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Repeated transcranial direct current stimulation in prolonged disorders of consciousness: A double-blind cross-over study

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ABSTRACT

Objective: To evaluate effects of 5 sessions of transcranial direct current stimulation (tDCS) over the left dorsolateral prefrontal cortex in patients with prolonged disorders of consciousness (DOC).
Methods: Seven patients in vegetative state (VS) and 6 in minimally conscious state (MCS), at ≥3 months after brain injury, were randomized into two groups: group 1 received one week of active tDCS and 1 week of sham stimulation, separated by 1 resting week; group 2 received active and sham stimulation in reverse order. We performed clinical and EEG evaluations before and after the first stimulation session, two hours after the last weekly stimulation, twice during the resting week, and during a 3-month follow-up.
Results: We observed small changes of patients' conditions after the first tDCS session and immediately after the 5 active stimulations. Substantial clinical and EEG changes were observed in 5/13 patients (3 in MCS and 2 in VS) starting after entire (active and sham) stimulation protocol and further progressing during the next months. No baseline features distinguished patients who improved from patients who did not improve.
Conclusions: Repeated tDCS did not exert remarkable short-term clinical and EEG effects in patients with prolonged DOC. Further studies should ascertain whether tDCS might promote clinical recovery in the long-term period.

1. Introduction

Several studies investigated therapeutic interventions to promote clinical recovery in brain-injured patients with disorders of consciousness (DOC). Most studies were based on attempts at enhancing consciousness by means of (generally off-label) pharmacological or neurosurgical treatments [1–4]. Only one randomized controlled study has been performed and reported positive effect of amantadine administration in patients with post-traumatic DOC [5]. At the moment no guideline for treatment of DOC has been established.

In last years, transcranial direct current stimulation (tDCS) has been proposed as a novel non-invasive therapeutic approach modulating activity of cortical networks in patients with Alzheimer's disease, Parkinson's disease or stroke [6–11]. Recently two studies investigated the effect of tDCS over the left dorsolateral prefrontal cortex (DLPFC) in patients with DOC. A first open observational study demonstrated that 3/3 patients in prolonged minimally conscious state (MCS) showed mild clinical improvements after 5 daily stimulation sessions, and 2 of them had recovered consciousness at 12-month follow-up; no patients in vegetative state (VS) improved at the end of the stimulation protocol [12]. The second study, using a randomized blinded design on a large cohort of patients, revealed a transient positive clinical effect in 13/30 patients in MCS and in 2/25 patients in VS within 2 h from a single tDCS session, but no impact on long-term outcome (12 months) [13]. A re-analysis on 8 of the 13 patients in MCS whose clinical conditions temporarily improved in the previous study suggested that the response to tDCS might be related to preservation of grey matter and residual metabolic activity in cortical and subcortical brain areas involved in attention and working memory (among which the left DLPFC), whereas baseline mean frequency bands on standard EEG did not distinguish responders from non-responders [14].

These two studies yielded some encouraging results but neither study reported a systematic follow-up of patients' conditions over the weeks after stimulation. Therefore, clinical usefulness of tDCS in patients with prolonged DOC has not been firmly established.

Abbreviations: CRS-R, coma recovery scale-revised; DLPFC, dorsolateral prefrontal cortex; ARS, active-rest-sham stimulation; DOC, disorders of consciousness; MCS, minimally conscious state; MCS−, minimally conscious state minus; MCS+, minimally conscious state plus; SRA, sham-rest-active stimulation; tDCS, transcranial direct current stimulation; VS, vegetative state.

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On the basis of available evidence, we performed a randomized sham-controlled double-blind cross-over, single-centre study with two main aims: 1) to evaluate the effects of a series of five tDCS stimulation sessions (run on consecutive days) on clinical status and EEG background activity in patients with prolonged DOC; 2) to ascertain whether specific patients' characteristics, clinical conditions and EEG features at baseline are associated with positive response to the tDCS stimulation. We recruited patients in MCS or VS at least 3 months after onset, and stimulated the left DLPFC in analogy with the two studies described above, and in line with the positive effect of such stimulation on sustained attention in normal individuals [15]. The possible effects of tDCS were evaluated after the first session (as in [13]), at the end of the stimulation sessions (as in [12]). All patients were followed-up during the 3 months following the stimulation protocol to ascertain long-term persistence of possible clinical changes (not assessed in previous studies).

2. Methods

2.1. Patients selection

We screened inpatients consecutively admitted to the Unit for Disorders of Consciousness at the Institute for Rehabilitation, Salvatore Maugeri Foundation (Telese, Italy). Inclusion criteria for the present study were: clinical diagnosis of VS or MCS according to standardized clinical diagnostic criteria [16–18], and stable level of responsiveness as assessed by the Italian version of coma recovery scale-revised (CRS-R) [19] on repeated clinical evaluations in the two weeks before study entry; traumatic, anoxic or vascular aetiology; time from onset ≥ 3 months; age ≥ 18 years. Exclusion criteria were: i) presence of pacemaker or metallic cerebral implant, according to safety criteria for transcranial electric stimulation in humans [20], ii) neuroimaging evidence of focal lesion on the left DLPFC, iii) premorbid history of psychiatric or neurodegenerative diseases; iv) severe medical conditions that might influence clinical diagnosis and EEG activity (e.g., severe hepatic insufficiency or renal failure, or sub-continuous or abundant epileptiform discharges on standard EEG recordings [21–23]). We also excluded patients in whom Na+ or Ca++ channel blockers (e.g., carbamazepine or verapamil) or *N*-methyl-D-aspartate receptor antagonists (e.g., memantine) could not be discontinued in order to avoid possible interaction with the effects of tDCS [24]. In presence of relevant acute medical complications in eligible patients (e.g., hyperthermia, infections, acute respiratory insufficiency) tDCS was postponed to when clinical conditions were stabilized.

2.2. Experimental design

In a double-blind sham-controlled cross-over design, all enrolled patients were randomly assigned to one of two groups: i) in the first group (active-rest-sham, ARS) 5 active stimulation sessions in 5 consecutive working days (from Monday to Friday) were followed by 1 week of no stimulation (resting), and by 5 sham stimulation sessions in 5 consecutive working days; ii) in the second group (sham-rest-active, SRA) the sham stimulation was administered first, followed by the resting week and by the series of real tDCS sessions (Fig. 1).

Patients underwent clinical evaluation at the following 3 time-points during the weeks of active and sham stimulation: 1) before the first session of stimulation; 2) two hours after the first stimulation; 3) at day 5, two hours after the last stimulation. In the resting week, patients were evaluated twice (on Monday and Friday). Finally, at the end of the stimulation protocol, all patients underwent systematic follow-up clinical evaluations once a week for 12 weeks.

EEG was recorded at the same 3 time-points during the weeks of active or sham stimulation (before the first session, two hours after the first stimulation, and two hours after the last session), twice during the resting week (in the same days of clinical assessments), at the end of the 3-month follow-up period, or at any change of the clinical diagnosis during this period (Fig. 1).

The clinical and EEG evaluations during the 3-week stimulation protocol and at the follow-up time-points were performed at patients' bed in the morning after customary nursing procedures, to minimize possible circadian clinical variability and to ensure patients' best vigilance

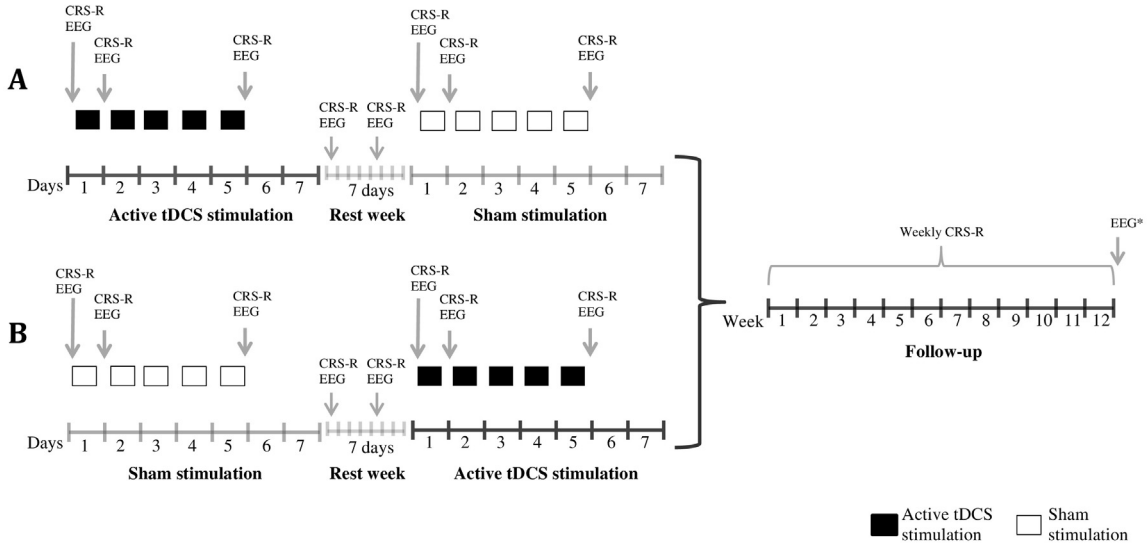

Fig. 1. Outline of the experimental design and of the clinical and EEG evaluations carried out. All patients underwent one week of five active tDCS in 5 consecutive working days, one week of no stimulation (resting), and one week of 5 sham stimulations in 5 consecutive working days. Patients were randomized into two groups: patients of the first group (A) were assigned to the active-rest-sham stimulation sequence, patients of the second group (B) were assigned to the sham-rest-active stimulation sequence. Then, both of both groups were followed until 3 months after the end of stimulation protocol. *Denotes that EEG was repeated at the end of the follow-up, and in case of changes of clinical diagnosis during the follow-up period. tDCS = transcranial direct current stimulation; CRS-R = coma recovery scale revised.

Table 1
Demographic, anamnestic, clinical and neuroimaging characteristics of patients at study entry.

Pt	Age at onset	Sex	Aetiology	Time post-onset (Months)	Clinical diagnosis	CRS-R total score (Subscales)	Neuroimaging findings (MRI or CT)
1	30	F	Traumatic	8	VS	5 (1-0-2-1-0-1)	Diffuse frontal and white matter damage
2	83	F	Vascular	13	VS	5 (1 + 0 + 1 + 1 + 0 + 2)	Right hemisphere lesion, diffuse cortical atrophy
3	76	M	Vascular	3	VS	3 (1 + 0 + 0 + 1 + 0 + 1)	Left superior parietal lesion, diffuse atrophy
4	68	M	Anoxic	3	VS	2 (1 + 0 + 0 + 0 + 0 + 1)	Severe diffuse cortical and subcortical atrophy
5	48	M	Anoxic	10	VS	4 (1 + 0 + 0 + 1 + 0 + 2)	Severe diffuse cortical and subcortical atrophy
6	24	F	Anoxic	57	VS	6 (1 + 0 + 2 + 1 + 0 + 2)	Severe diffuse cortical and subcortical atrophy
7	18	M	Anoxic	3	VS	7 (1 + 1 + 2 + 1 + 0 + 2)	Severe diffuse cortical and subcortical atrophy
8	59	F	Vascular	34	MCS−	8 (1 + 2 + 2 + 1 + 0 + 2)	Severe diffuse white matter damage
9	71	M	Vascular	7	MCS+	11 (3 + 3 + 2 + 1 + 0 + 2)	Bilateral ponto-mesencephalic lesions, diffuse atrophy
10	75	F	Vascular	18	MCS+	12 (3 + 3 + 2 + 2 + 0 + 2)	Bilateral parietal and temporal lesions
11	70	F	Vascular	6	MCS−	10 (2 + 3 + 2 + 1 + 0 + 2)	Bilateral parietal and thalamus-capsular lesions
12	38	M	Anoxic	84	MCS−	10 (2 + 3 + 2 + 1 + 0 + 2)	Severe diffuse cortical and subcortical atrophy
13	49	M	Anoxic	14	MCS−	12 (2 + 3 + 4 + 1 + 0 + 2)	Severe diffuse cortical and subcortical atrophy

Note: F = female; M = male; CRS-R = coma recovery scale-revised; VS = vegetative state; MCS− = minimally conscious state minus; MCS+ = minimally conscious state plus.

state. Chronic pharmacological therapy potentially influencing clinical state (e.g., anti-epileptic or benzodiazepine) and physiotherapy were not changed during entire study.

Patients developing relevant clinical conditions (e.g. hyperthermia, respiratory insufficiency) during the 3-week period of the protocol were excluded from the study.

The professionals who administered tDCS, clinically evaluated patients and analysed EEG recordings were blind as regards patient randomization and delivering of sham or active stimulation.

2.3. tDCS protocol

In active anodal stimulation, tDCS was delivered at 2 mA intensity by two 35-cm^2 sponge electrodes soaked in a saline water solution with the anode positioned over the left DLPFC (F3 according to the 10–20 international EEG system) and the reference cathode positioned on the right supraorbital region (FP2). Impedances were kept under 5 kΩ and voltage under 26 V. Stimulation lasted 20 min. In the sham stimulation, session duration and electrode placement was the same as in the active stimulation, but direct current was only delivered during the first and the last 30 s of the session. Stimulation was delivered by Brainstim tDCS stimulator, E.M.S. s.r.l., Bologna, Italy; before each session, the stimulator was programmed in advance by a clinician not involved in clinical and EEG evaluations.

2.4. Clinical evaluation

All patients were assessed by skilled hospital staff using the Italian version of CRS-R at study entry, during stimulation protocol and in the follow-up period [19]. This scale takes into account the Aspen Workgroup criteria [18], and includes six subscales addressing auditory, visual, motor, oromotor/verbal, communication and arousal functions. For each subscale specific operational criteria distinguish patients in VS from patients in MCS, such that presence of intentional (non-reflexive) responses on a single subscale can suffice to identify patients in MCS [25]; the total score might represent an index of responsiveness level, but is not used for diagnostic purposes.

We also applied the recent clinical criteria to sub-categorize MCS into MCS minus (MCS −, i.e., patients with low-level intentional behaviour, such as visual pursuit or localization of noxious stimulation) and

Table 2
Clinical evaluation before and 2 h after single active tDCS and sham stimulation.

First group Patient	Active stimulation CRS-R Pre (subscales)	Diagnosis Pre	CRS-R Post (subscales)	Diagnosis Post	Sham stimulation CRS-R Pre (subscales)	Diagnosis Pre	CRS-R Post (subscales)	Diagnosis Post
1	5 (1-0-2-1-0-1)	VS	5 (1-0-2-1-0-1)	VS	5 (1-0-2-1-0-1)	VS	5 (1-0-2-1-0-1)	VS
2	5 (1-0-1-1-0-2)	VS	**6** (1-0-**2**-1-0-2)	VS	6 (1-0-1-1-0-2)	VS	6 (1-0-1-1-0-2)	VS
3[a]	3 (1-0-0-1-0-1)	VS	**4** (**0**-**1**-0-1-0-**2**)	VS	6 (2-1-1-1-0-1)	VS	6 (2-1-1-1-0-1)	VS
4	2 (0-0-0-1-0-1)	VS	2 (0-0-0-1-0-1)	VS	3 (0-0-0-1-0-2)	VS	3 (0-0-0-1-0-2)	VS
7[a]	7 (1-1-2-1-0-2)	VS	**6** (1-**0**-2-1-0-2)	VS	7 (1-1-2-1-0-2)	VS	7 (1-1-2-1-0-2)	VS
9	11 (3-3-2-1-0-2)	MCS+	11 (3-3-2-1-0-2)	MCS+	10 (3-3-2-1-0-1)	MCS+	**11** (3-3-2-1-0-**2**)	MCS+
12	10 (2-3-2-1-0-2)	MCS−	10 (2-3-2-1-0-2)	MCS−	10 (2-3-2-1-0-2)	MCS−	10 (2-3-2-1-0-2)	MCS−
13[a]	12 (2-3-4-1-0-2)	MCS−	**11** (2-3-**3**-1-0-2)	MCS−	12 (2-3-4-1-0-2)	MCS−	12 (2-3-4-1-0-2)	MCS−

Second group Patient	Sham stimulation CRS-R Pre (subscales)	Diagnosis Pre	CRS-R Post (subscales)	Diagnosis post	Active stimulation CRS-R Pre (subscales)	Diagnosis Pre	CRS-R Post (subscales)	Diagnosis post
5	4 (1-0-0-1-0-2)	VS	4 (1-0-0-1-0-2)	VS	4 (1-0-0-1-0-2)	VS	4 (1-0-0-1-0-2)	VS
6	6 (1-0-2-1-0-2)	VS	6 (1-0-2-1-0-2)	VS	6 (1-0-2-1-0-2)	VS	6 (1-0-2-1-0-2)	VS
8[a]	8 (1-2-2-1-0-2)	MCS−	**7** (1-2-2-1-0-**1**)	MCS−	7 (1-2-2-1-0-1)	MCS−	**8** (1-2-2-2-0-**2**)	MCS−
10	12 (3-3-2-2-0-2)	MCS+	**13** (3-**4**-2-2-0-2)	MCS+	12 (3-3-2-2-0-2)	MCS+	12 (3-3-2-2-0-2)	MCS+
11 [a]	10 (2-3-2-1-0-2)	MCS−	**9** (2-**2**-2-1-0-2)	MCS−	9 (2-2-2-1-0-2)	MCS−	**10** (2-**3**-2-1-0-2)	MCS−

Note: tDCS = transcranial direct current stimulation; CRS-R = coma recovery scale-revised; VS = vegetative state; MCS− = minimally conscious state minus; MCS+ = minimally conscious state plus.

[a] Dcenotes patients showing improvements in clinical diagnosis during the protocol or at follow-up. Changes of clinical diagnosis and of Coma Recovery Scale-Revised total and subscore with respect to baseline are highlighted in bold.

MCS plus (MCS +, i.e., patients with high-level behavioural interactions, such as command following) [26].

2.5. EEG acquisition and analysis

We used a portable EEG device (Nicolet video-EEG system) to record a 30-min (at least) standard EEG from 11 electrodes placed on the scalp according to the international 10–20 system (O1, O2, Pz, C3, C4, Cz, T3, T4, Fz, Fp1, and Fp2).

Standard procedure of eye-closed recording was used with filter settings 0.5–70 Hz, and notch filter on. Two skilled clinical neurophysiologists reviewed EEG recordings and classified EEG background activity on the basis of frequency and amplitude of dominant cortical activity present in >50% of recordings, into one of five severity categories, according to criteria recently proposed for patients with prolonged DOC (Appendix 1) [27].

2.6. Statistical analysis

To compare demographic and clinical features in subgroups of patients as a function of clinical diagnosis (patients in VS vs. patients in MCS), and as a function of clinical improvements ("improved" vs. "non-improved"), we used two-tailed unpaired *t*-tests for continuous variables and Fisher's exact test or Chi-square for categorical variables, as appropriate. All analyses were performed with SAS statistical package (version 9.1).

2.7. Standard protocol approvals, registrations, and patient consent

The present study was conducted after approval of the local Ethics Committee. Written informed consent was obtained from the legal guardians of all patients.

3. Results

3.1. Patients' characteristics

We enrolled 15 inpatients, but 2 of them (1 in VS and 1 in MCS) showed relevant acute medical complications (acute respiratory and renal insufficiency) during the 3-week protocol and were excluded from the study. Thus, our final sample included 13 patients (6 females; 6 patients with vascular, 6 with post-anoxic, and 1 with post-traumatic aetiology; age range: 18–83 years; time post-onset range: 3–84 months; CRS-R total range: 2–12), 7 in VS and 6 in MCS (see Table 1 for clinical details). The two diagnostic groups did not differ for time from onset or age, whereas the CRS-R total score significantly differed between the two groups ($p < 0.001$). Four patients were classified to be in MCS − (CRS-R total score range: 8–12) and 2 in MCS + (CRS-R total score range: 11–12).

Eight patients received active stimulation first (ARS group) and 5 received sham stimulation first (SRA group). No adverse effects possibly related to tDCS were observed.

3.2. Clinical findings

No change in clinical diagnosis, and only small increase or decrease of the CRS-R total score were observed after the first single active or sham stimulation in either experimental groups (Table 2). The number of patients showing increase or decrease did not differ as a function of clinical diagnosis, or of experimental subgroup (ARS or SRA).

At the end of active stimulation week (Table 3) we found small improvements of CRS-R total score, not associated with changes in clinical diagnosis, in 3 patients of the ARS group (Cases 1, 2 and 3; such variations persisted during the resting week), and in 3 patients of the SRA group (Cases 8, 10 and 11). Thus, the pattern of clinical changes was substantially similar in the two experimental groups.

Table 3
Clinical evaluation performed before and after five days of active and sham stimulation, during one week of resting and during 3-month follow-up

First group		Active stimulation			Rest		Sham stimulation			Behavioural changes during the follow-up					
										First month			Third month		
Pt.	Diag T0	CRS-R Pre (subscales)	CRS-R Post (subscales)	Diag. post	CRS-R (subscales)	Diag.	CRS-R Pre (subscales)	CRS-R Post (subscales)	Diag. post	CRS-R (subscales)	Diag.		CRS-R (subscales)	Diag.	
1	VS	5 (1-0-2-1-0-1)	**8** (**2**-1-2-1-0-**2**)	VS	8 (2-1-2-1-0-2)	VS	5 (1-0-2-1-0-1)	5 (1-0-2-1-0-1)	VS	**8** (**2**-1-2-1-0-**2**)	VS				
2	VS	5 (1-0-1-1-0-2)	**6** (1-0-**2**-1-0-2)	VS	6 (1-0-2-1-0-2)	VS	6 (1-0-1-1-0-2)	6 (1-0-1-1-0-2)	VS						
3[a]	VS	3 (1-0-0-1-0-1)	**4** (1-0-0-1-0-**2**)	VS	4 (1-0-0-1-0-2)	VS	**6** (**2**-**1**-**1**-1-0-**1**)	**11** (2-**4**-**2**-1-0-**2**)	**MCS−**	**17** (3-**4**-**4**-**3**-**1**-**2**)	**MCS+**		**20** (**4**-**5**-4-3-**2**-2)	**eMCS**	
4	VS	2 (0-0-0-1-0-1)	2 (0-0-0-1-0-1)	VS	2 (0-0-0-1-0-1)	VS	**3** (0-0-0-1-0-**2**)	**2** (0-0-0-1-0-**1**)	VS	**3** (0-0-0-1-0-**2**)	VS		**6** (**2**-0-**1**-1-0-2)	VS	
7[a]	VS	7 (1-1-2-1-0-2)	7 (1-1-2-1-0-2)	VS	7 (1-1-2-1-0-2)	VS	7 (1-1-2-1-0-2)	**6** (1-**0**-**2**-1-0-2)	VS	**10** (**2**-**3**-2-1-0-2)	**MCS−**		**17** (**3**-**5**-**5**-**2**-0-2)	**MCS+**	
9	MCS +	11 (3-3-2-1-0-2)	11 (3-3-2-1-0-2)	MCS +	11 (3-3-2-1-0-2)	MCS +	**10** (3-3-2-1-0-**1**)	**11** (3-3-2-1-0-**2**)	MCS +						
12	MCS −	10 (2-3-2-1-0-2)	10 (2-3-2-1-0-2)	MCS −	10 (2-3-2-1-0-2)	MCS −	10 (2-3-2-1-0-2)	10 (2-3-2-1-0-2)	MCS −						
13[a]	MCS −	12 (2-3-4-1-0-2)	**11** (2-3-**3**-1-0-2)	MCS −	**12** (2-3-**4**-1-0-2)	MCS −	12 (2-3-4-1-0-2)	**15** (**3**-**4**-4-1-**1**-**2**)	**MCS+**	**17** (3-4-**6**-1-1-2)	**eMCS**				
Second Group		Sham stimulation			Rest		Active stimulation								
Pt.	Diag. T0	CRS-R Pre (subscales)	CRS-R Post (subscales)	Diag. post	CRS-R (subscales)	Diag.	CRS-R Pre (subscales)	CRS-R Post (subscales)	Diag. post	CRS-R (subscales)	Diag.		CRS-R (subscales)	Diag.	
5	VS	4 (1-0-0-1-0-2)	4 (1-0-0-1-0-2)	VS	4 (1-0-0-1-0-2)	VS	4 (1-0-0-1-0-2)	4 (1-0-0-1-0-2)	VS						
6	VS	6 (1-0-2-1-0-2)	6 (1-0-2-1-0-2)	VS	6 (1-0-2-1-0-2)	VS	6 (1-0-2-1-0-2)	6 (1-0-2-1-0-2)	VS						
8[a]	MCS −	8 (1-2-2-1-0-2)	**7** (1-2-2-1-0-**1**)	MCS −	**8** (1-2-2-1-0-**2**)	MCS −	**7** (1-2-2-1-0-**1**)	**10** (**2**-**3**-2-1-0-2)	MCS −	**15** (**3**-3-**4**-**2**-**1**-2)	**MCS+**				
10	MCS +	12 (3-3-2-2-0-2)	12 (3-3-2-2-0-2)	MCS +	12 (3-3-2-2-0-2)	MCS +	12 (3-3-2-2-0-2)	**13** (3-**4**-2-2-0-2)	MCS +	**15** (3-4-**4**-2-0-2)	MCS +		**18** (**4**-**5**-4-2-1-2)	MCS +	
11[a]	MCS −	10 (2-3-2-1-0-2)	10 (2-3-2-1-0-2)	MCS −	**9** (2-**2**-2-1-0-2)	MCS −	9 (2-2-2-1-0-2)	**12** (2-**3**-2-**2**-**1**-2)	MCS −	**17** (**4**-**5**-**4**-**1**-1-2)	**MCS+**		**21** (4-5-**5**-**3**-**2**-2)	**eMCS**	

Note: Pt. = patient, and [a]denotes those showing improvements in clinical diagnosis during the protocol or at follow-up. Diag. = Diagnosis; T0 = study entry; CRS-R = coma recovery scale-revised; VS = vegetative state; MCS − = minimally conscious state minus; MCS + = minimally conscious state plus; eMCS: emergence from minimally conscious state. Changes of clinical diagnosis and of CRS-R total and sub-score with respect to baseline are highlighted in bold.

Table 4
EEG background activity recorded before and after five days of active and sham stimulations, during one week of resting, and during 3-month follow-up.

First group		Active stimulation			Rest	Sham stimulation			Changes during the follow-up			
									First month		Third month	
Patient	Diagnosis T0	EEG pre	EEG post	Diagnosis post	EEG	EEG pre	EEG post	Diagnosis post	EEG	Diagnosis	EEG	Diagnosis
1	VS	DS	NE	VS	DS	DS	NE	VS				
2	VS	MoA	MoA	VS	MoA	MoA	MoA	VS				
3[a]	VS	MoA	MoA	VS	MoA	NE	MoA	MCS−	MoA	MCS+	**MiA**	eMCS
4	VS	LV	LV	VS	LV	LV	LV	VS				
7[a]	VS	LV/A	LV	VS	LV	LV	LV	VS	**DS**	MCS−	**MoA**	MCS+
9	MCS+	MoA	NE	MCS+	DS	DS	MoA	MCS+				
12	MCS−	MoA	NE	MCS−	NE	NE	NE	MCS−				
13[a]	MCS−	MiA	**MoA**	MCS−	MiA	MiA	**MoA**	MCS+	**N**	eMCS		

Second Group		Sham stimulation			Rest	Active stimulation			Changes during the follow-up			
Patient	Diagnosis T0	EEG pre	EEG post	Diagnosis post	EEG	EEG pre	EEG post	Diagnosis post	EEG	Diagnosis	EEG	Diagnosis
5	VS	LV	LV	VS	LV	LV	LV	VS				
6	VS	LV	LV	VS/UWS	LV	LV	LV	VS				
8[a]	MCS−	MoA	MoA	MCS−	MoA	MoA	MoA	MCS−	**MiA**	MCS+	**N**	MCS+
10	MCS+	MoA	**MiA**	MCS+	MiA	MiA	MiA	MCS+	**N**	MCS+		
11[a]	MCS−	DS	DS	MCS−	NE	DS	DS	MCS−	**MiA**	MCS+	**N**	eMCS

Note: [a]denotes those showing improvements in clinical diagnosis during the protocol or at follow-up. T0 = study entry; CRS-R = coma recovery scalerevised; VS = vegetative state; MCS-= minimally conscious state minus; MCS+= minimally conscious state plus; eMCS: emergence from minimally conscious state. Changes of clinical diagnosis and of CRS-R total and sub-score with respect to baseline are highlighted in bold. EEG = electroencephalogram; LV = low voltage; MoA = moderately abnormal; MiA = mildly abnormal; DS = diffuse slowing; N = normal EEG; NE = not evaluable because of artefacts.

At the end of sham stimulation week, the pattern of clinical changes clearly differed in the two experimental groups. In the ARS group we observed improvements in CRS-R score in 3 patients, associated to changes in clinical diagnosis in 2 of them (Cases 3 and 13), and also observed small decreases of CRS-R score, without clinical changes, in 2 patients. In the SRA group we only observed a small decrease in CRS-R score in 1 patient (Case 8), without change in clinical diagnosis.

During the follow-up period, in the ARS group we observed further improvements in two patients (Cases 3 and 13), who both emerged from MCS (10 weeks and 3 weeks after the end of stimulation protocol, respectively). One patient in VS (Case 7) showed a substantial clinical change during the early weeks of the follow-up period, reaching MCS− and then progressing to MCS+ after 11 weeks, whereas two other VS patients (Cases 1 and 4) presented only an increase of CRS-R score without regaining signs of awareness.

In the SRA group, 2 patients (Cases 8 and 11) switched from MCS− to MCS+ one month after the end of the stimulation protocol, and one of them (Case 11) emerged from MCS about two months after the end of the stimulation protocol. The third patient (Case 10) showed a substantial increase of CRS-R total score, but her clinical diagnosis did not change.

3.3. EEG findings

Over the entire 15-week study, substantial changes of EEG background activity were only observed in patients whose clinical diagnosis improved (Table 4). In three patients (Cases 7, 8, and 11) amelioration of EEG background activity was observed concurrently with changes of clinical diagnosis, whereas in two patients (Cases 3 and 13) EEG background activity substantially improved only when they emerged from MCS during the follow-up period. In one patient (Case 10), we found a substantial improvement of EEG organization (reaching a normal background activity) concurrently with relevant increase of CRS-R score, not associated with changes in clinical diagnosis.

3.4. Comparison between patients who improved and patients who did not improve

The 5 patients who showed an improvement of clinical diagnosis (2 patients in VS, 3 in MCS; see clinical sketches in Appendix 2) during the entire 15-week study did not differ from patients who did not improve for age (54.4 ± 22.8 vs. 54.6 ± 22.5; $p = 0.36$), gender (male/female: 3/2 vs. 4/4), aetiology (anoxic/vascular: 2/3 vs. 4/3; $p = 0.5$), CRS-R total score at study entry (8.0 ± 3.4 vs. 6.9 ± 3.6; $p = 0.71$), or time post injury (12.0 ± 13.1 vs. 25.0 ± 29.3 months; $p = 0.47$), although patients who improved had shorter disease duration.

Moreover, EEG background activity recorded at baseline did not differ between the two groups ($p = 0.55$; Table 4).

4. Discussion

The findings of the present randomized double-blinded sham-controlled cross-over study did not support effectiveness of a single tDCS, or of repeated tDCS over the left DLPFC. Relevant clinical changes were observed in some patients with prolonged DOC during the 3-month follow-up, when both groups had received repeated tDCS and sham stimulation. Indeed, in both experimental groups such clinical changes were observed starting from 2 to 4 weeks after active stimulation and persisted in the two subsequent months. Patients of the ARS group began to show relevant clinical improvements (quite paradoxically) after the week of sham stimulation (i.e., 2 weeks after active stimulations), whereas patients of the SRA group started to show relevant clinical improvements during the follow-up period (i.e., 2–4 weeks after active stimulations).

The lack of remarkable changes in the present study after the first real tDCS contrasts with the recent study reporting a transient recovery of a new intentional behaviour in 13/30 patients in MCS and in 2/25 patients in VS within 2 h from a single tDCS [13]. One potentially relevant difference between the two studies was related to time post-injury: the "responders" in Thibaut et al.'s study [13] underwent tDCS at a time post-injury (median: 2.3 months) much shorter than that of the present sample (median: 14 months). It is therefore possible that transient clinical amelioration after single tDCS is more likely to occur at short time post-onset, whereas stimulation might be effective at longer time post-onset only if repeated. We might speculate that a single stimulation, sufficient to increase neuronal excitability in targeted brain regions (via receptor-dependent excitability enhancement) [28,29]; see [30] for a study on DOC patients, could not exert relevant clinical effects in patients with prolonged DOC, in whom extensive and severe disruptions of cortical neuronal networks are present [31].

As recalled above, we observed substantial clinical improvements and increases in CRS-R total score in some patients after the end of the entire stimulation period, particularly in patients in MCS with shorter time post-injury. The clinical improvements in the two groups seemed to show specific temporal relationships with active tDCS, i.e. starting 2–4 weeks after active stimulations. These findings would be partially consistent with those reported in a previous study on 10 patients with prolonged DOC, in which changes in clinical status or improvements of CRS-R total score were observed in 3/3 patients in MCS 1 week after a 2-week period of repeated tDCS [12]. The longer period of direct observation in our study with respect to Angelakis et al.'s study [12] (12 weeks vs. 1 week after active stimulation) allowed us identifying enduring clinical changes at an unexpected time window, for which no appropriate control group was available. Since at that time both groups had received active and sham stimulation, it is not possible to exclude that clinical improvements were related to spontaneous recovery, which cannot be considered exceptional even in patients with prolonged DOC of non-traumatic aetiology [32].

The visual analysis of standard EEG did not detect relevant variations immediately after tDCS, but improvements in background activity were observed concurrently with changes in clinical diagnosis or recovery of further intentional responses, as assessed by the CRS-R total score. These findings are in line with the recent observations that EEG background activity is often coherent with clinical status and might help clinicians in monitoring clinical evolution in patients with DOC [27]. However, the visual analysis of baseline EEG was not sufficient to identify patients who showed clinical improvements in our study, in analogy with Thibaut et al. [14]. Quantitative analysis of EEG activity might help to recognize patients potentially liable to respond to non-invasive brain stimulation, as observed in conscious post-traumatic brain injured patients [33].

This study has several limitations. First, our experimental cross-over protocol was designed to test the effects of tDCS immediately after the first stimulation and within one week after the end of repeated real tDCS. This experimental protocol was directly derived from available studies in which tDCS was administered in DOC [12,13], and allowed us to treat all eligible patients (offering equal potential therapeutic opportunities to all of them). However, our study lacked of a control group suitable to establish whether the relevant clinical changes we observed later than the expected time could be ascribed to long-term effects of tDCS. Therefore, further specifically designed studies are necessary for demonstrating the possible changes related to repeated tDCS at the appropriate time window, adopting for instance cross-over designs with one-month resting period between active and sham stimulation. Second, in this single-centre study we could enrol a small number of patients, and only one of them had post-traumatic aetiology. This allowed us to use inferential statistics only for exploratory purposes, and limited generalization of our findings. Third, we might have underestimated the short-term effects of active stimulation on cerebral activity, since visual analysis of standard EEG might not be able to detect minimal and focal changes in EEG patterns. Last, we could not implement neuroimaging to identify patients with potential response to tDCS. These two last limitations should be addressed by studies including technologically advanced assessment tools (e.g. structural-functional MR and quantitative EEG evaluations), but we have to underline that our study paved the way for such further investigations addressing long-lasting effectiveness of repeated non-invasive brain stimulation in patients with prolonged DOC.

5. Conclusion

Notwithstanding the above limitations, the present study provided relevant findings about the clinical and neurophysiological effects of tDCS in patients with prolonged DOC. We did not observe relevant clinical or EEG changes in the short-term period after a single or repeated daily anodal stimulation over the left DLPFC. We observed, instead, long-term clinical and neurophysiological improvements starting 2–4 weeks after the active stimulation period and enduring at least until 3 months later. These results should be treated cautiously, as our study was not properly designed for unveiling long-term effects of tDCS. Further multi-centric studies on larger sample of patients are needed to ascertain possible long-lasting therapeutic effect of tDCS in stabilized DOC patients. In this perspective our study could serve as a starting point for cooperative long-term experimental trials, in which longer stimulation period might be used.

Conflict of interest statement

The authors have not competing interest to declare.

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Appendix 1. EEG background activity categories according to recent classification criteria [26]

EEG background activity category	EEG features
Normal	Presence of predominant posterior alpha rhythm and of the APG, without focal or hemispheric slowing or epileptiform abnormalities
Mildly abnormal	Presence of predominant posterior theta activity (≥20 μV), symmetric or not, with frequent (10–49% of recording) posterior alpha rhythms
Moderately abnormal	Presence of predominant posterior theta activity (≥20 μV), symmetric or not, poorly organized APG, even with rare (<1% of recording) or occasional (1–9% of recording) posterior alpha rhythms
Diffuse slowing	Presence of predominant diffuse theta or theta/delta rhythms at amplitude ≥ 20 μV, without APG
Low voltage	Presence of predominant EEG activity (theta or delta) <20 μV over most brain regions

Note: APG: anterior-posterior gradient.

Appendix 2. Clinical sketches of patients whose clinical diagnosis improved

Patient 3 was a 76-year-old man who suffered a severe haemorrhagic brain injury. Three months later he was in VS (CRS-R total score: 3). After the week of active stimulation, his score increased to 4 (for an increase in the arousal scale), without change in his clinical status, and remained stable during the subsequent resting week. At the beginning of the week of sham stimulation, Patient 3 was still in a VS with a CRS-R total score of 6 (the patient was able to localize sounds, regained visual startle, and abnormal posturing to noxious stimuli, but showed a decrease in arousal), whereas at the end of sham stimulation sessions his clinical diagnosis changed in MCS −, thanks to recovery of ability to localize and to reach for objects (CRS-R total score: 11). Patient 3's level of consciousness improved further during the next three weeks, when he was diagnosed to be in MCS +, as he was able to follow commands and to manipulate objects, and showed verbalization and non-functional communication (CRS-R total score: 17). These clinical conditions changed further in the subsequent follow-up period, and 70 days after the end of stimulation Patient 3 emerged from MCS, as he recovered functional communication (CRS-R total score: 20).

Patient 7, aged 18 year, sustained a severe anoxic brain injury following a cardiac arrest. At study entry, 3 months after onset, he was in

VS with CRS-R total score of 7. No change in the score was observed at the end of active stimulation sessions and during the resting week, but Patient 7's score decreased to 6 at the end of sham stimulation sessions, as visual startle had disappeared. However, two weeks later Patient 7 could localize sounds and regained visual pursuit, compatible with diagnosis of MCS− (CRS-R total score: 10). Clinical diagnosis progressed to MCS+ by 77 days after the end of stimulation, when Patient 7 showed reproducible movements to command and object recognition (CRS-R total score: 17).

Patient 8 was a 59-year-old woman who showed sustained visual fixation, compatible with diagnosis of MCS− at study entry, 34 months after a severe ischemic stroke (CRS-R total score: 8). The score fluctuated between 7 and 8 after sham stimulation sessions, during the resting week and at the beginning of the week of real stimulation, due to changes in arousal level. At the end of the active tDCS sessions Patient 8 was still in a MCS−, but her CRS-R total score had increased to 10, as she was able to localize sounds, recovered visual pursuit and had higher arousal level. Three weeks later Patient 8 recovered the ability to follow reproducible movements to commands, to manipulate objects, and non-functional communication, consistent with diagnosis of MCS− (CRS-R total score: 15), but did not show further evolution in her clinical condition or total score during the following 2 months.

Patient 11 was a 70-year-old woman in a MCS− at study entry, 6 months after a severe haemorrhagic stroke (CRS-R total score: 10). Her conditions did not change after the week of sham stimulation. During the resting week, Patient 11's visual tracking abilities disappeared without changes in clinical diagnosis (CRS-R total score: 9). Instead, the CRS-R total score increased to 12 at the end of the week of active stimulations, since Patient 11 recovered visual pursuit and showed vocalizations with non-functional intentional communication, but her clinical diagnosis did not change. After one month she regained her ability to execute consistent movements to command, to recognize and manipulate objects, attaining diagnosis of MCS+ (CRS-R total score: 17). Her clinical conditions further improved during the next 2 months, when she emerged from MCS, showing functional communication (CRS-R total score: 21).

Patient 13 was a 49-year-old man in a MCS− at baseline, 14 months after a severe anoxic brain injury (CRS-R total score: 12). The score lowered to 11 at the end of the week of real stimulation study, and then returned to 12 during the resting week, due to variations in the ability to manipulate objects, without changes in clinical diagnosis. At the end of the week of sham stimulation he recovered the ability to move his limbs following commands, to reach and manipulate objects, and non-functional communication, consistent with diagnosis of MCS+ (CRS-R total score: 15). Three weeks later Patient 13 was able to use objects appropriately, thus emerging from MCS (CRS-R total score: 17). No further changes were observed during the subsequent follow-up period.

References

[1] C. Tucker, K. Sandhu, The effectiveness of zolpidem for the treatment of disorders of consciousness, Neurocrit. Care. 24 (2016) 488–493.
[2] A. Estraneo, A. Pascarella, P. Moretta, V. Loreto, L. Trojano, Clinical and electroencephalographic on-off effect of amantadine in chronic non-traumatic minimally conscious state, J. Neurol. 262 (2015) 1584–1586.
[3] J.T. Giacino, J.J. Fins, A. Machado, N.D. Shiff, Central thalamic deep brain stimulation to promote recovery from chronic posttraumatic minimally conscious state: challenges and opportunities, Neuromodulation 15 (2012) 339–349.
[4] F.E. Pisa, G. Cosano, M. Giangreco, T. Giorgini, E. Biasutti, F. Barbone, Group for the Study of Medication Use in Centers for Post-acute Brain Injury Rehabilitation, Prescribing practice and off-label use of psychotropic medications in post-acute brain injury rehabilitation centres: a cross-sectional survey, Brain Inj. 29 (2015) 508–516.
[5] J.T. Giacino, J. Whyte, E. Bagiella, K. Kalmar, N. Childs, A. Khademi, B. Eifert, D. Long, D.I. Katz, S. Cho, S.A. Yablon, M. Luther, F.M. Hammond, A. Nordenbo, P. Novak, W. Mercer, P. Maurer-Karattup, M. Sherer, Placebo-controlled trial of amantadine for severe traumatic brain injury, N. Engl. J. Med. 366 (2012) 819–826.
[6] R. Ferrucci, F. Mameli, I. Guidi, S. Mrakic-Sposta, M. Vergari, S. Marceglia, F. Cogiamanian, S. Barbieri, E. Scarpini, A. Priori, Transcranial direct current stimulation improves recognition memory in Alzheimer disease, Neurology 71 (2008) 493–498.
[7] G.J. Elder, J.P. Taylor, Transcranial magnetic stimulation and transcranial direct current stimulation: treatments for cognitive and neuropsychiatric symptoms in the neurodegenerative dementias? Alzheimers Res. Ther. 10 (6) (2014) 74.
[8] R. Manenti, M. Brambilla, A. Benussi, S. Rosini, C. Cobelli, C. Ferrari, M. Petesi, I. Orizio, A. Padovani, B. Borroni, M. Cotelli, Mild cognitive impairment in Parkinson's disease is improved by transcranial direct current stimulation combined with physical therapy, Mov. Disord. 31 (2016) 715–724.
[9] P.S. Boggio, R. Ferrucci, S.P. Rigonatti, P. Covre, M. Nitsche, A. Pascual-Leone, F. Fregni, Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease, J. Neurol. Sci. 249 (2006) 31–38.
[10] E.K. Kang, M.J. Baek, S. Kim, N.J. Paik, Non-invasive cortical stimulation improves post-stroke attention decline, Restor. Neurol. Neurosci. 27 (2009) 645–650.
[11] B. Elsner, J. Kugler, M. Pohl, J. Mehrholz, Transcranial direct current stimulation (tDCS) for improving activities of daily living, and physical and cognitive functioning, in people after stroke, Cochrane Database Syst. Rev. 21 (2016) 3, CD009645. .
[12] E. Angelakis, E. Liouta, N. Andreadis, S. Korfias, P. Ktonas, G. Stranjalis, D.E. Sakas, Transcranial direct current stimulation effects in disorders of consciousness, Arch. Phys. Med. Rehabil. 95 (2014) 283–289.
[13] A. Thibaut, M.A. Bruno, D. Ledoux, A. Demertzi, S. Laureys, tDCS in patients with disorders of consciousness: sham-controlled randomised double blind study, Neurology 82 (2014) 1112–1118.
[14] A. Thibaut, C. Di Perri, C. Chatelle, et al., Clinical response to tDCS depends on residual brain metabolism and grey matter integrity in patients with minimally conscious state, Brain Stimul. 8 (2015) 1116–1123.
[15] J.T. Nelson, R.A. McKinley, E.J. Golob, J.S. Warm, R. Parasuraman, Enhancing vigilance in operators with prefrontal cortex transcranial direct current stimulation (tDCS), NeuroImage 85 (2014) 909–917.
[16] The Multi Society Task force on the Persistent Vegetative State, Statement on medical aspects of the persistent vegetative state, N. Engl. J. Med. 330 (1994) 1499–1508.
[17] Royal College of Physicians, The vegetative state: guidance on diagnosis and management, Clin. Med. 3 (2003) 249–254.
[18] J.T. Giacino, S. Ashwal, N. Childs, R. Cranford, B. Jennett, D.I. Katz, J.P. Kelly, J.H. Rosenberg, J. Whyte, R.D. Zafonte, N.D. Zasler, The minimally conscious state: definition and diagnostic criteria, Neurology 58 (2002) 349–353.
[19] A. Estraneo, P. Moretta, A. De Tanti, G. Gatta, J.T. Giacino, L. Trojano, Italian CRS-R Multicentre Validation Group, An Italian multicentre validation study of the coma recovery scale-revised, Eur. J. Phys. Rehabil. Med. 51 (2015) 627–634.
[20] M. Bikson, P. Grossman, C. Thomas, A.L. Zannou, J. Jiang, T. Adnan, A.P. Mourdoukoutas, G. Kronberg, D. Truong, P. Boggio, A.R. Brunoni, L. Charvet, F. Fregni, B. Fritsch, B. Gillick, R.H. Hamilton, B.M. Hampstead, R. Jankord, A. Kirton, H. Knotkova, D. Liebetanz, A. Liu, C. Loo, M.A. Nitsche, J. Reis, J.D. Richardson, A. Rotenberg, P.E. Turkeltaub, A.J. Woods, Safety of transcranial direct current stimulation: evidence based update 2016, Brain Stimul. 9 (2016) 641–661.
[21] L.J. Hirsch, Classification of EEG patterns in patients with impaired consciousness, Epilepsia 52 (2011) 21–24.
[22] L.J. Hirsch, LaRoche SM, N. Gaspard, C.D. Hahn, M.B. Westover, Critical Care EEG Monitoring Research Consortium, American Clinical Neurophysiology Society's standardized critical care EEG terminology: 2012 version, J. Clin. Neurophysiol. 30 (2013) 1–27.
[23] A. Pascarella, L. Trojano, V. Loreto, L. Bilo, P. Moretta, A. Estraneo, Long-term outcome of patients with disorders of consciousness with and without epileptiform activity and seizures: a prospective single centre cohort study, J. Neurol. 263 (2016) 2048–2056.
[24] C.J. Stagg, M.A. Nitsche, Physiological basis of transcranial direct current stimulation, Neuroscientist 17 (2011) 37–53.
[25] A. Estraneo, P. Moretta, V. Cardinale, A. De Tanti, G. Gatta, J.T. Giacino, L. Trojano, A multicentre study of intentional behavioural responses measured using the Coma Recovery Scale-revised in patients with minimally conscious state, Clin. Rehabil. 29 (2015) 803–808.
[26] M.A. Bruno, S. Majerus, M. Boly, A. Vanhaudenhuyse, C. Schnakers, O. Gosseries, P. Boveroux, M. Kirsch, A. Demertzi, C. Bernard, R. Hustinx, G. Moonen, S. Laureys, Functional neuroanatomy underlying the clinical subcategorization of minimally conscious state patients, J. Neurol. 259 (2012) 1087–1098.
[27] A. Estraneo, V. Loreto, I. Guarino, V. Boemia, G. Paone, P. Moretta, L. Trojano, Standard EEG in diagnostic process of prolonged disorders of consciousness, Clin. Neurophysiol. 127 (2016) 2379–2385.
[28] M.A. Nitsche, W. Paulus, Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans, Neurology 57 (2001) 1899–1901.
[29] M.A. Nitsche, K. Fricke, U. Henschke, A. Schlitterlau, D. Liebetanz, N. Lang, S. Henning, F. Tergau, W. Paulus, Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans, J. Physiol. 553 (2003) 293–301.
[30] A. Naro, R.S. Calabrò, M. Russo, A. Leo, P. Pollicino, A. Quartarone, P. Bramanti, Can transcranial direct current stimulation be useful in differentiating unresponsive wakefulness syndrome from minimally conscious state patients? Restor. Neurol. Neurosci. 33 (2015) 159–176.
[31] P. Guldenmund, A. Soddu, K. Baquero, A. Anhaudenhuyse, M.A. Bruno, O. Gosseries, S. Laureys, F. Gómez, Structural brain injury in patients with disorders of consciousness: a voxel-based morphometry study, Brain Inj. 30 (2016) 343–352.
[32] A. Estraneo, P. Moretta, V. Loreto, L. Santoro, L. Trojano, Clinical and neuropsychological long-term outcomes after late recovery of responsiveness: a case series, Arch. Phys. Med. Rehabil. 95 (2014) 711–716.
[33] F. Ulam, C. Shelton, L. Richards, L. Davis, B. Hunter, F. Fregni, K. Higgins, Cumulative effects of transcranial direct current stimulation on EEG oscillations and attention/working memory during subacute neurorehabilitation of traumatic brain injury, Clin. Neurophysiol. 126 (2015) 486–496.