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Increase in EEG Alpha-to-theta Ratio After transcranial Direct Current Stimulation (tDCS) in Patients with Disorders of Consciousness: A Pilot Study

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Abstract

Background: Transcranial direct current stimulation (tDCS) has emerged as a potentially effective intervention for improving consciousness levels in patients with disorders of consciousness (DoC). Most studies demonstrating benefits have targeted the left dorsolateral prefrontal cortex (DLPFC). However, inconsistent results have been reported across studies, and the brain effects of the stimulation remain unclear.

Objective: This work aimed to investigate the effects of a tDCS treatment on brain reactivity at rest in patients with DoC.

Methods: A 10-session tDCS treatment was administered over the left DLPFC in a group of patients with DoC. The effect of this stimulation was tested by combining the conventional behavioral assessment, conducted with the Coma Recovery Scale-Revised (CRS-R), with a quantitative analysis of the resting-state electrical brain activity, measured by electroencephalography (EEG).

Results: Following treatment, there was a slight improvement in CRS-R scores. More importantly, an increase in the ratio between alpha and theta power over posterior scalp regions was observed, even in patients who did not show changes in CRS-R scores.

Conclusions: These findings suggest that adding a simple quantitative EEG analysis alongside conventional clinical assessment post-tDCS could enhance the detection of changes in brain reactivity.

Keywords

disorders of consciousness, tDCS, DLPFC, EEG, brain reactivity, coma recovery scale-revised, CRS-R

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Introduction

Undoubtedly, new technologies and the effectiveness of intensive care have significantly reduced the mortality of patients suffering from neurocritical diseases, such as those with disorders of consciousness (DoC) resulting from severe brain injuries. Consequently, the number of patients experiencing these disorders has considerably increased (Schnakers, 2020; Wade, 2018). When the loss of consciousness is prolonged, patients may continue to show signs of coma or may develop unresponsive wakefulness syndrome (UWS), previously known as a vegetative state (VS; Bernat, 2006; Giacino et al., 2014). In some cases, they shift from this condition into a minimal consciousness state (MCS) and, later, may show signs of emergence from MCS (eMCS). In the coma state, there is

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a complete failure of the arousal system, with an inability to awaken even in response to vigorous sensory stimulation. Unresponsive wakefulness syndrome (i.e., vegetative state, thereafter called UWS/VS) is characterized by spontaneous or stimulus-induced eye opening, without signs of awareness of the environment. In contrast, patients with MCS show repeatable, although fluctuating, signs of awareness, such as the presence of intentional (non-reflexive) responses, but they are unable to communicate functionally. The behaviour of MCS patients may range from localizing noxious stimuli to reaching for objects, from detecting sound direction to following commands, from sustaining visual fixation to tracking moving stimuli, and from producing contingent to intelligible verbalizations (Giacino et al., 2002). Emergence from MCS starts when patients can follow a command and regain the capacity for functional communication or object use.

The clinical management of patients with DoC remains challenging and therapeutic options are limited (Thibaut et al., 2019). In recent years, the use of non-invasive brain stimulation techniques, in addition to pharmacological, neurosurgical, and rehabilitation interventions, has been tested. Most studies have focused on the effect of treatments based on transcranial direct current stimulation (tDCS; see Barra et al., 2022; Dong et al., 2023; Feng et al., 2020; Hu et al., 2023; Ma et al., 2023; Thibaut et al., 2019; Zhang & Song, 2018 for reviews). This technique can modulate neural excitability during and/or after delivering a weak electrical current on specific cortical regions by positioning two electrodes over the scalp, an anode and a cathode (Romero Lauro et al., 2014). In particular, the anodal stimulation is known to shift neural membrane potentials toward greater depolarization, resulting in increased neural firing rates and cortical excitability. Conversely, cathodal stimulation moves the membrane potential toward greater hyperpolarization, thereby decreasing neural firing rates and decreasing cortical excitability (Lefaucheur et al., 2017). tDCS has been shown to be a safe and effective tool that boosts brain plasticity, with the clear advantage of being inexpensive and simple to use. More importantly, in the case of multiple-session stimulation, tDCS can induce long-term effects by acting on metaplasticity.

Among the areas targeted for tDCS intervention in patients with DoC, numerous randomized controlled trials have shown that targeting the left dorsolateral prefrontal cortex (DLPFC) is the most beneficial (see the abovementioned reviews). This evidence suggests that tDCS has the potential to enhance the responsiveness of patients with DoC, at least in some patients; however, the mechanisms underlying these changes are still not fully understood.

One of the most recent studies testing the effect of a multiple-session tDCS on the level of consciousness is that of Martens et al. (2018). The authors applied a home-based tDCS protocol (at 2 mA for 20 min, five days a week) over the left DLPFC of patients with chronic MCS. Patients underwent ten real and ten sham sessions in a cross-over design. The potential therapeutic effect of the treatment was tested with the Consciousness Recovery Scale-Revised (CRS-R; Giacino et al., 2004). This behavioral scale is the most extensively used tool for clinically examining the level of consciousness. It consists of hierarchically ordered items that assess the patient's response to a various type of stimuli or commands, presented in a standardized manner. For instance, they assess the head or eyes orientation toward a sound presented behind the patient. The scale expressly incorporates the Aspen Workgroup diagnostic criteria for VS, MCS, and eEMC (Giacino et al., 2002), allowing the examiner to diagnose based directly on bedside behavioral observations. At the end of the treatment, the authors found an improvement in the CRS-R total score in the real stimulation relative to the sham protocol as long as patients received at least 80% of the planned sessions (Martens et al., 2018).

More recently, the same protocol was applied for twenty sessions in a multicentre randomized control study conducted in hospitalized settings (Thibaut et al., 2023). Patients received real or sham tDCS over the left DLPFC in a between-groups design. No differences between the effect of real and sham tDCS were found during sessions and immediately after them. However, an increase in the CRS-R total score was seen for MCS patients at 3-month follow-up.

We might attribute the contrasting results provided by these two studies to their reliance on the CRS-R exclusively, which is based on the examiner's observation of the patient and does not account for possible motor and communication impairments. Moreover, it does not provide direct measures of brain reactivity. To overcome these limitations and reconcile contrasting evidence, some studies have combined the use of CRS-R with an electroencephalography (EEG) assessment, although this requires a longer setup.

It is well known that the integrity of thalamocortical connections is essential for maintaining wakefulness and consciousness (Edlow et al., 2021; Llinás et al., 1999). Examination of spontaneous EEG rhythms of patients with DoC at rest has revealed that the EEG power spectrum is concentrated in very low frequencies, likely reflecting a deafferentation of the cortex from subcortical structures. Specifically, in MCS, a peak of activity in the theta range (3–7 Hz) has been found, whereas in normal wakefulness, the EEG power spectrum contains a complex mix of frequencies with a peak in the alpha range (8–12 Hz) (Chennu et al., 2014; Schiff et al., 2014).

First, Estraneo et al. (2017) conducted a qualitative EEG analysis immediately before and after a 5-session tDCS stimulation over the left DLPFC (at 2 mA for 20 min, five days a week). Although improvements in the CRS-R total score were found in some patients (MCS vs. UWS/VS with shorter time post-injury), the visual analysis of EEG at rest did not show changes. The authors concluded that this analysis was not sufficient to detect variations.

Subsequent studies employed quantitative EEG analyses. A study conducted by Cavinato et al. (2019), with a cross-over design, found that soon after ten sessions of tDCS over the left DLPFC (stimulation protocol similar to Estraneo et al., 2017), the group of patients with prolonged MCS, but not the UWS/VS patients, showed an improvement in the score on a behavioral scale after the real but not the sham stimulation. Importantly, they also showed an increase in frontal and posterior high-frequency (beta) EEG power and a decrease in low-frequency (delta) power.

More recently, Han et al. (2022) examined the EEG power after applying ten sessions of real tDCS with a stimulation protocol similar to Cavinato et al. (2019). They found an increase in the alpha (10–13 Hz) range after a single tDCS session already, and an increase in the upper theta (6–8 Hz) range after the entire treatment, in those patients who showed an improvement in the CRS-R scores relative to baseline (responders) but not in patients who did not show this improvement (non-responders). Remarkably, the findings were independent of the MCS or UWS/VS status. Similarly, Hermann et al. (2020) found that after a single 20-min session of anodal tDCS of 2 mA over the left DLPFC, responders, in comparison with non-responders, showed an increase in the alpha power over parietal cortices. Furthermore, they showed an increase in power and long-range cortico-cortical functional connectivity from 4 to 10 Hz (theta and lower alpha bands).

Taken together, the EEG findings show that an improvement in the level of consciousness after an active tDCS treatment might induce an increase in higher frequency power (alpha and beta bands) and a decrease in lower frequency power (delta and theta bands). This suggests that the tDCS treatment over the left DLPFC had an impact on the power ratio between higher and lower frequencies.

In the present study, we treated a group of patients with DoC with the previously validated multiple-session tDCS protocol over the left DLPFC and tested the effect of the stimulation by conducting a quantitative analysis of the spontaneous EEG rhythms before and after the treatment. Specifically, we extracted power spectrum differences in every band (delta, theta, alpha, and beta). We extended previous research by focusing the analyses on the ratio of power between the higher frequency ranges and the lower frequency ranges. Namely, we examined the theta/delta, alpha/theta, and beta/alpha ratios. This analysis has the advantage of detecting variations in intrinsic brain activity even when the power changes concurrently in multiple frequencies (Ambrosini & Vallesi, 2016) and would represent a straightforward and easy-to-use clinical tool. The underlying hypothesis was that a transition to a higher level of consciousness would imply an increase in power in higher-frequency bands relative to lower-frequency bands.

Methods

Participants

The study was conducted in accordance with the ethical standards for experiments on human subjects stated in the Declaration of Helsinki. The research protocol was approved by the local Ethics Committee (Lazio 1, protocol n. 107), and written informed consent was obtained from the patient's relatives or legal guardians.

We enrolled a series of patients who were consecutively admitted to the "Buccheri La Ferla – Fatebenefratelli" hospital in Palermo (Italy) with acute brain injuries from various causes and who were, at the time of assessment, in either a vegetative state (UWS/VS) or a minimally conscious state (MCS). The patients were hospitalized in the Physical and Rehabilitation Medicine unit specialized in neurological conditions, providing long-term care and rehabilitation therapies. The presence of a disorder of consciousness was clinically determined and confirmed by the CRS-R assessment. Patients were included if they had a diagnosis of UWS/VS or MCS caused by a brain injury (trauma, stroke, anoxia) and were older than 18 years. The exclusion criteria were severe medical complications occurring during the hospital stay, pacemakers, metallic head implants, incomplete cranial bone, premorbid deafness, history of acquired brain injury, severe medical conditions, or neurological/neurodegenerative diseases (such as dementia and epilepsy).

A total of 19 patients were recruited for this research. Among them, 12 patients comprising various aetiologies of brain injuries were included in the study. Data are reported in Table 1.

Table 1. Patients' Characteristics. MCS, Minimal Consciousness Status; UWS/VS, Unresponsive Wakefulness Syndrome/Vegetative Status.

ID	Age	Sex	Diagnosis	Etiology	Time From Brain Injury (months)
1	53	F	MCS	Traumatic	1.5
2	41	F	MCS	Vascular	19
3	51	F	MCS	Vascular	20.9
4	35	F	UWS/VS	Anoxic	0.8
5	72	M	MCS	Traumatic	1.1
6	54	F	UWS/VS	Anoxic	1.7
7	48	F	UWS/VS	Traumatic	0.7
8	30	M	UWS/VS	Traumatic	1.5
9	30	M	UWS/VS	Anoxic	83.4
10	59	F	UWS/VS	Anoxic	1.5
11	73	M	MCS	Traumatic	1.3
12	82	F	UWS/VS	Vascular	4.6

Clinical Measures

The Coma Recovery Scale-Revised (CRS-R) was administered to patients by an expert neuropsychologist (SF). The scale includes 23 items grouped in six subscales that assess auditory, visual, motor, oromotor/verbal, communication, and arousal/vigilance processes. Each subscale is scored on a Likert scale, ranging from 0 to 2–6 points. The total score ranges from 0 to 23, with higher scores indicating higher neurological function and a better prognosis. The lowest score represents reflexive activity, while the highest score describes cognitively mediated behaviors. The scale was administered at the patient's bedside, before and after the tDCS protocol.

tDCS Protocol

The tDCS protocol was administered to patients by an expert neuropsychologist (MLF). Direct current stimulation was delivered at 2 mA for 20 min, with a ramp-up/down time of 30 s, through a battery-driven electric stimulator (BrainStim, E.M.S., Bologna, Italy). The battery was connected with two $5 \times 5\ cm^2$ rubber electrodes inserted in dedicated saline-soaked sponges. The anode (active electrode) was positioned over the left DLPFC (F3 site, according to the International 10–20 EEG system), and the cathode (return electrode) was positioned over the supraorbital right site (Fp2). Electrode impedance was kept below 10 kΩ.

EEG Recording and Pre-processing

The EEG signal was acquired from 12 electrodes, applied over the scalp by an expert neurophysiology technician (AB), and distributed from frontal to occipital sites according to the 10–20 international system (Fp1, Fp2, F3, F4, C3, C4, P3, Pz, P4, O1, O2). The electrodes located at Cz and Fpz served as the online reference and ground, respectively. Electrode impedance was adjusted until it was kept below 5 kΩ. The signal data were digitized at a sampling rate of 128 Hz (EB-Neuro system, Florence, Italy). No online filters were applied. The EEG recording started as soon as the patients rested and lasted 10 min, or a little longer if movement artifacts were observed. The patients were behaviorally awake and resting, with their eyes closed. Spontaneous eye opening was included in the recording, while, in the case of the appearance of visible drowsiness or sleep signs in the EEG, the recording was stopped.

The EEG data were processed with the EEGLAB toolbox (Delorme & Makeig, 2004). First, the data were filtered with a low-pass filter at 45 Hz (cutoff) using a zero-phase Kaiser-windowed sinc FIR filter (beta = 5.653, transition bandwidth = 1 Hz; see Widmann et al., 2015). Subsequently, the same filter with a high-pass cutoff at 0.5 was applied.

The raw EEG trace was visually inspected, and segments with large artifacts, mainly due to spontaneous movements, were manually removed. Then, the artifact components in the signal were identified by an Independent Component Analysis (ICA). On average, 4.1 components (SD = 1.48, range = 1–6) were removed, mostly due to head and/or eye movements and muscle contractions. Afterwards, the EEG trace was segmented into 2-s epochs. A further visual inspection was performed, and epochs with residual noise were excluded from subsequent analyses (on average, 2.1% of segments, SD = 2.2, range = 0–2.7, for each patient).

The relative spectral power of the electrical activity was computed by the *spectopo* function (frequency range = 1–45 Hz; resolution = 0.75 Hz). The mean difference between post- and pre-tDCS sessions was computed in the delta (1–4 Hz), theta (4–7.5 Hz), alpha (7.5–12.5), beta (12.5–30 Hz) frequency band. In addition, the theta/delta, alpha/theta, and beta/alpha ratios were computed.

Two patients were excluded from the EEG analysis because of frequent involuntary movements; one patient was excluded because of technical issues in signal acquisition.

Experimental Procedure

The tDCS treatment included a 20-min stimulation repeated for ten separate days, within two consecutive weeks. The CRS-R assessment was performed before the first and after the last tDCS session, respectively. The study was a quasi-experimental research with a post- vs. pre-test design. The EEG recording was performed after the pre-CRS session and after the post-CRS session. During tDCS, EEG, and CRS-R assessments, the patient was positioned in a comfortable position in the bed. Throughout the study, the patients continued the conventional treatment scheduled by the hospital.

Statistical Analyses

Given the non-normal distribution of the CRS-R scores, a Wilcoxon signed-rank test was performed to test pre- vs. post-tDCS differences. Based on previous literature and to highlight the anterior-posterior gradient, two representative electrodes were chosen for the EEG power analysis, i.e., Fz and Pz, and submitted to a 2 (pre- vs. post-tDCS) × 2 (Fz vs Pz) repeated-measures ANOVA, for each frequency band. Statistical analyses were implemented in SPSS 23.

Results

No tDCS-related serious adverse effects were observed. In one case, the treatment was interrupted at the sixth

Table 2. Mean Scores (SD) on the Coma Recovery Scale-revised (CRS-R) Subscales.

CRS Subscales	Pre	Post	*p*
Auditory	0.63 (0.67)	1.54 (1.13)	*.059*
Visual	1.18 (1.47)	1.82 (1.78)	.18
Motor	1.63 (1.03)	2.09 (1.51)	.13
Oromotor/verbal	0.73 (0.47)	1.27 (0.9)	*.059*
Communication	0.09 (0.3)	0.45 (0.69)	.102
Arousal/vigilance	1.45 (0.69)	1.73 (0.9)	.083
Total score	5.73 (3.1)	8.91 (6.17)	**.046**

P-values < .1 are in italics; p-values < .05 are in bold.

session because the patient showed discomfort. After tDCS, five patients showed an improvement in the CRS-R score; among them, three showed signs of emergence from MCS.

Clinical Measures

As shown in Table 2, overall mean scores on the total as well as single subscales increased after stimulation. The Wilcoxon test revealed a significant effect of time (pre vs. post) on the total CRS-R score (Z = 2, $p = .046$). However, differences in scores on the single subscales did not reach statistical significance.

EEG

When considering single frequency bands, no significant pre- vs. post-tDCS differences emerged. However, when considering the alpha/theta power ratio, the ANOVA yielded a significant interaction between time (pre vs. post) and electrode (Fz vs. Pz) ($F_{(1,8)} = 5.18$, $p = .05$). The LSD post-hoc analyses revealed a significant increase in the alpha/theta power over Pz relative to Fz in the post-tDCS ($p = .038$) but not in the pre-tDCS ($p = .667$) session. Figure 1 shows the scalp distribution of the post-pre difference in the alpha/theta ratio.

To explore the relationship between the increase in alpha/theta over the parietal sites and the increase in the CRS-R clinical scale, a non-parametric correlation was performed. The pre- vs. post-tDCS difference in the alpha/theta power ratio did not correlate significantly with the pre- vs. post-tDCS difference in the CRS-R total score (Spearman's rho = .451, $p = .223$).

Individual responses are plotted in Supplementary Materials. The data of each patient are available at https://osf.io/sm6b5/?view_only=f279e041e1104b33b43bf3d812c3067c

Discussion

In the present study, we investigated whether ten sessions of anodal tDCS (20 min at 2 mA) over the left DLPFC could improve brain activity in a group of patients with DoC. To examine the effect of the stimulation, we combined a conventional behavioral assessment (i.e., the CRS-R score) with the analysis of spontaneous resting-state EEG rhythms collected before and after the treatment. Unlike previous studies, we used a simple EEG quantitative analysis approach, which assesses the ratio of power across frequency bands. Specifically, we analysed the theta/delta, alpha/theta, and beta/alpha ratios. In addition, unlike previous studies (Cavinato et al., 2019; Hermann et al., 2020), we did not group patients according to the CRS-R score, given that this behavioral measure might not always capture differences in patients' reactivity.

After the treatment, we observed a slight but significant increase in the total CRS-R score at the group level. Furthermore, we detected an increase in the alpha/theta ratio at a posterior/parietal scalp site (Pz). Overall, these findings suggest that tDCS effectively modulated brain activity, with posterior/parietal regions being affected by stimulation of a frontal region.

These findings are consistent with previous studies that used the same tDCS protocol over the left DLPFC and reported an increase in the score on CRS-R (Barra et al., 2022; Dong et al., 2023; Feng et al., 2020; Hu et al., 2023; Ma et al., 2023; Thibaut et al., 2019; Zhang & Song, 2018). Furthermore, our results align with the increase of alpha power over the parietal cortex observed in studies with single-session anodal tDCS over the left DLPFC (Hermann et al., 2020). We extended previous results by demonstrating that the examination of the ratio of EEG power between low- and high-frequencies after a multiple-session tDCS treatment can reveal changes in background brain activity, even when differences in

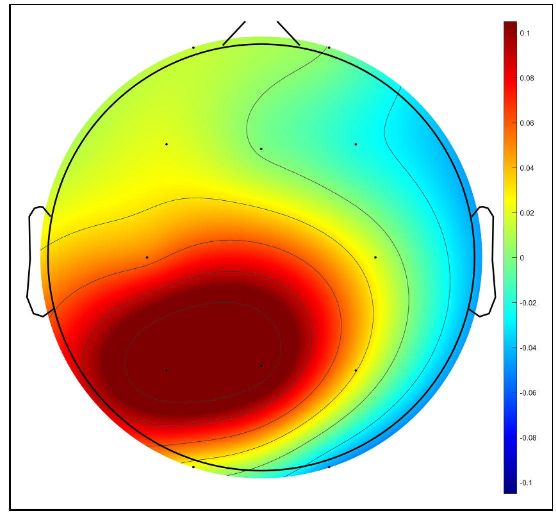

Figure 1. Power Spectral Density of the Alpha/Theta Ratio in the Post-tDCS Period Relative to the Pre-tDCS Period. EEG Power is Expressed in dB.

single frequency bands do not emerge. Consistent with earlier EEG studies in DoC patients, and considering the weak background alpha power in patients with DoC, we may speculate that the tDCS could have induced a spectral shift from the theta to the alpha band (Chennu et al., 2014; Thibaut et al., 2018). Notably, the increase in alpha power relative to theta is consistent with evidence linking alpha functional connectivity to the degree of consciousness recovery in patients with DoC (Chennu et al., 2017).

A similar re-balance between low- and high-frequency power has been observed in patients with traumatic brain injury, without DoC, undergoing neurorehabilitation. For example, Ulam et al. (2015) reported a decrease in delta activity after the first session anodal stimulation to the left DLPFC, followed by an increase in alpha activity after ten consecutive sessions. The study suggested that changes in EEG power across different frequency bands may reflect modifications in cortical excitability associated with neurorehabilitation outcomes. Moreover, previous studies in healthy volunteers have shown increased cortical oscillation activity in posterior alpha bands following anodal DLPFC tDCS (Keeser et al., 2011b; Zaehle et al., 2011).

The fact that posterior regions were modulated by an anterior stimulation suggests that tDCS acted on a fronto-parietal network node. These findings are in line with previous EEG evidence demonstrating changes in fronto-parietal connectivity in the theta band after ten sessions of tDCS over the left DLPFC in patients with DoC (Han et al., 2022). Accordingly, a functional magnetic resonance study with healthy volunteers has shown that a single session of tDCS applied for 20 min at 2 mA, with the anode positioned over the left DLPFC and the cathode over the right supraorbital region, induces significant changes in the brain connectivity within the fronto-parietal networks (Keeser et al., 2011a). Interestingly, in the context of DoC, studies have found that the level of consciousness and the associated recovery are mediated by changes in intrinsic functional connectivity of resting state brain networks, particularly those involving long-range connections (King et al., 2013). These networks encompass lateral fronto-parietal areas responsible for awareness of external inputs and medial brain areas for awareness of internal processes (Cavaliere et al., 2016; Vanhaudenhuyse et al., 2011; Wu et al., 2015). Therefore, tDCS likely exterted a direct effect on cortical excitability, potentially counteracting underactivation in lateral fronto-parietal regions (e.g., Bai et al., 2017). Additionally, the study of Zhang et al. (2022) complements our findings by demonstrating that patients who responded to anodal stimulation over Pz exhibited increase EEG power in the alpha and beta bands at frontal and parietal sites.

The absence of a significant correlation between the increase in alpha/theta power and the change in the CRS-R score suggests that not all patients who exhibited increased alpha/theta power showed a corresponding increase in the CRS-R scores. This discrepancy indicates that changes in brain activity may not always be observable at a behavioral level. Similar incongruities between behavioral scales and neuroimaging methods in assessing patients' responsiveness have been discussed in the literature (Stafford et al., 2019).

Several limitations in our study should be acknowledged before generalizing the results. Firstly, the lack of a sham control group means we cannot exclude the possibility that the observed effects were influenced by other factors, such as spontaneous recovery. Given the quasi-experimental design, it is important that the results will be integrated in the future with data from a controlled study to assess their relevance for neurorehabilitation practice. Additionally, the informative value of a single EEG recording may be questionable since EEG patterns can vary over time due to fluctuations in wakefulness or evolving clinical conditions (Kulkarni et al., 2007). The small sample size of patients undermines the robustness of the results and necessitates further replication with larger cohorts. Moreover, the limited sample size precludes comparisons between patients with different diagnoses and aetiologies of DoC.

Despite these limitations, our study reaffirms that left DLPFC-tDCS represents a promising tool for boosting other rehabilitation treatments aimed at promoting brain activity and aiding patients' recovery from DoC. Furthermore, it suggests that analyzing the alpha/theta ratio over posterior brain regions in standard EEG recordings could provide a simple as well as straightforward measure to assess brain reactivity in patients with DoC.

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Supplemental Material

Supplemental material for this article is available online.

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