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Cognitive and neurophysiological effects of bilateral tDCS neuromodulation in patients with minimally conscious state

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The minimally conscious state (MCS) is a clinical condition characterized by severely reduced but present awareness of self and the environment. Transcranial direct current stimulation (tDCS) has shown promising potential. The aim of this quasi-randomised control study was to investigate the effects of bilateral of tDCS applied to the right and left dorsolateral prefrontal cortex (DLPFC) on neurophysiological and cognitive outcomes in 28 patients with MCS. Participants were quasi-randomly assigned to one of two groups: experimental group with tDCS over both DLPFC, and a control group, which received sham tDCS. Neurophysiological assessments included event-related potentials (ERPs) analysis (N200 and P300) and EEG beta band study. Clinical outcomes were measured using ad hoc psychometric battery, including Coma Recovery Scale-Revised (CRS-R), Levels of Cognitive Functioning Scale (LCFS), and Functional Independence Measure (FIM). The findings revealed a significant improvement in ERP latencies and increased beta band rhythms in the experimental group, indicating enhanced neural responsiveness to cognitive stimuli. Additionally, significant improvements were observed in clinical measures of awareness and functional capacity. These findings suggest that tDCS may represent a promising therapeutic option for enhancing both neurophysiological responses and cognitive functioning in patients with MCS.

The minimally conscious state (MCS) is a clinical condition characterized by a reduced but present awareness of both self and the surrounding environment[1]. Unlike other disorders of consciousness (DoC), MCS is defined by the presence of consistent, albeit limited, voluntary and coherent behavioural responses to external stimuli, although these responses may be inconsistent[2]. MCS typically emerges following severe acquired brain injuries, such as traumatic brain injury, ischemic or haemorrhagic stroke, and cardiac arrest, which disrupt the neural networks governing awareness and consciousness[3]. Neurophysiologically, MCS is associated with remaining cortical and subcortical activation that supports minimal cognitive and perceptual processes[4]. This condition is often unstable, with outcomes ranging from improvement and stabilization to deterioration, making recovery unpredictable and challenging to forecast[5,6].

Therapeutic strategies for MCS aim to stimulate awareness and cognitive functions through a combination of intensive neurological rehabilitation, neuromodulation techniques, sensory stimulation, cognitive-behavioural intervention, and assistive technologies[7,8]. Among these intervention, transcranial direct current stimulation (tDCS) has garnered considerable attention as a potentially effective non-invasive neuromodulation approach to promote recovery in MCS patients[9,10]. tDCS involves the application of a weak electric current through electrodes placed on the scalp, modulating cortical excitability and influencing synaptic plasticity[11]. This technique is considered safe, relatively straightforward to administer, and has emerged as a promising therapeutic option in neurological rehabilitation[12].

The clinical interest in tDCS has expanded due to its demonstrated ability to enhance a range of cognitive functions, from sensory perception to complex cognitive processing, in both healthy individuals and patients with neurological or psychiatric disorders[13,14]. Recent studies have investigated the efficacy of tDCS in patients

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with DoC, yielding promising results, particularly in those with MCS[15–17]. A recent multicentric randomized controlled trial by Thibaut et al., reported that while no significant improvements were observed at the group level, patients with MCS and traumatic etiology exhibited a more pronounced response to tDCS, reinforcing the role of etiology and baseline consciousness level in neuromodulation outcomes[18]. Anodal stimulation of the left dorsolateral prefrontal cortex (DLPFC) has shown potential in improving cognitive functions and increasing behavioural responsiveness in some MCS patients[19]. These findings suggest that tDCS may modulate fronto-parietal networks involved in awareness and attention[19,20].

However, the comparative effectiveness of anodal stimulation targeting the left versus the right DLPFC, as well as the underlying electrophysiological mechanisms, remain unclear. Most research has focused on the left DLPFC, with fewer studies addressing the effects of stimulation on the right DLPFC[21–24]. For instance, a randomized controlled trial by Wu et al. compared anodal stimulation of the left and right DLPFC, revealing asymmetrical in effects: while left DLPFC stimulation enhanced global cortical excitability, right DLPFC stimulation led to more localized activation without significant improvements in consciousness[25]. Conversely, functional imaging studies, such as the one conducted by Liu et al., suggest that the right DLPFC may play a pivotal role in enhancing consciousness. This could be attributed to its involvement in top-down noradrenergic activation and its connections with the brainstem reticular formation, a key structure for maintaining arousal. Additionally, the right hemisphere's dominance in attentional processes supporting the hypothesis that right DLPFC stimulation may positively influence DoC[24,26].

Despite these promising insights, the comparative effectiveness of simultaneous or alternating anodal stimulation of both the left and right DLPFC in MCS patients has yet to be thoroughly explored. We hypothesize that a bilateral stimulation approach could achieve a more balanced and optimal modulation of cortical excitability, potentially addressing the limitations of unilateral protocols and enhancing therapeutic outcomes.

The purpose of this pilot study was to investigate the effects of a bilateral tDCS targeting the right and left DLPFC on neurophysiological and cognitive outcomes in a cohort of patients with a diagnosis of MCS.

Methods

Study design and population

A total of 28 patients with MCS were recruited from the U.O.C. Neurorehabilitation Unit of IRCCS Neurolesi "Bonino-Pulejo" in Messina, Italy, from January 2024 to July 2024, and evenly assigned to an experimental group (EG, n = 14) and a control group (CG, n = 14), as explained in Fig. 1. Of the 35 patients initially screened, 30 met the inclusion criteria and were enrolled in the study. The five excluded patients did not meet the eligibility criteria due to the following reasons: three patients had a history of epilepsy, one patient had a metallic implant incompatible with tDCS, and one patient was under pharmacological treatment with CNS-acting drugs at the time of screening. Participants were quasi-randomly allocated to one of the two groups. During the intervention phase, one participant from each group discontinued participation due to personal reasons, resulting in 14 participants completing the protocol in both groups. All 28 remaining participants were included in the final analysis. As up mentioned, this study employed a quasi-randomized control trial design to allocate patients to two groups based on enrolment order. The quasi-randomization was chosen to ensure practical and balanced group allocation within the constraints of the clinical setting. To minimize bias, the allocation sequence was implemented using sequentially numbered, opaque, sealed envelopes, prepared by an independent researcher not involved in the study. Participants were enrolled by the attending clinical team, while group assignment

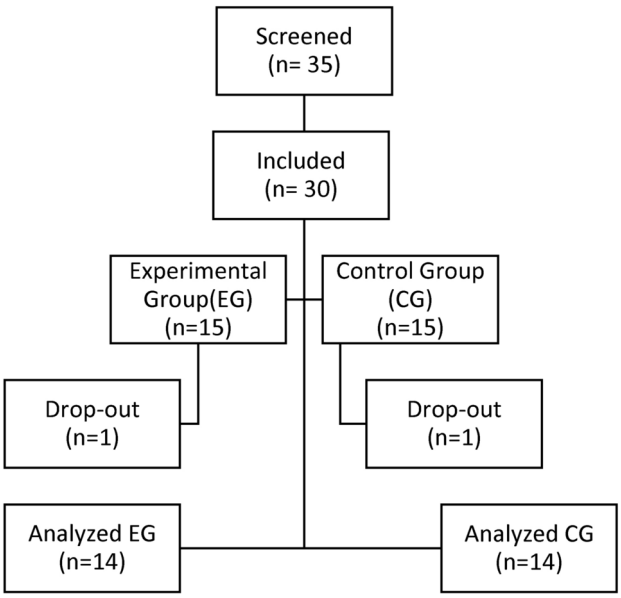

Fig. 1. Flow-diagram of patients' enrolment.

was performed by an investigator blinded to treatment details. To further ensure validity, both participants and caregivers were blinded to group allocation, and outcome assessors were shielded from allocation knowledge.

Although a quasi-randomized design introduces potential limitations, such as allocation dependency on enrolment order, rigorous steps were taken to mitigate bias and maintain the study's internal validity.

This study was conducted in accordance with the principles set forth in the Declaration of Helsinki and received approval from the Ethics Committee of the IRCCS Neurolesi Bonino-Pulejo in Messina (protocol approval number 21/2023, 7/08/2023). This study was registered on ClinicalTrials.gov under the identifier NCT06236503.

The sample size was based on the feasibility of recruiting participants from the available population within the given timeframe and resource constraints. As this was a pilot study, the primary goal was to gather preliminary data to estimate effect sizes and inform the design of future trials.

Before entering the study, each caregiver/tutor of MCS patients was carefully informed about the purpose of the research and the data collection procedures. Information was presented in a clear and comprehensible manner to ensure caregivers/tutor could make informed decision about their relative's participation. After receiving such explanations, each caregiver/tutor provided their written informed consent.

Inclusion criteria included: (1) no use of proscribed central nervous system (CNS)-acting drugs or neuromuscular blockers within a defined washout period (see Supplementary Table 1) (2) periods of spontaneous eye opening, indicative of preserved sleep-wake cycles, and (3) diagnosis of MCS based on the Coma Recovery Scale-Revised (CRS-R). Participants with metallic brain implants, or pacemakers were excluded.

Prior to the stimulation, a one-week monitoring period was conducted to ensure clinical stability through behavioural assessment and routine laboratory test. Training was completed in all subjects without any adverse events and no harms or side effects. No significant differences were observed at baseline (T0) between the two groups regarding demographic variables, aetiology, time post-injury and lesion characteristics. The participants had a mean age of 58.13 years (SD = ± 8.33) (see Table 1). The distribution of pharmacological treatments, including dopaminergic agents, antiepileptics, benzodiazepines, and antidepressants, is summarized in Supplementary Table 2.

Outcome measures

EEG, ERP measurements, and tDCS setup

EEG recordings were conducted bedside with a sampling rate of 500 Hz, using a low-pass filter set between 0.1 Hz and 70 Hz. Each EEG session lasted approximately 10 min and was performed at rest in a quiet environment to minimize external stimulation. Since patients with MCS may have limited voluntary control over eye opening and closing, the natural state of the eyes (open or closed) was noted during the recording. This approach ensured that the analysis accounted for individual variability in eye state while minimizing artifacts. Data acquisition was performed with SCAN software (version 4.3, Neuroscan, Compumedics, El Paso, TX, USA) and NuAMP amplifiers (https://advancedmedicalequipment.com/Support/Old/scan4.3.html accessed on 20/01/2024). The EEG signal was recorded using 19 scalp electrodes (Ag/AgCl) arranged in a bipolar montage. Electrodes were positioned according to the standard 10–20 system described by Jasper[27], with a focus on the fronto-temporal regions. Electrode impedances were maintained at 10 kΩ or less throughout the recording. These parameters were chosen to optimize signal quality while minimizing noise and artefacts.

Quantitative analysis of EEG data was performed using custom algorithms developed in MATLAB (The MathWorks Inc., Natick, MA, USA)[28]. Before analysis, EEG recordings were carefully inspected for artifacts. Segments affected by excessive muscle activity (EMG contamination), movement, or noise were excluded from the analysis, ensuring that only high-quality data were used. Power Spectral Density (PSD) was computed using the Welch method[29] to transform signals from the time domain to the frequency domain. This analysis focused on total absolute power and absolute power within specific frequency bands for each electrode. The beta band (14–29 Hz) was selected due to its relevance to cognitive functions such as attention, working memory, and decision-making. In this study, beta band activity was analysed using time-frequency analysis to provide detailed insights into cortical activation patterns during stimulation.

Patients	EG (N = 14)	CG (N = 14)	*p*-value
Age	58.13 ± 8.33	57.33 ± 11.06	0.8
Educational level			
Middle school	8 (57.14%)	7 (50.00%)	
High school	6 (42.86%)	7 (50.00%)	
Gender			
Male	10 (71.42%)	10 (71.42%)	1
Female	4 (28.53%)	4 (28.53%)	
Etiology			
Vascular	9 (64.28%)	9 (64.28%)	1
Traumatic	5 (35.72%)	5 (35.72%)	
Time post-injury (months)	12.4 ± 5.3	11.8 ± 4.9	0.76
Lesion characteristics	Frontal: 5, Parietal: 3, Multi-lobar: 6	Frontal: 4, Parietal: 4, Multi-lobar: 6	0.82

Table 1. Socio-demographic clinical description of the study sample.

Neurophysiological responses were assessed through a comprehensive analysis of EEG recordings and event-related potentials (ERPs), focusing on N200 and P300 latencies. An auditory oddball paradigm was used to elicit ERP responses, consisting of frequent standard tones (1000 Hz, 80% probability) and infrequent deviant tones (2000 Hz, 20% probability). Auditory stimuli were delivered binaurally via headphones at an intensity of 90 dB SPL. The interstimulus interval (ISI) was fixed at 1000 ms, and the ERP recording itself lasted approximately 12 min. However, due to the clinical condition of the patients, the entire session was planned to last up to 60 min. This timeframe included patient preparation, electrode placement, impedance checks, real-time artifact monitoring, necessary pauses for patient comfort, and post-recording signal verification. To ensure blinded data processing, the experimenter in charge of EEG/ERP analysis was not informed about the patients' clinical diagnosis or group allocation (active or sham tDCS). Patients underwent multisensory stimulation using the Neurowave device (Neurowave, Khymeia Srl, Padova, Italy; https://khymeia.com/it/products/neurowave accessed on 20/01/2024), which delivers automated and personalized stimuli (images, videos, sounds, and memories) tailored to the patient's experience. Each stimulation session lasted 60 min and included visual and auditory stimuli of 500 ms each, with an interval of 800 ms between stimuli and a rare stimulus occurrence probability set at 20%. ERP measurements, focusing on P300 and N200 components, were conducted using electrodes placed at Fz, Cz, and Pz, covering the centre-parietal and vertex regions, according to the 10–20 system. Electroencephalographic activity was monitored using electro-oculograms recorded with electrodes placed laterally at the outer canthus and above and below the left eye. Data were acquired with a sampling frequency of 256 Hz, filtered with a passband of 0.15–30 Hz, and with the application of a notch filter to reduce electrical noise.

The N200 component, typically observed around 200 ms after stimulus presentation, is associated with cognitive processes such as conflict detection, error monitoring, and cognitive control. Changes in its latency can reflect improvements in the brain's ability to detect and process deviations from expected stimuli. The P300 component, observed approximately 300 ms after stimulus onset, is widely recognized as a marker of attentional resource allocation and decision-making. Shortened P300 latency suggests enhanced cognitive processing speed and more efficient allocation of attentional resources to relevant stimuli.

The tDCS device used in this study, the BrainSTIM device manufactured by EMS S.r.l. of Bologna, Italy (http://brainstim.it/index.php?lang=it#&jjj=1737370906209 accessed on 20/01/2024), was specifically designed to deliver consistent and reliable transcranial stimulation for neurophysiological research. The stimulation was carried out using two sponge electrodes, each with a diameter of 25 mm and previously saturated with a saline solution. The constant current was delivered via a battery-powered stimulator, ensuring safety and precision throughout the procedure. Participants were carefully monitored for any adverse effects or discomfort throughout the intervention. Commonly reported side effects in tDCS studies include tingling sensations, mild headache, skin irritation, and fatigue. During our study, mild skin redness at the stimulation site was observed in a few participants, with one patient exhibiting more pronounced redness due to particularly sensitive skin. This was effectively managed by increasing the amount of conductive gel applied before stimulation, which resolved the issue without further complications.

No other adverse events were reported. A summary of potential side effects, their typical incidence in previous studies, and the monitoring strategies used in this study is provided in Supplementary Table 3.

Clinical assessment

The clinical assessment, conducted at the bedside, explored cognitive recovery, independence in daily living, and consciousness levels, offering a comprehensive overview of each patient's progress.

The CRS-R[30] offers an in-depth assessment of consciousness and recovery by examining six specific functions: auditory, visual, motor, oral-motor/verbal, communication, and arousal. The items are organized hierarchically, with lower scores reflecting reflexive activities and higher scores indicating cognitively mediated behaviours.

The Levels of Cognitive Functioning Scale (LCF)[31] was employed to monitoring and assessment of cognitive recovery by classifying the patient's difficulties and residual abilities on a ten-point scale, where Level 1 indicates a complete absence of response to any stimulus and Level 8 represents an independent patient with possible adaptations.

The Functional Independence Measure (FIM)[32] comprises 18 items divided into 13 motor (motFIM) and 5 cognitive (cognFIM) categories and explores the patient's social, psychological, and physical functioning in detail, evaluating the degree of independence in daily activities and providing a comprehensive measure of self-sufficiency.

Procedure

Patients were recruited based on medical evaluation and provided informed consent to participate in the study, which was signed by their primary caregiver. Neurophysiological assessments were conducted at two-time points: T0 (pre-treatment) and T1 (day 21). These assessments included beta band EEG and the measurement of N200 and P300 ERPs, and the clinical evaluation. Following this initial evaluation, participants were assigned to one of two groups using a quasi-randomized approach, based on the order of enrolment. As mentioned earlier, assessments and treatments were conducted at the patient's bedside, ensuring privacy and an appropriate setting for all participants by utilizing dedicated single rooms.

The experimental group received tDCS stimulation applied to the right and left DLPFC, with parameters including 2 mA intensity, a current density of 2.5 mA/cm^2. Stimulation was administered following a fixed order, with the first 2 weeks targeting the left DLPFC (F3) and the subsequent 2 weeks targeting the right DLPFC (F4), according to the EEG 10-20 system. Stimulation was administered at an intensity of 2 mA (with a current density of 2.5 mA/cm^2) for a duration of 20 min. The stimulation protocol was applied five times a week for two consecutive weeks, totalling 10 sessions for each side of the DLPFC, so the bilateral treatment was administered daily (5 days per week) over a period of 4 weeks, for a total of 20 sessions. The control group received sham

stimulation, replicating the procedure without active stimulation, for the same number of sessions. During and after each tDCS session, participants were carefully monitored for any adverse effects or discomfort. Any reported adverse symptoms were promptly recorded and managed according to established protocols.

At the end of the treatment period, all patients underwent the same neurophysiological and clinical assessments conducted at baseline. This allowed for a comparison of pre-and post-treatment data to evaluate the efficacy of the tDCS protocol. The evaluators responsible for the post-intervention assessments were blinded to the participants' group assignments.

Statistical analysis

Data analysis was conducted using IBM SPSS Statistics, Version 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp, Armonk, NY, USA). The significance level for statistical tests was set at $p < 0.05$. For the analysis of neurophysiological measures, we employed a MANOVA model for repeated measures with a between-subject factor (group: experimental and control) and a within-subject factor (phases: T0—pre-intervention baseline, T1—post-treatment) for both neurophysiological (N200, P300 and Beta band) and clinical measures (CRS-R, LCF and FIM). In case of significant effects, the effect size of the test was reported, computed, and categorized according to eta squared η2.

Furthermore, to provide a comprehensive assessment, we applied paired t-tests within each group (CG T0–T1 and EG T0–T1) and independent t-tests between the two groups (CG vs. EG at T0 and at T1) for clinical outcome measures. We assessed the assumption of normality using the Shapiro–Wilk test and examined the homogeneity of group variances using Levene's test. The data analysed had a normal distribution, the test was not significant, so Student t-tests, using the Bonferroni correction, were used for post-hoc testing of group differences in time and performance. Power analysis using Cohen's d as the effect size parameter was applied.

Finally, to verify whether the increments in physiological measures were consistent with each other, differences between T0 and T1 were calculated and analysed using Pearson's correlation coefficient (r). Additionally, we explored the relationship between physiological and clinical measures to understand the coherence and potential interactions between these variables.

Results

This pilot study revealed significant effects of bilateral tDCS on both neurophysiological and clinical measures in patients with MCS. The experimental group exhibited notable improvements in event-related potentials, beta band power, and clinical measures of awareness and functional independence. These outcomes, both neurophysiological and clinical, are detailed in the following sections.

Neurophysiological results

For N200 latency, repeated measures ANOVA revealed a significant effect of the Phase factor ($F(1, 26) = 50.27$, $p < 0.001$, $\eta^2 = 0.11$), indicating a difference between pre-treatment and post-treatment results. The Group by Phase interaction was also significant ($F(1, 26) = 25.72$, $p < 0.01$, $\eta^2 = 0.09$), suggesting that the observed change in N200 latency was influenced by the type of intervention. Contrasts showed that the change in N200 latency for the EG (C1 = μExp, Post − μExp, Pre) was statistically significant ($p < 0.05$). Conversely, the corresponding difference for the CG (C2 = μCtrl, Post − μCtrl, Pre) did not reach statistical significance ($p = 0.27$).

Regarding P300 latency, a significant effect of the Phase factor was found ($F(1, 26) = 59.76$, $p < 0.001$, $\eta^2 = 0.12$), showing a distinction between pre-treatment and post-treatment results. The Group by Phase interaction was also significant ($F(1, 26) = 42.27$, $p < 0.05$, $\eta^2 = 0.11$). Contrasts revealed that the difference in P300 latency for the EG (C1 = μExp, Post − μExp, Pre) was significant ($p < 0.05$), while CG (C2 = μCtrl, Post − μCtrl, Pre) showed no significant change ($p = 0.21$).

For beta band power, repeated measures ANOVA indicated a significant effect of the Phase factor ($F(1, 26) = 32.61$, $p < 0.01$, $\eta^2 = 0.09$), suggesting a difference between pre-treatment and post-treatment measurements. The Group by Phase interaction was significant ($F(1, 26) = 18.22$, $p < 0.05$, $\eta^2 = 0.09$). Contrasts showed a significant increase in beta band power in the EG (C1 = μExp, Post − μExp, Pre; $p < 0.01$), while the CG did not present a significant difference (C2 = μCtrl, Post − μCtrl, Pre; $p = 0.13$).

In conclusion, the EG showed significant changes in N200 and P300 latency, as well as beta band power from pre-treatment to post-treatment, while no significant changes were observed in the CG. These findings suggest that the intervention had a measurable impact on neurophysiological responses, as illustrated in Table 2 and Fig. 2.

Clinical outcomes

For the LCF, repeated measures ANOVA revealed a significant Phase effect ($F(1, 26) = 18.34$, $p < 0.01$, $\eta^2 = 0.09$), indicating improvement between pre-treatment and post-treatment results. The Group by Phase interaction was also significant ($F(1, 26) = 5.98$, $p < 0.05$, $\eta^2 = 0.089$). Contrasts showed a significant increase in LCF scores in the EG (C1 = μExp, Post − μExp, Pre; $p < 0.01$), while no significant change was found in the CG (C2 = μCtrl, Post − μCtrl, Pre; $p = 0.26$). Similarly, for the FIM, a significant Phase effect was observed ($F(1, 26) = 25.75$, $p < 0.01$, $\eta^2 = 0.11$), indicating a difference between pre-treatment and post-treatment results. The Group by Phase interaction was significant ($F(1, 26) = 10.32$, $p < 0.05$, $\eta^2 = 0.12$). Contrasts showed a significant improvement in FIM scores for the EG (C1 = μExp, Post − μExp, Pre; $p < 0.01$) but not for the CG (C2 = μCtrl, Post − μCtrl, Pre; $p = 0.28$). For the CRS-R, a significant Phase effect was also found ($F(1, 26) = 21.47$, $p < 0.01$, $\eta^2 = 0.10$). The Group by Phase interaction was significant ($F(1, 26) = 11.29$, $p < 0.05$, $\eta^2 = 0.09$). Contrasts showed significant improvement in CSR-R scores for the EG (C1 = μExp, Post − μExp, Pre; $p < 0.01$), whereas the CG did not show a significant change (C2 = μCtrl, Post − μCtrl, Pre; $p = 0.46$). A single-subject analysis revealed that 7 out of 14 patients (50%) in the experimental group showed an improvement in the total CRS-R score following tDCS.

	Pre-treatment	Post-treatment			
	M (± SD)	M (± SD)	t	*p*	d
Experimental group					
N200	233.33 (± 16.95)	213.83 (± 12.88)	7.22	0.001**	0.78
P300 latency	402.07 (± 12.43)	377.57 (± 12.37)	6.81	0.001**	0.79
Beta (13–30 Hz)	15.11 (± 2.51)	16.99 (± 2.21)		0.001**	0.85
LCF	2.30 (± 0.67)	3.60 (± 1.03)	8.23	0.01**	0.89
FIM	23.14 (± 5.53)	26.88 (± 7.21)	6.05	0.01**	0.91
CSR-R	7.21 (± 1.21)	8.95 (± 0.99)	4.11	0.01**	0.87
Control group					
N200	232.38 (± 14.75)	228.53 (± 15.33)	1.11	0.19	0.78
P300 latency	406.07 (± 15.88)	404.57 (± 13.55)	0.98	0.52	0.79
Beta (13–30 Hz)	15.53 (± 2.31)	15.28 (± 2.21)	1.05	0.22	0.85
LCF	2.37 (± 0.58)	2.40 (± 1.07)	0.23	0.11	0.89
FIM	23.86 (± 5.53)	24.02 (± 8.21)	1.05	0.27	0.91
CSR-R	7.09 (± 0.99)	7.60 (± 0.99)	0.23	0.46	0.88

Table 2. Means and standard deviations for P300, N200 latencies, beta band power, and LCF and FIM measures scores across the two groups [Experimental Group (EG) and Control Group (CG)] at different time points (pre- and post-treatment: T0 and T1).

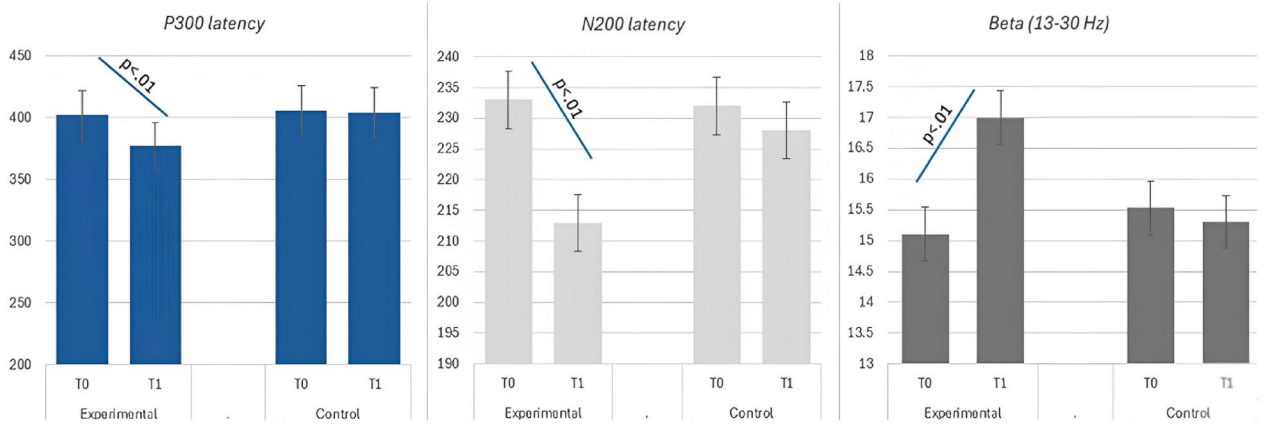

Fig. 2. Changes in the P300 latency, N200 latency, and Beta band power (13–30 Hz) measured at two time points (T0 and T1) in the Experimental Group (EG) and Control Group (CG). The figure shows the significant differences between T0 and T1 for the experimental group.

The most prominent changes were observed in the Arousal, Visual, and Motor subscales, which are known to be more sensitive to neuromodulation interventions[33,34]. Specifically, the Arousal subscale increased from a mean of 1.06 (SD = 0.33) at baseline to 1.44 (SD = 0.31) post-treatment, while the Visual subscale improved from 1.79 (SD = 0.33) to 2.24 (SD = 0.35), and the Motor subscale increased from 1.81 (SD = 0.27) to 2.25 (SD = 0.27). These findings suggest that the Visual and Motor subscales exhibited the greatest improvements, while the Arousal subscale showed a more moderate increase. CRS-R subscales analysis is provided in Supplementary Table 4. This trend aligns with previous studies indicating that visual and motor functions are particularly responsive to neuromodulation-based rehabilitation strategies. Among the patients who improved, the most frequently emerging clinical signs were visual pursuit, command following, and intentional communication, which align with previous findings on tDCS efficacy in disorders of consciousness[35]. These findings reinforce the potential role of tDCS in enhancing cognitive and motor recovery in this population.

Moreover, we did observe a slight positive trend in clinical parameters among patients with traumatic brain injury compared to those with vascular lesions. While this observation is purely descriptive and requires further investigation, it aligns with existing literature suggesting greater neuroplastic potential and recovery prospects in traumatic brain injury cases compared to vascular lesions, likely due to differences in the extent and distribution of damage, as well as the greater regenerative capacity of perilesional tissue in trauma patients[36].

In conclusion, results indicate that the EG showed significant changes in all clinical measures from pre-treatment to post-treatment, while no significant changes were observed in the CG. These findings suggest that the intervention had a measurable impact on clinical responses, as illustrated in Fig. 3.

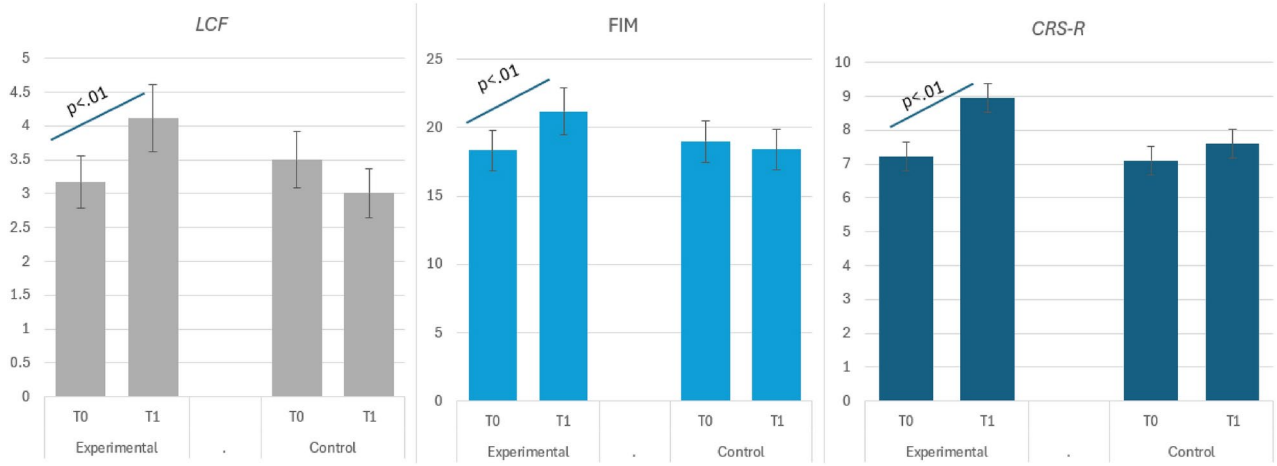

Fig. 3. Changes in LCF, FIM, and CRS-R measured at two time points (T0 and T1) in the Experimental Group (EG) and Control Group (CG). The figure highlights the significant differences between T0 and T1 in the experimental group.

	N200	P300 latency	Beta (13–30 Hz)	LCF	FIM
N200	–				
P300 latency	0.48**	–			
Beta (13–30 Hz)	−0.46**	−0.65**	–		
LCF	−0.39**	−0.38**	0.52**	–	
FIM	−0.53**	−0.49**	0.49**	0.61**	–

Table 3. Pearson correlations between neurophysiological and behavioural measures.

Correlation analysis

To explore the relationship between the neurophysiological measures, the change scores were calculated by subtracting T0 from T1 values for N200, P300, and beta power. Pearson correlation analysis was then performed on these change scores to assess the relationships among alterations in the neurophysiological parameters. The results revealed several significant relationships among the change scores (see Table 3). A significant positive correlation was observed between the change in P300 latency and the change in N200 latency (r (28) = 0.483, $p < 0.01$, p < 0.01, with a medium effect size, $d = 0.50$), indicating that increases in N200 latency were associated with increases in P300 latency. Conversely, the change in P300 latency was negatively correlated with the change in beta power (r (28) = −0.455, $p < 0.01$, with a medium effect size, $d = 0.47$), suggesting that as P300 latency decreases, beta power tended to increase. Additionally, the change in N200 latency was negatively correlated with the change in beta power (r (28) = −0.647, $p < 0.01$, with a large effect size, $d = 0.75$), indicating that decreases in N200 latency were associated with increases in beta power. These findings suggest interrelation between the neurophysiological measures of N200 and P300, with changes in latency correlating with changes in beta power. This pattern may indicate a linked underlying mechanism affecting these ERP components and EEG rhythms in response to the intervention.

The results also revealed significant correlations between neurophysiological measures (N200 and P300 latency) and behavioural measures (LCF and FIM). Pearson correlation coefficients showed a negative relationship between N200 change scores and LCF (r (28) = −0.39, $p < 0.05$, with a medium effect size, $d = 0.42$). This suggests that decreases in N200 latency were associated with higher LCF scores. Similarly, N200 latency was negatively correlated with FIM (r (28) = −0.53, $p < 0.01$), indicating that increases in N200 latency were associated with decreases in functional independence, with a large effect size ($d = 0.64$). P300 latency change scores also exhibited a negative correlation with both LCF (r (28) = −0.38, $p < 0.05$), and FIM (r (28) = −0.49, $p < 0.01$). These correlations suggest that increased P300 latency is associated with reductions in both levels of consciousness and functional independence. The effect sizes for these correlations were medium ($d = 0.41$), and large ($d = 0.57$) respectively. In contrast, beta power change scores were positively correlated with LCF (r (28) = 0.52, $p < 0.01$), and FIM (r (28) = 0.49, $p < 0.01$). These results indicate that increases in beta power are associated with improvements in consciousness levels and functional independence, with medium effect sizes ($d = 0.55$ for LCF and $d = 0.57$ for FIM).

Overall, these findings suggest that changes in neurophysiological measures are significantly associated with behavioural outcomes, indicating a possible link between neural activity alterations and clinical improvements following the intervention. This reinforces the potential of using ERPs and EEG parameters as objective markers for tracking functional recovery.

Discussion

This pilot study examined the effect of tDCS applied to the right and left DLPFC on neurophysiological and clinical parameters in patients with MCS. The DLPFC was selected due to its critical role in higher-order cognitive functions, including attention, memory, and decision-making, making it a target for interventions aimed at enhancing awareness and cognitive recovery[20,23,31,32]. The main hypotheses were that tDCS could improve the latencies of N200 and P300 event-related potentials, increase beta band power, and positively influence clinical measures of awareness and functional independence, as assessed by LCF, FIM and CRS-R.

The results obtained largely support these hypotheses, with significant improvements observed in both neurophysiological and clinical parameters, providing evidence for the efficacy of bilateral tDCS in this patient population. We observed a significant reduction in the latencies of N200 and P300 event-related potentials, with N200 latency decreasing by an average of 18 ms and P300 latency by 22 ms, indicating enhanced cognitive processing speed and resource allocation. The reduced latency of N200 suggests enhanced cognitive processing speed and an improved ability to detect environmental changes[37]. The reduced P300 latency reflects better processing of significant stimuli and improved allocation of cognitive resources, facilitating decision-making[34–36].

The increase in beta band power, with an observed rise of 25% in spectral power, suggests that tDCS enhanced neuronal activity in cortical areas involved in attention regulation and cognition[38]. For MCS patients, this result can be interpreted as an improvement in the ability to maintain alertness and engage in cognitive activities, albeit in a limited manner. Clinical measures also showed significant improvements, with average increases of 2 points in LCF, 3 points in FIM, and 4 points in CRS-R scores, reflecting better functional independence and awareness. These results suggest that tDCS facilitated better interaction with the environment and more coherent responses to external stimuli. This suggests improved awareness and a promising impact of tDCS on functional abilities, particularly in cognitive domains, compared to the CG. These results align with previous evidence demonstrating the efficacy of tDCS in modulating neurophysiological and clinical activities in MCS patients[9,16,18].

Relatively few studies have explored the neurophysiological changes induced by tDCS in patients with DoC. This has left significant gaps in understanding, particularly regarding its effects on specific EEG frequency bands and ERP components, such as N200 and P300. Notably, Estraneo et al., identified several prognostic factors associated with better recovery in patients with DoC, including younger age, shorter time post-injury, higher baseline CRS-R scores, and EEG responsiveness to eye-opening stimuli[33]. These factors may partially explain the variability observed in our results and reinforce the need for a multimodal approach to accurately assess patient prognosis and treatment efficacy. Bai et al. observed an increase in fronto-parietal coherence in the theta band and a decrease in the gamma band following tDCS, suggesting that stimulation might alter interactions between brain regions and neural synchronization in specific frequencies[39]. Guo et al. reported a decrease in centre-parietal coherence in the delta band following tDCS of the posterior parietal cortex, indicating changes in synchronization associated with sleep and recovery states[40]. Cavinato et al. found an increase in power and coherence in the alpha and beta bands in frontal and parietal regions in MCS patients, with alpha bands associated with relaxation and beta bands with attention and cognitive activity[41]. Hermann et al. observed an increase in power and connectivity in the theta-alpha band, which is implicated in attention and memory[42]. Finally, Carrera et al. found an increase in power in the alpha and theta bands in patients with severe brain injuries, although with no significant group-level effects, suggesting individual variability in response to tDCS[43]. These findings highlight how tDCS may modulate neural activity across various frequency bands, supporting its potential to promote recovery in MCS patients.

However, most studies have overlooked a thorough analysis of the effects of tDCS on the beta band, despite its established relevance to cognitive processes such as sustained attention, working memory, and decision-making. The beta band, associated with processes like attention and memory, was analysed using spectral power methods to provide insights into cortical activation during stimulation and its link to cognitive improvements[44]. In this study, beta band activity was analysed using spectral power calculation methods, providing detailed insights into cortical activation patterns during stimulation and their potential link to cognitive improvements. In MCS patients, analysing this band could provide a more detailed understanding of the residual levels of these cognitive functions and offer insights into the cognitive processes still active. Since the beta band is often associated with alertness and active attention, variations in its power might reflect changes in awareness and responsiveness to stimuli[45]. Given that patients with MCS exhibit reduced but not absent consciousness, beta band analysis could reveal crucial clues about brain activation levels. Additionally, the study of ERPs, particularly the P300 and N200 components often evoked in oddball paradigms, where infrequent target stimuli facilitate the detection of deviations in the EEG signal, could provide further insights into the effectiveness of tDCS[46]. The P300 potential, typically observed around 300 ms after the stimulus, is indicative of the allocation of attentional resources and cognitive processing of task-relevant stimuli[47]. The N200 potential, appearing approximately 200 ms following the stimulus, is associated with conflict detection, cognitive control, and error monitoring[35,36]. Both are of particular importance in the analysis of MCS patients, as they offer a window into residual cognitive abilities that might not be detectable through behavioural observations alone. In MCS patients, external behaviours can be extremely limited or absent, making it challenging to assess whether the patient retains forms of perception, attention, or awareness. Specifically, the latencies of the P300 and N200 could be crucial in this context because they provide precise indications of the speed and efficiency of cognitive processing, revealing the presence of attentional capacities and error monitoring even when external behaviours are limited or absent.

However, our study adds new insights by emphasizing the benefits of bilateral stimulation over unilateral approaches. This finding aligns with prior evidence suggesting the complementary roles of the right and left DLPFC in cognitive processes and recovery. Wu et al. suggested more pronounced effects with left DLPFC stimulation[48], while Liu et al. indicated a crucial role of right DLPFC in improving consciousness[26]. Our data, which show benefits of bilateral stimulation, might suggest a more balanced approach. Nevertheless, further

research is needed to confirm these results and optimize stimulation strategies. It would be valuable to explore how bilateral stimulation compares to unilateral stimulation in terms of efficacy and optimal parameters for enhancing awareness and functional abilities in MCS patients.

An important aspect to consider is the potential variability in response to tDCS across different etiologies of MCS, particularly between patients with TBI and those with vascular lesions. Neural plasticity mechanisms differ substantially between these two groups, influencing recovery trajectories and responsiveness to neuromodulation interventions. While our study did not perform a specific subgroup analysis, future research should investigate whether etiology-specific differences impact the efficacy of tDCS in MCS patients. Moreover, recent advancements in precision non-invasive brain stimulation, as discussed by Cappon and Pascual-Leone[49], highlight the importance of optimizing tDCS protocols by considering individual brain connectivity patterns and lesion characteristics. The concept of lesion network localization suggests that functional outcomes are not solely determined by lesion location but also by the impact of lesions on broader neural networks. Integrating these principles into tDCS applications could refine stimulation parameters to enhance treatment outcomes. Additionally, emerging approaches such as multifocal tDCS and real-time monitoring of neurophysiological markers offer promising avenues for personalized interventions, potentially improving the effectiveness of tDCS in MCS patients.

One of the principal strengths of this study is the inclusion of a control group, which mitigates potential biases and enhances the validity of the findings. The control group received sham stimulation to simulate the procedure without delivering active tDCS, ensuring that any observed effects could be attributed to the intervention. Additionally, blinding procedures were employed for post-treatment evaluations to further minimize bias and maintain the integrity of the results.

Moreover, the combined analysis of neurophysiological and clinical measures provided a comprehensive assessment of the effects of tDCS across different levels of functioning. The significant correlations between neurophysiological changes and clinical improvements provide valuable insights into the underlying mechanisms. Specifically, decreases in N200 latency were negatively correlated with LCF ($r = -0.39$, $p < 0.05$) and FIM scores ($r = -0.53$, $p < 0.01$), indicating that faster cognitive processing was associated with improved functional independence and consciousness. Similarly, reductions in P300 latency were negatively correlated with LCF ($r = -0.38$, $p < 0.05$) and FIM ($r = -0.49$, $p < 0.01$), further supporting the link between enhanced cognitive efficiency and clinical improvements. Conversely, increases in beta power were positively correlated with both LCF ($r = 0.52$, $p < 0.01$) and FIM ($r = 0.49$, $p < 0.01$), suggesting that enhanced cortical activity contributes to functional and behavioural recovery. These findings highlight the coherence between neurophysiological alterations and clinical outcomes, reinforcing the potential of ERPs and EEG measures as objective markers of recovery.

The study's relatively small sample size is a notable limitation, potentially affecting the generalizability of the results. The sample consisted of 28 patients, which limits broader conclusions but provides a foundation for future research in this domain. However, as a pilot study, it aimed to evaluate the potential effects of neuromodulation when applied bilaterally in patients with DoC. To enhance the evidence on the effectiveness of tDCS, future studies with larger sample sizes and longitudinal designs are recommended to assess the durability of observed effects and improve the generalizability of the findings. Additionally, the duration of the tDCS intervention may have been insufficient to observe long-term changes. Future research should also explore various stimulation configurations, including frequency, duration, and intensity, to identify the most effective protocols. Furthermore, investigating the underlying brain mechanisms and individual variability could provide a more detailed understanding of the benefits of tDCS and enable the personalization of intervention strategies. Further exploration of prolonged effects of the optimization of stimulation duration is warranted. Lastly, the variability in individual responses to tDCS has not been fully explored; future studies might investigate factors influencing treatment response and the long-term clinical and neurophysiological outcomes, comparing the effect of bilateral tDCS versus unilateral intervention in patients with diagnosis of MCS.

Conclusion

In conclusion, this pilot study sheds some lights on the promising effects of tDCS in improving neurophysiological and clinical measures in patients with MCS. The results suggest that tDCS may represent an innovative and potentially effective therapeutic strategy for enhancing awareness and functional abilities in this population. Integrating tDCS into rehabilitation protocols for MCS patients has the potential to serve as a valuable adjunct to traditional therapies, optimizing clinical outcomes and improving patient quality of life. Neurophysiological measures, including N200 and P300 latencies and beta band power, emerge as reliable and objective indicators for monitoring progress and tailoring interventions. Furthermore, the results of this study could inform the development of clinical guidelines for the application of tDCS, supporting the adoption of evidence-based and more effective rehabilitation practices for patients with MCS.

Data availability

The data that support the findings of this study are available on request from the corresponding author, F.I. The data are not publicly available due to restrictions, their containing information that could compromise the privacy of research participant.

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Author contributions
Conceptualization, A.G., F.I.; methodology, A.G., F.I., R.A.F.; software, R.A.F.; validation, all authors; formal analysis, A.G., R.A.F, F.I.; investigation, R.D.L., A.G., F.I; resources, A.Q., R.S.C.; data curation, A.G., R.A.F, R.D.L., F.I.; writing-original draft preparation, A.G., R.D.L.; writing-review and editing, F.I., R.S.C.; visualization, all authors; supervision, F.I., R.S.C.; project administration, A.G., R.S.C.; funding acquisition, A.Q.; A.G. and F.I. contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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