Journal of Integrative Neuroscience

J. Integr. Neurosci. **2023**; 22(1): 15
https://doi.org/10.31083/j.jin2201015

Original Research

Transcranial Direct Current Stimulation Improves Some Neurophysiological Parameters but not Clinical Outcomes after Severe Traumatic Brain Injury

Weiming Sun[1,2,3,4,*,†], Guanxiu Liu[2,4,†], Xiangli Dong[4,5], Yang Yang[1,3], Guohua Yu[2,4], Xing Sun[2,4], Zhen Feng[2,4], Chaolin Ma[1,3,*]

[1]School of Life Science, Nanchang University, 330031 Nanchang, Jiangxi, China
[2]Department of Rehabilitation Medicine, The First Affiliated Hospital of Nanchang University, 330036 Nanchang, Jiangxi, China
[3]Institute of Life Science, Nanchang University, 330031 Nanchang, Jiangxi, China
[4]Jiangxi Medical College, Nanchang University, 330031 Nanchang, Jiangxi, China
[5]Department of Psychosomatic Medicine, The Second Affiliated Hospital of Nanchang University, 330006 Nanchang, Jiangxi, China
*Correspondence: sunweiming@email.ncu.edu.cn (Weiming Sun); chaolinma@ncu.edu.cn (Chaolin Ma)
[†]These authors contributed equally.
Academic Editor: Gernot Riedel
Submitted: 28 July 2022 Revised: 30 August 2022 Accepted: 7 September 2022 Published: 11 January 2023

Abstract

Background: Disorders of consciousness (DOC) are one of the clinical hallmarks of severe traumatic brain injury (TBI). DOC impair patient life quality and increase the burden on their families and society. **Methods**: A double-blind, randomized, controlled clinical trial was conducted to determine the efficacy of routine rehabilitation combined with transcranial direct current stimulation (tDCS) in DOC patients after TBI. A total of 78 DOC patients were randomly divided after TBI into two groups: participants in the treatment group received routine rehabilitation combined with an active tDCS protocol. In contrast, participants in the control group received routine rehabilitation combined with a sham tDCS protocol. An anode was placed over the left dorsolateral prefrontal cortex and a cathode was placed over the right supraorbital area. The stimulation intensity was 2 mA. Both tDCS protocols lasted for eight consecutive weeks (20 minutes per day, six days per week). Patients were followed up for a further eight weeks. Glasgow Outcome Scale (GOS), Glasgow Coma Scale (GCS), brainstem auditory evoked potentials, somatosensory evoked potentials and electroencephalogram were measured at weeks zero, two, four, six, eight and sixteen from the start of tDCS. **Results**: Neither the GOS nor GCS scores differed significantly between the two groups, while brainstem auditory evoked potentials, somatosensory evoked potentials and electroencephalogram scores did. **Conclusions**: This study found that tDCS improves some neurophysiological parameters but not clinical outcomes of DOC patients after TBI. **Clinical Trial Registration**: Chinese Clinical Trial Registry, ChiCTR1800014808 (The version is V.1.0). Registered on February 7, 2018. http://www.chictr.org.cn/showproj.aspx?proj=25003.

Keywords: transcranial direct current stimulation; rehabilitation; disorders of consciousness; traumatic brain injury; randomized controlled trial

1. Introduction

One of the leading causes of disability and death among young people around the world, particularly in South-East Asia and the Western Pacific, is traumatic brain injury (TBI), which is caused by a force that directly or indirectly affects the brain [1,2]. Each year, approximately 5.48 million people suffer from severe TBI [2]. For a variety of reasons, recently the incidence of TBI in China has risen sharply, as has mortality [3]. Currently, the latter is between 2.7% and 21.8% [3]. Disorders of consciousness (DOC) are one of the clinical hallmarks of severe TBI and it affects many patients with severe TBI. DOC refer to the serious impairment in a patient's recognition and perception of their status and surroundings, including coma, vegetative state (VS)/unresponsive wakefulness syndrome (UWS) and a minimally conscious state (MCS) [4]. Coma may be defined as a state of profound unawareness from which the patient cannot be aroused. Crucially, eyes are closed and a normal sleep-wake cycle is absent. This usually lasts only a few days or weeks following acute brain injury [5]. Some patients awaken from coma (i.e., open their eyes) but remain unresponsive (i.e., show only reflex movements without response to command). This is UWS, and is also referred to as VS [6]. Such patients may open their eyes but exhibit only reflex (i.e., non-intentional) behaviors and are therefore considered unaware of themselves and their surroundings. Patients in MCS show unequivocal signs of non-reflex cortically mediated behaviors [7], occurring inconsistently, yet reproducibly, in response to environmental stimuli [8]. All patients are in coma immediately after a severe TBI and only a few patients will successively develop a vegetative state. The mortality rate is increased with the length of coma [4].

Detecting consciousness in unresponsive patients by means of clinical examination is challenging. Since 1974, the Glasgow Coma Scale has provided a practical method for bedside assessment of impairment of the level of consciousness, the clinical hallmark of acute brain injury [9]. The Glasgow Outcome Scale has been used widely to quantify outcomes in severe TBI trials [6]. Paradigms to detect consciousness by means of positron emission tomography (PET) functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) have been developed during the past two decades to supplement the clinical evaluation of DOC [10]. Neurophysiological tests (EEG and evoked potentials) are useful for assessing the degree of awareness especially in the difficult context of withdrawal of life-sustaining treatments [11]. In traumatic brain injury, brainstem auditory evoked potentials (BAEP) is a good prognostic indicator for traumatic brain injury, while the absence of all waves beyond wave 1 predicts an unpromising prognosis [12]. The use of somatosensory evoked potentials (SEPS) for positive prognosis in the presence of N20 has been proposed to investigate long-latency SEPS components (P25, N35, N70). Their amplitudes were positively associated with a good prognosis, but with a broad confidence interval [13]. Although previous studies have failed to demonstrate the usefulness of single components of the early SEPS and short latency BAEP for predicting the clinical outcome after TBI [14], they can be used as an integral part of the clinical and prognostic assessment of patients with DOC [15].

There are many therapies for recovering consciousness, including pharmacology, surgery, rehabilitation and alternative medical methods. Despite several previous reviews that systematically evaluated the potential effective treatments for DOC patients [16], such treatment is still far from establishing any convincing clinical guidelines. Given the limited evidence of its effect, non-invasive brain stimulation, with little known clinical harm and adverse effect, including transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), is considered a relatively more acceptable therapeutic strategy. tDCS provides a noninvasive brain stimulation technique in which a 1–4 mA direct current is applied through the scalp to regulate cortical excitability [17,18]. The stimulation can be either anodal or cathodal depending on the direction of the current flow. It has been found that anodal tDCS increases the cortical excitability of motor cortex [19], while cathodal tDCS reduces cortical excitability. Additionally, it may affect nearby areas of the brain as well as the stimulation area under the electrode [20] and may also alter the functional connectivity between different brain regions [21]. Moreover, the changes in cortical excitability following a single stimulation can last for up to an hour if the duration and intensity of the stimuli are sufficient [22]. tDCS has been shown to have both short-term and long-term effects on patients with DOC [23,24]. There is evidence that a single tDCS session can change the neurophysiological indices in patients with prolonged DOC [25]. Thibaut *et al*. [23] conducted a randomized double-blind controlled trial to stimulate the left dorsolateral prefrontal cortex of DOC patients and found that the consciousness state of 13 (43%) MCS patients and two (8%) VS/UWS patients improved. However, there are few reports on the effects of tDCS on evoked potentials in DOC patients. Here, a hypothesis is proposed that repetitive tDCS may alter the evoked potentials of DOC patients after TBI and assist in recovery from coma. A randomized controlled trial test was performed to verify this. Moreover, recent studies show that the effects of tDCS are influenced by gender. Generally, the effects of tDCS are more pronounced in females [26,27]. Currently, most trials exploring gender differences in tDCS efficacy have focused on healthy populations, but there are few reports focused on DOC patients. So gender differences were also studied in DOC patients treated with repetitive tDCS.

2. Materials and Methods

2.1 Subjects

Subjects who met the following criteria were enrolled in this study [28]: (1) Male or female coma patients aged 18–65 years, (2) Coma caused by severe TBI, (3) Coma that lasted more than one week, (4) Magnetic resonance imaging (MRI) of the head showed no obvious displacement, structural damage, extensive brain structure necrosis and obvious brain stem (not including the pyramidal tract) or thalamic lesions where the lesion scope of each cortex did not exceed 30% of one side of a brain region, (5) Condition and vital signs were stable, and (6) Family members voluntarily agreed to let the participant participate in the study and signed an informed consent.

Participants who received any of the following treatments during the evaluation period were excluded: (1) Anesthetics, psychotropics, muscle relaxants, sedatives, sleeping pills, Ca^{2+} and Na^{+} channel blockers, (2) Ventilator dependent (3) the course of DOC was longer than one year, (4) Any material contraindicated by MRI appears in the body such as pacemakers, dentures, metal prostheses, etc., or open craniocerebral injury or skull defects that would contraindicate electromagnetic stimulation; seizures or history of seizure, (5) Complicated with serious diseases, (6) Progressive disorders of the nervous system, (7) Pregnancy, (8) Local skin injury or inflammation, (9) Hemostasis, coagulation dysfunction, and anticoagulation therapy users, as well as participants taking anticoagulants; (10) Acute massive cerebral infarction and (11) Hyperalgesia in the stimulus area.

Neurologists evaluated potential participants based on the inclusion and exclusion criteria. Candidates for this study must have met all listed requirements. Eligible individuals were assessed by a neurologist for DOC diagnosis and assessment and their families provided informed consent.

2.2 Methods

The trial lasted a total of 16 weeks and was divided equally between intervention and follow-up. Eighty hospitalized DOC patients after TBI were randomized equally into the treatment or control groups. The participants in the treatment group received routine rehabilitation combined with an active tDCS protocol and the participants in the control group received routine rehabilitation combined with a sham tDCS protocol. Routine rehabilitation is an intervention widely used in clinic to improve a participant's level of consciousness, including hyperbaric oxygen, cerebellar nuclear stimulation, limb electrical stimulation and passive limb range-of-motion training. All patients in this study received the above interventions.

Both tDCS protocols lasted for eight consecutive weeks (20 minutes per day, six days per week). The anode was placed over the left dorsolateral prefrontal cortex (DLPFC) centering at F3, and the cathode was placed over the upper edge of the right orbit centering at FP2 (International 10–20 system). During and after each tDCS treatment, adverse events and side effects were measured. One participant found a slight redness of the skin on his forehead after treatment which recovered within an hour. After repeated examinations during the test, the participant's vital signs were stable. After verification, the operation process, equipment status and treatment parameters were correct. Finally, this family withdrew informed consent and the patient withdrew from the trial. None of the other participants had skin damage under the electrodes.

Participants were followed for a further eight weeks. In this period of time, medical staff followed up with the participants' caregivers by telephone every two weeks. The prognosis and consciousness status of all participants were evaluated from Glasgow Outcome Scale (GOS), Glasgow Coma Scale (GCS), BAEP, SEPS and EEG measured at weeks zero, two, four, six, eight and sixteen from the start of tDCS. A flowchart of participant enrollment is shown in Fig. 1.

An independent statistician from the Evidence-Based Medicine Center, Nanchang University's first affiliated hospital, performed blinding and randomization.

This study used randomization and allocation of hidden blocks. All participants were randomly assigned to either the intervention or control group in a 1:1 ratio. An independent statistician, who was not involved in the trial, derived the order of randomization using the statistical software SPSS 21.0 (IBM Corp., Chicago, IL, USA). Additionally, results assessors and statisticians were blind to the random ratings. The allocation of eligible participants was also concealed from their caregivers and rehabilitation therapists after assessing the demographic data of the participants.

Owing to the double-blind nature of this study, for the "third-party" non-participating personnel who managed and supervised the implementation of the blind method: (1) Caregivers of participants were not allowed to open the envelopes indicating the order in which they were involved in the study. The treatment prescription of the treatment and control groups were respectively defined as mode A and mode B. The treating practitioner only knew that they used either mode A or B, but did not know the specific content of a given prescription. Therefore, they did not know which treatment a participant received. (2) Mode A was the active tDCS and mode B was the sham tDCS; treatment outcomes were assessed by third-party assessors who were unaware of any given grouping. (3) Two unblinding steps were conducted. The first step was group unblinding. Each patient's group (group A or B) was revealed, but it was not known which group received active or sham tDCS. Statistical analysis was then conducted to clarify any difference between groups A and B. In a second step, groups A and B were defined as experimental group (active tDCS) or control group (sham tDCS), respectively. Further analysis was then conducted.

2.3 Intervention

2.3.1 Basic Treatments

All participants were given basic treatment in accordance with the Guidelines for the Management of Severe Traumatic Brain Injury [29]. The treating physician managed each participant according to the guidelines and the participant's condition, including administration of medication and prevention of complications.

2.3.2 Hyperbaric Oxygen

Hyperbaric oxygen therapy is used to treat hypoxic diseases and related disorders by breathing above atmospheric pressure pure or high concentration oxygen. All participants were treated in a hyperbaric oxygen chamber (YC3200/0.3-22VII). According to previous studies [30], the following parameters were set in the chamber: (1) Pressure treatment adjusted to 1.8–2.0 atmospheres absolute; (2) Plus and minus pressure time was 25 minutes; (3) After the pressure in the oxygen chamber was adjusted by the regulator, the participant wore a mask and inhaled pure oxygen twice every 30 minutes and inhaled cabin air or pure oxygen for 10 minutes between the two pure oxygen inhalations.

2.3.3 Cerebellar Nuclear Stimulation

Cerebellar nuclear stimulation is the use of a specific range of low-frequency modulation currents on the human body via a cerebellar top nuclear power stimulation device (headband) and limb neuromuscular electrical stimulation device, using its electrophysiological effect to promote brain blood circulation and limb neuromuscular training. The cerebellar nuclei of all participants were stimulated with an electronic stimulator (CVFT-MG201, Shanghai Qiankang Medical Technology Co., LTD, Shanghai, China). Based on normal sensory threshold and tolerance, to improve the brain's posterior circulation, the therapist applied a 30-minute electrical stimulus (0~15 mA) to a cres-

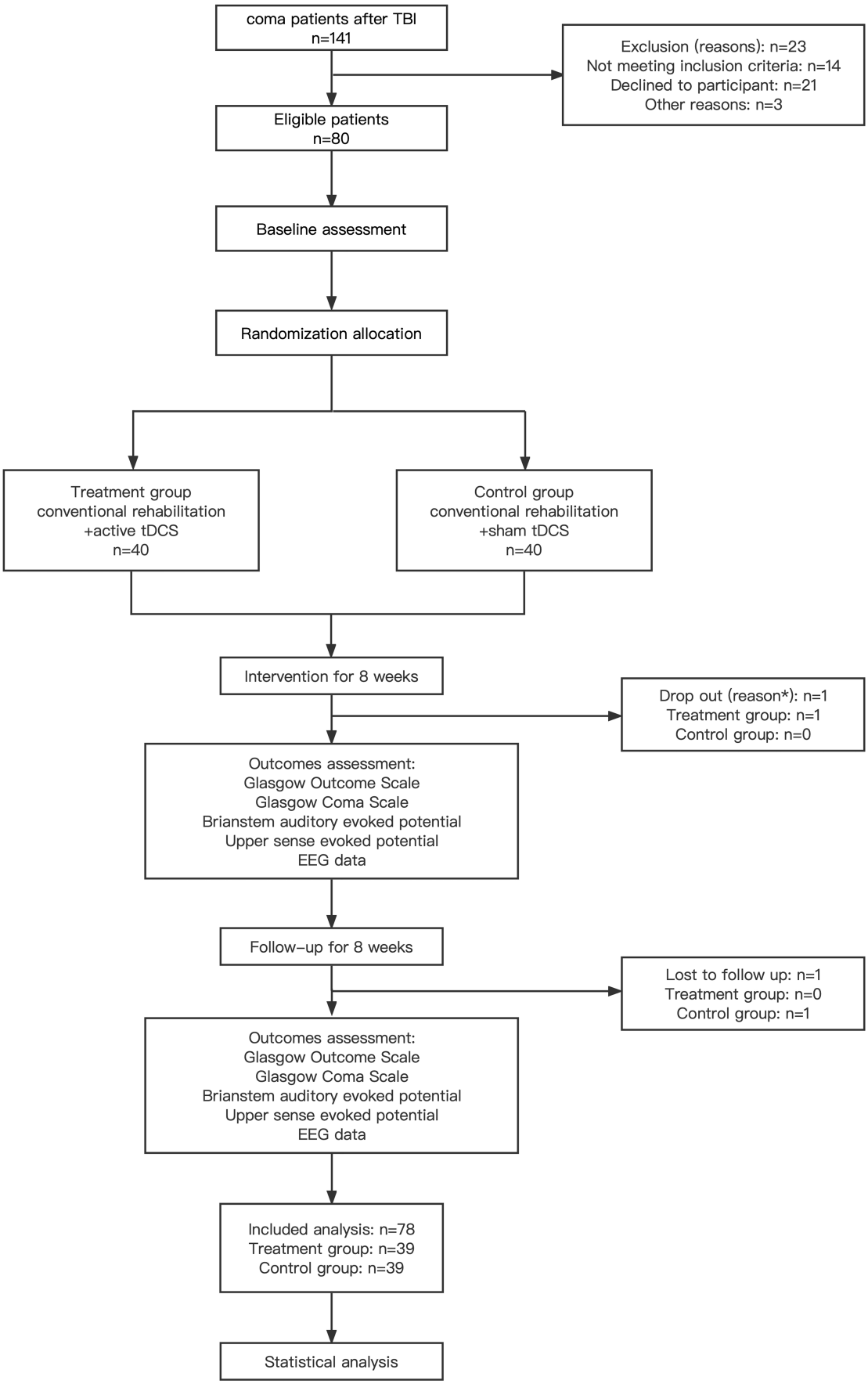

Fig. 1. Participant enrollment. Flowchart.

cent electrode behind the participant's ear.

2.3.4 Limb Electrical Stimulation

Limb electrical stimulation employs a low-frequency pulse current, through a preset program to stimulate one or more groups of muscles, induce muscle movement or simulate normal autonomous movement and to improve or restore the stimulated muscles or muscle group function. All participants received limb electrical stimulation to the tibialis anterior muscle. Two 4 cm $\times$ 4 cm electrodes were used to stimulate the muscle in the longitudinal direction. The current intensity was based on slight muscle contraction, with each treatment duration being 30 minutes.

2.3.5 Passive Limb Range-of-Motion Training

All participants undertook passive range motion training under the guidance of rehabilitation therapists. The therapist performed full joint exercises on the participant's shoulders, elbows, hips and knees, each for 30 minutes.

2.3.6 Active tDCS

Participants in the treatment group received the active tDCS protocol. The anodal electrode was positioned over the left DLPFC centering at F3, while the cathodal electrode was placed over the right supraorbital area centering at FP2 (International 10–20 system). The stimulation device used was a MBM-I (Jiangxi Huaheng Jingxing Medical Technology Co., LTD, China). The current intensity was 2 mA, the anode electrode size was a square of 5 cm and the cathode electrode size was a square of 7 cm. The following treatment parameters were used: (1) 20 minutes per treatment, (2) Daily and (3) Six times per week (See schematic, diagram, Fig. 2A).

2.3.7 Sham tDCS

Participants in the control group received the sham tDCS with the same electrode positions as active tDCS. The tDCS occurred for only 30 seconds during the initial and end stages, with no current for 19 minutes during the sham treatment. Other parameters were consistent with those of the treatment group (see schematic diagram, Fig. 2B).

2.4 Follow up

Medical staff followed up with the participants' caregivers by telephone every two weeks after completion of the tDCS protocol and evaluated the prognosis and consciousness status of all participants in the hospital at week 16.

2.5 Trial Outcomes

2.5.1 Primary Outcomes

Glasgow Outcome Scale

The GOS was used to evaluate the recovery and outcome of participants who suffered TBI. According to indicators such as whether participants recovered sufficiently to undertake work, study and self-care, the severity of the disability was divided into five grades: (1) Good recovery, (2) Moderate disability, (3) Severe disability, (4) Vegetative state and (5) Death. The higher the grade, the more severe the condition.

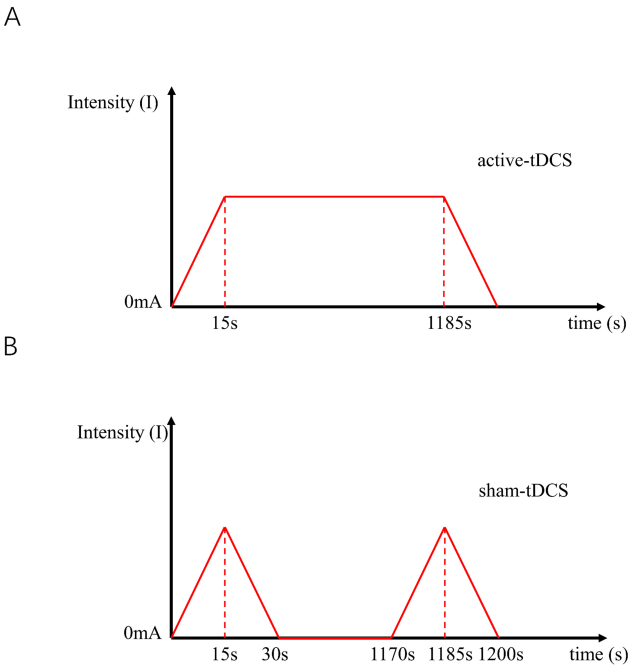

Fig. 2. Schematic diagram of active (A) and sham (B) tDCS.

2.5.2 Secondary Outcomes

Glasgow Coma Scale

The GCS was first introduced by Teasdale and Jennett [31]. It is a short scale with a total of 15 points, including three items: eye-movement, verbal and motion responses. If the GCS score is 13 to 15 points and coma onset after an injury is shorter than 20 minutes, a participant is defined to have mild TBI; if the score is between 9 and 12 points and coma onset is 20 minutes to 6 h after injury the participant has moderate TBI; when scored between 6 and 8 points and coma onset is longer than 6 h, the participant is considered to have extremely severe TBI.

2.5.3 Brainstem Auditory Evoked Potential

BAEP has been used for more than two decades as a sensitive indicator of lesions in the brain stem auditory pathway. This test is widely used to assess brain function in acute critical diseases and can also be used to assess the severity of TBI [32]. The recording electrode was positioned at the top of the skull (Cz point), the reference electrode was over the inner side of the earlobe on the same side as the sound stimulus (A1 and A2 points), and the grounding electrode was placed in the middle of the forehead (F point). The impedance of the skin-electrode was less than 5 KΩ, the stimulus intensity was 100 dB above the hearing threshold and the average stacking was $\times$1000. The BAEP grading standard was initially proposed by Green-

Table 1. The Hall grading standard of abnormal BAEP condition.

Grade	Description	Score
I	Normal Normal latency and amplitude for major waves (l, III, V) Normal interwave latencies (I-IIII-V) Normal wave V/I amplitude ratio	3
II	Mildly abnormal Major waves clearly Discernable, but Prolonged interwave intervals and/or Reduced amplitude or major waves and/or Reduced wave V/I amplitude ratio	2
III	Moderately abnormal Waves III and/or V not repeatedly recorded	1
IV	Markedly abnormal Only Wave I, or No wave	0

Table 2. The Greenberry standard of abnormal SEPS condition.

Grade	Description	Score
I	Normal All waveforms are basically normal	3
II	Mildly abnormal Lack of waveform component after 50 ms Prolonged incubation period	2
III	Moderately abnormal Only P15 and N20 Lacked waveform components after 20 ms	1
IV	Markedly abnormal Only P15, or No wave	0

Table 3. Hockaday's EEG grading criteria for DOC.

Grade	Description	Score
I	Within normallimits Alpha rhythm Predominant alpha with rare theta	3
II	Mildly abnormal Predominant theta with rare alpha Predominanttheta with rare delta	2
III	Moderately abnormal Delta, mixed with theta and rare alpha Predominant delta. with no other activity	1
IV	Markedly abnormal A nearly flat recor No EEG at all	0

berg [33]. Later, many clinicians adopted this standard for clinical research and improvements and many new methods have also been formulated. Hall [34] provided one of the most detailed BAEP grading standards (Table 1). The lower the score, the more severe the TBI coma.

2.5.4 Somatosensory Evoked Potentials

DOC can be predicted with high accuracy by the SEPS, which stimulates the upper skin or peripheral nerves [28]. Evoked potentials produced by this stimulation mode are also called upper sense evoked potentials (USEP) [28]. Recording electrodes were placed on the top of the head (C3 ', C4 '), Erb's point, cervical spinous VII (C7), reference electrode on the forehead (FPz), square wave pulse electrical stimulation of bilateral wrist median nerve and lateral elbow muscle cutaneous nerve running site. The stimulation intensity was 5–15 mA and the scanning duration was 50 ms. SEPS recording should be done in a quiet state, the room temperature should not be too high or too low. Grading was also according to the Greenberry standard (Table 2) [35]. The lower the score, the more severe the TBI coma.

2.5.5 EEG Data

EEG is an effective tool for measuring, assessing and predicting brain function. A NATION8128 ELECTRO encephalograph was used to measure EEG changes in participant brain function. Electrode placement was according to the International 10–20 system and 16-lead recordings were made. The nasal root, external foramen and occipital tuberosity were divided into ten equal parts, with the point at the top of the head. The electrode position was determined by the intersection of the radius and concentric circles centered at the top of the head. Electroencephalography should be recorded in a quiet state at a room temperature that is neither too high nor too low. The score was based on Hockaday's [36] EEG grading criteria for DOC (Table 3). The lower the score, the more severe the TBI coma.

2.6 Sample Size

Sample size calculations were based on improvements to the GCS score. A similar study found that GCS scores following both active and sham tDCS were (12.44 ± 2.51) and (10.43 ± 1.90) (n = 38), respectively [37]. Active tDCS combined with a conventional rehabilitation strategy improved the GCS by 2.01 points on average for participants compared to those in a control group. Improvement measured according to the same sample size were obtained from an estimation formula:

$$n = \frac{2(\mu\alpha + \mu\beta)^2\sigma^2}{\delta^2}$$

With a type I error of 5% ($\alpha = 0.05$) and 90% power ($\beta = 0.10$), 33 participants per group were estimated to be required. Given a dropout rate of 20% during the study, at least 40 participants per group were considered to be sufficient.

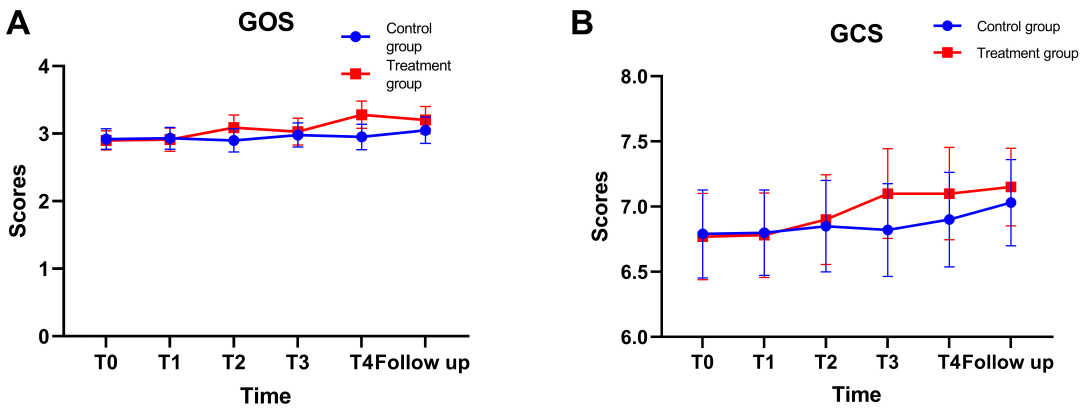

Fig. 3. GCS and GOS analysis of experimental results. *T0, T1, T2, T3 and T4 refer to weeks 0, 2, 4, 6 and 8 from the start of tDCS, respectively. Follow-up refer to week 16 from the start of tDCS. (A) Changes in GOS scores of the two groups from week 1 to week 16. There was no statistical difference between the two groups during weeks 1 to 4, however the treatment group began to score higher at the beginning of week 4. During follow-up, the GOS scores of the treatment group decreased, while the scores of the control group were statistically similar. (B) Changes in GCS scores of the two groups from week 1 to week 16. There was no difference between the two groups during weeks 1 to 16 and the treatment group began to score higher at the beginning of week 6. However, no statistical difference was found during follow-up.

2.7 Statistical Analysis

SPSS21.0 software (IBM Corp., Chicago, IL, USA) was used to analyze the data. Continuous variables of the normal distribution are reported as mean and standard deviation in the descriptive analysis, while it was median and quartile ranges that were reported for data that were not normally distributed. The *t*-test was used to analyze continuous variables and the Fisher exact test for categorical variables to compare baseline characteristics between groups. If statistical significance was identified, the inequality factors were treated as confounding variables in the final efficacy analysis. For comparison of the primary or secondary outcomes between groups, a *t*-test was used to analyze continuous data, and the Fisher's exact test was used to analyze categorical data. If necessary, the general linear model or the Logit model was used to adjust for confounding effects. Repeat measurements were analyzed using Analysis of Variance (ANOVA). In the subgroup analysis, the main results were stratified according to the gender of the participants.

3. Results

Include a concise summary of the data presented in all display items (figures and tables). Excessive elaboration of data shown in display items should be avoided. Numerical data should be analyzed using appropriate statistical tests described in the Experimental Design and Statistical Analysis section. Authors must provide detailed information for each statistical test applied. Report exact p values rather than ranges (e.g., $p = 0.048$ rather than $p < 0.05$).

3.1 The Demographic Data

A total of 78 participants were included in this study, 39 in each of the treatment and control groups (Table 4). One participant was withdrawn from the treatment group following an ethics and informed consent request by the family. One participant in the control group could not be reached during the follow-up. The remaining 78 participants completed eight weeks of tDCS treatment and eight weeks of follow-up from January 2019 to December 2021.

3.2 Glasgow Outcome Scale

Participants did not statistically differ for GOS scores at the baseline ($t = 0.19$, $p = 0.85$). GOS levels of both control and treatment groups are increased from T0 (Control 2.92 ± 0.47 *vs.* Treatment 2.90 ± 0.44) to T4 (Control 2.95 ± 0.58 *vs.* Treatment 3.28 ± 0.62). This indicates an aggravated severity of a disability. Compared to the control group, participants in the treatment group increased more on GOS, but an *F*-test revealed no statistical difference ($F = 0.89$, $p = 0.21$). The control group showed fewer increases in scores but the same *F*-test results as the treatment group ($F = 0.30$, $p = 0.91$). *t*-test results showed no statistical difference between the two groups ($t = 1.43$, $p = 0.15$). As a result of eight weeks of follow-up, the scores for the treatment group had a slight decrease. Additionally, *t*-test results revealed no statistical difference between the groups ($t = 1.09$, $p = 0.29$). The results of the GOS scores are presented in Fig. 3A and Table 5.

3.3 Glasgow Coma Scale

GCS results were similar to the GOS scores. At baseline, participants did not differ for the GOS test ($t = 0.09$, $p = 0.93$). GCS levels of both control and treatment groups

Table 4. Demographic information of participants.

	Control group	Treatment group	χ^2/t	p
Age (years)	55.33 ± 9.63	56.49 ± 8.56	0.382	0.732
Gender (Male/Female)	19/20	18/21	0.402	0.998
Education (Years)	10.22 ± 3.32	9.96 ± 3.03	0.397	0.868
Time since coma (Days)	21.85 ± 7.41	22.12 ± 8.35	0.133	0.695

*At baseline, the two groups did not differ significantly in demographics.

Table 5. GOS analysis of experimental results.

	T0	T1	T2	T3	T4	Follow-up	F	p
Control	2.92 ± 0.47	2.93 ± 0.50	2.90 ± 0.53	2.98 ± 0.55	2.95 ± 0.58	3.05 ± 0.60	0.30	0.91
Treatment	2.90 ± 0.44	2.91 ± 0.53	3.09 ± 0.57	3.03 ± 0.61	3.28 ± 0.62	3.20 ± 0.62	0.89	0.21
t	0.19	0.17	1.52	0.38	1.43	1.09		
p	0.85	0.86	0.13	0.70	0.15	0.29		

*T0, T1, T2, T3 and T4 refer to weeks 0, 2, 4, 6 and 8 from the start of tDCS, respectively. Follow-up refer to week 16 from the start of tDCS.

Table 6. GCS analysis of experimental results.

	T0	T1	T2	T3	T4	Follow-up	F	p
Control	6.79 ± 1.04	6.80 ± 1.01	6.85 ± 1.08	6.82 ± 1.10	6.90 ± 1.12	7.03 ± 1.02	0.24	0.95
Treatment	6.77 ± 1.02	6.78 ± 1.00	6.90 ± 1.06	7.10 ± 1.06	7.09 ± 1.09	7.15 ± 0.92	0.64	0.66
t	0.09	0.09	0.21	1.15	0.80	0.55		
p	0.93	0.93	0.84	0.26	0.43	0.59		

*T0, T1, T2, T3 and T4 refer to weeks 0, 2, 4, 6 and 8 from the start of tDCS, respectively. Follow-up refer to week 16 from the start of tDCS.

Table 7. BAEP analysis of experimental results.

	T0	T1	T2	T3	T4	Follow-up	F	p
Control	2.05 ± 0.50	2.06 ± 0.49	2.15 ± 0.47	2.26 ± 0.46	2.22 ± 0.44	2.20 ± 0.36	2.48	0.03
Treatment	2.04 ± 0.50	2.07± 0.49	2.23 ± 0.45	2.43 ± 0.30	2.52 ± 0.42	2.45 ± 0.38	3.41	0.01
t	0.09	0.09	0.77	1.93	3.08	2.98		
p	0.93	0.93	0.44	0.57	<0.01	<0.01		

*T0, T1, T2, T3 and T4 refer to weeks 0, 2, 4, 6 and 8 from the start of tDCS, respectively. Follow-up refer to week 16 from the start of tDCS.

increased from T0 (Control 6.79 ± 1.04 *vs*. Treatment 6.77 ± 1.02) to T4 (Control 6.90 ± 1.12 *vs*. Treatment 7.09 ± 1.09). This indicates an improved degree of brain injury. After week two, scores of the treatment group increased, but the F-test showed no statistical difference (F = 0.64, p = 0.66). Scores of the control group also increased, but exhibited similar F-test results (F = 0.24, p = 0.95). There was no statistical difference between the two groups based on t-test results (t = 0.80, p = 0.43). After eight weeks of follow-up, the result did not change (t = 0.55, p = 0.59). The results of the GCS scores are presented in Fig. 3B and Table 6.

3.4 Brainstem Auditory Evoked Potential

Electrophysiological results were slightly different from the scores on the above scales. At baseline, the two groups were not statistically different (t = 0.09, p = 093). BAEP levels of both control and treatment groups are increased from T0 (Control 2.05 ± 0.50 *vs*. Treatment 2.04 ± 0.50) to T4 (Control 2.22 ± 0.44 *vs*. Treatment 2.52 ± 0.42). This indicates an improved degree of brain injury. BAEP scores in the treatment group continued to increase and an F-test showed a statistical difference within the group (F = 3.41, p = 0.01). Scores of the control group also decreased and similar F-test results were obtained (F = 2.48, p = 0.03). t-test results showed a statistical difference between the two groups (t = 3.08, p = 0.02). After eight weeks of follow-up, the difference was still significant (t = 2.98, $p < 0.01$). BAEP analysis is given in Fig. 4A and Table 7.

3.5 Upper Sense Evoked Potentials

At baseline, the two groups were not significantly different (t = 0.18, p = 0.86). USEP levels of both control and treatment groups are increased from T0 (Control 1.46 ± 0.50 *vs*. Treatment 1.44 ± 0.48) to T4 (Control 1.60 ± 0.49

Table 8. SEPS analysis of experimental results.

	T0	T1	T2	T3	T4	Follow-up	*F*	*p*
Control	1.46 ± 0.50	1.49 ± 0.49	1.56 ± 0.62	1.56 ± 0.58	1.60 ± 0.49	1.61 ± 0.50	2.57	0.02
Treatment	1.44 ± 0.48	1.44 ± 0.50	1.59 ± 0.47	1.62 ± 0.47	1.84 ± 0.44	1.83 ± 0.38	3.11	0.01
t	0.18	0.45	0.24	0.50	2.28	2.02		
p	0.86	0.66	0.81	0.62	0.03	0.04		

*T0, T1, T2, T3 and T4 refer to weeks 0, 2, 4, 6 and 8 from the start of tDCS, respectively. Follow-up refer to week 16 from the start of tDCS.

Table 9. EEG analysis of experimental results.

	T0	T1	T2	T3	T4	Follow-up	*F*	*p*
Control	1.26 ± 0.44	1.28 ± 0.45	1.29 ± 0.46	1.25 ± 0.47	1.40 ± 0.48	1.38 ± 0.49	2.40	0.04
Treatment	1.24 ± 0.43	1.22 ± 0.47	1.41 ± 0.49	1.46 ± 0.50	1.63 ± 0.50	1.59 ± 0.49	2.56	0.03
t	0.20	0.58	1.12	1.91	1.99	1.90		
p	0.84	0.27	0.27	0.05	0.04	0.06		

*T0, T1, T2, T3 and T4 refer to weeks 0, 2, 4, 6 and 8 from the start of tDCS, respectively. Follow-up refer to week 16 from the start of tDCS.

vs. Treatment 1.84 ± 0.44). This indicates an improved degree of brain injury. Scores in the treatment group (F = 3.11, p = 0.01) and control group (F = 2.57, p = 0.02) increased significantly. According to the statistic quoted (p = 0.03) there is a statistical difference between the two groups compared (at an alpha = 0.05). After eight weeks of follow-up, the difference remained (t = 2.02, p = 0.04). The results of the USEP are given in Fig. 4B and Table 8.

3.6 EEG Data

At baseline, there was no statistical difference between the EEG data of the two groups (t = 0.20, p = 0.84). EEG scores of both control and treatment groups are increased from T0 (Control 1.26 ± 0.44 *vs*. Treatment 1.24 ± 0.43) to T4 (Control 1.40 ± 0.49 *vs*. Treatment 1.63 ± 0.50). This indicates improved degree of brain injury. The increases in the control group were shown to be statistically different by the F-test (F = 2.56, p = 0.03). The treatment group also shows significant intra-group differences (F = 2.40, p = 0.04). t-test results showed a statistical difference between the two groups at week eight (t = 1.99, p = 0.04). However, after eight weeks of follow-up, the difference between the two groups disappeared (t = 1.90, p = 0.06). Results of EEG analysis are given in Fig. 4C and Table 9.

3.7 Gender Subgroup Analysis

In this study, gender subgroup analyses determined that male and female participants in the treatment and control groups did not differ significantly in either the GCS or GOS scores. With respect to BAEP and SEPS data, females in the treatment group had higher scores, but the difference was insignificant. Little difference was also found in EEG scores between males and females (Fig. 5). Overall, no statistical difference in response to tDCS was found between males and females in this study.

3.8 Side Effects

Ten percent (4/40) of participants' left frontal skin showed slight redness after active tDCS, but they recovered within an hour. After repeated examinations during the test, the vital signs of participants were stable with little fluctuation. Following verification, the operation process, equipment status and treatment parameters were correct. The family of one participant insisted on withdrawing them from the study. The participant was withdrawn due to revocation of consent. The study indicated that tDCS was relatively safe under the condition of appropriate intensity, with no malfunction either of the equipment or the procedural standards.

4. Discussion

This study found that tDCS had no obvious therapeutic effect on patients with TBI, which was inconsistent with previous findings of repeated stimulation of the left dorsolateral prefrontal cortex (DLPFC) [38–41]. In this study, no stratified statistical analyses was undertaken for patients with UWS and MCS, as was the case with most previous studies. It is conjectured here that the lack of therapeutic effect may be related to the time after injury: At the start of treatment, the average duration of coma was three weeks, by which time the patient's status had changed to UWS or MCS. Due to the short time of injury and the small increase in GCS score of the patients, it can be inferred that most of the patients are in the state of UWS. Multiple studies have reported clinical improvement immediately after treatment in some patients with MCS but not UWS [38,39]. Therefore, it is concluded that tDCS stimulation failed to achieve a satisfactory effect of improving the state of consciousness from behavioral changes estimated by GCS and GOS, possibly because of the very low state of consciousness of most patients (i.e., UWS). In addition, studies have shown

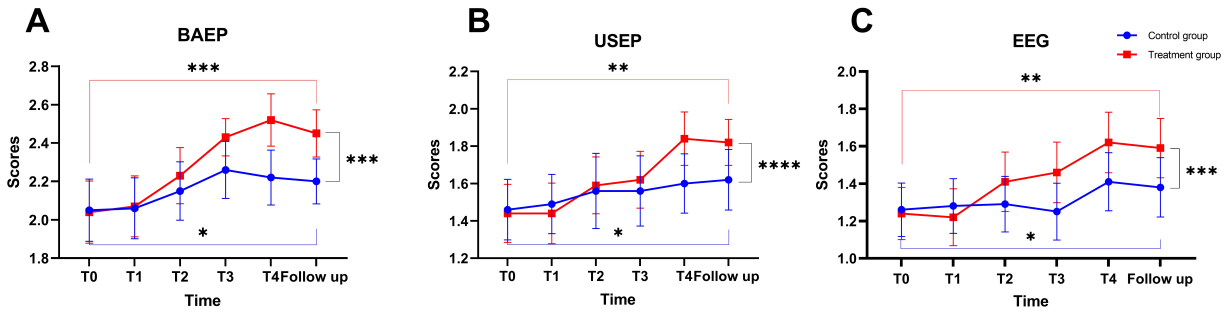

Fig. 4. Data analysis of experimental results. *T0, T1, T2, T3 and T4 refer to weeks 0, 2, 4, 6 and 8 from the start of tDCS, respectively. Follow-up refer to week 16 from the start of tDCS. (A) BAEP scores increased in both groups, with the treatment group increasing more. (B) Both groups showed statistically significant increases in SEPS scores, but those in the treatment group were greater. (C) Both groups showed a significant effect on EEG scores with the treatment group showing the greater increase.

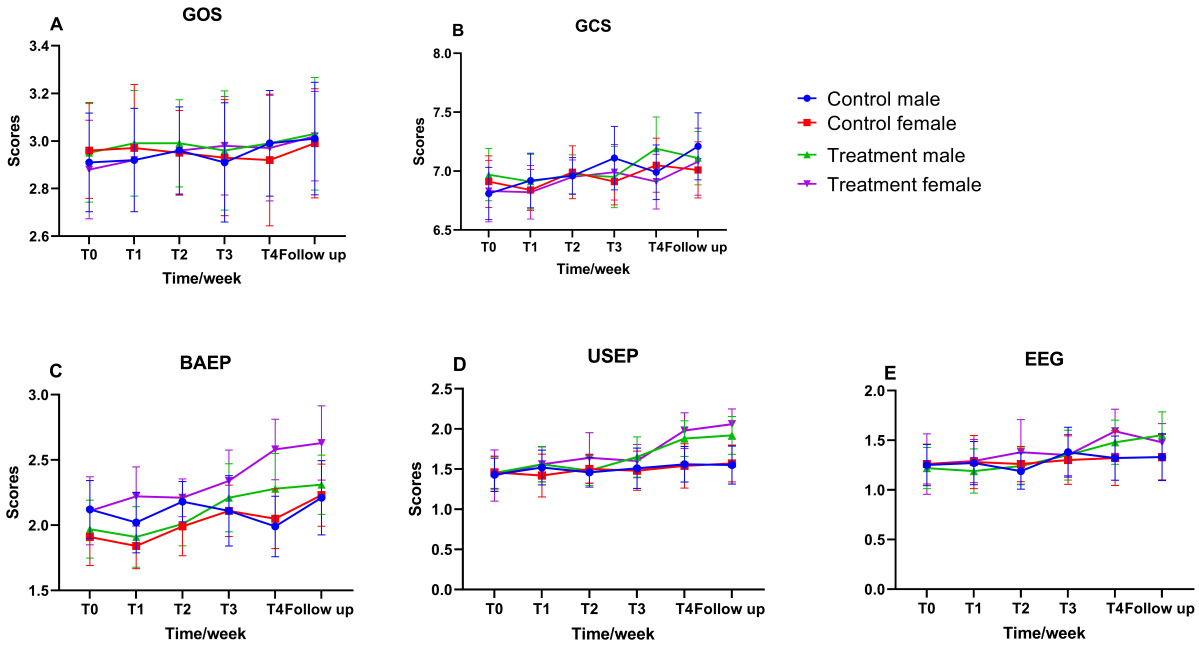

Fig. 5. Data analysis of gender subgroups. *T0, T1, T2, T3 and T4 refer to weeks 0, 2, 4, 6 and 8 from the start of tDCS, respectively. Follow-up refer to week 16 from the start of tDCS. (A) Statistically there were no differences between males and females between the two groups for the GOS scores. (B) Females in both groups had higher scores. However, no statistical differences were found between females and males in the two groups for the GCS. (C) BAEP data of female participants increased more in the treatment group, however, no statistical difference was found. (D) No statistical difference was found between males and females in the SEPS data. (E) No statistical difference was found in the EEG data between males and females in the two groups.

that although the specificity of judging the prognosis of patients with coma or vegetative state according to GCS score is high, the risk of false positive prognostic results is also high, and this phenomenon is especially common in cases of severe TBI [42].

In the present work, these neuroelectrophysiological indicators were used to evaluate the efficacy, which made the results more objective. The BAEP score of the treatment group improved more than that of the control group from the second week, and reached the highest level at the eighth week (2.52 $\pm$ 0.42). The SEPS score of the treatment group improved more than that of the control group from the fourth week and reached the highest in the eighth week (1.84 $\pm$ 0.44). And the EEG scores decreased slightly in the second week, were improved more in the treatment group than in the control group starting

at the fourth week, and peaked at the eighth week (1.63 $\pm$ 0.50). Similar to this study, Carriere *et al*. [25] found that a single tDCS session could generate neurophysiological changes, but had no relevant clinical effect in DOC patients. Cavinato *et al*. [43] found that tDCS could induce changes in cortical EEG oscillations, modulating the travel of alpha and beta waves between anterior and posterior brain areas when some cognitive functions were preserved. BAEP is a non traumatic neuroelectrophysiological detection technique that utilizes computer technology to stimulate the auditory nerve through short acoustic sounds from headphones, with potentials recorded via the scalp [44]. Many studies showed that BAEP has a high application value in evaluating the degree of brain function damage and predicting the prognosis of patients [45–47]. Su *et al*. [48] found that BAEP combined with other neuroelectrophysiological examinations can accurately and objectively determine brain function after cardiopulmonary resuscitation. Sand *et al*. [49] showed that if coma is caused by a brain trauma, cerebrovascular episode or other neurological disease, information about which sensory brainstem pathways are damaged can be obtained from SEPS and BAEP. SEPS are potentials recorded in different parts of the somatosensory ascending pathway by stimulating the terminal sensory nerves of the limbs [50]. Arciniegas *et al*. [51] showed that SEPS are useful early prognostic markers with high specificity (N20) and sensitivity (N70). Moreover, N70 has additional potential value for improving the prediction of good long-term functional outcomes. Liesiene *et al*. [52] found that prognosis of patients with DOC may be worse if pathological BAEP and correlate with pathological dynamic changes in EEG and TBI, diagnosed during CT. EEG is an examination technique of brain bioelectrical activity, which measures the spontaneous and rhythmic bioelectrical activity in order to understand the brain function status of subjects [53]. Scarpino *et al*. [54] showed that specific EEG patterns were independent predictors of improved consciousness at discharge in UWS patients. Some studies showed that EEG provides accurate prognostic information in the early phase of coma [55–57]. Evoked potential examination plays an increasingly important role in predicting the rehabilitation and prognosis of patients with craniocerebral injury and disturbance of consciousness [58].

In terms of electrophysiologial parameters, there was a significant statistical difference between the treatment and control group, which indicated that the treatment effect in the treatment group was better than in the control group. When examining the results within the groups, the scores after 8 weeks of treatment were significantly higher than those before treatment. The results revealed that repeated tDCS treatment can effectively improve the electrophysiological activity of patients with DOC after TBI and promote the recovery of consciousness. Conversely, an independent sample *t*-test was used to compare the mean scores of the GCS and GOS grading indexes. The differences between the control and treatment groups before treatment were not statistically significant. However, the results after 8 weeks of treatment revealed that the scores in the treatment group were significantly higher than those in the control group. And within each group, the scores after 8 weeks of treatment were significantly higher than those before treatment. The results revealed that tDCS may have a long-lasting tDCS effect. Some changes can only be observed after a long period of tDCS treatment. In summary, the study results revealed that tDCS can promote the recovery of consciousness in patients with DOC after TBI and can be used as a rehabilitative treatment for patients with a disturbance in consciousness.

It was surmised that neurophysiological changes were possibly caused by increased excitability of the corresponding brain regions and induced by the stimulation of DLPFC by anodic tDCS. Currently, different electrode placements have been tested in patients with DOC after TBI, but the most effective location may be the DLPFC. Anodal tDCS on the left DLPFC of MCS patients has been shown to improve conscious behavior, whether after a single or repeated stimulation [25,59]. fMRI has also shown that tDCS modulate functional connectivity between the PFC and thalamus [60]. Moreover, electrophysiological responses and electric fields were significantly correlated over frontal cortical areas [61], which represents strong electrophysiological evidence that DLPFC- tDCS directly affects DOC. Additionally, it has been reported that the cumulative effect of repeated tDCS treatment may modulate cortical excitability through normalization of EEG patterns [43,62]. It has been reported that short time application of tDCS at a cortical level exerts a sub-threshold modulation of neuronal resting membrane potential, modulates the firing rate of neurons in response to an input, as observed in animal studies [63] and may act on neuronal recruitment [64]. Anodic tDCS can induce long-term enhancing-like effects when used to prepare rodent brain slices [65]. These changes appear to contribute to the regulation and normalization of cortical function during and after tDCS.

Currently, most trials exploring gender differences in tDCS efficacy have focused on healthy populations, whereas, this study involved relatively comatose participants after TBI and found no significant gender-based difference. Disease may have masked gender differences in tDCS outcomes. Further, the trial was a small sample, single-center clinical trial, which may also be a factor.

5. Limitation and Outlook

The present study found that neither GCS nor GOS scores significantly differed between the treatment and control groups. It is speculated that the treatment frequency and duration are related. Adjustment of treatment parameters may also have a more pronounced effect. Additionally, the scales, both GCS and GOS, used to measure the results

of this experiment may not have been appropriate. GOS is used to predict the prognosis of coma rather than measure the level of consciousness. Similarly, the GCS total score was also found to be inaccurate in reflecting the level of consciousness, which is a key indicator of the severity of injury [66]. CRS-R, a more sensitive scale for measuring consciousness, could allow for more accurate reporting [8].

In current clinical trials for the treatment of DOC after TBI, there are no standard inclusion criteria for the possible range of brain injury. Limited inclusion criteria may affect the generalizations of corresponding results of a finite group. Future sample sizes should be expanded to further explore whether the therapeutic effect of tDCS is affected by the extent of brain injury.

Further studies of the efficacy of tDCS in improving DOC should be confirmed in more centers and with larger sample trials. A greater number of hospitals should be recruited to give access to the larger sample sizes required by multi-center trials. Additionally, more accurate treatment parameters, more sensitive outcome scales and more accurate analysis of male and female differences should improve the quality of future trials. Further, special attention should be paid to the recording, treatment and statistics of adverse reactions during treatment. Optimization of participant inclusion criteria and detailed subgroup analysis may also be necessary. Only in this way can tDCS come to be used more accurately and safely in clinical practice.

6. Conclusions

Notwithstanding the above limitations, the present study provided relevant findings about the clinical and neurophysiological effects of tDCS in patients with DOC after TBI. We did not observe relevant clinical changes after repeated tDCS, neither the GOS nor GCS scores differed significantly between the two groups. We observed, instead, BAEP scores, SEPS and EEG scores are improved. Although no significant changes in clinical outcomes were observed, the improvement in these neurophysiological parameters is encouraging. The study results revealed that tDCS can promote the recovery of consciousness in patients with DOC after TBI and can be used as a rehabilitative treatment for patients with a disturbance in consciousness.

Author Contributions

CLM and ZF provided conceptualization to the study. XLD, GHY and YY provided methodology, formal analysis, software, investigation and validation. GHY helped with resources. WMS, GXL and XS wrote original draft, reviewed and edited. WMS supervised the whole study. All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

The research study has been approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University: Clinical Medicine Ethics Review [2015]043. All family members of study participants had been informed of the trial details and their consent obtained. Before the trial, we conducted a session to inform the participants and their families about the principles, precautions, and adverse reactions of the trial.

Acknowledgment

We thank the rehabilitation department of The First Affiliated Hospital of Nanchang University, and all patients who participated in the study. Thank Lang Shuai, Mingzhu Xu and Qi Ding for supervising and participating in the clinical trial.

Funding

The study was supported in part by grants from the National Natural Science Foundation of China (31760276 and 31960171), the Jiangxi Natural Science Foundation (20171BAB204019 and 20192ACB20022), and the Jiangxi Provincial Special Fund for Postgraduate Innovation (YC2021-B021).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Giacino JT, Trott CT. Rehabilitative Management of Patients with Disorders of Consciousness. Journal of Head Trauma Rehabilitation. 2004; 19: 254–265.

[2] Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung Y, Punchak M, *et al*. Estimating the global incidence of traumatic brain injury. Journal of Neurosurgery. 2019; 130: 1080–1097.

[3] Li Y, Gu J, Zhou J, Xia X, Wang K, Zheng X, *et al*. The epidemiology of traumatic brain injury in civilian inpatients of Chinese Military Hospitals, 2001–2007. Brain Injury. 2015; 29: 981–988.

[4] Giacino JT, Fins JJ, Laureys S, Schiff ND. Disorders of consciousness after acquired brain injury: the state of the science. Nature Reviews Neurology. 2014; 10: 99–114.

[5] Posner JB, Saper CB, Schiff N, Plum F. Plum and Posner's diagnosis of stupor and coma. Oxford University Press. 2007; 79: 110–110.

[6] 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 Medicine. 2010; 8: 68.

[7] Naccache L. Minimally conscious state or cortically mediated state? Brain. 2018; 141: 949–960.

[8] 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. European Journal of Neurology. 2020; 27: 741–756.

[9] Yamal J, Hannay HJ, Gopinath S, Aisiku IP, Benoit JS, Robertson CS. Glasgow Outcome Scale Measures and Impact on Analysis and Results of a Randomized Clinical Trial of Severe Trau-

matic Brain Injury. Journal of Neurotrauma. 2019; 36: 2484–2492.
[10] Kondziella D, Friberg CK, Frokjaer VG, Fabricius M, Møller K. Preserved consciousness in vegetative and minimal conscious states: systematic review and meta-analysis. Journal of Neurology, Neurosurgery & Psychiatry. 2016; 87: 485–492.
[11] Pruvost-Robieux E, Marchi A, Martinelli I, Bouchereau E, Gavaret M. Evoked and Event-Related Potentials as Biomarkers of Consciousness State and Recovery. Journal of Clinical Neurophysiology. 2022; 39: 22–31.
[12] André-Obadia N, Zyss J, Gavaret M, Lefaucheur J, Azabou E, Boulogne S, *et al*. Recommendations for the use of electroencephalography and evoked potentials in comatose patients. Neurophysiologie Clinique. 2018; 48: 143–169.
[13] Zandbergen EGJ, Koelman JHTM, de Haan RJ, Hijdra A, PROPAC-Study Group. SSEPs and prognosis in postanoxic coma: only short or also long latency responses? Neurology. 2006; 67: 583–586.
[14] Morgalla MH, Tatagiba M. Long-term Outcome Prediction after a Traumatic Brain Injury Using Early Somatosensory and Acoustic Evoked Potentials: Analysis of the Predictive Value of the Different Single Components of the Potentials. The Neurodiagnostic Journal. 2014; 54: 338–352.
[15] Johnson EL, Kaplan PW. Clinical neurophysiology of altered states of consciousness: Encephalopathy and coma. Handbook of Clinical Neurology. 2019; 161: 73–88.
[16] Pietrzak E, Pullman S, McGuire A. Using Virtual Reality and Videogames for Traumatic Brain Injury Rehabilitation: a Structured Literature Review. Games for Health Journal. 2014; 3: 202–214.
[17] Shinde AB, Lerud KD, Munsch F, Alsop DC, Schlaug G. Effects of tDCS dose and electrode montage on regional cerebral blood flow and motor behavior. NeuroImage. 2021; 237: 118144.
[18] Zaninotto AL, El-Hagrassy MM, Green JR, Babo M, Paglioni VM, Benute GG, *et al*. Transcranial direct current stimulation (tDCS) effects on traumatic brain injury (TBI) recovery: A systematic review. Dementia & Neuropsychologia. 2019; 13: 172–179.
[19] Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. The Journal of Physiology. 2000; 527: 633–639.
[20] Grefkes C, Fink GR. Reorganization of cerebral networks after stroke: new insights from neuroimaging with connectivity approaches. Brain. 2011; 134: 1264–1276.
[21] Kunze T, Hunold A, Haueisen J, Jirsa V, Spiegler A. Transcranial direct current stimulation changes resting state functional connectivity: a large-scale brain network modeling study. NeuroImage. 2016; 140: 174–187.
[22] Jacobson L, Koslowsky M, Lavidor M. TDCS polarity effects in motor and cognitive domains: a meta-analytical review. Experimental Brain Research. 2012; 216: 1–10.
[23] Thibaut A, Bruno M-, Ledoux D, Demertzi A, Laureys S. TDCS in patients with disorders of consciousness: Sham-controlled randomized double-blind study. Neurology. 2014; 82: 1112–1118.
[24] Schnakers C, Monti MM. Disorders of consciousness after severe brain injury: therapeutic options. Current Opinion in Neurology. 2017; 30: 573–579.
[25] Carrière M, Mortaheb S, Raimondo F, Annen J, Barra A, Binda Fossati MC, *et al*. Neurophysiological Correlates of a Single Session of Prefrontal tDCS in Patients with Prolonged Disorders of Consciousness: A Pilot Double-Blind Randomized Controlled Study. Brain Sciences. 2020; 10: 469.
[26] Thomas C, Ghodratitoostani I, Delbem ACB, Ali A, Datta A. Influence of gender-related differences in transcranial direct current stimulation: a Computational Study. 2019 41St Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). 2019; 5196–5199.
[27] León JJ, Sánchez-Kuhn A, Fernández-Martín P, Páez-Pérez MA, Thomas C, Datta A, *et al*. Transcranial direct current stimulation improves risky decision making in women but not in men: a sham-controlled study. Behavioural Brain Research. 2020; 382: 112485.
[28] Li S, Dong X, Sun W, Zhao N, Yu G, Shuai L. Effects of transcranial direct current stimulation on patients with disorders of consciousness after traumatic brain injury: study protocol for a randomized, double-blind controlled trial. Trials. 2019; 20: 596.
[29] Carney N, Totten AM, O'Reilly C, Ullman JS, Hawryluk GW, Bell MJ, *et al*. Guidelines for the management of severe traumatic brain injury, fourth edition. Neurosurgery. 2017; 80: 6–15.
[30] Sankaran R, Radhakrishnan K, Sundaram KR. Hyperbaric oxygen therapy in patients with hypoxic ischemic encephalopathy. Neurology India. 2019; 67: 728–731.
[31] Teasdale G, Jennett B. Assessment and prognosis of coma after head injury. Acta Neurochirurgica. 1976; 34: 45–55.
[32] Habib SH, Habib SS. Auditory brainstem response: An overview of neurophysiological implications and clinical applications -A Narrative Review. Journal of Pakistan Medical Association. 2021; 71: 2230–2236.
[33] Greenberg RP, Mayer DJ, Becker DP, Miller JD. Evaluation of brain function in severe human head trauma with multimodality evoked potentials. Part 1: Evoked brain-injury potentials, methods, and analysis. Journal of Neurosurgery. 1977; 47: 150–162.
[34] Hall JW, Huang-Fu M, Gennarelli TA. Auditory Function in Acute Severe Head Injury. The Laryngoscope. 1982; 92: 883–890.
[35] Amantini A, Grippo A, Fossi S, Cesaretti C, Piccioli A, Peris A, *et al*. Prediction of 'awakening' and outcome in prolonged acute coma from severe traumatic brain injury: evidence for validity of short latency SEPs. Clinical Neurophysiology. 2005; 116: 229–235.
[36] Hockaday JM, Potts F, Epstein E, Bonazzi A, Schwab RS. Electroencephalographic changes in acute cerebral anoxia from cardiac or respiratory arrest. Electroencephalography and Clinical Neurophysiology. 1965; 18: 575–586.
[37] Hongling L. Transcranial direct current stimulation on the clinical curative effect of patients with disturbance of consciousness. Annals of Physical and Rehabilitation Medicine. 2018; 61: e226–e227.
[38] Hermann B, Raimondo F, Hirsch L, Huang Y, Denis-Valente M, Pérez P, *et al*. Combined behavioral and electrophysiological evidence for a direct cortical effect of prefrontal tDCS on disorders of consciousness. Scientific reports. 2020; 10: 1–16.
[39] Angelakis E, Liouta E, Andreadis N, Korfias S, Ktonas P, Stranjalis G, *et al*. Transcranial Direct Current Stimulation Effects in Disorders of Consciousness. Archives of Physical Medicine and Rehabilitation. 2014; 95: 283–289.
[40] Martens G, Lejeune N, O'Brien AT, Fregni F, Martial C, Wannez S, *et al*. Randomized controlled trial of home-based 4-week tDCS in chronic minimally conscious state. Brain Stimulation. 2018; 11: 982–990.
[41] Estraneo A, Pascarella A, Moretta P, Masotta O, Fiorenza S, Chirico G, *et al*. Repeated transcranial direct current stimulation in prolonged disorders of consciousness: a double-blind cross-over study. Journal of the Neurological Sciences. 2017; 375: 464–470.
[42] Chen R, Bolton CF, Young GB. Prediction of outcome in patients with anoxic coma: a clinical and electrophysiologic study. Critical Care Medicine. 1996; 24: 672–678.
[43] Cavinato M, Genna C, Formaggio E, Gregorio C, Storti SF, Manganotti P, *et al*. Behavioural and electrophysiological ef-

fects of tDCS to prefrontal cortex in patients with disorders of consciousness. Clinical Neurophysiology. 2019; 130: 231–238.
[44] Benichoux V, Ferber A, Hunt S, Hughes E, Tollin D. Across Species "Natural Ablation" Reveals the Brainstem Source of a Noninvasive Biomarker of Binaural Hearing. The Journal of Neuroscience. 2018; 38: 8563–8573.
[45] Di Stefano V, Ferrante C, Telese R, Caulo M, Bonanni L, Onofrj M, *et al*. Brainstem evoked potentials and magnetic resonance imaging abnormalities in differential diagnosis of intracranial hypotension. Neurophysiologie Clinique. 2019; 49: 217–226.
[46] Mendez CV. Mild Traumatic Brain Injury: Neuroimaging of Sports-Related Concussion. Journal of Neuropsychiatry. 2005; 17: 297–303.
[47] Debatisse D, Pralong E, Guerit JM, Bisdorff A. Recording click-evoked myogenic potentials (CEMPs) with a setup for brainstem auditory evoked potentials (BAEPs). Neurophysiologie Clinique/Clinical Neurophysiology. 2005; 35: 109–117.
[48] Su YY, Yang QL, Pang Y, Lv XP. Evaluation of coma patients after cardiopulmonary resuscitation. Chinese Medical Journal. 2005; 118: 1808–1811.
[49] Sand T, Kvaløy MB, Wader T, Hovdal H. Evoked potential tests in clinical diagnosis. Tidsskrift for den Norske laegeforening. 2013; 133: 960–965.
[50] Toleikis JR. Intraoperative Monitoring Using Somatosensory Evoked Potentials. A position statement by the American Society of Neurophysiological Monitoring. Journal of Clinical Monitoring and Computing. 2005; 19: 241–258.
[51] Arciniegas-Villanueva AV, Fernández-Diaz EM, Gonzalez-Garcìa E, Sancho-Pelluz J, Mansilla-Lozano D, Segura T. Functional and Prognostic Assessment in Comatose Patients: A Study Using Somatosensory Evoked Potentials. Frontiers in Human Neuroscience. 2022; 16: 904455.
[52] Liesiene R, Kevalas R, Uloziene I, Gradauskiene E. Search for clinical and neurophysiological prognostic patterns of brain coma outcomes in children. Medicina. 2008; 44: 273–279.
[53] Gao Y, Ren L, Li R, Zhang Y. Electroencephalogram-Electromyography Coupling Analysis in Stroke Based on Symbolic Transfer Entropy. Frontiers in Neurology. 2018; 8: 716.
[54] Scarpino M, Lolli F, Hakiki B, Atzori T, Lanzo G, Sterpu R, *et al*. Prognostic value of post-acute EEG in severe disorders of consciousness, using American Clinical Neurophysiology Society terminology. Neurophysiologie Clinique. 2019; 49: 317–327.
[55] Juan E, Kaplan PW, Oddo M, Rossetti AO. EEG as an Indicator of Cerebral Functioning in Postanoxic Coma. Journal of Clinical Neurophysiology. 2015; 32: 465–471.
[56] Oddo M, Rossetti AO. Early multimodal outcome prediction after cardiac arrest in patients treated with hypothermia. Critical care medicine. 2014; 42: 1340–1347.
[57] Kafashan M, Ryu S, Hargis MJ, Laurido-Soto O, Roberts DE, Thontakudi A, *et al*. EEG dynamical correlates of focal and diffuse causes of coma. BMC Neurology. 2017; 17: 197.
[58] Lew HL, Poole JH, Castaneda A, Salerno RM, Gray M. Prognostic Value of Evoked and Event-related Potentials in Moderate to Severe Brain Injury. Journal of Head Trauma Rehabilitation. 2006; 21: 350–360.
[59] Thibaut A, Wannez S, Donneau A, Chatelle C, Gosseries O, Bruno M, *et al*. Controlled clinical trial of repeated prefrontal tDCS in patients with chronic minimally conscious state. Brain Injury. 2017; 31: 466–474.
[60] Stagg CJ, Lin RL, Mezue M, Segerdahl A, Kong Y, Xie J, *et al*. Widespread Modulation of Cerebral Perfusion Induced during and after Transcranial Direct Current Stimulation Applied to the Left Dorsolateral Prefrontal Cortex. Journal of Neuroscience. 2013; 33: 11425–11431.
[61] Hermann B, Raimondo F, Hirsch L, Huang Y, Denis-Valente M, Pérez P, *et al*. Combined behavioral and electrophysiological evidence for a direct cortical effect of prefrontal tDCS on disorders of consciousness. Scientific Reports. 2020; 10: 4323.
[62] Ulam F, Shelton C, Richards L, Davis L, Hunter B, Fregni F, *et al*. Cumulative effects of transcranial direct current stimulation on EEG oscillations and attention/working memory during subacute neurorehabilitation of traumatic brain injury. Clinical Neurophysiology. 2015; 126: 486–496.
[63] Bindman LJ, Lippold OCJ, Redfearn JWT. The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. The Journal of Physiology. 1964; 172: 369–382.
[64] Nitsche MA, Seeber A, Frommann K, Klein CC, Rochford C, Nitsche MS, *et al*. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. The Journal of Physiology. 2005; 568: 291–303.
[65] Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, *et al*. Direct Current Stimulation Promotes BDNF-Dependent Synaptic Plasticity: Potential Implications for Motor Learning. Neuron. 2010; 66: 198–204.
[66] Bodien YG, Barra A, Temkin NR, Barber J, Foreman B, Vassar M, *et al*. Diagnosing Level of Consciousness: The Limits of the Glasgow Coma Scale Total Score. Journal of Neurotrauma. 2021; 38: 3295–3305.