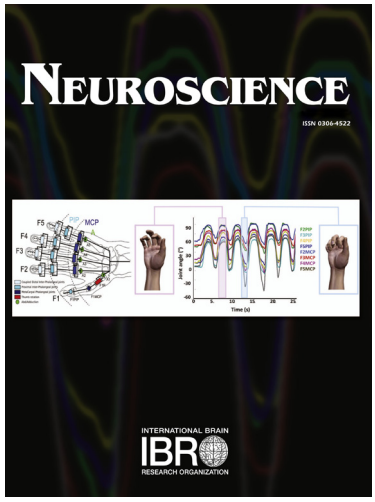

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ELECTRICAL STIMULATION OF THE FRONTAL CORTEX ENHANCES SLOW-FREQUENCY EEG ACTIVITY AND SLEEPINESS

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Running title: Modulation of spontaneous cortical synchronization by tDCS

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Abstract

Our aim was to enhance the spontaneous slow-frequency EEG activity during the resting state by using oscillating transcranial direct currents (tDCS) with a stimulation frequency that resembles the spontaneous oscillations of sleep onset. Accordingly, in this preliminary study, we assessed EEG after-effects of a frontal oscillatory tDCS with different frequency (0.8 *vs*. 5 Hz) and polarity (anodal, cathodal, and sham).

Two single-blind experiments compared the after effects on the resting EEG of oscillatory tDCS [Exp.1=0.8 Hz, 10 subjects (26.2±2.5 years); Exp.2=5 Hz, 10 subjects (27.4±2.4 years)] by manipulating its polarity.

EEG signals recorded (28 scalp derivations) before and after stimulation [slow oscillations (0.5-1 Hz), delta (1–4 Hz), theta (5–7 Hz), alpha (8–12 Hz), beta 1 (13–15 Hz) and beta 2 (16–24 Hz)] were compared between conditions as a function of polarity (anodal *vs*. cathodal *vs*. sham) and frequency of stimulation (0.8 *vs*. 5 Hz).

We found a significant relative enhancement of the delta activity after the anodal tDCS at 5 Hz compared to that at 0.8 Hz. This increase, even though not reaching the statistical significance compared to sham, is concomitant to a significant increase of subjective sleepiness, as assessed by a visual analog scale. These two phenomena are linearly related with a regional specificity, correlations being restricted to cortical areas perifocal to the stimulation site.

We have shown that a frontal oscillating anodal tDCS at 5 Hz results in an effective change of both subjective sleepiness and spontaneous slow-frequency EEG activity. These changes are critically associated to both stimulation polarity (anodal) and frequency (5 Hz). However, evidence of frequency-dependence seems more unequivocal than evidence of polarity-dependence.

Key words: oscillatory transcranial direct current stimulation (osc-tDCS), resting EEG, EEG synchronization, sleepiness, sleep onset, frontal cortex

1 INTRODUCTION

Transcranial current stimulation (tCS) is a general term referring to non-invasive brain stimulation techniques defined by the specific characteristics of the current used (Paulus, 2011). In the most frequently used protocols, a direct current (tDCS) is applied. At the neuronal level, the current polarity modulates the membrane potential, producing somatic depolarization and increased firing rates in the neural populations underlying the anode, and the opposite effects under the cathode (Bindman et al., 1964; Purpura and McMurtry, 1965; Bikson et al., 2004; Fröhlich and McCormick, 2010).

Recently, tCS protocols have been extended to include time-varying currents (AC). Using a sinusoidally varying current, as in transcranial alternating current stimulation (tACS), spontaneous brain oscillations can be modulated in a frequency-specific manner (Fröhlich and McCormick, 2010; Ozen et al., 2010). Indeed, results from *in vitro* and *in vivo* animal studies indicate that weak sinusoidal electric fields entrain the activity of cortical and hippocampal neurons by modulating their neuronal membrane potential coherently with the frequency of field fluctuations (Radman et al., 2007; Fröhlich and McCormick, 2010; Ozen et al., 2010; Ali et al., 2013). tDCS and tACS can be combined resulting in oscillatory-tDCS (osc-tDCS) protocols, in which a direct current is superimposed onto an alternating current polarizing the stimulation (Groppa et al., 2010). Altogether these protocols allow exploiting both DC effect on cortical excitability (Groppa et al., 2010) and AC synchronizing effect on rhythmic neuronal activity (Marshall et al., 2006; Kirov et al., 2009). Hence, the osc-tDCS protocols seem to be a candidate to simultaneously manipulate both cortical excitability and spontaneous brain rhythms.

It has been suggested that the induction of oscillating currents enables a direct interaction with the ongoing oscillatory cortical activities according to the principle of resonance (Bergmann et al., 2009). Therefore, besides the polarity profile of the current (i.e., tDCS vs. osc-tDCS vs. tACS), its frequency should also be considered as a relevant factor affecting extent and direction of the induced changes. The frequency of stimulation may be crucial when the interest is promoting EEG synchronization, which implies an enhancement of spontaneous slow-frequency activity during waking and sleep states. Empirical evidences of such an effect are rather mixed. It has been reported that an anodal osc-tDCS at 0.75 Hz on frontal sites increased <1 Hz (Marshall et al., 2006; Marshall et al., 2011) and alpha (Marshall et al., 2006; Marshall et al., 2011; Del Felice et al., 2015) rhythms during sleep, while a stimulation at 5 Hz decreased <1 Hz activity (Marshall et al., 2006; Marshall et al., 2011). However, other authors failed to show substantial effects of the same procedure on the EEG activity (Eggert et al., 2013; Sahlem et al., 2015). These studies delivered anodal osc-tDCS at 0.75 Hz during

sleep, mostly with an EEG background dominated by slow-wave activity (1-4 Hz) and slow oscillations (<1 Hz). During wakefulness, Kirov and coworkers (2009) used the same protocol (i.e., anodal osc-tDCS at 0.75 Hz) and reported an enhanced theta activity, more pronounced during quiet than during attentive wakefulness. This seems of interest since theta activity is a marker of sleepiness (Finelli et al., 2000; De Gennaro et al., 2007; Marzano et al., 2007; Gorgoni et al., 2014), and the large increase during quiet wakefulness (Kirov et al., 2009) might be interpreted in terms of enhancing an actual spontaneous rhythm.

Quite surprisingly, osc-tDCS with a frequency in the theta range has never been used during wakefulness to affect the ongoing oscillatory activities, in accordance with the principle of resonance (Bergmann et al., 2009). Accordingly, we decided to explore in two experiments the EEG after-effects of an osc-tDCS comparing different stimulation frequencies and polarity during the resting state. As a manipulation of polarity and frequency, we compared anodal *vs.* cathodal and 0.8 Hz *vs.* 5 Hz frequency stimulations, respectively.

The basic aim was "promoting" the EEG synchronization during wakefulness with a stimulation frequency that resembles the endogenous oscillatory activity. According to the regional differences in the process of synchronization (Marzano et al., 2013), we studied the frontal areas as the locus of stimulation. Indeed, the synchronization process, during the spontaneous sleep onset, begins during wakefulness with a progressive increase in lower frequencies centered on frontal areas (Marzano et al., 2013). Moreover, it has been shown that the frontal cortical regions are the preferential sites of origin for both slow oscillations (Massimini et al., 2004) and theta oscillations (Iramina et al., 1996; Asada et al., 1999; Ishii et al., 1999).

2 EXPERIMENTAL PROCEDURES

2.1 Methods

2.1.1 Participants

Twenty healthy female subjects (18-30 years) participated in two different experiments after having given their informed written consent. Ten subjects (mean age: 26.2 ± 2.5 years) participated in Exp. 1, and ten subjects (mean age: 27.4 ± 2.4 years) participated in Exp. 2. All participants were medication-free and met the following inclusion criteria: no presence or history of epilepsy, no neurological or psychiatric disorder and intracranial metal implants, no daytime nap habits or excessive daytime sleepiness or other sleep disturbances as assessed by a clinical interview. During the week before the experimental sessions, participants were asked to keep constant their wake-sleep cycle by sleeping about 7 h per night with a regular schedule, and to fill out a daily sleep log in order to control their compliance. During the

morning of the experimental sessions they were not allowed to consume coffee, tea, chocolate, or any kind of neuroactive drugs. All subjects were tested in the luteal phase of the menstrual cycle in order to avoid possible confounding effect of the cyclical ovarian hormones.

The study was approved by the Institutional Ethics Committee of the Department of Psychology of University of Rome Sapienza and of the IRCCS San Raffaele Pisana, and was conducted in accordance with the Declaration of Helsinki.

2.1.2 Experimental design

The experiments consisted of 3 within-subject experimental sessions, two active conditions (anodal and cathodal osc-tDCS) and a sham condition, separated by an interval of at least 1 week. The sequence of sessions was counterbalanced across subjects, and participants were blind to the condition.

Regardless of the different experimental conditions, the timeline of the experimental sessions was identical in both the experiments (Fig 1B).

Subjects arrived at the laboratory at 12:00 h, had a small lunch, and underwent preparation for the EEG recordings and stimulation. Then, they were asked to sit relaxed on a comfortable chair in a soundproof, temperature-controlled, and electrically shielded room with constant dim light. Each session started at 14:00 h and included: a) 5-min EEG recordings immediately before the stimulation (EEG pre-stimulation); b) Stimulation protocol (10 min); c) 5-min EEG recordings immediately after the stimulation (EEG post-stimulation). EEG was recorded in a resting eyes-closed condition. During recordings, subjects were asked to imagine fixating a point on the wall in front of them. The polysomnographic signals were continuously monitored. When signs of excessive drowsiness were detected (e.g., slow pendular eye movements and eye blinks), the subject was addressed by the experimenter and asked to respond.

In the second experiment, a self-reported measure of sleepiness was introduced to evaluate if the possible stimulation effects on the EEG were associated with a variation of subjective sleepiness. Participants filled in the Visual Analog Scale for global vigor (VASgv) (Monk, 1987).

[Please Insert Fig.1 about here]

2.1.3 Subjective sleepiness

The VASgv (Monk, 1987) is a measure of subjective alertness, which combines the scores on four scales (alert, sleepy, weary and effort) to obtain a global vigor score between 0 and 40. Subjects indicated the extent to which the adjective described their current state (from “not at all” to “very much”) by making a vertical mark on a 10-cm line for each scale. The

measurement in centimeters was converted to the same number of points ranging from 0 to 10 points. According to the aims of the current study, only data from the sleepiness scales (i.e. "sleepy" item, sleepiness-VAS) were considered.

In each experimental session of Exp. 2, sleepiness-VAS scores were collected just before the start of the pre-stimulation EEG recording and immediately after the end of the post-stimulation EEG recording.

2.1.4 EEG recordings

The EEG signals were recorded from 28 sintered Ag–AgCl electrodes mounted in an elastic cap (Easycap, Falk Minow, Munich) at scalp locations C3, C4, Cp1, Cp2, Cp5, Cp6, Cz, F3, F4, F7, F8, Fc1, Fc2, Fc5, Fc6, Fp1, Fp2, Fz, O1, O2, Oz, P3, P4, P7, P8, Pz, T7, T8 (according to the 10–20 system) with linked mastoid references (A1 and A2, Fig. 1A). Horizontal eye movements were detected by recording electro-oculogram (EOG), and the electromyogram (EMG) was recorded by two submental electrodes for off-line artefact detection. The ground electrode was placed between Fz and Cz, at Fcz. Electrode resistance was kept below 5 kΩ. Signals were recorded using the BrainAmp MR plus system (Brain Products GmbH, Gilching) with a sampling rate of 250 Hz, amplified in the range of ±3.2768 mV at a resolution of 0.1 μV, and filtered between 0.16 and 70 Hz. EEG data were digitally stored on hard disk for further offline analyses.

2.1.5 Osc-tDCS

Exp. 1 – In three separated sessions, participants received one of three different osc-tDCS protocols: anodal Slow Oscillatory-tDCS (anodal SO-tDCS), cathodal SO-tDCS, or sham. The stimulation was applied via two conductive-rubber circular electrodes (diameter: 1.2 cm) connected to a battery-operated stimulator system (BrainSTIM, EMS medical). The stimulation electrodes was placed in sponges saturated with tap water and high conductivity gel.

In the two active conditions (anodal and cathodal SO-tDCS), a sinusoidal oscillating current with frequency of 0.8 Hz was applied for 10 min (10 sec ramp in and 10 sec ramp out). Current intensity ranged from a minimum of 0 mA to a maximum of 0.6 mA (maximum current density: 0.531 mA/cm^2).

In the anodal SO-tDCS, the electrode with the positive polarity, representing the stimulation electrode, was placed 1 cm anterior to Fz (Fig 1A), while the electrode with negative polarity, the reference electrode, was placed on the right deltoid muscle (Cogiamanian, 2007). The reference electrode arrangement has been decided in order to disentangle the single-polarity contribution to the cortical effects, and to describe it without confounding biases arising from

two simultaneous cortical stimulations of opposite polarities involved when the reference electrode is placed on the scalp [e. g., on supraorbital region (Nasseri et al., 2015)].
In the cathodal SO-tDCS condition, the polarity of stimulation and reference electrodes were reversed.
In the sham condition, electrodes placement and current features were identical to the active conditions, but the stimulator was turned off after 10 sec.

Exp. 2 – In Exp. 2, the stimulations protocols of the first experiment were exactly replicated except for the stimulation frequency that was set at 5 Hz. The three experimental conditions were: anodal θ-tDCS, cathodal θ-tDCS, and sham.
None of the participants reported adverse effects during or after the stimulation protocols, but a slight and short lasting tingling under the stimulation electrode at the beginning of the session. Participants noticed no differences among the two active and sham conditions, as assessed by a post-experiment debriefing.

2.2 Data Analysis

2.2.1 Resting EEG

The power spectra of the 28 EEG derivations were computed by a Fast Fourier transform (FFT) routine on 2-s artefacts-free epochs with a 1 Hz bin resolution (0.5 Hz only for the first bin) in the range 0.50-29 Hz. Ocular and muscle artefacts were detected by offline visual inspection of 2-s EOG, EMG, and EEG epochs. EEG epochs affected by artefacts on specific channels, once detected were rejected for all the scalp locations. This duration was chosen to maximize the duration of EEG recording free of EMG or EOG artifacts. The mean percentage of waking EEG epochs remaining after the artefact rejection was 62.4% ± 23.12%.
Data analysis was performed using the software package MATLAB (The Math Works, Inc., MA, USA) and its signal analysis and statistics toolbox.
The EEG power values for each frequency bin were averaged across the following EEG bands: slow oscillation (0.5-1 Hz), delta (1–4 Hz), theta (5–7 Hz), alpha (8–12 Hz), beta 1 (13–15 Hz) and beta 2 (16–24 Hz).
Relative EEG power spectra changes resulting from each tDCS protocol (Exp. 1: Anodal SO-tDCS, Cathodal SO-tDCS, Sham; Exp. 2: Anodal θ-tDCS, Cathodal θ-tDCS, Sham) were expressed as ratio between post- and pre-stimulation EEG spectral power, and calculated for each cortical derivation and frequency band.

2.2.2 Effect of polarity

Relative EEG power changes from both the stimulation protocols were submitted to one-way repeated measure Analyses of Variance (ANOVAs) comparing the 3 conditions (Anodal, Cathodal, Sham). ANOVAs were carried out separately for each scalp location and frequency band.

Since cortical activity measures at different scalp locations and at different time are correlated to each other, we corrected the repeated measure analyses for multiple comparisons with an adjusted (or partial) Bonferroni method which takes into account the mean inter correlation coefficients among all dependent variables [details on the applied Dubey/Armittage-Parmaris procedure can be found in Sankoh et al. (1997)].

Considering the mean correlation between the dependent variables (i.e. the relative EEG power changes of each cortical derivation and frequency band in the three experimental conditions) in Exp. 1 (r = 0.12) and in Exp. 2 (r=0.10) and the number of not independent comparisons (28 scalp locations 6 frequency bands 3 polarities = 504), α level was adjusted to 0.00020 and to 0.00019, respectively (Perneger, 1998; Sankoh et al., 1997). Post-hoc tests have been carried out by paired t-tests. If significant effects were found at the *omnibus* ANOVA, significance of post-hoc tests was left at the standard $p \leq 0.05$.

2.2.3 Effect of frequency

To assess the effect of frequency, EEG power values of the two experiments were expressed as the ratio between the relative EEG power changes in active and sham conditions (active/sham). Then, these ratios were submitted to two-way mixed design ANOVAs, Frequency (0.8 Hz vs. 5 Hz) x Polarity (Anodal vs. Cathodal), with the second factor as a repeated measure. ANOVAs were carried out separately for each scalp location and frequency band. Considering the mean correlation between the dependent variables (r=0.20) and the number of not independent comparisons (28 scalp locations 6 frequency bands = 168), α level was adjusted to 0.0009 (Perneger, 1998; Sankoh et al., 1997). In this case, post-hoc tests have been carried out by unpaired t-tests ($p \leq 0.05$).

2.2.4 Subjective sleepiness

Subjective estimates of sleepiness collected during the second experiment, expressed as ratios between post- and pre- stimulation of sleepiness-VAS scores, were analyzed by one-way ANOVA comparing the three conditions (anodal, cathodal, sham). Post-hoc tests have been carried out by paired t-tests ($p \leq 0.05$).

3 RESULTS

3.1 Polarity of stimulation (anodal vs. cathodal vs. sham)

3.1.1 Experiment 1: stimulation at 0.8 Hz

The topography of EEG power changes (expressed as post/pre SO-tDCS) in the three stimulation conditions of Exp. 1 (anodal, cathodal and sham; Fig. 2A) exhibits the largest variation after anodal SO-tDCS, with a marked increase in the alpha band, maximal at the Pz electrode and a posterior to anterior gradient. In the other bands, the topographical changes mirror those of the alpha band, although to a lesser extent. Cathodal and sham conditions share some effects on the EEG topography, which can be considered as associated with the protocol itself, because of the lack of a real stimulation in the sham condition. These effects mainly consist of increases of power spectra in the left hemisphere at the temporo-parietal and occipital sites in the theta and alpha bands, and with an ipsilateral temporo-frontal maximum in the other EEG bands. Peculiar of the cathodal SO-tDCS, a power increase appears in the stimulation frequency band (slow oscillation) on the left fronto-temporal cortical sites. However, these differences were not significant at any cortical site or frequency band after the partial Bonferroni's correction for multiple comparisons (Fig 2B, C).

[Please Insert Fig. 2 about here]

3.1.2 Experiment 2: stimulation at 5 Hz

The topography of EEG power changes in the three stimulation conditions (anodal, cathodal and sham) after the θ-tDCS shows some modifications only after the anodal stimulation (Fig. 3A). This condition is characterized by an increase in slow oscillation and delta bands at the prefrontal areas and an enhancement of the alpha and theta activity, maximal at the parietal sites. In the higher frequency bands, the stimulation induces an increase over the frontal areas. Also in this case, the results from the statistical comparisons point to no statistical difference at the ANOVAs, as none reaches the significance level after the partial Bonferroni's correction, at any cortical site or frequency band (Fig 3B). However, it is worth noting that the largest difference at the *omnibus* ANOVAs was found for the delta activity at prefrontal sites (Fp1 $F_{2,9}$=5.64 p=0.012.; Fp2 $F_{2,9}$=7.47, p=0.004). This difference was due to a higher delta activity after anodal compared to both sham (Fp1: t_9=3.05, p=0.01; Fp2: t_9=2.88, p=0.01) and cathodal stimulation (Fp1: t_9=2.66, p=0.026; Fp2 t_9=3.90, p=0.004; Fig. 3C).

[Please Insert Fig. 3 about here]

3.2 Frequency of stimulation (5 Hz *vs.* 0.8 Hz)

EEG power ratios between active/sham conditions of the two experiments were analyzed to assess the differential effect of the osc-tDCS frequency on EEG power. The results of Frequency (0.8 Hz vs. 5 Hz) x Polarity (Anodal vs. Cathodal) ANOVAs at each scalp location and frequency band are reported in Fig 4A. Neither Frequency nor Polarity main effects were

significant after the partial Bonferroni's correction. Instead, a significant interaction was found for the delta band at Fp2 ($F_{1,18}$=20.48; p=0.0004; η^2=0.09) surviving the partial Bonferroni's corrrection. This effect is explained by an increased delta power after the anodal stimulation at 5 Hz compared to that at 0.8 Hz (t_9=3.57, p=0.002; Fig. 4B). In order to further confirm the reliability and robustness of this finding, we have also applied the False Discovery Rate (Storey et al., 2004), which confirmed the results with only one q-value ≤ 0.05 associated to the interaction Frequency x Polarity in the corresponding ANOVA at Fp2 (q-value= 0.02).

[Please Insert Fig. 4 about here]

3.3 Subjective sleepiness

The three conditions (anodal, cathodal, sham) of the second experiment were compared with respect to the changes in subjective estimates of sleepiness. The difference was significant ($F_{2,18}$=4.45, p=0.03). Specifically, the anodal stimulation was associated to larger increases of sleepiness compared to both sham (t_9=2.38; p=0.04) and cathodal (t_9=2.52; p=0.03) conditions (Fig 5A). The difference between the last two conditions was not significant (t_9=0.38; p=0.71).

[Please Insert Fig. 5 about here]

3.4 EEG changes after anodal stimulation at 5 Hz and sleepiness

The anodal stimulation at 5 Hz was associated to both an increased delta power over the right prefrontal area when compared to that at 0.8 Hz and an increased subjective sleepiness when compared to the sham condition. The lack of subjective sleepiness measures in the Experiment 1 did not allow us to compare the effects of the two stimulation frequencies on this variable. Notwithstanding this, we have evaluated the existence of a correlation between the changes in Delta band after the 5-Hz anodal stimulation and the associated changes in subjective sleepiness. To this aim, changes in sleepiness-VAS scores and changes of delta power were correlated by calculating Spearman's Rho coefficients for each scalp location. Both measures were expressed as ratios between anodal/sham conditions.

Bonferroni's correction adjusted for the mean correlation between the dependent variables (i.e. anodal/sham ratios of changes in sleepiness-VAS scores and Delta power, r=0.65) was applied (Sankoh et al., 1997; Perneger, 1998), and the α level was corrected to 0.02.

The results depicted in Fig 5B show significant correlations for Fp2 (rho_9=0.73; p=0.016) and F8 (rho_9=0.81; p=0.004), indicating that enhanced delta EEG activity over prefrontal sites after the 5-Hz anodal stimulation is strongly related to a concomitant increased sleepiness.

4 DISCUSSION

Our two independent experiments were aimed at assessing the after-effects of an oscillatory tDCS on the spontaneous resting EEG activity. According to the general goal of enhancing EEG synchronization during wakefulness, we have evaluated the effects of two basic parameters of osc-tDCS, polarity (anodal *vs*. cathodal) and frequency (0.8 *vs.* 5 Hz), in healthy subjects in an eyes-closed condition. The main finding is a significant enhancement of the delta activity after the anodal osc-tDCS at 5 Hz compared to 0.8 Hz. Instead, the increase in low frequency EEG activity after the 5 Hz stimulation does not reach the statistical significance when compared to the sham condition. Nevertheless, the enhancement of the delta activity after the anodal osc-tDCS at 5 Hz is concomitant to a significant increase of sleepiness relative to the control condition. Moreover, the two phenomena are linearly correlated, with a high regional specificity, being limited to the prefrontal areas. In other words, the relation is restricted to the cortical areas stimulated by the 5 Hz osc-tDCS, and is characterized by a clear antero-posterior gradient.

4.1 The experimental manipulation of sleepiness and prefrontal SWA

Bergmann and coworkers (Bergmann et al., 2009) hypothesized that the effectiveness of transcranial stimulations to affect the cortical physiology depends on the ongoing oscillatory cortical activities according to the principle of resonance. For instance, the effectiveness of different tACS stimulation frequencies (over the occipital cortex) to induce phosphenes depends on the predominant endogenous oscillation (Kanai et al., 2008), being most effective in the beta range with eyes open, and in the alpha range with eyes closed. Although Bergmann and coworkers (Bergmann et al., 2009) suggested the basic principle of resonance, they actually used an osc-tDCS at 0.75 Hz, and found no stimulation-specific changes in subjective sleepiness. This failure was interpreted as due to the fact that SO-tDCS occurred in a frequency corresponding to that endogenously arising during NREM sleep (i.e., <1 Hz activity). Similarly, Kirov and coworkers (Kirov et al., 2009) enhanced waking endogenous theta activity (besides slow-frequency and beta activity) using an identical protocol. Coherently with the idea that effectiveness of trascranial stimulation depends on the endogenous oscillations, their enhancement was more pronounced during quiet than during attentive wakefulness. However, they didn't find any change in sleepiness ratings when compared sham and active conditions.

Therefore, the current finding is the first effective manipulation of the endogenous EEG

oscillations via tDCS with a concomitant increase of sleepiness.

4.2 The magnitude of the changes in EEG activity and sleepiness

Although our 5 Hz osc-tDCS is more effective than the 0.8 Hz osc-tDCS in inducing a local delta activity increase, the actual extent of the induced changes is relatively small. Actually, 5 Hz anodal stimulation increases delta activity over Fp2 site by 55.01% and 31.09% compared to sham and anodal at 0.8 Hz stimulations, respectively. In our opinion, two different factors may explain the small statistical effect. Undoubtedly, there is a problem of power, and these effects should be replicated in larger samples. Secondly, delta activity over Fp2 also increases by 9.03% independently of the stimulation, and sleepiness increases by 38.30% independently of the stimulation, although not significantly (t_9=1.56; p=0.15). Summarizing, the effectiveness of osc-tDCS may coexist with spontaneous increases of sleepiness and slow-frequency activity. This is coherent with the reported increase of sleepiness across session time independent of stimulation mode (Bergmann et al., 2009). The authors proposed that this increase could be a consequence of the monotonous sensory input associated with the experimental setting, rather than due to the stimulation itself, since they had no control condition to rule out a specific stimulation effect (Bergmann et al., 2009).

According to the principle of resonance, that is oscillatory transcranial stimulations affect cortical physiology depending on the ongoing oscillatory activity, increased sleepiness and slow-frequency activity due to the experimental setting may act as both *permissive* and *confounding* factor. In other words, spontaneous slow-frequency activity presumably associated to an increased sleepiness may act as a *permissive* factor, since the magnitude of induced frequency-dependent changes seems affected by the resonance with the frequency of oscillatory tDCS. On the other hand, the existence of a spontaneous sleepiness as expressed by an increased slow-frequency EEG activity may obscure experimentally-induced changes in EEG activity.

4.3 The effect of polarity

According to the current views, the polarity of tDCS modulates the direction of the stimulation effects on cortical activity in the area underlying the electrodes (Bindman et al., 1964; Purpura and McMurtry, 1965; Bikson et al., 2004; Fröhlich and McCormick, 2010). However, the underlying neuronal mechanisms of osc-tDCS are yet poorly understood. Bergmann et al. (2009) proposed that shifting the membrane potential repeatedly back and forth in an oscillating manner might be the basic principle by which osc-tDCS affects the endogenous rhythms as expressed in the scalp EEG rhythms. In this context, we had no *a*

priori hypothesis on the effect of polarity. Our findings, like those during sleep (Marshall et al., 2006; Marshall et al., 2011; Del Felice et al., 2015), point to a larger effectiveness of anodal compared to cathodal stimulation. However, unlike other studies during sleep (Marshall et al., 2006; Marshall et al., 2011; Del Felice et al., 2015) and during wakefulness (Kirov et al., 2009) that did not compare active anodal and cathodal conditions, our results directly point to a larger effectiveness of anodal osc-tDCS.

Moreover, these studies (Marshall et al., 2006; Kirov et al., 2009; Marshall et al., 2011; Del Felice et al., 2015) adopted a bi-cephalic frontal montage with reference electrodes placed on ipsilateral mastoids. On one hand, the references placement on mastoids areas involves a stimulation with the opposite polarity of the adjacent cortical areas (Miranda et al., 2006) that is not entirely negligible and makes it difficult to disentangle the single-polarity contribution to the cortical effects. For this reason, we used the right deltoid muscle as a non-cephalic reference in order to better discriminate the specific contribution of polarity to the cortical effects, and to describe it without confounding biases arising from two simultaneous cortical stimulations of opposite polarity. On the other hand, the bi-cephalic montage also implies the concurrent frontal stimulation of both the hemispheres, resulting in a more widespread stimulation and a greater total amount of current applied compared to the one administered in our study. Both these features –namely the more widespread stimulation and the greater amount of current- enhance the entrainment of cortical activity, especially in case of mismatch between stimulation and spontaneous activity frequency (Radman et al., 2007; Fröhlich and McCormick, 2010; Reato et al., 2010; Ali et al., 2013). Our choice of using a monopolar electrodes montage disentangles the specific polarity contribution to nearly selective cortical effects restricted to the right prefrontal sites but may have minimized the magnitude of the induced changes, due to the larger distance between the two stimulation electrodes (Moliadze et al., 2010). For all these reasons, we suggest, in future tCS studies aimed to assess the frequency-dependence of the stimulation effects but not interested in evaluating the specific contribution of the stimulation polarity, to use a transcranial alternating current stimulation (tACS). Being a not polarized stimulation, tACS allows to adopt a bipolar cephalic montage overpassing the problem of the bias introduced by a polarizing cephalic reference on the interpretation of the stimulation effects. In this case, the magnitude of stimulation effects should increase, due to the reduced distance between the electrodes, and the stimulation effectiveness at entraining cortical activity should be maximized, due to the application of a less spatially localized stimulation.

4.4 Limits of the study

The major limit of the study is represented by the small sample size for each experiment, and by the between-group comparisons with respect to the effects of the frequency of stimulation (5 *vs.* 0.8 Hz). According to the inter-subject variability in the efficacy of tDCS (Nitsche et al., 2008) and the inter-subject variability of EEG profiles, these differences may have affected our results.

Given the preliminary nature of the study, our results should be confirmed in future studies with larger samples and fully within-subject studies.

A second major limit of the study is the lack of the subjective sleepiness assessment in the first study (osc-tDCS at 0.8 Hz). This flaw did not allow us to confirm the frequency-specificity of the stimulation effects on sleepiness, an important issue that should be addressed in future studies.

5 CONCLUSIONS

To the best of our knowledge, this is the first study in which a frontal anodal osc-tDCS results in an effective manipulation of both sleepiness and spontaneous slow-frequency EEG activity. These changes are critically associated to both stimulation polarity (anodal) and frequency (5 Hz). However, evidence of frequency-dependence seems more unequivocal than evidence of polarity-dependence. The lack of any significant difference in the direct comparisons between anodal *vs.* cathodal conditions, and the questions raised by the choice of an extra-cephalic reference, suggest some caution. In this respect, we suggest that future studies should consider the adoption of a tACS protocol, which does not need an extra-cephalic reference and avoid the confounding bias arising from two simultaneous cortical stimulations of opposite polarity, although this procedure may lead to a decrease in regional specificity.

Beside the polarity-dependence, some questions still remain open. The first question regards the detection of "genuine" oscillatory activity in the resting EEG, since the presence of a spectral peak resulting from a Fourier Transform of the EEG signal does not necessarily imply an underlying oscillatory activity at that frequency, given that non-oscillatory and transient signals can produce power changes at specific frequencies. Hence, future studies should also consider methods able to detect oscillatory activity within EEG signals containing a "background" non-rhythmic portion (Caplan et al., 2001; Marzano et al., 2011; Moroni et al., 2012; Marzano et al., 2013).

The second question regards the time-course of the after-effects induced by osc-tDCS. Our results are limited to a 5-min interval subsequent to 10-min of osc-tDCS. We have also evaluated if the effect of 5-Hz anodal stimulation changed across the five 1-min intervals, without finding appreciable changes (data not shown). The effects on slow-frequency EEG

activity reported by Kirov and coworkers (Kirov et al. 2009) using a 0.75-Hz anodal stimulation were short-lasting, since they were found across the five 1-min intervals immediately succeeding the stimulation epochs, disappearing 30 and 60 min after. According to the current findings and to the role of SWA as the main homeostatic marker of sleep pressure (Borbély, 1982), future studies should also investigate potential after-effects of tDCS on subsequent sleep.

The last question regards the optimal site of stimulation. According to the regional differences in the process of synchronization and sleep onset (Marzano et al., 2013), all the studies used frontal areas as the locus of the transcranial stimulations. However, growing evidence suggests that EEG synchronization of sleep onset starts more than 10 min in advance in thalamic (Magnin et al., 2010) or hippocampal (Sarasso et al., 2014) regions. Therefore, one can wonder if an inter-hemispheric transcranial stimulation of the temporal areas may better enhance spontaneous slow-frequency EEG.

At this stage, our study provides the first evidence on the 5-Hz anodal tDCS as promising tool for the manipulation of sleepiness through the modulation of spontaneous cortical synchronization. The possibility of an experimental manipulation of sleep pressure by transcranial stimulation represents a useful tool with possible applications in both basic and clinical sleep research, when an increase in sleep pressure is desirable. This chance, indeed, opens fascinating perspectives since it might be possible to induce sleep. A short latency to sleep onset is the highly desired condition for many patients suffering from sleep disorders and mainly for patients with primary insomnia.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Substantial contributions to the conception and design of the work: LDG, PMR

Acquisition, analysis of data: ADA, EDS, MG, FF

Interpretation of data: LDG, PMR, MF, FF, ADA

Drafting the work and revising it critically for important intellectual content: LDG, PMR, FF, MF, ADA, MG, EDS

Final approval of the version to be published: LDG, PMR, FF, MF, ADA, MG, EDS

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: LDG, PMR, FF, MF, ADA, MG, EDS

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LEGENDS TO THE FIGURES

Figure 1. Experimental design

A. EEG recording montage (grey circles) and stimulation electrodes montage (black circles);
B. Experimental design [VASgv = Visual Analog Scale for global vigor (Monk, 1987)].

Figure 2. Effect of polarity: Changes as a function of SO-tDCS

Topographic maps of mean EEG changes associated with anodal, cathodal and sham SO-tDCS and statistical maps of comparisons between the three experimental conditions. Values are colour coded and plotted at the corresponding position on the planar projection of the scalp surface and are interpolated (biharmonic spline) between electrodes. Maps are plotted for the following frequency bands: slow oscillation (0.50–1 Hz), delta (1–4 Hz), theta (5–7 Hz), alpha (8–12 Hz), beta 1 (13–15 Hz), and beta 2 (16–24 Hz).
Panel A. Topographic distribution of mean relative EEG changes (Post-/Pre-stimulation spectral power) in anodal, cathodal and Sham conditions; values > 1 indicate a mean increase of spectral power after the stimulation.
Panel B. Results of one-way *omnibus* ANOVAs comparing the three conditions.
Panel C. Results of *post-hoc* comparisons (paired t-tests); positive coefficients indicate a greater increase of spectral power in the anodal compared to cathodal, in the anodal compared to sham, and in the cathodal compared to sham stimulation, respectively.

Figure 3. Effect of polarity: Changes as a function of θ-tDCS

Topographic maps of mean EEG changes associated with anodal, cathodal and sham θ-tDCS (5 Hz) and statistical maps of comparisons between the three experimental conditions. Values are colour coded and plotted at the corresponding position on the planar projection of the scalp surface and are interpolated (biharmonic spline) between electrodes. Maps are plotted for the following frequency bands: slow oscillation (0.50–1 Hz), delta (1–4 Hz), theta (5–7 Hz), alpha (8–12 Hz), beta 1 (13–15 Hz), and beta 2 (16–24 Hz).
Panel A. Topographic distribution of mean relative EEG changes (Post-/Pre-stimulation spectral power) in anodal, cathodal and sham conditions; values > 1 indicate a mean increase of spectral power after the stimulation.
Panel B. Results of one-way *omnibus* ANOVAs comparing the three conditions.
Panel C. Results of *post-hoc* comparisons (paired t-tests); positive coefficients indicate a greater increase of spectral power in the anodal compared to cathodal, in the anodal compared to sham, and in the cathodal compared to sham stimulation, respectively.

Figure 4. Effect of frequency: 5 Hz *vs.* 0.8 Hz osc-tDCS

Statistical maps of the comparisons between θ-tDCS vs. SO-tDCS conditions. Planned comparisons have been carried our separately for anodal and cathodal stimulations. Values are colour coded and plotted at the corresponding position on the planar projection of the scalp surface and are interpolated (biharmonic spline) between electrodes. Maps are plotted for the following frequency bands: slow oscillation (0.50–1 Hz), delta (1–4 Hz), theta (5–7 Hz), alpha (8–12 Hz), beta 1 (13–15 Hz), and beta 2 (16–24 Hz).

Panel A: Results of two-way mixed design ANOVAs, Frequency (0.8 Hz *vs.* 5 Hz) x Polarity (Anodal *vs.* Cathodal), with the second factor as a repeated measure; the main effects are reported in the first two rows, and the interactions are depicted in the third row.

Panel B: Results of the planned comparisons (unpaired t-tests) between 0.8 *vs.* 5 Hz frequency of osc-tDCS for anodal and cathodal condition, respectively. Positive coefficients indicate a greater increase of spectral power after a 5-Hz compared to 0.8 Hz stimulation.

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Figure 5. The relation between changes in subjective sleepiness and EEG changes induced by θ-tDCS

Panel A. Changes of subjective sleepiness ratings after sham, cathodal, and anodal oscillatory trascranial stimulation, expressed as percentages (post/pre*100).

Panel B. Topographic distribution of the correlation coefficients (Spearman's rho) between changes in subjective sleepiness and delta EEG changes induced by θ-tDCS (left side), and associated probability (right side). Values are colour coded and plotted at the corresponding position on the planar projection of the scalp surface and are interpolated (biharmonic spline) between electrodes. Positive t test values indicate a greater increase (or a lesser decrease) of spectral power in active condition than in sham, and vice versa for negative values. After the correction for multiple comparisons, correlation coefficients are significant for Fp2 (rho_9=0.73; p=0.016) and F8 (rho_9=0.81; p=0.004).

Figure 1

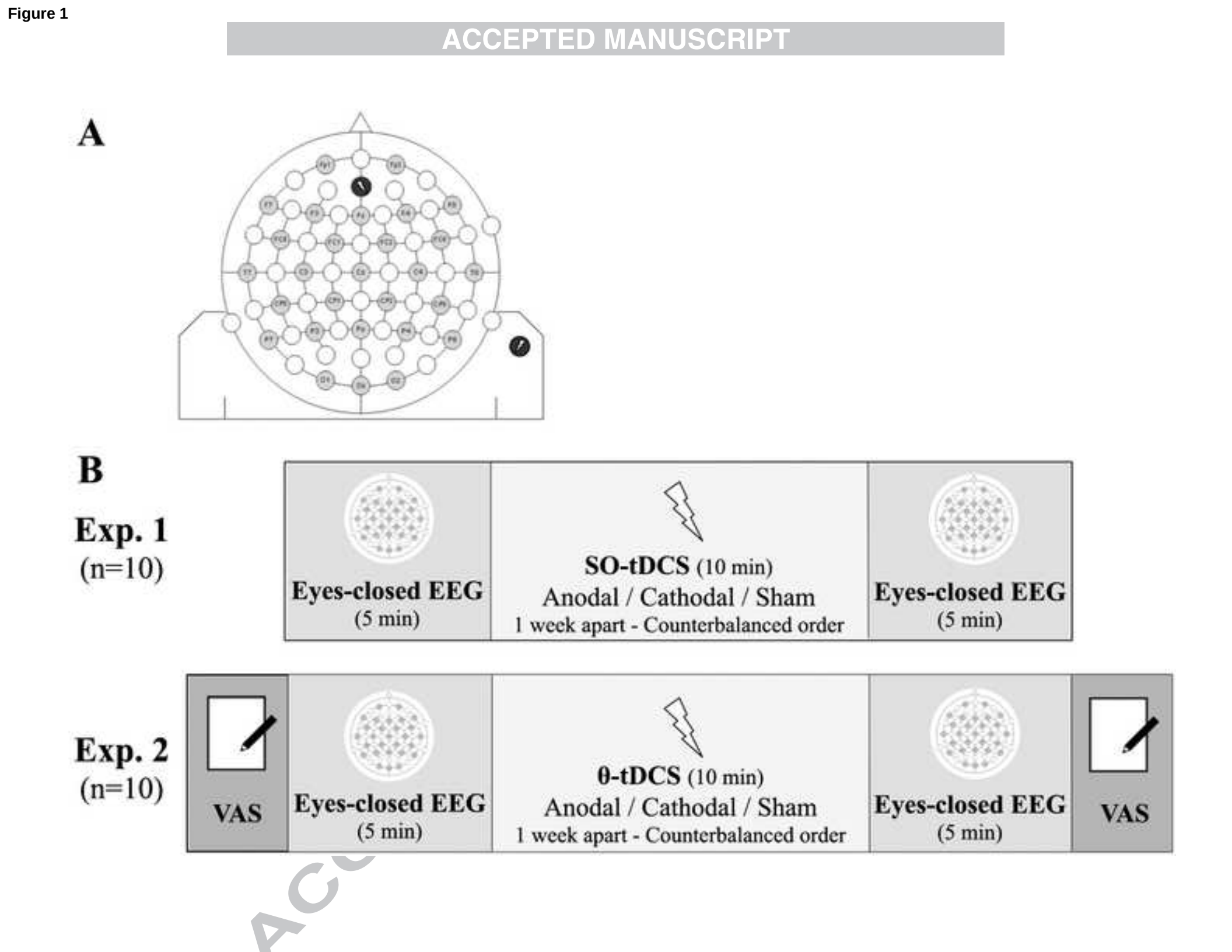


Figure 2

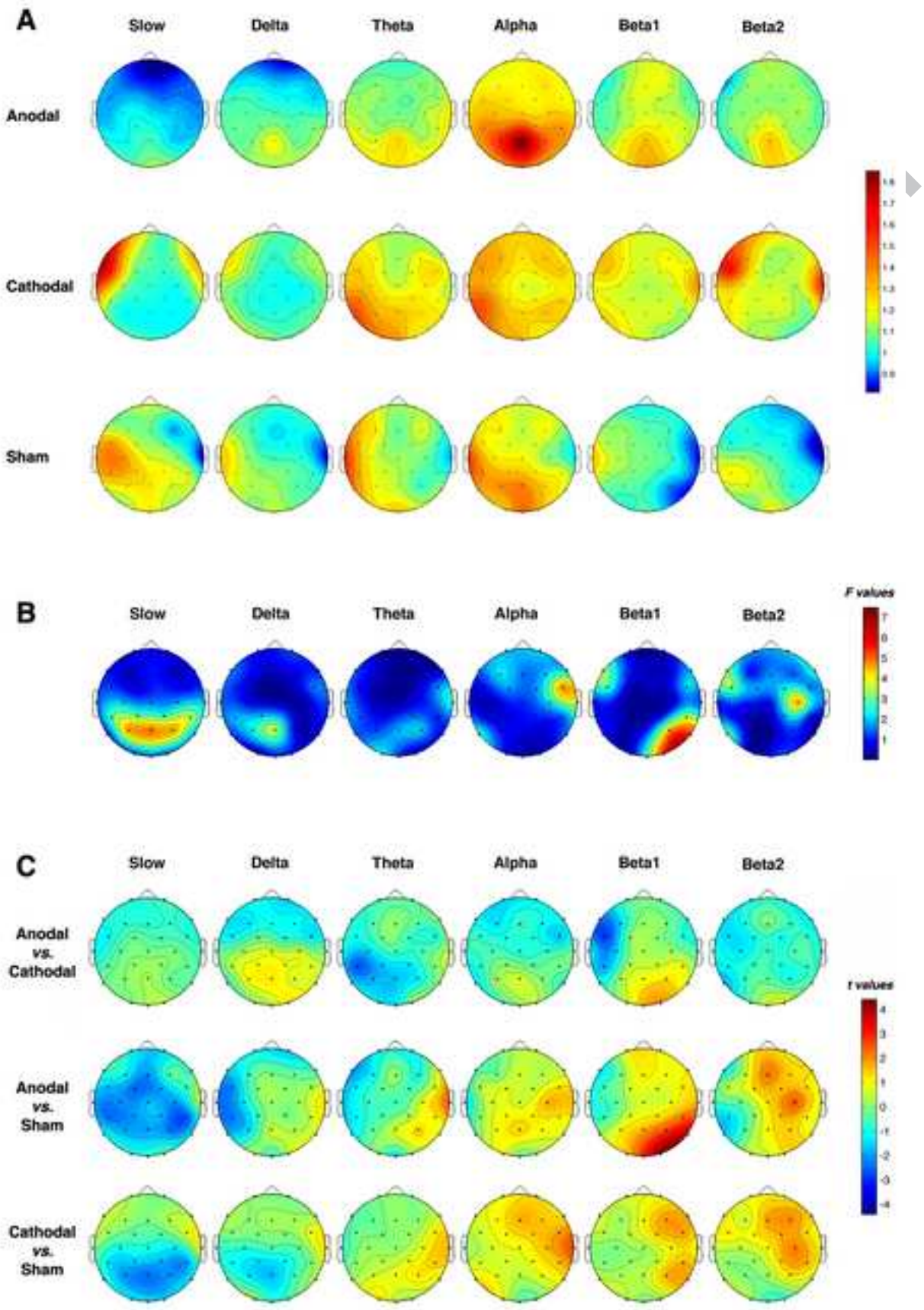


Figure 3

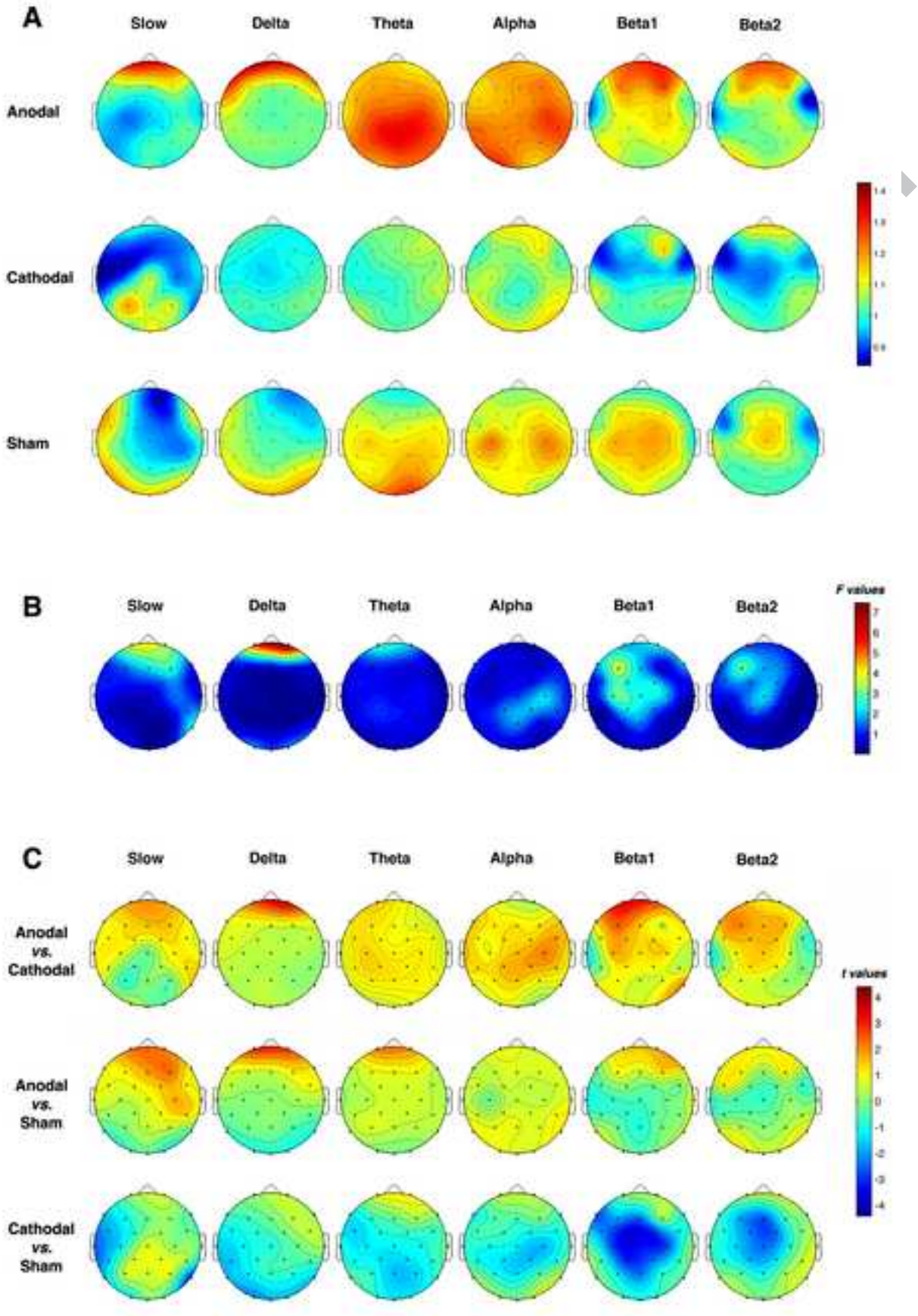


Figure 4

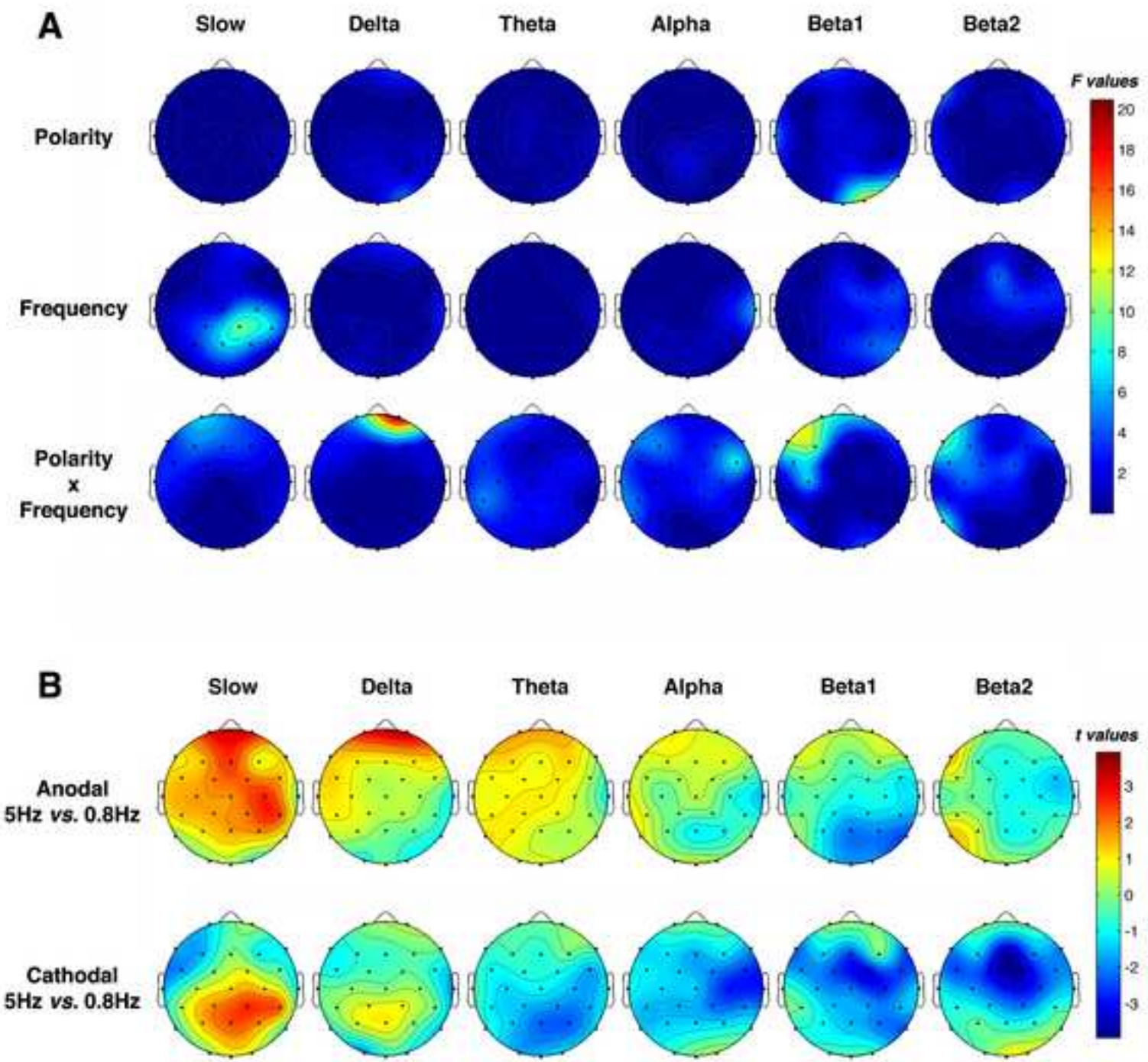
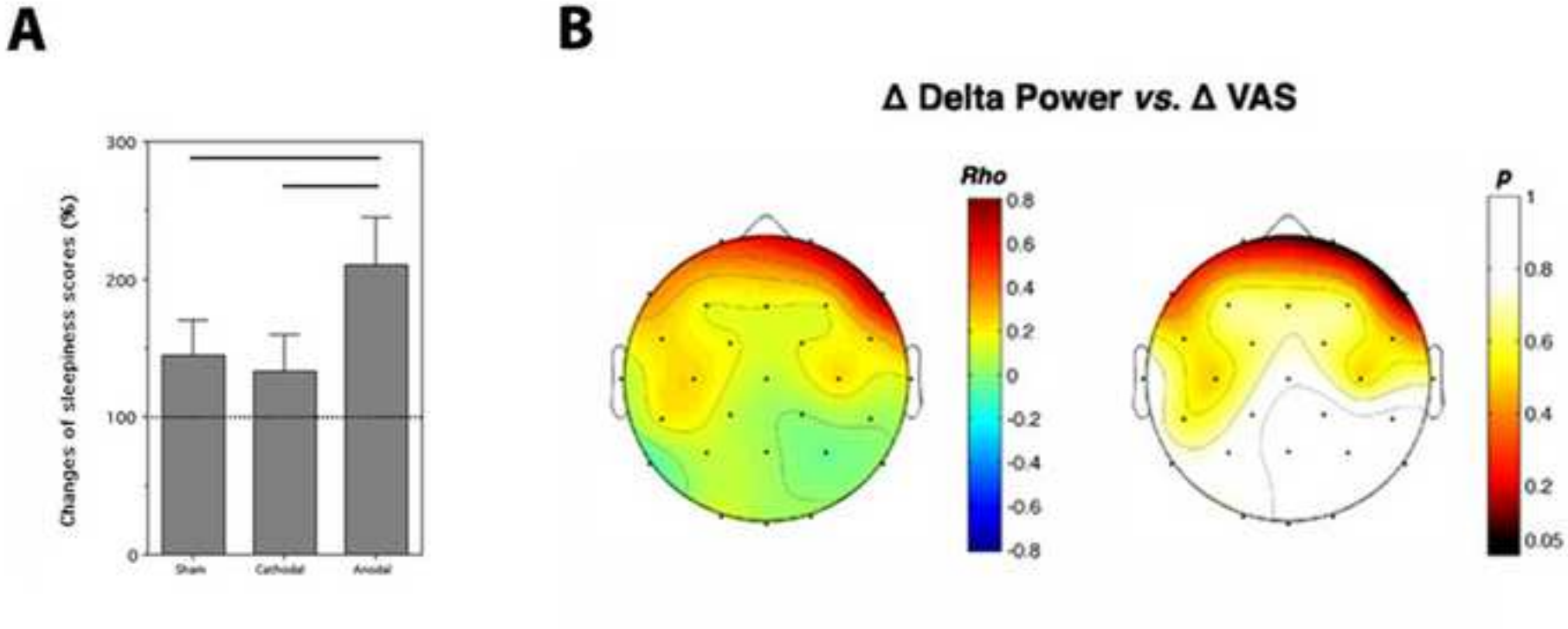


Figure 5



Highlights

- 5 Hz more than 0.8 Hz anodal tDCS is effective in inducing EEG synchronization
- 5 Hz anodal tDCS as compared to sham induces an enhancement of sleepiness
- Cortical topography of delta EEG changes is regionally related to sleepiness