be related to the improvement in motor memory consolidation. Given that non-responders and responders mainly differed in spindle activity increase for very fast frequencies (15–16 Hz), we restricted our correlation analysis to this frequency window. Indeed, we found a significant negative correlation between the verum-related change in response time and spindle activity for the very fast spindle frequency range, indicating that the increase in fast sleep spindle activity predicted reduction in tapping time (increase in speed) due to verum stimulation (Figure 5C). This negative correlation was found globally but only reached trend level or significance for mainly parietal and occipital electrodes; Pearson correlation of merged parieto-occipital cluster (four electrodes), $r(13) = -0.65$, $p = 0.009$ (cluster survives supra-threshold cluster analysis; see the Supplemental Experimental Procedures). No significant correlation was found for delta-theta activity reduction in tapping time due to verum stimulation (Figure S6). The number of applied stimulations during the verum night (Table S3) was not related to the FB-tACS-related motor memory improvement, $r(14) = -0.09$,

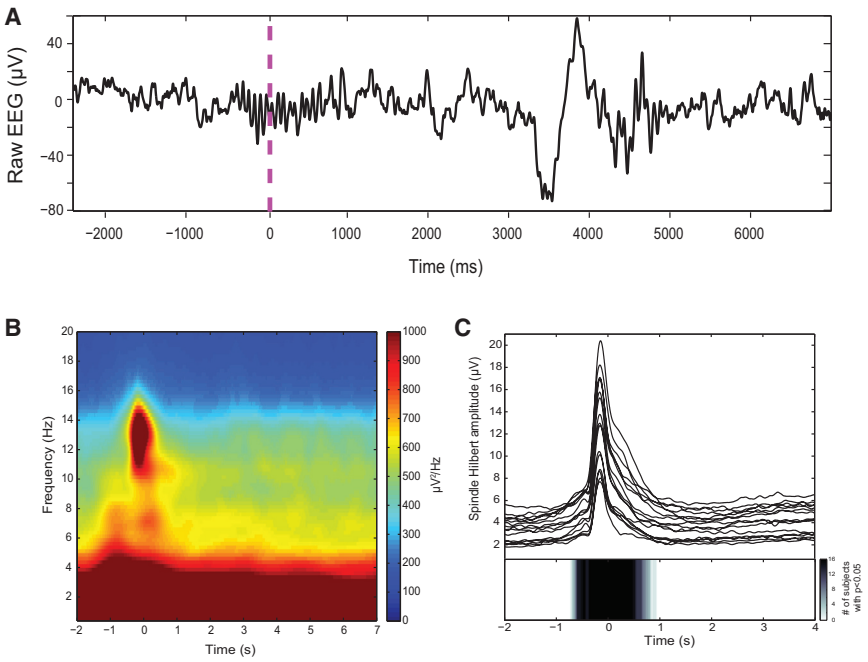

Figure 3. Spindle FB-tACS Only Applies tACS when 11–16 Hz Spindle Activity Is Prevailing in the EEG

(A) Single EEG trace of a representative participant with a detected spindle (pink dashed line) using our online spindle detection algorithm. The algorithm we used detected a spindle using two criteria. The first one was the spindle activity threshold, and the second one was the number of peaks above this threshold. When the algorithm detected five peaks above the threshold (refers to time point 0 ms, pink dashed line), stimulation started for 1.5 s in the verum condition. Online spindle detection was used to control the stimulation start, ensuring cortical stimulation exclusively during NREM spindles. Trace was obtained from a sham night (Fz-CPz, only triggering, no stimulation).

(B) Spectrogram of Fz-CPz of a representative participant during sham night shows that tACS triggers were present during sleep spindles, as indicated by increased spindle activity (10–16 Hz) around 0 (represents onset of tACS for verum condition).

(C) Spindle (11–16 Hz) Hilbert amplitude averaged spindle triggers of Fz-CPz during sham night. Each line represents a participant (n = 16). The lower panel illustrates within-subject statistics. An unpaired one-sided t test (right-tailed) was performed for the spindle Hilbert amplitude at each time point of the illustrated epoch to the overall mean of the epoch (−2.5 to 7.5 s around trigger) for all correct NREM spindle triggers. Gray-black bars illustrate the number of participants showing significant increased spindle amplitude at the respective time point compared to the mean of the whole epoch. Around 0 ms ("stimulation onset"), all participants showed significantly increased, prevailing spindle activity compared to the rest of the epoch.

See also Tables S1 and S4.

p = 0.75. Considering that we only encountered a spindle increase in N2, we further performed the same analysis including only the number of stimulations during N2. Again, no significant correlation was found, r(14) = −0.05, p = 0.86.

Spindle Characteristics and Sleep-Dependent Motor Memory Consolidation Are Similarly Correlated during the Sham Night

To further confirm the role of fast sleep spindles in motor memory consolidation, we finally examined whether a similar relationship exists between motor memory consolidation and different NREM sleep spindle characteristics (e.g., density) in the absence of stimulation (sham). Overnight change in response time was negatively correlated with spindle density and duration, again for the same frequency bins (15–16 Hz for density and 14.5–16 Hz for duration) and posterior electrodes (Figure 6). This finding convincingly confirms that the characteristics of fast spindles, specifically density and duration, are important for sleep-dependent motor memory consolidation.

DISCUSSION

We established a successful framework to investigate the functional role of specific transient brain oscillations in cognitive processes by applying targeted, individualized, and feedback-controlled weak electrical brain stimulation. We found that spindle FB-tACS can enhance sleep spindle activity in a broad frequency range during NREM N2 sleep without increasing other sleep rhythms or time spent in individual sleep stages. Furthermore, spindle FB-tACS enhanced motor sequence consolidation by means of increased speed, and fast sleep spindle activity played a functional role in this gain. Therefore, we provide the first direct demonstration of the functional role of sleep spindle activity in motor memory consolidation.

Sleep spindles have previously been hypothesized to benefit memory formation [7, 8]. For instance, sleep-dependent improvements in declarative and procedural learning paradigms correlated with sleep spindle characteristics [35, 37, 38]. Furthermore, spindles were increased during sleep following the training of these learning paradigms compared to a control condition [39–42]. In further support of a central role of sleep spindles in memory processes, patients with schizophrenia show a pronounced reduction in sleep spindles that correlates with deficits in sleep-dependent motor memory consolidation [33, 34]. However, these studies were restricted to correlations, leaving it unclear whether learning-associated changes in sleep spindle dynamics are an epiphenomenon or, indeed, play a functional role in memory consolidation. Previous attempts in manipulating sleep in humans (e.g., auditory stimulation, pharmacology, or slow-oscillatory direct current stimulation) were only successful in enhancing sleep spindles as a side effect of enhancing slow oscillations [9, 10, 12, 13] or the time spent in sleep stages, such as slow-wave sleep [11]. In addition, tES approaches so far have only enhanced declarative memory but failed to improve procedural tasks [43], even though one of the studies reported increases in sleep spindle measures along with enhanced slow oscillations/slow-wave sleep [10]. A possible explanation for this missing effect on procedural memory is that the reported significant increase in sleep spindles was only found for slow-frequency spindles, but not for

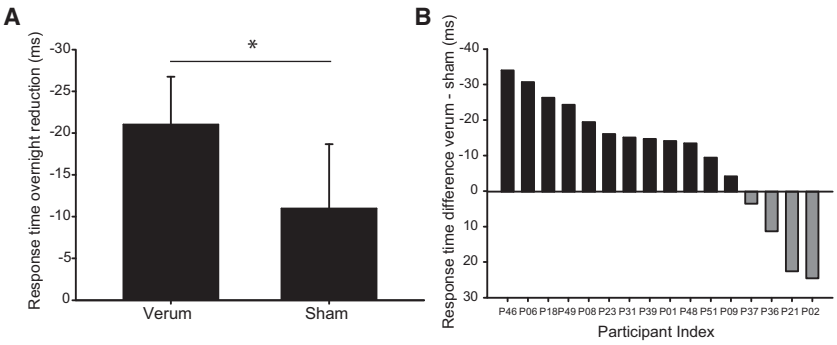

Figure 4. Spindle FB-tACS Increases Motor Sequence Tapping Speed

(A) Spindle FB-tACS caused superior speed improvement (reduction in response time) compared to a night with the sham condition as verified with a robust linear mixed-model analysis. *n = 16; F(1, 11.8) = 5.7, p = 0.035. Bars illustrate mean + SEM.

(B) Difference of overnight speed gain (verum − sham) for each individual. Black bars illustrate participants with superior overnight speed gain during verum compared to sham (responders, n = 12), and gray bars indicate participants with inferior overnight speed gain during verum compared to sham (non-responders, n = 4).

See also Figure S2 and Table S2.

fast-frequency spindles [10]. In addition, all studies using tES to modulate NREM sleep and enhance memory consolidation applied either slow-oscillatory transcranial direct current stimulation (tDCS)/tACS (0.75 Hz) or tDCS [43] and were, therefore, not optimized to selectively target sleep spindles. We are the first here to selectively enhance sleep spindle activity, along with motor memory consolidation, using FB-tACS throughout nocturnal sleep and, therefore, provide a functional role of these oscillations in cognitive processes.

Spindle FB-tACS specifically enhanced sleep-dependent speed gains, and not accuracy, in a motor sequence tapping paradigm reflected in a significant decrease of response time but not error rate. This is in accordance with previous studies that mainly found a robust effect of sleep on speed measures (e.g., [10, 11, 36, 44, 45]). However, most of these studies used number of correct sequences per trial as a measure for speed. Our results revealed that the number of correct trials is not independent of the error rate and, therefore, relates to the accuracy of the performance. In addition, some studies also indicate a beneficial effect of sleep on the error rate (accuracy) [46]. In other words, changes/variations in error rate might be reflected in the number of correct sequences and could, therefore, mask/confound sleep and intervention condition effects on speed measures. By including error rate as a covariate in our model, stimulation condition had a significant effect on number of correctly tapped sequences, showing that spindle FB-tACS selectively enhanced sleep-dependent speed benefits, but not accuracy. Collectively, our findings argue for the use of more "pure" measures of speed in motor sequence tapping tasks, e.g., by focusing on the response time of correctly tapped sequences or controlling for the error rate in future models. Of note, future studies will be needed to investigate more complex "real-life" motor tasks that benefit from sleep and to relate those findings to sleep spindles/FB-tACS. To elucidate the specific changes that sleep spindles have on motor memory, the use of simple motor tasks can be of advantage. In our case, the task design enabled us to differentiate speed from accuracy. Nevertheless, from a translational point of view, it remains to be investigated whether "real-life" memory impairments could benefit from our stimulation approach.

It has recently been shown that tACS effects on cognitive performance are dependent on basal cognitive performance [47]. Indeed, we also see a significant negative correlation, $r(14) = -0.86$, $p = 0.004$, for sham motor memory consolidation and FB-tACS-related increases, indicating that the more participants already benefit from sleep in motor sequence memory during the sham night, the less they further improve due to FB-tACS stimulation. However, as Santarnecchi et al. [47] addressed in their publication, we cannot rule out that the correlation we see is confounded by regression to the mean. Future studies are needed to support this specific finding.

Analysis of spindle activity during tACS was not possible due to the stimulation artifact. However, spindle activity was enhanced in a specific window after tACS. We hypothesize that our stimulation increases properties of the next spindle that followed the initially detected/and stimulated one (either probability of having a spindle, duration, or amplitude of the spindle). Intriguingly, the increase starts around 3.5 s after the previous spindle started. According to literature, sleep spindles seem to have a prominent reoccurrence rate of around 3–5 s [48–50]. This also points to the idea that the spindle following the stimulation might have been affected or more likely to be started. Since we have clear analysis limitations due to strong, multiple artifacts in our verum night (and, therefore, not a continuous recording that can be investigated), we further investigated correlations of motor memory benefits and spindle characteristics during the sham night. Our findings show that mainly spindle density and duration are correlated with the sleep benefit on motor sequence tapping speed, which gives a first hint that changes in sleep spindle duration and/or density might have given rise to our verum-related motor memory improvement. Further studies are needed to determine the effect of our stimulation on the stimulated spindle itself and specific spindle characteristics in a dataset that can be continuously analyzed. This might be achieved by performing animal experiments with intracranial electrodes where spiking activity can be obtained during stimulation (e.g., [23]) or by developing and applying highly sophisticated tACS artifact removal algorithms that can perfectly separate neuronal activity from artifact in the same frequency range. Long-lasting effects of tACS have been reported, but previous reports did not find any long-lasting effects of tACS during wakefulness for very short applications of tACS (e.g., 1 s [51, 52]). However, they focused on alpha activity during wakefulness, and a different mechanism might hold true for sleep. A recent report from our group [53] showed that stimulation effects that outlast stimulation depended on the specific state in wakefulness. Moreover, using a computational model, the study suggested that recurrent

Table 1. Sleep Architecture Comparison between Sham and Verum Conditions

	Mean (SEM)		Statistics		
	Sham	Verum	Factor Condition (p)	Interaction Condition × Session (p)	Factor Session (p)
Total sleep time (min)	447.0 (4.5)	447.6 (4.1)	>0.1	>0.1	>0.1
Sleep efficiency (%)	93.1 (0.9)	93.3 (0.9)	>0.1	>0.1	>0.1
Sleep latency (min)	10.6 (2.0)	12.8 (3.2)	>0.1	>0.1	>0.1
WASO (%)	5.3 (0.8)	4.6 (0.6)	>0.1	>0.1	>0.1
Stage 1 (%)	3.4 (0.6)	3.1 (0.3)	>0.1	>0.1	>0.1
Stage 2 (%)	50.2 (1.7)	49.9 (1.9)	>0.1	>0.1	>0.1
Stage 3 (%)	19.4 (1.7)	18.7 (1.7)	>0.1	>0.1	>0.1
NREM sleep (%)	69.6 (1.3)	68.6 (1.2)	>0.1	>0.1	>0.1
REM sleep (%)	20.1 (0.9)	21.6 (0.9)	>0.1	>0.1	>0.1

n = 16. WASO, wake after sleep onset.

connections in thalamo-cortical and cortico-cortical loops led to outlasting effects of stimulation. A similar mechanism is plausibly in play in the present study. During sleep, short stimulation bursts may activate the thalamo-cortical loop, which leads to a more pronounced subsequent spindle, or increase the likelihood of occurrence of the next spindle after stimulation. Along this line, we could speculate that we only see a spindle activity increase in N2, but not N3, because the stimulation was not strong enough to modulate thalamo-cortical system properties during N3, since this state is characterized by more delta/slow waves and fewer sleep spindles than N2 [5, 54]. Future studies in animal models will be needed to explore spindle changes after tACS and to establish the mechanism of this tACS induced after the effect in spindle activity using cortical and subcortical invasive recordings. The stimulation-induced overnight gains in motor sequence learning were mediated by fast sleep spindle activity, which is in line with previous literature showing a correlation of motor memory exclusively with fast spindle characteristics [39]. We replicate this correlation in the sham night with different spindle characteristics and found the same frequency bins and electrodes of spindle density and duration significantly correlating with motor memory consolidation. Several studies hypothesized that slow- and fast-frequency spindles might serve different functions [5, 39, 55]. Each spindle type shows a different topography with slower spindle frequencies (around 12 Hz) being preferentially visible over frontal areas, whereas fast sleep spindles (around 14 Hz) are more pronounced over centro-parietal regions [5, 54, 56, 57]. Considering that our correlations with motor memory consolidation were restricted to the fast spindle frequency range, our results underline the assumption that slow and fast sleep spindles might serve different functions. Therefore, our results highlight the importance of separating slow and fast frequencies for future analyses of sleep spindles. The correlation between spindle activity and memory consolidation is seen mainly over posterior regions. Of note, the negative correlations are global but only reach significance in the posterior regions. Thus, it is important to take our spatial findings with caution. Fronto-parietal regions (e.g., SMA [supplementary motor area], M1, precuneus), cerebellum, and basal ganglia are implicated in motor sequence learning [58, 59]. Interestingly, frontal regions seem to be more activated during early stages of learning, while parietal regions seem to get more involved during later stages [58, 60]. In addition, Shadmehr and Holcomb showed that, hours after performing a visuomotor task (consolidation), there is a shift in the neural representation of the internal model by means of activation in posterior parietal regions instead of frontal regions [61]. Thus, our correlation, which is strongest at parietal regions, might point to the main parietal involvement in motor sequence consolidation. However, it is still unclear which specific cortical regions might be involved in sleep-dependent memory consolidation. Based on our findings, future studies should specifically target posterior brain regions using faster frequencies (e.g., 15 Hz tACS) to optimally benefit motor memory consolidation.

In addition, the question arises whether synchronization of frontal oscillatory activity might play a role for the efficacy of our applied stimulation on behavior. It still remains to be investigated whether spindles synchronized across cortical regions are essential for memory consolidation to occur or whether only spindles localized to brain regions involved in performing the task are necessary. Future studies are needed to investigate this idea by, e.g., using only left or right hemispheric stimulation or out-of-phase stimulation.

Besides sleep spindles, slow waves have been proposed to play an important role in memory consolidation [8]. However, we found a superior sleep-dependent speed gain for verum condition, despite a spindle FB-tACS-induced decrease in delta and theta power, pointing to a limited role of slow waves in this specific process. Along this line, some studies have suggested a role of spindles in motor memory consolidation in dissociation from effects mediated by the slow waves. Using tones to reduce slow waves and REM sleep without changing sleep spindles, Genzel et al. [62] were able to preserve the consolidation of procedural and declarative memory. Enhancing slow-wave activity (SWA; 0.5–4 Hz) but decreasing spindle activity using the GABA reuptake inhibitor Tiagabine led to diminished memory consolidation in a motor sequence tapping task [63]. Finally, patients with schizophrenia who show reduced motor sequence consolidation also exhibit a pronounced decrease in sleep spindles with negligible changes in SWA [31–34, 64]. Here, we show that selective spindle enhancement had no effect on declarative memory consolidation, despite this hypothesis from previous studies [8]. A possible explanation for this missing effect could

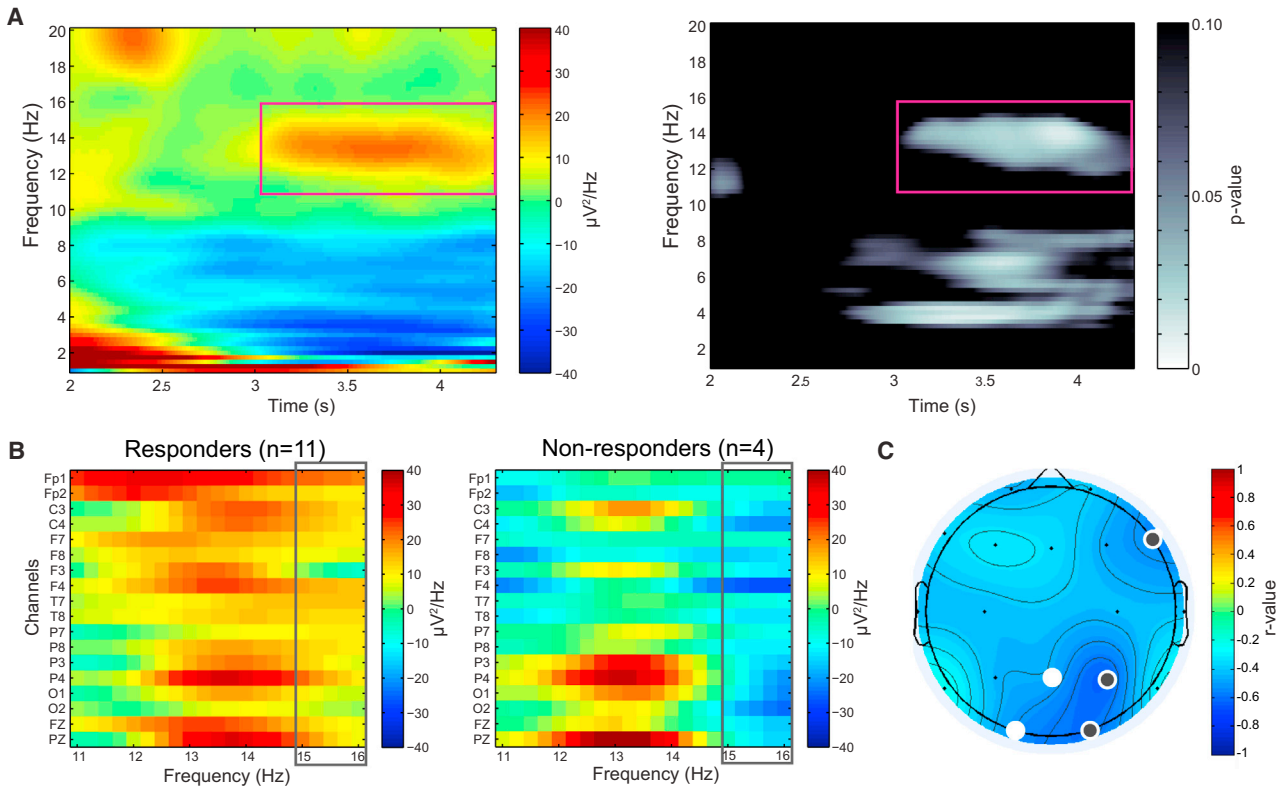

Figure 5. FB-tACS Increases Spindle Activity during NREM Stage 2 Sleep that Is Related to Stimulation-Induced Motor Sequence Tapping Speed Gains

(A) Difference of spectrograms (verum − sham) averaged for all channels for longest artifact-free interval during NREM stage 2 (N2, 2–4.3 s) and corresponding p values of a paired t test between sham and verum conditions (p values >0.1 are black; pink rectangles highlight window with increased spindle activity).

(B) Detailed analysis of increased spindle activity window during N2 (11–16 Hz; pink window in A). Spectrogram values were averaged over time for the selected time window and plotted for each frequency bin and channel. This analysis was done for responders (n = 11; superior speed gain in motor sequence task for verum condition compared to sham) and non-responders (n = 4) separately.

(C) Topographical representation of Pearson correlation coefficients between the spindle activity difference (n = 15; pooled for responders and non-responders) for 15–16 Hz (black rectangle in B) and the difference (verum − sham) in overnight speed gain (Figure 4B). Superior speed gain in verum condition compared to sham is reflected in a negative number as superior speed means reduced response time. Thus, negative correlation coefficients show that more spindle activity increase is related to a more pronounced sleep-dependent response time decrease (speed increase) in the verum condition compared to sham. Electrodes (black dots) that showed a significant correlation (Pearson) are indicated with gray dots ($p < 0.05$), and electrodes that showed a trend level are indicated with white dots ($p \geq 0.05$ and $p < 0.1$). The size of the cluster (four neighboring electrodes with gray and white dots) was significant after a supra-threshold cluster analysis was performed (see the Supplemental Experimental Procedures).

See also Figures S3–S6 and Table S3.

be the reduction in delta activity, because sleep spindles might only be beneficial for this memory type in combination with slow waves [65]. However, further studies are needed to delineate the importance of coalescence of slow waves and spindles for declarative memory, e.g., by applying spindle tACS time locked to slow-wave up states.

Our results further suggest that sleep spindles and slow waves cannot be independently modulated. Along this line, previous studies have shown that specific sleep spindle characteristics and slow waves are inversely related [5, 54, 66–70]. For instance, spindle density and spindle frequency are reduced in early NREM sleep, in the middle of NREM cycles and in N3, when SWA is maximal [54]. Further studies underlining this notion found less spindle activity in the recovery night after sleep deprivation that is marked by increased SWA [5, 68] or reported negative correlations between spindle measures (e.g., sigma activity) and SWA during NREM sleep [69, 70]. Our results further support and extend the notion that SWA and sleep spindles share a reciprocal relationship.

Collectively, spindle FB-tACS revealed the functional relationship between fast sleep spindles and motor memory consolidation. Thus, our findings serve as an important starting point to develop neuro-therapeutics for treating motor memory impairments afflicting patients with psychiatric and neurological disorders [33, 34, 64] and older individuals [71]. Future studies, however, are needed to further find optimized stimulation parameters by means of ideal stimulation location (centro-parietal instead of frontal) and (spindle) frequency applied (e.g., 15 Hz instead of 12 Hz). In addition, future research is needed to evaluate whether other frequencies temporally far from spindle activity worsen the performance. In a broader context, our results provide convincing evidence that targeted and individualized stimulation

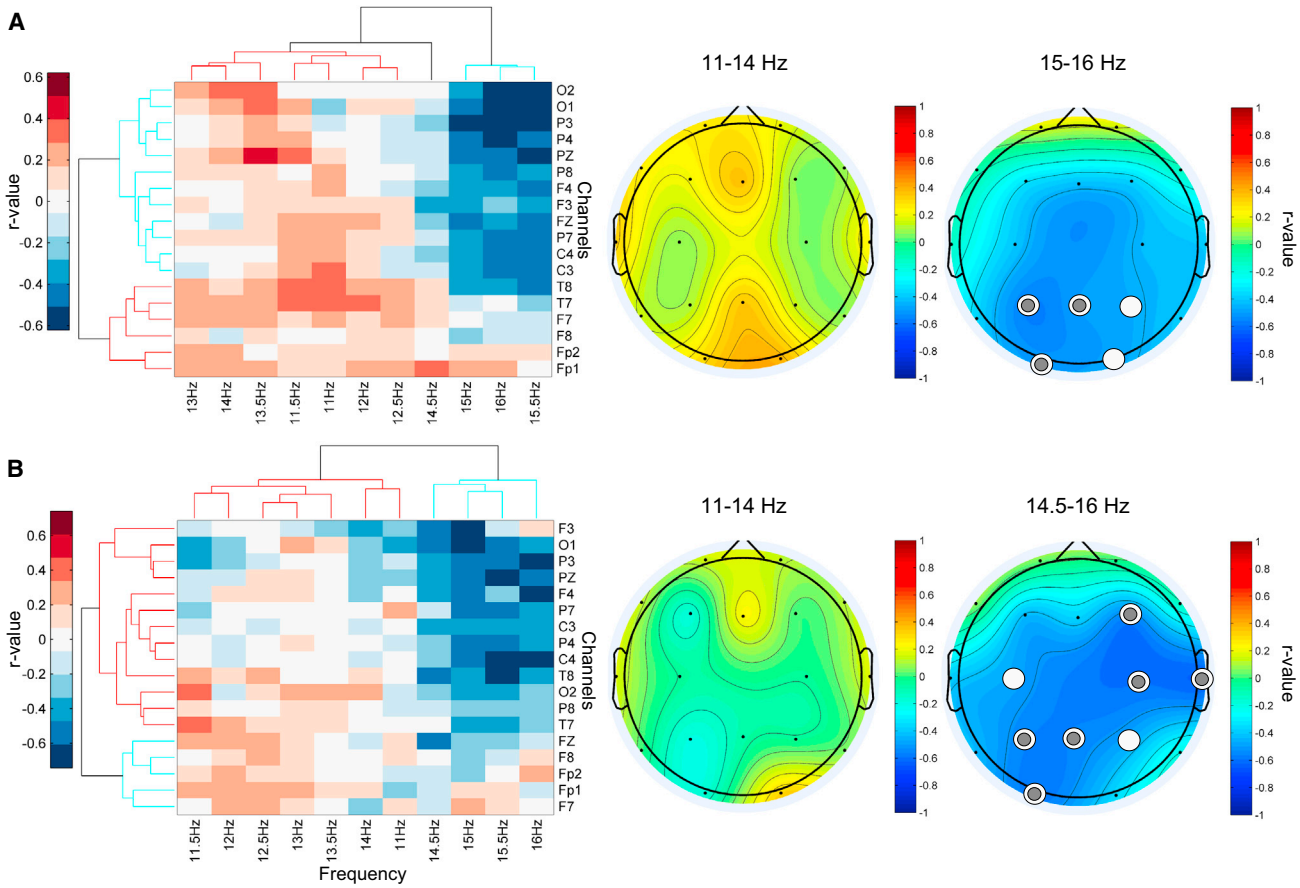

Figure 6. Relationship between Sleep-Dependent Motor Memory Consolidation and Spindle Characteristics in Absence of Stimulation

(A and B) Two-dimensional hierarchical cluster trees (dendrogram) and heat plots of the r values of the correlation between sleep-dependent reduction in response time (speed gain) during the sham night and (A) spindle density, and (B) spindle duration. Colored branches illustrate clusters with an Euclidean distance below 1.3. Negative correlation coefficients show that more pronounced appearance of the respective spindle characteristic was reflected in sleep-dependent response time decrease (speed increase). Right column illustrates corresponding correlation coefficient (r) topographical plots of clustered frequency bands (based on clustering in dendrogram). Electrodes (black dots) that showed significant correlations (Pearson correlations) are marked with gray dots ($p < 0.05$) and electrodes that showed a trend-level with white dots ($n = 16$; $p \geq 0.05$ and $p < 0.1$). The size of the cluster in (A) (six neighboring electrodes with gray and white dots) was trend level after performing a supra-threshold cluster analysis (see the Supplemental Experimental Procedures); the cluster in (B) (eight neighboring electrodes) was significant.

approaches are fundamental for selectively boosting transient brain oscillations. Furthermore, our study provides a model paradigm for establishing the functional role of transient brain oscillations in human behavior. Our FB-tACS design is a radical departure from the former stimulation approach, because it takes individual, endogenous network activity into account. Stimulation success likely depends on the underlying network activity as has been convincingly shown in in vivo, in vitro, and computational studies [16, 23–26]. This is why feedback-controlled approaches provide a promising starting point for individualized treatment paradigms that successfully target pathological network dynamics with non-invasive brain stimulation.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.06.044.

AUTHOR CONTRIBUTIONS

C.L., B.V.V., and F.F. designed the study. C.L., M.R.B., and F.F. designed online spindle detection algorithm and feedback-controlled stimulation. C.L., M.R.B., and J.M.M. conducted data collection. C.L., M.B., and S.A. performed data analysis. All authors contributed to writing the manuscript.

CONFLICTS OF INTEREST

The UNC has filed provisional patents on tACS-related technology with F.F. as the lead inventor. No licensing has occurred. F.F. is the founder and majority shareholder of Pulvinar Neuro.

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