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Behavioral and electrophysiological effects of network-based frontoparietal tDCS in patients with severe brain injury: A randomized controlled trial

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ABSTRACT

Background: Transcranial direct current stimulation (tDCS) may promote the recovery of severely brain-injured patients with disorders of consciousness (DOC). Prior tDCS studies targeted single brain regions rather than brain networks critical for consciousness recovery.
Objective: Investigate the behavioral and electrophysiological effects of multifocal tDCS applied over the frontoparietal external awareness network in patients with chronic acquired DOC.
Methods: Forty-six patients were included in this randomized double-blind sham-controlled crossover trial (median [interquartile range]: 46 [35 – 59] years old; 12 [5 – 47] months post injury; 17 unresponsive wakefulness syndrome, 23 minimally conscious state (MCS) and 6 emerged from the MCS). Multifocal tDCS was applied for 20 min using 4 anodes and 4 cathodes with 1 mA per electrode. Coma Recovery Scale-Revised (CRS-R) assessment and 10 min of resting state electroencephalogram (EEG) recordings were acquired before and after the active and sham sessions.
Results: At the group level, there was no tDCS behavioral treatment effect. However, following active tDCS, the EEG complexity significantly increased in low frequency bands (1–8 Hz). CRS-R total score improvement was associated with decreased baseline complexity in those bands. At the individual level, after active tDCS, new behaviors consistent with conscious awareness emerged in 5 patients. Conversely, 3 patients lost behaviors consistent with conscious awareness.
Conclusion: The behavioral effect of multifocal frontoparietal tDCS varies across patients with DOC. Electrophysiological changes were observed in low frequency bands but not translated into behavioral changes at the group level.

1. Introduction

Transcranial direct current stimulation (tDCS), a neuromodulation method that may increase neuronal excitability, can enhance responsiveness of some patients with disorders of consciousness (DOC) when applied over the prefrontal cortex (Thibaut et al., 2019). While previous studies focused on stimulating individual cortical regions, multifocal stimulation may be more relevant for DOC because recovery of consciousness is likely reliant on networks (Laureys et al., 2000). Two networks have been identified as potential mediators of consciousness: the default mode network, functionally related to internal awareness (Vanhaudenhuyse et al., 2010; Snyder and Raichle, 2012) and the frontoparietal executive control network, which processes external stimuli (Golland et al., 2007; Fox et al., 2005; Vanhaudenhuyse et al.,

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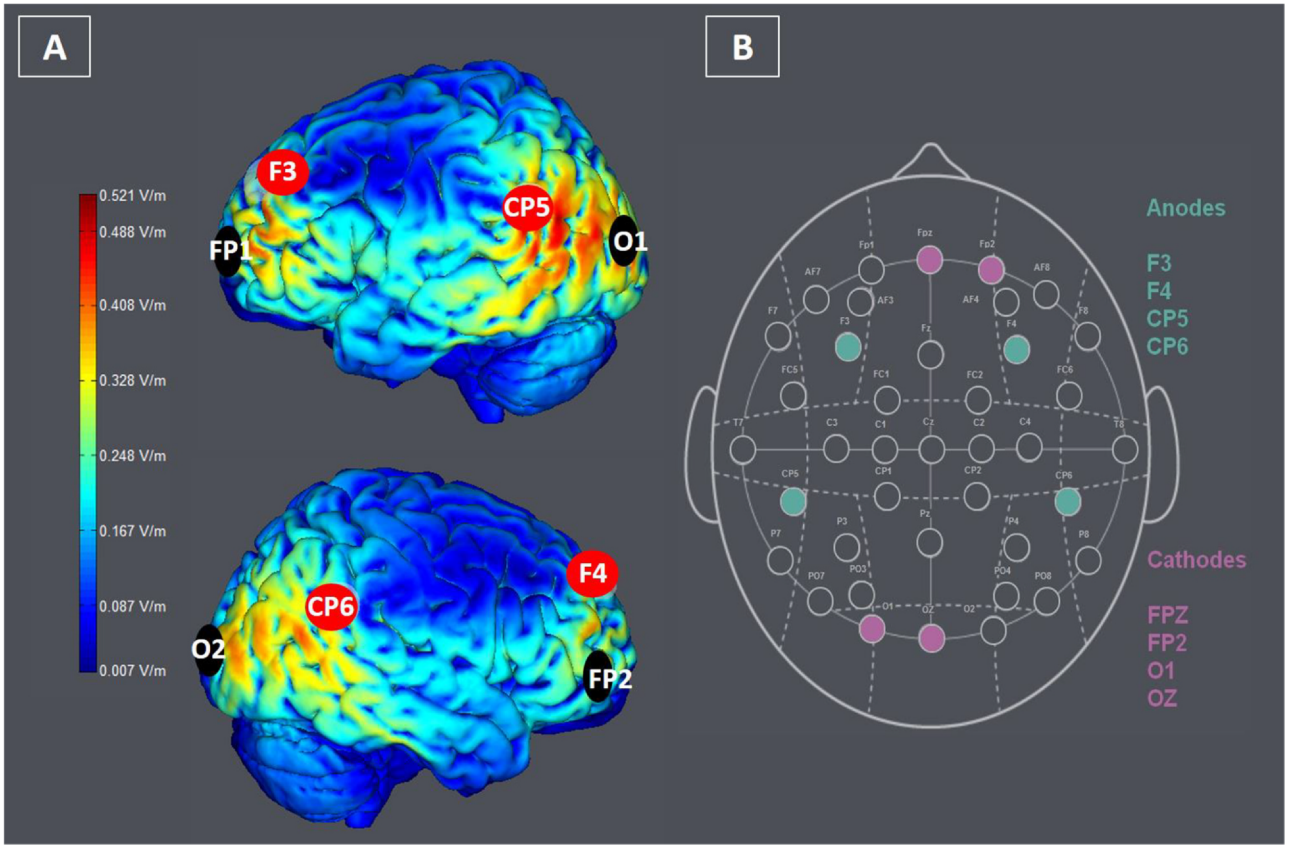

Fig. 1. E-field modelling with anodes in red and cathodes in black (A) and tDCS montage used (B). Taken from Neuroelectrics® Instrument Controller (NIC). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2011). In this context, using novel tDCS devices that allow concurrent stimulation of multiple brain regions within the frontoparietal network may enhance patients' interaction with their environment. Furthermore, the effects of tDCS on electroencephalographic (EEG) measures of complexity are unknown. A scoping review presented an algorithm that could quantify consciousness using EEG signals (Ruffini, 2017). The Lempel-Ziv-Welch (LZW) algorithm which depicts the 'randomness' of the neural signal and the integrity of inter-neural connectivity (Tononi and Edelman, 1998; Méndez et al., 2012), has indeed been shown to be correlated with levels of consciousness (e.g., general anesthesia (Schartner et al., 2015a), sleep (Mateos et al., 2018; Schartner et al., 2015b) and could be used to evaluate the neurophysiological effects of tDCS in DOC.

To test the effects of multifocal tDCS on recovery of conscious behaviors after severe brain injury, we stimulated four key regions of the frontoparietal network in a double-blind, sham controlled trial. We also evaluated the electrophysiological effects of tDCS.

2. Methods

2.1. Study design

This was a single-center double-blind, sham-controlled, cross-over study design, registered on ClinicalTrials.gov (NCT02626403) and conducted in accordance with CONSORT guidelines. All patients' legal representative provided written informed consent to participate in the study in accordance with local Research Ethics Committee approval.

2.2. Population

We prospectively enrolled a consecutive sample of post-comatose patients, 16 to 75 years of age, who were at least 28 days post-injury and diagnosed with unresponsive wakefulness syndrome (UWS; only reflexive movements (Laureys et al., 2010), minimally conscious state (MCS; fluctuating but reproducible signs of consciousness (Giacino et al., 2002) or emergent from the MCS (EMCS; able to functionally communicate and/or use objects (Giacino et al., 2002). Patients were admitted to the University Hospital between February 2015 and August 2017 for evaluation of level of consciousness and prognosis using advanced neuroimaging and electrophysiological techniques. Prior to study enrollment, the best of three consecutive behavioral assessments (using the Coma Recovery Scale-Revised – CRS-R (Giacino et al., 2004) conducted within a one-week period established the baseline diagnosis. The EMCS group consisted of patients demonstrating functional communication or object use on two consecutive assessments. Patients had to be medically stable (e.g., absence of infection, untreated epilepsy, ventilation), free of sedative drugs and Na^+, Ca^{2+} blockers or N-methyl-D-aspartate (NMDA) receptor antagonists to be eligible for the study. We excluded patients with premorbid neurological (e.g., Alzheimer, stroke) or psychiatric disease, patients in the acute phase of recovery (i.e., < 28 days post-acute brain injury), non-French or English speakers and patients with a metallic cerebral implant, cranioplasty or shunt.

2.3. tDCS intervention

Subjects participated in two sessions, active (a-tDCS) and sham (s-tDCS), spaced by two to six days. Direct current was applied with the Starstim 8 (Neuroelectrics, Spain), a tDCS stimulator capable of simultaneously measuring EEG activity (Giovannella et al., 2018). The montage was comprised of 8 gelled electrodes (3.14 cm^2 Ag/AgCl), 4 anodes and 4 cathodes. Stimulation was delivered over the bilateral frontoparietal areas through the anodes placed on F3-F4 and CP5-CP6 according to the international 10–20 EEG system (Herwig et al., 2003).

Cathodes were placed over the prefrontal and occipital areas on Fp2-Fpz and O1-Oz, based on an electrical field simulator and optimizer (Ruffini et al., 2014), targeting the highest field over the bilateral frontoparietal network, as shown in Fig. 1. Intensity was set to 1 mA per anode, for a total of 4 mA of current delivered per session. For a-tDCS, current was applied for 20 min, preceded by a 30-second ramp-up period and followed by a 30-second ramp-down period. For s-tDCS, 1 mA was applied through each anode for 30 s, preceded by a 30-second ramp-up and followed by a 30-second ramp-down and 19 min and 30 s of no stimulation. Impedances were monitored by the device and kept at < 10 kΩ and voltage < 30 V.

2.4. Randomization and masking

Patients were randomized to receive either a-tDCS or s-tDCS first. A random number generator was used for each enrolled patient to assign conditions in a 1:1 manner. This was performed by a researcher who was not involved in any assessments; thus, the investigators and patients were blinded to the allocation. Behavioral assessments and EEG recordings were carried out when the tDCS device was in "double-blind mode", depicting identical information for a-tDCS or s-tDCS.

2.5. Behavioral and electrophysiological assessments

During the study period, level of consciousness was assessed with the CRS-R by trained and experienced neuropsychologists before and immediately after each combined EEG-tDCS session (Fig. 2). CRS-R characteristics are described in Appendix e-1. Ten minutes of resting EEG was recorded with eyes open (patients were verbally or tactily stimulated when drowsy) using the Startsim 8 with the same gelled electrodes (i.e., Fp1, Fpz, F3, F4, CP5, CP6, O1, Oz). The sampling frequency was 500 Hz. Two additional sticky electrodes were placed on both mastoids as reference. The order of assessments was as follows: CRS-R (~30 min) → EEG (10 min) → a-tDCS/s-tDCS (20 min) → EEG (10 min) → CRS-R (~30 min).

2.6. Outcomes

Our primary outcome measure was the tDCS behavioral treatment effect (i.e., CRS-R total score change a-tDCS [ΔCRS-R active] vs. s-tDCS [ΔCRS-R sham]) at the group-level. Our secondary outcomes included the influence of diagnosis on the behavioral treatment effect and the tDCS electrophysiological treatment effect considering EEG band power and complexity in three frequency bands of interest (see below). We also identified individual behavioral response patterns following a- and s-tDCS based on changes in MCS/EMCS behaviors (see Appendix e-2). Finally, we investigated the relationships between our baseline EEG metrics (band power and complexity) and behavioral outcomes (i.e., ΔCRS-R).

2.7. Analyses

2.7.1. Behavioral data

Statistical preliminary analyses on the behavioral data were performed using R 3.5.1 (Core and Team, 2008). Baseline characteristics (age, gender, time since onset, etiology, baseline CRS-R score and baseline diagnosis) between randomized groups (a-tDCS first and s-tDCS first) were tested for comparability using Wilcoxon Rank Sum test for continuous variables, Fisher test for dichotomous variables and Chi-square test for categorical variables. Descriptive statistics (median, interquartile range [IQR]) were used to characterize the study sample. For the calculation of the tDCS treatment effect, we used a Linear Mixed Effects Model as described in (Senn, 2002) using the lme4 R package. The following regressors were included: (1) random subject-specific intercept; (2) baseline CRS-R; (3) carryover effect; (4) period effect; (5) treatment effect (group level); (6) interaction between diagnosis and treatment. Baseline CRS-R total scores were considered as subject specific random intercept. The code used for this model can be found in Supplementary Material.

We then proceeded to identify potential individual tDCS-responders by categorizing our sample in five different groups: 1) "tDCS + a": patients who showed a new sign of consciousness (i.e., MCS or EMCS)

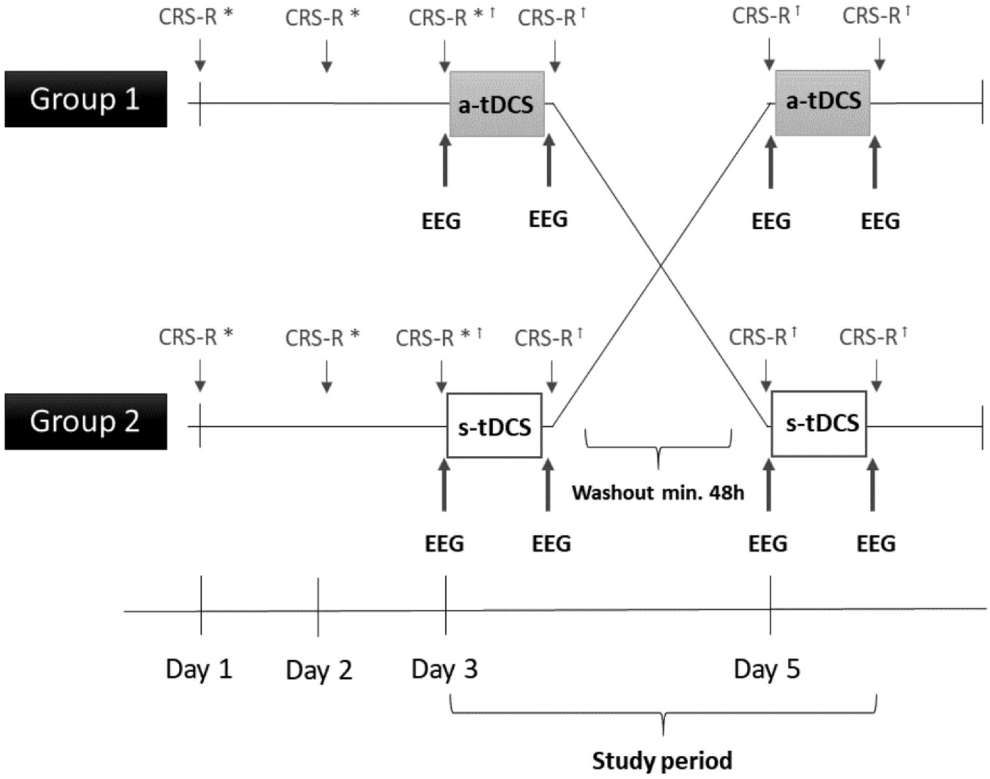

Fig. 2. Study protocol. CRS-R *= assessments taken into account for the baseline diagnosis. CRS-R = assessments taken into account for the individual tDCS response. a-tDCS = active stimulation; s-tDCS = sham stimulation; CRS-R = Coma Recovery Scale-Revised; tDCS = transcranial direct current stimulation;
EEG = electroencephalography.

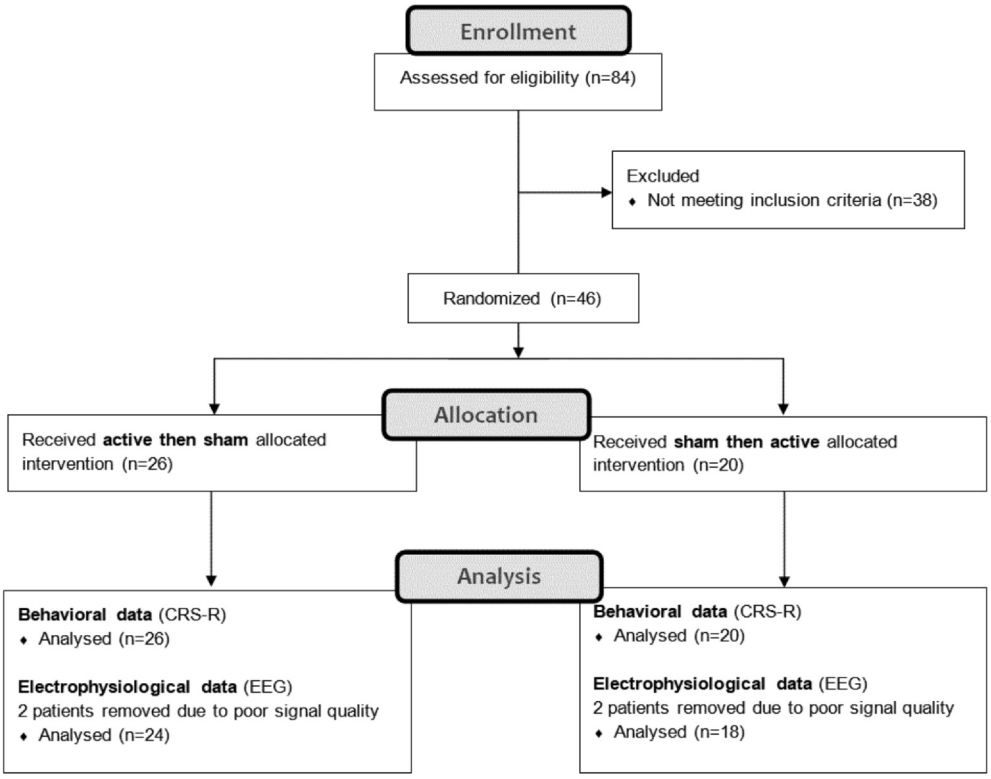

Fig. 3. CONSORT Flowchart of the study participants.

following a-tDCS never observed otherwise (i.e., not observed before a-tDCS, not observed before s-tDCS, not observed after s-tDCS); 2) "tDCS-a": patients who lost a sign of consciousness following a-tDCS otherwise always present (present before a-tDCS, present before s-tDCS, present after s-tDCS); 3) "tDCS + s": patients who showed a new sign of consciousness after s-tDCS never observed otherwise (not observed before s-tDCS, not observed before a-tDCS, not observed after a-tDCS); 4) "tDCS-s": patients who lost a sign of consciousness following s-tDCS otherwise always present (present before a-tDCS, present before s-tDCS, present after s-tDCS); and 5) "tDCS = ": patients who did not gain or lose any sign of consciousness following a- or s-tDCS. The 13 CRS-R signs of consciousness are described in Appendix e-2. The four CRS-R assessments conducted during the study period were considered for this classification: before a-tDCS, after a-tDCS, before s-tDCS and after s-tDCS. We then coded the patients as either having a clinically relevant behavioral change (i.e., tDCS + a; tDCS-a; tDCS + s; tDCS-s) or not (i.e., 'tDCS = ') and checked for potential differences between these two categories using Fisher's exact test (for gender and etiology), Pearson's Chi-squared test (for diagnosis) and Kruskal-Wallis rank sum test (for age and time since injury).

2.7.2. EEG data

Since the samples used for behavioral (n = 46) and electrophysiological outcomes (n = 42) differed, we first checked for differences between samples in terms of age, gender, time since onset, etiology and diagnosis using the same tests described above.

The EEG analyses were conducted on Matlab 2016b and Python 2.7 with in-house scripts. The EEG data were pre-processed in the following way: the signals were band-pass filtered into delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), low beta (beta1: 13–23 Hz) and high beta (beta2: 23–35 Hz) bands using an Infinite Impulse Response Butterworth filter. The data were segmented into 5-sec epochs with 50% overlap, as a compromise for sufficient number of cycles for all bands and sufficient number of clean epochs, while dealing with the non-stationary nature of the EEG data. Epochs with amplitude larger than 75uV in each frequency band were considered artifacts (muscular) and automatically excluded from the analysis. Additional channel rejection was performed for each frequency band, based on the median absolute deviation (MAD). Specifically, channels larger than 2.5 MAD values were considered noisy and automatically excluded from the analysis. Moreover, all channels with amplitudes < 2uV were also considered artifacts and automatically excluded from the analysis. The final clean signals were demeaned and detrended as well as re-referenced to the common average of the clean remaining channels per epoch.

The relative band power (with respect to 1–35 Hz) was extracted by computing the power on the filtered signals and integrating over the discrete temporal domain. The LZW was estimated for each frequency band separately as each EEG rhythm is associated with different underlying cognitive functions (Buzsáki, 2006). The LZW complexity metric was calculated as described in Appendix e-3. It was computed to capture the spatial global signal complexity (i.e., across all channels) for each frequency band, epoch and patient, and then averaged across all epochs to get one complexity value per frequency band for each subject. To calculate the difference between a-tDCS and s-tDCS, the LZW metric was expressed as the percentage of change (POC) with respect to the baseline condition as described in Appendix e-3.

Statistical analyses on the electrophysiological data were performed using R 3.5.1 and the R-package GLMMadaptive (Core and Team, 2008). Descriptive statistics (median, IQR) were used to characterize the outcomes. We analyzed the relative band power and signal complexity changes in three bands of interest for [0, 1] DOC: alpha, theta and delta. Relative band power measures are, by definition, bounded by the) interval and can be conveniently described using a Beta distribution (a continuous probability distribution). We apply the same distributional assumption to the signal complexity measures (even if in some rare and extreme cases one could observe values above 1). We therefore used a Generalized Linear Mixed Model with a Beta response and a logit link. We included the following regressors: (1) random effect (intercept); (2) baseline EEG; (3) carryover effect; (4) period effect; (5)

Table 1
. Individual demographic and clinical characteristics of the study sample.

ID	Age(sex)	Diag.	TSO(days)	Etiology	TreatmentAllocation	CRS-R presham	CRS-R postsham	Δs-tDCS	CRS-R preactive	CRS-R postactive	Δ a-tDCS	Behavioral response
1	50(F)	UWS	188	nTBI	active/sham	5 (1–0-1–1–0–2)	5 (1–0-1–1–0–2)	0	4 (1–0–1–1–0–1)	5 (1–0-1–1–0–2)	1	tDCS =
2	55(M)	MCS	2557	TBI	active/sham	14 (2–4-5–1-0–2)	12 (0–4-5–1-0–2)	−2	13 (2–3-5–1-0–2)	13 (2–3-5–1-0–2)	0	tDCS =
3	38(M)	MCS	328	TBI	active/sham	8 (0–0-5–1-0–2)	8 (0–0-5–1-0–2)	0	10 (0–3-5–1-0–1)	11 (0–3-5–1-0–2)	1	tDCS =
4	47(M)	UWS	32	nTBI	sham/active	5 (1–0-1–1–0–2)	6 (1–1–1–1-0–2)	1	5 (1–0-1–1–0–2)	5 (1–0-1–1–0–2)	0	tDCS =
5	39(F)	UWS	338	nTBI	sham/active	4 (1–0-1–0-0–2)	5 (1–0-1–1–0–2)	1	5 (1–0-1–1–0–2)	4 (1–0–1–1–0–1)	−1	tDCS =
6	36(F)	EMCS	2110	TBI	sham/active	23 (4–5-6–3-2–3)	23 (4–5-6–3-2–3)	0	23 (4–5-6–3-2–3)	23 (4–5-6–3-2–3)	0	tDCS =
7	31(M)	MCS	2603	TBI	active/sham	9 (2–3-1–1-0–2)	10 (2–3-2–1-0–2)	1	7 (0–3-1–1-0–2)	7 (0–3-1–1-0–2)	0	tDCS =
8	57(M)	EMCS	1401	TBI	active/sham	16 (3–4-2–3-2–2)	16 (3–4-2–3-2–2)	0	8 (3–0-1–3-1–0)	11 (3–1-1–3-2–1)	3	tDCS =
9	35(F)	MCS	4053	TBI	active/sham	5 (0–1-2–1-0–1)	7 (0–3-2–1-0–1)	2	7 (0–2-2–1-0–2)	7 (0–2-2–1-0–2)	0	tDCS + s
10	35(M)	MCS	4600	nTBI	active/sham	8 (1–3-1–1-0–2)	9 (2–3-1–1-0–2)	1	9 (2–3-1–1-0–2)	9 (2–3-1–1-0–2)	0	tDCS =
11	43(M)	MCS	11,719	TBI	active/sham	10 (2–3-2–1-0–2)	10 (2–3-2–1-0–2)	0	7 (0–3-2–1-0–1)	7 (0–3-2–1-0–1)	0	tDCS =
12	43(M)	UWS	41	nTBI	active/sham	5 (1–0-2–1–0–1)	5 (1–0-2–1–0–1)	0	4 (1–0–1–1–0–1)	4 (1–0–1–1–0–1)	0	tDCS =
13	56(M)	UWS	85	nTBI	sham/active	5 (1–0-2–1–0–1)	5 (1–0-2–1–0–1)	0	6 (1–0–2–1–0–2)	6 (1–0–2–1–0–2)	0	tDCS =
14	45(F)	UWS	43	nTBI	active/sham	5 (1–0-1–1–0–2)	6 (1–1–1–1-0–2)	1	4 (1–0–1–1–0–1)	4 (1–0–1–1–0–1)	0	tDCS =
15	59(M)	MCS	104	TBI	active/sham	7 (1–3-1–1-0–1)	7 (1–3-1–1-0–1)	0	5 (1–0-1–1–0–2)	8 (1–3-1–1-0–2)	3	tDCS =
16	20(F)	MCS	621	nTBI	sham/active	11 (3–0-5–1-0–2)	6 (1–1-2–1-0–1)	−5	5 (0–0-1–2-0–2)	6 (1–1-2–1-0–1)	1	tDCS =
17	26(M)	MCS	3561	TBI	active/sham	8 (1–3-2–1-0–1)	9 (2–3-1–1-0–2)	1	9 (2–3-1–1-0–2)	14 (4–5-0–2-1–2)	5	tDCS + a
18	46(M)	MCS	1394	TBI	active/sham	10 (3–3-2–1-0–1)	11 (3–3-1–2-0–2)	1	9 (3–3-1–1-0–1)	9 (3–3-1–1-0–1)	0	tDCS =
19	38(M)	MCS	201	nTBI	sham/active	5 (1–0-2–1–0–1)	5 (1–0-2–1–0–1)	0	6 (1–1-2–1-0–1)	9 (1–3-2–1-0–2)	3	tDCS + a
20	62(M)	MCS	170	nTBI	active/sham	14 (2–1-5–3-1–2)	13 (2–0-5–3-1–2)	−1	17 (3–3-5–3-1–2)	15 (3–3-5–3-0–1)	−2	tDCS =
21	65(M)	UWS	129	nTBI	active/sham	4 (1–0–1–1–0–1)	5 (1–0-1–1–0–2)	1	4 (1–1-0–1-0–1)	4 (1–0–1–1–0–1)	0	tDCS =
22	57(M)	MCS	246	nTBI	sham/active	5 (1–1–1–1-0–1)	3 (0–0–1–1-0–1)	−2	6 (0–3–1–1-0–1)	6 (1–2-1–1-0–1)	0	tDCS =
23	46(F)	UWS	148	nTBI	sham/active	6 (1–0–2–1–0–2)	5 (1–0-1–1–0–2)	−1	5 (1–0-1–1–0–2)	5 (1–0-1–1–0–2)	0	tDCS =
24	60(M)	MCS	2145	TBI	active/sham	3 (0–0-2–1-0–0)	3 (0–0-2–1-0–0)	0	9 (2–0-5–1-0–1)	13 (2–4-5–1-0–1)	4	tDCS + a
25	59(M)	UWS	29	nTBI	sham/active	4 (1–0–1–1–0–1)	4 (1–0–1–1–0–1)	0	4 (1–0–1–1–0–1)	4 (1–0–1–1–0–1)	0	tDCS =
26	30(M)	MCS	1190	nTBI	active/sham	5 (1–1-0–1-0–2)	6 (1–1–1–1-0–2)	1	10 (3–4-0–1-0–2)	6 (3–0-0–1-0–2)	−4	tDCS =
27	26(M)	EMCS	116	TBI	active/sham	16 (4–5-4–1-0–2)	16 (4–4-5–1-0–2)	0	18 (4–5-5–1-2–1)	16 (3–5-4–1-2–1)	−2	tDCS-a
28	48(F)	MCS	191	nTBI	sham/active	9 (1–1-5–1-0–1)	15 (2–4-5–2-0–2)	6	10 (1–1-5–2-0–1)	10 (1–1-5–2-0–1)	0	tDCS + s
29	60(M)	MCS	98	nTBI	active/sham	11 (2–1-4–2-0–2)	8 (2–1-1–2-0–2)	−3	10 (1–3-2–2-0–2)	10 (1–3-2–2-0–2)	0	tDCS =
30	60(F)	EMCS	361	TBI	active/sham	19 (3–5-6–1-2–2)	19 (3–5-6–1-2–2)	0	16 (3–3-5–1-2–2)	18 (4–4-6–1-1–2)	2	tDCS + a/-a
31	36(M)	UWS	806	nTBI	sham/active	4 (1–0–1–1–0–1)	4 (1–0–1–1–0–1)	0	3 (0–0–1–1-0–1)	5 (1–0-2–1–0–1)	2	tDCS =
32	32(M)	MCS	557	TBI	active/sham	8 (0–0-5–2-0–1)	8 (0–0-5–2-0–1)	0	11 (3–0-5–2-0–1)	11 (3–0-5–2-0–1)	0	tDCS =
33	20(M)	MCS	388	TBI	sham/active	15 (3–3-5–2-0–2)	10 (3–3-1–1-0–2)	−5	9 (2–3-1–1-0–2)	9 (2–3-1–1-0–2)	0	tDCS =
34	32(F)	UWS	371	TBI	sham/active	5 (1–0-1–1–0–2)	5 (1–0-1–1–0–2)	0	6 (1–1–1–1-0–2)	7 (1–1–1–2-0–2)	1	tDCS =
35	60(F)	UWS	304	nTBI	active/sham	5 (1–1–1–1-0–1)	6 (1–1–1–1-0–2)	1	6 (1–1–1–1-0–2)	6 (1–1–1–1-0–2)	0	tDCS =
36	67(F)	UWS	283	nTBI	active/sham	5 (1–1–1–1-0–1)	6 (2–1–1–1-0–1)	1	5 (1–1–1–1-0–1)	5 (1–1–1–1-0–1)	0	tDCS =
37	74(F)	UWS	47	nTBI	sham/active	4 (1–1-0–1-0–1)	4 (1–1-0–1-0–1)	0	5 (1–0-2–1–0–1)	5 (1–0-2–1–0–1)	0	tDCS =
38	70(F)	MCS	1811	nTBI	sham/active	8 (0–0-5–2-0–1)	14 (2–3-5–2-0–2)	6	16 (4–4-5–2-0–1)	12 (0–4-5–2-0–1)	−4	tDCS =
39	44(F)	UWS	586	nTBI	active/sham	5 (1–0–1–2-0–1)	6 (1–1–1–2-0–1)	1	8 (1–1-2–2-0–2)	7 (1–1-2–2-0–1)	−1	tDCS =
40	48(F)	MCS	476	TBI	active/sham	9 (1–3-2–2-0–1)	9 (1–3-1–2-0–2)	0	11 (3–3-1–2-0–2)	9 (1–3-1–2-0–2)	−2	tDCS =
41	59(F)	UWS	37	nTBI	active/sham	4 (0–1–1–1-0–1)	4 (0–1–1–1-0–1)	0	4 (0–1–1–1-0–1)	4 (0–1–1–1-0–1)	0	tDCS =
42	21(F)	EMCS	169	TBI	sham/active	14 (4–3-5–1-0–1)	17 (4–4-5–1-2–1)	3	16 (3–4-5–2-0–2)	18 (3–5-5–1-2–2)	2	tDCS + a
43	77(F)	MCS	2069	TBI	active/sham	12 (3–3-2–2-0–2)	18 (4–3-6–2-1–2)	6	14 (4–3-2–2-1–2)	11 (3–3-2–1-0–2)	−3	tDCS + s
44	60(M)	EMCS	346	TBI	sham/active	22 (4–5-6–2-2–3)	22 (4–5-6–2-2–3)	0	22 (4–5-6–2-2–3)	22 (4–5-6–2-2–3)	0	tDCS =
45	28(M)	MCS	1564	TBI	sham/active	12 (3–5-2–1-0–1)	9 (2–3-2–1-0–1)	−3	8 (1–3-2–1-0–1)	10 (2–3-2–1-0–2)	2	tDCS =
46	31(M)	UWS	359	TBI	sham/active	5 (0–0–2–1-0–2)	7 (1–1-2–1-0–2)	2	5 (0–0–2–1-0–2)	5 (0–0–2–1-0–2)	0	tDCS =

CRS-R scores are depicted as follows: Total Score (Auditory subscore – Visual subscore – Motor subscore – Oromotor/Verbal subscore – Communication subscore – Arousal subscore). Diag. = diagnosis based on 3 consecutive CRS-R assessments; F = Female; M = Male; diag. = diagnosis; UWS = Unresponsive Wakefulness Syndrome; MCS = Minimally Conscious State; EMCS = Emergence from the MCS; TSO = Time Since Onset; TBI = Traumatic Brain Injury: nTBI = non-Traumatic Brain Injury; CRS-R = Coma Recovery Scale-Revised; Δ = post – pre. In the last column, tDCS + a = patients showing a new sign of consciousness only after a-tDCS; tDCS + s = patients showing a new sign of consciousness only after s-tDCS; tDCS-a = patients losing a sign of consciousness only after a-tDCS and "tDCS=" = patients not gaining nor losing a sign of consciousness taking into account the 4 CRS-R assessments (pre and post a-tDCS and s-tDCS) conducted during the study period.

treatment effect (group level); (6) interaction between diagnosis and treatment and; (7) delta CRS-R (baseline/post). Noteworthy, in some instances where a baseline EEG value was unavailable , we used the other available baseline EEGs to impute it. The code used for this model can be found in Supplementary Material.

As further explorative analyses, we investigated the relationship between baseline EEG band power and complexity values and ΔCRS-R active in the whole sample, as well as in conscious (MCS, EMCS) and unconscious patients (UWS) separately, using Spearman's correlation test.

3. Results

As presented in the CONSORT Flow Diagram (Fig. 3), 46 out of 84 admitted patients were included.

The sample comprised 17 patients in UWS, 23 in MCS, and 6 in EMCS with both traumatic (n = 22) and non-traumatic (n = 24) etiologies. The median [IQR] age was 46 [35 – 59] years; median [IQR] time post injury was 12 [5 – 47] months. Individual demographic data and CRS-R total scores of the stimulation conditions can be found in Table 1.

Patients adequately tolerated all the tDCS sessions (i.e., no burns, skin damage or clinical signs of pain or discomfort) and no patients dropped-out. There were no significant differences between allocation groups (a-tDCS – s-tDCS vs. s-tDCS – a-tDCS) regarding age (p = 0.308), gender (p = 0.766), etiology (p = 0.497), time since onset (p = 0.317), baseline CRS-R score (p = 0.680) and baseline diagnosis (p = 0.172). Data is available on https://doi.org/10.5281/zenodo.495611.

3.1. Behavioral data

Regarding the changes in the CRS-R total score, no carry-over effect was observed between a-tDCS and s-tDCS (p = 0.807). At the group level, there was no significant tDCS treatment effect (p = 0.222), neither in the diagnostic subgroups (UWS, p = 0.425; MCS, p = 0.180). This is presented in Table 2 summarizing the Linear Mixed Model used.

At the single-subject level, we identified 5 patients who gained a sign of consciousness only following a-tDCS (i.e., tDCS + a; gained systematic response to command in two cases; object recognition in two cases; object localization in one case; visual pursuit in one case), two patients who lost a sign of consciousness only following a-tDCS (i.e., tDCS-a; lost systematic response to command and functional communication, respectively) and three patients who gained a sign of consciousness only following s-tDCS (i.e., tDCS + s; gained object localization; visual pursuit and functional object use, respectively). Noteworthy, one patient (P30) both gained a new sign of consciousness only following a-tDCS and lost another sign of consciousness only following a-tDCS and is therefore in both "tDCS + a" and "tDCS-a" subgroups (i.e., tDCS + a/-a). The remaining 37 patients neither gained nor lost a sign of consciousness after a-tDCS (i.e., "tDCS = "). There were no significant differences between the group of 9 patients who had clinically relevant behavioral changes (i.e., tDCS + a, tDCS-a, tDCS + s) and the group of 37 patients without clinically relevant behavioral changes (i.e., 'tDCS = ') regarding age (p = 0.54); gender (0.95); time since injury (p = 0.34) or etiology (p = 0.06). There was a significant difference for the diagnosis (p = 0.02), with significantly more UWS patients in the tDCS = group (17/17 UWS patients were in the tDCS = group compared to 20/29 (E)MCS patients).

We further checked this classification by considering an additional CRS-R assessment performed prior to study inclusion. None of the CRS-R sub-scores did challenge this classification meaning that when a sign of consciousness was gained (or lost) following tDCS, it was not present (or absent) on this additional baseline CRS-R assessment either. There was a notable exception for P30 who initially was in both tDCS + a and tDCS-a groups as he gained a sign of consciousness – systematic response to command – but lost another one – functional communication – after a-tDCS; this patient would be a tDCS-a responder only considering the additional baseline assessment. The individual CRS-R score variability between these three baseline assessments is presented in Table e-2. At the whole-sample level (n = 46), there were no significant difference regarding the CRS-R total score on this additional baseline CRS-R assessment and the two baseline CRS-R conducted during the study period (KW χ^2 = 0.78; p = 0.68). This data is available in Supplementary Material (Table e1 and e-2).

Table 2
. Linear Mixed Model for the CRS-R behavioral results. Groups in parenthesis are compared to the baseline. Baselines conditions are: treatment = active; diagnosis = EMCS; period = 0 (first period, the other level is 1). *** p < 0.001.

	Behavioral results (CRS-R total scores)		
Predictors	*Estimates*	*std. Error*	*p*
Intercept	5.176 ***	1.453	< **0.001**
Baseline	0.696 ***	0.072	< **0.001**
Treatment (sham)	1.355	1.109	0.222
Sequence (1)	−0.102	0.416	0.807
Period (1)	0.310	0.296	0.295
Treatment (sham) : Diagnosis (MCS)	−1.686	1.257	0.180
Treatment (sham) : Diagnosis (UWS)	−1.030	1.290	0.425

3.2. EEG data

Four EEGs could not be recorded due to too bad signal quality (impedances were too high and could not be reduced): subject 1 (UWS, nTBI), 16 (MCS, nTBI), 21 (UWS, nTBI) and 31 (UWS, nTBI). Therefore, the EEG analyses were performed on 42 patients (14 UWS, 22 MCS, 6 EMCS, 22 TBI, 20 nTBI, median [IQR] age: 46 [35 – 59] years; median [IQR] time post injury: 13 [5 – 54] months). This sample did not significantly differ from the full sample in terms of age (p = 0.95), gender (p = 0.76), time since injury (p = 0.97), etiology (p = 0.52) or diagnosis (p = 0.80). To assess the reliability of the EEG measures (band power and complexity), we used Bland-Altman plots to check the retest-reliability of the deltas (baseline – post). The plots did not present any systematic pattern between means and differences of EEG measures (both power and complexity). These results can be found in Supplementary Material (Figure e-12). The band power and complexity at baseline, after stimulation and the POC for both active and sham conditions for the whole sample are presented in Table S1. Baseline band power data and complexity are illustrated in Figures e-2 and e-3, respectively.

3.3. Relative band power

The Generalized Linear Mixed Model did not show any tDCS treatment effect on EEG relative band power in the alpha, theta and delta bands (see Table 3). We did not observe evidence for carry-over and period effects. We observe a strong evidence of the predictive power of the baseline measurements: patients with high baseline values, for instance, show on average high post stimulation values, which can be expected. There was no significant treatment – diagnosis interaction.

3.4. LZW complexity

The Generalized Linear Mixed Model showed again no significant, period or carryover effect (see Table 4). There was a tDCS treatment effect on the LZW complexity in the theta (p = 0.035) and delta bands (p = 0.002): the active session showed on average higher complexity values when compared to the sham session for the EMCS group.

Table 3
. Generalized Linear Mixed Model for EEG relative band power. Groups in parenthesis are compared to the baseline. Baselines conditions are: treatment = active; diagnosis = EMCS; period = 0 (first period, the other level is 1). *** p < 0.001.

	Alpha_power			Theta_power			Delta_power		
Predictors	*Estimates*	*std. Error*	*p*	*Estimates*	*std. Error*	*p*	*Estimates*	*std. Error*	*p*
Intercept	−3.050 ***	0.213	< **0.001**	−2.779 ***	0.132	< **0.001**	−1.688 ***	0.238	< **0.001**
Baseline	8.600 ***	1.190	< **0.001**	6.530 ***	0.539	< **0.001**	3.172 ***	0.379	< **0.001**
Treatment (sham)	0.178	0.096	0.065	−0.040	0.076	0.602	0.001	0.119	0.994
Delta CRS-R	−0.007	0.023	0.752	−0.010	0.017	0.534	0.025	0.024	0.293
Sequence (1)	0.043	0.058	0.462	0.073	0.040	0.067	−0.057	0.060	0.345
Period (1)	−0.032	0.050	0.523	−0.000	0.036	0.997	0.072	0.052	0.168
Treatment (sham) : Diagnosis (MCS)	−0.167	0.119	0.160	0.051	0.089	0.564	−0.133	0.139	0.339
Treatment (sham) : Diagnosis (UWS)	−0.090	0.136	0.506	0.101	0.101	0.316	−0.071	0.149	0.633

There was a negative main effect of the UWS diagnosis on the delta complexity, meaning that the UWS group had lower delta complexity after a-tDCS compared to the EMCS group, which is expected.

3.5. Relationship between behavioral and electrophysiological outcomes

Regarding the relationship with the behavioral outcome (i.e., ΔCRS-R), there was a significant negative effect in the theta band (p = 0.001) and the delta band (p = 0.043) only for the LZW complexity. The model suggests that, on average, when ΔCRS-R (i.e., post tDCS *minus* pre tDCS) increases, the complexity (post tDCS) decreases. When further investigating the relationship between the baseline EEG values and the behavioral outcome, we did not find a significant correlation between ΔCRS-R active and baseline values for power or for complexity in the whole sample (p greater than 0.05). When stratifying by level of consciousness, we found a significant negative correlation between the baseline complexity in theta and the ΔCRS-R for the conscious patients (MCS & EMCS; r = -0.429; p = 0.02), as presented in Fig. 4, but not for the UWS patients.

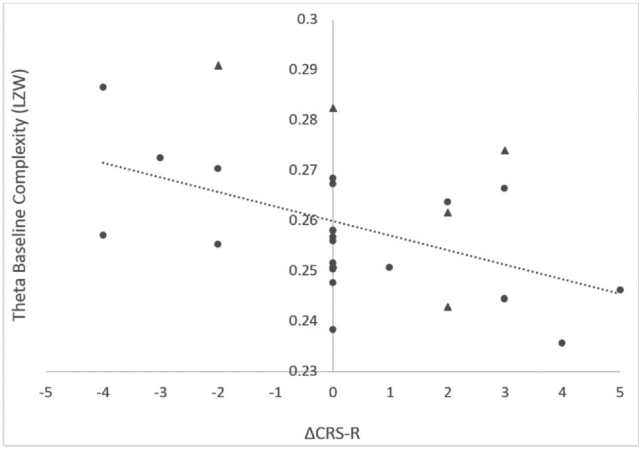

Fig. 4. Correlation between the baseline theta complexity values and the ΔCRS-R (i.e., CRS-R total score post active stimulation minus before active stimulation) in the MCS (n = 23; dots) and the EMCS (n = 6; triangles) patients. Spearman's rho = -0.429; p = 0.02.

4. Discussion

The aim of this study was to investigate the behavioral and electrophysiological effects of tDCS applied bilaterally on the frontoparietal network (i.e., external awareness network (Vanhaudenhuyse et al., 2011) in chronic patients in DOC (UWS, MCS) and in EMCS patients following a severe acquired brain injury.

The group-level behavioral effects were not significant and thereby inconsistent with previous studies that stimulated the left dorsolateral prefrontal cortex (DLPFC) in single (Thibaut et al., 2014) or repeated sessions (Thibaut et al., 2017; Martens et al., 2018; Estraneo et al., 2017; Angelakis et al., 2014). The absence of treatment effect at the group level could be related to the montage, which may have paradoxically reduced the benefits of tDCS as a result of inter-hemispheric competition. In stroke patients (Murase et al., 2004; Bütefisch et al., 2008), many montages target the affected hemisphere with the anode while decreasing the excitability of the unaffected side with the cathode, leading to significant recovery (Schlaug et al., 2008) and reduced inter-hemispheric imbalance (Di Lazzaro et al., 2014). Here, we stimulated both hemispheres with anodes which could have played a role in inhibiting rather than potentiating the inter-hemispheric balance, even though our population typically sustains damage to both hemispheres (Guldenmund et al., 2016). Another specificity of the tested population is the extent and the heterogeneity of the cortical and subcortical lesions. The tDCS current flow modelling does not take into account these lesions as it is based on a healthy brain and is therefore probably slightly different from one patient to another. Likewise, potential damaged areas could have included our target areas (i.e., bilateral frontoparietal cortices) further limiting tDCS efficacy (Thibaut et al., 2015). Additionally, the location of the cathodes over fronto-

Table 4
. Generalized Linear Mixed Model for EEG complexity. Groups in parenthesis are compared to the baseline. Baselines conditions are: treatment = active; diagnosis = EMCS; period = 0 (first period, the other level is 1). * p < 0.05; ** p < 0.01; *** p < 0.001.

	Alpha_LZW			Theta_LZW			Delta_LZW		
Predictors	*Estimates*	*std. Error*	*p*	*Estimates*	*std. Error*	*p*	*Estimates*	*std. Error*	*p*
Intercept	−1.784 ***	0.109	< **0.001**	−1.850 ***	0.134	< **0.001**	−1.988	1.265	0.116
Baseline	3.482 ***	0.263	< **0.001**	3.109 ***	0.499	< **0.001**	7.922	8.189	0.333
Treatment (sham)	−0.006	0.007	0.426	−0.030 *	0.014	**0.035**	−0.292 **	0.096	**0.002**
Delta CRS-R	−0.005	0.003	0.116	−0.018 *	0.006	**0.001**	−0.093 *	0.046	**0.043**
Diagnosis (MCS)	−0.007	0.010	0.469	−0.005	0.018	0.787	−0.265	0.204	0.195
Diagnosis (UWS)	0.006	0.011	0.600	−0.029	0.021	0.162	−0.503 *	0.219	**0.021**
Sequence (1)	−0.001	0.004	0.847	0.005	0.006	0.433	0.104	0.078	0.185
Period (1)	0.002	0.003	0.462	−0.008	0.006	0.180	0.007	0.041	0.870
Treatment (sham) : Diagnosis (MCS)	0.008	0.008	0.323	0.039 *	0.016	**0.016**	0.290 *	0.113	**0.010**
Treatment (sham) : Diagnosis (UWS)	−0.016	0.009	0.058	0.023	0.017	0.184	0.318 *	0.123	**0.010**

polar and occipital areas might also have interfered with network activation.

As part of the secondary outcomes, at the single-subject level, 9 patients (6 MCS; 3 EMCS; 20% of the sample, and 31% of conscious patients) presented clinically relevant behavioral changes (i.e., gain or loss of signs of consciousness) following a- or s-tDCS. In the tDCS + a subgroup (n = 5), reproducible and systematic response to command and visual sub-scores were mainly recovered (object recognition, object localization and visual pursuit). We also identified 2 patients who lost a conscious behavior after a-tDCS that was otherwise present. The present study is the first to report this type of response. The fact that some patients could lose conscious behaviors following tDCS needs to be confirmed by future research but raises concerns regarding the therapeutic efficacy of this multichannel bihemispheric tDCS montage. If these "paradoxical responders" can be consistently identified, they should be better characterized using structural and metabolic neuroimaging, which may identify potential exclusion criteria for future studies. The other 37 patients (80% of the sample) did not gain nor lose a sign of consciousness following a- or s-tDCS (i.e., tDCS =) and, unsurprisingly, all of the UWS patients were in that group. This is in line with previous studies showing that patients in UWS exhibit less clinically relevant behavioral changes after tDCS than those in MCS (Thibaut et al., 2014; Angelakis et al., 2014; Cavinato et al., 2019); as they also have a farther path to recovery.

It should however be acknowledged that individually characterizing clinically relevant behavioral changes after one tDCS session is intricate in nature. Indeed, inferring the presence of an actual new behavior based on only few baseline assessments cannot be accurately performed from a statistical standpoint. Moreover, patients who are recovering from a comatose period and gradually demonstrate behaviors compatible with consciousness recovery (e.g., MCS) will do so inconsistently and thereby fluctuate a lot, even in the absence of an external intervention such as tDCS. Better controlling for this fluctuation would require multiplying the baseline assessments as well as the stimulation sessions, for instance. This would also allow identifying consistent EEG or other neuroimaging patterns that would constitute an accurate biomarker for tDCS response at the individual level.

When looking at the EEG findings, exploring relative band power did not reveal any significant effect of a-tDCS over s-tDCS, at the group level. On the other hand, the LZW complexity values showed a significantly greater increase following a-tDCS in the theta and delta bands compared to s-tDCS, suggesting a measurable electrophysiological effect of tDCS. These electrophysiological effects did not translate into observable behavioral changes. Only a few studies used complexity metrics to evaluate the effects of tDCS and none used it for patients with DOC. The Perturbational Complexity Index (Casali et al., 2013) calculated using LZW is diminished as compared to healthy controls and can discriminate between conscious and unconscious patients (Casali et al., 2013; Casarotto et al., 2016), with which our present findings regarding diagnosis are in line. A previous DOC study compared left and right DLPFC tDCS and sham while measuring CRS-R and EEG changes. Although there were no notable behavioral changes in this small study (n = 15), the authors identified increased EEG functional connectivity in the beta band in the right frontal lobe following right DLPFC tDCS. However, in the lower frequency bands (delta and theta), increased connectivity was distributed across the cortex (Wu et al., 2019).

In the present study, there was a significant influence of the behavioral outcome (ΔCRS-R) in conscious patients on the LZW complexity values in the delta and theta frequency bands, with better post stimulation CRS-R scores and decreased complexity after tDCS. Regarding the changes in clinically relevant conscious behaviors, the small sample sizes of the identified sub-groups unfortunately did not allow conducting proper statistical comparisons of the respective relative power and complexity values. A previous EEG-tDCS DOC study however showed increased baseline spatial connectivity and higher network centrality in the theta band in DLPFC tDCS-responders as compared to non-responders (Thibaut et al., 2018). Regarding the baseline neurophysiological values measured here; there was a single significant correlation between baseline complexity and ΔCRS-R for the theta band in conscious patients only. The present study thus suggests decreased theta complexity is beneficial for behavioral improvement as measured by the CRS-R total score in the MCS and EMCS populations. This hypothesis needs to be further tested but could be explained by the fact that low theta complexity at baseline can potentiate a spectral shift from theta to alpha, which would induce a clinical improvement, as suggested by previous studies (Thibaut et al., 2018; Williams et al., 2013). A shift in the alpha band was not observed here which could be attributed to our relatively low dose of tDCS, the small number of responders and also the fact that the overall contribution of alpha in the relative band power is less important than the ones of delta and theta.

Some limitations in this study could affect the generalizability of the results. First, we applied a single session of tDCS while it is now known that tDCS effects are enhanced with repeated stimulations (Thibaut et al., 2017; Boggio et al., 2007; Marangolo et al., 2013). Second, the fluctuations in vigilance that are characteristic of this population (Candelieri et al., 2011; Piarulli et al., 2016) may have impacted the results, as suggested by behavioral changes observed after s-tDCS. As stated above, it is indeed impossible to eliminate the impact of behavioral fluctuation on the results as some patients lost and gained conscious behaviors repeatedly. However, it is important to note that at baseline there were no significant differences in CRS-R total score, EEG power and EEG complexity prior to a-tDCS vs. s-tDCS. Finally, the EEG acquisition setup only consisted of eight electrodes, as it was integrated with the tDCS system. This low-density EEG restricts the sensitivity of the analyses.

To conclude, at the group level, a single session of multifocal frontoparietal tDCS did not have behavioral effects in patients with DOC while significantly affecting the LZW complexity metric. The observation that some MCS and EMCS patients improved and other worsened underlines the inter-individual variability, characteristic of this population, which translated into the tDCS-response. This highlights the need for longer prospective protocols, customized montages and identification of biomarkers. To this latter end, we showed that baseline theta EEG activity may have a role to play and therefore contribute to building an individual response phenotype and, *in fine*, to optimizing the therapeutic approach for DOC.

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CRediT authorship contribution statement

Géraldine Martens: Data curation, Formal analysis, Investigation, Writing - original draft. **Eleni Kroupi:** Data curation, Formal analysis, Methodology, Writing - review & editing. **Yelena Bodien:** Methodology, Validation, Writing - review & editing. **Gianluca Frasso:** Data curation, Formal analysis, Methodology, Validation, Visualization, Writing - review & editing. **Jitka Annen:** Formal analysis, Writing - review & editingHelena Cassol: Investigation, Writing - review & editing. **Alice Barra:** Investigation, Writing - review & editing. **Charlotte Martial:** Investigation, Writing - review & editing. **Olivia Gosseries:** Conceptualization, Funding acquisition, Methodology,

Writing - review & editing. **Nicolas Lejeune:** Investigation, Resources, Writing - review & editing. **Aureli Soria-Frisch:** Conceptualization, Funding acquisition, Methodology, Resources, Software, Writing - review & editing. **Giulio Ruffini:** Conceptualization, Funding acquisition, Methodology, Resources, Writing - review & editing. **Steven Laureys:** Concecptualization, Funding acquisition, Methodology, Writing - review & editing. **Aurore Thibaut:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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