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Behavioural and electrophysiological effects of tDCS to prefrontal cortex in patients with disorders of consciousness

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HIGHLIGHTS

- Left dorsolateral prefrontal cortex tDCS was applied in patients with disorders of consciousness.
- EEG coherence and spectral analysis were used to determine brain changes.
- Therapeutic effects of tDCS rely on modulation of fronto-parietal connectivity in patients with residual consciousness.

ABSTRACT

Objectives: Left dorsolateral prefrontal cortex anodal transcranial direct current stimulation (tDCS) was applied in a group of patients with disorders of consciousness to determine the effects of modulation of spontaneous oscillatory brain activity.
Methods: 12 patients in an unresponsive wakefulness syndrome (UWS) and 12 in a minimally conscious state (MCS) underwent 2-weeks active and 2-weeks sham tDCS. Neurophysiological assessment was performed with EEG power spectra and coherence analysis directly before and after each session.
Results: An increase of power and coherence of the frontal and parietal alpha and beta frequency bands and significant clinical improvements were seen after the active tDCS in MCS patients. In contrast, UWS patients showed some local frontal changes in the slow frequencies. No treatment effect was observed after sham.
Conclusions: tDCS could induce changes in cortical EEG oscillations, modulating the travel of alpha and beta waves between anterior and posterior brain areas when some cognitive functions were preserved. This plays an important role in consciousness by integrating cognitive-emotional processing with the state of arousal. In unresponsive people, brain integration seems to be lost.
Significance: Our results further support the critical role of long-range fronto-parietal connections in consciousness and show the potential therapeutic utility of tDCS.

1. Introduction

Patients with unresponsive wakefulness syndrome (UWS) are clinically characterized by the absence of any discernible behavioural evidence of consciousness. They do not have any voluntary motor responsiveness, except stimulus induced stereotyped movements, and their autonomic functions can be preserved (Sarà et al., 2012). Sometimes UWS is transitional, evolving in a partial recovery of consciousness that can be attributable to the minimally conscious state (MCS). MCS is characterized by the presence of limited, but explicit behaviours, demonstrating evidence of limited awareness. Patients can present with visual tracking, noxious localization and movement/verbalization in response to command.

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Often, MCS patients can be difficult to differentiate from UWS, because their conscious behaviour may be slight and infrequent. Nevertheless, differentiation is crucial because patients in MCS have significantly better prognosis for recovery that could be promoted by appropriate therapeutic interventions.

At present, however, therapeutic approaches are limited and there are no evidence-based recommendations for them.

Several efforts have been made to define effective pharmacological interventions, such as aminergic and neurostimulant agents, or rehabilitation plans that include, among others, the recent use of transcranial brain stimulations.

Transcranial direct-current stimulation (tDCS) is a non-invasive technique that modulates the excitability of targeted cerebral areas through low direct current stimulation from the scalp using a pair of sponge electrodes. tDCS induces changes in the neuronal membrane resting potentials, influence the level of neuronal excitability, and modulate firing rates (Nitsche et al., 2011). These short and long-lasting effects can effectively affect attention, working memory and executive functions and have potential beneficial neurorehabilitation effects (Monti et al., 2013; Nitsche et al., 2012; Rothwell, 2012; Piccione et al., 2011; Calvo-Merino and Haggard, 2004).

However, the positive findings on cognitive tasks of tDCS collide with numerous recent reports showing inconsistent effects and a low reproducibility of this technique.

These variable and contradictory data could be due to the lack of standardization of stimulation/assessment protocols, being tDCS employed over numerous brain areas, in a variety of behavioural tasks, making comparisons across studies difficult. The same neuronal excitability changes induced by tDCS can vary based on stimulation polarity. Anodal stimulation is expected to depolarize the pyramidal neuron soma, which may increase excitability (Radman et al., 2009). Conversely, a cathodal stimulation can produce hyper-polarization, decreasing excitability. However, dendrites and axons polarization and inversions across single brain gyri produce fluctuation of current flow even locally under the electrode, making simplistic to refer to anodal stimulation as "depolarizing" and, viceversa, cathodal stimulation as "inhibiting".

All these aspects denote a high individual variability of tDCS effects (Woods et al., 2016). Certainly, tDCS represents a potential therapeutic tool for disorders characterized by electrophysiological and behavioural abnormalities. However, only a handful of very recent studies have focused on the efficacy of transcranial stimulation on improvement of patients with disorders of consciousness (DOCs) (Thibaut et al., 2014; Angelakis et al., 2014). In both the cited studies, a significant transient clinical effect immediately after treatment was observed in MCS patients, but not in patients with UWS. A possible explanation for this lack of specific literature could be related to the inconsistency of evidence-based treatment of DOCs and the insufficient knowledge of the specific mechanisms mediating the tDCS-induced effects in cortical excitability. In the attempt to clarify these points, power spectrum and coherence of spontaneous electroencephalogram (EEG) have been used to describe electrophysiological changes induced by tDCS (Vecchio et al., 2016; Accornero et al., 2014). EEG reflects the oscillatory activity within an area or across circuits, thereby aiding determination of the brain areas directly or indirectly affected by tDCS. Moreover, EEG can provide additional support for the development of uniform stimulation protocols in patients with disorders of consciousness giving information about the modulation of cortical excitability and behaviour induced by tDCS. Different EEG responses to transcranial stimulation can represent an important and non-invasive quantitative tool to discriminate different levels of consciousness.

In this multicenter study, we aimed to assess EEG coherence and power spectra changes, as well as behavioural evidence of recovery of consciousness after anodal tDCS over the dorsolateral prefrontal cortex (DLPFC) in patients with different levels of DOCs. In particular, we followed two groups of patients with unresponsive wakefulness syndrome and minimally conscious state.

2. Methods

2.1. Patients

Twenty-six DOC patients (mean age ± SD, 53 ± 19; 10 women and 16 men) following hypoxic-ischemic or traumatic brain damage were enrolled in the study. All participants were hospitalized and prospectively recruited in the Neurorehabilitation Departments of Foundation San Camillo Hospital (Venice, Italy), Sacro Cuore Don Calabria (Verona, Italy), and Casa dei Risvegli Luca de Nigris (Bologna, Italy). The inclusion criteria were as follows: patients in stable clinical condition with more than four months after the brain insult, single first-ever ischemic stroke or traumatic brain injury, clinically diagnosed with UWS/MCS. The exclusion criteria included history of seizure, epileptiform EEG alterations, implanted metal object and pacemakers, no concurrent use of medications altering effects of tDCS (benzodiazepines, anticonvulsants, dextromethorphan and pseudoephedrine) (Liebetanz et al., 2002; Nitsche et al., 2004). Patients with other neurological and psychiatric disorders were also excluded. Medication and therapies were kept unchanged throughout the whole experiment.

The level of consciousness was evaluated by means of the JFK Coma Recovery Scale-Revised (Giacino et al., 2004) and the Western NeuroSensory Stimulation Profile (WNSSP). The WNSSP is developed to evaluate cognitive function in patients with severe brain lesions, to monitor and predict changes of patients with altered state of consciousness. This scale consists of 32 items that evaluate patient's attention/awakening and his abilities, the expressive communication, and reactions to acoustic, visual, olfactory, tactile stimulations.

Before enrolment, all patients were evaluated by a trained and experienced blinded neurologist and ascribed to the group of UWS or MCS based on a cut-off score of 8 on the JFK-CR scale. This score provides the best balance between true positive and true negative rates, accurately classifying 93% of cases (Yelena et al., 2016).

Legal representatives gave written informed consent for the patients to participate in research. The study was approved by the Research Ethics Committee of San Camillo Foundation and was compliant with the declaration of Helsinki guidelines.

2.2. Study design and tDCS treatment

The study was designed as a randomized, double blind, crossover study. A computerized random-number generator was used to allocate the first stimulation session as real or sham tDCS, following a stratified randomization by study center.

The treatment protocol, as shown in Fig. 1, consisted of 20 minutes per day of stimulation over the left DLPFC. Our preference about the site and polarity of stimulation was dictated by the indication that anodal tDCS on the left DLPFC seems to enhance high-level processes requiring consciousness, and particularly in maintenance of sensory information in memory, visual perception and perceptual decision (Binder et al., 2004; Curtis and D'Esposito, 2003; Summerfield et al., 2006;Fregni et al., 2005; Mulguiney et al., 2011; Seidler et al, 2017). Furthermore, we opted to use an extracephalic reference (the contralateral shoulder) to result in a current flow targeting the source of frontal areas.

Anodal tDCS was delivered at an intensity of 2 mA using saline-soaked 5×7 cm sponge electrodes and a constant current

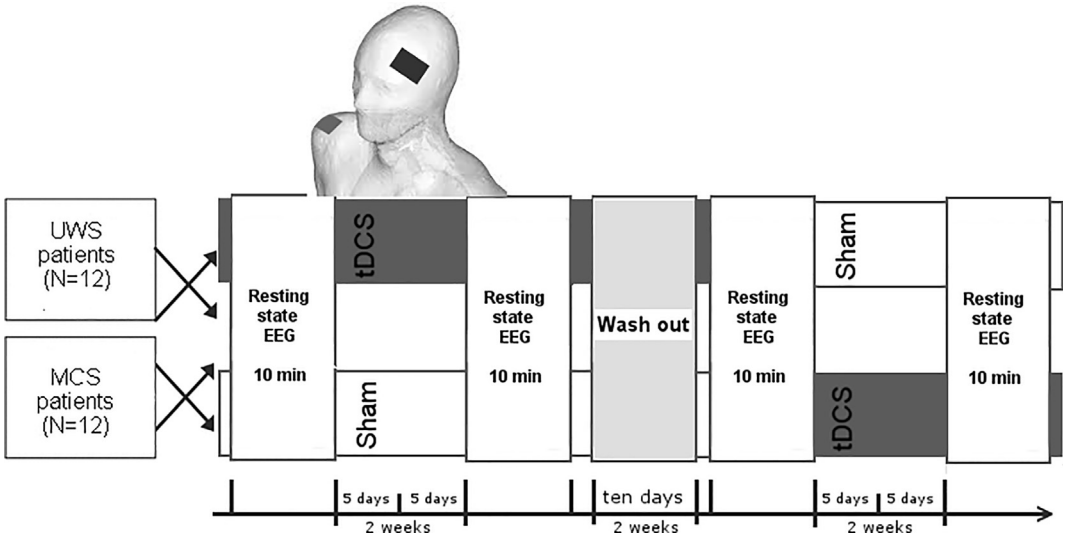

Fig. 1. Flow diagram of the stimulation protocol.

electrical stimulator, BrainStim (E.M.S. srl, Italy). The active stimulation electrode was placed over the left dorsolateral prefrontal cortex localized using the international 10–20 EEG system and defined as F3. The reference electrode was applied to the deltoid muscle of the opposite side in order to avoid any electrical influence on other brain areas.

In the case of sham tDCS, sponge electrodes were placed in the same position as in the actual stimulation. The device was set up with a stimulation time of 60 seconds to imitate the perceived sensations of tingling of real tDCS. This parameter was based on previous reports describing that skin sensations usually fades out in the first 60 seconds of tDCS (Paulus, 2003).

In both tDCS and sham, the current was initially increased in a ramp-like fashion over 30 s until reaching 2 mA and turned off slowly over 30 s.

The investigator applying tDCS was blinded to the stimulation condition. This was facilitated by the employed tDCS device that offers protocols of stimulation (real and sham) that can be customized and programmed via a dedicated computer software.

The treatment involved 2-week active and 2-week sham tDCS with a washout period of ten days between the two experiments. The order of stimulations was counterbalanced across subjects.

2.3. Clinical and neurophysiological assessment

Before starting each EEG session, patients were assessed along the JFK CRS-R and the WNSSP scales.

To investigate the neurophysiological effects of tDCS (active or sham), we recorded a 10-min electroencephalogram immediately before and after each stimulation session. EEG was started only if patients had their eyes open spontaneously, the eyelids were then kept closed by a hand until the end of recording. All patients opened their eyes spontaneously at the end of EEGs. This and a real time check of traces for drowsiness or sleep onset suggested an unchanged level of wakefulness throughout registration.

The EEG was recorded using 19 silver electrodes placed according to the 10–20 system montage (Galileo Mizar Sirius amplifiers, EbNeuro Spa, Italy). All channels were referenced to the mastoid, and the ground electrode was placed at the FPz. Eye movements (electrooculogram, EOG) were monitored by bipolar recording from electrodes at the right corner of the eyelid. Electrocardiogram was also detected by two electrodes under the clavicles, midclavicular line. The data were processed in Matlab 7 (MathWorks, Natick, MA) using scripts based on EEGLAB (http://www.sccn.ucsd.edu/eeglab). The EEG from each electrode site was amplified and digitized at 250 Hz. During pre-processing stage, the signals were filtered using a two-way least-squares FIR filter between 0.5–50 Hz. A notch filter in 47–50 Hz was applied to remove power line interference. The EEG data were segmented into single 2s-epoches, adjusted through ocular correction and rejection of artefact exceeding an amplitude of 150 μV and further filtered (0.5–50 Hz). Epochs containing artefacts due to eye blinks, ocular and head movements were rejected with an automatic algorithm. Thus, only EEG segments free of artefacts were accepted for fast Fourier transformation (FFT) using a resolution of 0.5 Hz and a Hanning window of 10% of the length. Each participant's data were averaged across the epochs for each electrode, and the mean absolute power was computed for each of the frequency bands: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), and gamma (30–50 Hz).

Computation of the coherence from the Fourier transformed data was calculated according to the following equation:

$$Coh_{xy}(f) = |P_{xy}(f)|^2 / P_{xx}(f) P_{yy}(f)$$

where $P_{xy}(f)$ is the cross power spectral density and $P_{xx}(f)$ and $P_{yy}(f)$ are the respective power spectral densities of two signals x and y (pre and post session). The EEG coherence was analyzed and divided into the five main frequency bands. The averaged coherence of each frequency band was used for the analysis.

2.4. Statistical analysis

In order to normalize the distribution of EEG power and coherence values, logarithmic transformation of absolute power and Fisher's Z transformation of each band in each derivation was implemented. The averaged EEG power and coherence were calculated for each participant and used for the statistical analysis of three regions of interest to emphasize the connections between frontal and parietal networks. We dissociated: (1) the frontal sites, where relevant current information is held and continuously updated, defined as Fp1, Fp2, F3, Fz and F4; (2) the posterior sites, where sensory information is stored, defined as P3, Pz, P4, O1 and O2 and (3) the fronto-parietal sites identified as the connections among F3, Fz, F4, P3, Pz and P4. Analysis were undertaken independently for power and coherence data and for each of the two groups of patients.

For the power analysis, each frequency band analysis was submitted separately to a repeated-measures' ANOVA, in which 'stimulation' (Active vs. Sham), 'condition' (Pre vs. Post) and 'site' (Frontal, Posterior and fronto-parietal) were repeated within-subject factors, while 'gender' (Male vs Female) was a controlling between-subject variable. Time from trauma, age and treatment order (Active first vs. Sham first) were introduced into the model as covariates. To verify model assumptions, sphericity was tested with the Mauchly test, whereas the Kolmogorov-Smirnov test was used to test the normality of the standardized residuals at each level of the within-subject factors. In case either sphericity or normality assumptions were not met, the Greenhouse correction and the Box-Cox transformation were applied respectively. For the coherence analysis, to gain normality data was first transformed using Fisher Z transformation. Hence, for each frequency band independently, a mixed model was run since the factor 'site' (Frontal vs Posterior vs Fronto-parietal) had levels with unequal number of observations e.g. there were more Fronto-parietal pairs of sensors. 'Stimulation' (Active vs. Sham), 'condition' (Pre vs. Post), 'site' (Frontal vs Posterior v Fronto-parietal), 'gender' (Male vs Female),Time from trauma (in days), age and treatment order (Active first vs. Sham first) were considered in the model as fixed effects, with the latter three acting as controlling variables, whereas the random effect was 'subject'.

In case significant differences between the means were found by the corresponding ANOVA test or mixed model, post-hoc analyses were performed using paired t-test.

In addition, a two-way nonparametric robust rank-based ANOVA type statistic (ATS) with interaction was applied to the JFK CRS-R and the WNSSP scales independently. The factors considered were 'stimulation' (Active vs. Sham) and 'condition' (Pre vs. Post). Pearson's correlations coefficients were calculated and tested to investigate the relationship between EEG coherence changes and the clinical disability assessed by the JFK CRS-R and the WNSSP scales. Bonferroni correction was applied. Statistical significance was defined as $P < 0.05$.

3. Results

Two of the 26 eligible patients were unable to complete the protocol of stimulation because of important infection of the pulmonary tract. The data of the remaining 24 patients were analysed. Twelve of those patients were in MCS (mean age of 47 ± 17 years), 3 were anoxic, 7 traumatic, and 2 had different etiology, such as cerebrovascular accident and subarachnoid hemorrhage, admitted 32 ± 31 months after the insult. The other 12 patients were assigned to the group of UWS (mean age of 53 ± 19 years), 2 were posttraumatic, 5 anoxic, 3 had subarachnoid

Table 1
Clinical and demographic data of patients in UWS/MCS included in the study. MCS: minimally conscious state; UWS: unresponsive wakefulness syndrome; tDCS: transcranial Direct Current Stimulation.

Patient no./gender/age, yrs	Diagnosis	Etiology	MRI/CT findings	Time from trauma	tDCS/Sham order
1/M/31	MCS	Trauma	Occipital fracture, tetraventricular hydrocephalus and bilateral frontal and parietal hemorrhages.	1y	Sham
2/F/62	MCS	Cerebrovascular accident	Right temporo-parietal ischemic-hemorrhagic foci, widespread cortico-subcortical ventricular atrophy. Ischemic lesions in the right midbrain.	1y, 2mo	tDCS
3/F/77	MCS	Subharacnoid hemorrage	Right parietal and occipital hemorrhage. Ex-vacuo hydrocephalus.	2y, 10mo	Sham
4/M/30	MCS	Trauma	Cerebral edema, post-traumatic subharacnoid hemorrage, left lenticular nucleus lesions, left sub-dural hematoma with midline shift.	7y	tDCS
5/F/28	MCS	Trauma	Frontal parenchimal atrophy. Right frontal and insular hemorrhages. Mild ventricular hydrocephalus.	2y, 4mo	Sham
6/F/58	MCS	Anoxia	Diffuse anoxic-ischemic lesion	4mo	tDCS
7/M/29	MCS	Trauma	BIlateral fronto-insular and temporal atrophy. Parenchimal alterations secondary to cortico-spinal atrophy. Thinning of the corpus callosum.	7mo	Sham
8/M/50	MCS	Anoxia	Diffuse cerebral edema.	9mo	tDCS
9/M/39	MCS	Trauma	Multiple contusions in the left temporal lobe and cerebral peduncle.	5y	tDCS
10/M/41	MCS	Trauma	Cortical contusions in the left temporo-parietal lobes	8y	Sham
11/M/71	MCS	Trauma	Left frontal and parietal contusions. Mild hydrocephalus in the area of the third and lateral centricles.	2y, 4mo	tDCS
12/F/50	MCS	Anoxia	Bilateral cortical sub-cortical ischemic lesions in the occipital lobes, insula, pre and post-rolandic areas, lenticular and caudate nuclei.	7mo	tDCS
13/M/48	UWS	Anoxia	Diffuse cerebral edema.	1y, 4mo	tDCS
14/M/43	UWS	Cerebrovascular accident	Diffuse left hemisphere hypodensity. Parathalamic hematoma.	11y	Sham
15/M/84	UWS	Subharacnoid hemorrage	Right frontal and temporal hemorrhages. Mild vintricular hydrocephalus.	11y	Sham
16/M/58	UWS	Anoxia	Diffuse cerebral edema.	1y, 10mo	tDCS
17/F/74	UWS	Anoxia	Diffuse cerebral edema.	6y	Sham
18/F/84	UWS	Anoxia	Diffuse cortical atrophy.	1y, 8mo	Sham
19/M/65	UWS	Subharacnoid hemorrage	Bilateral frontal lobes contusive lesions.	6y	Sham
20/M/45	UWS	Anoxia	Diffuse cerebral edema.	3y	tDCS
21/F/53	UWS	Subharacnoid hemorrage	Hydrocephalus in the area of the lateral and third ventricles. Diffuse right frontal parenchimal malacia. Right thalamic and occipital ischemic lesions.	3y, 5mo	tDCS
22/F/47	UWS	Trauma	Frontal and left temporal cortical-subcortical hypodensity. Right posterior temporal lesion. Hydrocephalus in the area of the lateral and third ventricles.	8mo	Sham
23/M/18	UWS	Trauma	Cortical sub-cortical atrophy. Ventricular hydrocephalus. Occipital sopratentorial ipodensity.	6mo	tDCS
24/F/78	UWS	Cerebrovascular accident	Anterior temporal malacia. Ventricular hydrocephalus.	5mo	Sham

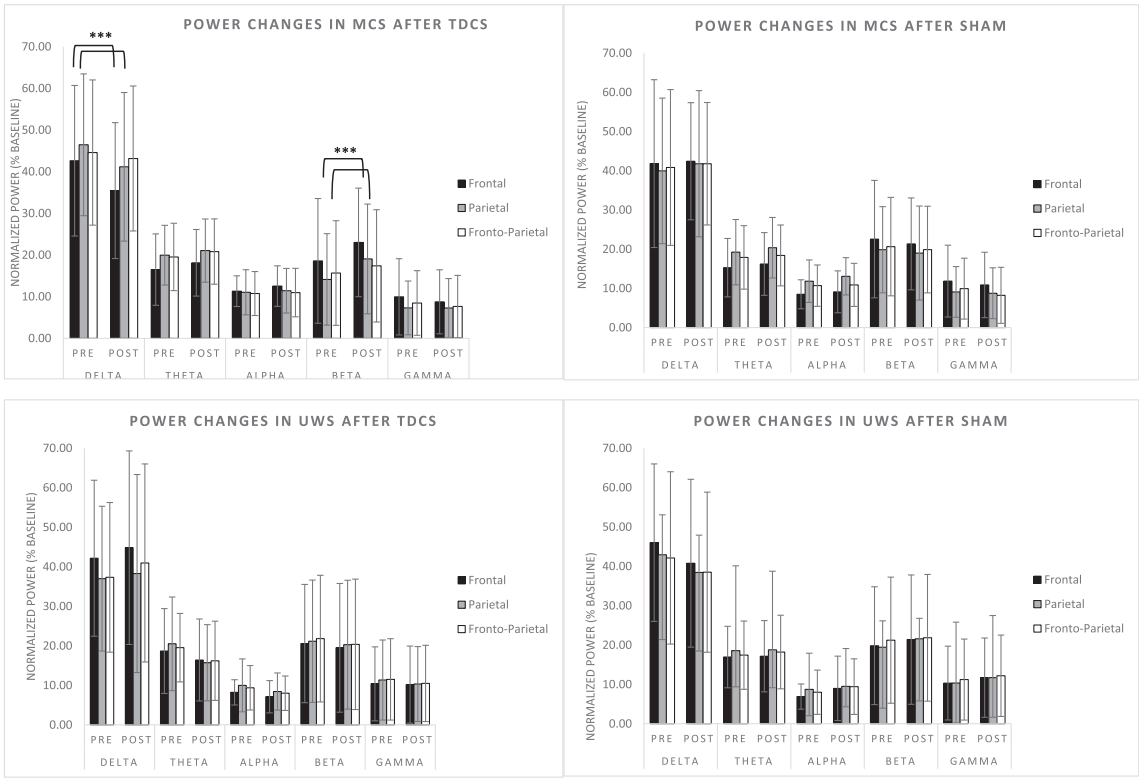

Fig. 2. Power-spectrum analysis in the two groups of patients (MCS and UWS) before and after tDCS stimulation and sham. Power spectra of the main electroencephalographic bands were studied in frontal, parietal and fronto-parietal electrodes. Bars show means ± Standard Deviation. Data were statistically analyzed by repeated measures' ANOVA, $^{*}p < 0.05$. MCS: minimally conscious state; UWS: unresponsive wakefulness syndrome; tDCS: transcranial direct current stimulation. MCS: minimally conscious state; UWS: unresponsive wakefulness syndrome; tDCS: transcranial direct current stimulation.

hemorrhage, and 2 cerebrovascular accident, with an interval since insult of 47 ± 45 months. Demographic and clinical features of participants are listed in Table 1.

3.1. Neurophysiological effect of tDCS

3.1.1. Changes in EEG power

An interaction 'stimulation' x 'condition' x 'site' was observed in the beta ($F_{1,55} = 8.54$; $p = 0.005$) and delta ($F_{1,55} = 101.84$; $p < 0.001$) frequency bands in the MCS group. Post-hoc analysis showed an increase of frontal and posterior beta power ($p < 0.01$; $p < 0.01$, respectively) and a decrease of delta power in the same areas ($p < 0.01$; $p < 0.01$, respectively) in the minimally conscious patients only after anodal tDCS. Conversely, patients with unresponsive wakefulness syndrome showed no significant changes in the power spectral analysis (see Fig. 2).

3.1.2. Changes in EEG coherence

Changes in the EEG coherence are shown in Fig. 3.

In the MCS group, the mixed model analysis revealed a significant three-way interaction 'stimulation' x 'condition' x 'site' ($p < 0.001$). Post-hoc analysis indicates stronger connections in the alpha frequency bands ($t = 2.09$, $p = 0.05$) over the posterior sites and higher fronto-parietal and posterior coherences in the beta frequencies ($t = 2.00$, $p = 0.05$; $t = 2.48$, $p = 0.03$, respectively) after real tDCS. Sham did not imply any variation of coherences (alpha: $t = 1.03$, $p = 0.33$; beta: $t = 0.96$, $p = 0.55$; gamma: $t = 0.96$, $p = 0.40$; theta: $t = 1.05$, $p = 0.35$; and delta: $t = 1.13$, $p = 0.31$).

The UWS patients showed a significant interaction 'stimulation' x 'condition' x 'site' ($p < 0.001$) showing higher frontal coherences in the delta frequencies ($t = 2.4$, $P = 0.03$) after the sessions of real tDCS.

Sham stimulation showed no significant changes in brain connections (alpha: $t = 0.57$, $p = 0.29$; beta: $t = 0.24$, $p = 0.81$; gamma: $t = 0.94$, $p = 0.38$; theta: $t = 1.40$, $p = 0.48$; and delta: $t = 0.54$, $p = 0.64$).

3.2. Clinical effect of tDCS

After real stimulation, MCS patients exhibited a significantly higher WNSSP total score ($t = 2.27$, $p = 0.04$). No clinical changes were observed in unresponsive participants or after sham, as shown in Table 2.

4. Discussion

In the present randomized sham-controlled crossover study, we investigated whether a treatment with anodal tDCS over the left dorsolateral prefrontal cortex could modify the clinical and electrophysiological features and outcome of a group of patients with DOCs. Our results can be summarized as follows: (1) a selective effect of anodal stimulation on power and coherence could be seen in the brain regions stimulated directly through tDCS (frontal areas) and in the posterior non-contiguous areas in patients in minimally conscious state; (2) some local changes could be detected over the anterior regions in the slow frequencies in the unresponsive patients; (3) sham stimulation did not reveal any significant electrophysiological changes in both groups of patients; (4) some significant clinical changes could be observed in MCS, but not in UWS patients, after real stimulation.

The ability of an external perturbation, such as tDCS or repetitive transcranial magnetic stimulation (rTMS), to rearrange and produce a common ongoing oscillatory activity and unmask intrinsic oscillations has been already described in numerous studies

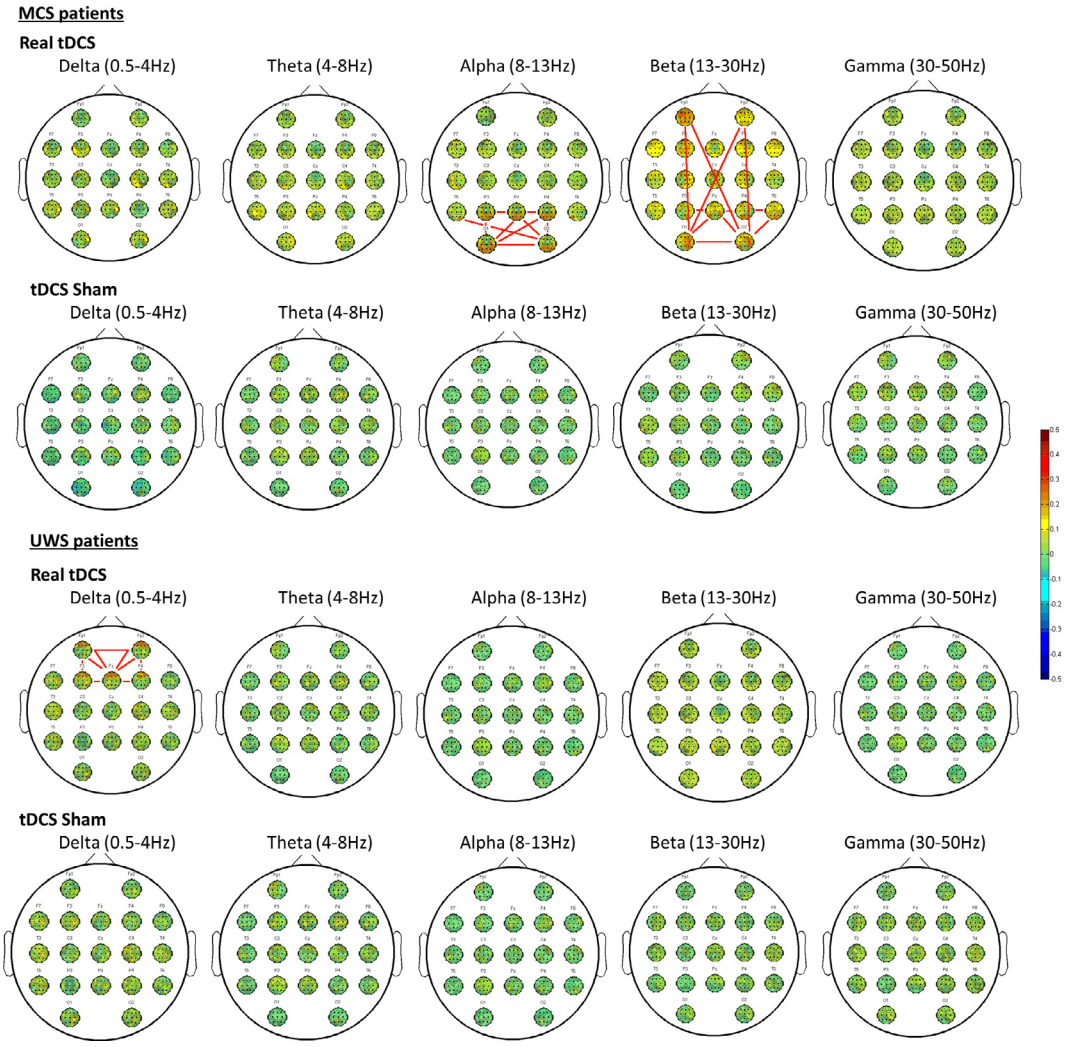

Fig. 3. Mapping of significant EEG coherence changes after real (a) and sham (b) tDCS at the five main frequency bands. Significant couplings between the electrodes are indicated by colors from dark blue (lower EEG coherence) to dark red (higher EEG coherence) indicated for each EEG band. MCS: minimally conscious state; UWS: unresponsive wakefulness syndrome; tDCS: transcranial direct current stimulation.

Table 2
tDCS effects on the clinical scales for patients with disorders of consciousness. CRS-R: Coma recovery scale-revised; WNSSP: Western Neuro Sensory Stimulation Profile; tDCS: transcranial Direct Current Stimulation; MCS: minimally conscious state; UWS: unresponsive wakefulness syndrome.

	CRS-R					WNSSP				
	Difference tDCS/Sham	Median	p 25	p 75	p value	Difference tDCS/Sham	Median	p 25	p 75	p value
MCS	0	0	0	1	0,91	3,9	3	0,75	7,5	0,02
UWS	0,2	0	0	0	0,32	1,8	0	−0,3	0,8	0,26

(Litwin-Kumar and Doiron, 2014; Brignani et al., 2008; Sauseng et al., 2005; Paus et al., 2001). However, it is still not fully understood whether these effects are mediated directly, or via excitability changes in the stimulated cortex. Previous investigations have postulated that anodal tDCS applied to preparation of rodent brain slice induced long-term potentiation-like effects due to NMDA-receptor activity and brain derived neurotrophic factor secretions (Fritsch et al., 2010). These changes, in conjunction with local modifications of the conductance of sodium and calcium channels, seem to contribute to the modulation and normalization of cortical oscillations during and after tDCS. Our results, in accordance to previous findings on patients with traumatic brain injury, report a power decrease of slow activity and increase of faster activity in frontal and parietal areas after anodal tDCS and suggest that the cumulative effect of consecutive tDCS sessions may regulate cortical excitability by normalizing EEG pattern (Ulam et al., 2015). In particular, Spitoni et al. described a selective synchronization in the alpha frequency band after anodal tDCS and interpreted this finding as an enhancement of the relaxed/alert wakefulness state (Spitoni et al., 2013). In accordance, we found that patients in MCS showed an increase of alpha coherence over regions far from the site of stimulation, such as the posterior areas. This behaviour is similar to that described in the healthy subjects of previous studies where the authors introduced the concept of "traveling alpha waves", suggesting that tDCS could have potentiated the travel of alpha waves between anterior and posterior brain areas (Klimesch et al., 2007; Mangia et al., 2014). The authors found that alpha band was linked to activity of several prefrontal

and parietal regions. These connections can be ascribed to the fronto-parietal network and the specific relationship between anterior and posterior sites reflects an intrinsic association of large-scale alpha phase locking with cognitive operations supported by this network (Sadaghiani et al., 2012). This inference implies that variations of local cortical excitability in one part of a specific functional network can influence the whole neural system associated with those specific functions beyond the site of stimulation and that the modulatory effects of tDCS on fronto-parietal connections could be specifically related to the stimulation of the DLPFC. In particular, the anterior cingulated cortex (ACC) has been proposed to play an important role in consciousness by integrating cognitive emotional processing with the state of arousal and the intent-to-act (Paus, 2001). The ACC is a key site of behavioural self-regulation and is closely connected with the parietal cortex (Posner et al., 2007).

Our study further demonstrates that anodal tDCS can rearrange and modulate the traveling alpha waves implied in the fronto-parietal network reflecting a spread of cortical activation induced by electrical perturbation.

In the same way, an increase of beta-frequency connections was observed over the fronto-parietal and posterior areas after real stimulation.

Beta activity appears on the EEG when there is mental effort or cognitive functioning (Ray and Cole, 1985). The trivial eye opening during resting state causes an enhancement of beta waves, and this seems to be related to mental and physical state changes, rather than a simple result of eye movements. Therefore, a perturbation induced by tDCS could change the brain to a ready state for efficient cognitive functioning and integration of long-range connections, as reported by other studies (Song et al., 2014).

This appears to be true only in patients in a minimally conscious state, where some residual cognitive resources can be detected. On the other hand, unresponsive patients showed only some local modulation of slow oscillatory activity in the frontal regions, where tDCS was applied. The excitatory effect of anodal stimulation on the local cerebral cortex seems to be caused by modifications of spontaneous neural excitability by hyperpolarization of the resting-membrane potential and local changes in ionic concentrations immediately under the electrode. These changes, however, did not spread in non-contiguous areas and remained circumscribed in the anterior regions, denoting a lack of integration of fronto-parietal functional networks.

Contextually to the modulation of network properties in terms of EEG cortical oscillations, we found some significant clinical changes in the patients with minimally conscious state after tDCS. Indeed, the patients showed significant changes in the score of Western Neurosensory Stimulation Profile after two-week sessions of active stimulation. Differently from the CRS-R scales, this measure is highly sensitive in identifying small behavioural changes in slow-to-recover patients with severe brain injury in response to different stimuli. From the clinical point of view, caregivers reported a reduced fluctuation of awareness and an increase of stability of the unambiguous signs of consciousness, distinctive of each patient. Other two recent studies reported clinical improvement of some MCS patients immediately after treatment, coming out in favour of tDCS as a promising non-invasive tool of neuromodulation to improve consciousness in MCS patients (Thibaut et al., 2014; Angelakis et al; 2014).

This study has several limitations. First, the use of an extracephalic cathode may have played a significant role in the clinical efficacy of the stimulation, that was present, but rather mild. While the dorsolateral prefrontal cortex is one of the most used site for anodal tDCS, placing the return electrode (cathode) on the contralateral part of the body is a relatively novel occurrence (Tseng et al., 2012). The choice of an extracerebral reference was made to facilitate the interpretation of results because a montage with both electrodes positioned onto the cerebral areas raises questions about which brain regions are primarily modulated as both electrodes can contribute to the overall effect of stimulation. Nevertheless, the distance between the anodal and return electrodes is negatively correlated with the tDCS induced after-effect magnitude and duration in healthy volunteers (Moliadze et al., 2010). Thus, the montage we used (left DLPF cortex vs. contralateral deltoid) could have affected the tDCS clinical efficacy by increased distance between electrodes compared to the other studies using intracephalic reference.

Second, we used a washout period of ten days, which could be relatively too short to wash out the effect of cumulative tDCS. The choice of this period was mainly due to hospital needs and planning. The lack of an understanding of tDCS mechanisms and the high heterogeneity of the stimulation protocols make it difficult to determine the optimum washout time for tDCS studies. In the literature, the length of this period ranges from 2 days (Spieser et al., 2015) to more than 3 months (Rroji et al., 2015). Pavlova et al., Amadi et al., Shah et al., and many other authors used a washout of seven days (or less) for multiple sessions of tDCS. In any case, the relatively short duration of washout has to be considered another important limitation in this study.

5. Conclusions

In conclusion, the results of the present study suggest that tDCS can modulate ongoing network dynamics through specific EEG frequencies, mainly in alpha and beta bands, likely due to changes in coupling of brain regions, in our context, anterior and posterior areas. This can be observed in patients exhibiting some form of consciousness, denoting partial preservation of fronto-parietal network and cognitive processing. In addition, the development of specific tools, such as the study of EEG coherence changes in the fronto-parietal network after anodal tDCS, could help to detect voluntary brain activity in patients with minimal behavioural output and test the efficacy of rehabilitation by correlates specifically oriented to the quantification of the neural functions related to consciousness. This could represent one of the key points to increase prognostic specificity and guarantee the better chance to recovery in each unresponsive patient.

The combination of tDCS and EEG recordings, applied during resting state, but even more in active cognitive conditions, such as responses to vocal commands or motor/visual imagery, might represent an innovative approach to modulate functional excitability and induce synchronization changes in disorders characterized by electrophysiological and behavioural abnormalities.

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Conflict of interest

None of the authors have potential conflicts of interest to be disclosed.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2018.10.018.

References

Accornero N, Capozza M, Pieroni L, Pro S, Davì L, Mecarelli O. EEG mean frequency changes in healthy subjects during prefrontal transcranial direct current stimulation. J Neurophysiol 2014;112:1367–75.

Angelakis E, Liouta E, Andreadis N, Korfias S, Ktonas P, Stranjalis G, et al. Transcranial direct current stimulation effects in disorders of consciousness. Arch Phys Med Rehabil 2014;95:283–9.

Binder JR, Liebenthal E, Possing ET, Medler DA, Ward BD. Neural correlates of sensory and decision processes in auditory object identification. Nat Neurosci 2004;7:295–301.

Brignani D, Manganotti P, Rossini PM, Miniussi C. Modulation of cortical oscillatory activity during transcranial magnetic stimulation. Hum Brain Mapp 2008;29 (5):603–12.

Clvo-Merino B, Haggard P. Transcranial magnetic stimulation. Applications in cognitive neuroscience. Rev Neurol 2004;38:374–80.

Curtis CE, D'Esposito M. Persistent activity in the prefrontal cortex during working memory. Trends Cogn Sci 2003;7:415–23.

Fregni F, Boggio PS, Nitsche M, Bermpohl F, Antal A, Feredoes E, et al. Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. Exp Brain Res 2005;166:23–30.

Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, et al. Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron 2010;66:198–204.

Giacino JT, Kalmar K, Whyte J. The JFK Coma Recovery Scale-Revised: measurement characteristics and diagnostic utility. Arch Phys Med Rehabil 2004;85:2020–9.

Klimesch W, Sauseng P, Hanslmayr S. EEG alpha oscillations: the inhibition-timing hypothesis. Brain Res Rev 2007;53:63–88.

Liebetanz D, Nitsche MA, Tergau F, Paulus W. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain 2002;125:2238–47.

Litwin-Kumar A, Doiron B. Formation and maintenance of neuronal assemblies through synaptic plasticity. Nat Commun 2014;5:5319.

Mangia AL, Pirini M, Cappello A. Transcranial direct current stimulation and power spectral parameters: a tDCS/EEG co-registration study. Front Hum Neurosci. 2014;8:601.

Moliadze V, Antal A, Paulus W. Electrode-distance dependent aftereffects of transcranial direct and random noise stimulation with extracephalic reference electrodes. Clin Neurophysiol 2010;121:2165–71.

Monti A, Ferrucci R, Fumagalli M, Mameli F, Cogiamanian F, Ardolino G, et al. Transcranial direct current stimulation (tDCS) and language. J Neurol Neurosurg Psychiatry 2013;84:832–42.

Mulquiney PG, Hoy KE, Daskalakis ZJ, Fitzgerald PB. Improving working memory: exploring the effect of transcranial random noise stimulation and transcranial direct current stimulation on the dorsolateral prefrontal cortex. Clin Neurophysiol 2011;122:2384–9.

Nitsche MA, LieBetanz D, Schlitterlau A, Henschke U, Fricke K, Frommann K, et al. GABAergic modulation of DC stimulation-induced motor cortex excitability shifts in humans. Eur J Neurosci 2004;19:2720–6.

Nitsche MA, LieBetanz D, Antal A, Lang N, Tergau F, Paulus W. Modulation of cortical excitability by weak direct current stimulation-technical, safety and functional aspects. Suppl Clin Neurophysiol 2011;56:255–76.

Nitsche MA, Koschack J, Pohlers H, Hullemann S, Paulus W, Happe S. Effects of frontal transcranial direct current stimulation on emotional state and processing in healthy humans. Front Psychiatry 2012;3:58.

Paulus W. Transcranial direct current stimulation (tDCS). Suppl Clin Neurophysiol 2003;56:249–54.

Paus T, Castro-Alamancos MA, Petrides M. Cortico-cortical connectivity of the human mid-dorsolateral frontal cortex and its modulation by repetitive transcranial magnetic stimulation. Eur J Neurosci. 2001;14:1405–11.

Piccione F, Cavinato M, Manganotti P, Formaggio E, Storti SF, Battistin L, et al. Behavioural and neurophysiological effects of repetitive transcranial magnetic stimulation on the minimally conscious state: a case study. Neurorehabil Neural Repair 2011;25:98–102.

Posner MI, Rothbart MK, Sheese BE, Tang Y. The anterior cingulate gyrus and the mechanism of self-regulation. Cogn Affect Behav Neurosci. 2007;7:391–5.

Radman T, Ramos RL, Brumberg JC, Bikson M. Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro. Brain Stimul 2009;2:215–28.

Ray WJ, Cole HW. EEG alpha activity reflects attentional demands, and beta activity reflects emotional and cognitive processes. Science 1985;228:750–2.

Rothwell JC. Clinical applications of noninvasive electrical stimulation: problems and potential. Clin EEG Neurosci 2012;43:209–14.

Rroji O, van Kuyck K, Nuttin B, Wenderoth N. Anodal tDCS over the primary motor cortex facilitates long-term memory formation reflecting use-dependent plasticity. PLoS One 2015;10:e012727.

Sadaghiani S, Scheeringa R, Lehongre K, Morillon B, Giraud AL, D'Esposito M, et al. α-band phase synchrony is related to activity in the fronto-parietal adaptive control network. J Neurosci 2012;32:14305–10.

Sarà M, Pistoia F. Bedside detection of awareness in the vegetative state. Lancet 2012;379:1702–3.

Sauseng P, Klimesch W, Stadler W, Schabus M, Doppelmayr M, Hanslmayr S, et al. A shift of visual spatial attention is selectively associated with human EEG alpha activity. Eur J Neurosci 2005;22:2917–26.

Seidler RD, Gluskin BS, Greeley B. Right prefrontal cortex transcranial direct current stimulation enhances multi-day savings in sensorimotor adaptation. J Neurophysiol 2017;117:429–35.

Song M, Shin Y, Yun K. Beta-frequency EEG activity increased during transcranial direct current stimulation. Neuroreport. 2014;25:1433–6.

Spieser L, van den Wildenberg W, Hasbroucq T, Ridderinkhof KR, Burle B. Controlling your impulses: electrical stimulation of the human supplementary motor complex prevents impulsive errors. J Neurosci 2015;35:3010–5.

Spitoni GF, Cimmino RL, Bozzacchi C, Pizzamiglio L, Di Russo F. Modulation of spontaneous alpha brain rhythms using low-intensity transcranial direct-current stimulation. Front Hum Neurosci. 2013;7:529.

Summerfield C, Egner T, Greene M, Koechlin E, Mangels J, Hirsch J. Predictive codes for forthcoming perception in the frontal cortex. Science 2006;314:1311–4.

Thibaut A, Bruno MA, Ledoux D, Demertzi A, Laureys S. tDCS in patients with disorders of consciousness: sham-controlled randomized double-blind study. Neurology 2014;82:1112–8.

Tseng P, Hsu TY, Chang CF, Tzeng OJ, Hung DL, Muggleton NG, et al. Unleashing potential: transcranial direct current stimulation over the right posterior parietal cortex improves change detection in low-performing individuals. J Neurosci 2012;32:10554–61.

Ulam F, Shelton C, Richards L, Davis L, Hunter B, Fregni F, et al. Cumulative effects of transcranial direct current stimulation on EEG oscillations and attention/working memory during subacute neurorehabilitation of traumatic brain injury. Clin Neurophysiol 2015;126:486–96.

Vecchio F, Pellicciari MC, Miraglia F, Brignani D, Miniussi C, Rossini PM. Effects of transcranial direct current stimulation on the functional coupling of the sensorimotor cortical network. Neuroimage 2016;140:50–6.

Woods AJ, Antal A, Bikson M, Boggio PS, Brunoni AR, Celnik P, et al. A technical guide to tDCS, and related non-invasive brain stimulation tools. Clin Neurophysiol 2016;127:1031–48.