TMS bursts can modulate local and networks oscillations during lower-limb movement in elderly subjects

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Highlights

1. TMS bursts amplifies task-related theta oscillations in the target (vertex) region.
2. TMS bursts increases task-related theta coherence between cortical network.
3. Post-task-related TMS bursts session increases resting-state alpha activity.

Abstract

High order lower-limb functions involve processing information via motor and cognitive control networks. Measuring oscillations is a key element in communication within and between cortical networks during high order motor functions. Increased midfrontal theta oscillations are related to improved lower-limb motor performances in patients with movement disorders. Non-invasive neuromodulation approaches have not been explored extensively to understand the oscillatory mechanism of lower-limb motor functions. Transcranial magnetic stimulation (TMS) bursts (4 pulses) were applied to the midfrontal lead (vertex) before a GO-Cue pedaling task, to examine the effects on local and network electroencephalogram oscillations in healthy elderly subjects. TMS bursts increased the theta activity in the local, as well as the associated network during the lower-limb pedaling task. TMS bursts increased the midfrontal cue-triggered post-imperative negative variation component. Furthermore, after task-related TMS bursts sessions, increased resting-state alpha activity was observed in the midfrontal region. TMS burst-induced changes have been associated with improvement in motor performance with cognitive control. Our study suggests the ability of midfrontal TMS bursts to directly modulate local and network oscillations in a frequency manner during lower-limb motor task. TMS bursts-induced modulation may provide insights into the functional roles of oscillatory activity during lower-limb movement in normal and disease conditions.

1. Introduction

Increasing evidence has shown the role of cortical oscillations in conveying information within motor and cognitive control networks (Buzsaki and Draguhn, 2004; Helfrich and Knight, 2016; Singh, 2018; Singh, et al., 2018). Within the motor and cognitive research field, midfrontal theta, beta, and gamma oscillations have been associated with motor tasks with cognitive recruitment such as gait or lower-limb movements (Seeber, et al., 2014; Peterson and Ferris, 2018; Singh, et al., 2020). Motor and cognitive control networks are impaired in movement disorders, such as in Parkinson's disease (PD) patients, and have been linked to attenuated midfrontal theta, and increased beta oscillations during lower-limb pedaling motor tasks (Singh, et al., 2020). Cognitive contributions to lower-limb movement are well-acknowledged in movement sciences. Additionally, the role of midfrontal theta oscillations in cognitive control has been studied extensively in neuropsychiatric and movement disorders patients (Schacter, 1977; Cavanagh and Frank, 2014; Singh, et al., 2018). Animal studies have shown increased theta oscillations around the anterior cingulate cortex and frontal cortex, similar to frontal midline theta oscillations in humans, during attentional motor tasks (Tsujimoto, et al., 2006; Han, et al., 2019). Therefore, midfrontal theta oscillations may be associated with executive attention. These studies have spurred a growing interest in the manipulation of midfrontal theta oscillations to improve attentional motor tasks. A better understanding of their causal physiological role can be achieved in the coding of movements involving attentional processes. Indeed, this endeavor has the potential to alleviate lower-limb motor problems in patients with movement disorders.

Non-invasive cortical stimulation techniques such as transcranial magnetic and electrical stimulation (TMS and tES) have emerged as promising neuromodulation methods, enabling direct modulation of oscillations in the local and related networks (Fregni, et al., 2005; Polania, et al.,

2018). TMS, specifically repetitive TMS (rTMS), appears to be a particularly effective neuromodulation tool to target spatially confined cortical regions and in modulating oscillations in the local region and associated neural networks (Hallett, 2007; Ziemann, 2011). In comparison to a single pulse, TMS bursts can induce long-term plastic changes in the motor and cognitive networks and modulate neuronal oscillations effectively, leading to an increased interest in therapeutic possibilities (Larson and Lynch, 1989; Morgan, et al., 2013). Recent findings have shown that TMS bursts in the motor cortical region can improve gait and balance problems in PD patients (Benninger, et al., 2011; Y. J. Xie, et al., 2020), however, the mechanisms by which TMS bursts exert neuromodulatory effects on lower-limb motor performance remain unknown.

The combination of scalp electroencephalogram (EEG) and TMS is an ideal method to understand oscillatory mechanisms and entrainment underlying motor performance, due to their good spatial and temporal resolution (Ilmoniemi and Kicic, 2010; Fecchio, et al., 2017). Nevertheless, it remains unclear whether TMS bursts (theta bursts) results in entrainment for theta frequency band in the local stimulated brain region. Furthermore, it is not known how locally modulated oscillations impact resultant activity in the associated cortical regions during lower-limb movement. To address these questions, we analyzed EEG recordings in healthy elderly subjects, while applying TMS bursts to the midfrontal region (at vertex). Using a TMS burst consisting of 4 pulses, we examined theta power and coherence in frontal cortical regions during a lower-limb pedaling motor task. TMS was delivered before a visual GO-Cue, followed by the participant completing a full rotation in pedaling task. Our results show that a TMS bursts increased theta power and coherence between frontal cortical regions in the theta-band during pedaling task. Therefore, results suggest that TMS bursts can induce entrainment in direct interactions with the underlying brain oscillations. Consequently, one of the underlying

mechanisms could be either the induction, enhancement, or generation of natural oscillation of the targeted generator (Thut, et al., 2011).

2. Methods

2.1. Participants

Eleven healthy elderly subjects (mean ± sem; age 70 ± 3 years; 6F/5M) participated under protocols approved by the University of Iowa Institutional Review Board and is in accordance with the Declaration of Helsinki. Subjects were recruited from the Iowa City community and the University of Iowa Seniors Together in Aging Research Registry. All subjects were screened for exclusionary criteria, using a standard questionnaire consisting of neurological and mental health history, psychoactive medication use, presence of a cardiac pacemaker or any other metal implant. All participants provided written informed consent to participate in the study. Only certified personnel approved by the Iowa Brain Stimulation Program administered TMS.

2.2. Lower-limb pedaling motor task and analysis

Similar to our previous study (Singh, et al., 2020), a pedaling motor task was used to study lower-limb motor control. This type of motor task induces minimal movement-related artifact and requires bilateral lower-limb coordination similar to gait. Further, this paradigm can be used to investigate feedback-based neuromodulation. Pedaling task involves 2 blocks, each consisting of 25 trials during which the subject remains seated. Fifty total trials provide adequate signal-to-noise ratios for subsequent EEG analysis, and also prevents fatigue in elderly participants. For each trial, a warning cue briefly appeared on a stimulus presentation computer screen. Afterwards, a TMS burst was delivered to the midfrontal region (above Cz, vertex) before the GO-Cue, triggered by

TTL input. The 2 s GO-Cue appeared 3 ± 0.6 s after the 4th TMS pulse discharge (Fig. 1A), with an inter-trial interval of 3 ± 0.1 s. The total duration of each trial averaged 10.4 ± 0.7 s across subjects, who were instructed to complete one full rotation following the appearance of the GO-Cue. A 3-axis accelerometer (Brain Products GmbH, Gilching, Germany) was attached to the subject's left ankle to collect acceleration signals in all three planes of movement (X-, Y-, and Z-axes), and averaged to generate a single signal. Linear speed for each trial was analyzed during the 0-2 s time window following the GO-Cue signal, as participants took approximately 2 s to complete one pedal rotation (Fig. 1B). Signals were detrended, and a 5 Hz low-pass filter was applied prior to computing the linear speed. Mean linear speed for each subject (g*s) was calculated using the average linear speed from all trials.

2.3. Active and sham TMS to the midfrontal region

Subjects were comfortably seated with their chin positioned in a chin rest. Both active and sham stimulation conditions were randomized ensuring a minimum 60 min between sessions. A TMS burst consisted of 4 consecutive arrhythmic biphasic pulses within 1 s. The occurrences of TMS pulses were different on each trail and for every participant. Only a single TMS burst per trial was applied. As previous studies have shown that rhythmic TMS can effectively cause local entrainment of the target oscillations (Thut and Miniussi, 2009; Thut, et al., 2011), an arrhythmic TMS pulse burst pattern was employed in the present study. Under normal physiology, bursty neurons fire in an arrhythmic fashion resulting in oscillations at a certain frequency (Anidi, et al., 2018; Torrecillos, et al., 2018). We did not investigate the effects of rhythmic TMS bursts in the current study.

We used a figure-of-eight TMS coil (MC-B70) connected to a MagVenture (MagVenture, Inc., Alpharetta GA) Stimulator (MagPro X100) in both active and sham stimulation conditions over the midfrontal ("Cz" vertex) region according the 10/20 electrode position. We selected midfrontal location for the TMS to modulate theta oscillations in the target (midfrontal; vertex) and connected regions (Singh, et al., 2018; Singh, et al., 2020). Even, previous studies have demonstrtated that stimulation over the vertex can increase leg motor cortex excitability bilaterally and induce most stable responses in the leg motor area (Rothwell, et al., 1991; Terao, et al., 1994; Kesar, et al., 2018; Ghosh, et al., 2019). We followed all the suggested TMS methodological checklist to improve the quality of data collection and reporting results (Chipchase, et al., 2012). Stimulator timing was controlled via TTL inputs using Arduino hardware and software, and custom Matlab software. For sham stimulation, the figure-of-eight coil was inverted relative to the target location, preventing the magnetic field from reaching the skull and stimulating the brain (Brasil-Neto, et al., 1992; Hoeft, et al., 2008). Earplugs were used to reduce acoustic stimulations related to the coil discharge noise, and to protect participant hearing. Although, bone vibrations due to the coil discharge could potentially contribute to auditory evoked potentials (Vernet and Thut, 2014), this concern was controlled by using the same coil for both sham and active conditions, thus mimicking the auditory and somatosensory effects of active TMS.

Stimulation intensity was set at 90% of the individuals resting motor threshold (RMT). RMT for the right abductor pollicis brevis (APB) muscle was determined on each subject, and is the minimum intensity at which TMS pulses applied to the left primary motor cortex yielded an activation of the APB in at least 50% of the attempts (total 10 attempts). To target the left primary motor cortex, the coil was placed over the C3 lead (above the EEG cap) perpendicular to the central sulcus.

2.4. EEG-TMS recording and preprocessing

Scalp EEG-TMS signals were collected during the lower-limb pedaling task using a 64-channel EEG actiCAP (Brain Products GmbH, Gilching, Germany) with a high-pass filter of 0.1 Hz with a sampling frequency rate of 10 kHz. EEG signals were also collected for 2-3 min during the eyes-open resting-state condition after completing pedaling task.

Reference and grounded channels were electrodes Pz and Fpz, respectively. Channels that were considered unreliable due to muscle artifacts or white noise (Fp1, Fp2, FT10, TP9, and TP10) were removed, and one channel on mid-cerebellar region was placed (Iz), leaving a total of 59 channels for pre-processing analysis. We used a customized EEG cap with I1 (left cerebellar) and I2 (right cerebellar) leads and without FT9, PO3, and PO4, allowing for the placement of cerebellar leads to collect oscillations, similarly to a recently published article (Pan, et al., 2020). However, as a limitation of this analysis, we did not confirm the source of the cerebellar oscillations. EEG signals were epoched around the GO-Cue (-0.6 s to 2.4 s) to exclude TMS-evoked artifacts. Epochs with excessively noisy EEG or muscle artifacts were excluded using a conjunction of the Fully Automated Statistical Thresholding for EEG artifact Rejection (FASTER) algorithm (Nolan, et al., 2010) and pop_rejchan from EEGLAB (Delorme and Makeig, 2004). Eye movement artifacts were removed following ICA. Subsequently, data was rereferenced to common average reference.

2.5. EEG postprocessing analysis

2.5.1. Task-related time-frequency analysis

Preprocessed (epoched) signals were used for time-frequency analysis by complex Morlet wavelets, as explained previously (Singh, et al., 2018; Singh, et al., 2019; Singh, et al., 2020).

Further, epochs were cut in –0.5 to +2.0 s window, frequency bands between 1 and 50 Hz in logarithmically-spaced bins were chosen, and power was normalized by conversion to a decibel (dB) scale. Similar to our prior studies, the baseline for each frequency consisted of the average power from –0.3 to –0.2 s prior to the onset of the GO-Cue (Singh, et al., 2019; Singh, et al., 2020). We limited our time-frequency analyses to electrode Cz and a-priori time-frequency Regions of Interest (tf-ROI). ROIs were preselected for the theta-band (3–7 Hz) and our time window of interest was 0.5–1.5 s following the GO-Cue. We used tf-ROIs mean power values for statistical comparisons, with the main analysis focused on a-priori tf-ROI since it represents the specificity. Power values were also analyzed in the alpha-band (7-13), beta-band (13–31 Hz), and gamma-band (31-50 Hz) and tf-ROIs topographic map was plotted and compared between active versus sham conditions.

2.5.2. Task-related spectral coherence analysis

Preprocessed signals were also used for time-frequency spectral coherence (magnitude-squared coherence) analysis to elucidate the temporal functional coupling or connectivity between two regions. We used –0.3 to –0.2 s window as a baseline to compute change in coherence. We then implemented spectral coherence algorithms explained in detail by Cohen (Cohen, 2014). Spectral coherence over trials between Fz and Cz (fronto-cortical network), C3 and C4 (inter-hemispheric motor cortical network), and Cz and Iz (cortico-cerebellar network) were computed. We used same priori tf-ROIs (as above) to export coherence values to compare active versus sham stimulation data, focusing on the comparisons within theta frequency band.

2.5.3. Post-imperative negative variation (PINV) analysis

The late event-related potential (ERP) post-imperative negative variation (PINV), is a marker of sustained attentional activity, that may represent the motor preparation (Verleger, et al., 1999; Bender, et al., 2005) and can be seen as negativity that continues after an imperative GO-Cue. To analyze GO-Cue-triggered PINV, signals were filtered from 0.1 to 20 Hz. Quantification of PINV was focused on mean amplitude in a 0-1 s time window (Hoormann, et al., 1998), and analysis was focused on FCz lead because Cz lead (target location) might be contaminated with TMS-evoked artifacts. Although TMS-induced contaminations are difficult to remove in ERPs analysis, (Ilmoniemi and Kicic, 2010), the wavelet-based time-frequency analysis approach utilized in the present study can remove or diminish these artifacts (Blanco, et al., 1995). Ultimately mean amplitudes of the GO-Cue-triggered PINV component after the active and sham TMS bursts were compared.

2.5.4. Resting-state analysis

EEG resting-state signals were preprocessed (similar to above) to improve signal to noise ratio. Further, a Welch's power spectral density estimate (pwelch) matlab function was applied on epoched (3.5 s) signals to compute spectral properties. Relative power at delta and alpha frequency bands was quantified compared to the power over 0.1–50 Hz. The resting-state mean relative power, after the task-related active and sham TMS bursts tests, was compared. Resting-state analysis was focused on the Cz electrode.

2.5.5. Statistical analysis

Paired t-tests were used to statistically compare behavior and electrophysiological data between active TMS and sham. Statistical analysis was focused on our a-priori hypothesis to

determine the specificity. In addition to this, we employed threshold-based correction on the full time-frequency plots in order to reveal any other reliable differences between active and sham TMS, and these differences are shown as black contour in time-frequency plots. The p value <0.05 was considered statistically significant.

3. Results

3.1. Change in pedaling speed after TMS burst

Previous studies have demonstrated that non-invasive neuromodulation may enhance motor and cognitive behaviors in healthy elderly subjects (Hummel, et al., 2010; Zimerman and Hummel, 2010). Although it appeared that a time-dependent increase in pedaling speed was observed following active TMS burst when compared to sham (see Fig.1 C), this comparison was not significant ($t_{(10)}=0.76$, $p=0.46$, Fig. 1D). Kinematic data show that TMS bursts may potentially improve motor task in patients with movement disorders (Siebner, et al., 1999; Y. J. Xie, et al., 2020).

3.2. Pedaling task-related oscillatory power changes at frontal region after TMS burst

Active TMS burst significantly increased the theta-band power when compared to sham ($t_{(10)}=-3.1$, $p=0.01$, Fig. 2A-D, see Fig. S1 for each subject) during pedaling task in healthy elderly subjects. Time-frequency plots show no changes in the other frequency bands power (Fig. 2C). Topographic figure confirms that change in the task-related theta power was more confined in the midfrontal region after active TMS burst (Fig. 2E). Our results distinctly demonstrate that TMS burst (4 pulses) can enhance the power in the burst frequency which is the theta range in our study.

3.3. Pedaling task-related changes in the cortical networks after TMS burst

Spectral coherence analysis between frontal leads (Fz-Cz) showed increased pedaling-task related coherence within the theta frequency range after active TMS (Fig. 3A-C, $p<0.05$, see the black contour in figure 3C). A significant increase in coherence in the tf-ROI theta-band was observed ($t_{(10)}=-2.3$, $p=0.04$, Fig. 3D) after active TMS burst as compared to sham. Furthermore, we analyzed spectral coherence between left and right motor cortical leads (C3-C4) to observe coherent activity in the inter-hemispheric motor cortical network; and between frontal and cerebellar leads (Cz-Iz) to observe coherent activity in the cortico-cerebellar network. Both inter-hemispheric motor cortical and cortico-cerebellar networks showed increased pedaling-task related coherence within the theta frequency range after active TMS burst (C3-C4: Fig. S2A-C; Cz-Iz: Fig. S3A-C; $p<0.05$, see the black contour). Nevertheless, tf-ROI showed a trend towards an increase in coherence in the theta-band in the inter-hemispheric motor cortical network ($t_{(10)}=-2.16$, $p=0.057$, Fig. S2D) and cortico-cerebellar network ($t_{(10)}=-2.0$, $p=0.07$, Fig. S3D) after active TMS burst as compared to sham. In line with previous reports, our results also suggest that TMS bursts may modulate the cortical network oscillations during lower-limb motor task (Ziemann, 2011; Shafi, et al., 2014; Noh, et al., 2015).

3.4. GO-Cue-Triggered PINV component after TMS burst

To explore how midfrontal activity was changed with TMS burst, component analysis of EEG activity at the midfrontal FCz lead was performed. TMS burst induced higher GO-Cue-triggered PINV as compared to sham ($t_{(10)}=-2.8$, $p=0.019$, Fig. 4A-C). However, analysis of the Cz lead did not show any significant changes in PINV component ($p=0.17$; Fig. S4). Previous studies have shown that smaller PINV is associated with reduced cognitive control and delaying

in preparatory activity in the sensory domain (Verleger, et al., 1999; Gomez, et al., 2007; Wynn, et al., 2010). These results suggest that TMS bursts may lead to improving preparatory activity by enhancing the PINV component in patients with motor and cognitive deficits.

3.5. Changes in resting-state oscillatory activity after task-related TMS bursts sessions

We analyzed resting state signals to investigate the overall changes in the spectral properties after the active and sham TMS bursts sessions (Fig. 5A). No significant difference in relative power was found in the theta-band in the midfrontal lead after active TMS bursts sessions compared to sham ($t_{(10)}$=2.19, p=0.053, Fig. 5B and C). However, we found decrease in relative power in the alpha-band ($t_{(10)}$=3.02, p=0.01, Fig. 5D and E) in the midfrontal lead. In line with current results, previous studies have also reported that following transcranial stimulation protocols, changes in resting-state alpha oscillations have been shown to modulate cognitive functions (Klimesch, 1999; Klimesch, et al., 2003; Thut and Miniussi, 2009).

4. Discussion

To investigate the immediate effects of TMS bursts on lower-limb motor task, it is crucial to identify the actions of TMS on local and network oscillations. Our results suggest that TMS can modulate brain oscillations in direct interaction with the underlying generator, via synchronized midfrontal lower-limb task-related theta activity when targeting the underlying theta (4-7 Hz) oscillations generator. We also demonstrate that TMS bursts can enhance theta oscillations in the associated network. Results suggest that TMS bursts may enhance the underlying naturally occurring theta oscillations rather than the imposition of artificial oscillations. The overall data support the hypothesis that TMS bursts may drive natural brain oscillations by entrainment, and this might be a possible mechanism via which TMS may act on the local and associated oscillatory

activities to influence motor behavior (Thut and Miniussi, 2009; Zimerman and Hummel, 2010; Noh, et al., 2015). Possible limitations to the current study include the low subject number involved as well as the use of a healthy population in contrast to a clinical population. Using a normal model alone may mean that threshold and burst patterns may not encompass the frequencies required for changes in a diseased population. Data interpretation will require confirmation with a larger dataset that includes a clinical population in the model.

Movement disorder patients with lower-extremity dysfunctions have shown impaired cognitive control and decreased frontal theta oscillations, and thus show impaired kinematics of lower-limb movement (Singh, et al., 2020). Attention is an important factor when performing lower-limb movements, especially, when walking under dual-task conditions (Bayot, et al., 2018; Li, et al., 2018). Midfrontal theta oscillations could play an underlying role in providing strong cognitive engagement during dual-tasks, contributing to the sensorimotor integration needed to perform lower-limb motor tasks. The current task-related EEG data reveal that TMS bursts can upregulate targeted theta oscillations in higher-order frontal regions of the attention network and may enhance lower-limb movements. Frontal beta oscillations also play a critical role in a top-down pathway to convey the information for preparatory and execution plans during lower-limb motor tasks, and these processes may malfunction in movement disorders (Wagner, et al., 2016; Singh, 2018). Increased beta-band power in the cortical and basal ganglia network are associated with motor abnormalities in patients with PD (Singh, 2018; Torrecillos, et al., 2018). Previous studies have shown the reduction in beta power in the cortical and sub-cortical regions during lower-limb motor task in patients with movement disorders (Singh, et al., 2011; Singh and Botzel, 2013; Singh, et al., 2013; Anidi, et al., 2018; Singh, et al., 2020). Critically, invasive deep brain stimulation (DBS) attenuates the beta-band activity and improves upper-limb movement in PD

patients (Whitmer, et al., 2012; de Hemptinne, et al., 2015). However, fewer DBS studies have also shown a therapeutic option to improve lower-limb movements or gait in patients with PD (T. Xie, et al., 2015; Anidi, et al., 2018). Similar to invasive studies, our non-invasive TMS bursts approach may decrease cortical beta-band power and improve gait or lower-limb performances in PD. The overall results may support our previous hypothesis that increased frontal theta activity and reduced beta activity are associated with improvement in lower-limb movement in PD patients with gait abnormalities (Singh, et al., 2020).

High order lower-limb movements are a network phenomenon; and aberrations in the cortico-cortical, motor cortical, and cortico-cerebellar networks have been observed in PD patients with gait abnormalities (Boonstra, et al., 2008; Gilat, et al., 2017; Singh, 2018). The DBS method in movement disorder patients has shown a correlation between improvement in gait pattern and normalization of the cortical and associated oscillatory networks (Ferraye, et al., 2008; T. Xie, et al., 2015). Similarly, non-invasive TMS protocols can modulate oscillations in the target and associated networks during motor and cognitive tasks (Thut, et al., 2011; Y. J. Xie, et al., 2020). In line with these studies, current results demonstrate the proficiency of TMS bursts protocol to modulate theta oscillations in the cortico-cortical and motor cortical networks, which may be correlated with changes in the motor behavior with cognitive load. Interestingly, frontal TMS bursts can also influence cortico-cerebellar oscillatory network, which might also be associated with improving gait pattern and balance (Morton and Bastian, 2004; Lee, et al., 2007). However, as in the present study, future studies examining the cerebellar contribution to this network may encounter difficulty, as electrodes placed over cerebellar regions are often contaminated with signals from the occipital lobes.

In addition to looking at event-related spectral changes with time, we also observed event-related positive or negative voltage deflections as PINV to assess neural activity related to both cognitive and motor sensory processes. An increased PINV feature is associated with improved cognitive control in neuropsychiatric and neurodegenerative patients (Verleger, et al., 1999; Gomez, et al., 2007; Wynn, et al., 2010). In the current study, a "GO-Cue" was an imperative cue for planning and execution of pedaling task. Our results show that TMS bursts can increase the amplitude of PINV component at the "GO-Cue", this kind of facilitation in cognition or attention processes may help further to accelerate information processing for motor planning and then execution.

Additionally, results show that TMS bursts did not modulate resting-state theta oscillations, suggesting the change in theta activity is most-likely associated with GO-Cue-triggered motor performance (Singh, et al., 2018; Singh, et al., 2020). However, an increase in resting-state alpha-band activity after the TMS bursts demonstrates the overall post-stimulation effect in the target region, which may also be related to improvement in motor and cognitive control systems (Klimesch, 1999; Thut and Miniussi, 2009). Notably, changes in alpha oscillations have been observed after transcranial neuromodulation in previous human studies (Klimesch, et al., 2003; Samaha, et al., 2017), and these oscillations have been implicated in top-down cortical processing (Compton, et al., 2011; Manza, et al., 2014).

Further evidence has shown that single pulse and rTMS protocols can improve motor symptoms in movement disorder patients (Siebner, et al., 1999; Chou, et al., 2015; Moisello, et al., 2015). These reports revealed advantages of different TMS protocols for measures of upper-limb motor symptoms but not lower-limb motor symptoms, suggesting a need for an alternative TMS approach. In comparison to previous reports, in which TMS effects were significant when high-

frequency TMS protocols were targeted in the motor cortical region (Siebner, et al., 1999; Moisello, et al., 2015), in the present study, we targeted midfrontal region with TMS bursts. Our TMS approach may increase the overall speed of the lower-limb pedaling task, and we speculate that this change in performance after TMS bursts could be attributed to the state of brain activity (e.g., degree of brain activation or interregional connectivity).

The current study may provide an efficient, non-invasive method to normalize brain oscillations that are often abnormal in conditions with motor disabilities. Specifically, by combining the TMS bursts protocol utilized with specific motor paradigms, this approach has the potential to investigate the causal links between midfrontal brain oscillations and lower-limb movement in humans (Singh, et al., 2020). Further, this method may provide an understanding of the neurophysiological basis of modulations in the local and associated oscillatory networks, potentially contributing to novel rehabilitation regimens in patient populations such as PD.

Author contributions

AIE: data collection and critical revision. JLS: manuscript preparation and critical revision. AS: designed the study, data collection, data analysis, manuscript preparation, and critical revision.

Declaration of Competing Interest

None of the authors have any potential conflicts of interest to disclose.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at

Research Data

All datasets that support the findings, and matlab codes to generate figures are available in the Data to Share repository.

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Figure Legends

Fig. 1. No evidence whether TMS burst may affect the speed of the lower-limb pedaling task. A) Schematic diagram of the trial of EEG-TMS pedaling task. B) Mean acceleration signal after active and sham TMS burst. C) Time-dependent changes in pedaling speed after active and sham TMS burst. D) Mean pedaling speed after active and sham TMS burst. B-C: thin blue and green lines represent the standard error of mean for sham and active stimulation, respectively. D: n.s. = non-significant; dashed line with error bar represents the mean and standard error of mean.

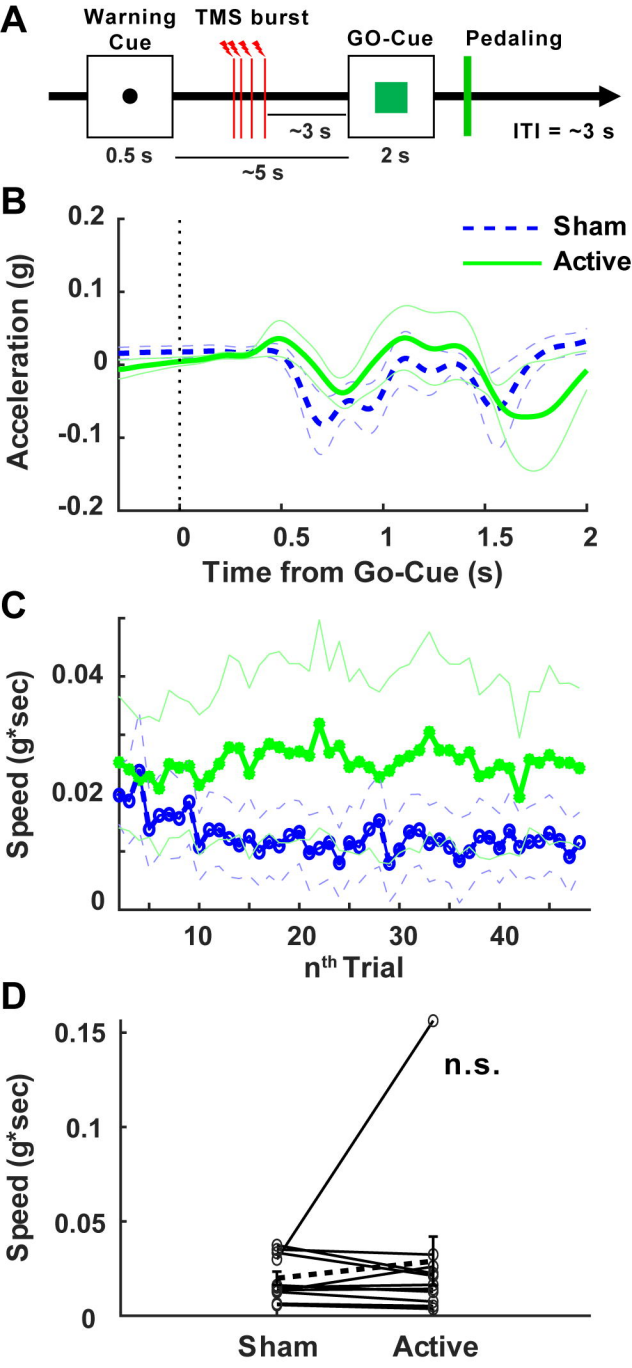
Fig. 2. TMS burst increased pedaling task-related theta-band activity in the midfrontal region. A-C) Time-frequency analysis showed increased theta power at midfrontal scalp electrode (vertex or ‘‘Cz”) during pedaling task after active TMS burst as compared to sham in healthy elderly subjects. D) Plot displayed the mean power values in tf-ROIs (theta-band power value under black box) during pedaling task. E) Topography plot (active TMS burst versus sham) indicated increased theta activity in the midfrontal region. C: $p<0.05$ outlined in bold lines. D: *$p<0.05$ vs sham stimulation; dashed line with error bar represents the mean and standard error of mean. E: diamonds show significant electrodes.

Fig. 3. Spectral coherence in the fronto-cortical network was increased within theta-band. TMS burst can increase spectral coherence in the fronto-cortical network (Fz-Cz) in the theta frequency band during pedaling task in healthy elderly subjects. A) Sham and B) active TMS burst induced changes in coherence from baseline during pedaling task. C) Difference in coherence between active and sham stimulation. D) Change in coherence from the baseline in the tf-ROIs: theta frequency band during active and sham TMS burst. C: $p<0.05$ outlined in bold lines and black

box represents the tf-ROIs in the theta frequency range. D: *p <0.05; dashed line with error bar represents the mean and standard error of mean.

Fig. 4. TMS burst can modulate midfrontal GO-Cue-triggered PINV component. A) GO-Cue-triggered FCz PINV component after active and sham TMS burst in healthy elderly subjects. B) Graph represents the mean amplitude of PINV component from 0-1 s window (shaded region in A). C) Topography plot (active TMS burst versus sham TMS burst) indicates increased PINV component in the frontal region. B:*p<0.05 vs sham stimulation; dashed line with error bar represents the mean and standard error of mean. C: diamonds show significant electrodes.

Fig. 5. TMS bursts sessions increased resting state midfrontal alpha-band activity. A) Resting-state spectra after task-related active and sham TMS bursts sessions in healthy elderly subjects. B-B-E) Graphs and topographic plots show no difference in theta activity but significant decreases in alpha activity after active TMS bursts sessions. B and D: *p<0.05 vs. sham; dashed line with error bar represents the mean and standard error of mean; n.s. = non-significant. C and E: topographic electrodes with significantly different relative power are indicated with a diamond.


A
Warning Cue
TMS burst
GO-Cue
Pedaling
~3 s
ITI = ~3 s
0.5 s
~5 s
2 s
B
Sham
Active
Acceleration (g)
0.2
0.1
0
-0.1
-0.2
0
0.5
1
1.5
2
Time from Go-Cue (s)
C
Speed (g*sec)
0.04
0.02
0
10
20
30
40
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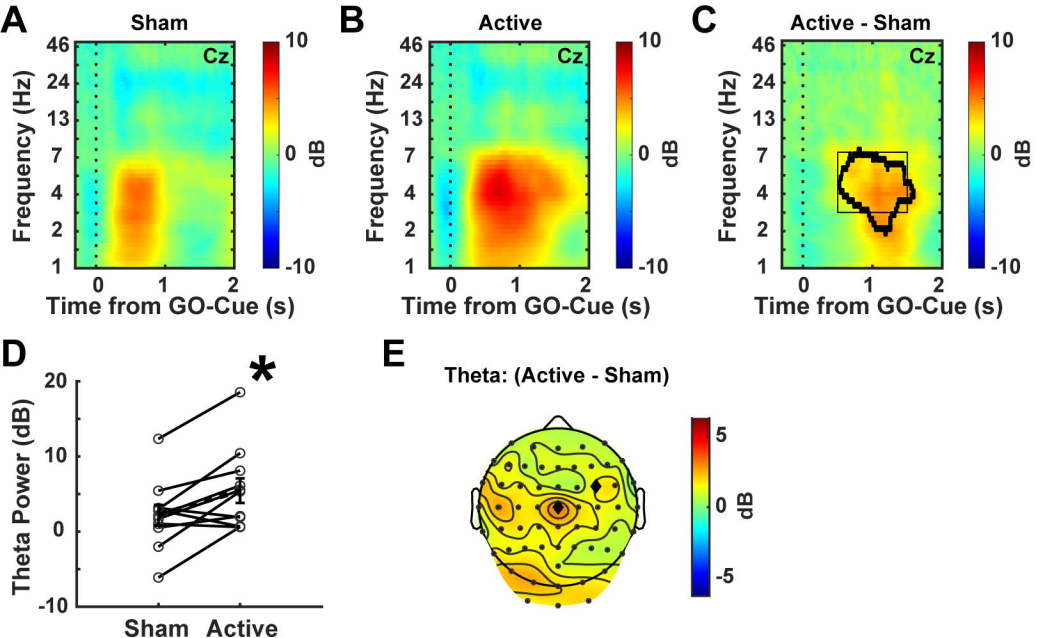
D
Speed (g*sec)
0.15
0.1
0.05
0
n.s.
Sham
Active


A
Sham
Cz
Frequency (Hz)
46
24
13
7
4
2
1
0
1
2
Time from GO-Cue (s)
10
0
-10
dB
B
Active
Cz
Frequency (Hz)
Time from GO-Cue (s)
dB
C
Active - Sham
Cz
Frequency (Hz)
Time from GO-Cue (s)
dB
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Theta Power (dB)
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-10
Sham
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Theta: (Active - Sham)
5
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-5
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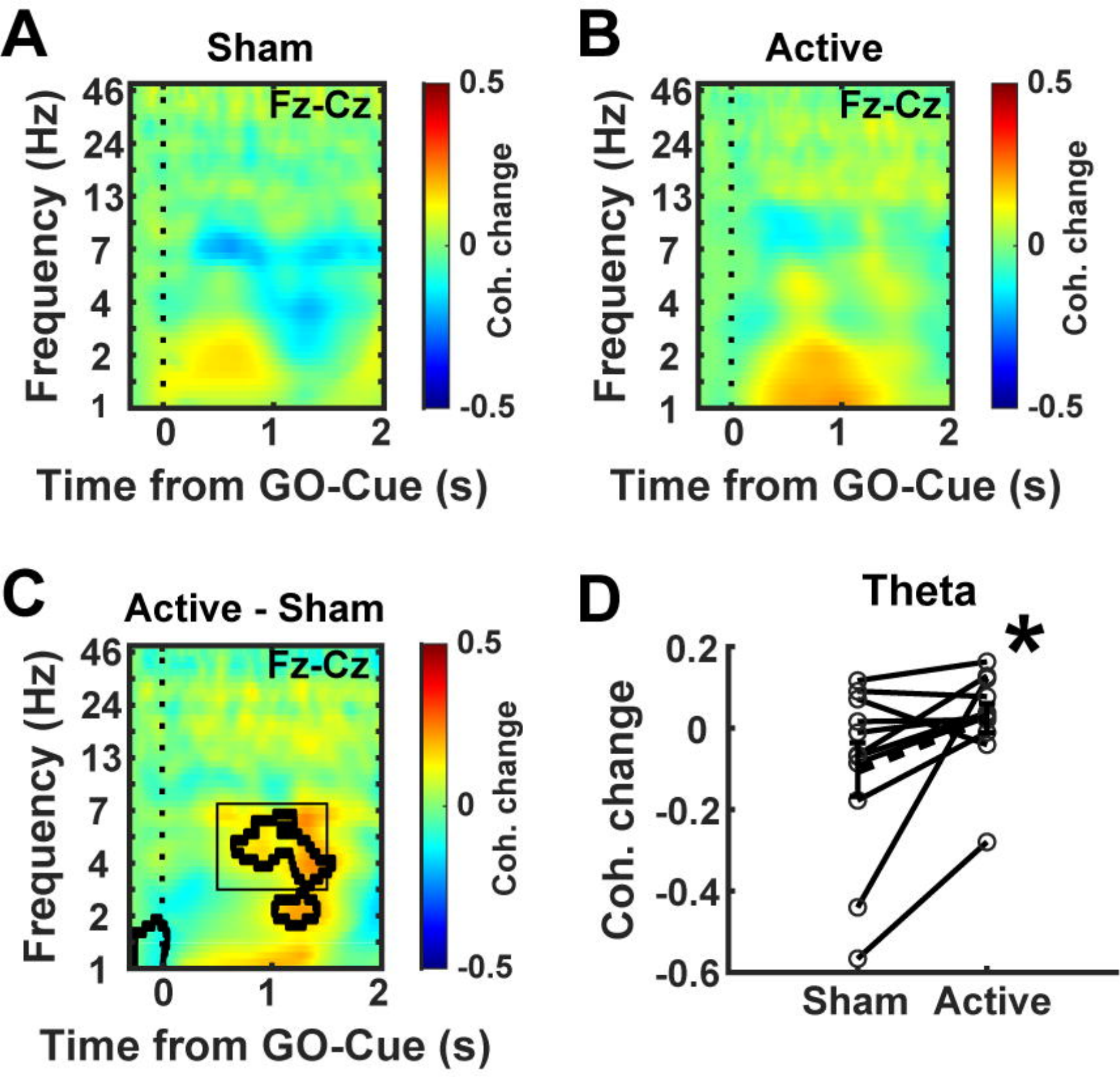
A
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Frequency (Hz)
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Time from GO-Cue (s)
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B
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Fz-Cz
Frequency (Hz)
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Time from GO-Cue (s)
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Coh. change
C
Active - Sham
Fz-Cz
Frequency (Hz)
46
24
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2
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2
Time from GO-Cue (s)
0.5
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-0.6
Sham
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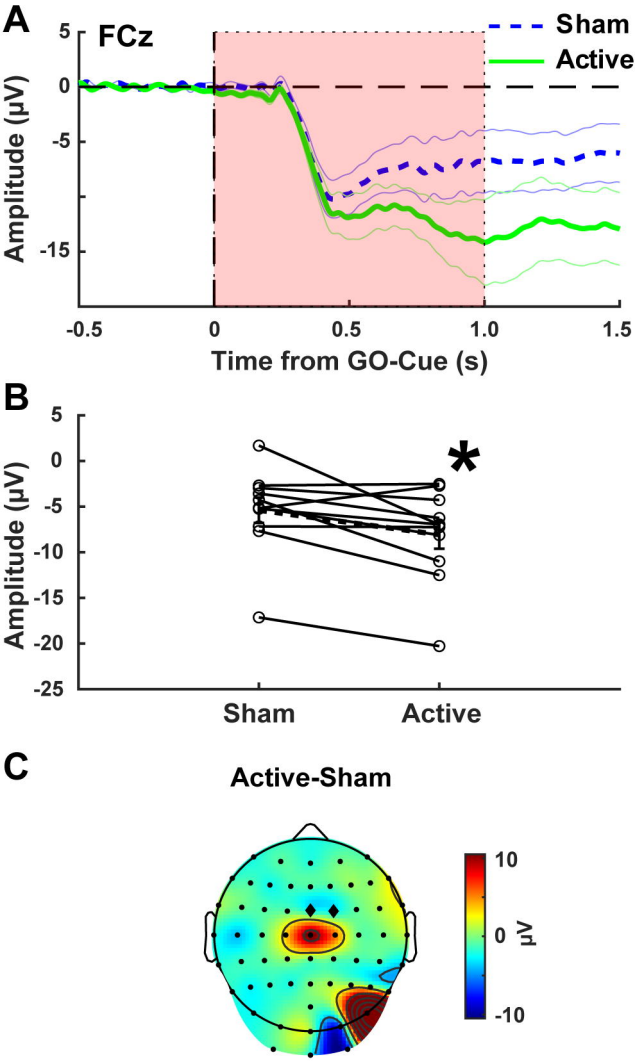
A
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Sham
Active
Amplitude (μV)
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0
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-15
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1.0
1.5
Time from GO-Cue (s)
B
Amplitude (μV)
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*
Sham
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-10
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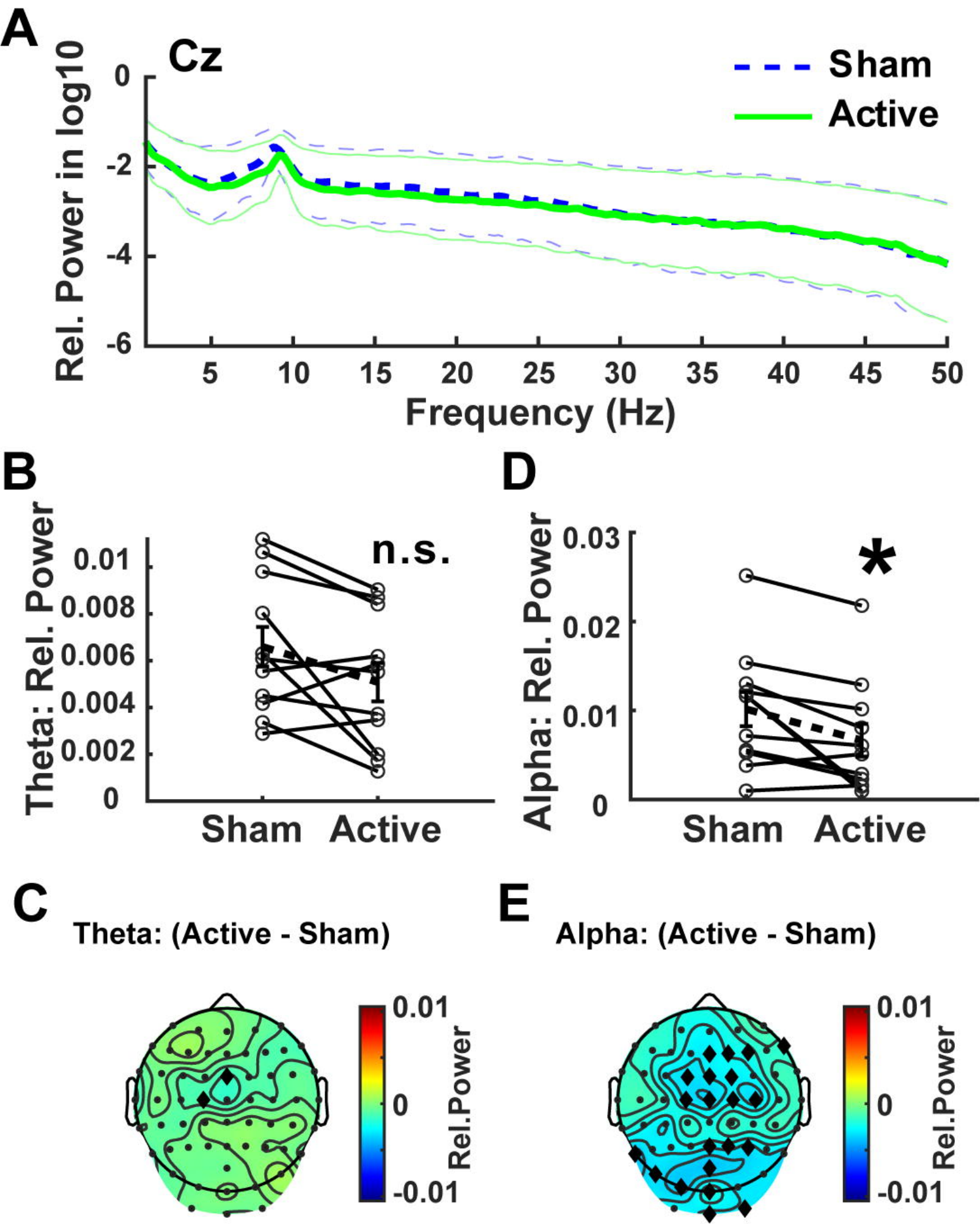
A
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Rel. Power in log10
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-2
-4
-6
5
10
15
20
25
30
35
40
45
50
Frequency (Hz)
B
Theta: Rel. Power
0.01
0.008
0.006
0.004
0.002
0
n.s.
Sham
Active
D
Alpha: Rel. Power
0.03
0.02
0.01
0
*
Sham
Active
C
Theta: (Active - Sham)
0.01
0
-0.01
Rel.Power
E
Alpha: (Active - Sham)
0.01
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-0.01
Rel.Power