Journal of NeuroEngineering and Rehabilitation

RESEARCH

Open Access

Perturbational complexity index in assessing responsiveness to rTMS treatment in patients with disorders of consciousness: a cross-over randomized controlled trial study

Chengwei Xu[1,4†], Zhanxing Yuan[3†], Zerong Chen[1†], Ziqin Liao[1], Shuiyan Li[1], Yanqi Feng[1], Ziqiang Tang[1], Jichan Nian[1], Xiyan Huang[1], Haili Zhong[1] and Qiuyou Xie[1,2,3,4*]

Abstract

Background Disorders of Consciousness (DoC) caused by severe brain injuries represent a challenging clinical entity, which is easy to misdiagnosis and lacks effective treatment options. Repetitive Transcranial Magnetic Stimulation (rTMS) is a non-invasive neuroelectric stimulation method that shows promise in improving consciousness for DoC, especially in minimally conscious state (MCS). However, there is little evidence of its effectiveness, especially in RCT studies.

Methods Twenty MCS patients participated in a double-blind, randomized, crossover, sham-controlled clinical study to evaluate the safety and efficacy of rTMS for MCS. Subjects were randomized into two groups: one group received rTMS-active for 10 consecutive days ($n=10$), and the other group received rTMS-sham for 10 consecutive days ($n=10$). After a 10-day washout period, the two groups were crossed over and received the opposite treatment. the rTMS protocol consisted of 2,000 pulses per day in the left dorsolateral prefrontal cortex (L-DLPFC), sent at 10 Hz. The stimulation intensity was 90% of the resting motor threshold. Coma Recovery Scale Revised (CRS-R), the main evaluation index, was evaluated before and after each phase in a double-blind manner. Meanwhile RS-EEG and TMS-EEG data were acquired and relative alpha power (RAP), and perturbational complexity index based on state transitions (PCIst) were caculated.

Results One-way ANOVA revealed significantly higher scores in rTMS-active treatment compared to rTMS-sham across various measures, including CRS-R total score, RAP, PCIst (all $P<0.05$). Among the 20 MCS patients, 7 (35%) were identified as responders following rTMS treatment. Compared to rTMS-sham, responder scores for CRS-R, RAP, and PCIst (all $P<0.05$) were significantly elevated after rTMS-active treatment. Conversely, there was no significant difference observed in non-responders. Furthermore, post-hoc analysis revealed that baseline PCIst was significantly

†Chengwei Xu, Zhanxing Yuan and Zerong Chen contributed equally to this work.

*Correspondence:
Qiuyou Xie
Xqy7180@163.com

Full list of author information is available at the end of the article

higher in responders than non-responders. Upon a 6-month follow-up, CRS-R scores significantly increased in all 20 patients ($P=0.026$). However, the responder group exhibited a more favorable prognosis compared to the non-responder group ($P=0.031$).

Conclusions Applying 10 Hz rTMS to L-DLPFC significantly increased consciousness level in MCS patients. PCIst is a neurophysiological index that has the potential to evaluate and predict therapeutic efficacy.

Trial registration www.ClinicalTrials.gov, identifier: NCT05187000.

Keywords Repetitive transcranial magnetic stimulation, Disorders of consciousness, Minimally conscious state, EEG, TMS-EEG, Perturbational complexity index, Randomized control trial

Introduction

Disorders of consciousness (DoC) refers to a range of awareness and cognitive disorders secondary to organic brain diseases following severe brain injuries [1–3]. With the advancement of critical care medicine, more patients with severe brain injuries survive comas, leading to prolonged disorders of consciousness, which increases mental pain and economic burdens on them and their families. This is accompanied by numerous social, ethical, and legal issues [4] and poses a major challenge for clinical neuroscience [5, 6].

DoC comprises vegetative state [7, 8], also called unresponsive wakefulness syndrome (UWS) [9] and minimally conscious state (MCS) [10]. The former is featured by the absence of self-awareness expression, showing only reflexive responses to stimuli and lacking awareness of self or surroundings [8]. In the latter case, there are clear signs of non-reflexes, cortical, cognitively mediated behavior, along with conscious recognition of self or surroundings [11, 12]. MCS could act as a bridge, with its behavioral responses and the effectiveness of cortical connections appearing to lie between those of individuals in a completely unconscious VS/UWS state and healthy individuals with full consciousness [4]. Therefore, diagnostic and rehabilitation therapy studies of MCS patients allow for a natural model for exploring the conscious cortex and important evidence for DoC clinical decision-making.

Until now, treatments for DoC have been limited. Pharmacotherapy remains a crucial way for clinical treatment [13, 14]. Amantadine facilitates the metabolism of low-activity brain regions, accelerating consciousness recovery in patients with DoC [15, 16]. Zolpidem can influence the GABAergic system of limbic circuits in the brain, with studies revealing its ability to partially restore the normal metabolism of cortical cells after brain injury. It could improve the consistency and complexity of behavioral responses in patients with DoC [17, 18]. Besides, intrathecal injection of baclofen (ITB), acting on the spinal cord, can aid in motor neuron regeneration and help improve consciousness [19, 20]. However, the effectiveness of drug treatment is not satisfactory.

Notably, in recent years, repetitive transcranial magnetic stimulation (rTMS), a non-invasive brain stimulation (NIBS) technique, has much to offer in modulating cortical excitability and enhancing neuroplasticity. Multiple applications can also lead to long-term potentiation (LTP) effects beyond the stimulation period [21], holding great popularity in the department of neurology and rehabilitation. The left dorsal lateral prefrontal cortex (L-DLPFC) represents a key component in the frontoparietal network which includes the executive control network (ECN) [22], involved in many advanced behavioral and cognitive processes [23, 24]. Currently, it emerges as one of the most promising targets of rTMS in restoring consciousness in DoC patients.

Several studies have targeted L-DLPFC to improve the level of consciousness in DoC patients, and to evaluate the efficacy of rTMS in terms of behavioral, neurophysiological, neuroimaging, and even hormonal level changes [25–31]. He et al. [30] used resting-state EEG as an evaluation and prediction indicator after transient 20 Hz rTMS treatment, showing that 10 patients with improved consciousness. They classified these patients as responders, which were characterized by more preserved alpha power and significant reduction in rTMS-induced delta power. Chen et al. [31] found in a RCT with 50 patients that 6 weeks of 10 Hz rTMS on L-DLPFC enhanced the level of consciousness in DoC patients. It implies that the elevation of neural connectivity levels may lay a foundation for successful HF-rTMS treatment for DOC patients.

TMS (single-pulse stimulation) combined with high-density EEG (TMS-EEG) can record the effects of TMS perturbation on the brain, which has become an effective way to measure brain activity [32]. Moreover, the PCI/PCIst derived from TMS-EEG can describe the spatio-temporal complexity of TMS-evoked potentials to quantify cortical effective connectivity [33], serving as an objective metric for assessing the efficacy of drugs or brain stimulation [34]. This provides a relatively convenient, objective, and applicable assessment method for DoC patients [35], bringing new hope for clinical diagnosis and treatment of DoC [36, 37].

As such, TMS/rTMS could be a promising approach for the DoC assessment and treatment. However, there remains no clear evidence from any treatment guidelines supporting the use of TMS/rTMS [3, 36]. This mainly results from limited sample sizes and pilot studies of published. Besides, some studies are insufficient in strong objective evaluation metrics to support their findings [13, 38].

To this end, we designed a crossover randomized double-blind controlled trial, which employed L-DLPFC as the target of rTMS, along with the help of MCS, a consciousness "bridge", and EEG, offering a quantification of the complexity of brain perturbations, investigating the application of TMS in disease recovery for DoC patients.

Materials and methods

Participants

Between November 2021 to September 2023, a total of 20 MCS patients (10 males and 10 females) were recruited into this study from the Department of Rehabilitation Medicine of Zhujiang Hospital of Southern Medical University.

All enrolled patients met the following specific criteria. Inclusion criteria: (1) Those aged 18–70 years old, with acquired brain injury of less than 1 year and greater than 28 days; (2) Those without previous neuropsychiatric-related diseases; (3) Those without ongoing use of sedative medications and medications potentially interfering with brain stimulation, such as Na^{+}/Ca^{2+} channel blockers or N-Methyl-D-Aspartate receptor antagonists; (4) Those with stable vital signs; (5) Those with family members voluntarily participating in this study and providing Informed Consent Form (ICF); (6) Those with structural integrity of the L-DLPFC and M1 areas, as confirmed by CT/MRI. Exclusion criteria: (1) Those participating in other IBS or NIBS trials; (2) Those with uncontrolled seizures, i.e., seizures within 4 weeks before enrollment; (3) Those with contraindications of rTMS or TMS-EEG, such as skull metal implantation, brain pacemaker, craniotomy at stimulating sites, etc. All enrolled participants or their families willingly provided their signatures on the ICF.

Study design

This study involved a cross-over randomized double-blind sham-controlled clinical trial. Participant were divided into two groups in a 1:1 ratio, with each group receiving a total of 10 intervention sessions using 10 Hz rTMS, actively targeting the left dorsolateral prefrontal cortex (L-DLPFC), and 10 sessions of rTMS-sham. A ten-day washout period was implemented between the active and sham treatments (Fig. 1A). The total score

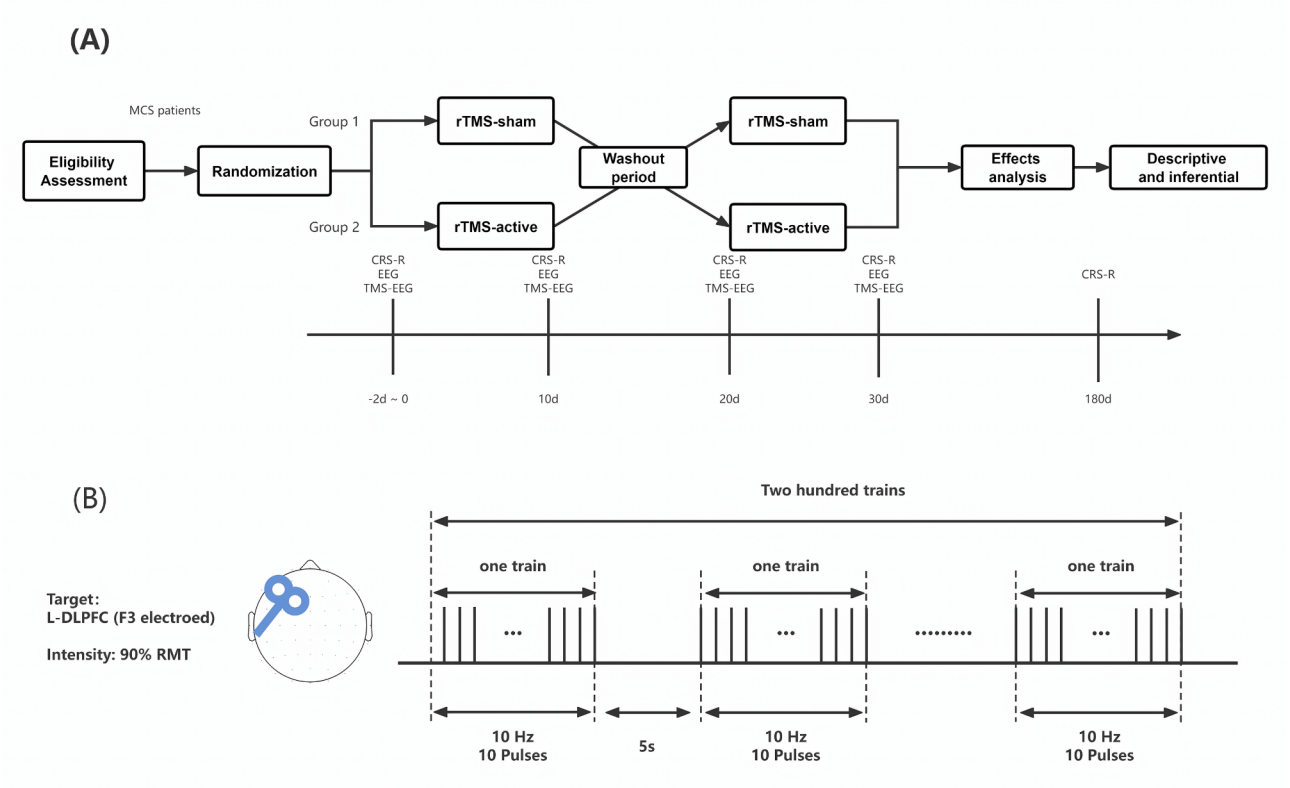

Fig. 1 Study protocol. (**A**) The crossover, randomized, double-blind, sham-controlled study protocol; (**B**) Details of rTMS parameters. CRS-R, Coma Recovery Scale-Revised; EEG, Electroencephalogram; TMS-EEG, TMS combined EEG; L-DLPFC, left dorsolateral prefrontal cortex; RMT, resting motor threshold

on the JFK Coma Recovery Scale Revised (CRS-R) [39] after the two-phase treatment was utilized as the primary outcome metric. Also, resting-state EEG (RS-EEG) and TMS-EEG data were recorded synchronously to calculate secondary outcome metrics, from relative alpha power (RAP) to perturbational complexity index based on state transitions (PCIst). The study was approved by the Ethics Committee of Zhujiang Hospital of Southern Medical University (2021-KY-092-01) and has been registered on ClinicalTrials.gov (NCT05187000). It complied with the requirements highlighted in the Declaration of Helsinki.

Randomization, blinding and allocation

Randomization was performed by a blinded staff member of the Data Monitoring Committee (DMC) at Zhujiang Hospital. He managed the electronic coding and randomized patients into two groups in a 1:1 ratio using the function of the statistical software SPSS 23.0 (IBM, USA). Blind codes were enclosed in a sealed, opaque envelope. rTMS coils labeled A or B, were wrapped in opaque white plastic paper. The physical therapists were informed by the DMC staff whether side A or B would be used first.

Qualified physical therapists from Zhujiang Hospital conducted the rTMS treatment. During the entire trial, they administered rTMS-active or rTMS-sham to patients independently and were not involved in any evaluations, while remaining unaware of them. Two attending physicians completed the patient's primary/secondary evaluation indexes. Throughout the entire treatment process, neither doctors, therapists nor patients were informed whether the intervention was rTMS-active or rTMS-sham.

rTMS protocol

The rTMS protocol involved 10 days of active and sham stimulation per patient, with a washout period of no less than 10 days. The stimulation intensity was determined by the resting motor threshold (RMT), i.e., a minimum of 5 out of 10 TMS single pulses in the M1 area evoked myoelectricity with an amplitude of 50μ V in the first dorsal interosseous muscle. The RMT was measured before the first rTMS session of each stage. During treatment, the patient was placed in a semi-recumbent position, and the international 10–20 electrode distribution system was utilized to position the figure-of-eight coil surface at a 45-degree tangential angle to the patient's L-DLPFC (at the F3 electrode) [31, 40]. After initially confirming the target, we used a marker to make an offline mark on the patient's scalp, which was used as the stimulation target in subsequent sessions. Stimulation parameters included a frequency of 10 Hz, intensity at 90% of the RMT, 1-second train duration, and 5-second inter-train interval, with 20 min per session. Treatment occurred once a day for a course of 10 sessions. Effective stimulation consisted of 200 effective stimulation series and 2,000 pulses at 90% of RMT (Fig. 1B). rTMS treatment was performed in full accordance with safety guidelines [41].

In this study, rTMS intervention was implemented using the NTK-TMS-II300 transcranial magnetic stimulator from Jiangxi Brain Modulation Technology Development Co. Ltd. in China. The device can generate a magnetic induction intensity ranging from 1.5 T to 8 T, with a pulse width of 0.32ms±10%. The coil employed was a Model IIB502 97-mm figure-of-eight coil. There are two identical surfaces in this coil, i.e., surface A outputs rTMS-active, and surface B outputs rTMS-sham.The former exhibits a high-frequency pulsed magnetic field generated by rTMS acting on the cerebral cortex, whereas the latter doesn't. It only presents noise and vibrations, similar to those produced during pulse transmission.

Behavioral assessment

CRS-R currently proves the most sensitive scale in all behavioral tests which keep the main clinical assessments to evaluate the level of consciousness in patients with DoC and remains widely used to distinguish from EMCS, MCS+, MCS- or VS/UWS. It comprises 6 subscales addressing auditory, visual, motor, oromotor/verbal, communication, and arousal processes, in which the score from the lowest to the highest represents the reflexive activity to cognitively mediated behaviors on each subscale [39]. In this study, CRS-R was given by two experienced physician and physical therapist within 48 h before enrollment, 24 h after the first round of rTMS, 24 h after the end of the washout period, and 24 h after the completion of the second round of rTMS interventions. These assessments aimed to evaluate the score increase/decrease and the diagnostic changes, respectively. The CRS-R assessment was executed when the patients were in their best condition throughout the day. Patients were classified as responders or non-responders based on whether or not they progressed into the next stage (e.g., MCS- to MCS+or EMCS, MSC+to EMCS). At 6 months post-treatment, follow-up outcomes were obtained through a structured telephone follow-up (with video calls used if necessary) based on CRS-R.

EEG data acquisition and analysis

RS-EEG was performed using a TMS-compatible 64-channel event correlation system from Neuroscan Australia (Compumedics Neuroscan, Neuroscan 8050), in conjunction with a SynAmps2 EEG signal amplifier. And a TMS-compatible 64-conducting polar cap was placed in accordance with the International 10–20 system (Neuroscan, Quik-Cap) for data acquisition. Conductive paste was applied before the test, and the impedance of each electrode was maintained below 5 kΩ. A bandpass filter was set from DC to 1000 Hz, and the

online sampling rate was set at 2500 Hz. During the data acquisition, if sleep features (such as sleep spindle wave or K-complex wave in the EEG) appeared, data acquisition was immediately suspended and the patient was awakened using the standard CRS-R normative procedure [39].

Preprocessing of resting-state EEG data was conducted using EEGLAB 14.0.2.5b in the Matlab environment (version: 2016a, MathWorks Inc, Natick, USA). The raw EEG data were first performed band-pass filter within 1 to 45 Hz and downsampled to 500 Hz. Afterwards, the EEG was divided into 5s segments and removed segments with obvious artifacts. Bad channels were removed with a limit of 10% of the total channels to ensure data quality. Then, the independent component analysis (ICA) was used to remove non-EEG components, such as blinks and muscle activity. Finally, the channels that were removed in previous steps were interpolated, and an average reference was then performed. Data were considered invalid if less than 80% of the final data segments were retained.

To calculate the relative alpha power (RAP), the cleaned EEG data segments were further analyzed using power spectral density (PSD) estimation. The Welch's method was applied, with a Hamming window of 5s length and 50% overlap. The PSD was computed for each 5s segment and averaged across segments. The power within the 8–14 Hz frequency band was extracted for each channel and normalized to the total power within the 1–45 Hz band to obtain the RAP.

RAP (8–14 Hz) was then computed for group-level analysis. RAP is considered the key frequency band for distinguishing between MCS and VS [42, 43], whereas patients with DoC show lower RAP compared to normal subjects [43]. For a given channel, the RAP is calculated as follows:

$$RAP = \frac{P(8,14)}{P(1,45)} \times 100\% \quad (1)$$

where P(8,14) and P(1,45) denote the EEG band energies in the range of 8–14 Hz and 1–45 Hz, respectively.

TMS-EEG data acquisition and analysis

TMS-EEG data acquisition was carried out using a Magstim BiStim^2 stimulator with a figure-of-eight coil. The intensity of the TMS pulses was tailored to the needs of the test and was set concerning RMT of the individual. The intensity of TMS-EEG evaluation was set at 100% of the RMT of the patient, and the bandpass filter was set from DC to 1000 Hz, with an online sampling rate of 2500 Hz. Patients were instructed to wear headphones that produced the same spectral mixture of noise as generated during TMS stimulation. This measure aimed to counteract the auditory response to TMS stimulation. Thin sponges were positioned at the contact area between the stimulation coil and the scalp to minimize the effect of vibration of the TMS coil. The trigger interval of the TMS pulses was randomly varied between 2 and 2.4 s. Before each recording session, the signal was checked in advance. To obtain accurate evoked potentials, the left M1 area was designated as the stimulation point, and a total of 200 pulses were triggered. During TMS-EEG data collection process, we used electromyography (EMG) to observe the activity of the first dorsal interosseous muscle, which ensured the accurate stimulation.

TMS-EEG data preprocessing was completed using the TMS-EEG signal analyser (TESA) toolkit under EEGLAB 14.0.2.5b in Matlab environment (version: 2016a, MathWorks Inc, Natick, USA) [44]. Firstly, the pulses were detected and marked by TMS pulse features, and the TMS-EEG recordings were divided into segments 500 ms before and after each TMS pulse. The channels exhibited prominent noise for most of the time were removed, with a limit of 10% of the total channels to ensure data quality. Then, the data from −2 ms to 10 ms were removed to avoid large-value magnetic field noise and the missing data were interpolated using a cubic function. Subsequently, the data were downsampled to 1000 Hz. The segments with obvious artifacts were removed, and all patients data were retained at least 80% of original segments. The bandpass filter (1–100 Hz) and notch filter (48–52 Hz) were performed. After that, fast ICA was performed and components with TMS-induced artifacts were removed. Any remaining peak artifacts were replaced using cubic interpolation. Then, fast ICA was performed again and the components with blink, eye movement, muscle movement and other non-EEG artifacts were removed. Finally, the channels that were removed in previous steps were interpolated, and TMS-EEG data were re-referenced to a common average.

The TMS-evoked perturbation complexity index (PCI) of the patients was subsequently computed to assess the patient's level of consciousness. PCI is an index describing the spatiotemporal complexity of the TMS-evoked potential [45], and is highly specific for identifying the patient's level of conscious activity. In this study, a derivative version of PCI, known as PCIst, was chosen, which estimated the complexity of TMS perturbations by signal decomposition and recurrence quantification analysis (RQA) [46]. Wang et al. [47] conducted a TMS-EEG collection of 30 healthy participants and 181 patients with DoC and computed the value of PCIst, suggesting a significant and positive correlation between PCIst and the level of consciousness. The PCIst is calculated through the following process:

Initially, N low-dimensional components are obtained through singular value decomposition. For the n-th component (n=1, 2, …, N), distance matrices of data points

are computed separately for the response and baseline periods. These matrices are binarized based on a threshold value ε, resulting in the response transition matrix and baseline transition matrix for the nth component respectively. The complexity of the n-th component is defined as the maximum weighted difference between the average number of state transitions (ANST) during the response period and the baseline period (denoted respectively as $ANST_n^{\text{res}}$ and $ANST_n^{\text{base}}$). This complexity is represented as $\Delta ANST_n$, and the calculation formula of $\Delta ANST_n$ is:

$$\Delta ANST_n = T_R \left[ANST_n^{res}(\varepsilon_n^*) - k \times ANST_n^{base}(\varepsilon_n^*) \right] \quad (2)$$

where T_R represents the number of samples during the response period, and $\varepsilon_n{}^*$ is the threshold value that maximizes the value of $\Delta ANST_n$.

Finally, the PCIst is defined as the sum of the complexities ($\Delta ANST_n$) of each signal component:

$$PCIst = \sum_{n=1}^{N} \Delta ANST_n \quad (3)$$

In this study, the baseline period for calculating PCIst was set from −250 ms to -50 ms, the response period was set from 0 to 300ms, the singular value decomposition process selected principal components with the sum of the variance share greater than 99% and the signal-to-noise ratio (SNR) greater than 1.2, and k is set to 1.2. Moreover, the PCIst was calculated using EEG data from all channels to capture a comprehensive measure of cortical complexity.

Basic treatment and routine rehabilitation

All patients received routine medication, examination, nursing care and rehabilitation programs for DoC at the Department of Rehabilitation Medicine. The programs primarily included passive limb range-of-motion training, electrical limb stimulation, barometric therapy, respiratory therapy, swallowing therapy, gastrointestinal rehabilitation and hyperbaric oxygen therapy.

Statistical analysis

The data were analyzed using SPSS 23.0 statistical software. Two independent-sample t-test and chi-square test were utilized for the baseline characteristic analysis between the two groups. Considering the crossover design, we initially assessed the significance of the stage effect between the two sequences to evaluate the carryover effect (i.e., the effect of the first treatment affecting the second treatment period) before each evaluation index. If the carryover effect was not significant at the 10% level to rule out a carryover effect of different phases, further analysis was performed. The univariate general linear model ANOVA (Analysis of Variance) was used to compare the main effects between rTMS type (active and sham stimuli), phase, and patient, and to calculate the CRS-R total score, RAP and the PCIst, respectively. Finally, we evaluated the prognosis by assessing CRS-R scores for all patients at 6 months post-treatment. All statistical assumptions were tested using two-sided tests, and the statistical significance test level was set at $P<0.05$, with parameter confidence intervals set at 95%.

Results

Initially, a total of 25 hospitalized patients were screened. Yet, 3 patients failed TMS-EEG acquisition due to excessive head movement during detection, and 2 patients' family members did not agree to sign the ICF. Thus, 20 MCS patients successively completed all treatments and were included in the final analysis (Fig. 2). Among them, there were 6 with Traumatic Brain Injury (TBI), 10 with non-Traumatic Brain Injury (nTBI), and 4 with Hypoxic-ischemic Encephalopathy (HIE) with an average age of 50±14.24 years. There were no significant differences in age (t = -1.382, p=0.184), time since injury (t=1.052, p=0.307), and baseline CRS-R score (t = -0.408, p=0.688) between the two sequential groups (rTMS-active - rTMS-sham vs. rTMS-sham - rTMS-active). No study-related adverse events occurred.

A two-stage baseline CRS-R showed that the stage effect was not statistically significant between the two sequential groups (t = -1.207, p=0.235), indicating no carryover effects. Furthermore, the univariate general linear model ANOVA suggested that total CRS-R scores were significantly higher in MCS patients after rTMS-active than rTMS-sham (F=4.615, p=0.046) (Fig. 3A). Next, we calculated RAP and PCIst after active and sham rTMS stimulation in all patients. ANOVA suggested that rTMS-active showed significantly higher RAP (F=6.154, p=0.023) (Figs. 3B and 4A), and higher PCIst at the group level, compared to rTMS-sham (F=4.961, p=0.039) (Fig. 3C). Demographic and clinical characteristics results are detailed in Table 1.

Based on the change in CRS-R scores after rTMS-active, a total of 7 patients were considered responders (35%), and they gained at least one indication of the next stage of consciousness after rTMS-active. Responders exhibited improvement primarily in the visual (8), auditory (3), and motor (7), followed by the oral-motor (2) and communication (2). These responders significantly improved CRS-R scores at the group level with rTMS-active compared to rTMS-sham (F=7.141, p=0.044). In terms of neuroelectrophysiology, responders showed a significant increase in RAP (F=5.202, p=0.048) and PCIst (F=6.890, p=0.047) (Fig. 5) compared to rTMS-sham after rTMS-active treatment. In contrast, no

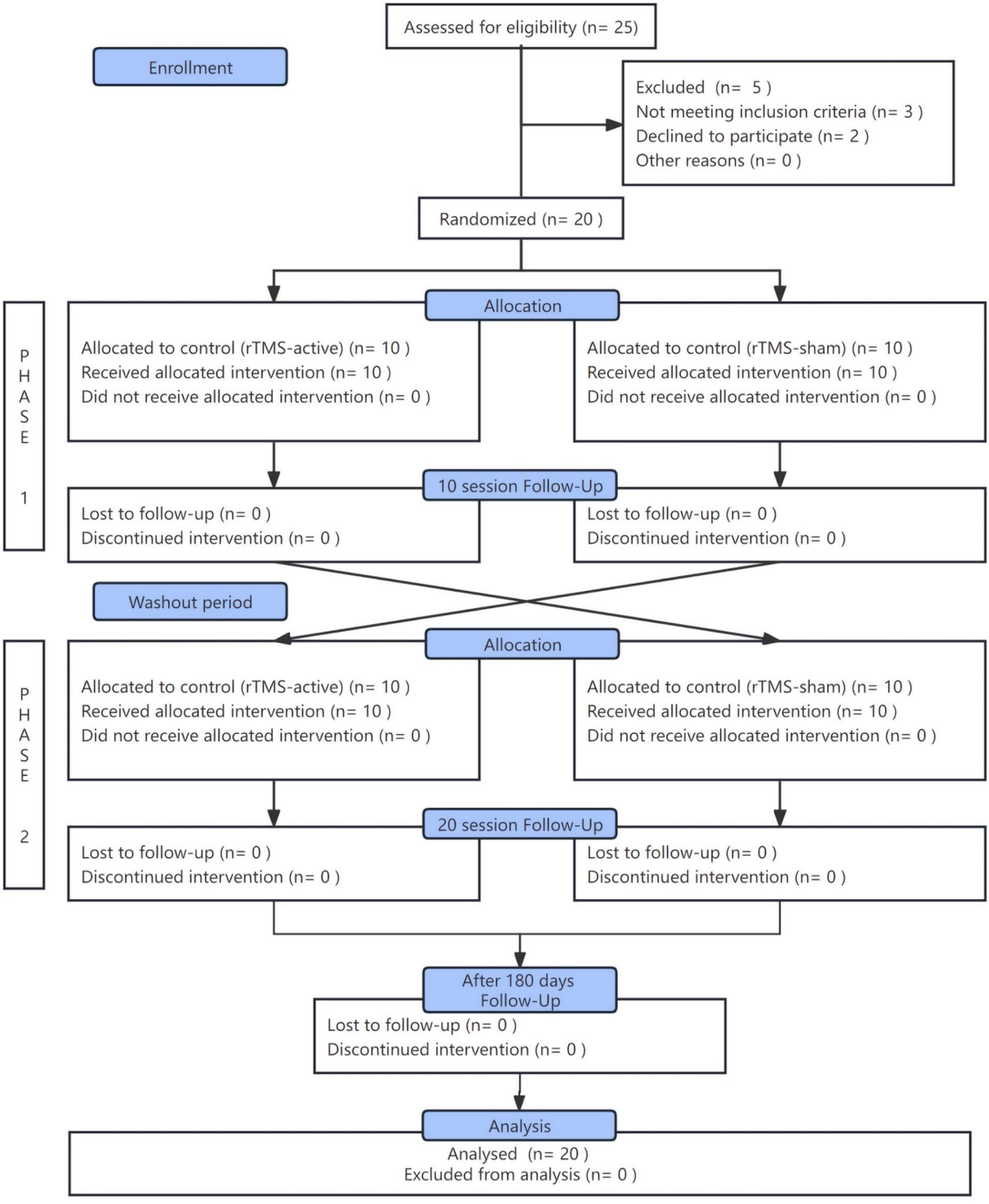

Fig. 2 Flow diagram of the trial. Randomization, trial-group assignment, and follow-up in the trial

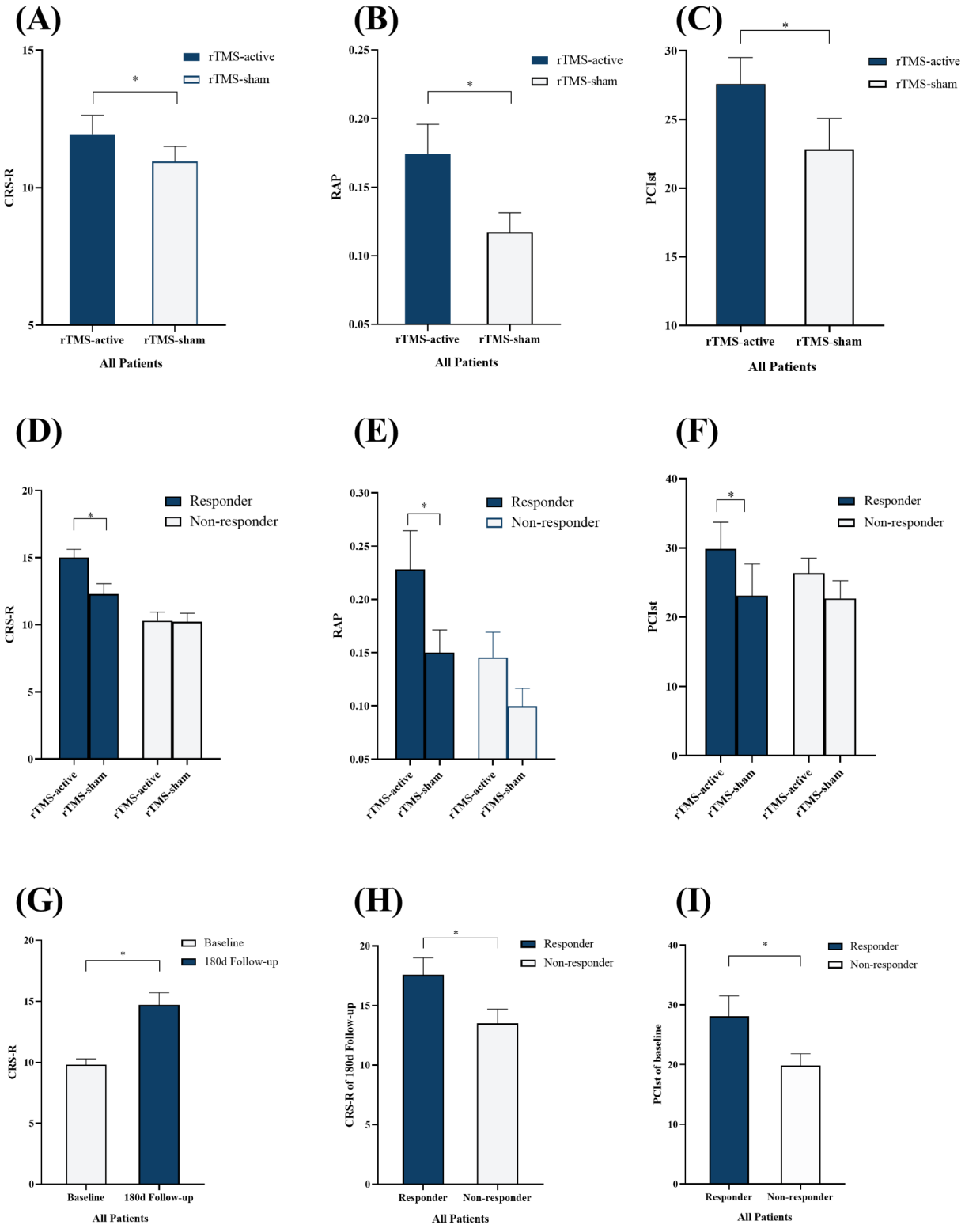

Fig. 3 (**A**-**C**) Comparisons the difference between real and fake stimulation on CRS-R, RAP and PCIst for all patients respectively; (**D**-**F**) Comparisons the difference between real and fake stimulation on CRS-R, RAP and PCIst for Responder and non-responder respectively; (**G**) Comparisons the difference between all patients when the baseline and 180 days follow-up; (**H**) Comparisons the difference in CRS-R between Responder and non-responder at 180-day follow-up; (**I**) Post-hoc analysis compares the difference in PCIst between Responder and non-responder at baseline

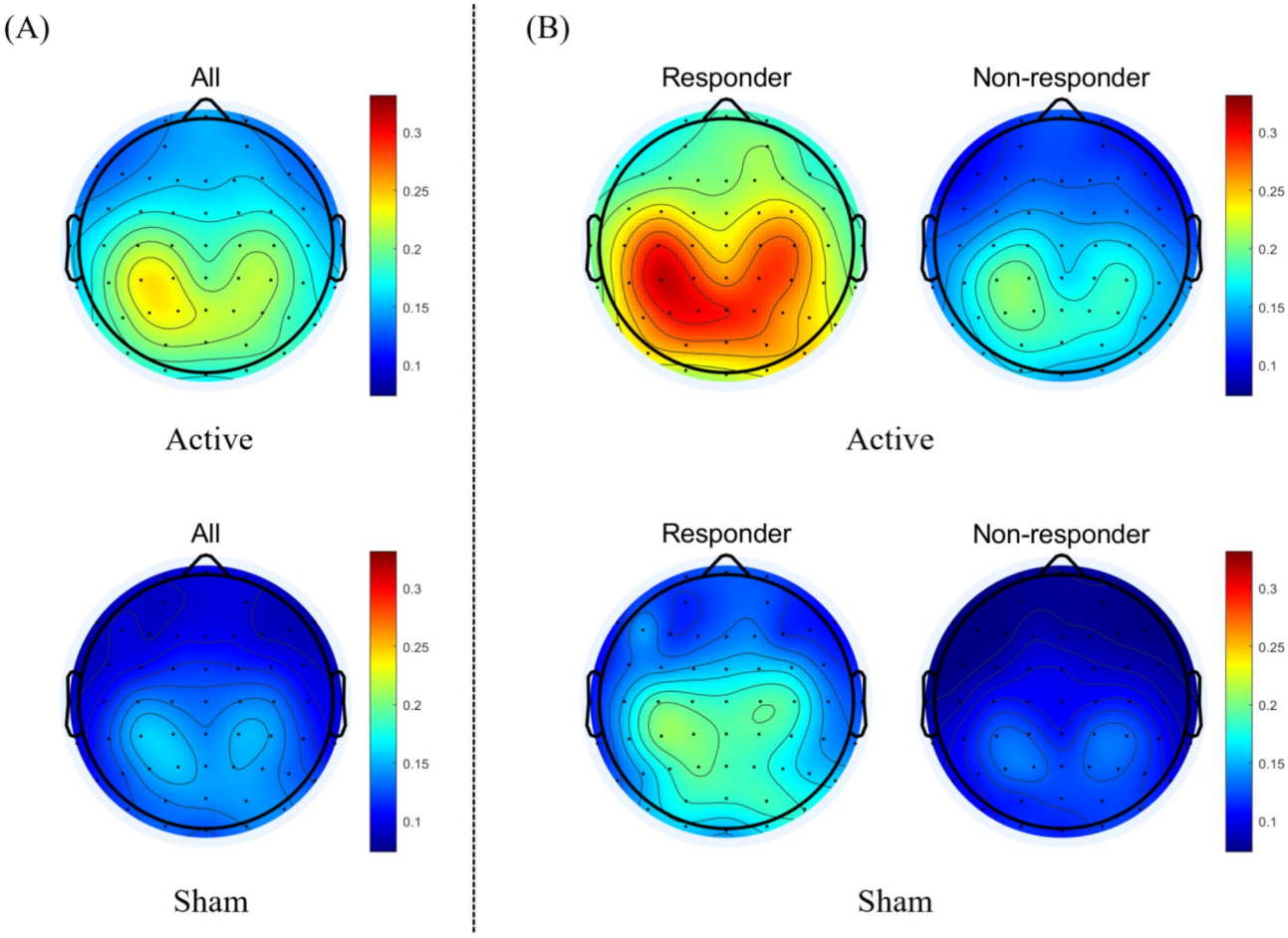

Fig. 4 RAP whole-brain topographic map differences. (**A**) Differences in RAP whole-brain topography in all patients after active/sham stimulation; (**B**) Differences in RAP whole-brain topography between responders and non-responders after real/sham stimulation

significant difference was observed in non-responders between rTMS-active and rTMS-sham (Fig. 3D-F). Besides, we did not observe evidence of any significant differences between Responders and non-responders in age ($P>0.05$), gender ($P>0.05$), time to injury ($P>0.05$), and the carryover effects of each baseline score or EEG. Notably, our subsequent post-hoc analysis showed that PCIst at baseline was significantly higher in the responder group than in the non-responder group ($F=0.066$, $p=0.044$), whereas the other outcome metrics (CRS-R and RAP) did not show any significance (Fig. 3G-I).

In the ongoing prognostic analysis, all 20 patients were successfully followed up. An independent-samples t-test showed a significant increase in CRS-R score at 6 months (t = -2.310, $p=0.026$) for these patients. However, compared to the non-responder group, the responder group revealed a better prognosis ($t=2.342$, $p=0.031$).

Discussion

We investigated the effect of 10 Hz rTMS acting on L-DLPFC in combination with conventional rehabilitation for 10 consecutive days on the level of consciousness and brain function in patients with MCS in a double-blind randomized controlled trial. We first ruled out the potential carryover effect associated with a crossover design trial. Thus, the present study performed behavioral and neuroelectrophysiology analyses without stage differences in DoC patients. To the best of our knowledge, we are the first one to suggest that rTMS-active targeting the L-DLPFC significantly enhances the level of awareness and cortical complexity in MCS patients compared to rTMS-sham, both behaviorally and neurophysiologically, and contributing to a better prognosis at the next 6-month follow-up. Besides, the present study produced 7 responders out of 20 MCS patients. Compared to rTMS-sham, they showed progress in at least one subscale score of CRS-R scores after rTMS-active, and a significant increase in RAP and PCIst, implying a new height level of awareness and cortical response. In

Table 1 Demographic and clinical information of pariticipants

ID	Age(sex)	Etiology	Post-injury (months)	Treatment Allocation	CRS-*R* presham	CRS-*R* postsham	Δ rTMS-s	CRS-*R* preactive	CRS-*R* postactive	Δ rTMS-a	rTMS responder
1	19(M)	HIE	7	active/sham	7(1-0-5-1-0-0)	7(1-0-5-1-0-0)	0	7(1-0-5-1-0-0)	7(1-0-5-1-0-0)	0	Non-responder
2	50(F)	Hemorrhage	2.5	active/sham	8(0-3-2-1-0-2)	9(1-3-2-1-0-2)	1	7(1-0-2-2-0-2)	7(1-0-2-2-0-2)	0	Non-responder
3	34(M)	Hemorrhage	3	active/sham	10(2-3-2-1-0-2)	10(2-3-2-1-0-2)	0	8(1-3-2-1-0-1)	10(2-3-2-1-0-2)	2	Non-responder
4	53(M)	Hemorrhage	1	sham/active	7(1-1-3-1-0-1)	8(1-1-3-1-0-2)	1	7(1-1-3-1-0-1)	9(1-3-3-1-0-1)	2	Responder
5	68(F)	Hemorrhage	8	active/sham	15(2-3-5-2-0-2)	15(2-3-5-2-0-2)	0	13(2-3-5-1-0-2)	17(2-4-6-2-1-2)	4	Responder
6	59(F)	TBI	4.2	sham/active	13(2-3-5-1-0-2)	13(2-3-5-1-0-2)	0	13(2-3-5-1-0-2)	16(3-3-6-2-0-2)	3	Responder
7	49(M)	TBI	3.3	sham/active	9(1-3-2-1-0-2)	9(1-3-2-1-0-2)	0	9(1-3-2-1-0-2)	10(1-3-3-1-0-2)	0	Responder
8	66(F)	Hemorrhage	3.1	active/sham	14(1-3-6-2-0-2)	14(1-3-6-2-0-2)	0	10(1-1-5-1-0-2)	14(1-3-6-2-0-2)	4	Responder
9	59(F)	Hemorrhage	4.7	sham/active	10(2-3-2-1-0-2)	13(2-3-2-3-1-2)	3	13(2-3-2-3-1-2)	14(3-3-2-3-1-2)	1	Responder
10	52(M)	TBI	2.1	active/sham	10(1-3-2-2-0-2)	10(1-3-2-2-0-2)	0	8(1-3-2-1-0-1)	10(1-3-2-2-0-2)	2	Non-responder
11	49(M)	Hemorrhage	4.6	sham/active	13(2-3-5-1-0-2)	13(2-3-2-3-1-2)	0	13(2-3-2-3-1-2)	14(2-3-5-2-0-2)	2	Non-responder
12	62(M)	Hemorrhage	1.3	sham/active	9(0-1-5-1-0-2)	10(1-1-5-1-0-2)	1	9(0-1-5-1-0-2)	9(0-1-5-1-0-2)	0	Non-responder
13	68(M)	HIE	2	active/sham	13(2-3-5-1-0-2)	13(2-3-5-1-0-2)	0	11(2-1-5-1-0-2)	13(2-3-5-1-0-2)	0	Responder
14	48(M)	Hemorrhage	1.4	sham/active	11(0-3-5-1-0-2)	11(0-3-5-1-0-2)	0	11(0-3-5-1-0-2)	13(0-4-6-1-0-2)	2	Responder
15	18(M)	HIE	7.9	active/sham	10(1-3-2-2-0-2)	10(1-3-2-2-0-2)	0	8(1-1-2-2-0-2)	10(1-3-2-2-0-2)	2	Responder
16	52(M)	TBI	1.5	sham/active	8(0-3-2-1-0-2)	8(0-3-2-1-0-2)	0	8(0-3-2-1-0-2)	9(1-3-2-1-0-2)	1	Non-responder
17	49(M)	HIE	2.2	active/sham	15(3-3-5-2-0-2)	15(3-3-5-2-0-2)	0	14(3-3-5-2-0-1)	15(3-3-5-2-0-2)	1	Non-responder
18	59(F)	Hemorrhage	6	sham/active	11(2-3-3-1-0-2)	11(2-3-3-1-0-2)	0	11(2-3-3-1-0-2)	14(3-4-5-1-0-2)	3	Responder
19	33(F)	TBI	5.2	active/sham	8(1-1-3-2-0-1)	9(1-1-3-2-0-2)	1	9(1-1-3-2-0-2)	17(3-4-5-2-1-2)	8	Responder
20	53(F)	TBI	4.9	sham/active	9(1-3-2-1-0-2)	11(1-3-3-2-0-2)	2	9(1-3-2-1-0-2)	9(1-3-2-1-0-2)	0	Non-responder

CRS-R scores are described as follows: Total score (Auditory subscore–Visual subscore–Motor subscore–Oromotor/Verbal subscore–Communication subscore–Arousal subscore); F=Female; M=Male; HIE=hypoxic-ischemic encephalopathy; TBI=Traumatic Brain Injury; CRS-R=Coma Recovery Scale-Revised; rTMS-a=rTMS-active=rTMS-s, rTMS-sham. Δ=post–pre. In the last column, Responder=patients showing new signs of consciousness after rTMS; Non-responder=patients not showing any new sign of consciousness taking into account the CRS-R assessments (pre and post rTMS-active and rTMS-sham) conducted during the study period.

contrast, the other non-responders showed no significant improvements, which may be associated to whether the cerebral cortex of MCS patients can respond to rTMS (see the discussion below) [48].

rTMS represents a prominent NIBS method, generating pulsed magnetic fields that can penetrate extracerebral tissues (scalp, bone, meninges) at specific frequencies and sequences within the target area. It can act directly on central nerve system tissues, resulting in superficial axonal depolarization of the electric field and activating networks in the cortex [49]. This study utilized rTMS to directly stimulate key nodes of the ECN and employs EEG/TMS-EEG to investigate changes in the overall cortical complexity [50]. The present study focused on rTMS's direct stimulation of hub ECN, which involves EEG/TMS-EEG to explore the alterations in overall cortical complexity [51]. Although rTMS's direct impact on the excitability of the cortex on the surface of brain networks, the significant perspective of treatment

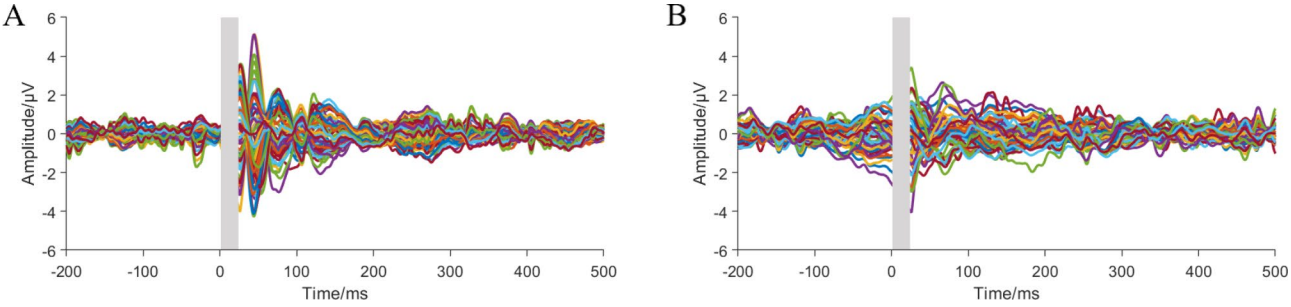

Fig. 5 TMS-evoked activity by butterfly plots of responder (**A**) and non-responder (**B**) groups

proves the plasticity-causing mechanism triggered by multiple interventions [52].

At present, the physiopathologic mechanisms underlying DoC remain poorly understood [53]. Previously, the foundation for directing rTMS in the treatment of DoC patients was mostly derived from stroke-related theories, such as the interhemispheric competition model, vicariation model, and bimodal-balance recovery model [54]. However, these theories focus on functional reorganization after focal brain damage, whereas DoC patients mostly exhibit arousal/awareness dysfunction due to severe brain damage [55]. DoC patients serve as natural models for exploring the concept of consciousness. By studying their treatment responses and cortical activity, we can gain insights into the nature, mechanisms, and localization of consciousness. With the continuous exploration in consciousness neuroscience, theories represented by global workspace theory (GWT) and mesocircuit model have emerged as a research topic.

From the cortico-cortical and thalamo-cortical perspectives, it is believed that the hotspot of consciousness is located in the front of the brain, and the consciousness generated by the "ignition" works as a central hub in the workspace [14, 56]. Building on above theories, current studies are inclined to rTMS therapeutic targets towards the L-DLPFC, the hub of the ECN and the mesocircuit model. Additionally, the L-DLPFC is located on the surface of the cerebral cortex, allowing rTMS to directly activate orienting neurons on the horizontal plane of the cortex [48]. In 2014, Naro et al. [25] first found that one single application of rTMS facilitate transient enhancement of consciousness and restoration of connectivity in some cortical areas in 10 patients with UWS due to hypoxia. Also, He et al. [30] included 25 DoC patients, employing immediate resting-state EEG after a single 20 Hz rTMS stimulation as an evaluation and predictor. Their study revealed an consciousness improvement in 10 patients classified as responders, characterized by a more preserved alpha power and a significant reduction in delta power after rTMS treatment.

To more thoroughly assess the effects of rTMS treatment beyond merely the behavioral level, we employed TMS-EEG, an effective tool for measuring cortical activity. Currently, TMS-EEG receives extensive concentration in central nervous system disorders researches [32, 57]. This technique allows researchers to observe and analyze the functional integration (i.e., connectivity) and information exchanges between different brain regions. Thus, researchers can more deeply explore the functional organization and information processing characteristic of the brain across different states of consciousness [58] Some studies have demonstrated the effectiveness of TMS-EEG applications in assessing consciousness levels and evaluating treatment effects in DoC patients [26, 59, 60]. To date, many methods have been developed to quantitatively analyze the complex network properties of TMS-EEG, among which the PCI and its derivative version PCIst are some of the most widely used. As they demonstrated remarkable sensitivity in distinguishing different levels of consciousness [61, 62]. The critical property of PCI is the ability to effectively quantify the integration and differentiation of brain networks [45]. This ability to reveal the dynamic interactions of neural networks is the basis for effectively distinguishing different levels of consciousness [63].

In this study, the level of consciousness (or cortical responsiveness to TMS) characterized by PCIst reflects the fundamental level of brain function. The present study showed that the overall PCIst level in patients was significantly improved after rTMS-active compared to rTMS-sham, and the corresponding CRS-R also indicated a significant improvement in the level of consciousness. This suggests that PCIst not only can quantify the level of cortical response in DoC patients, but also can serve as a powerful indicator for evaluating improvements in brain function after treatment.

Furthermore, the relationship between the level of brain function, i.e. the level of cortical responsiveness, and the responsiveness of actual treatment is also of great concern in this study. Bodart et al. [64] assessed the level of consciousness in patients using PCI and Fluorodeoxyglucose Positron Emission Tomography (FDG-PET), demonstrating that patients with VS/UWS exhibiting higher complexity and metabolism may have

a better prognosis, even though they did not show any overt consciousness. Wu et al. [65] showed that the level of consciousness, characterized by functional connectivity strength, predicted treatment prognosis in patients with brain injury. These studies indicate the importance of considering individual differences in cortical response when evaluating efficacy. Our study highlights that patients with higher cortical complexity in MCS patients may have a more positive response to treatment.

In our study, PCIst was found significantly higher after rTMS-active than rTMS-sham in the responder group, while no significant difference found in the non-responder group. This indicated that PCIst, as a quantitative index of cortical response, may be beneficial in predicting the response of DoC patients to rTMS treatment. Specifically, the significant increase of PCIst in the responder group unveiled that the cortex of these patients demonstrated a higher level of complexity and integration after rTMS treatment, which is in line with an increase in their CRS-R scores. In contrast, no significant alterations in PCIst were found in the non-responder group, indicating that these patients showed limited cortical response to rTMS treatment and limited improvement in the level of consciousness.

Afterwards, this study conducted a post-hoc analysis to explore the difference between responders and non-responders at baseline, hoping to further explore the difference to rTMS treatment. Our findings suggest that PCIst values were significantly higher in the responder group than in the non-responder group ($p=0.044$). Our results imply that PCIst not only distinguishes DoC patients with different levels of consciousness [47], but also has a positive predictive effect on the recovery of MCS patients.

Subsequently, we explored the alterations in brain activity patterns of MCS patients treated with rTMS based on RS-EEG. The EEG spectrum analysis was used, which has been widely used in DoC studies for extracting information on neuronal rhythmic activity in different frequency bands and functional states of the brain [66]. The alpha band activity actively participates in the operation of complex conscious activities and cognitive functions, which is particularly important in the process of recovering consciousness [67]. In DoC patients, improved alpha power is positively associated with increased CRS-R scores [68–70]. Moreover, our previous study also showed a significant improvement in the relative power of the alpha frequency band in VS/UWS patients after rTMS treatment, corresponding to an enhanced state of consciousness [71].

This study further demonstrates that compared to rTMS-sham, MCS patients show a significant increase in RAP after receiving rTMS-active treatment. This represents a positive modulation by rTMS on the alpha rhythm activity of the brain in MCS patients, corresponding to behavioral scores. Meanwhile, we performed the above analysis on the responder and non-responder groups respectively. The results showed a significant increase in the RAP of the responders, whereas non-responders showed no significant increase. This suggests that responders can enhance brain consciousness activities characterized by the alpha rhythm through rTMS treatment.

Accurate diagnosis of DoC is important for effective clinical treatment. Currently, the recommended approach for assessing levels of consciousness involves multimodal assessments (including CRS-R, EEG/ERP, fMRI and PET-CT). This comprehensive method aims to achieve more accurate confirmation of both consciousness level and functional status, which are critical for influencing subsequent clinical decisions.Even then, The obtained diagnoses still represent the patient's level of consciousness at a specific moment. Consequently, some studies have proposed multimodal assessments at multiple time points to evaluate the recovery potential/prognosis of patients [72]. However, it demands high patient compliance, medical diagnostic conditions, and costs, which would add more pressure on the patient's family. In this study, we found PCIst to be a significant indicator at baseline, in distinguishing responder and non-responder groups, based on their response to rTMS treatment. It provides a potential neuro-biomarker to predict the recovery potential of patients for subsequent studies.

Ensuring safety is the primary prerequisite for administering rTMS therapy to patients with DoC. As such, we excluded patients who had epilepsy or related complications (such as paroxysmal sympathetic excitation, fever, or sleep deprivation) within the last 2 weeks based on a previous study. The safety parameters were established following the latest rTMS safety guidelines [41]. Throughout the whole process, none of patients experience any adverse events, which is sufficient to demonstrate the safety of the protocol and holds practical significance for enhancing clinical guidelines. This study not only demonstrated the feasibility of this protocol in patients with VS/UWS, but also the effectiveness when combined with other routine rehabilitation therapies.

The success of a cross-over double-blind RCT can establish the overall treatment effects, while mitigating individual patient differences. Our team continued the study of rTMS on MCS patients, which was built on the previous finding from our previous study of VS/UWS [71]. In this study, PCIst was used as an objective evaluation tool for RCT for the first time, and a significant difference in PCIst was found between responder and non-responder identified by CRS-R at baseline after 10 consecutive rTMS-active sessions. In addition, we also conducted a post-hoc analysis to examine the statistical

power of the study, which was 0.811, demonstrating the adequacy of the sample size. These results will offer new insights for subsequent rTMS or even NIBS intervention, precise diagnosis, or prognostic studies of DoC.

Limitations

However, this study still has some limitations. Firstly, rather than using rTMS combined with MRI navigation techniques, we used 10–20 international EEG systems with F3 electrodes to localize the L-DLPFC. While an absolutely precise location cannot be guaranteed, this method is closer to clinical treatment and fewer hospitals and institutions are equipped with navigation systems. Therefore, our findings can directly provide guidance for the clinical treatment of rTMS in patients with DoC. Secondly, although MCS is the most representative category of patients in DoC, future studies should include more but not limited to VS/UWS, locked-in syndrome, and cognitive-motor dissociation patients to obtain more comprehensive clinical evidence for the rehabilitation of consciousness disorders. We will focus on addressing these issues in the next stage of research.

Conclusion

In conclusion, this crossover, double-blind RCT offers new evidence for clinical application of rTMS in the treatment of MCS patients. Specifically, the application of 10 Hz rTMS on the L-DLPFC can significantly enhance CRS-R, RAP, and PCIst in MCS patients. This suggests that this treatment protocol may be the right approach to improve the level of awareness and cerebral functioning in MCS patients. Post hoc analysis revealed that the PCIst of the responder was significantly higher than that of the non-responder at baseline. This findings could serve as a promising indicator for determining the level of consciousness/brain function in patients with DoC and might emerge as a potential neuro-biomarker for predicting the potential of patient recovery.

Abbreviations

DoC	Disorders of Consciousness
rTMS	Repetitive transcranial magnetic stimulation
NIBS	non-invasive brain stimulation
CRS-R	Coma Recovery Scale-Revised
VS	Vegetative State
UWS	Unresponsive Wakefulness Syndrome
MCS	Minimally Conscious State
EMCS	Emerge Minimally Conscious State
LTP	Long-Term Potentiation
RMT	Resting motor threshold
TESA	TMS-EEG signal analyser
PCI	Perturbational complexity index
PCIst	Perturbational complexity index based on state transitions
RAP	Relative alpha power
TBI	Traumatic brain injury
nTBI	Non-traumatic brain injury
HIE	Hypoxic-ischemic encephalopathy
EEG	Electroencephalogram
ICA	Independent Component Analysis
GWT	Global Workspace Theory
L-DLPFC	Left dorsal lateral prefrontal cortex
ECN	Executive Control Network
DMN	Default mode network
CRC	Clinical Research Center
SMU	Southern Medical University
DMC	Data Monitoring Committee

Acknowledgements

The authors would like to thank the Department of Rehabilitation Medicine, Zhujiang Hospital of Southern Medical University for the availability of the Central Laboratory of Disorders of Consciousness and Clinical Research Center, Zhujiang Hospital of Southern Medical University, for the support. Special thanks to the physical therapists in department of rehabilitation medicine, Jieyang people's Hospital, Ms. Wanchun Wu, for their kind assistance with participant recruitment and data collection.

Author contributions

Chengwei Xu and Qiuyou Xie conceived and designed the study protocol and contributed to the draft of the manuscript. Chengwei Xu, Zhanxing Yuan, and Zerong Chen wrote the manuscript and participated in the coordination and implementation of the study. Ziqin Liao and Qiuyou Xie revised the study protocol and wrote several sections of the manuscript. Shuiyan Li, Yanqi Feng, Ziqiang Tang, Jichan Nian, Xiyan Huang and Haili Zhong helped develop the study measures and data collection. All authors contributed to the manuscript' s draft and approved the final manuscript.

Funding

The study was supported by the grants from National Natural Science Foundation of China (82171174, 82371184) and the Key Technologies R&D Program of Guangdong Province (2023B0303020003).
The funding organizations played no further role in study design, data collection, analysis, interpretation, or paper writing.

Data availability

The data analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by Ethical Committee of Zhujiang Hospital of Southern Medical University. The patients/participants provided their written informed consent to participate in this study.

Consent for publication

Participants give their consent for publication of their image if require.

Competing interests

The authors declare no competing interests.

Author details

[1]Joint Research Centre for Disorders of Consciousness, Department of Rehabilitation Medicine, Zhujiang Hospital of Southern Medical University, Guangzhou, China
[2]Department of hyperbaric oxygenation, Zhujiang Hospital of Southern Medical University, Guangzhou, China
[3]School of Biomedical Engineering, Southern Medical University, 1023 Shatai SouthRoad, Guangzhou, Guangdong 510515, China
[4]School of Rehabilitation Sciences, Southern Medical University, 1023 Shatai SouthRoad, Guangzhou, Guangdong 510515, China

Received: 5 February 2024 / Accepted: 2 September 2024
Published online: 19 September 2024

References

1. First MB, Reed GM, Hyman SE, Saxena S. The development of the ICD-11 clinical descriptions and diagnostic guidelines for Mental and behavioural disorders. World Psychiatry. 2015;14:82–90.
2. Walter J. Consciousness as a multidimensional phenomenon: implications for the assessment of disorders of consciousness. Neurosci Conscious. 2021;2021:niab047.
3. Eapen BC, Georgekutty J, Subbarao B, Bavishi S, Cifu DX. Disorders of consciousness. Phys Med Rehabil Clin N Am. 2017;28:245–58.
4. Fins JJ. Disorders of consciousness, past, Present, and Future. Camb Q Healthc Ethics. 2019;28:603–15.
5. Schnakers C. Update on diagnosis in disorders of consciousness. Expert Rev Neurother. 2020;20:997–1004.
6. Giacino JT, Katz DI, Schiff ND, Whyte J, Ashman EJ, Ashwal S, et al. Practice guideline update recommendations summary: disorders of consciousness: report of the Guideline Development, Dissemination, and implementation Subcommittee of the American Academy of Neurology; the American Congress of Rehabilitation Medicine; and the National Institute on Disability, Independent Living, and Rehabilitation Research. Neurology. 2018;91:450–60.
7. Jennett B, Plum F. Persistent vegetative state after brain damage. RN. 1972;35:ICU1–4.
8. Multi-Society Task Force on PVS. Medical aspects of the persistent vegetative state (1). N Engl J Med. 1994;330:1499–508.
9. Laureys S, Celesia GG, Cohadon F, Lavrijsen J, León-Carrión J, Sannita WG, et al. Unresponsive wakefulness syndrome: a new name for the vegetative state or apallic syndrome. BMC Med. 2010;8:68.
10. Kondziella D, Bender A, Diserens K, van Erp W, Estraneo A, Formisano R, et al. European Academy of Neurology guideline on the diagnosis of coma and other disorders of consciousness. Eur J Neurol. 2020;27:741–56.
11. Giacino JT, Ashwal S, Childs N, Cranford R, Jennett B, Katz DI, et al. The minimally conscious state: definition and diagnostic criteria. Neurology. 2002;58:349–53.
12. Naccache L. Minimally conscious state or cortically mediated state? Brain. 2018;141:949–60.
13. Thibaut A, Schiff N, Giacino J, Laureys S, Gosseries O. Therapeutic interventions in patients with prolonged disorders of consciousness. Lancet Neurol. 2019;18:600–14.
14. Giacino JT, Fins JJ, Laureys S, Schiff ND. Disorders of consciousness after acquired brain injury: the state of the science. Nat Rev Neurol. 2014;10:99–114.
15. Giacino JT, Whyte J, Bagiella E, Kalmar K, Childs N, Khademi A, et al. Placebo-controlled trial of amantadine for severe traumatic brain injury. N Engl J Med. 2012;366:819–26.
16. Rappaport M, Dougherty AM, Kelting DL. Evaluation of coma and vegetative states. Arch Phys Med Rehabil. 1992;73:628–34.
17. Thonnard M, Gosseries O, Demertzi A, Lugo Z, Vanhaudenhuyse A, Bruno M-A, et al. Effect of zolpidem in chronic disorders of consciousness: a prospective open-label study. Funct Neurol. 2013;28:259–64.
18. Machado C, Estévez M, Rodriguez-Rojas R. Zolpidem efficacy and safety in disorders of consciousness. Brain Inj. 2018;32:530–1.
19. Pistoia F, Sacco S, Sarà M, Franceschini M, Carolei A. Intrathecal baclofen: effects on spasticity, pain, and consciousness in disorders of consciousness and locked-in syndrome. Curr Pain Headache Rep. 2015;19:466.
20. Margetis K, Korfias SI, Gatzonis S, Boutos N, Stranjalis G, Boviatsis E, et al. Intrathecal baclofen associated with improvement of consciousness disorders in spasticity patients. Neuromodulation. 2014;17:699–704. discussion 704.
21. Hallett M. Transcranial magnetic stimulation: a primer. Neuron. 2007;55:187–99.
22. Di Perri C, Amico E, Heine L, Annen J, Martial C, Larroque SK, et al. Multifaceted brain networks reconfiguration in disorders of consciousness uncovered by co-activation patterns. Hum Brain Mapp. 2018;39:89–103.
23. Petrides M. The role of the mid-dorsolateral prefrontal cortex in working memory. Exp Brain Res. 2000;133:44–54.
24. Lemaire J-J, Pontier B, Chaix R, El Ouadih Y, Khalil T, Sinardet D, et al. Neural correlates of consciousness and related disorders: from phenotypic descriptors of behavioral and relative consciousness to cortico-subcortical circuitry. Neurochirurgie. 2022;68:212–22.
25. Naro A, Russo M, Leo A, Bramanti P, Quartarone A, Calabrò RS. A single Session of Repetitive Transcranial magnetic stimulation over the Dorsolateral Prefrontal Cortex in patients with unresponsive wakefulness syndrome: preliminary results. Neurorehabil Neural Repair. 2015;29:603–13.
26. Bai Y, Xia X, Kang J, Yin X, Yang Y, He J, et al. Evaluating the Effect of Repetitive Transcranial Magnetic Stimulation on disorders of consciousness by using TMS-EEG. Front Neurosci. 2016;10:473.
27. Xia X, Bai Y, Zhou Y, Yang Y, Xu R, Gao X, et al. Effects of 10 Hz Repetitive Transcranial Magnetic Stimulation of the Left Dorsolateral Prefrontal Cortex in disorders of consciousness. Front Neurol. 2017;8:182.
28. He RH, Wang HJ, Zhou Z, Fan JZ, Zhang SQ, Zhong YH. The influence of high-frequency repetitive transcranial magnetic stimulation on endogenous estrogen in patients with disorders of consciousness. Brain Stimul. 2021;14:461–6.
29. Fan J, Zhong Y, Wang H, Aierken N, He R. Repetitive transcranial magnetic stimulation improves consciousness in some patients with disorders of consciousness. Clin Rehabil. 2022;36:916–25.
30. He R, Fan J, Wang H, Zhong Y, Ma J. Differentiating responders and non-responders to rTMS treatment for disorder of consciousness using EEG after-effects. Front Neurol. 2020;11:583268.
31. Chen J-M, Chen Q-F, Wang Z-Y, Chen Y-J, Zhang N-N, Xu J-W, et al. Influence of high-frequency repetitive transcranial magnetic stimulation on Neurobehavioral and Electrophysiology in patients with disorders of consciousness. Neural Plast. 2022;2022:7195699.
32. Tremblay S, Rogasch NC, Premoli I, Blumberger DM, Casarotto S, Chen R, et al. Clinical utility and prospective of TMS–EEG. Clin Neurophysiol. 2019;130:802–44.
33. Gosseries O, Thibaut A, Boly M, Rosanova M, Massimini M, Laureys S. Assessing consciousness in coma and related states using transcranial magnetic stimulation combined with electroencephalography. Ann Fr Anesth Reanim. 2014;33:65–71.
34. Wang Y, Dang Y, Bai Y, Xia X, Li X. Evaluating the effect of spinal cord stimulation on patient with disorders of consciousness: a TMS-EEG study. Comput Biol Med. 2023;166:107547.
35. Ilmoniemi RJ, Kicić D. Methodology for combined TMS and EEG. Brain Topogr. 2010;22:233–48.
36. Lefaucheur J-P, André-Obadia N, Antal A, Ayache SS, Baeken C, Benninger DH, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). Clin Neurophysiol. 2014;125:2150–206.
37. Somaa FA, de Graaf TA, Sack AT. Transcranial Magnetic Stimulation in the treatment of neurological diseases. Front Neurol. 2022;13:793253.
38. Vitello MM, Rosenfelder MJ, Cardone P, Niimi M, Willacker L, Thibaut A, et al. A protocol for a multicenter randomized and personalized controlled trial using rTMS in patients with disorders of consciousness. Front Neurol. 2023;14:1216468.
39. Giacino JT, Kalmar K, Whyte J. The JFK Coma Recovery Scale-Revised: measurement characteristics and diagnostic utility. Arch Phys Med Rehabil. 2004;85:2020–9.
40. Herwig U, Satrapi P, Schönfeldt-Lecuona C. Using the international 10–20 EEG system for positioning of transcranial magnetic stimulation. Brain Topogr. 2003;16:95–9.
41. Rossi S, Antal A, Bestmann S, Bikson M, Brewer C, Brockmöller J, et al. Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert guidelines. Clin Neurophysiol. 2021;132:269–306.
42. Schiff ND, Nauvel T, Victor J. Large-scale Brain Dynamics in disorders of consciousness. Curr Opin Neurobiol. 2014;0:7–14.
43. Edlow BL, Claassen J, Schiff ND, Greer DM. Recovery from disorders of consciousness: mechanisms, prognosis and emerging therapies. Nat Rev Neurol. 2021;17:135–56.
44. Rogasch NC, Sullivan C, Thomson RH, Rose NS, Bailey NW, Fitzgerald PB, et al. Analysing concurrent transcranial magnetic stimulation and electroencephalographic data: a review and introduction to the open-source TESA software. NeuroImage. 2017;147:934–51.
45. Casali AG, Gosseries O, Rosanova M, Boly M, Sarasso S, Casali KR, et al. A theoretically based index of consciousness independent of sensory processing and behavior. Sci Transl Med. 2013;5:198ra105.
46. Comolatti R, Pigorini A, Casarotto S, Fecchio M, Faria G, Sarasso S, et al. A fast and general method to empirically estimate the complexity of brain responses to transcranial and intracranial stimulations. Brain Stimul. 2019;12:1280–9.
47. Wang Y, Niu Z, Xia X, Bai Y, Liang Z, He J, et al. Application of fast Perturbational Complexity Index to the diagnosis and prognosis for disorders of consciousness. IEEE Trans Neural Syst Rehabil Eng. 2022;30:509–18.
48. Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards M, et al. A common polymorphism in the brain-derived neurotrophic factor gene (BDNF)

modulates human cortical plasticity and the response to rTMS. J Physiol. 2008;586:5717–25.
49. Klomjai W, Katz R, Lackmy-Vallée A. Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). Ann Phys Rehabil Med. 2015;58:208–13.
50. Li Y, Li L, Huang H. Effect of non-invasive brain stimulation on conscious disorder in patients after brain injury: a network meta-analysis. Neurol Sci. 2023;44:2311–27.
51. Lioumis P, Kičić D, Savolainen P, Mäkelä JP, Kähkönen S. Reproducibility of TMS-Evoked EEG responses. Hum Brain Mapp. 2009;30:1387–96.
52. Antal A, Luber B, Brem A-K, Bikson M, Brunoni AR, Cohen Kadosh R, et al. Non-invasive brain stimulation and neuroenhancement. Clin Neurophysiol Pract. 2022;7:146–65.
53. Gosseries O, Di H, Laureys S, Boly M. Measuring consciousness in severely damaged brains. Annu Rev Neurosci. 2014;37:457–78.
54. Lin Y-L, Potter-Baker KA, Cunningham DA, Li M, Sankarasubramanian V, Lee J, et al. Stratifying chronic stroke patients based on the influence of contralesional motor cortices: an inter-hemispheric inhibition study. Clin Neurophysiol. 2020;131:2516–25.
55. Charles L, Van Opstal F, Marti S, Dehaene S. Distinct brain mechanisms for conscious versus subliminal error detection. NeuroImage. 2013;73:80–94.
56. Dehaene S, Naccache L. Towards a cognitive neuroscience of consciousness: basic evidence and a workspace framework. Cognition. 2001;79:1–37.
57. Hui J, Tremblay S, Daskalakis ZJ. The current and future potential of Transcranial magnetic stimulation with Electroencephalography in Psychiatry. Clin Pharmacol Ther. 2019;106:734–46.
58. Sarasso S, Rosanova M, Casali AG, Casarotto S, Fecchio M, Boly M, et al. Quantifying cortical EEG responses to TMS in (Un)consciousness. Clin EEG Neurosci. 2014;45:40–9.
59. Bai Y, Xia X, Kang J, Yang Y, He J, Li X. TDCS modulates cortical excitability in patients with disorders of consciousness. NeuroImage: Clin. 2017;15:702–9.
60. Bai Y, Lin Y, Ziemann U. Managing disorders of consciousness: the role of electroencephalography. J Neurol. 2021;268:4033–65.
61. Casarotto S, Comanducci A, Rosanova M, Sarasso S, Fecchio M, Napolitani M, et al. Stratification of unresponsive patients by an independently validated index of brain complexity. Ann Neurol. 2016;80:718–29.
62. Sinitsyn DO, Poydasheva AG, Bakulin IS, Legostaeva LA, Iazeva EG, Sergeev DV, et al. Detecting the potential for consciousness in unresponsive patients using the Perturbational Complexity Index. Brain Sci. 2020;10:917.
63. Virmani M, Nagaraj N. A novel perturbation based compression complexity measure for networks. Heliyon. 2019;5:e01181.
64. Bodart O, Gosseries O, Wannez S, Thibaut A, Annen J, Boly M, et al. Measures of metabolism and complexity in the brain of patients with disorders of consciousness. NeuroImage: Clin. 2017;14:354–62.
65. Wu X, Zou Q, Hu J, Tang W, Mao Y, Gao L, et al. Intrinsic functional connectivity patterns predict consciousness level and recovery outcome in Acquired Brain Injury. J Neurosci. 2015;35:12932–46.
66. Chennu S, Finoia P, Kamau E, Allanson J, Williams GB, Monti MM et al. Spectral Signatures of Reorganised Brain Networks in Disorders of Consciousness. Ermentrout B, editor. PLoS Comput Biol. 2014;10:e1003887.
67. Mahjoory K, Cesnaite E, Hohlefeld FU, Villringer A, Nikulin VV. Power and temporal dynamics of alpha oscillations at rest differentiate cognitive performance involving sustained and phasic cognitive control. NeuroImage. 2019;188:135–44.
68. Lechinger J, Bothe K, Pichler G, Michitsch G, Donis J, Klimesch W, et al. CRS-R score in disorders of consciousness is strongly related to spectral EEG at rest. J Neurol. 2013;260:2348–56.
69. Stefan S, Schorr B, Lopez-Rolon A, Kolassa I-T, Shock JP, Rosenfelder M, et al. Consciousness indexing and outcome prediction with resting-state EEG in severe disorders of consciousness. Brain Topogr. 2018;31:848–62.
70. Babiloni C, Sarà M, Vecchio F, Pistoia F, Sebastiano F, Onorati P, et al. Cortical sources of resting-state alpha rhythms are abnormal in persistent vegetative state patients. Clin Neurophysiol. 2009;120:719–29.
71. Xu C, Wu W, Zheng X, Liang Q, Huang X, Zhong H, et al. Repetitive transcranial magnetic stimulation over the posterior parietal cortex improves functional recovery in nonresponsive patients: a crossover, randomized, double-blind, sham-controlled study. Front Neurol. 2023;14:1059789.
72. Gallucci A, Varoli E, Del Mauro L, Hassan G, Rovida M, Comanducci A et al. Multimodal approaches supporting the diagnosis, prognosis and investigation of neural correlates of disorders of consciousness: a systematic review. Eur J Neurosci. 2023.

Publisher's note