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ORIGINAL ARTICLE

Sham-controlled randomized multicentre trial of transcranial direct current stimulation for prolonged disorders of consciousness

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Abstract

Background and purpose: Transcranial direct current stimulation (tDCS) has been shown to improve signs of consciousness in a subset of patients with disorders of consciousness (DoC). However, no multicentre study confirmed its efficacy when applied during rehabilitation. In this randomized controlled double-blind study, the effects of tDCS whilst patients were in rehabilitation were tested at the group level and according to their diagnosis and aetiology to better target DoC patients who might repond to tDCS.

Methods: Patients received 2 mA tDCS or sham applied over the left prefrontal cortex for 4 weeks. Behavioural assessments were performed weekly and up to 3 months' follow-up. Analyses were conducted at the group and subgroup levels based on the diagnosis (minimally conscious state [MCS] and unresponsive wakefulness syndrome) and the aetiology (traumatic or non-traumatic). Interim analyses were planned to continue or stop the trial.

Results: The trial was stopped for futility when 62 patients from 10 centres were enrolled (44 ± 14 years, 37 ± 24.5 weeks post-injury, 18 women, 32 MCS, 39 non-traumatic). Whilst, at the group level, no treatment effect was found, the subgroup analyses at 3 months' follow-up revealed a significant improvement for patients in MCS and with traumatic aetiology.

Conclusions: Transcranial direct current stimulation during rehabilitation does not seem to enhance patients' recovery. However, diagnosis and aetiology appear to be important factors leading to a response to the treatment. These findings bring novel insights into possible cortical plasticity changes in DoC patients given these differential results according to the subgroups of patients.

KEYWORDS
anoxia, coma, disorders of consciousness, minimally conscious sate, rehabilitation, stroke, transcranial direct current stimulation, traumatic brain injury, vegetative state

INTRODUCTION

Recent discoveries relying on the inherent plasticity of the brain suggest a wide range of therapeutic possibilities. In the past 10 years, a number of studies have reported that some patients in unresponsive wakefulness syndrome/vegetative state (UWS/VS) (eye opening without signs of consciousness) and in minimally conscious state (MCS) (eye opening with reproducible purposeful behaviours, no functional communication) could spontaneously improve even several years after the brain injury [1–3]. Studies of treatments improving clinical responses and recovery of patients with disorders of consciousness (DoC) have shown that deep brain stimulation of the intralaminar nuclei of the thalamus [4] and some pharmacological agents such as amantadine [5], apomorphine [6] or zolpidem [7] could improve behavioural signs of consciousness in some patients with DoC. Beside pharmacological interventions and deep brain stimulation, during the last 20 years the rediscovery and development of non-invasive brain stimulation has been witnessed. Trials investigating the behavioural effects of techniques such as transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation have flourished [8, 9]. Briefly, tDCS allows the brain activity of a specific cortical region to be modulated by sending a weak electrical current (usually from 1 to 2 mA) between two electrodes, the anode (excitatory) and the cathode (inhibitory) [10]. Mechanistically, tDCS has been shown to modulate neuronal excitability during the stimulation (online effects), as well as to induce long-term potentiation or depression plasticity-like effects (offline effects) [11].

For non-communicative patients, tDCS represents a promising tool to promote brain plasticity [12] as it is a safe, inexpensive and straightforward technique that could easily be integrated in rehabilitation programmes. In a first sham-controlled double-blind randomized crossover study, the effect of a single prefrontal tDCS was evaluated in a heterogeneous population of 55 patients with DoC (UWS/VS and MCS), acute–subacute (<3 months) and chronic, with traumatic brain injury (TBI) and non-TBI aetiologies [13]. At the group level, a treatment effect, as measured with the Coma Recovery Scale Revised (CRS-R), was observed in the MCS but not in the UWS/VS patient subgroup. In addition, no tDCS related side effects were observed. In another sham-controlled trial, tDCS was applied for 10 days over 2 weeks either over the sensorimotor cortex or over the left dorsolateral prefrontal cortex (DLPFC) in 10 chronic patients

with DoC [14]. The results highlighted that, in some patients, the effect could last up to 12 months post-tDCS. Since these first two clinical trials in patients with DoC, tDCS has gained attention as reflected by the numerous randomized controlled trials (RCTs) that have been conducted testing various dosages and montages (for a review see Barra et al. [15]), showing that, so far, repeated sessions of tDCS targeting the left DLPFC seem to be the most beneficial for patients in MCS.

The clinical translation of tDCS was first tested in a home-based randomized study looking at the behavioural effects of 20 consecutive sessions of tDCS in 27 chronic MCS patients [16]. The results demonstrated that, as long as patients received at least 80% of the planned sessions, the treatment effect of 4 weeks of tDCS was significant, suggesting that the amount of tDCS applied can be another determinant of responsiveness.

Despite important efforts in investigating the potential therapeutic effects of tDCS to improve the recovery of patients with DoC, there is a lack of multicentre RCTs to evaluate the large-scale effects of tDCS, as highlighted in a recent gap analysis paper [17]. Therefore, the aim of this multicentre study was to determine the safety and effectiveness of tDCS—using the same parameters as in previous studies showing efficacy—in promoting recovery of signs of consciousness in patients diagnosed in prolonged UWS/VS and in MCS whilst being in post-acute rehabilitation. In addition, the aim was to determine the long-term effects of tDCS (3-month follow-up) as well as whether the diagnosis and the aetiology might influence the results.

METHODS

Study design

This study was a prospective parallel randomized triple-blind sham-controlled multicentre trial, with two arms (active and sham tDCS).

Standard protocol approvals, registrations and patient consents

Written informed consents were obtained by the representative of each patient in accordance with the Declaration of Helsinki. This multicentre study was registered (ClinicalTrials.gov NCT03114397) and approved by the central ethics committee of the University Hospital of Liège, and by each local ethics committee as needed.

Participants and eligibility criteria

Patients with DoC (UWS or MCS) were prospectively enrolled in 10 rehabilitation centres across five countries in Europe (see Data S1) based on the following criteria.

Inclusion criteria

The inclusion criteria were as follows: (1) central nervous system pharmacological therapy stable for at least a week before inclusion; (2) stable diagnosis of UWS/VS or MCS (no diagnosis change based on two CRS-R assessments performed within 1 week); (3) adult (16 years old to 80 years old); (4) acquired brain injury (e.g., trauma, stroke, hypoxia); (5) between 4 weeks and 24 months post-injury; (6) no previous history of severe acquired brain injury; (7) structural magnetic resonance imaging or computed tomography scan performed before enrolment to localize the brain lesion.

Exclusion criteria

The exclusion criteria were as follows: (1) craniotomies encompassing the frontal region (electrode location); (2) ventriculo-peritoneal shunt under the stimulated area (left prefrontal cortex); (3) pacemaker; (4) metallic cerebral implant; (5) patients under sedative drugs, sodium or calcium channel blockers (e.g., carbamazepine) or *N*-methyl-D-aspartate receptor antagonists (e.g., dextromethorphan); (6) severe medical conditions that might influence clinical diagnosis and sub-continuous, continuous or abundant epileptiform discharges on standard electroencephalogram (EEG) recordings; (7) severe hepatic insufficiency or renal failure.

Assessments

The primary outcome measure was the CRS-R [18], a sensitive and validated neurobehavioural scale including all diagnosis criteria for the MCS. It is composed of six subscales assessing the auditory, visual, motor and verbal functions, as well as communication and arousal. The total score is computed by additioning the scores of each subscale with the highest scores corresponding to more complex behaviours and lower scores to reflexive behaviours.

The Glasgow Outcome Scale (GOSE) was used to assess the functional long-term outcome of patients [19]. It is a short 5-point scale with the lower scores corresponding to the worse outcomes (death or UWS/VS) and the highest scores to the best outcomes (moderate disability or normal occupational and social activities).

Procedures

Twenty (active or sham) tDCS sessions were applied five times per week for 4 weeks.

Active stimulation parameters

The duration was 20 min. For electrode placement, the anode was over the left DLPFC (F3 according to international 10–20 EEG

electrode placement) and the cathode was over the right supraorbital area (Fp2). Each electrode size was 25 cm^2 (rubber electrode in dedicated sponges); the current intensity was 2 mA; a total of 20 sessions was delivered; and the ramp up/down was 15 s. Impedances were kept under 5 kΩ and voltage under 26 V.

Sham condition

The sham condition had the same duration, electrode size and placement as the active condition. The ramp up/down was 15 and 30 s of stimulation was delivered at the beginning of the 20 min session to simulate the initial sensation of the active current.

Dosing schedule

All participants were assigned to receive either active or sham tDCS at the same period of the day, preferably in the morning, throughout the experiment. All participants received tDCS whilst being awake, preferably in a sitting position rather than supine, and without any other type of intervention. If the patient started to close his/her eyes, tactile and auditory stimulations were provided to ensure eyes were constantly open.

Randomization and blinding

A computer-generated randomization sequence was assigned in a 1:1 ratio to deliver either active or sham stimulation. Randomization was performed centrally at the University of Liège. A third person, not involved in data collection, was responsible for the treatment allocation. The allocation of the participants to one of the two groups (active or sham) was concealed from the researchers until the randomization occurred. The investigators, patients and patients' relatives and caregivers were blinded to the treatment.

Three stimulation devices were used: DC Stimulator PLUS (NeuroConn©, Germany), Starstim 8 (Neuroelectrics©, Spain) and Brainstim (Newronika©, Italy). For the NeuroConn device, the local investigator, not involved in data acquisition and assessments, provided a code corresponding to the active or the sham intervention to the researcher responsible for data acquisition and assessments. For the Neuroelectrics device, a built-in double-blind mode with two pre-set active and sham protocols (randomly labelled A or B) was used. The allocation codes were provided by the local investigator, not involved in data acquisition and assessments. For both the DC Stimulator PLUS and the Starstim 8, a blind mode implemented in the software did not allow the person in charge of the data acquisition to visualize the parameters of stimulation. For the Newronika device, a researcher not involved in the clinical assessments was responsible to set up the tDCS device depending on the group allocation for each patient. The researcher responsible for the clinical assessments was blinded to the group allocation.

Outcome measures

The primary outcome measure is the level of consciousness/responsiveness as measured with the CRS-R total score at enrolment and weekly from week 1 through week 4 post-enrolment. The secondary outcome measure is the CRS-R total score at enrolment and after 1, 2, 4, 8, 12 and 16 weeks follow-up (Figure 1).

A change in diagnosis (i.e., UWS/VS to MCS−, MCS+ or emergence from MCS (EMCS); MCS− to MCS+ or EMCS; MCS+ to EMCS) after the treatment period (week 4) and at the 3-month follow-up was also reported.

In an exploratory manner, the treatment effect was evaluated by subgroup per diagnosis and aetiology, at week 4 and at 3 months' follow-up. The GOSE was also collected and compared between the two groups at 2 and 3 months' follow-up.

Adverse events occurring during the stimulation session were reported weekly from enrolment through termination using a standardized form. Demographic and clinical data relating to the past and current medical history were collected via review of the medical record or discussion with family members and clinicians familiar with the case to supplement the data acquired from the medical chart. Note that quantitative EEGs were also collected as a secondary outcome measure. However, as the majority of centres could not collect such data, only the behavioural results are reported in the present work.

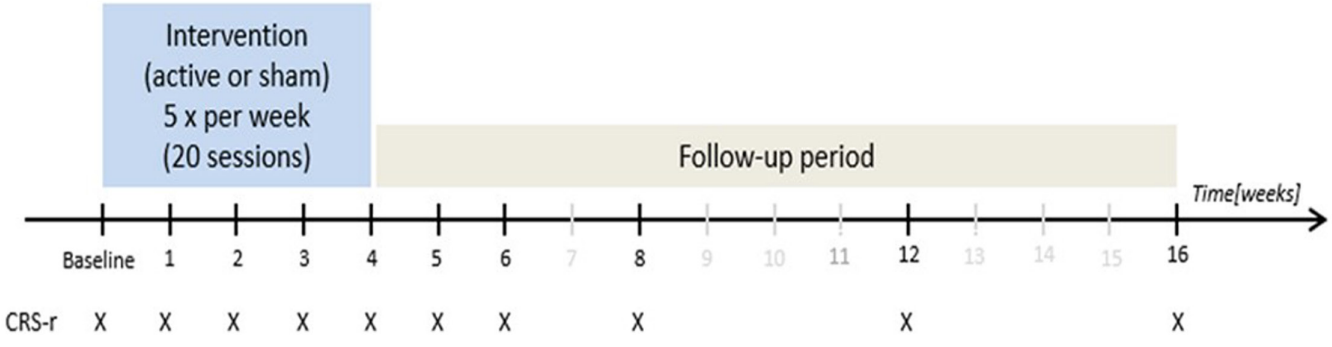

FIGURE 1 Study protocol. Each patient received five transcranial direct current stimulation (tDCS) sessions a week for 4 weeks, followed by a 12-week follow-up period. Coma Recovery Scale Revised (CRS-R) was performed at baseline, then once a week during the intervention period, and at 2 weeks, 1, 2 and 3 months follow-up.

Protocol violations

Protocol violations included a change in central acting medication, infections, surgical interventions, functional deterioration, two consecutive missing sessions or more than four missing sessions, appearance of seizure, and transfer to another institution not permitting the stimulation sessions or behavioural assessments to be pursued. Subjects who violated the protocol were excluded from the study. A detailed procedure and the case report form can be found in Data S1. All subjects randomized in the study were included in the statistical analysis, regardless of protocol violations or treatment compliance.

Study power

Sample size was estimated based on information from the previous studies including patients in both UWS/VS and MCS. Sample size calculations were based on the following assumptions: based on our previous tDCS study showing an effect size of 0.57 at 1-week follow-up, and for a two-sided α of 0.05 and a power of 80%, at least 52 subjects per group were needed. The sample size was then increased by 20% to account for possible dropouts, thus increasing to 62 patients per group, 124 total. Interim analyses were planned when 62 participants would have completed the study in order to run the power calculation, adjust the sample size if needed or stop the trial early for futility. The trial would be stopped for success in the case that significant results for our primary outcome would be found. On the other hand, the results would be considered futile if the conditional power based on the data of the 62 randomized patients would be below <35% (as used previously; e.g. Mank et al. [20]). The power calculation was performed using the non-parametric Mann–Whitney test to compare the change between the baseline and end-of-treatment measurements between the active and the sham groups.

Analyses

The intention-to-treat approach was used to analyse our data. Quantitative parameters are summarized using mean, standard deviation (SD), median, first and third quartiles (P25–P75) and minimal and maximal values (min–max), whereas qualitative parameters are summarized using numbers (*n*) and frequencies (%). The normality of quantitative parameters was investigated using descriptive and graphical techniques (comparison of mean and median values, histogram and quantile–quantile plots) and tested using the Shapiro–Wilk test. Homogeneity of the covariates, namely diagnosis (UWS/VS or MCS), aetiology (TBI or non-TBI) and time since injury (<12 or >12 months) between the two treatment groups was tested using the χ^2 test or Fisher's exact test if the conditions for the χ^2 test were not met.

The evolution of the CRS-R total score during the 4 weeks of treatment, but also during the follow-up period, was analysed using generalized estimating equations models. Different correlation structures such as compound symmetry, independent or unstructured were evaluated. Models were compared using the quasi likelihood under the independence model criterion (QIC) and QICu (smaller is better). The details of these results can be found in Data S1. Effects of time, of treatment, but also an interaction effect (time*treatment), were tested, with adjustment for the covariates mentioned above. These same analyses were performed for subgroups: (1) according to the diagnosis (MCS and UWS/VS patients) and (2) according to the aetiology (TBI and non-TBI patients). A log transformation of the CRS-R total score was considered to meet the conditions for the application of these models (normality and homoscedasticity of residuals).

In an exploratory manner, the differences in the CRS-R total scores at baseline compared to both the end of the stimulation session (week 4) and the end of the follow-up (month 3) (i.e., Δs) were examined. These changes (Δs) were then compared based on the diagnosis (active and sham UWS/VS and MCS) and the aetiology (active and sham TBI and non-TBI) with a Kruskal–Wallis test. In the case of rejection of the null hypothesis, a Mann–Whitney test was used to look at the differences between the active and the sham groups for each subgroup (UWS/VS, MCS, TBI and non-TBI). Finally, the GOSE at 2 and 3 months follow-up was compared between the active and sham group with a Mann–Whitney test.

All randomized patients were included in our analyses (intention to treat) and missing data were not replaced as all dropouts were due to causes independent of the treatment (e.g., transfer to another institution, change of medication, infection).

The results were considered significant at the $\alpha = 5\%$ uncertainty level ($p < 0.05$). The analyses were performed using SAS version 9.4, R version 4.0.2 and G*Power 3.1.9.4 software.

RESULTS

Out of 483 patients screened in the 10 rehabilitation centres, 62 patients (18 women, 44 ± 14 years old, 36 ± 25.5 weeks post-injury, 32 MCS, 24 anoxia, 23 TBI, 13 stroke, two others) were enrolled and randomized in the study between September 2017 and July 2021. Patients were enrolled at 37 ± 24.5 weeks post-injury, were receiving 2.6 ± 0.8 h of rehabilitation per day and the median and interquartile range (IQR) 25 and 75 CRS-R at baseline was 7 (6–11). Thirty-three were allocated to the active stimulation (eight women, 42 ± 12 years old, 41 ± 13.5 weeks post-injury, 17 MCS, 13 TBI, 15 anoxia, four stroke, one other) and 29 to the sham group (11 women, 45.5 ± 12 years old, 30 ± 15 weeks post-injury, 15 MCS, 10 TBI, nine anoxia, nine stroke, one other). Individual demographic data and CRS-R scores of the 62 patients are reported in Table 1. No differences in demographic data were found between the active and the sham groups at baseline for diagnosis ($p = 0.62$), aetiology ($p = 0.69$) and time since injury ($p = 0.55$) and baseline CRS-R total scores ($p = 0.50$). Baseline characteristics of each subgroup can be found in Table 2.

No tDCS-related serious adverse effects were identified. One patient had a seizure and one died during the 4 weeks of

TABLE 1 Sociodemographic characteristics of the study population and CRS-R scores at baseline, after 4 weeks of treatment (week 4) and at 1 month, 2 months and 3 months after the end of treatment (month 1 FU, month 2 FU, month 3 FU).

								CRS-R total score, diagnosis				
ID	Group	Inclusion site	Age (years) gender	Aetiology, time since injury (weeks)	Centrally acting medication	Rehabilitation (h/day)	CT or [MRI] report	Baseline	Week 4	Month 1 FU	Month 2 FU	Month 3 FU
1	1	ICSM1	44 F	Non-TBI Anoxia (CA), 85	Selegilina 10 mg/day	3	Diffuse atrophy, ventricular enlargement	6, UWS	6, UWS	6, UWS	6, UWS	6, UWS
2	1	ICSM2	17 M	Non-TBI Anoxia (CA), 98	Lioresal 25 mg 1 × 3/day	3	Hypodensity of the right basal frontal, bilateral occipital regions and brainstem	19, MCS+	19, MCS+	19, MCS+	19, MCS+	19, MCS+
3	1	NEURORHB1	20 M	TBI, 25	Diazepam 5 mg at night Levetiracetam 500 mg c/12 h Amantadine 200 mg/day	4	[MRI] Left haemorrhagic temporal contusion (craniectomy). Diffuse cortical (bilateral superior frontal) and subcortical (corpus callosum, right cerebellum, right thalamus-mesencephalic) alteration. Ventricular enlargement	9, MCS−	11, MCS−	11, MCS−	11, MCS−	11, MCS−
4	0	ICSM3	61 M	Non-TBI Anoxia (CA), 16	Amantadine 100 mg Lioresal 25 mg 3/day	3	Hypodensity basal and thalamic nucleus; diffuse cortical supratentorial and subtentorial alteration	6, UWS	6, UWS	6, UWS	–	–
5	0	ICSM4	68 M	Non-TBI Vascular, 28	Selegiline 10 mg/day	3	Hypodensity right cerebral white matter; ventricular enlargement	10, MCS−	10, MCS−	10, MCS−	–	–
6	1	ICSM5	34 F	Non-TBI Anoxia, 52	Selegiline 10 mg/day	3	Hypodensity basal ganglia and periventricular white matter	7, UWS	7, MCS−	7, UWS	7, UWS	7, UWS
7	0	BE1	65 M	Non-TBI Haemorrhagic stroke, 39	Amantadine 200 mg Keppra 200 mg	2	Right fronto-temporo-occipital haemorrhage	8, MCS−	–	–	–	–
8	1	NEURORHB2	46 F	Non-TBI Anoxic encephalopathy, 14	Valproate 600 mg 3/day Levetiracetam 1500 mg 2/day	4	[MRI] Hypointensities in lentiform and caudate nuclei	6, UWS	6, UWS	7, UWS	7, UWS	7, UWS
9	0	BE2	56 F	Non-TBI Anoxia (CA) 62	Levetiracetam 200 mg	2	[MRI] DAI; hippocampus atrophy, ventricular enlargement	5, UWS	3, UWS	5, UWS	5, UWS	5, UWS
10	0	CUNEO1	39 M	Non-TBI Aneurysmal subarachnoid haemorrhage, 16	None	3	NA	8, MCS−	9, MCS−	7, MCS−	8, MCS−	8, MCS−
11	1	CUNEO2	52 F	Non-TBI Aneurysmal subarachnoid haemorrhage, 13	None	3	NA	9, MCS−	9, MCS−	9, MCS−	9, MCS−	9, MCS−
12	0	ICSM6	45 F	Non-TBI Vascular, 36	Selegiline 10 mg/day	3	[MRI] Left thalamic haemorrhage	10, MCS+	10, MCS+	10, MCS+	10, MCS+	10, MCS+

TABLE 1 (Continued)

ID	Group	Inclusion site	Age (years) gender	Aetiology, time since injury (weeks)	Centrally acting medication	Rehabilitation (h/day)	CT or [MRI] report	CRS-R total score, diagnosis				
								Baseline	Week 4	Month 1 FU	Month 2 FU	Month 3 FU
13	1	BE3	40 M	TBI, 83	Propranolol 20 mg Baclofen 25 mg Tramadol 50 mg Trazodone 100 mg Escitalopram 10 mg	1	[MRI] DAI, corpus callosum and cerebellum	8, MCS−	11, MCS+	11, MCS+	11, MCS+	11, MCS+
14	0	BE4	68 F	TBI, 15	Levetiracetam 300 mg Amantadine 2175 mg	1	[MRI] Left parieto-temporal and basal ganglia alteration	13, MCS−	–	–	–	–
15	1	FSL 1	34 M	TBI, 36	Levetiracetam 1500 mg/12 h Neurontin 200 mg/121 h	3	[MRI] Bilateral white matter hypointensities	10, MCS−	10, MCS−	9, MCS−	10, MCS−	10, MCS−
16	1	ICSM7	56 M	TBI, 95	Selegiline 10 mg/daily Lioresal 251 mg 3/day0	3	Left posterior pons and callosal lesions, cortical atrophy	8, MCS−	9, MCS−	9, MCS−	7, MCS−	10, MCS−
17	1	BE5	63 M	Non-TBI Anoxia (CA), 42	Baclofen 25 mg Clonazepam 10 mg Tramadol 50 mg 4/day	2	Left fronto-temporo-parietal and right parietal subcortical ischaemic lesions; bitemporal and biparietal cortical ischaemic lesions	7, UWS	7, UWS	7, UWS	7, UWS	7, UWS
18	0	BE6	31 M	Non-TBI Meningitis, 22	Trazadone 150 mg	2	Left inferior cerebellar ischaemia, right basal ganglia and discrete diffuse cerebral oedema	12, MCS+	–	–	–	–
19	0	BE7	23 M	TBI, 20	Levetiracetam 300 mg	2	Bilateral frontal haematoma, right temporal lesion, bilateral lesions of basal ganglia, lesion of the splenium of corpus callosum, lesion of posterior part of right midbrain, DAI	5, UWS	–	–	–	–
20	1	CUNEO3	47 M	Non-TBI Haemorrhagic stroke, 13	None	3	[MRI] Left thalamic haemorrhage	11, MCS−	–	–	–	–
21	0	ICSM8	38 M	Non-TBI Anoxia, 29	Amantadine 200 mg/day	3	DAI, ventricular enlargement	6, UWS	6, MCS−	6, MCS−	6, UWS	7, MCS−
22	1	FSL2	52 F	Non-TBI Anoxia, metabolic hypoglycaemic, 28	Amantadine 200 mg/day Pregabalin 300 mg/day Duloxetine 60 mg/day Melevodopa + carbidopa 300/75/day	3	Diffuse hypodensity in periventricular white matter. Multiple small lesions in bilateral frontoparietal regions. Cortical and subcortical atrophy	5, UWS	6, UWS	6, UWS	6, UWS	9, MCS−
23	1	ICSM9	60 M	Non-TBI Vascular, 34	Amantadine 200 mg/day	3	DAI	5, UWS	6, MCS−	6, MCS−	5, UWS	5, MCS−
24	0	ICSM10	51 M	Non-TBI Ischaemic stroke, 31	Amantadine 200 mg/day	3	Pons lesion	6, UWS	–	–	–	–

(Continues)

TABLE 1 (Continued)

ID	Group	Inclusion site	Age (years) gender	Aetiology, time since injury (weeks)	Centrally acting medication	Rehabilitation (h/day)	CT or [MRI] report	CRS-R total score, diagnosis				
								Baseline	Week 4	Month 1 FU	Month 2 FU	Month 3 FU
25	1	FSL3	51 M	Non-TBI Anoxia (CA), 26	Lacosamide 50 mg/day Pregabalin 300 mg/day	3	[MRI] Focal bilateral cortical parieto occipital, mesial cerebellar, lenticulo capsular nucleus, talamus ponto bulbar and corpus callosum lesions. Cortical and subcortical atrophy	4, UWS	3, UWS	3, UWS	3, UWS	3, UWS
26	1	FLS4	20 M	TBI, 32	Amantadine 200 mg/day Levetiracetam 500 mg/12 h	3	DAI with brainstem involvement	8, MCS−	8, MCS−	8, MCS−	8, MCS−	8, MCS−
27	1	NEURORHB3	21 M	TBI, 86	Amantadine 200 mg/day	2	[MRI] Diffuse white matter and bilateral thalamus alterations	12, MCS−	12, MCS−	12, MCS−	12, MCS−	12, MCS−
28	1	CUNEO4	69 M	TBI, 17	Levetiracetam 500 mg 2/day	2	[MRI] DAI	9, MCS−	10, MCS−	11, MCS−	11, MCS−	11, MCS−
29	0	CUNEO5	53 M	Non-TBI Haemorrhagic stroke, 15	None	2	[MRI] Left parieto-temporal and basal ganglia lesions	5, UWS	6, UWS	7, UWS	6, UWS	7, UWS
30	1	CUNEO6	55 M	Non-TBI Anoxia (CA), 41	None	2	[MRI] Bilateral white matter hypointensities	6, UWS	–	–	–	–
31	1	SKBA1	43 M	TBI, 13	None	2	[MRI] DAI, subdural haematoma right frontal, subarachnoid haemorrhage	9, MCS−	–	–	–	–
32	1	BE8	72 F	Non-TBI Haemorrhagic stroke, 94	Amantadine 200 mg/day	0.5	DAI and atrophy in cerebellum, thalamus and fronto-parietal areas	11, MCS+	10, MCS+	–	–	–
33	0	SKBA2	48 M	TBI, 33	None	2	Oen TBI, left subdural haematoma, right epidural frontobasal haematoma, hydrocephalus	12, MCS+	19, EMCS	11, MCS−	8, UWS	8, UWS
34	0	NEURORHB4	38 M	TBI, 67	Levetiracetam 1000 mg 2/day	4	[MRI] Left subdural haematoma, left parietal contusion (craniectomy), ventricular enlargement	10, MCS−	10, MCS−	10, MCS−	10, MCS−	10, MCS−
35	1	BE9	24 M	TBI, 14	Levetiracetam 300 mg Propranolol 40 mg	1	Left posterior hemisphere and left cerebellum lesions	17, MCS+	23, EMCS	23, EMCS	23, EMCS	23, EMCS
36	0	FSL5	43 F	Non-TBI Anoxic (CA), 13	Amantadine 200 mg/day	3	Diffuse bulbo-pontine ischaemic lesion. Cortical and subcortical atrophy	3, UWS	5, UWS	5, UWS	5, UWS	5, UWS
37	1	TZB1	49 M	Non-TBI Hypoxic–ischaemic encephalopathy, 14	None	1.5	[MRI] Cerebral hypoxic damage	7, MCS+	–	–	–	–
38	1	NEURORHB5	38 M	Non-TBI Anoxic encephalopathy, 30	Levetiracetam 500 mg 2/day	4	[MRI] Cortico-subcortical atrophy	8, UWS	8, UWS	8, UWS	8, UWS	8, UWS

TABLE 1 (Continued)

ID	Group	Inclusion site	Age (years) gender	Aetiology, time since injury (weeks)	Centrally acting medication	Rehabilitation (h/day)	CT or [MRI] report	CRS-R total score, diagnosis				
								Baseline	Week 4	Month 1 FU	Month 2 FU	Month 3 FU
39	0	NEURORHB6	55 M	TBI, 32	Amantadine 200 mg/day	4	[MRI] Left subdural haematoma (craniectomy), frontobasal and temporobasal contusions. Cortical and subcortical atrophy. Ventricular enlargement	14, MCS−	–	–	–	–
40	1	RCN1	24 M	TBI, 68	None	3	Left posterior pons and callosal lesions, cortical atrophy	7, UWS	7, MCS−	7, UWS	7, UWS	7, UWS
41	0	BE10	53 F	Non-TBI Haemorrhagic stroke, 22	None	2	Left brainstem (pons and midbrain) and right parieto-temporal lesions	6, MCS−	5, UWS	–	–	–
42	0	TZB2	61 F	Non-TBI Hypoxic brain injury, 13	Levothyroxin 100 µg Ibuprofen 400 mg Levetiracetam 500 mg Metocolopramide 10 mg Baclofen 10 mg Fentanyl 25 µg/day	1.7	[MRI] No evidence of recent cerebral infarction or recent hypoxic brain damage	5, UWS	6, UWS	–	–	–
43	0	TZB3	36 M	Non-TBI Hypoxic encephalopathy, 14	Lorazepam 0.5 mg 3/day	1.65	No evidence of acute intracranial haemorrhage or territorial ischaemia	7, UWS	6, UWS	–	–	8, UWS
44	0	NAMRC1	21 F	TBI, 97	Valproate 750 mg 2/day	2	[MRI] DAI, right thalamus and frontal lobe lesions	15, MCS+	17, EMCS	17, MCS+	–	–
45	1	TZB4	39 F	TBI, 13	None	2.15	DAI, normal tension hydrocephalus	3, UWS	5, UWS	6, UWS	–	–
46	1	NAMRC2	36 M	Non-TBI Anoxia (CA), 13	None	2	[MRI] Multiple small fronto-basal brain lesions, DAI	4, UWS	4, UWS	–	–	–
47	0	BE11	50 F	TBI, 22	Tramadol 50 mg Trazadone 100 mg Sertaline 100 mg	2	No evidence of acute intracranial haemorrhage or territorial ischaemia	16, MCS+	13, MCS+	–	–	–
48	0	TZB5	29 M	Non-TBI Hypoxic, 13	Quetiapine 50 mg Clonazepam 2 mg	5	No evidence of hypoxic encephalopathy, no bleeding	3, UWS	4, UWS	–	–	–
49	0	TZB6	37 M	Non-TBI Intracerebral haemorrhage, 14	Clonazepam 1 mg Quetiapine 25 mg	5	Extensive post-haemorrhagic parenchymal defect left parieto-temporal involving basal ganglia	5, UWS	3, UWS	–	6, UWS	–
50	1	NEURORHB7	24 M	Non-TBI Anoxia, 76	Levodopa 625 mg/day Baclofen 10 mg 3/day	4	[MRI] Bilateral white matter hypointensities. Ventricular enlargement	7, UWS	7, UWS	7, UWS	7, UWS	7, UWS
51	1	BE12	48 M	Non-TBI Anoxia (CA), 43	Tramadol 37.5 mg Prazepam 5 mg Lormetazepam 1 mg	2	Anoxic lesions in bilateral basal ganglia	15, MCS+	17, MCS+	16, MCS+	–	–
52	0	NAMRC3	33 M	TBI, 16	Phenytoin 100 mg 3/day	1	[MRI] Multiple small fronto-basal brain lesions, DAI	6, MCS−	6, MCS−	–	–	–

(Continues)

TABLE 1 (Continued)

ID	Group	Inclusion site	Age (years) gender	Aetiology, time since injury (weeks)	Centrally acting medication	Rehabilitation (h/day)	CT or [MRI] report	CRS-R total score, diagnosis				
								Baseline	Week 4	Month 1 FU	Month 2 FU	Month 3 FU
53	1	NAMRC4	26 M	TBI, 13	None	2	No evidence of acute intracranial haemorrhage or territorial ischaemia	14, MCS−	16, MCS+	–	–	–
54	1	RCN2	54 F	Non-TBI Anoxia, 17	None	3	Diffuse axonal injury; cortical and subcortical atrophy	7, UWS	7, UWS	7, UWS	–	–
55	1	FSL6	48 M	TBI, 42	Biperidene 4 mg/day Pregabalin 300 mg/day	3	DAI, cortical and subcortical atrophy	13, UWS	14, MCS+	14, MCS+	14, MCS+	16, MCS+
56	0	BE13	36 M	TBI, 15	Tramadol 100 mg Lormetazepam 2 mg	2	DAI infra- and supra-tentorially. Microbleeds on occipital left and temporal right regions	14, MCS−	18, EMCS	20, EMCS	18, EMCS	–
57	0	NEURORHB8	24 M	TBI, 58	Levetiracetam 500 mg c/12 h Tizanidine 2 mg/8 h	4	[MRI] Right frontal contusion (craniectomy). Diffuse cortical (left temporal); subcortical (c. callosum, white matter, left thalamus) alterations. Ventricular enlargement	12, MCS+	12, MCS+	12, MCS+	12, MCS+	10, MCS−
58	0	FSL7	65 F	Non-TBI Anoxia, 58	Levetiracetam 1000 mg/12 h Diazepam 3 mg/24 h Lacosamide 50 mg/24 h Pregabalin 225 mg/24 h Tizanidine 2 mg/24 h	3	Diffuse ischaemic bilateral white matter damage. Basal ganglia, corpus callosum and cortico-subcortical atrophy	5, UWS	5, UWS	5, UWS	5, UWS	5, UWS
59	1	BE14	44 M	Non-TBI Anoxia, 24	Lormetazepam 1 mg Trazodone 100 mg Prazepam 10 mg 3/day	2	Widespread anoxic lesions, right frontal, cerebellum and bilateral basal ganglia lesions	6, UWS	7, UWS	6, UWS	6, UWS	6, UWS
60	0	TZB7	61 M	Non-TBI, 19	None	5	Large parenchymal haemorrhage basal ganglia involving rostral thalamus	3, UWS	4, UWS	3, UWS	–	–
61	1	FLS8	36 M	Non-TBI Anoxia (CA), 58	Levetiracetam 1500 mg 2/day Clonazepam 00.5 mg 3/day Phenytoin 200 mg 2/day Phenobarbital 100 mg 2/day	3	[MRI] Multiple, small and bilateral temporal-occipital-parietal cortex and fronto-basal brain lesions	6, UWS	6, UWS	6, UWS	6, UWS	–
62	0	NEURORHB9	31 F	Non-TBI Anoxia (CA), 35	Levetiracetam 1500 mg 2/day Lacosamide 100 mg 2/day Clonazepam 0.5 mg 3/day Baclofen 25 mg 3/day Piracetam 800 mg 3/day	4	[MRI] Bilateral periventricular white matter hypointensities. Bifrontal (predominantly right) white matter hypointensities. Bilateral hypointensities in lentiform nuclei. Ventricular enlargement	6, UWS	6, UWS	6, UWS	6, UWS	6, UWS

Abbreviations: CA, cardiac arrest; CRS-R, Coma Recovery Scale Revised; CT, computed tomography; DAI, diffuse axonal atrophy; EMCS, emergence from MCS; F, female; FU, follow-up; M, male; MCS, minimally conscious state; MRI, magnetic resonance imaging; NA, not available; TBI, traumatic brain injury; UWS, unresponsive wakefulness syndrome.

TABLE 2 Demographic data for the entire group and per subgroup for diagnosis (UWS vs. MCS) and aetiology (TBI vs. NTBI).

		Age (years) mean ± SD	Diagnosis	Gender	TBI/NTBI	Time since injury (weeks) mean ± SD	Baseline CRS-R median (IQR 25–75)
Group ($n=62$)	Active ($n=33$)	42 ± 12	17 MCS 16 UWS	8 women 25 men	13 TBI 20 NTBI	41 ± 23.5	8 (6–10)
	Sham ($n=29$)	45.5 ± 12	15 MCS 14 UWS	11 women 18 men	10 TBI 19 NTBI	30 ± 15	6 (5–12)
	p value	0.34	0.62	0.49	0.69	0.55	0.50
MCS ($n=32$)	Active ($n=17$)	40.5 ± 14	–	2 women 15 men	11 TBI 6 NTBI	44.3 ± 28.5	10 (9–13)
	Sham ($n=15$)	45 ± 12	–	6 women 9 men	9 TBI 6 NTBI	34.5 ± 16.5	12 (9–13.5)
	p value	0.46	–	0.07	0.78	0.76	0.80
UWS ($n=30$)	Active ($n=16$)	44 ± 9.5	–	6 women 10 men	2 TBI 14 NTBI	39 ± 18.5	6 (5–7)
	Sham ($n=14$)	46 ± 12	–	5 women 9 men	1 TBI 13 NTBI	25.5 ± 13	5 (5–6)
	p value	0.65	–	0.62	0.626	0.22	0.06
TBI ($n=23$)	Active ($n=13$)	35.5 ± 12.5	11 MCS 2 UWS	1 women 12 men	–	41.5 ± 26	9 (8–12)
	Sham ($n=10$)	39.5 ± 12.5	9 MCS 1 UWS	4 women 6 men	–	37.5 ± 22	12.5 (10.5–14)
	p value	0.58	0.71	0.06	–	0.23	0.17
NTBI ($n=39$)	Active ($n=20$)	46 ± 9.5	6 MCS 14 UWS	7 women 13 men	–	40.5 ± 22	7 (6–8.25)
	Sham ($n=19$)	48.5 ± 11.5	6 MCS 13 UWS	7 women 12 men	–	26 ± 11.5	6 (5–7.5)
	p value	0.56	0.916	0.91	–	0.82	0.14

Note: Comparisons between the active and sham group for all variables are also presented. No significant differences between groups for any of the variables were observed.Abbreviations: CRS-R, Coma Recovery Scale Revised; IQR, interquartile range; MCS, minimally conscious state; NTBI, non-traumatic brain injury; TBI, traumatic brain injury; UWS, unresponsive wakefulness syndrome.

tDCS; however, both patients were allocated to the sham group. Regarding adverse effects, skin redness was reported for three patients, irritation of the skin for one patient and sleepiness for another patient. Regarding adherence, out of 20 sessions of tDCS, three patients missed two sessions and one missed three sessions. No patient had to be excluded due to missing sessions (see Protocol violations). In addition, one session had to be interrupted after a few minutes on three occasions (three different patients) due to impedance issues. For one patient, two sessions were terminated before the end of the session and for three other patients three sessions were terminated prematurely due to impedance issues.

Twelve patients dropped out during the intervention period (six in the active and six in the sham groups; 19% dropout) and an additional 16 patients during the follow-up period (five in the active and 11 in the sham groups; total of 45% dropout). In sum, 82% of the patients allocated to the active arm and 79% of the patients allocated to the sham arm completed the 4 weeks of tDCS and 66% versus 41% completed all study time points. Details of the time and reason for dropout are provided in the study flowchart (see Figure 2).

Based on our primary outcome (i.e., treatment*time interaction during 4 weeks of treatment at the group level), the calculated effect size (non-parametric test for two independent samples) was 0.140. Given this effect size, the conditional power was 8.2%, which is under the cut-off of 35% that was set to stop the trial. Therefore, all the analyses were performed on the 62 patients enrolled.

Regarding the repeated measures model, the values of QIC did not show important differences between independent or compound symmetry correlation structure. Also, according to values of QICu, there was no significant difference between the three models. At the group level, the generalized linear mixed model did not reveal any treatment*time interaction effect during the 4 weeks of tDCS ($\chi^2=1.52$, $p=0.22$, primary outcome) nor during the 3 months' follow-up phase ($\chi^2=0.27$, $p=0.60$, secondary outcome) (Figure 3).

The results remain the same after adjusting for covariates. The calculated effect size after 4 weeks of tDCS was 0.140. Similarly, none of the subgroup analyses revealed any significant treatment*time interaction effect for the 4 weeks of tDCS (UWS/VS, $\chi^2=0.99$, $p=0.32$; MCS, $\chi^2=0.37$, $p=0.54$; TBI, $\chi^2=0.50$, $p=0.48$; non-TBI, $\chi^2=0.81$, $p=0.37$) nor during the 3-month follow-up (UWS/VS, $\chi^2=1.47$, $p=0.23$; MCS, $\chi^2=1.84$, $p=0.18$; TBI, $\chi^2=1.68$, $p=0.92$; non-TBI, $\chi^2=1.16$, $p=0.28$).

When looking at the difference between the measures at baseline and at the end of the treatment period (week 4) as well as at the end

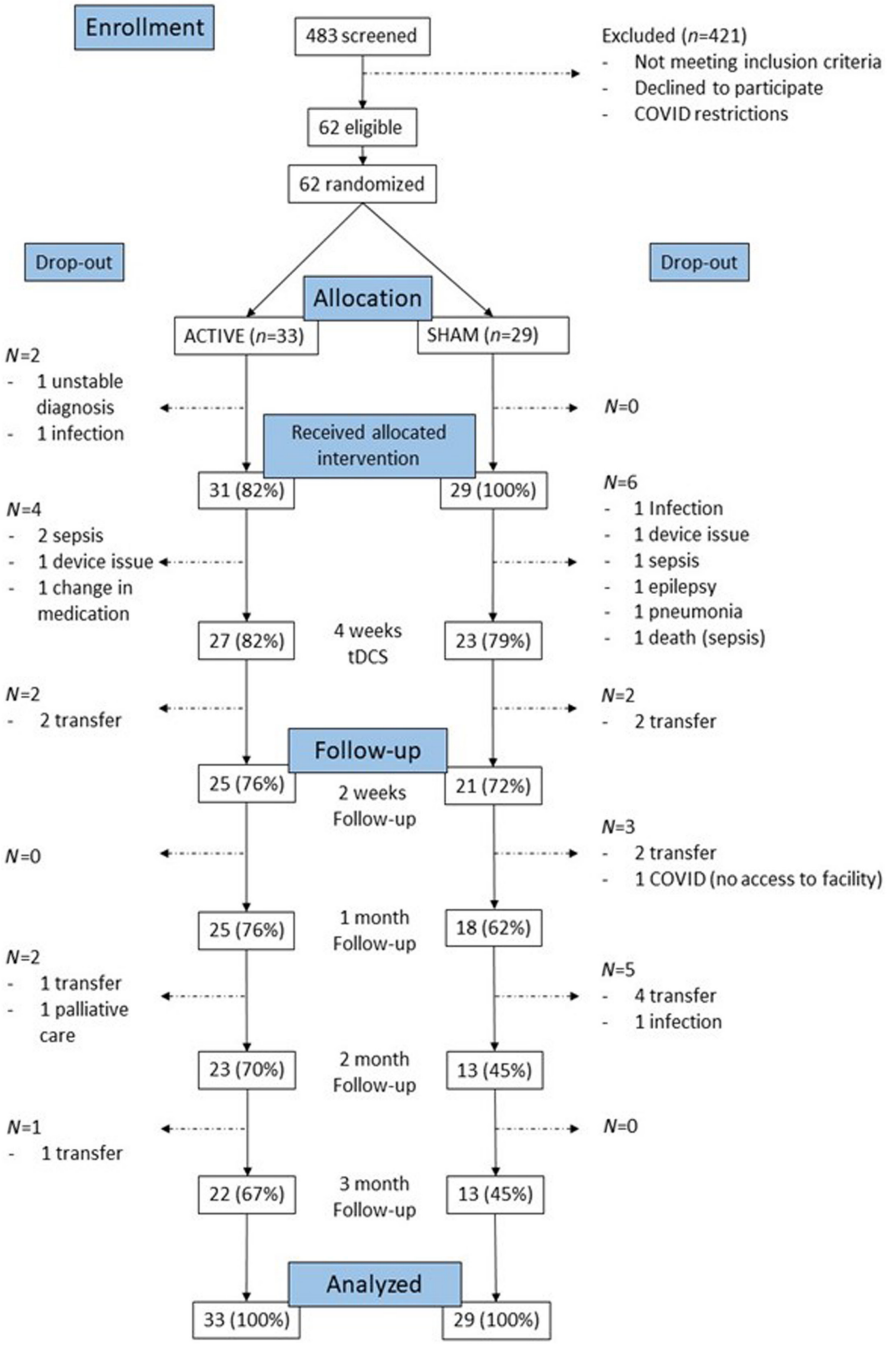

FIGURE 2 Study flowchart.

of the follow-up period (3-month follow-up) (Figure 4), group analyses did not reveal any differences ($Z=0.761$, $p=0.446$, and $Z=1.231$, $p=0.218$). Subgroup analyses for both the diagnosis (UWS/MCS) and the aetiology (TBI/no-nTBI) revealed a significant difference (UWS/MCS: $\chi^2=10.043$, $p=0.018$, and TBI/non-TBI: $\chi^2=9.154$, $p=0.012$) at the 3-month follow-up but no differences after 4 weeks of treatment ($\chi^2=2.809$, $p=0.442$, and $\chi^2=7.112$, $p=0.068$). Post hoc analyses demonstrated an improvement in the CRS-R total scores (median, IQR) for the active compared to the sham group for MCS patients ($Z=2.465$; $p=0.014$—improvement of 2 [0–3] for the active group and 0 [−2–0] for the sham group) and for TBI patients ($Z=2.279$; $p=0.023$—improvement of 2 [0–3] for the active group and −2 [−4–0] for the sham group) at 3 months (Figure 4).

No difference on the GOSE was found at 2 or at 3 months follow-up ($Z=0.567$, $p=0.571$, and $Z=0.624$, $p=0.533$).

No baseline differences between the active and sham group for any of the subgroups were found ($p>0.05$). Details of all results can be found in Data S1.

DISCUSSION

In this prospective, randomized, double-blind trial for patients with DoC, no significant difference was found between the groups of patients receiving active versus sham tDCS (effect size 0.140). The results of a planned interim analysis performed when half of the

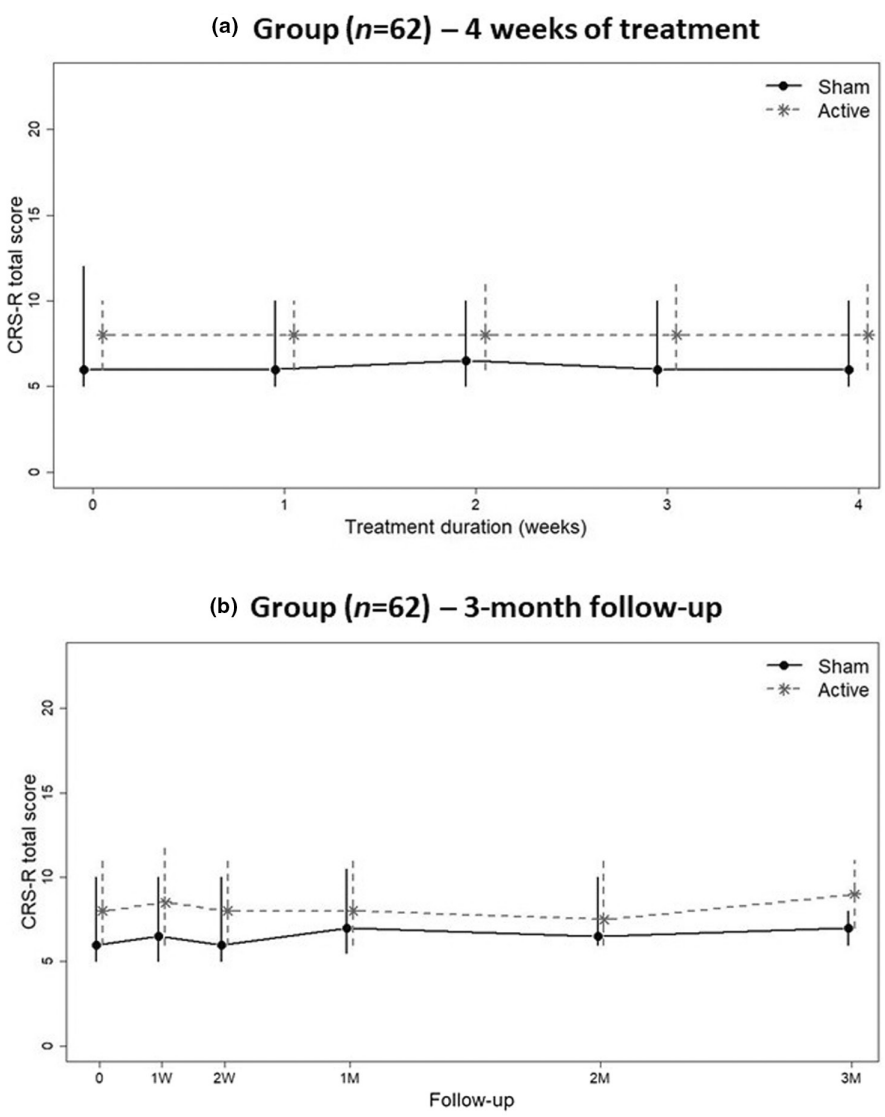

FIGURE 3 Results of the generalized linear mixed model during the 4-week intervention phase and the 3-month follow-up. (a) The median and interquartile range of the log transformation of the CRS-R total score during the intervention period (4 weeks). (b) The median and interquartile range of the log transformation of the CRS-R total score during the follow-up period (3 months).

predefined sample would have completed the study (i.e., $n = 62$) met the futility criterion for stopping the trial.

Nonetheless, the safety and the feasibility was demonstrated of 4 weeks of tDCS when applied in rehabilitation facilities in patients with prolonged DoC. Behaviourally, even if a treatment effect was not observed at the group level (including both UWS/VS and MCS and all etiologies), a significant improvement was found for the subgroup of patients in MCS and patients who had a TBI when assessed at the 3-month follow-up.

In the therapeutic management of patients with DoC, non-invasive brain stimulation represents a promising complement to pharmacological approaches to promote brain activity and patients' recovery. In the present multicentre study, it was shown that tDCS could be easily implemented as an additional treatment to rehabilitation programmes for patients with DoC. Indeed, adherence to the protocol was very good and the dropout rate during the stimulation period was similar to previous RCTs (i.e., 20%). Importantly, no severe adverse events were reported when tDCS was applied for a relatively long period of time in a population of patients prone to various medical complications. Our findings confirm the safety and feasibility of the technique in patients with severe brain injuries, which represents a first step for the clinical translation and the use of tDCS in patients with DoC whilst in rehabilitation. tDCS stands out as a particularly suitable candidate to complement current rehabilitation techniques thanks to its ease of use and its standard session duration (i.e., 20 min), which could be applied during physiotherapy or occupational therapy sessions.

Behaviourally, at the group level, no difference was observed between the active and the sham group as reported in a few earlier studies [21]. Whilst it is known that MCS patients tend to respond better to tDCS compared to UWS/VS, the present study still aimed to include both MCS and UWS/VS. Indeed, it is now widely admitted that a significant proportion of UWS/VS present a brain activity compatible with the diagnosis of MCS (i.e., MCS*, covert consciousness or cognitive motor dissociation) which is associated with a better outcome [22–25]. However, such therapeutic effect in VS/UWS (possibly with covert consciousness) was not observed in our study. In addition, regardless of the group allocation, whilst patients were included less than 2 years after their brain injury, only one UWS/VS (out of 30, 3%), regained signs of consciousness

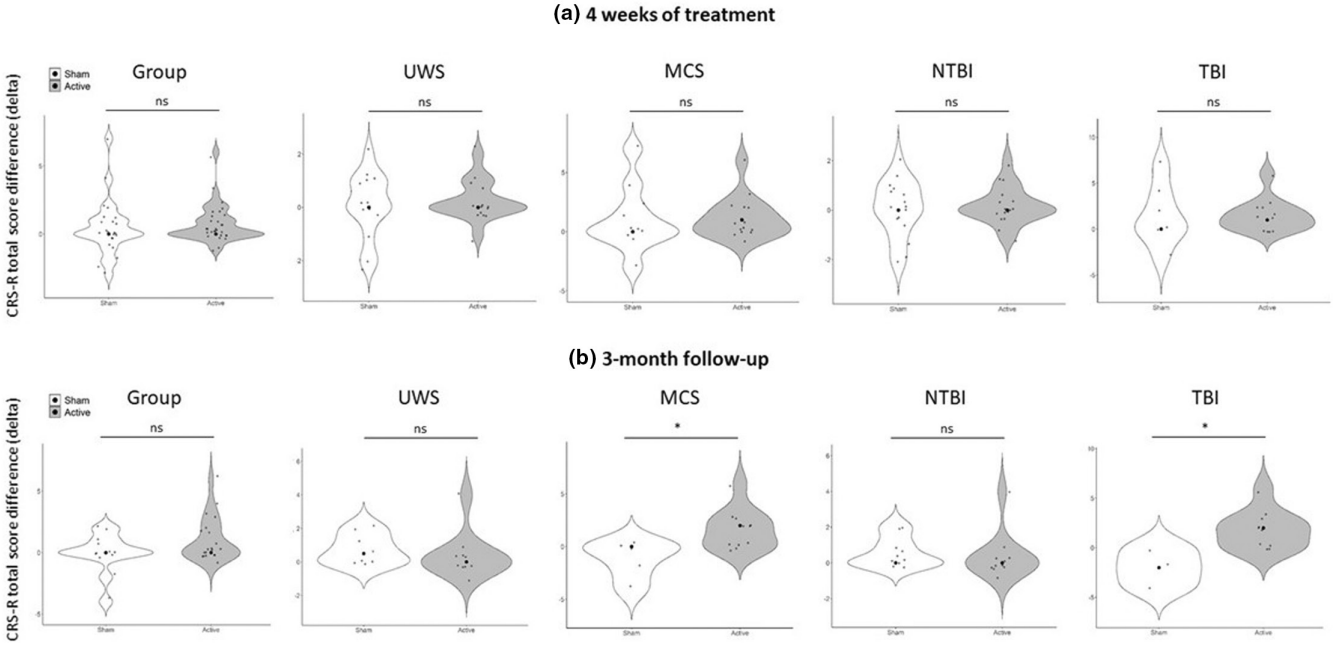

FIGURE 4 Results of the subgroup analyses. (a) The violin plots represent the difference between the CRS-R total scores at baseline and at the end of the treatment period (4 weeks) for the sham group in white and the active group in grey. (b) The violin plots represent the difference between the CRS-R total scores at baseline and at the end of the follow-up period (3 months) for the sham group in white and the active group in grey. The small dots represent the difference for each individual and the bigger dots represent the median for each group. MCS, minimally conscious state; NTBI, non-traumatic brain injury; TBI, traumatic brain injury; UWS, unresponsive wakefulness syndrome.

during the stimulation period, which highlights the limited chances of recovery of unresponsive patients in prolonged DoC (i.e., >28 days post-injury [26]).

When looking at the subgroup of patients in MCS, a significant improvement was found, not during the 4-week stimulation period but at the 3 months' follow-up, showing a better recovery for the group of MCS patients allocated to the active compared to the sham group that could not be accounted for by other variables such as time since injury, aetiology or age. More specifically, whilst the control group did not improve at all (no changes in consciousness/responsiveness level as measured with the CRS-R total score) over the 3 months' follow-up period, the active group gained a median of 2 points on the CRS-R (IQR 0–3), which is compatible with previous RCTs targeting the prefrontal region [13,16,27] and a previous meta-analysis showing that tDCS has greater effects in MCS patients compared to UWS/VS [9]. At the individual level, regardless of the allocation, out of 32 patients in MCS at enrolment, four of them emerged and one regained language-related behaviours (i.e., MCS plus, all TBI), corresponding to a 16% rate of diagnosis change. However, no difference between the active and the sham groups could be observed with regard to the change in diagnosis.

Similarly, patients who suffered from a TBI demonstrated a significant improvement at the 3-month follow-up in the active compared to the sham group, in comparison with those from other aetiologies. More specifically, whilst the sham group decreased by 2 points (IQR 0 to −4), the active group improved by 2 points (IQR 0–3). As for patients in MCS, it is well known that patients with TBI have a better prognosis compared to other aetiologies such as anoxia or stroke [2]. In addition, patients with TBI are often younger than patients with non-TBI (stroke or cardiac arrest often occurring in older people), as in our study (mean of 37 ± 15 vs. 47 ± 13 years; exploratory a posteriori analysis revealed a significant difference; $p = 0.01$), and also have higher chances of improving as age is a prognostic marker of good recovery [2]. Indeed, cortical plasticity and excitability seem to be reduced in older (healthy) individuals as shown in several studies using paired associative stimulation to induce neuroplastic changes [28,29]. In the specific case of tDCS, a recent study explored the age-dependent plasticity alterations following tDCS [30]. The authors found that, whilst tDCS induced a significant improvement of cortical excitability in young and middle-aged adults (i.e., increase in motor evoked potential), no change was observed for the elderly (>65 years old). In this context, non-TBI (i.e., older) patients might not benefit from tDCS as its neuroplastic effects seem to be limited in older adults [30].

This study encompasses some limitations that need to be taken into account before generalizing our results. First, the rate of dropout (43.5% at 3 months follow-up) is an important limitation to our study which can be explained by the fragility of the population (as mentioned above) and the duration of the whole protocol (i.e., 4 months). Transfer to another facility was the main reason for dropout in the follow-up phase. Besides, infection and change of medications were additional reasons for dropouts, which are difficult to avoid during a 4-month period in this population. Conducting trials in a later phase of the disease (e.g., when the patient is in a long-term care facility or at home) might allow

more stable patients to be enrolled, less likely to be transferred, to develop an infection or to change medication, thus limiting the rate of dropout. Another limitation is the heterogeneity of the population since various aetiologies with very heterogeneous cortical and subcortical brain lesions were included, which could have had an impact on the effects of tDCS as previously shown in healthy participants [31] and stroke [32]. In this context, individualized setups based on each patient's structural or functional brain lesions could provide better results by optimizing the current field on the chosen brain target [33]. Specific software is now available to simulate a transcranial electrical field, such as ROAST [34] or SimNIBS [35] or Shamo [36]. However, these approaches must first be tested and validated in patients with DoC before being tested in the clinic. Regarding the outcomes, behavioural effect was our primary outcome but the aim was also to collect EEG data. However, fewer than half the centres were able to collect EEG data despite having the equipment on site. This underlines the poor feasibility of implementing EEG measurements into daily clinical routine in rehabilitation settings with tight schedule constraints and important workload for clinicians; EEG requires indeed a longer setup than tDCS to obtain satisfying signal quality.

CONCLUSION

This study is the first large multicentre international trial conducted with tDCS on patients with DoC. As stated above, these multicentre trials are critical to answer clinical questions from the field and improve the external validity of tDCS studies. However, given the fragility of this specific population of patients, many challenges were faced when conducting the present clinical trial. Despite these limitations, based on our results, the use of tDCS for TBI and MCS patients is advocated, as part of the rehabilitation strategies for DoC patients.

DISCLOSURE

The authors report no disclosures relevant to the manuscript.

AUTHOR CONTRIBUTIONS

AT, AE, EN, RL, RF, AB, EK, LL, CK, FM, SL, OG, NL and GM designed the study. SF, EN, JF, GM, CK, MR, GL, SK, HJ, NL and GM collected behavioural data. AT, FF and ND ran the statistical analyses. AT, GM and NL wrote the manuscript. All authors contributed to data interpretation, revised the manuscript critically for intellectual content, and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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