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Brain stimulation in cognition and disease

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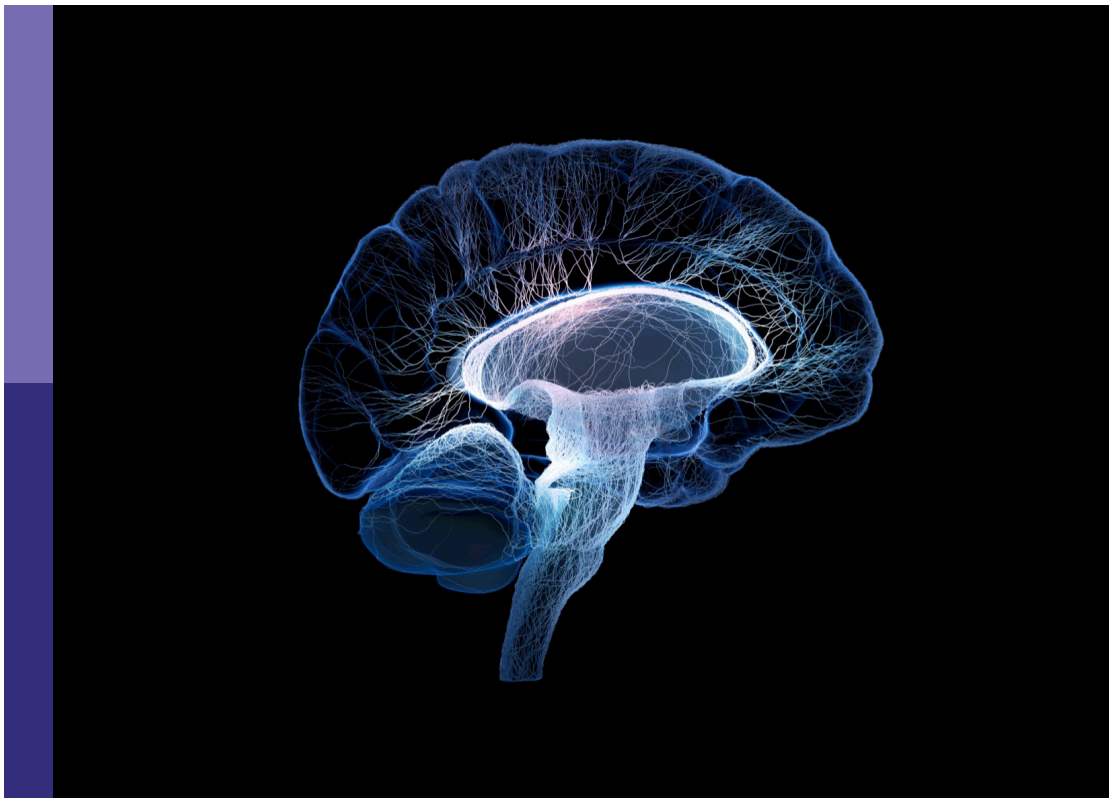
Published in
Frontiers in Neuroscience



ISSN 1664-8714
ISBN 978-2-8325-2583-8
DOI 10.3389/978-2-8325-2583-8

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Brain stimulation in cognition and disease

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Citation

Yu, K., Wang, J., Jiang, H., Mourad, P., eds. (2023). *Brain stimulation in cognition and disease*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2583-8

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frontiers | Frontiers in Neuroscience

TYPE Editorial
PUBLISHED 16 May 2023
DOI 10.3389/fnins.2023.1213272

OPEN ACCESS

EDITED AND REVIEWED BY
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RECEIVED 27 April 2023
ACCEPTED 02 May 2023
PUBLISHED 16 May 2023

CITATION
Yu K, Wang J, Jiang H and Mourad PD (2023) Editorial: Brain stimulation in cognition and disease. *Front. Neurosci.* 17:1213272. doi: 10.3389/fnins.2023.1213272

Editorial: Brain stimulation in cognition and disease

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KEYWORDS

brain stimulation, cognition, neurological disease, deep brain stimulation, transcranial direct current stimulation, transcranial alternating current stimulation, transcranial magnetic stimulation, transcranial ultrasound stimulation

Editorial on the Research Topic
Brain stimulation in cognition and disease

Introduction

Brain stimulation techniques have emerged as a promising avenue for both understanding the intricacies of cognitive processes and addressing neurological disorders. Our Research Topic of *Brain stimulation in cognition and disease* hosted by *Frontiers in Neuroscience* therefore focuses on the advancements, innovations, challenges, and future directions of brain stimulation technologies in cognition and disease.

Thanks to the effort of all the authors and invited reviewers, we have collected 11 articles with seven original research and four review papers. Their topics range from invasive to non-invasive brain stimulation modalities, from preclinical studies to clinical practices, from mechanistic insights of brain stimulation to treating brain diseases. Here, we provide commentary that highlights and offers a view toward the future of the exciting work offered by our Research Topic.

Brain stimulation for improving cognition

Non-invasive brain stimulation (NIBS) shows great potential for improving cognition, by targeting specific cognitive functions guided by electrophysiological biomarkers.

In a rodent study, Xie et al. proposed a novel closed-loop transcranial ultrasound stimulation (TUS) protocol for targeted neuromodulation in the CA1 region of hippocampus. They reported differential effects from TUS triggered at the peak vs. the trough of the CA1 theta oscillatory activity, observing changes in many measures of theta- and gamma-band activity, including coupling between the bands. Theta rhythm has been associated with attention, information processing, decision making, memory consolidation, etc., while gamma activity can relate to execution of motor and memory tasks. Therefore, their closed-loop TUS protocol may 1-day improve specific memory and cognitive functions in humans.

In a human study, Guo et al. demonstrated that multitarget high-definition transcranial direct current stimulation (HD-tDCS) applied over the right inferior frontal gyrus and pre-supplementary motor area improved response inhibition and neural efficiency compared to single target HD-tDCS. Guo et al. also showed that repeated multitarget HD-tDCS plus cognitive training further improved response inhibition, especially in the high-performance subject group. Future studies should obtain fine-grained segmentation of the interested brain regions in order to develop a personalized multitarget stimulation protocol.

Wang et al. reviewed the current state of research on NIBS techniques for the treatment of stroke survivors. A key finding of this review is that repetitive transcranial magnetic stimulation (rTMS) and tDCS each offer significant promise for improving cognitive function. However, the authors also emphasize the substantial heterogeneity in the included studies regarding stimulation parameters, outcome measures, and patient characteristics, which limits the generalizability of the findings, important topics for improving future research in this area.

Moretti et al. offer a cautionary tale regarding two promising NIBS technologies: rTMS and transcranial alternating current stimulation (tACS) as applied over right posterior parietal cortex. They observed no significant effect from either stimulation modality on temporal and visuospatial attention. Despite the null findings, the authors' work refines our knowledge of the boundaries of these NIBS techniques by emphasizing the importance of optimizing the targeting and NIBS parameters to obtain effective neuromodulation.

A little studied but very exciting application of NIBS is optimization of inter-brain neuromodulation for improving teamwork. Lu et al. performed a review of this topic, emphasizing the neural mechanisms of teamwork and potential transcranial electrical stimulation (TES) related technologies to improve teamwork. While much of the available literature focuses on military pilots, improved teamwork has important applications beyond this cohort. The authors discussed the characteristics and existing usage of TES. They found that inter-brain synchronization (IBS) might underlie consistent behaviors or intentions between persons, hence use of TES to enhance IBS might promote cooperation. To further increase IBS, the authors proposed using hyper-tACS together with hyper-scanning technology to enhance teamwork.

Brain stimulation for treating brain disease

The usage of brain stimulation for treating neurological and psychiatric diseases has also grown significantly and researchers continue to seek improvements in existing techniques and development of novel stimulation approaches for better clinical outcome.

Lin et al. demonstrated a rescue procedure involving bilateral subthalamic nucleus (STN) deep brain stimulation (DBS) together with posteroventral pallidotomy (PVP) for dystonia patients experiencing secondary failure of DBS in the globus pallidus internus (GPi). All six participants in the study experienced reduced motor benefits from bilateral GPi DBS 12–24 months after standard DBS. Their approach provided significant improvement in both the movement and disability scores with PVP + bilateral STN DBS that lasted for at least 12–24 months. Further study with more patients may 1-day show that their protocol can offer an important treatment option for these patients.

Wu et al. explored the potential synergistic benefit of combining high-frequency repetitive transcranial magnetic stimulation (HF-rTMS) and cervical nerve root magnetic stimulation (CNRMS) to improve motor function in the upper extremities of stroke patients. The observed promising immediate post-intervention effects motivate future research that examines the long-term effect of their novel approach. This preliminary study holds promise for enhancing rehabilitation strategies for stroke survivors and offers valuable insights into the mechanisms underlying motor recovery.

Xu et al. investigated the effect of rTMS on serum levels of serum amyloid A (SAA) and testosterone in a real-world setting. The authors found that patients with depression benefit most from combined rTMS treatment with medications. Future research is needed in the form of double-blind, randomized control trials that examines the relationship between SAA level and rTMS depression outcome.

Ma et al. provided a systematic review and meta-analysis of the effect of tDCS for patients with disorders of consciousness, showing a significant increase in GCS (Glasgow coma scale) scores and CRS-R (Coma Recovery Scale—Revised) scores due to repeated application of tDCS, especially for patients in a minimal conscious state (MCS). While supportive, the authors could not identify optimal stimulation parameters due to the limited number of eligible studies and wide range of stimulation protocols.

He et al. provided a mechanistic overview of tACS, *in vivo*, as a means of optimizing its parameters to improve its efficacy and broaden its applications. The authors argued that future directions for tACS need to take into account, in a systematic fashion, the frequency, spatial, mechanism-specificity of tACS as well as robustness and replicability of associated findings.

Conclusion

The future of brain stimulation in cognition and disease is rich with possibilities. Multidisciplinary collaborations between neuroscientists, engineers, and clinicians can drive innovation and accelerate the translation of research findings into practical applications. The dissemination of knowledge through open access journals, such as *Frontiers in Neuroscience*, is vital for fostering collaboration and ensuring that the benefits of brain stimulation research reach a wide audience. We believe the publications collected here will

become an important resource for those interested in this research realm.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

JW was funded by grants from NIH (R01NS117822, R01NS037019, R37NS077657, and P50NS123109), Engdahl Family Foundation, and a research grant from the Michael J. Fox Foundation.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers | Frontiers in Neuroscience

ORIGINAL RESEARCH
published: 14 June 2022
doi: 10.3389/fnins.2022.903977

Offline Parietal Intermittent Theta Burst Stimulation or Alpha Frequency Transcranial Alternating Current Stimulation Has No Effect on Visuospatial or Temporal Attention

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OPEN ACCESS

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Specialty section:
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

Received: 24 March 2022
Accepted: 17 May 2022
Published: 14 June 2022

Citation:
Moretti J, Marinovic W, Harvey AR, Rodger J and Visser TAW (2022) Offline Parietal Intermittent Theta Burst Stimulation or Alpha Frequency Transcranial Alternating Current Stimulation Has No Effect on Visuospatial or Temporal Attention. Front. Neurosci. 16:903977. doi: 10.3389/fnins.2022.903977

Non-invasive brain stimulation is a growing field with potentially wide-ranging clinical and basic science applications due to its ability to transiently and safely change brain excitability. In this study we include two types of stimulation: repetitive transcranial magnetic stimulation (rTMS) and transcranial alternating current stimulation (tACS). Single session stimulations with either technique have previously been reported to induce changes in attention. To better understand and compare the effectiveness of each technique and the basis of their effects on cognition we assessed changes to both temporal and visuospatial attention using an attentional blink task and a line bisection task following offline stimulation with an intermittent theta burst (iTBS) rTMS protocol or 10 Hz tACS. Additionally, we included a novel rTMS stimulation technique, low-intensity (LI-)rTMS, also using an iTBS protocol, which uses stimulation intensities an order of magnitude below conventional rTMS. Animal models show that low-intensity rTMS modulates cortical excitability despite sub-action potential threshold stimulation. Stimulation was delivered in healthy participants over the right posterior parietal cortex (rPPC) using a within-subjects design (n = 24). Analyses showed no evidence for an effect of any stimulation technique on spatial biases in the line bisection task or on magnitude of the attentional blink. Our results suggests that rTMS and LI-rTMS using iTBS protocol and 10 Hz tACS over rPPC do not modulate performance in tasks assessing visuospatial or temporal attention.

Keywords: rTMS, iTBS, transcranial alternating current stimulation (tACS), attention, line bisection, attentional blink

INTRODUCTION

Non-invasive brain stimulation is a growing field with wide clinical and basic science applications due to its ability to transiently and safely change brain excitability and oscillatory activity (Dayan et al., 2013; Lefaucheur et al., 2020). Research has shown that several brain stimulation techniques, including repetitive transcranial magnetic stimulation (rTMS) and transcranial

electrical stimulation (tES), can modulate cognition in patients or healthy individuals by facilitating or disrupting mental processes. This has been suggested to arise *via* multiple potential mechanisms including induction of action potentials, changes to membrane potential and entrainment of endogenous brain oscillations (Miniussi et al., 2013). In particular, longer-term offline plastic changes are thought to be facilitated with rTMS through simultaneous depolarisation of pre- and post-synaptic neurons (Lenz et al., 2015), likely through rTMS induction of action potentials. However, brain stimulation in rodent models using a novel low-intensity (LI-) rTMS technique has shown that stimulation delivered at intensities below the action potential threshold (1–150 mT) can also induce behavioural and cellular changes, suggesting that direct induction of action potentials may not be necessary to induce such changes (Moretti and Rodger, 2022). Transcranial electrical stimulation also uses sub-action potential threshold stimulation and is able to induce various neuromodulatory effects on motor and cognitive function (Kuo and Nitsche, 2012; Flöel, 2014). Therefore LI-rTMS may be an intermediate approach combining the high focality of rTMS and the lower intensity stimulation of tES.

Low-intensity stimulation has several potential benefits including fewer side effects (e.g., headaches), reduced power requirements and the potential for more compact and portable design. LI-rTMS allows for these benefits while maintaining the focality of rTMS making it a desirable tool for translation. However, unlike conventional rTMS [which we will refer to as high-intensity (HI-) rTMS] and transcranial alternating current stimulation (tACS), LI-rTMS has not previously been used in humans, although there have been studies with sub-threshold pulsed magnetic fields, which is similar to LI-rTMS, that showed low intensity stimulation could modulate mood in humans (Rohan et al., 2004, 2014; Martiny et al., 2010). To explore LI-rTMS effects in humans for the first time, we included a LI-rTMS condition and assessed whether it could influence cognition.

We also aimed to compare LI-rTMS alongside HI-rTMS and tACS to explore the effects of different brain stimulation techniques in neuromodulation. There are not many studies in the literature which combine rTMS and tES in the same experiment to allow for direct comparisons between stimulation techniques. Single-session stimulation with HI-rTMS and tACS has previously been reported to induce cognitive change, including various aspects of attention (for reviews see Luber and Lisanby, 2014; Santarnecchi et al., 2015; Reteig et al., 2017). We test LI-rTMS in contrast with HI-rTMS, which has similar focality and includes a magnetic field, and tACS, which, like LI-rTMS, is a subthreshold stimulation, but uses widespread electrical, alternating current stimulation applied directly to the scalp. tACS was chosen as a comparative tES technique in order to match the alternating frequency and biphasic waveform of rTMS, as opposed to direct current stimulation. Therefore in this study, we assessed the impact of LI-rTMS, HI-rTMS, and tACS on human visuospatial attention in a within-subject design to compare relative efficacy.

Another aspect of cognitive modulation is the frequency protocol used to induce effects. Theta burst stimulation (TBS) is a complex patterned rTMS frequency often used in studies, with bursts of 3 pulses at 50 Hz applied at a frequency of 5 Hz. The bursts can be applied continuously for a set time [continuous (c)TBS], or intermittently in 2 s periods at a rate of 0.1 Hz [intermittent (i)TBS] to produce effects that are generally inhibitory or excitatory, respectively. Applying cTBS or iTBS to induce motor excitability changes is more efficient compared to simple patterned rTMS protocols (1 Hz, 10 Hz, etc.). The short application time of TBS protocols (3 min) also makes it an attractive stimulation technique. Despite the short stimulation time, cortical excitability changes induced by iTBS and cTBS have been observed for up to 60 and 50 min, respectively, after stimulation ends (Wischnewski and Schutter, 2015). Several studies have explored the use of iTBS and cTBS in cognitive domains to determine whether it is similarly effective for neuromodulation, with mixed results (e.g., Esterman et al., 2017; Gan et al., 2019; Mariner et al., 2021; Schintu et al., 2021; Whybird et al., 2021). We explore whether HI- and LI-rTMS applied using iTBS protocol are effective in enhancing visuospatial attention. We chose to assess attention as it is a higher-order cognitive process (Posner and Petersen, 1990) with several levels of processing susceptible to modulation by brain stimulation. Attention collectively refers to processes involved in the selection of environmental information to support behaviour. Here we focus on two of these processes–spatial and temporal attention–which are used to direct cognitive resources to specific locations in space or specific periods of time. We assessed participants' spatial attention using the line bisection (Landmark) task and temporal attention with an attentional blink (AB) task across three sessions with different stimulation types.

The stimulation site, over the rPPC, was kept consistent between groups with the Cz as the reference electrode with tACS. We hypothesised that excitatory offline HI- and LI- rTMS over the right posterior parietal cortex would induce a leftward shift in spatial bias in the line bisection task and reduce the attentional blink in the AB task in line with previous studies (e.g., line bisection: Fierro et al., 2000; Hilgetag et al., 2001; Kim et al., 2005; Thut et al., 2005; Nyffeler et al., 2008; attentional blink: Cooper et al., 2004). In contrast, alpha frequency (10 Hz) is associated with inhibition of visual perception and attention, therefore alpha frequency tACS is thought to inhibit visual attention (Jensen and Mazaheri, 2010; Foxe and Snyder, 2011; Clayton et al., 2015; c.f. Clayton et al., 2019). Therefore, we hypothesised that rPPC tACS would induce a rightward shift in spatial bias and inhibit temporal attention, possibly increasing the attentional blink.

MATERIALS AND METHODS

This study was approved by the University of Western Australia Human Research Ethics Committee (RA/4/20/6005) and all participants gave informed consent. Twenty-four participants (15 female, 9 male, all self-reported as right-handed, mean age = 19.5 years, *SD* = 2.7) with normal or corrected-to-normal vision participated in the study. The exclusion criteria used for selection conformed to the guidelines for rTMS (Rossi et al., 2009) and tES research (Antal et al., 2017). Participants were undergraduate university students and received partial course

credit in exchange for their participation. Four participants withdrew from the experiment: one without an explanation, one due to an injury between sessions affecting their vision and two due to adverse side effects following HI-rTMS session. Adverse side effects included a headache for one participant and "tightness" in the jaw for the other, possibly in reaction to the repeated tapping sensations. Available data from these participants from previous sessions with no adverse effects were still included.

Participants received three types of stimulation (HI-rTMS, LI-rTMS, or tACS) in separate sessions (counterbalanced) separated by at least a week to prevent carry-over effects using a cross-over, within-subject design. In order to minimise the number of repeat visits required and increase retention, participants received both sham and active stimulation in each session. Sham was delivered first to avoid carry-over effects of stimulation. Participants were informed that they would receive both sham and active stimulation each session but were blinded to the order. Each session followed the same sequence (**Figure 1A**). For the HI-rTMS session, there was an additional thresholding step at the beginning of the session to determine the participant's phosphene threshold.

A post-stimulation questionnaire was administered to assess for possible side-effects and whether participants thought they received a sham or active stimulation. All experiments were run on a Windows computer using specialised software programmed in PsychoPy (Peirce et al., 2019). Stimuli were presented on a 24-inch monitor running at a refresh rate of 60 Hz with a viewing distance of 55 cm.

Line Bisection Task

The line bisection task was similar to Kim et al. (2005; see **Figure 1B**). Stimuli were white, horizontal lines transected or bisected by a white 2.2 degree vertical line on a black background. All lines were 0.1 degree thick. The horizontal line was one of 5 lengths (36–40 degrees) with each length presented equally often. When the horizontal line was transected the elongated side was longer by 1 degree, and the vertical transecting line remained in the centre of the screen.

A single trial consisted of a fixation cross which appeared for 1000 ms followed by a line stimulus presented for 100 ms. The line stimulus was then masked for 1000 ms by a noise mask (50.6 degrees × 20.92 degrees) consisting of randomly generated white or grey solid circles of various sizes. Before each block, participants were instructed to report either which side of the line was longer or which side was shorter. The question alternated each block, and each task alternated which question began the first block. Participants were instructed to respond quickly without sacrificing accuracy by pressing the left and right arrow keys with their right index and middle finger, respectively. If participants did not respond within 1000 ms of mask onset, the trial was considered an error and the next trial was initiated.

Prior to each session, two blocks of 30 practice trials were completed, each consisting of 15 left-elongated, and 15 right-elongated lines presented for 200 ms to allow participants to familiarise themselves with the task. This was followed by the main task consisting of four blocks of 40 trials (10 lines transected with left-side elongation, 10 lines transected with right-side elongation, 20 evenly bisected lines presented in random order).

Attentional Blink Task

The Attentional Blink task was similar to Cooper et al. (2004; see **Figure 1C**). Letter stimuli were presented in black, 48 pt Helvetica font on a grey background. A single trial consisted of a fixation cross presented for 1000 ms followed by a stream of 17 letters presented for 20 ms each with an 80 ms blank inter-letter interval. The first target (T1) was a white letter that could appear randomly in positions 4, 5, 6, 7, or 8 in the stream. The second target (T2) was a black letter X that could appear 1, 2, 3, 5, or 7 positions (lags) after the white letter. The white letter (T1) was chosen from a subset of letters: N, Z, B, E, L, T, W, and M. Non-target letters were chosen from the remaining letters of the alphabet (except X). After the stream was complete, participants were prompted to report the identity of the white letter by pressing the matching key with their left hand and to report whether there had been an X presented by pressing marked arrow keys with their right hand. Participants were instructed to emphasise accurate responding. Following participant responses, there was a 500 ms blank interval before a new trial began.

The experimental tasks consist of 2 blocks of 55 trials. Forty trials included T2, presented equally often at each lag. The order of the trials were randomised for each block. Before beginning the task, participants were told that at least 50% of the trials contained an X, in order to reduce a bias towards reporting the absence of T2. Prior to beginning the experimental task, participants completed two blocks of 125 trials as practice to thoroughly familiarise themselves with the task requirements.

Stimulation

Determining Phosphene Threshold

For rTMS we used the MagPro R30 Stimulator (Magventure, Denmark) with a 75 mm Figure-of-8 coil (MC-B65-HO-2). At the beginning of the session single TMS pulses are delivered to the back of the head under dim lighting to determine the phosphene threshold of the participant based on the methods of Kammer et al. (2001). We conduct a searching procedure for a phosphene "hot spot" over the right hemisphere, beginning 3 cm dorsal and 5 cm lateral from the inion. We deliver single TMS pulses at a high intensity [up to 80% maximum stimulator output (MSO)] and systematically move the coil until the participant reliably reports seeing phosphenes following the TMS. Once the hot spot is located, we adjust the TMS intensity down in steps of 5%, and then 1% MSO, delivering 10 consecutive pulses at each intensity level. The lowest intensity at which 5 out of 10 pulses are reported to induce a phosphene in the participant's vision is determined to be the phosphene threshold. If a participant failed to reliably see phosphenes, stimulation at 50% MSO for HI-rTMS was used, or the next highest intensity that was comfortable for the participant.

Repetitive Transcranial Magnetic Stimulation

For the rTMS stimulation we delivered 600 pulses (biphasic sine waves, 3 min) using the iTBS protocol at either 90% phosphene threshold [34–53% maximum stimulator output

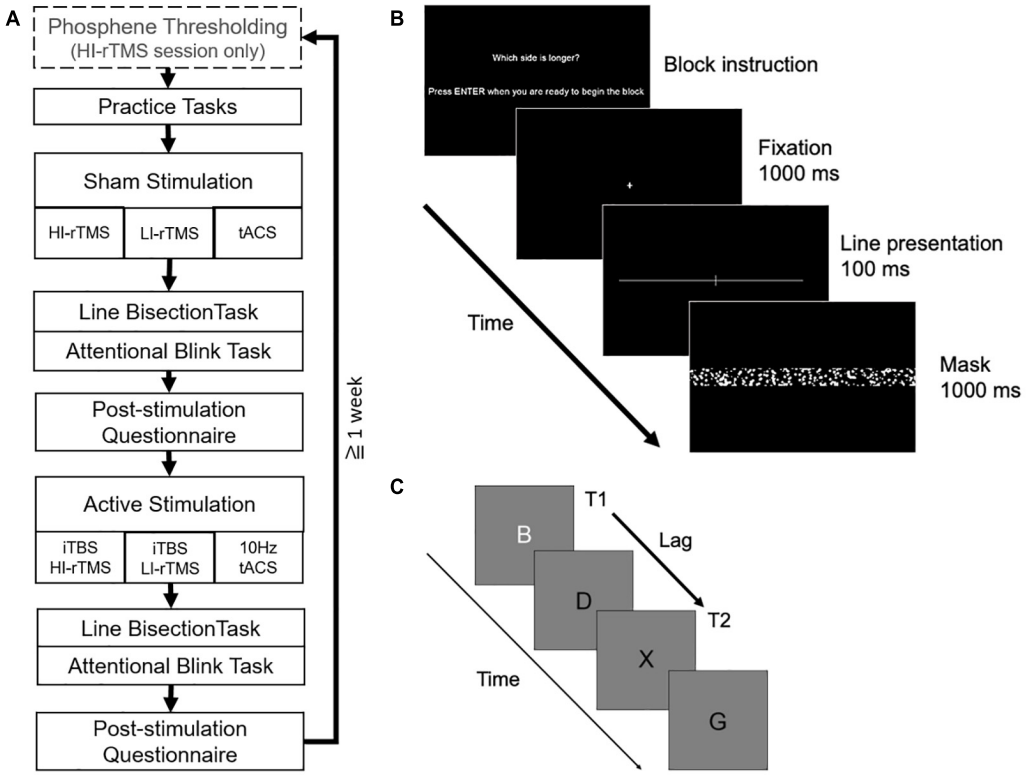

FIGURE 1 | (A) Overall order and structure of each experimental session. **(B)** Example of a trial in the line bisection task. **(C)** Example letter sequence in the attentional blink task (T2 shown at lag 2 position).

(MSO)] (HI-rTMS) or 7% MSO (LI-rTMS) over the right posterior parietal cortex (rPPC) (electrode site P4). Seven percent MSO was equivalent to approximately 50 mT at the estimated distance of the cortical surface (2.5 cm from the scalp), based on magnetic field measurements from the coil. This intensity was chosen to match LI-rTMS parameters that have previously been delivered in animal models (Heath et al., 2018).

In each session participants received a sham and active stimulation. For the sham stimulation, the coil was set to 0% MSO and held above electrode site P4 by the experimenter, with a speaker playing a recording of the appropriate rTMS protocol to mimic the auditory sensation.

Transcranial Alternating Current Stimulation

A multichannel neuromodulation system (Soterix Medical, United States, Model: MXN-5) was used to deliver 20 min (with 30 s ramp up/down) of 10 Hz tACS at 2 mA peak-to-peak amplitude (biphasic sine waves) to the rPPC. Two 5 × 7 cm rubber electrodes in saline-soaked sponges were placed above electrode sites P4 and Cz with electrode gel for added conduction and secured in place with bandages. There was no overlap between the two electrodes. The induced e-field produced with the electrode positioning was modelled using Soterix software (**Figure 2**). The Cz was chosen as the reference electrode based on previous tES studies that examined attention when stimulating rPPC (Sparing et al., 2009; Loftus and Nicholls, 2012; Filmer et al., 2015; Hopfinger et al., 2017). Participants received a sham and active stimulation. For sham stimulation, current was ramped up over 30 s and immediately ramped down over 30 s at both the beginning and end of stimulation.

RESULTS

Data Analysis

For the AB task one participant was excluded due to self-reported inability to see T2 at any point during a session ($n = 23$).

Remaining data were analysed using generalised logistic mixed models at the trial level. For the line bisection task, bias scores were calculated by coding responses to bisected lines as "0" when the response indicated that the left side appeared longer, and "1" when the response indicated that the right side appeared longer.

Line Bisection Task

Task Accuracy

Accuracy on the line bisection task for unevenly transected lines was analysed using generalised mixed model with fixed effects set as elongated side, Stimulation Type, and Active vs. Sham stimulation (see **Table 1**) and subject as a random effect. There was a significant main effect for Stimulation Type ($\chi^2 = 9.54$, $p = 0.008$), but no other significant effects or interactions

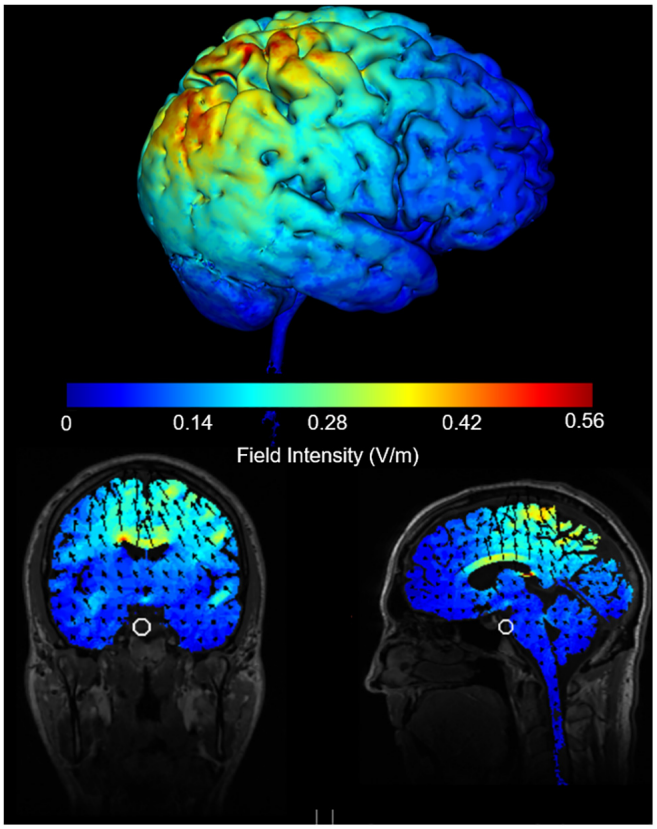

FIGURE 2 | Induced e-field modelling of tACS parameters when electrodes are positioned at Cz and P4 and delivering 2 mA peak to peak intensity.

($\chi^2 \leq 5.44, p > 0.066$). Follow up analyses indicated that accuracy during the HI-rTMS sessions was significantly lower compared to tACS sessions ($z = -2.66$, $p = 0.008$) and LI-rTMS sessions ($z = -2.73$, $p = 0.006$). However, accuracy during the tACS and LI-rTMS sessions did not differ ($z = -0.06$, $p = 0.949$).

Spatial Bias

Initial Bias

One-sample *t*-tests showed bias scores for evenly bisected lines during following sham stimulation were not significantly different from 0.5 for HI-rTMS and LI-rTMS sessions, but there was a slight but significant rightward bias for the tACS session [HI-rTMS: $M = 0.4997$, $t(1484) = -0.026$, $p = 0.979$; LI-rTMS: $M = 0.4968$, $t(1716) = -0.265$, $p = 0.791$; tACS: $M = 0.5404$, $t(1657) = 3.30$, $p < 0.001$]. This suggests none of the participant conditions showed the conventional leftward spatial bias (pseudoneglect) (Milner et al., 1992; Learmonth et al., 2015) prior to stimulation.

TABLE 1 | Mean accuracy (%) when responding to transected line stimuli in the line bisection task.

Stimulation type		Mean accuracy (SD) (%)	
		Left elongated	Right elongated
HI-rTMS	Sham	57.1 (5.0)	61.7 (4.9)
	Active	58.2 (4.9)	63.3 (4.8)
LI-rTMS	Sham	66.7 (4.7)	64.8 (4.8)
	Active	65.2 (4.9)	63.6 (4.8)
tACS	Sham	61.1 (4.9)	71.2 (4.5)
	Active	65.2 (4.8)	62.5 (4.9)

Numbers in brackets represent standard deviation.

Effect of Stimulation

Bias scores were analysed with a generalised linear mixed model with fixed factors of Stimulation Type, Active vs. Sham Stimulation and Block and subject included as a random effect (**Figure 3**). Block was included as a variable in order to assess for any delayed effects of stimulation (Gamboa et al., 2010; Gan et al., 2019). There was a significant main effect of Stimulation Type ($\chi^2 = 14.9$, $p < 0.001$), but no significant main effects of factors Active vs. Sham Stimulation ($\chi^2 = 0.806$, $p < 0.369$) or Block ($\chi^2 = 4.89$, $p = 0.180$). There was also a significant interaction for Stimulation Type*Block ($\chi^2 = 12.8$, $p = 0.046$) and Active vs. Sham Stimulation *Stimulation Type *Block interaction ($\chi^2 = 15.9$, $p = 0.014$). In order to understand the nature of the interaction, we followed up with simple effect comparisons, contrasting sham vs. active stimulation between the same block for each Stimulation Type (i.e., comparing Sham

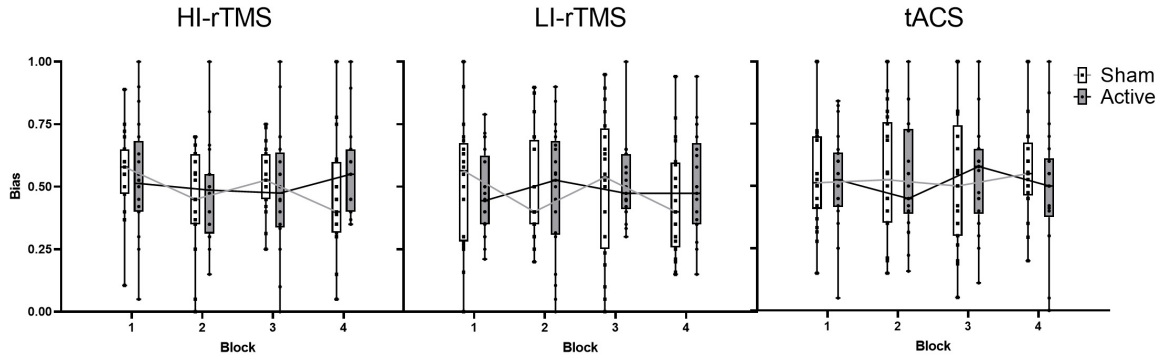

FIGURE 3 | Spatial bias scores for sham and active stimulation for each stimulation type across blocks of the line bisection task. No significant effects or interactions present. Individual points represent mean spatial bias for individual participants. For the bias score: 0 = absolute leftward bias, 1 = absolute rightward bias.

HI-rTMS Block 1 with Active HI-rTMS Block 1; see **Table 2**). There were significant effects for HI-rTMS block 3 and 4; LI-rTMS block 4 and tACS block 4, but none survived adjustment for multiple comparison using Holm–Sidak corrections. We also performed simple effect comparisons for the Stimulation Type*Block interaction, comparing stimulation types across each block. The only comparison that survived Holm–Sidak multiple comparison correction was a difference between HI-rTMS and tACS in block 1 ($z = -2.99$, $p = 0.035$). The simple effects also suggest that the main effect of Stimulation Type cannot be interpreted as there was no pairwise comparison between two stimulation types that was significantly different across all blocks. Thus, inter-session performance was relatively stable.

Attentional Blink

T1 Accuracy

T1 accuracy (**Table 3**) was analysed using generalised linear mixed model with Stimulation Type, Active vs. Sham stimulation and Lag included as fixed factors, and subject included as a random effect. As can be seen in the Table, overall accuracy was close to ceiling. Nevertheless, there was a significant main effect of Stimulation Type ($\chi^2 = 9.87$, $p = 0.007$) and a main effect of Active vs. Sham stimulation ($\chi^2 = 3.86$, $p = 0.049$), but no main effect of Lag ($\chi^2 = 5.47$, $p = 0.361$) and no significant interactions ($\chi^2 \leq 8.15$, $p > 0.258$). Follow up comparisons indicated that accuracy during the HI-rTMS session (92.1% ± 1.47) was significantly higher compared to both LI-rTMS (90.4% ± 1.73; $z = 2.97$, $p = 0.003$) and tACS sessions (90.7 ± 1.69; $z = 2.50$, $p = 0.0012$). T1 accuracy during LI-rTMS and tACS sessions did not differ from each other ($z = -0.479$, $p = 0.632$). The difference between Sham and Active stimulation, although significant, was quite small, and not necessarily meaningful, with mean accuracy reduced by 1% following active stimulation (Sham T1 Accuracy: 91.6% ± 1.53; Active T1 Accuracy: 90.6% ± 1.68). The lack of an interaction effect with Stimulation Type also indicates that the stimulation effect was not specific to, or more pronounced for a particular stimulation technique.

TABLE 2 | Simple effect comparisons for the Active vs. Sham Stimulation *Stimulation type *Block interaction for spatial bias.

Block	Stimulation type	Contrast	z	$p_{unadjusted}$	$p_{adjusted}$
1	HI-rTMS	Active vs. Sham	−1.312	0.190	0.815
	LI-rTMS	Active vs. Sham	−0.793	0.428	0.955
	tACS	Active vs. Sham	−1.028	0.304	0.921
2	HI-rTMS	Active vs. Sham	0.147	0.883	0.986
	LI-rTMS	Active vs. Sham	−0.106	0.915	0.986
	tACS	Active vs. Sham	−0.602	0.547	0.958
3	HI-rTMS	Active vs. Sham	−2.028	0.043	0.356
	LI-rTMS	Active vs. Sham	−0.532	0.595	0.958
	tACS	Active vs. Sham	0.835	0.404	0.955
4	HI-rTMS	Active vs. Sham	2.433	0.015	0.166
	LI-rTMS	Active vs. Sham	1.970	0.049	0.364
	tACS	Active vs. Sham	−2.135	0.033	0.309

Adjusted p-values use Holm–Sidak corrections for multiple comparison.

T2|T1 Accuracy

In order to assess the group effects of stimulation on temporal attention, T2 accuracy calculated only on trials when T1 is correct (T2|T1 Accuracy) was analysed using a generalised linear mixed model with fixed factors of Stimulation Type, Active vs. Sham Stimulation and Lag, with subject included as a random effect. There was a significant effect of Lag ($\chi^2 = 986$, $p < 0.001$) and a significant interaction with Stimulation Type * Lag ($\chi^2 = 19.7$, $p < 0.032$), indicating a robust attentional blink with Lag 1 sparing (**Figure 4**). There were no other significant main effects or interactions, $\chi^2 \leq 4.91$, $p > 0.092$. The interaction between Stimulation Type * Lag suggest that there was some slight difference in attentional blink between sessions, but since there was no interaction with Active vs. Sham Stimulation, it is not connected with application of active stimulation.

Sensation and Blinding During Stimulation

For HI-rTMS, 85% of participants correctly guessed when they received the sham stimulation, and 90% correctly guessed the active stimulation. For LI-rTMS, 57% correctly guessed the sham stimulation, but only 38% correctly guessed the

TABLE 3 | Mean accuracy (%) when responding to T1 in the attentional blink task.

Stimulation type		Mean accuracy (SD) (%)					
		T2 absent	**Lag 1**	**Lag 2**	**Lag 3**	**Lag 5**	**Lag 7**
HI-rTMS	Sham	89.5 (30.7)	90.8 (29.0)	91.1 (28.5)	88.5 (32.0)	88.8 (31.6)	91.1 (28.5)
	Active	89.4 (30.8)	91.7 (27.7)	86.5 (34.3)	90.6 (29.2)	87.2 (33.5)	88.9 (31.5)
LI-rTMS	Sham	88.5 (31.9)	89.4 (30.9)	89.1 (31.3)	89.1 (31.3)	87.5 (33.1)	87.2 (33.5)
	Active	87.1 (33.5)	88.4 (32.1)	86.0 (34.7)	84.2 (36.5)	87.5 (33.1)	84.8 (35.9)
tACS	Sham	86.5 (34.2)	87.8 (32.8)	89.3 (31)	90.2 (29.8)	86.3 (34.4)	86.3 (34.4)
	Active	90.2 (29.8)	86.3 (34.4)	87.8 (32.8)	87.8 (32.8)	87.2 (33.5)	84.2 (36.5)

Numbers in brackets represent standard deviation.
Accuracy for T1 was significantly lower with stimulation.

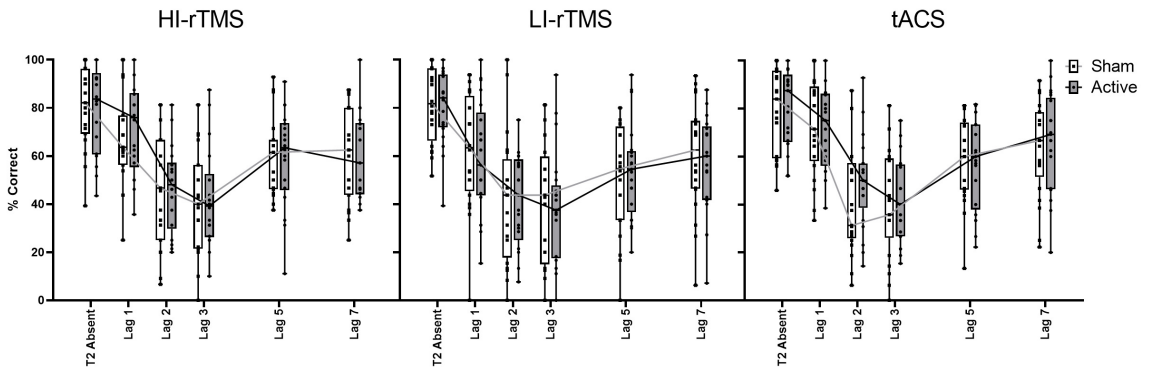

FIGURE 4 | Percentage accuracy for reporting T2| T1 across lag positions for each stimulation type. The attentional blink occurred with Lag 1 sparing. Accuracy did not differ between stimulation parameters for T2| T1. Individual points represent mean accuracy for individual participants.

active stimulation. For tACS, 41% correctly guessed the sham stimulation, while 36% correctly guessed the active stimulation. Tapping and tingling sensations were reported following HI-rTMS and tACS, respectively, in some participants. No physical sensation was reported following LI-rTMS. The HI-rTMS sham was not as effective as tACS or LI-rTMS, however, as there were no stimulation type effects in tasks it does not appear that there were disproportionate sham or expectancy effects.

DISCUSSION

In this study we assessed whether offline LI-rTMS or HI-rTMS delivering iTBS and 10 Hz tACS would induce shifts in visuospatial attention in a line bisection task and alter temporal attention in an AB task. Overall, offline brain stimulation did not change performance in either task, with the exception of a small reduction in T1 accuracy during the attentional blink task following active stimulation.

The lack of significant differences following stimulation in task performance related to attention was unexpected as several studies report changes to cognition following stimulation, particularly for the line bisection task (e.g., line bisection: Fierro et al., 2000; Hilgetag et al., 2001; Kim et al., 2005; Thut et al., 2005; Nyffeler et al., 2008; attentional blink: Cooper et al., 2004). On the face of it, one might speculate that the absence of stimulation effects may reflect the absence of pseudoneglect at a group level, potentially suggesting a lack of sensitivity to spatial bias. However, we think this explanation is unlikely for two reasons. First, our task was based on Kim et al.'s (2005) study, which showed a robust pre-stimulation leftward bias and thus should be sensitive to stimulation effects on spatial bias if present in our sample. Second, despite the lack of evidence for pseudoneglect at a group level, our statistical analyses accounted for individuals' biases and their change over time to maximise statistical sensitivity to modulation. Notably, our other attentional task–the attentional blink–showed a robust group-level attention effect but also no changes in temporal attention following stimulation, making the suggestion that the absence of stimulation effects depends on the presence of group level attention affects prior to stimulation less plausible.

It is also possible that our study design, comparing task results following sham and active stimulation, may overlook effects elicited due to sham intervention. If such effects occurred it may have resulted in ceiling effects that active stimulation could not improve upon. For example, in a study of discrimination sensitivity, there was an instance of a sham intervention effect where active transcranial direct current stimulation (tDCS) stimulation compared against baseline resulted in a significant effect, but comparison against sham did not (Benwell et al., 2015). However, this pattern was not found for attentional bias which was examined in the same study. Moreover, other studies have included separate baseline vs. sham comparisons in similar cognitive tasks and not shown a significant sham intervention

effect (Kim et al., 2005; Giglia et al., 2011; Learmonth et al., 2017). Therefore, we believe previous studies suggest that the contribution of a sham-elicited effect is unlikely in our design; however, this needs further investigation. Below, we discuss our results in relation to the current brain stimulation literature and consider possible contributing factors to our non-significant results in greater detail.

Contributing Factors–Stimulation Protocols

Theta Burst Stimulation

We differed from several previous experimental designs in that we applied HI- and LI-rTMS delivering iTBS rather than a simple patterned rTMS protocol such as 10 or 1 Hz stimulation (e.g., Hilgetag et al., 2001; Kim et al., 2005). However, previous studies have shown iTBS and cTBS can induce cognitive effects (for review see Demeter, 2016). Specifically, after stimulation over the right parietal cortex, cTBS has been shown to induce spatial attention deficits in healthy participants (Nyffeler et al., 2008; Cazzoli et al., 2009; Rizk et al., 2013; Varnava et al., 2013; Chechlacz et al., 2015; Schintu et al., 2021) and alleviate deficits in neglect patients (Nyffeler et al., 2009; Cazzoli et al., 2015; Fu et al., 2015; Yang et al., 2015). cTBS stimulation was also more effective at alleviating deficits than simple patterned protocols for neglect patients (Fu et al., 2015). Cerebellar iTBS was also shown to improve performance in an AB task (Esterman et al., 2017), while cerebellar cTBS increased the AB (Arasanz et al., 2012). Unfortunately, iTBS over the PPC has not been extensively assessed in cognitive studies, although one recent study assessing TBS over the parietal cortex showed changes to inhibition, sequence learning and working memory, but not spatial attention in a simple cue task following both iTBS and cTBS (Whybird et al., 2021). Another recent study compared iTBS over the left PPC with sham, high definition-tDCS and a cTBS protocol in tasks assessing working memory, divided attention, and generalised attention (Stroop task) (Gan et al., 2019). All active stimulation conditions improved reaction times in the generalised attention task, but there was no significant effect on divided attention or working memory. iTBS also had the largest effect size, followed by tDCS and cTBS (Gan et al., 2019). Both Gan et al. (2019) and Whybird et al. (2021) show that iTBS can induce cognitive changes, however, it is not yet established which areas of cognition iTBS can reliably modulate. Whybird et al. (2021) demonstrated modulation of working memory, but Gan et al. (2019) showed no significant modulation of working memory. Despite the contrasting working memory results, both studies show reduced reaction time in inhibition related tasks following left PPC stimulation (emotional Stroop task: Gan et al., 2019; NoGo task: Whybird et al., 2021).

For this study we were interested in whether an iTBS protocol was able to be an effective cognitive enhancement tool. Although iTBS often has the opposing action to cTBS, it may be that iTBS over the rPPC does not induce the opposing behavioural effects evidenced by cTBS in previous spatial attention studies. Disruption of cognition also tends to be more easily induced than cognitive enhancement (Luber and Lisanby, 2014), which could explain the propensity for cTBS but not iTBS effects, especially if attention is already operating at high efficacy. Although when iTBS did appear to induce changes, it was more effective than cTBS (Gan et al., 2019). Compared to simple patterned protocols (i.e., 10 Hz, 1 Hz), iTBS can induce stronger and longer lasting effects compared to simple patterned protocols in measurements of synaptic plasticity (Huang et al., 2005) and has also been more effective than simple protocols in other cognition studies (Fu et al., 2015; Wu et al., 2021). However, this may not be the case for the tasks included in this study. The lack of significant changes to spatial attention in this study are in line with the lack of changes to attention cuing, a spatial attention task, seen in Whybird et al. (2021).

Transcranial Alternating Current Stimulation vs. Transcranial Direct Current Stimulation

We included offline tACS as a comparison with HI- and LI-rTMS in order to compare whether biphasic stimulation *via* application of alternating current directly onto the scalp would differ in strength of effect compared to magnetic stimulation. We theorised that differences could possibly provide information about differences in mechanisms between rTMS and tES, particularly between LI-rTMS and tES as both induce sub-action potential threshold levels of electrical stimulation. We therefore chose tACS for this study in order to have alternating current stimulation across all three stimulation types. One potential limitation of this choice, is that tDCS is more commonly used to induce cognitive effects. However, tACS has previously been shown to affect attention and various other forms of cognition (for review see Klink et al., 2020). For example, Yaple and Vakhrushev (2018) reported changes to temporal attention in an attentional blink task following 20 Hz tACS. Schuhmann et al. (2019) also reported a shift in spatial attention in cued attention and detection tasks with 10 Hz tACS and Otsuru et al. (2019) documented changes to spatial bias and temporal discrimination in a temporal order judgement task with 10 Hz tACS. Nonetheless, since there is less evidence for spatial and temporal attention modulation with tACS, it remains possible that tDCS could have been a more effective stimulation method.

Offline Stimulation

Another difference with many other tACS and tDCS studies is that we applied tACS offline, rather than online, in order to match the timing of rTMS stimulation and to facilitate a comparison between electrical vs. magnetic stimulation. However, a potential problem with this choice is that one of the main proposed mechanisms of tACS is its ability to entrain alpha wave brain oscillation during a cognitive task to induce cognitive effects (Dayan et al., 2013; Miniussi et al., 2013), and previous positive results used online stimulation (Yaple and Vakhrushev, 2018; Otsuru et al., 2019; Schuhmann et al., 2019). In addition, Veniero et al. (2017) initially showed that tACS during the line bisection task (online) but not preceding the task (offline) was able to shift spatial attention, although they could not replicate the result. That said, offline tACS can induce cognitive effects in other domains (e.g., memory and perception, see Klink et al., 2020 for review), and can enhance alpha oscillations,

apparently *via* spike-timing dependent plasticity rather than direct entrainment (Vossen et al., 2015). Nonetheless, for future studies, if the aim is modulation of attention, it may be better to use online interventions.

Stimulation Methodology

An additional consideration is advancements in brain stimulation techniques which can refine stimulation protocols. For example, although the use of 10–20 EEG positions to target stimulation sites are quick and easy to administer, it has limited accuracy. A study comparing methods of determining stimulation sites found that the EEG coordinate approach using "P4" was associated with the lowest behavioural effect size in a number comparison task, while fMRI- and MRI-guided neuronavigation was most effective (Sack et al., 2009). Therefore using MRI-guided rTMS would allow for more precise and consistent stimulation site targeting which could lead to greater likelihood of significant stimulation findings. However, the need for MRI scanning and specialised equipment means this option is highly dependent on the resources available to the researcher. Furthermore, applying tACS using individual alpha frequency rather than fixed frequency may be a more successful way to induce tACS effects. Individual alpha frequency tACS has been associated with long-lasting after effects due to plastic changes (e.g., Vossen et al., 2015) and is increasingly the preferred method for applying tACS. However, comparisons between fixed and individualised alpha frequency tACS are still needed to compare the efficacy of the two techniques.

Stimulation Intensity

Another factor of stimulation is the intensity chosen for each stimulation. This is a source of variation across all brain stimulation studies, with no uniform approach, particularly since various brain regions may respond differently to differing intensities and "the more, the better" is often not the case. Without a specific dose-response curve, it is difficult to conclude whether the intensity dosage was optimal for each stimulation, however, selection of intensities reflected previous studies.

For HI-rTMS, iTBS is usually applied between 70 and 90% of an individual's active or resting motor threshold (Turi et al., 2021). A limitation with regards to intensity comparisons is that we are in the minority as studies who use phosphene thresholds as a way to individualise stimulation intensities (Turi et al., 2021). There is some criticism for whether motor thresholds or phosphene thresholds are appropriate for guiding amplitude selection in non-motor or non-visual areas (Stewart et al., 2001; Boroojerdi et al., 2002; Beynel et al., 2019). Furthermore, a meta-analysis of online rTMS studies' effects on cognition found that use of fixed versus thresholded rTMS intensities did not differ in terms of rTMS effects (Beynel et al., 2019).

For tACS, stimulation intensity is not usually individualised and similar to HI-rTMS, the intensity applied varies, usually between 1 and 2 mA. There are not any robust comparison studies that assess the "optimal" intensity for attention tasks, but different intensities could possibly affect outcomes. Perhaps 2 mA was not the optimal intensity for tACS, however, 2 mA has previously induced significant outcomes in cognitive studies (e.g., Kasten and Herrmann, 2017; Kasten et al., 2020). As discussed further in Section "Previous Replication Failures," there is evidence for intensity-dependent effects in spatial attention (Benwell et al., 2013), but it was not replicated in a follow up study (Learmonth et al., 2015). Studies investigating the biophysics of various tACS intensities could shed light on the interaction between intensity and functional effects. For example, a recent study in non-human primates reported that higher intensities of tACS (comparing 0.5, 1, and 1.5 mA) were able to entrain more cells to induce spike-timing dependent changes, and also increase "burstiness" of neurons (Johnson et al., 2020). Note, this study only looked at short-term stimulation (2 min) so the relevance to longer stimulation used in most tACS studies, offline effects, and functional outcomes are still to be investigated.

Finally, with regards to LI-rTMS, intensity plays a large part in its consideration as a possible new stimulation approach. Animal models using a range of intensities have shown biological and functional effects (Moretti and Rodger, 2022). Our intensity is on the higher range to match with what has been most effective behaviourally in animals (Heath et al., 2018), however, there is still a lot that is unknown about any "optimal" intensity. Future studies to explore dosage parameters and determine the minimum effective intensity, both in humans and animals, would be useful. Since LI-rTMS is subthreshold, we approached the intensity choice in a similar way to tACS and in line with previous LI-rTMS animal models–using a fixed intensity. Part of this reasoning was to remain in line with the animal models and follow a translational pipeline approach, but this differs from convention in HI-rTMS. Although individualising intensity can help normalise stimulation across intra-individual differences in physiological excitability, there are drawbacks with regards to relating thresholded intensities to basic and preclinical research (Turi et al., 2021). Plus, as discussed above, using fixed vs. thresholded intensity approaches do not necessarily predict different rTMS effects (Beynel et al., 2019). In addition, since LI-rTMS remains below the action-potential threshold, individual excitability on the scale of motor or phosphene thresholds are not necessarily relevant to mechanisms of action of LI-rTMS. Similar to tACS, intensity may be important on a cellular level, but using an aggregate measure of cortical excitability is less relevant to LI-rTMS. To be able to step away from the practice of individualised rTMS intensities would make application of LI-rTMS easier and require less expertise for stimulation delivery, more in line with tES approaches. With all of this in consideration, the fixed intensity approach may still be a limitation. Further exploration of dosage-response curves with LI-rTMS comparing the fixed and individualised approaches in the future could help elucidate this.

Previous Replication Failures

At a group level, previous studies had found that 10 Hz rTMS over the rPPC increased visuospatial attention in the left, contralateral hemispace and increased leftward biases in healthy participants (Kim et al., 2005). Multiple sessions of 10 Hz rTMS also improved hemispatial neglect in stroke patients, when assessed with a line bisection task (Kim et al., 2013). Inhibitory rTMS (1 Hz) also facilitated visuospatial attention in the unilateral hemisphere (e.g., Hilgetag et al., 2001),

demonstrating how excitatory and inhibitory stimulation of different hemispheres can effect visuospatial attention in similar ways. Shifts in visuospatial attention have been reported with offline tDCS in a polarity-dependent manner (Sparing et al., 2009; English et al., 2018), online tDCS (Giglia et al., 2011; e.g., Benwell et al., 2015) and online tACS (Schuhmann et al., 2019). Modulation of temporal attention has also been demonstrated with improved attentional blink following TMS over the rPPC (Cooper et al., 2004) and online 20 Hz tACS of frontal and parietal regions (Yaple and Vakhrushev, 2018). Online 10 Hz tACS has also shown evidence for improved temporal discrimination following stimulation on either side of the PPC and leftward shift in spatial bias following rPPC stimulation in a temporal order judgement task (Otsuru et al., 2019).

However, there are also several examples of an absence of cognitive change following brain stimulation. For example, Learmonth et al. (2017), followed up reports of significant modulation of spatial attention in a line bisection task following tDCS seen by Benwell et al. (2015), using a within-subject study design. They were unable to reproduce the same positive results, reporting no significant changes to spatial bias following bi-parietal online tDCS for 15 min. Learmonth et al. (2017) were also unable to replicate an interaction found by Benwell et al. (2015) that a rightward shift in visuospatial attention depended on participant's baseline task performance and tDCS intensity (1 vs. 2 mA). Similarly, Veniero et al. (2017) ran two experiments assessing tDCS and 10 Hz tACS on spatial attention bias. In their first experiment, they were unable to replicate a shift in spatial attention with cathodal tDCS previously reported by Giglia et al. (2011) and Benwell et al. (2015), but did show significant change in bias during online 10 Hz tACS. However, when they attempted to replicate the 10 Hz tACS experiment in a separate sample using a within subjects design, they were unable to reproduce the shift in spatial attention. When they combined the two experimental samples, the previous 10 Hz tACS result also disappeared with the increase in sample size (Veniero et al., 2017).

Our protocol differed in several ways compared to various online tDCS and tACS experiments detailed above, and therefore is not a direct replication attempt. However, the unreliable nature of brain stimulation-induced cognitive changes, particularly with crossover study designs further underlines the difficulty of interpreting the results of studies that apply new stimulation parameters. Interpretation requires evaluation of the reason behind negative results when using exploratory neurostimulation techniques to better determine whether they reflect a true lack of neuromodulatory effects, or are instead the result of other confounding factors which can underlie unreliable or inconsistent modulatory effects reported in both brain stimulation literature and broader cognitive research (Draheim et al., 2021).

Limitations–Additional Measures of Individual Variation

Another limit to establishing consistent effects of brain stimulation is the high rate of inter-individual variability. There has been an increasing push to identify predictors and biomarkers that can help predict whether an individual will respond favourably to brain stimulation which could help guide patient or participant selection. For example, functional and structural connectivity have been identified as possible determinants of stimulation effects in individuals. Mariner et al. (2021) showed that at a group level cTBS did not show the expected rightward shift in visuospatial attention with a Landmark test. However, EEG connectivity, specifically connectivity between the rPPC and left temporal-parietal region, was a significant predictor and likely determinant of whether cTBS was able to influence spatial attention on the individual level. Other studies also link inter-individual variability in visuospatial attention following cTBS with changes in functional connectivity and structural connectivity particularly related to the posterior corpus callosum (Schintu et al., 2021). Schintu et al. (2021) discuss the possibility that differences in connectivity change stimulation outcomes due to differential effects on inhibition or excitation of interhemispheric pathways that modulate visuospatial attention (Koch et al., 2011). For example, individuals with more robust callosal pathways may have less effective inhibition of the interhemispheric PPC pathway following cTBS than individuals with weaker connections (Schintu et al., 2021). Therefore an individual's baseline structural and functional connectivity, which can be influenced by several factors such as sex, genetic, and environmental influences [e.g., training in music (de Manzano and Ullén, 2018) or motor skills (Scholz et al., 2009)] (for review see Lebel and Deoni, 2018) may determine how susceptible they are to stimulation effects.

Availability of γ-aminobutyric acid (GABA) and glutamate, as measured by magnetic resonance spectroscopy, have also been suggested as biomarkers for tDCS effects (Filmer et al., 2019). Training in a response selection task was disrupted by cathodal tDCS over the left the prefrontal cortex. The degree to which training was disrupted was associated with individuals' concentration of GABA and glutamate in the prefrontal cortex. Individual levels of cortical inhibition, suggested by the ratio between GABA and glutamate concentrations (i.e., more GABA than glutamate), had larger disruptions in task training. The disruption in task training and association with neurochemical availability was only evident with cathodal, not anodal or sham stimulation (Filmer et al., 2019). Interestingly, although they did not assess changes on an individual level, Vidal-Piñeiro et al. (2015) demonstrated that a single stimulation of iTBS, but not cTBS over the left inferior parietal lobe was able to increase GABA concentration in the posterior cingulate cortex, a distal region to the stimulation site. The change in distal GABA concentration and a non-significant change in combined glutamate/glutamine concentration was significantly associated with intrinsic connectivity between inferior parietal lobe and posterior cingulate cortex before TBS. This further suggests that individual functional connectivity modulates brain stimulation effects. Finally, other factors such as genetic variation among plasticity-related genes are also beginning to be explored as contributors to inter-individual differences in brain stimulation responses, e.g., BDNF Val66Met polymorphisms (Cheeran et al., 2008; Abellaneda-Pérez et al., 2022). In sum, multiple levels of variation, down to the genetic level likely influence brain

connectivity and consequent responses to brain stimulation, and future work should assess such individual variations in order to attempt to bolster consistency across stimulation studies.

Future Directions

It may be that cognitive changes assessed solely through experimental tasks were not sensitive enough to pick up on any subtle changes to attention induced by the stimulation. Assessing changes to excitability using motor evoked potentials or EEG may be more suitable as a barometer of whether LI-rTMS can induce changes in humans, and easier to compare quantitatively against HI-rTMS or tES. As demonstrated by Mariner et al. (2021), including EEG can also allow connectivity analysis to be used to further assess determinants behind inter-individual variability, and would allow the ability to use individualised alpha-frequency tACS methods rather than fixed frequency tACS to possibly increase tACS efficacy or after-effects. Comparing effects of online stimulation may also be more likely to produce significant changes and allow more suitable comparison between tES and LI-rTMS, in order to assess the effects of sub-threshold stimulation on cognition and behaviour. However, HI-rTMS is difficult to administer online as it can induce muscle twitching and is accompanied by a loud clicking sound which could distract participants from the task. Due to the exploratory nature of this study in relation to LI-rTMS it may also be that attention was not the most suitable behaviour to assess LI-rTMS effects, although our choice was guided by animal models which have suggested some attention-related effects of LI-rTMS (Poh et al., 2018; Moretti et al., 2021). LI-rTMS may be able to modulate behaviour in other tasks, although it is not yet clear which tasks would be most suitable. For example, it may be that sub-threshold stimulation using LI-rTMS acts under principles of stochastic resonance which was suggested after LI-rTMS modulation of visual evoked potentials in mice (Makowiecki et al., 2018) and is similar to theories proposed for tES (Miniussi et al., 2013). Therefore, perception tasks and inclusion of online LI-rTMS may be a good starting point to look at potential behavioural changes through the lens of optimising signal-to-noise ratio of neural activity.

CONCLUSION

This study was the first to assess the effects of LI-rTMS on cognition in humans. LI-rTMS was tolerated extremely well, however, we did not observe any significant changes to spatial or temporal attention. We also did not observe changes to spatial or temporal attention following offline rTMS delivering iTBS and 10 Hz tACS. Since we were unable to modulate attention as has been seen in previous studies using rTMS and tES we cannot yet draw conclusion on how LI-rTMS compares with conventional stimulation currently used in humans and the possible mechanisms underlying these techniques. Our null results following HI-rTMS and tACS provide evidence supporting ineffective modulation of attention when applying iTBS and offline tACS.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Western Australia Human Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JM: conceptualisation, methodology, writing – original draft, review and editing, visualisation, formal analysis, investigation, and software. WM: writing – review and editing, resources, and formal analysis. AH: writing – review and editing and supervision. JR and TV: writing – review and editing, supervision, conceptualisation, and methodology. All authors contributed to the article and approved the submitted version.

FUNDING

JM was supported by an Australian Government Research Training Program (RTP) scholarship, and Byron Kakulas Prestige scholarship. JR was supported by a fellowship from Multiple Sclerosis Western Australia (MSWA) and the Perron Institute for Neurological and Translational Science.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
published: 13 July 2022
doi: 10.3389/fnins.2022.931265

Transcranial Electrical Stimulation Offers the Possibility of Improving Teamwork Among Military Pilots: A Review

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Effective teamwork among military pilots is key to successful mission completion. The underlying neural mechanism of teamwork is thought to be inter-brain synchronization (IBS). IBS could also be explained as an incidental phenomenon of cooperative behavior, but the causality between IBS and cooperative behavior could be clarified by directly producing IBS through extra external stimuli applied to functional brain regions. As a non-invasive technology for altering brain function, transcranial electrical stimulation might have the potential to explore whether top-down enhancement of the synchronization of multiple brains can change cooperative behavioral performance among members of a team. This review focuses on the characteristic features of teamwork among military pilots and variations in neuroimaging obtained by hyper-scanning. Furthermore, we discuss the possibility that transcranial electrical stimulation could be used to improve teamwork among military pilots, try to provide a feasible design for doing so, and emphasize crucial aspects to be addressed by future research.

Keywords: teamwork, hyper-scanning, tACS, IBS, fNIRS, military pilot

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

Received: *28 April 2022*
Accepted: *24 June 2022*
Published: *13 July 2022*

Citation:
Lu H, Zhang Y, Huang P, Zhang Y, Cheng S and Zhu X (2022) Transcranial Electrical Stimulation Offers the Possibility of Improving Teamwork Among Military Pilots: A Review. Front. Neurosci. 16:931265. doi: 10.3389/fnins.2022.931265

INTRODUCTION

With the rapid development of network information technology and the deepening of data resource sharing, the mode of modern war has become one of combined arms strategies implemented through multi-unit cooperation. The use of electronic countermeasures and stealth operations make combat missions more complex and changeable, making it difficult for a single combat unit (such as a single soldier or a single aircraft) to be competent for all relevant tasks. Instead, effective operation must involve the cooperation of combat units (Min, 2013). The premise of coordination is to form a combat team with team members as the core. So long as they operate as a "team" rather than as a "group," synergistic benefits can be achieved at lower cost, such that "$1 + 1 > 2$." Generally, modern air force combat units always take the form of action teams consisting of two or more aircraft (Ohlander et al., 2016a). The level of coordination between team members in air battle has been found to play an important role in the successful completion of a military mission, and it is significantly positively correlated with team performance (Ohlander et al., 2009). In recent years, there has arisen not only human–human team cooperation, but also human–machine cooperation, which has rapidly developed into a new mode of combat (Stowers et al., 2021). Such a hybrid team similarly provides a combination of human decision-making and a machine information sharing chain, greatly improving the effectiveness of air combat (Jian, 2017). Teamwork thus plays an important role in military flight operations, and it is of great significance to maximize team cooperation in order to achieve military objectives. It

is particularly important to find a way to effectively improve teamwork in both peacetime and wartime. As a representative non-invasive brain intervention technology, transcranial electrical stimulation has been proven to improve individual cognitive functions such as attention, execution, and risk decision-making by changing neuronal excitability or inducing neural synchronization and oscillation through low-intensity current (Guo et al., 2018; Kronberg et al., 2020; Lipka et al., 2021; Lu et al., 2021, 2020). Davis and Smith (2019) have discussed the risks and benefits of transcranial electrical stimulation technology in military applications and has affirmed the military advantages of transcranial electrical stimulation (such as cognitive improvement in combat, enhancement of survivability for emergency, and so on), believing that this technology could have a great potential in improving military combat effectiveness in the future. Improving the cognitive ability of individual soldiers might have a positive impact on teamwork. However, studies of teams often require a holistic analysis of individuals in a collaborative context, and exploring possible roles for transcranial electrical stimulation in intra-team cooperation is critical for both current needs and military preparedness. It has been proven that transcranial electrical stimulation technology cannot only enhance cognitive ability but also have different degrees of positive impact on social interactions between multiple individuals (Peled-Avron et al., 2019; Pan et al., 2021). Although the range of studies has been limited, the conclusions of the existing studies indicate that transcranial electrical stimulation technology could be a crucial way to improve the capabilities of military pilot teams in the future.

This review summarizes and discusses prior relevant studies, which are divided into the following groups. First, we introduce the concept and features of military pilot teamwork. Second, imaging studies on the potential neural mechanisms of teamwork among military pilots are summarized. Third, this paper reviews the research on the improvement of teamwork among military pilots, and in particular the effect of transcranial electrical stimulation technology on improving teamwork. Finally, we indicate the limitations of current research and propose future prospects for the improvement of teamwork in military pilots by transcranial electrical stimulation.

CONCEPT AND FEATURES OF MILITARY PILOT TEAMWORK

As the basic unit of an organization, a team is composed of two or more individuals. In order to achieve a common team goal, team members maximize the team's benefits through orderly division of labor. This form of organization plays an important role in the survival of animals in nature and in the operation of human social activities (Salas et al., 1992; Anderson, 2001). Not only lions and wolves (Scheel and Packer, 1991; Anderson, 2001; Pennisi, 2017), but also medical and military teams are all typical teams (Doyle et al., 2020; Martin et al., 2022). Salas et al. (2005) put forward the "Big Five" model of teamwork on the basis of prior studies. This model contains five core factors (team leadership, mutual performance monitoring, backup behavior, adaptability, and team orientation) and three additional factors (shared mental model, closed-loop communication, and mutual trust). This model has enjoyed wide support over the past decade, and it has been studied and applied in various fields such as medical treatment, rescue, aviation, the military, and air traffic control (Driskell et al., 2018; Svensson et al., 2020). Thus far, it has been verified that the teamwork model proposed by Salas et al. (2005). is applicable to teams of military pilots (Ohlander et al., 2018). Ohlander et al. (2019) integrated the five core elements of the "Big Five" teamwork model with the three coordinating factors and found that the importance of mutual performance monitoring, closed-loop communication, shared mental model, adaptability, mutual trust, team orientation, team leadership, and backup behavior decreased in turn, after interviewing a group of experienced active fighter pilots. Further research found that fighter pilot teamwork should be analyzed within a full mission cycle, which includes building the flight team before a mission (choosing members, appointing a team leader, task allocation, etc.), team discussion (division of labor, implementation rules, etc.), performance of the flight mission, reflection and discussion after the mission, and finally team dissolution (Ohlander et al., 2016a,b). As shown in **Figure 1**, the importance of the factors at each stage is different. At the beginning of team building, mutual trust and team orientation are most important, as they are the prerequisite for the successful completion of team tasks. In the early stages of the task, group members often discuss and exchange ideas, familiarize themselves with the task process, and establish a shared mental model. The stage of task execution requires close communication between team members, backup behavior, mutual performance monitoring, and adaptability for emergencies. After the task, a meeting is held to update, based on experience, the existing shared mental model.

In summary, military pilots often fly as a team to successfully complete military missions. The "Big Five" team model can explain the weights of different factors at different stages of a mission, but the interviews were based only on subjective data. They lack the support of biological evidence, which limits the possibilities for in-depth understanding of the occurrence and development of team cooperation. The essence of collaboration among team members is still a complex social interaction behavior, including the most basic cooperation, interpersonal learning, trust, etc. Such social interaction between individuals has been proven to be key to the success of teamwork (Lechler, 2001). Therefore, using mature neuroimaging technology to explore the neural mechanisms of team cooperation can provide a valuable reference to reasonably adjust the team structure, cooperation strategy, and team member training.

NEUROIMAGING STUDIES ON THE POTENTIAL NEURAL MECHANISMS OF TEAMWORK IN MILITARY PILOTS

Team behavior among military pilots is a type of social interaction: in essence, information exchange and sharing between individual nervous systems. However, some information is lost due to natural physical isolation during transmission, such

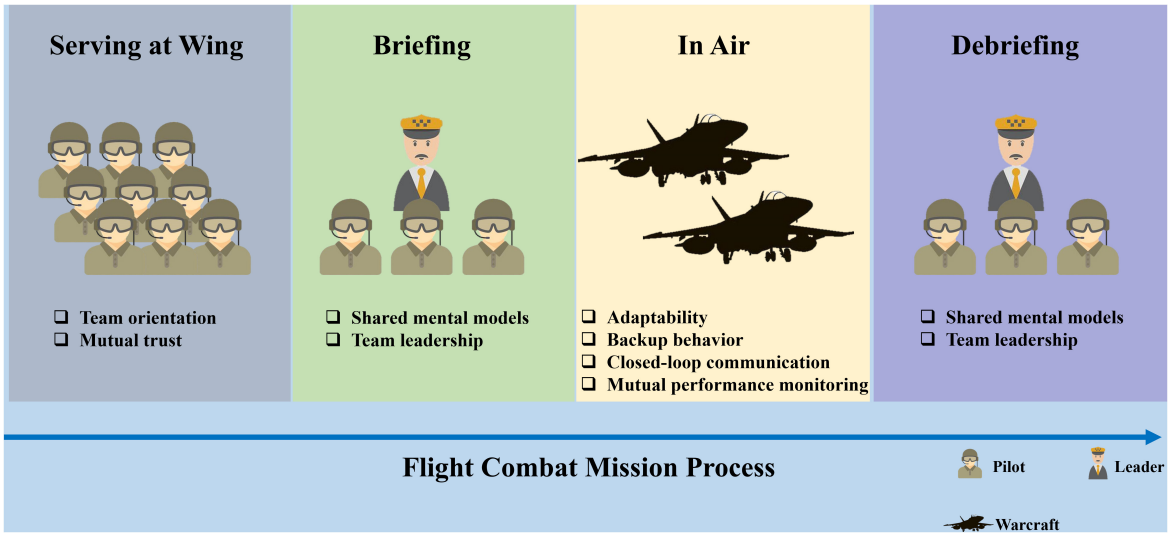

FIGURE 1 | Teamwork factors required at different flight combat mission stages. Adapted from Ohlander et al. (2019).

as sense perceptions, behavior, language, etc. (Kingsbury and Hong, 2020). Therefore, the study of teamwork among military pilots should not be limited to the observation of behavioral performance. In order to obtain more adequate information, we need to explore the neural mechanisms underlying teamwork. Previous imaging studies in cognitive neuroscience have usually focused on a single individual or single brain – for example, a cognitive function or emotional response that is accompanied by changes in the activation of a specific brain region or changes in the functional connectivity of multiple brain regions. Neuroimaging studies of multiple individuals in groups have only emerged in the last two decades. Hyper-scanning refers to a technology that can support real-time signal transmission, recording, and analysis between two or more brains, which can be used to explore the neural mechanisms of social interaction (Hari and Kujala, 2009; Nguyen et al., 2021a). Functional magnetic resonance imaging (fMRI), electroencephalography (EEG), functional near-infrared spectroscopy (fNIRS), and other brain imaging techniques can be used for hyper-scanning studies (Czeszumski et al., 2020). Blood oxygenation level dependent (BOLD) signals of cerebral blood flow are used in fMRI to perform tomography with high spatial accuracy but low temporal resolution; this technology indirectly reflects the activity of brain neurons. Hyper-scanning studies by fMRI to date have lacked real social interaction and sufficient ecological validity. Such studies are generally designed around subjects' subjective imagination (Shibata et al., 2011), network communication between subjects, etc. (Redcay et al., 2010). Compared with fMRI, EEG has had a wider application in hyper-scanning through the real-time acquisition of EEG signals from multiple individuals with high temporal resolution, which can capture transient electrophysiological signals of brain activities under rapid stimulation. However, the weak spatial resolution of traditional EEG cannot accurately observe the activation of brain regions, and limited movement tolerance weakens the possibility of using such an experimental design in real activities (Liu et al., 2018). Fortunately, the recent studies have already shown that electrophysiological source imaging (ESI) based on EEG would provide improved spatiotemporal precision for further application of EEG (He et al., 2018; Edelman et al., 2019; Seeber et al., 2019), and that motion artifacts of EEG could be promisingly rejected by using dry flexible electrodes, in-ear EEG, optimized algorithms of signal processing and so on (Seok et al., 2021). fNIRS has been applied for recording hemoglobin concentrations in particular brain regions by near-infrared light; it has been widely used in the study of infant neurodevelopment due to its strong tolerance for movement (Teresa and Marisa, 2015). Thus, fNIRS will play an important role in the future of social interaction studies thanks to its moderate spatial resolution, temporal resolution, motion tolerance, and portable operation (Mayseless et al., 2019; Reindl et al., 2019; Li et al., 2021). Therefore, a teamwork neuroimaging study based on fNIRS hyper-scanning will be emphasized here. It's worth mentioning that optically pumped magnetometers (OPMs) enabled wearable magnetoencephalography (MEG) is a new and competitive approach to assess brain function (Boto et al., 2018), which would be considered have potential to provide a guidance for the neural mechanisms of teamwork in the future.

Inter-brain synchronization (IBS) usually occurs when individuals in a social interaction have shared behaviors or intentions (i.e., cooperation) (Mayseless et al., 2019). Generally, the index for assessing IBS is coherence calculated by oxy-hemoglobin (HbO) concentration which has higher signal-to-noise ratio than deoxy-hemoglobin (HbR) concentration in fNIRS studies, and so IBS is also referred to as interpersonal brain coherence (Cui et al., 2012). IBS is an indicator of the degree of consistency of brain activity, obtained by hyper-scanning two or more individuals in a group (Xu et al., 2012). IBS of the bilateral dorsolateral prefrontal cortex during cooperative behavior among team members is stronger than that during competitive

behavior, and such a synchronization effect increases over time (Lu and Hao, 2019). Liu et al. (2021) conducted a nine-person drumming experiment with three experimental modes: random drumming, group focus drumming, and metronome focus drumming. They found that the self-reported interdependence was higher in the group focus drumming mode and was accompanied by higher IBS of the temporoparietal junction and the medial prefrontal cortex, representing an understanding of others' thoughts and intentions. These results provided imaging evidence for the important role of shared mental models in team cooperation. Interestingly, team creativity was higher in the cooperative condition than in the competitive condition, and the increased creativity was associated with enhanced IBS of the right dorsolateral prefrontal cortex and the right temporo-parietal lobe (Lu et al., 2019). Therefore, IBS of functional brain regions seems to be a potential neural mechanism of teamwork and is closely associated with team creativity. In addition, IBS may be influenced by factors such as intimacy, gender, profession, social experience, etc. IBS between father and child in the bilateral dorsolateral prefrontal cortex and the left temporoparietal junction were significantly increased during cooperative tasks (Nguyen et al., 2021b). A prior study of IBS between mother and child further showed that children's responsiveness can promote their commitment compliance through the mediating effect of IBS of the temporoparietal junction (Zhao et al., 2021). This evidence indicates that the enhancement of IBS in the corresponding brain region is promoted by a healthy parent-child relationship, which is of great significance for the psychological development of children. IBS could be affected by gender, in that the IBS of the prefrontal lobe is higher in heterosexual cooperation than in homosexual cooperation, and this neural synchronization is directional (female to male) (Cheng et al., 2015; Pan et al., 2017). Occupation is also one of the factors that influence IBS. Athletes majoring in team sports have shown better cooperative behaviors than other subjects, accompanied by significant IBS in the dorsolateral prefrontal region (Li et al., 2020). Individuals whose social experiences differed from each other had better cooperative behavior and greater IBS than those with similar social experiences (Sun et al., 2021). Meanwhile, the team creativity of individuals with low creativity was equal to that of individuals with high creativity, and IBS intensity of the frontal lobe of the former was higher than that of the latter (Hua et al., 2018).

An enhancement of IBS during cooperative behavior between pilots was also observed in previous studies. Similar to Ohlander et al.'s (2016b) evaluation of changes in the core elements of fighter pilot teamwork during flight, IBS in functional brain regions also changed at different stages of flight tasks. It has been found, when using scanning technology for real-time monitoring of brain signals of each of two pilots during a simulated flight mission, that IBS of the frontal and parietal cortex calculated by EEG signals in alpha or theta band, was strongest when two pilots fly in the most difficult phases (take-off and landing) requiring the highest level of cooperation, and that IBS of the frontal and parietal cortex was weak or even zero in the other process of flight (Astolfi et al., 2012; Toppi et al., 2016). Therefore, IBS in functional brain regions seems to be a valid neural indicator of teamwork. However, it should be noted that these indicators have merely been shown to accompany cooperative tasks, and whether they could be used as a scientific explanation of cooperative behavior remains to be further determined. Classic cognitive neuroscience studies have a similar limitation in that correlations between time-dependent behavioral changes and neurological indicators cannot be used as a basis for causal inference. This question will be discussed in detail later.

RESEARCH ON THE IMPROVEMENT OF TEAMWORK AMONG MILITARY PILOTS

Effective teamwork has been proven to play an important role to deal with unexpected instances in the public health sector's response to the COVID-19 crisis (Tomer et al., 2021). The same is true for military pilots, and the question of how to ensure strong teamwork to maximize the effectiveness of the team is particularly crucial. At present, research on the improvement of teamwork can broadly be classified into optimization of team structure, improvement of communication among members, skill training, motivation, and enhancement of brain area function.

Team structure is extremely important for the whole team, and a reasonable team structure can often determine whether a task is successfully completed. For example, a medical team in the intensive care unit mainly includes attending doctors, medical interns, nurses, pharmacists, dietitians, and other staff members. Only with the complementary advantages of these staff can teamwork be maximized and the safety of critically ill patients be guaranteed Coleman and Pon (2013). In addition, the team as a whole should be established on the premise of effective communication around shared team goals or mental models, as effective communication between members can ensure that information is fully and accurately transmitted within the team. One study has found that the communication ability of team members was significantly improved, resulting in increased satisfaction of their patients, after communication training in an outpatient environment (Dodge et al., 2019). With regard to skill training, a team member not only contributes to the common goal, but also gives full play to one's unique advantage. Therefore, professional skill training not only improves individual ability unilaterally, but also reduces the probability of weaknesses of the team. Motivation factors have also been found to play a key role in the application of team training to improve teamwork (Tabassi et al., 2012). All of the above are classic behavioral methods, which directly promote teamwork behavior by changing the external performance of individuals or the whole team. Cognitive neuroscience generally believes that stable changes in behavior depend on variations in neural mechanisms, but the aforementioned methods promote teamwork by changing the environment (i.e., team structure) or behavioral habits (i.e., communication, skills, etc.), rather than directly intervening in the target brain regions. In addition, this kind of method requires more training resources, training time, experience, etc. Based on the aforementioned studies of neuroimaging related to teamwork behavior, IBS appears to be the underlying neural mechanism of teamwork. Therefore, we suspect that the synchronization of

neural oscillations between members supports the occurrence and development of collaborative behaviors. Can IBS in the corresponding brain regions of individuals be changed by external intervention, and can teamwork be affected thereby? In theory, such a top-down approach is easy to implement by using non-invasive brain stimulation technology. This can help us to solve two problems: proving the causal relationship between IBS and cooperation behavior, quantifying the neural and behavioral benefits induced by external stimuli, and exploring the promoting effects of different stimulus parameters on teamwork.

Concept, Classification, and Characteristics of Transcranial Electrical Stimulation

Transcranial electrical stimulation is a safe, non-invasive technology that delivers low-intensity current to the cerebral cortex to change brain functions by forming a current pathway through scalp electrodes (Reed and Cohen Kadosh, 2018). The clinical applications of transcranial electrical stimulation are extremely wide, extending to conditions such as compulsive behavior, migraine attack, dementia, Alzheimer's, etc. (Brunoni et al., 2012; Khedr et al., 2019; Antal et al., 2020; Grover et al., 2021; Moussavi et al., 2021). Cognitive improvements in healthy individuals have also been observed after transcranial electrical stimulation (Metuki et al., 2012; Katz et al., 2017; Berger et al., 2018; Reinhart and Nguyen, 2019; Borwick et al., 2020). Transcranial electrical stimulation is divided into transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS), and transcranial random noise stimulation (tRNS). tDCS stimulates the target brain region with low-intensity direct current (0.5–2 mA) to change the excitability of neurons (Nitsche et al., 2002). tACS mainly induces synchronous oscillations of neurons in target brain regions through different frequency currents (Herrmann et al., 2016). tRNS, in a sense, is also a special "alternating current stimulation" to change neuronal excitability by delivering stimulation with random frequencies and amplitudes within a specific stimulation range, which has been proved to produce more promising benefit on auditory perception than other transcranial electrical stimulation (Fertonani and Miniussi, 2017; Prete et al., 2017, 2018). However, tDCS and tACS are most widely used in the study of cognitive improvement at present, so the subsequent introduction will mainly focus on these two methods. High-precision transcranial electrical stimulation has a current path composed of multiple electrodes and high directivity, so it has great advantages in stimulation accuracy and current density compared to traditional transcranial electrical stimulation (DaSilva et al., 2015; Turski et al., 2017; Pa Rlikar et al., 2021). In addition, the following parameters can affect the intervention effect in transcranial electrical stimulation experiments. First, the range of current intensities in transcranial electrical stimulation experiments is generally not higher than 2 mA; generally speaking, current intensities at the upper end of that range bring better intervention benefits. Second, the excitability of the cortex is inhibited under the cathode while increased under the anode during tDCS (Shin et al., 2015). Third, the selected brain region should be covered by the current field, which can be simulated using computer software (Lu et al., 2021, 2020). The last parameter is the frequency of tACS. In general, synchronous neural oscillations are more likely to occur when the stimulus frequency is consistent with the internal frequency of neurons in the functional brain regions (Herrmann et al., 2013; Takeuchi and Izumi, 2021).

The Improvement of Teamwork by Transcranial Electrical Stimulation

It has been found, according to the underlying neural mechanism of teamwork, that it is feasible to modulate brain function through external stimulation to change cooperative behavioral performance between team members. However, as shown in **Figure 2**, there are different intervention models between tDCS and tACS (hyper-tACS) for improving cooperative behavior. The main characteristic of IBS is synchronous nerve oscillations between multiple brains, while the stimulation of tDCS is characterized by direct current interference in a particular brain region, and seems unable to directly induce the synchronization of the corresponding brain region through neural entraining. Enticott et al. (2012) found that tDCS intervention of brain regions (inferior frontal gyrus) involved in the mirror neuron system in healthy individuals enhanced interpersonal motor resonance. The mirror neuron system plays an important role in imitation activities, interpersonal learning, and other behaviors (Oberman et al., 2007; Mainieri et al., 2013; Meng Yuan et al., 2018), and autism is considered in part to be related to dysfunction in the mirror neuron system (Hamilton, 2008). A previous study has shown that cathode tDCS significantly reduced musicians' assessment of musical creativity, which was related to the mediating effect of empathy (Colombo et al., 2021). Anodal tDCS intervention on the right inferior frontal gyrus of healthy subjects is thought to induce imitative behavior in social interaction (Hogeveen et al., 2015). Therefore, although tDCS cannot directly regulate the neural oscillation rhythm in a particular brain region across multiple brains, it could modulate the cooperative behavior of subjects by intervening in the mirror neuron system, which is closely related to cooperation and teamwork. Additionally, as a crucial part in rapid instructed task learning related with teamwork (Meiran et al., 2016), working memory was also proved to be effectively improved by tDCS and other transcranial electrical stimulation, which would provide a way to enhance teamwork (Ke et al., 2019; Nissim et al., 2019; Zeng et al., 2022). However, the nature of cooperative behavior still probably lies in the occurrence and development of multi-brain IBS. Using tACS technology would provide more possibilities for future research into the influence of different frequency and phase parameters on teamwork behavior.

Novembre and Iannetti (2021a,b) argued that the phenomenon of multi-brain IBS observed by hyper-scanning cannot be clearly explained in relation to social interaction: is it actual causality, or mere contingency? Therefore, regulating interbrain synchronization directly through multi-brain stimulation (MBS), such as hyper-tACS, and taking IBS as an independent variable in the study is critical to understanding the

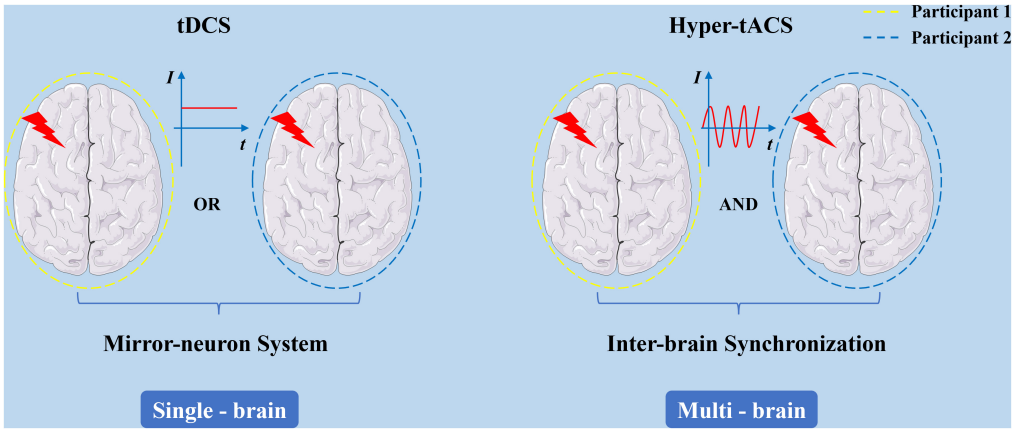

FIGURE 2 | Different intervention patterns of tDCS and hyper-tACS in promoting cooperative behavior.

neural mechanism of social interaction behaviors. Hyper-tACS is used to stimulate one or several functional brain regions to induce the coupling of neural oscillations between multiple brains, with the goal of promoting social interaction behaviors such as collaborative writing and interpersonal learning (Novembre et al., 2017; Pan et al., 2021). The effects modulated by hyper-tACS are always phase-frequency specific. A study found that 6 Hz in-phase hyper-tACS located on the prefrontal lobe could be successfully applied to induce spontaneous synchronous movement between teachers and students. A song teaching effect was also promoted, while interventions at other frequencies or phases did not produce similar effects (Pan et al., 2021). 20 Hz in-phase hyper-tACS on left motor cortex has been found to increase the synchronization of interpersonal movement, while the same results were not present for other frequencies or for anti-phase or false stimulation (Novembre et al., 2017). However, it has been found that such immature hyper-tACS technology does not produce significant changes in promoting synchronicity under the two-person drumming task, although this may be related to the choice of stimulus program (Szymanski et al., 2017). Therefore, hyper-tACS does provide a possibility for the improvement of teamwork or cooperation behavior, but there are still urgent problems to be solved in the future, such as the specific settings of parameters, selection of stimulus programs, synchronous imaging acquisition, compatibility of hardware and software, etc.

RESEARCH PROSPECTS FOR TRANSCRANIAL ELECTRICAL STIMULATION TECHNOLOGY TO PROMOTE TEAMWORK AMONG MILITARY PILOTS

Based on the evaluation of team cooperation among military pilots and related enhancement technology, and taking into account the advantages and disadvantages of methods used in prior studies, this review puts forward possible design ideas for future research on teamwork among military pilots, as shown in **Figure 3**. As a neuroimaging technique suitable for multiple participants, hyper-scanning based on fNIRS should be used to record information about synchronous oscillation in the target brain regions. Next, the connection between behavioral performance during a task (under three conditions: cooperative, competitive, or neutral) and IBS should be analyzed before and after hyper-tACS intervention. The underlying causality would thus be clarified. In addition, the enhancement of military pilot teamwork could be explored based on a credible improvement strategy according to the effective parameters of hyper-tACS, as obtained by laboratory investigation. However, the details of the study design must be optimized by feedback, based on the benefits obtained.

Future studies should mainly focus on two aspects:

Quantification of Behavior and Neural Mechanism in the Teamwork of Military Pilots

Although the teamwork environment of military pilots has characteristics such as high pressure, high noise, narrow scope of activities, high mental load, fast decision-making, and difficult situational awareness, teamwork among military pilots still conforms to the "Big Five" teamwork model. Therefore, despite being a special form of teamwork, military pilot teamwork is still a kind of social interaction. However, prior studies on social interaction behavior were still based on simple laboratory research. Military pilots are faced with a complex and changeable environment when performing tasks, and replicating that environment could be key to quantifying such cooperative behavior with improved accuracy and ecological validity. Virtual reality, simulated aircraft, and flight operation games are all new behavioral quantitative tools with high ecological validity (Bauer and Klingauf, 2006; Hans et al., 2016; Villafaina et al., 2021). Compared to the traditional laboratory paradigm, such tools could provide more vivid operating conditions and increased participation for participants. In addition, the selection of suitable neuroimaging tools (such as fNIRS) with great

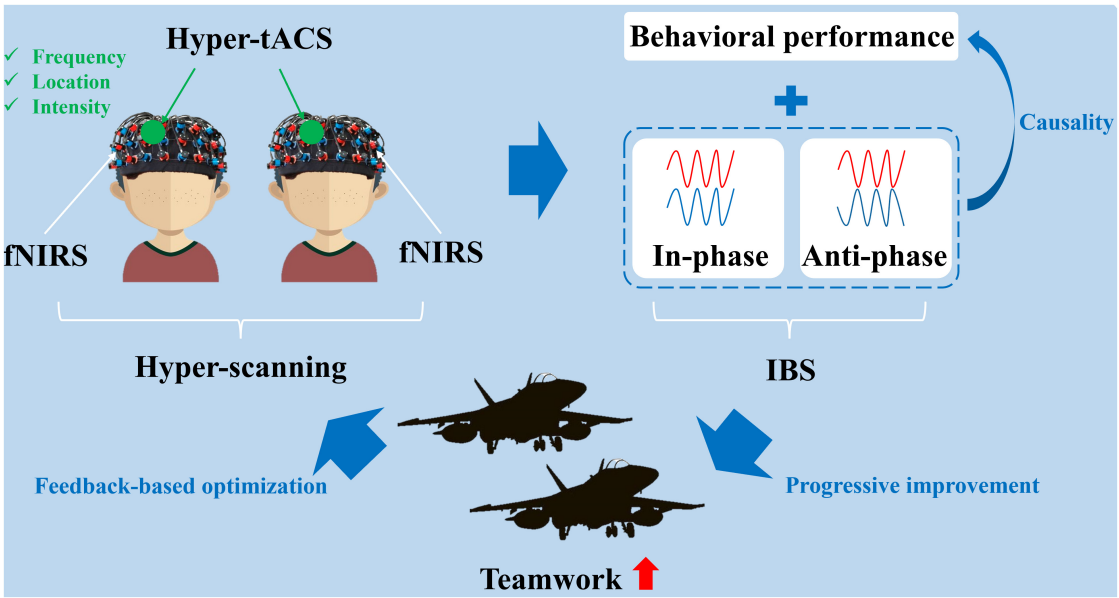

FIGURE 3 | Possible technical routes for research on military pilot teamwork enhancement.

movement tolerance could ensure the collection and analysis of neuroimaging data in such an environment. Portable fNIRS would be more suitable since it is lighter, cheaper, wireless, and has better adaptability in most social scenes compared to traditional wire-based fNIRS (Agro et al., 2016; Gozde, 2017). In particular, fNIRS equipped on each member of the team would provide more comprehensive monitoring of brain regions in the study of interactions between pilots in military fighter formations. The data analysis method for fNIRS also must be selected carefully according to the actual conditions of study. In general, the common method is based on averaging the target signal during the time window before conducting wavelet coherence or Granger causality analysis (Hu et al., 2021), but time information would be lost because the tasks always undergo dynamic changes. This is problematic because different stages of a task are accompanied by different states of IBS. Therefore, a dynamic IBS analysis method would retain time-level information and explore dynamic changes in IBS over time (Li et al., 2021). Accurately quantifying behavioral performance and neuroimaging changes in military pilots during teamwork tasks would help us establish effective evaluation schemes and data sets, and improve the screening validity during team member selection. The difficulty to be overcome in future research would be to select appropriate evaluation methods and parameters to create the prerequisite conditions for follow-up interventions to promote teamwork.

Selection of Hyper-Transcranial Alternating Current Stimulation Scheme to Promote Military Pilot Teamwork

Team structure, professional skills, motivation, and other factors are the most routine and basic approaches for the promotion of teamwork among military pilots (Zhiqiang, 2015; Aitoro, 2019). These methods are carried out from the early stages of pilot training. However, "tacit understanding" training among the members of the flying formation is absent, and this could cause a failure of cooperative behavior among pilots and increase the difficulty of task completion. Due to the tension of a training mission, military pilots cannot afford to spend time on interactive cooperative behavior training, but hyper-tACS could enable a military pilot to obtain high compatibility and more quickly adapt to their partner. The question of how to maximize the intervention effect is also worth exploring in future studies, especially with regard to the selection of stimulus sites, frequency, intensity, and phase of hyper-tACS. Some complex cognitive processes have been shown to be the result of cross-frequency coupling between brain regions (such as the inhibitory prefrontal cortex's regulation of the motor cortex), so it may be necessary to adopt different frequency-coupled stimulus modes during hyper-tACS intervention (Riddle et al., 2021). In addition, there are lingering concerns about the safety of transcranial electrical stimulation. A large number of studies have shown that even repeated stimulation is safe and reliable compared with sham stimulation as long as the operational requirements of electrical stimulation were conducted in strict accordance with safety protocols (Turski et al., 2017; Nikolin et al., 2019). Choe et al. (2016) conducted electrical stimulation in the laboratory on 32 healthy subjects undergoing flight training to explore its influence on flight performance and the relevant data of EEG and fNIRS. Thus, the safety of transcranial electrical stimulation is guaranteed under proper operation. In view of the current model of air combat, how military pilots engage in optimal teamwork plays a key role in successful completion of the mission. Therefore, future research should focus on solving the

problem of how to improve the teamwork behavior of military pilots using a plan that has been optimized based on feedback from the evaluation results.

CONCLUSION

In this review, we have clarified the model of teamwork among military pilots and provided an underlying explanation for the neural mechanism of teamwork. However, although IBS is known to be closely related to cooperative behavior, the question of causality is not clear. Thus, we hypothesize that transcranial electrical stimulation could be applied to directly stimulate brain regions related to teamwork to enhance IBS among multiple members in a team, and the causal link between IBS and cooperative behavior would then be clarified. Furthermore, it is crucial for military pilots to improve their teamwork by either tDCS or hyper-tACS. We therefore provided a feasible study design as a basis for an enhancement strategy. The hyper-scanning and hyper-tACS could provide a possible way for military pilots to enhance their capability for teamwork and would help us better explore the relationship between synchronous oscillation and cooperative behavior. We hope this review can provide some theoretical inspiration for future research on improving the combat effectiveness of military pilot teams, and we put forward suggestions on the basis of current research to improve relevant study designs in the future.

AUTHOR CONTRIBUTIONS

HL and YjZ completed the writing of the manuscript and manuscript revision. PH, YnZ, and SC conducted the search and collation of literature. XZ provided the financial support and writing guidance. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the "13th Five-Year" Major Project of PLA (AWS17J012).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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TYPE Original Research
PUBLISHED 29 July 2022
DOI 10.3389/fnins.2022.905247

OPEN ACCESS

EDITED BY
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Zhejiang University, China

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SPECIALTY SECTION
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

RECEIVED 26 March 2022
ACCEPTED 11 July 2022
PUBLISHED 29 July 2022

CITATION
Guo Z, Gong Y, Lu H, Qiu R, Wang X, Zhu X and You X (2022) Multitarget high-definition transcranial direct current stimulation improves response inhibition more than single-target high-definition transcranial direct current stimulation in healthy participants.
Front. Neurosci. 16:905247.
doi: 10.3389/fnins.2022.905247

Multitarget high-definition transcranial direct current stimulation improves response inhibition more than single-target high-definition transcranial direct current stimulation in healthy participants

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Prior studies have focused on single-target anodal transcranial direct current stimulation (tDCS) over the right inferior frontal gyrus (rIFG) or pre-supplementary motor area (pre-SMA) to improve response inhibition in healthy individuals. However, the results are contradictory and the effect of multitarget anodal stimulation over both brain regions has never been investigated. The present study aimed to investigate the behavioral and neurophysiological effects of different forms of anodal high-definition tDCS (HD-tDCS) on improving response inhibition, including HD-tDCS over the rIFG or pre-SMA and multitarget HD-tDCS over both areas. Ninety-two healthy participants were randomly assigned to receive single-session (20 min) anodal HD-tDCS over rIFG + pre-SMA, rIFG, pre-SMA, or sham stimulation. Before and immediately after tDCS intervention, participants completed a stop-signal task (SST) and a go/nogo task (GNG). Their cortical activity was recorded using functional near-infrared spectroscopy (fNIRS) during the go/nogo task. The results showed multitarget stimulation produced a significant reduction in stop-signal reaction time (SSRT) relative to baseline. The pre-to-post SSRT change was not significant for rIFG, pre-SMA, or sham stimulation. Further analyses revealed multitarget HD-tDCS significantly decreased SSRT in both the high-performance and low-performance subgroups compared with the rIFG condition which decreased SSRT only in the low-performance subgroup. Only the multitarget condition significantly improved neural efficiency as indexed by lower △oxy-Hb after stimulation. In conclusion, the present study provides important

preliminary evidence that multitarget HD-tDCS is a promising avenue to improve stimulation efficacy, establishing a more effective montage to enhance response inhibition relative to the commonly used single-target stimulation.

KEYWORDS

high-definition transcranial direct current stimulation (HD-tDCS), response inhibition, right inferior frontal gyrus (rIFG), pre-supplementary motor area (pre-SMA), fNIRS

Introduction

Response inhibition refers to the ability to inhibit inappropriate or irrelevant responses so that one can make flexible and goal-directed behavioral responses to changes in the environment, which is an important part of executive function (Verbruggen and Logan, 2008, 2009; Diamond, 2013). Response inhibition is involved in many everyday activities, such as a driver stopping from pressing the accelerator in order to not hit a pedestrian. Prior studies have shown that response inhibition is related to decision-making (Xu et al., 2020), working memory (Alderson et al., 2017), impulse control (Mayer et al., 2020), etc. Additionally, many psychiatric disorders are associated with deficits in response inhibition (Hughes et al., 2012; Steele et al., 2014; van Rooij et al., 2015; Gowda et al., 2019; Alizadehgoradel et al., 2020; Sun et al., 2021). In recent years, studies have increasingly focused on the neural substrates of response inhibition and have demonstrated that it is based on the right hemispheric fronto-basal ganglia network, including the right inferior frontal gyrus (rIFG), the pre-supplementary motor area (pre-SMA), and the basal ganglia (Aron and Poldrack, 2006; Aron et al., 2016; Hannah and Aron, 2021). The importance of the rIFG and pre-SMA in response inhibition is well supported by investigations of traumatic brain injury and transcranial magnetic stimulation (TMS) (Aron et al., 2003; Chambers et al., 2006; Floden and Stuss, 2006). In summary, the rIFG and pre-SMA are two critical brain regions for the effective execution of response inhibition, and methods aimed at simultaneously promoting the activity of these brain regions provide a new direction for improving response inhibition and treating patients with impaired response inhibition ability.

Transcranial direct current stimulation (tDCS) is a promising method to regulate cortical activity and enhance cognitive ability. Although there are some impact factors limiting the reliability of causal relationship revealed by tDCS, such as limited spatial precision and unwanted brain area activation, tDCS is still a good way to provide causal evidence for the links between brain function and corresponding behavioral changes (Filmer et al., 2014; Gbadeyan et al., 2016; Yavari et al., 2018). tDCS is non-invasive, safe, tolerable, and easy to operate (Bikson et al., 2016; Valiengo et al., 2020; Weidler et al., 2020). It transmits a weak direct current through electrodes placed on the scalp and influences the activity of the cerebral cortex (Nitsche and Paulus, 2000). Generally, anodal stimulation will increase the excitability of the cortex via subthreshold depolarization and long-term potentiation (LTP)-like plasticity, while cathodal stimulation decreases excitability via hyperpolarization and long-term depression (LTD)-like plasticity (Nitsche and Paulus, 2000, 2001; Pisoni et al., 2018).

Currently, tDCS has been widely used in studies on response inhibition, but the results are heterogeneous. Prior studies have revealed elevated response inhibition after anodal stimulation on the rIFG (Jacobson et al., 2011; Stramaccia et al., 2015; Li et al., 2019) and pre-SMA (Hsu et al., 2011; Kwon and Kwon, 2013a,b; Yu et al., 2015) in healthy young participants, indicating that the rIFG and pre-SMA are important targets for enhancing response inhibition using tDCS. However, contradictory results have also been reported, claiming that single-target tDCS over rIFG or pre-SMA is ineffective (Dambacher et al., 2015; Bender et al., 2017; Thunberg et al., 2020). Additionally, the majority of the tDCS studies targeting rIFG or pre-SMA in healthy participants employed conventional tDCS; few studies used high-definition tDCS (HD-tDCS) (Hogeveen et al., 2016). HD-tDCS, an optimized form of conventional tDCS, can produce more prominent behavioral and neurophysiological effects with more superior spatial precision compared with conventional tDCS, supporting its more widespread application (Kuo et al., 2013; Sehatpour et al., 2021). Taken together, this underscores the need to develop more potent protocols using HD-tDCS to improve response inhibition and clarify the validity of single-target tDCS.

It is well known that the normally effective execution of brain function is based on neural networks rather than on isolated brain regions (Ester and Kullmann, 2021). Simultaneous HD-tDCS with identical polarity on multiple functionally related brain regions – in other words, multitarget stimulation – can regulate cortical excitability more efficiently and enhance tDCS effects more prominently than single-target stimulation (Fischer et al., 2017; Hill et al., 2018;

Ester and Kullmann, 2021; Gregoret et al., 2021). Multitarget HD-tDCS has been applied to studies of motor ability and working memory and the results have demonstrated that multitarget stimulation is more effective (Dagan et al., 2018; Hill et al., 2018). However, currently, studies of multitarget HD-tDCS for response inhibition have not been carried out. Therefore, this study aims to investigate the effects of multitarget HD-tDCS on enhancing response inhibition and compare them with the effects of single-target HD-tDCS.

To better understand the neural mechanism of tDCS-induced behavioral changes in response inhibition, relevant neurophysiological tools such as fMRI, positron emission tomography (PET), electroencephalography (EEG), and functional near-infrared spectroscopy (fNIRS) should be used in conjunction with behavioral tasks. However, the application of fMRI and PET is limited by their large bulk and immobility, and the accuracy of EEG signal is easily disturbed with the artifacts, so fNIRS may be the ideal tool for tDCS research (Yaqub et al., 2018; Yang et al., 2019; Friehs et al., 2021b), and it has been used to monitor hemodynamic changes induced by tDCS intervention (Yaqub et al., 2018; Lu et al., 2020). fNIRS is an optical and non-invasive neuroimaging method, with the advantages of greater tolerance to motion artifacts, high adaptability, portability, low cost, and participant-friendliness. Hence, fNIRS can overcome some limitations of the aforementioned imaging technologies. It can measure the concentrations of oxyhemoglobin and deoxyhemoglobin in brain tissue in a more natural situation (Scholkmann et al., 2014; Pinti et al., 2020; Veit et al., 2021). In recent years, fNIRS has been employed in the study of response inhibition and the results have revealed increased oxyhemoglobin concentrations in the prefrontal cortex during response inhibition (Herrmann et al., 2005; Hudak et al., 2017). However, few response inhibition studies have applied fNIRS to measure the neural activity of relevant brain regions before and after tDCS.

In order to overcome the limitations of previous studies, this study was designed to examine the effects of multitarget anodal HD-tDCS on improving response inhibition and to determine whether anodal stimulation of the rIFG or pre-SMA actually enhances response inhibition compared to sham stimulation. We hypothesized that HD-tDCS applied to rIFG + pre-SMA, rIFG, or pre-SMA could all enhance response inhibition compared with sham stimulation. We further anticipated that multitarget HD-tDCS could be more effective at improving response inhibition. As far as we know, this is the first study to examine the effect of multitarget anodal HD-tDCS on response inhibition and to compare the effects of different stimulation montages.

Materials and methods

Participants

A total of 92 healthy college students (mean age = 20.58 ± 1.54 years, range = 18 – 24 years, 43 males) participated in the experiment and were randomly divided into four groups: (1) multitarget anodal HD-tDCS (rIFG + pre-SMA condition), n = 22; (2) anodal HD-tDCS on the rIFG (rIFG condition), n = 24; (3) anodal HD-tDCS on the pre-SMA (pre-SMA condition), n = 22; and (4) sham stimulation, n = 24. All participants had normal or corrected-to-normal vision and were right-handed as assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). Inattention and impulsivity were assessed by the Adult ADHD Self-report Scale (ASRS); only participants with an average score of 17 and below were included, as individuals with a sum score on either subscale of 17 or higher were considered likely to have ADHD (Kessler et al., 2005; Yeh et al., 2008). All participants were naive to the nature of the study and were screened to ensure that the final sample included only neurologically and psychiatrically healthy individuals without any contraindications (e.g., metal implants in the head, pregnancy, a history of seizures, etc.) to tDCS, and none of the participants reported taking any psychotropic medication. G*Power 3.1.9.6 was used to compute *a priori* sample size, and a minimum sample N of 48 (12 per group) was needed with a medium effect size of f = 0.25, a power of 1 - β = 0.80, and an α-value of 0.05 (Cohen, 1992; Faul et al., 2007).

TABLE 1 Basic characteristics of participants (numbers or means and standard deviations).

Variable	rIFG + pre-SMA	rIFG	pre-SMA	sham	F/χ^2	p
n	22	24	22	24		
Gender (male/female)[a]	11/11	11/13	10/12	11/13	0.124	0.989
Age (years)[b]	20.82 (1.47)	20.38 (1.66)	20.41 (1.76)	20.71 (1.27)	0.457	0.713
Education (years)[b]	15.68 (1.17)	15.29 (1.71)	15.41 (1.59)	15.75 (1.54)	0.483	0.695
ASRS-inattention[b]	11.41 (3.67)	9.46 (3.78)	11.64 (3.86)	10.63 (2.78)	1.790	0.155
ASRS-hyperactivity/impulsivity[b]	7.95 (4.13)	7.58 (4.03)	8.00 (3.95)	8.04 (4.29)	0.063	0.979

ASRS, Adult ADHD Self-report Scale; rIFG, right inferior frontal gyrus; pre-SMA, pre-supplementary motor area; a, χ^2 test; b, one-way analysis of variance; $p < 0.05$ was considered significant.

The groups were matched in basic characteristics (**Table 1**). Participants gave their written informed consent before the experiment. The study was approved by the Tangdu Hospital Ethics Committee and abided by the Declaration of Helsinki.

Design and procedure

The experiment followed a single-blind, randomized, between-subject, and sham-controlled design (**Figure 1**). The two important tasks used to study response inhibition are the stop-signal task (SST) and the go/nogo task (GNG) (Cunillera et al., 2016). In this study, we employed both SST and GNG in order to increase the robustness of the results. To detect neural changes, we chose to collect fNIRS data during behavioral tasks. However, we only recorded fNIRS signals during GNG but not during SST because the design of fNIRS recording was drawing upon previous studies, which utilized SST and GNG as behavioral assessment but only recorded fNIRS during GNG (Hudak et al., 2017). Additionally, collecting fNIRS signals during both SST and GNG takes more time to record, which may make participants feel uncomfortable because they need to keep still in the process. Before the experiment, participants participated in a brief interview to collect basic demographic information, complete the ASRS, and screen for their eligibility for tDCS. Each participant completed a pretest including SST and GNG in a counterbalanced order and fNIRS data were recorded during the GNG. Then they were randomly assigned to receive one of the four types of single-session stimulation. After tDCS application, they immediately received a posttest identical to the pretest as well as a questionnaire to evaluate side effects and blinding efficacy. Tasks were programmed and run on E-prime 3.0 software (Psychology Software Tools, Inc., Sharpsburg, PA). Before starting each task, participants were given instructions on how to complete it. The whole experiment was performed within 120 min.

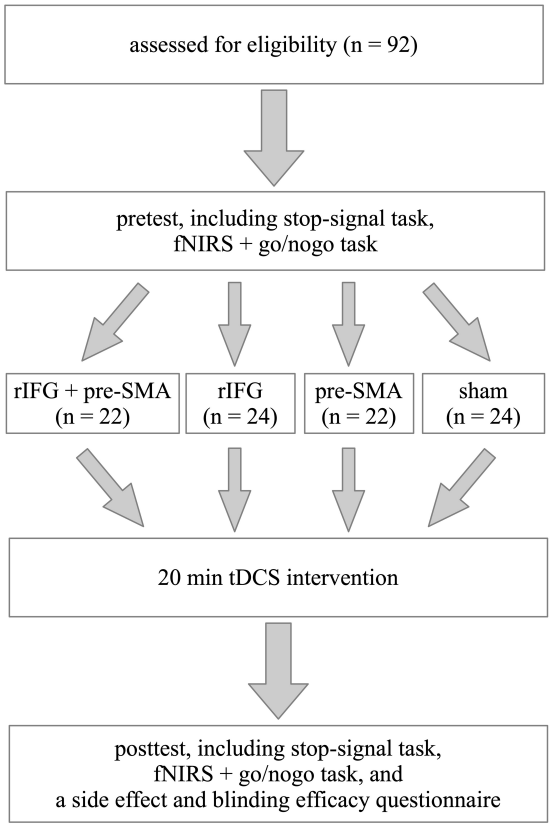

FIGURE 1
Schematic illustration of the study design. The experiment followed a single-blinded, randomized, between-subject, sham-controlled, and pretest-posttest design.

High-definition transcranial direct current stimulation

High-definition transcranial direct current stimulation (HD-tDCS) was applied by a Soterix Medical MXN-9 High-Definition Transcranial Electrical Stimulator (Soterix Medical, Inc., New York, United States). This study followed all procedures for using HD-tDCS as demonstrated previously (Villamar et al., 2013). The electrodes were localized using the 10-10 EEG system (Jurcak et al., 2007). The montage was determined and the corresponding electric field and current flow were generated (**Figure 2A**) using HD-Targets and HD-explore software (Soterix Medical, Inc., New York, United States). This method has been widely used in prior studies and has proven to be effective (Nikolin et al., 2015; Hogeveen et al., 2016; Reinhart and Nguyen, 2019; Maldonado and Bernard, 2021). The parameters of each electrode in each verum stimulation condition are listed below (**Table 2**). Participants in the sham stimulation condition were pseudo-randomized to receive one of the three verum stimulation montages (Hill et al., 2018). The pseudo-randomization is different from randomization because it is generated by some algorithms. In this study, participants in the sham stimulation condition were sorted according to the ascending order of their names and were labeled with number 1, 2, and 3 in order. Number 1, 2, and 3 represented the participant received the rIFG + pre-SMA condition, rIFG condition, and pre-SMA condition, respectively. All conditions were conducted with the same electrode placement as the multitarget condition with only the currents changed for blinding purposes (Schneider et al., 2021; Zhou et al., 2021). The panel of the instrument was not visible to the participants. HD-tDCS was delivered at 2.5 mA for multitarget stimulation and 1.25 mA for single-target stimulation. These intensities have been proven safe and reliable enough to improve cognitive performance (Villamar et al., 2013; Hogeveen et al., 2016; Abellaneda-Perez et al., 2021; Zhou et al., 2021). Verum stimulation was applied for

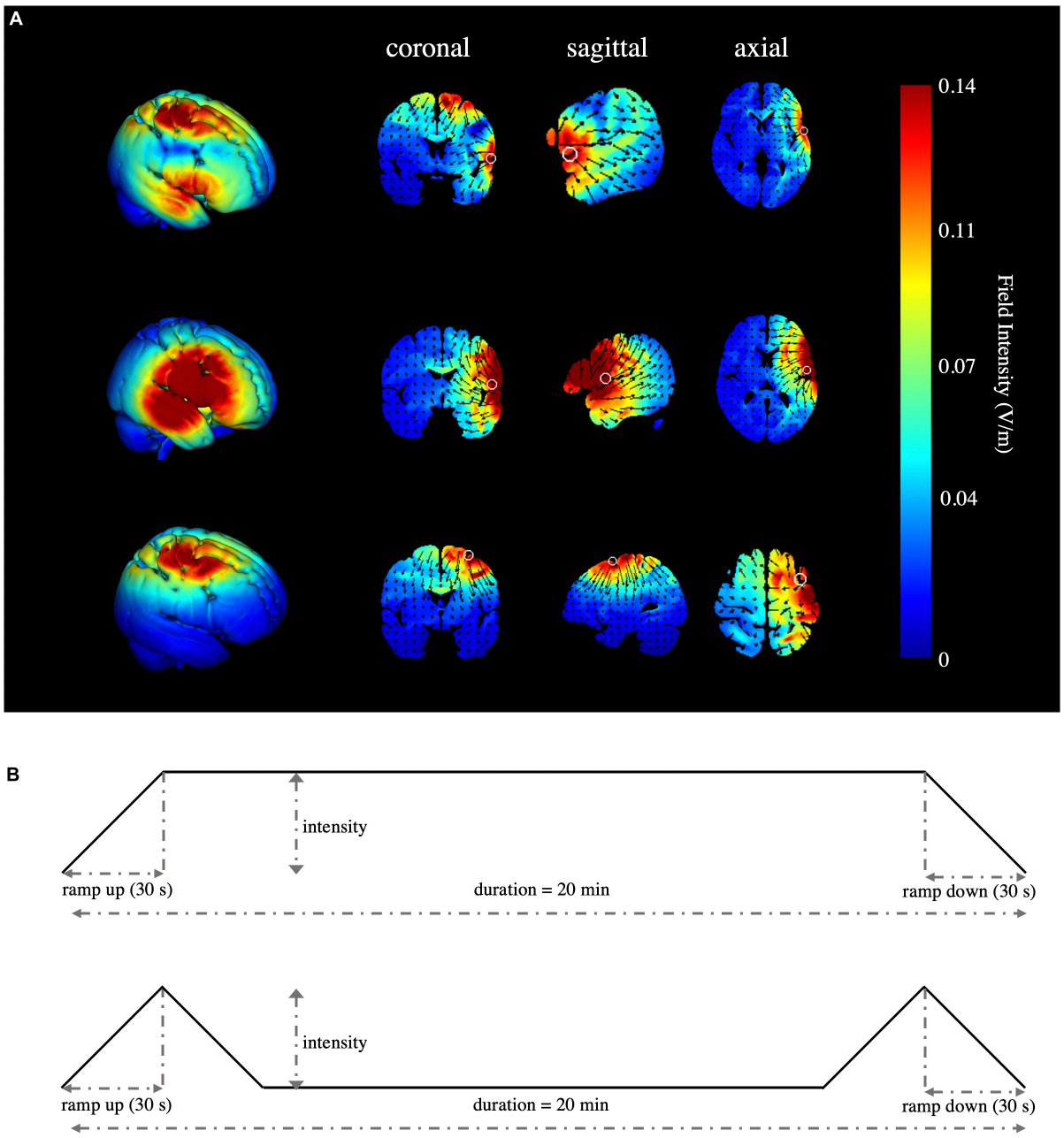

FIGURE 2
Simulated stimulation conditions and time progression of tDCS. **(A)** Simulated electric field and current flow of rIFG + pre-SMA (top), rIFG (middle), and pre-SMA (bottom). The color bar represents the field intensity and the arrow points in the direction of current flow. Column 1 is a 3D view, while column 2 to column 4 range from coronal to axial slices. **(B)** Schematic illustration of the duration of tDCS, ramp-up, and ramp-down periods for verum (top) and sham stimulation (bottom). The current intensity was delivered at 1.25 mA for single-target HD-tDCS, 2.5 mA for multitarget HD-tDCS, and 1.25 mA or 2.5 mA in a pseudo-random order for the sham stimulation condition.

20 min with a ramp up and ramp down of 30 s each. Sham stimulation consisted of a 30 s ramp up and a 30 s ramp down at the beginning and the end, respectively, with no current during the intervening time (**Figure 2B**), facilitating blinding by mimicking the sensations of verum tDCS without actual neurological changes (Di Rosa et al., 2019; Sharma et al., 2021). After stimulation, participants were asked whether they received verum or sham stimulation and how confident they were based on a scale from 0 (complete guess) to 10 (absolutely sure), Additionally, another 11-point scale was used to evaluate the intensity of any sensations (e.g., itching, tingling, metallic taste, or burning) they felt during the stimulation,

TABLE 2 Location and current intensity (mA) of each electrode for each verum stimulation condition, according to the international 10-10 system.

Electrode location	rIFG + pre-SMA	rIFG	pre-SMA
Fz	−0.51	0.00	−0.32
C2	1.48	0.00	1.25
FC4	−0.41	−0.32	−0.31
C4	−0.52	−0.31	−0.31
P4	−0.36	0.00	−0.31
FT8	1.02	1.25	0.00
FT10	−0.53	−0.31	0.00
TP8	−0.17	−0.31	0.00
Total current	2.50	1.25	1.25

with 0 = no sensation and 10 = strongest sensation imaginable (Hill et al., 2017).

Stop-signal task

We employed a valid and reliable behavioral task, the stop-signal task (SST), to investigate response inhibition (Logan et al., 1984; Verbruggen and Logan, 2008; Verbruggen et al., 2019), in which participants responded to a go stimulus (also referred to as the primary task). Occasionally, the go stimulus was unpredictably followed by a stop signal at irregular intervals; the stop signal instructed participants to withhold their response. The SST settings we applied were consistent with the current consensus (Verbruggen et al., 2019). On the prepotent go stimuli (75% of total trials), participants were required to press "F" on the keyboard with their right index finger in response to left arrows and press "J" with their right ring finger in response to right arrows as quickly and accurately as possible. However, on a minority of trials (25%), a small red square (stop signal) was presented above the arrow after an interval (stop signal delay, SSD), indicating the need to cancel the planned response. The SSD started at 250 ms and was dynamically adjusted by a tracking procedure (50 ms increment/decrement for successful stopping/unsuccessful stopping, range = 0 – 1250 ms) to ensure that each participant successfully inhibited about 50% of the stop trials. Details about the task procedures and the duration of fixation, stimulus presentation, and blank are displayed in **Figure 3A**. Besides a practice block of 48 trials (25% stop-signal trials), there were 200 trials in the test block, including 150 go trials and 50 stop-signal trials, all presented in a randomized order. We estimated the covert latency of the inhibition process by using the stop-signal reaction time (SSRT) as calculated by the mean method, which subtracted the mean SSD from the mean reaction time in all correct go trials when the overall stop accuracy converged at 0.5 (Logan et al., 1984; Verbruggen and Logan, 2009; Hogeveen et al., 2016; Bartholdy et al., 2019; Chen et al., 2020), with shorter SSRTs indicating superior response inhibition. In addition to SSRT, stop accuracy (the probability of correctly withholding responses on stop trials) and goRT (mean RT on correct go trials) were also assessed.

Go/nogo task

The go/nogo task was designed to induce response inhibition. In the current study, we recorded fNIRS during GNG, and thus we redesigned the task in a block-design paradigm (**Figure 3B**). The task began with a go block with subsequent blocks alternating between go and nogo (four repetitions each, and 12 trials per block) separated by rest blocks, with each block lasting 30 s (Herrmann et al., 2005; Hudak et al., 2017). Prior to the actual fNIRS measurement, participants were given instructions for the following two task blocks, and they were told to sit in a relaxed position and keep still to avoid head movements. A 5 s cue appeared before each rest block to alert participants whether the next block was a go or nogo block (Nagashima et al., 2014; Lu et al., 2020). In the go block trials, participants were asked to respond as quickly and accurately as possible to each stimulus (number 1, 2, and 4) by pressing "J" on the keyboard with their right index finger. In the nogo block trials, the participants had to press "J" as quickly and accurately as possible in response to the go stimuli (number 1, 2, and 4), whereas they were instructed to withhold their response to the nogo stimulus (number 3) following the fixation cross. For the go blocks, all 12 trials were go stimuli, and for nogo blocks, both go and nogo stimuli had the same occurrence probability of 0.5. In total, the go/nogo task maintained a ratio of 75% go and 25% nogo trials (Herrmann et al., 2005; Nagashima et al., 2014; Rodrigo et al., 2014). For each trial, a fixation cross appeared in the center of the screen for 1000 ms, and then the number stimulus was presented for a maximum of 500 ms or until reaction. Once the participant responded to the stimulus, it disappeared immediately and a blank screen appeared. The stimulus and blank were together presented for 1500 ms (**Figure 3C**) (Herrmann et al., 2005). In addition to goRT and nogo accuracy (the possibility of successful inhibition in nogo trials), inverse efficiency score (IES) was analyzed and adopted as the primary outcome. IES may be a better indicator to measure GNG performance in consideration of the tradeoff between speed and accuracy, with a lower value reflecting higher performance (Bruyer and Brysbaert, 2011; Zhao et al., 2018). IES was calculated by dividing goRT by the percentage of all correct responses (the number of correct go trials and nogo trials divided by the total number of trials).

Functional near-infrared spectroscopy

Changes in oxygenated (oxy-Hb) and deoxygenated (deoxy-Hb) hemoglobin were measured using the LABNIRS fNIRS

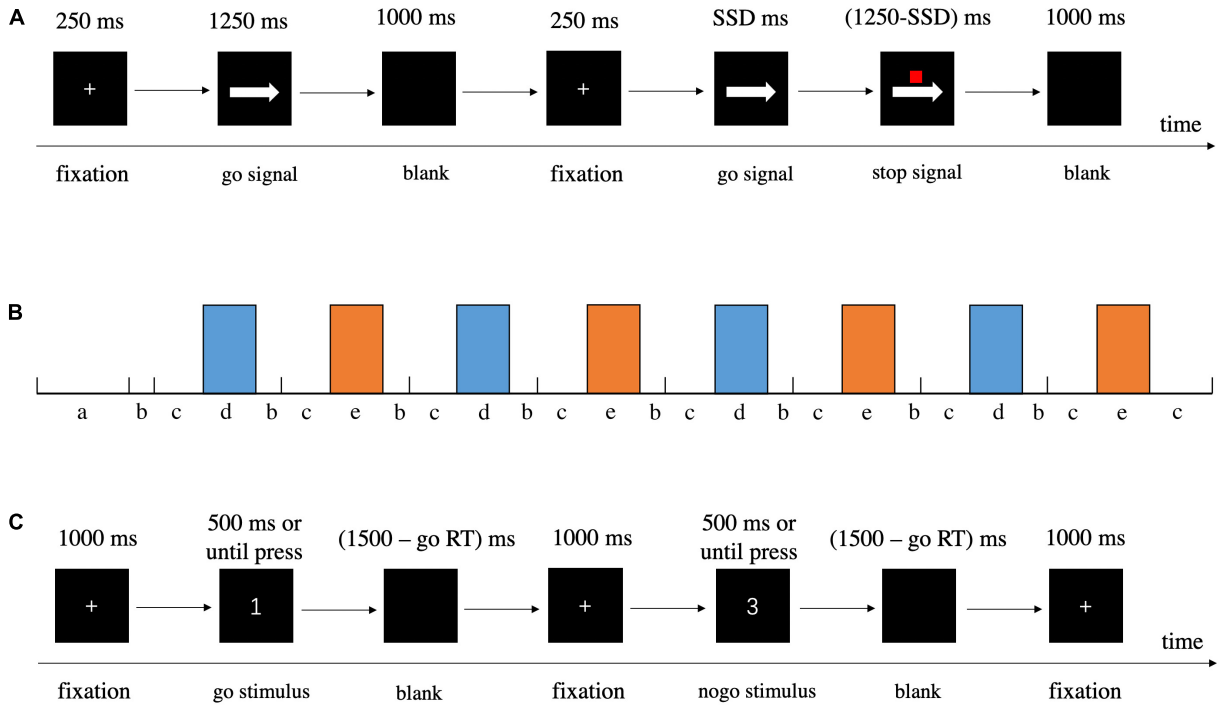

FIGURE 3
Detailed information about procedures of behavioral tasks. **(A)** SST. **(B)** Schematic illustration of block design for GNG, a = instruction, b = cue, c = rest, d = go block, e = nogo block. **(C)** GNG.

system (Shimadzu Co., Kyoto, Japan) during GNG. We used 11 sources (emitting light at 780 nm, 805 nm, and 830 nm) and 11 detectors to form 34 measurement channels over the right cerebral cortex, including the regions of interest (i.e., the pre-SMA and rIFG), with a raw sample rate of 27.78 Hz. Participants were fitted with a headcap with optode holders to set the source-detector distance at 3 cm. For consistency of optode placement across participants, channel 1 was located at the Cz point of the international 10–20 EEG system (Jasper, 1958) and the uppermost edge of the probe set overlapped with Cz-Oz (**Figure 4A**). To determine the anatomical locations of optodes and channels, we used a digitizer (Fastrak, Polhemus, Colchester, VT, United States) to capture the 3D coordinates of optode positions based on head landmarks (nasion, Cz, and left and right preauricular points) in real-world space and registered fNIRS coordinates of channels and optodes on the standard Montreal Neurological Institute (MNI) template using the software package NIRS-SPM (**Figures 4B,C**) (Ye et al., 2009; Orcioli-Silva et al., 2021). Finally, we estimated the corresponding relationship between fNIRS channels and the anatomical structural labels in the Brodmann areas and LPBA40 according to the channels' coordinates (Tsuzuki et al., 2007; Ye et al., 2009; Nagashima et al., 2014). We stipulated in advance that if the percentage of overlap exceeded 50%, the channel represented the corresponding brain area. Finally, each region of interest (ROI) consisted of corresponding channels. Nine channels labeled the pre-motor and supplementary motor cortex in the Brodmann areas (channel 1/2/5/6/8/9/12/19/26) and 2 channels represented the right inferior frontal gyrus using LPBA40 (channel 24/27).

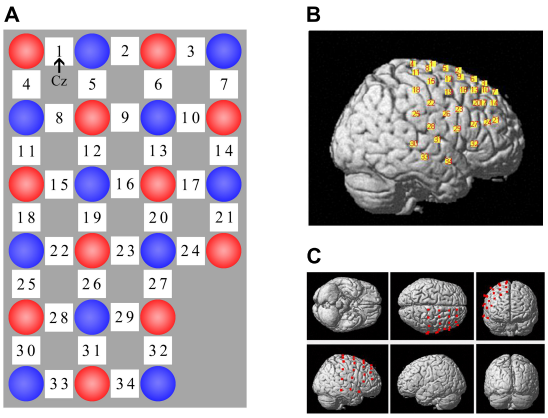

FIGURE 4
fNIRS channel layout. **(A)** Optode arrangement, red circle = source, blue circle = detector, white square = channel. Channel 1 was located at Cz and the uppermost edge (i.e., channel 1 – 3) of the probe set overlapped with Cz-Oz. **(B)** Spatial registration of channels on a rendered brain. **(C)** Different views of optode locations.

Data preprocessing

We preprocessed raw fNIRS data in the Homer2 fNIRS analysis package (Huppert et al., 2009) based on Matlab R2013b software. At first, raw data was down-sampled to 9.26 Hz after being imported into Homer2 and was visually inspected to ensure there was no totally bad signal channel. Next, optical intensity was converted to optical density (OD) by the hmrIntensity2OD function. Motion artifacts were identified by a hmrMotionArtifactByChannel function and corrected by a spline interpolation method in every participant (Scholkmann et al., 2010; Zhang et al., 2021). Then the fNIRS signals were bandpass-filtered with cutoff frequencies of 0.01 and 0.1 Hz to eliminate physiological noise (e.g., heartbeat and respiration) and correct drift artifacts throughout the experimental process. According to the modified Beer-Lambert law, the filtered OD signal was transformed to relative concentration signal data for oxy-Hb, deoxy-Hb, and total-Hb. Finally, we used a 5-s period prior to the onset of each block as a baseline to standardize hemodynamic changes during the 30 s task block and calculated a block-averaged relative concentration change for the two conditions (go and nogo) over the time range. We decided to analyze only oxy-Hb data because oxy-Hb is more reliable and sensitive to brain activity changes relative to deoxy-Hb or total-Hb (Hoshi et al., 2001; Nagashima et al., 2014; Ehlis et al., 2016; Lu et al., 2020; Zhuo et al., 2022). But we also provided the relevant deoxy-Hb and total-Hb data in the **Supplementary Material**.

With regard to the behavioral data, 11 participants were excluded from further analysis in the SST because they showed (1) stop accuracy <0.25 or >0.75 (Congdon et al., 2012), which might result from participants' strategic behavior and not complying with the task instruction, such as waiting for the stop signal to show (obtaining a high stop accuracy) and pressing the key too fast throughout the task (obtaining a low stop accuracy); (2) goRT > 1000 ms (Enge et al., 2014) or (3) violation of the independent race model, implying the mean RT on unsuccessful stop trials is greater than goRT (Verbruggen and Logan, 2009). After exclusion, the SST analysis was based on n = 20 for the multitarget condition, n = 22 for the rIFG condition, n = 20 for the pre-SMA condition, and n = 19 for the sham stimulation group. One participant was excluded from the GNG and fNIRS analyses due to an error rate exceeding 40% (Enge et al., 2014). Therefore, the final sample for the GNG and fNIRS data analyses included 91 participants (n = 22, 24, 22, and 23 for groups 1–4, respectively).

Statistical analyses

IBM SPSS (version 26.0) software was used to conduct statistical analyses. The fNIRS data were analyzed by creating oxy-Hb contrasts for the nogo block minus the go block (△oxy-Hb = oxy-Hb_{nogo} − oxy-Hb_{go}) (Veit et al., 2021). △oxy-Hb signals from channel 1/2/5/6/8/9/12/19/26 were averaged to represent pre-SMA activity, and those from channel 24/27 were averaged to yield rIFG activity. The data of △deoxy-Hb and △total-Hb are provided in the **Supplementary Material**. Categorical variables were examined by the chi-square test. One-way analysis of variance (ANOVA) was used to test data measured once and baseline performance, and the Kruskal–Wallis test was used for data with a skewed distribution. Whether the tracking procedure in SST obtained a stop accuracy of approximately 0.5 was verified using one-sample t-tests. The effects of tDCS stimulation were assessed with repeated measures ANOVA (RM-ANOVA) with time (pretest and posttest) as the within-subject factor and stimulation condition (multitarget, rIFG, pre-SMA, and sham stimulation) as the between-subject factor. *Post hoc* analyses were performed using Bonferroni-corrected pairwise comparisons. Considering the limitation of the classic frequentist approach, we used Bayesian analysis to further investigate the non-significant interaction effect in order to strengthen the robustness of our results. This was performed using JASP software (version 0.14.1.0) with a default Cauchy prior distribution with γ = 0.707. The Bayes factor BF_{10} represented the ratio of the possibility that the data favored the alternative hypothesis (H_1) compared to the null hypothesis (H_0). A BF_{10} superior to 3 indicated at least moderate evidence for H_1. A BF_{10} between 1/3 and 3 indicated anecdotal evidence for H_0 and H_1, while a BF_{10} score between 1/10 and 1/3 represented moderate evidence for H_0 and inferior to 1/10 signified strong evidence for H_0 (Wagenmakers et al., 2011, 2018a,b). Further analysis explored the effect of each tDCS condition on the SSRT. Participants were allocated to the high-performance (HP) and low-performance (LP) subgroups in each condition by a median split method based on baseline SSRT (Whelan et al., 2012). An independent samples t-test was employed to compare the SSRT of the HP and LP subgroups in each condition. Further analysis was performed using a 2 (time: pretest and posttest) × 2 (subgroup: HP and LP) RM-ANOVA. In all analyses, $p < 0.05$ was considered statistically significant. In addition, for ANOVAs, effect sizes were reported as partial eta-squared (η^2_p).

Results

Behavioral data

Baseline

As shown in **Table 3**, the one-way ANOVAs revealed no significant difference ($ps > 0.05$) in any of the indices for SST and GNG between the four groups before tDCS intervention, thereby ensuring that any performance changes

TABLE 3 Means and standard deviations of behavioral task performance at baseline.

Task	rIFG + pre-SMA	rIFG	pre-SMA	sham	F	*p*
Stop-signal task						
SSRT	291.31(33.43)	267.11(33.65)	274.34(31.87)	277.12(29.9)	2.036	0.116
stop accuracy	0.51(0.06)	0.51(0.04)	0.50(0.05)	0.53(0.06)	0.936	0.428
goRT	543.19(185.40)	515.47(159.84)	497.63(156.17)	591.42(212.05)	1.023	0.387
Go/nogo task						
IES	388.42(67.52)	365.88(66.65)	378.46(59.73)	380.91(67.34)	0.476	0.700
goRT	371.41(56.07)	352.07(55.38)	364.58(56.22)	359.23(45.52)	0.539	0.657
nogo accuracy	0.93(0.07)	0.92(0.06)	0.92(0.06)	0.90(0.09)	1.028	0.384

goRT, mean reaction time on correct go trials; IES, inverse efficiency score; SSRT, stop-signal reaction time; rIFG, right inferior frontal gyrus; pre-SMA, pre-supplementary motor area. Besides the accuracy indicators, the units of the other measurements were milliseconds (ms). $p < 0.05$ was considered significant.

between the pretest and the posttest would be attributable to the tDCS stimulation.

Stop-signal task

One-sample *t*-tests indicated there was no significant difference between stop accuracy and 0.5 either in pretest or posttest for group (*p*s > 0.05). The number "0.5" refers to the optimal stop accuracy ensured by the aforementioned tracking procedure. RM-ANOVA showed a significant interaction effect between time and stimulation condition ($F_{(3,77)} = 4.196$, $p = 0.008$, $\eta^2_p = 0.141$) for SSRT. *Post hoc* analysis revealed a significant decrease in SSRT after multitarget tDCS ($p = 0.005$). However, no significant difference was found after rIFG ($p = 0.057$), pre-SMA ($p = 0.109$), or sham stimulation ($p = 0.717$) (Figure 5A). The main effects were not significant (*p*s > 0.05). The baseline SSRT was significantly shorter in the HP subgroup relative to the LP subgroup for each condition after independent samples *t*-tests (*p*s < 0.001). Further analysis revealed that the main effect of time ($F_{(1,18)} = 7.547$, $p = 0.013$, $\eta^2_p = 0.295$) was significant in the multitarget condition, manifesting significant smaller SSRT after stimulation (mean = 267.39 ms, SD = 37.81 ms) compared with pre-stimulation (mean = 291.31 ms, SD = 33.43 ms) regardless of subgroup. For the rIFG condition, the interaction effect between time and subgroup ($F_{(1, 20)} = 4.56$, $p = 0.045$, $\eta^2_p = 0.186$) and the effect of subgroup ($F_{(1, 20)} = 4.56$, $p = 0.003$, $\eta^2_p = 0.357$) were significant. *Post hoc* analysis showed only the LP subgroup experienced reduced SSRT ($p = 0.009$) (Figure 5B). Only the subgroup effect was significant in the pre-SMA condition ($F_{(1, 18)} = 26.429$, $p < 0.001$, $\eta^2_p = 0.595$), which indicated that no subgroups significantly changed SSRT after stimulation. For sham stimulation, the interaction effect was significant ($F_{(1, 17)} = 4.877$, $p = 0.041$, $\eta^2_p = 0.223$), but *post hoc* tests showed no significant difference in SSRT between pretest and posttest for both subgroups (*p*s > 0.05) (Figure 5B). There were no significant interaction effects for stop accuracy or goRT (*p*s > 0.05), and none of the main effects reached significance (*p*s > 0.05). Bayesian analysis showed moderate evidence for the null hypothesis that there was no interaction effect for stop accuracy ($BF_{10} = 0.13$) or for goRT ($BF_{10} = 0.13$).

Go/nogo task

The main effect of time was significant for IES due to a pre-to-post decrease for all conditions ($F_{(1, 87)} = 14.948$, $p < 0.001$, $\eta^2_p = 0.147$), but the main effect of condition and the interaction effect were not significant (*p*s > 0.05). Bayesian analysis showed a BF_{10} of 0.07 for the IES interaction term, indicating strong evidence for the null hypothesis. RM-ANOVA revealed a significant time effect for goRT driven by a decrease in RT after intervention under all conditions ($F_{(1, 87)} = 27.645$, $p < 0.001$, $\eta^2_p = 0.241$). There was no main effect of condition and no interaction effect for goRT (*p*s > 0.05). Bayesian analysis revealed moderate evidence to support the absence of an interaction term ($BF_{10} = 0.11$). None of the main effects or interaction effect reached significance for nogo accuracy (*p*s > 0.05). Strong evidence in favor of no interaction effect was established by $BF_{10} = 0.09$.

Functional near-infrared spectroscopy data

The baselines of △oxy-Hb were matched between the four tDCS conditions in both the pre-SMA ($F_{(3, 87)} = 2.546$, $p = 0.061$, $\eta^2_p = 0.081$) and rIFG ($F_{(3, 87)} = 0.274$, $p = 0.844$, $\eta^2_p = 0.009$). In the pre-SMA region, an interaction effect between time and stimulation condition was found ($F_{(3, 87)} = 3.023$, $p = 0.034$, $\eta^2_p = 0.094$), and *post hoc* analysis indicated that △oxy-Hb significantly decreased after the multitarget stimulation ($p = 0.026$). Although no significant differences in the other three groups were detected (*p*s > 0.05), a pre-to-post decrease in △oxy-Hb was observed under the rIFG and pre-SMA conditions but not in the sham stimulation condition (Figure 5C). The main effects of time and condition did not reach significance (*p*s > 0.05). In the rIFG region, RM-ANOVA revealed no significant main effects or an interaction effect for △oxy-Hb (*p*s > 0.05) (Supplementary Figure 1).

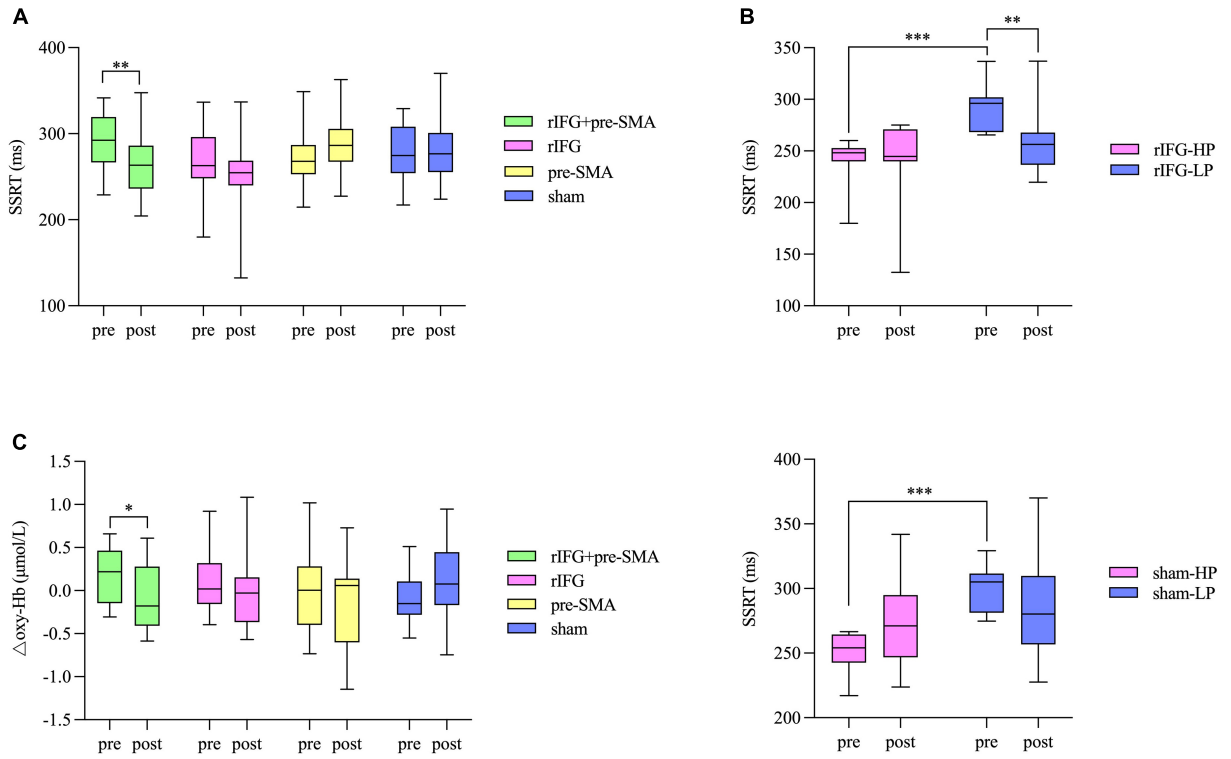

FIGURE 5
Box and whisker plots, showing the effects of HD-tDCS on the outcome measures. **(A)** Behavioral performance in SST. **(B)** Significant interaction effects between subgroup and time for the rIFG condition (top) and sham condition (bottom) in the further analysis for SST. HP, high-performance subgroup; LP, low-performance subgroup. **(C)** Changes in △oxy-Hb from pretest to posttest in the pre-SMA ROI. Boxes extend from the 25 to 75th percentiles with a horizontal line representing the median. Whiskers show the min to max values. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Bayesian analysis revealed strong evidence supporting the null hypothesis that there was no interaction effect ($BF_{10} = 0.08$).

Side effects, blinding efficacy, and electric field modeling

All participants tolerated the tDCS procedure well and there were no serious side effects reported. The ratings of the intensity of sensations between groups were similar ($F_{(3, 88)} = 0.521$, $p = 0.669$, $\eta^2_p = 0.017$). There were 21, 23, 19, and 22 participants in conditions one to four respectively who reported that they received real stimulation. No significant difference ($\chi^2 = 1.764$, $p = 0.656$) was found in the number of participants speculating whether they received real or sham stimulation, and the confidence scores were insignificant according to the Kruskal–Wallis test ($p = 0.589$). The electric field simulation confirmed the focal electric field over the pre-SMA and rIFG for the multitarget condition, rIFG for the rIFG condition, and pre-SMA for the pre-SMA condition. The rIFG stimulation condition produced much higher electric field intensity in the targeted cortex than either the multitarget or pre-SMA conditions (Figure 2A).

Discussion

Response inhibition is a critical part of executive function, and thus it is worthwhile to investigate how response inhibition ability can be more effectively improved through tDCS. The present study aimed to investigate the effect of different stimulation montages on response inhibition as assessed by behavioral and neuroimaging methods. Behavioral data showed a significant decrease in SSRT after multitarget HD-tDCS that did not exist in the other conditions. Further analysis showed that significant reductions in SSRT were present in both the LP and HP subgroups after multitarget stimulation, as well as in the LP subgroup after rIFG stimulation. fNIRS data showed that only multitarget stimulation produced a significantly lower △oxy-Hb in the pre-SMA. However, pre-SMA tDCS modulated neither SSRT nor fNIRS signals significantly from pretest to posttest. The other indices in SST or GNG were not substantially altered for the real stimulation conditions relative to the sham

stimulation condition. All null hypotheses of interaction effects between time and stimulation condition were confirmed by Bayesian analysis.

To the best of our knowledge, this is the first study to provide evidence that multitarget stimulation is an effective way to improve response inhibition and is more potent than the commonly used single-target HD-tDCS. SSRT decreased significantly after rIFG + pre-SMA stimulation, indicating improved response inhibition. However, the pre-to-post changes in SSRT did not reach significance under the other three conditions. It has been reported that tDCS effects are dependent on initial performance, with greater tDCS effects observed in those with poor baseline performance; better baseline performance is related to higher neural excitability, which is difficult to elevate further (Wu et al., 2021a,b). The HP subgroup and the LP subgroup were divided using a median-split method in the present study (Whelan et al., 2012). After statistical comparisons, the results showed the HP subgroup had a significantly shorter SSRT relative to LP subgroup for each condition. Further analysis revealed that multitarget tDCS improved SSRT in both the HP subgroup and the LP subgroup compared with the rIFG condition which improved SSRT only in the LP subgroup. Coupled with the fact that none of the changes were seen in either subgroup for the pre-SMA tDCS condition, the results indicate that multitarget HD-tDCS yielded the most pronounced effects of all the conditions tried. This result is consistent with published studies indicating that multitarget stimulation produces larger effects relative to single-target tDCS (Vaseghi et al., 2015; Dagan et al., 2018). Additionally, the electric field modeling results showed the rIFG condition yielded greater electric field intensity in the targeted cortex compared with the multitarget condition and pre-SMA condition. However, the measurement results illustrated the multitarget stimulation is more beneficial to improving response inhibition. The results seemed contradictory because of the assumption that electric field intensity in a brain area directly associates with the behavioral effect of tDCS (Evans et al., 2020). One highly possible explanation may lie in that efficient execution of brain function is based on networks of brain areas rather than individual brain regions (Hoogman et al., 2017; Ester and Kullmann, 2021); and multitarget stimulation tries to modulate the associated brain network and may result in additive effects of tDCS on performance compared with single-target stimulation (Brem et al., 2018; Ester and Kullmann, 2021; Friehs et al., 2021a; Gregoret et al., 2021). Additionally, there is some evidence for the potentially inverted U-shaped nature of tDCS interactions with behavior performance, in which an intensity may lead to better performance when it lies closer to the peak of the inverted-U curve (Ehrhardt et al., 2021). According to the electric field modeling results, if the site of action of stimulation was just the IFG, the multitarget condition seemed to apply a much lower stimulation intensity to the IFG than the IFG stimulation condition; this stimulation intensity produced by multitarget condition may lie near the peak of the inverted-U curve. In this way, the inverted U-shaped intensity response curve may partly account for the difference between the rIFG condition and multitarget condition. Consequently, the present study verifies our hypothesis and provided preliminary evidence that multitarget tDCS is a more effective montage for enhancing response inhibition and fills a research gap on enhancing response inhibition using a multitarget montage. However, additional studies are warranted to confirm whether it is the particular case with our selected stimulation intensities.

We found that the rIFG condition was effective in improving response inhibition, although only for the low-performance participants. The favorable effect of rIFG stimulation relative to sham stimulation is consistent with previous results (Jacobson et al., 2011; Stramaccia et al., 2015; Li et al., 2019). However, single-target tDCS over the pre-SMA did not improve response inhibition. On the one hand, this result contradicts previous studies reporting significant reductions in SSRT after anodal tDCS over the pre-SMA (Kwon and Kwon, 2013a,b; Yu et al., 2015). One factor that might account for the discrepancy could be the different stimulation parameters employed in these studies (Mayer et al., 2020; Schneider et al., 2021). Prior studies used conventional tDCS with large pad electrodes ranging from 16 to 35 cm^2 (Kwon and Kwon, 2013a,b; Yu et al., 2015), leading to low spatial resolution and distributed current. Consequently, it is highly possible that other brain regions related to response inhibition were stimulated in a complex way (Chen et al., 2021). However, the present study used HD-tDCS with small circular electrodes (1.2 cm diameter), and the center anode was surrounded by return electrodes, yielding greater spatial precision relative to conventional tDCS (Kuo et al., 2013; Sehatpour et al., 2021). Hence, HD-tDCS reduces the confounding impact of other brain regions relative to conventional tDCS, making the causal relationship between brain stimulation and relevant behavioral changes more convincing. In addition, the placement of electrodes might also lead to inconsistency because the anode electrode was placed over C2 in the present study, and the center of the pad electrode was put over Fz (Yu et al., 2015) or 4 cm anterior to Cz (Kwon and Kwon, 2013a,b) in previous studies. On the other hand, the absence of improvement in SSRT is consistent with the results of some other studies (Bender et al., 2017; Fujiyama et al., 2021), suggesting that more studies are needed to figure out the effect of anodal tDCS over the pre-SMA.

Corresponding cortical activity is critical for the execution of response inhibition and is directly related to cerebral blood flow. We measured hemodynamic responses in the rIFG and pre-SMA during a go/nogo task using fNIRS and found that △oxy-Hb was significantly reduced in the pre-SMA after multitarget stimulation compared to baseline. There was also a decrease of △oxy-Hb in both the rIFG and pre-SMA stimulation

conditions, although it was not significant. This decrease could be considered as a biomarker of improved neural efficiency representing a more efficient neural network, defined as the quantity of performance-related changes accomplished by per neuron activity (Zarahn et al., 2007; Enriquez-Geppert et al., 2013). Neural efficiency has been discussed in prior studies, which found that behavioral performance was unchanged or improved even though the corresponding brain activity was decreased using tDCS (Holland et al., 2011; Lu et al., 2020; Orcioli-Silva et al., 2021). We found that HD-tDCS intervention might reduce the amount of energy the brain needs to finish the same GNG without changing performance. In particular, the multitarget HD-tDCS significantly decreased △oxy-Hb, demonstrating substantially improved neural efficiency, which is further proof of the advantage of using multitarget stimulation. However, in contradiction to prior studies that showed brain activity in the rIFG (Herrmann et al., 2005; Rodrigo et al., 2014), we did not observe a significant change of △oxy-Hb in that region even when using multitarget HD-tDCS. One possible explanation is that the probe set covering the rIFG was located at the border of the rIFG, so there were only two channels representing the rIFG. Therefore, the fNIRS in our study may have failed to measure the activity of the rIFG reliably and reflect the true changes. Besides, the channels representing pre-SMA in our study included relatively lateral channels (e.g., channels 19 and 26), meaning the pre-SMA may not be specific enough. This may occur because there only exists the "Pre-Motor and Supplementary Motor Cortex" anatomical label in the standard brain template when we anatomically labeled fNIRS channels, which limits us to further segment the brain region. We had to regard this label as the pre-SMA label. This practice is in line with previous studies which also used the pre-SMA to refer to the "Pre-Motor and Supplementary Motor Cortex" (Wang et al., 2021).

Although the present study provides preliminary evidence for the advantages of multitarget tDCS for improving response inhibition, some limitations should be considered. First, the participants were all young healthy adults in our study; consequently, the results should be cautiously generalized to other groups with different ages. Considering neural anatomical differences, the effect of tDCS over the rIFG and pre-SMA on response inhibition has been shown to be age-dependent (Fujiyama et al., 2021), so future studies are warranted to further elucidate age-related differences in the results of tDCS application. Second, there was no follow-up assessment and thus the sustainability of the effects of multitarget tDCS remains unclear; this is a vital issue for the use of tDCS in practical applications. Some studies have reported that a single session of conventional tDCS-induced (1 mA, 13 min) excitability changes could last for 90 min (Nitsche and Paulus, 2001) and single-target HD-tDCS (2 mA, 20 min) has shown a lasting after-effect for more than 2 h (Kuo et al., 2013). Hence, additional studies with follow-up measurements are needed to illuminate the duration of the after-effect of multitarget HD-tDCS. Third, the brain activity in the rIFG has yet to be clarified. For future work, more accurate probe placement should shed more light on the neurophysiological changes that take place after tDCS intervention. Fourth, the brain region of the pre-SMA needs to be more specific. It is recommended for future studies to utilize MRI to obtain more fine-grained segmentation of the brain region. Besides, the present study adopted a single-blind and between-group design, which might weaken the power of the results (Lu et al., 2020; Friehs et al., 2021b). Therefore, more rigorous experimental designs are recommended for future studies. Moreover, the electric field intensity differed in different stimulation conditions. Although it did not impact the interpretation of the findings in this study, future studies should carefully consider to normalize to produce roughly equivalent electric field intensity at the cortex. Finally, the multitarget HD-tDCS protocol in this study, including electrode positions and current intensity, was determined based on a generic head model rather than personalized adjustment. However, due to the inter-individual variability of cortical excitability changes in response to stimulation, the standardized "one size fits all" application of the multitarget HD-tDCS stimulation protocol may not be generalized well to other clinical individuals (Mizutani-Tiebel et al., 2022). Personalized application of multitarget stimulation protocol should be further explored in future studies.

Conclusion

The present study has demonstrated that multitarget HD-tDCS improved response inhibition. Both high-performance and low-performance participants showed a significant reduction in SSRT after multitarget stimulation, whereas only the low-performance participants yielded a significantly decreased SSRT after the rIFG stimulation. We did not observe any significant improvements in SSRT after the pre-SMA stimulation and sham stimulation. Other indicators in behavioral tasks were not significantly altered for the verum stimulation conditions compared with the sham stimulation condition. fNIRS signals recorded during GNG showed a decrease in △oxy-Hb under all three verum tDCS conditions in the pre-SMA region, interpreted as sharpened neural efficiency, but the decrease reached statistical difference only for multitarget tDCS. This study thus provides preliminary evidence that multitarget HD-tDCS over the rIFG and pre-SMA is likely to be the most potent protocol for enhancing response inhibition ability in healthy individuals. It also lays a solid theoretical basis for clinical utility and provides new progress for the treatment of response inhibition deficits.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Tangdu Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZG, YG, XZ, and XY conceived the study design. ZG, YG, HL, RQ, and XW performed the participants' recruitment and data collection. ZG and YG performed data analysis. ZG wrote the draft of the manuscript. XZ obtained funding and contributed to the manuscript revision. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Major Project of Medicine Science and Technology of PLA (AWS17J012).

Acknowledgments

We would like to thank Liu Yang, Shuyi Liang, and Diyan Lu for their help during participants' recruitment and data collection and MogoEdit (https://www.mogoedit.com) for its English editing during the preparation of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.905247/full#supplementary-material

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frontiers | Frontiers in Neuroscience

TYPE Original Research
PUBLISHED 18 August 2022
DOI 10.3389/fnins.2022.924617

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

RECEIVED 20 April 2022
ACCEPTED 28 July 2022
PUBLISHED 18 August 2022

CITATION
Lin S, Wang L, Shu Y, Guo S, Wang T, Li H, Zhang C, Sun B, Li D and Wu Y (2022) Rescue procedure for isolated dystonia after the secondary failure of globus pallidus internus deep brain stimulation.
Front. Neurosci. 16:924617.
doi: 10.3389/fnins.2022.924617

Rescue procedure for isolated dystonia after the secondary failure of globus pallidus internus deep brain stimulation

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Introduction: Globus pallidus internus (GPi) deep brain stimulation (DBS) is widely used in patients with dystonia. However, 10–20% of patients receive insufficient benefits. The objectives of this study are to evaluate the effectiveness of bilateral subthalamic nucleus (STN) DBS along with unilateral posteroventral pallidotomy (PVP) in patients with dystonia who experienced unsatisfactory GPi-DBS and to address the reported rescue procedures after suboptimal DBS or lesion surgery in dystonia patients.

Methods: Six patients with isolated dystonia who had previously undergone bilateral GPi-DBS with suboptimal improvement were included. Standardized assessments of dystonia using the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) and quality of life using SF-36 were evaluated before surgery and 1, 6 months, and last follow-up (LFU) after surgery. STN bilateral OFF (bi-OFF), unilateral ON (uni-ON), and bilateral ON (bi-ON) states were recorded at LFU. Specific items were used to find publications published before 10 April 2022 regarding rescue procedures after suboptimal DBS or lesion surgery in patients with dystonia for reference. Eleven original studies including case reports/series were identified for discussion.

Results: Substantial clinical benefits were achieved in all six patients. Significant amelioration was achieved during the 1-month (6.5 ± 7.45; p = 0.0049), 6-month (5.67 ± 6.3; p = 0.0056) follow-ups, and at LFU (4.67 ± 4.72; p = 0.0094) when compared with the baseline (LFU of GPi DBS with on status) (17.33 ± 11.79) assessed by BFMDRS. The percentage of improvement reached 70.6, 74.67, and 77.05%, respectively. At LFU, significant differences were found between the stimulation bi-OFF and uni-ON (11.08 ± 8.38 vs. 9 ± 8.52, p = 0.0191), and between the stimulation bi-OFF and bi-ON (11.08 ± 8.38 vs. 4.67 ± 4.72, p = 0.0164). Trends depicting a better improvement in stimulation bi-ON compared with uni-ON (4.67 ± 4.72 vs. 9 ± 8.52, p = 0.0538) were observed.

Conclusion: Our results suggest that bilateral STN-DBS plus unilateral PVP may be an effective rescue procedure for patients with isolated dystonia who experienced suboptimal movement improvement following GPi-DBS. However, given the heterogeneity of patients and the small sample size, these findings should be interpreted with caution.

KEYWORDS

rescue procedures, deep brain stimulation, dystonia, globus pallidus internus, subthalamic nucleus, pallidotomy

Introduction

Isolated dystonia refers to a clinically and genetically heterogeneous group of movement disorders characterized by sustained and repetitive muscle contractions that often results in abnormal posturing and no other neurological abnormalities apart from tremor. The etiology of isolated dystonia can be classified as inherited, acquired, and idiopathic (Albanese et al., 2013). Most affected individuals experience educational withdrawal and social isolation, leading to a significant reduction in their quality of life. Current evidence indicates that the pathophysiology of isolated dystonia involves the dysfunction of the corticostriatal-thalamocortical circuit (Balint et al., 2018).

Deep brain stimulation (DBS) is a minimally invasive procedure for patients with dystonia, whether it is inherited or idiopathically isolated. And it is suitable for those resistant to systematic medications and botulinum toxin injections (Rodrigues et al., 2019). The globus pallidus internus (GPi) is a viable therapeutic target for DBS, and multiple studies have demonstrated that bilateral stimulation at GPi could effectively and safely improve the clinical symptoms and quality of life of patients with isolated dystonia (Kupsch et al., 2006; Meoni et al., 2017; Lin et al., 2019). The randomized controlled trial published in 2012 reported that GPi DBS could improve the dystonia severity of primary generalized or segmental dystonia by 47.9% at 6 months and 61.1% at 3 years (Volkmann et al., 2012). However, 10–20% of patients show improvement below 25–30% (Pauls et al., 2017). The therapeutic failure was either primary (i.e., patients who had never shown any response) or secondary (i.e., patients who experienced a loss of response after initial improvement) (Pauls et al., 2017). Additionally, the management of some patients remains difficult despite the exclusion of reversible and common complications, such as improper lead positioning, hardware issues, and inadequate programming.

One dual-target, crossover sham-controlled study (Schjerling et al., 2013) in 2013 examined 12 patients with dystonia (10 primary and 2 secondary) whose electrodes were implanted bilaterally in the GPi and subthalamic nucleus (STN). The report found that the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) movement scores were larger with four electrodes in service compared to bilateral stimulation at either target. These findings suggest that the simultaneous stimulation of GPi and STN may generate an additional value. However, the combination strategy means that the implantation of four electrodes and two sets of implanted pulse generators (IPGs), which will remarkably increase the economic burden and new trauma for additional IPG, is not applicable to a subset of patients.

Unilateral posteroventral pallidotomy (PVP) is an alternative surgical option for dystonia. Several studies demonstrate the comparable efficacies between PVP and GPi-DBS. Previous investigations have shown a much higher risk of employing a bilateral PVP than a unilateral procedure, although the efficacy of bilateral PVP in dystonia could reach a 50–90% alleviation in BFMDRS scores (Eltahawy et al., 2004; Horisawa et al., 2021). Recently, one study from Horisawa's team reported the safety and efficacy of unilateral PVP for primary dystonia in all midline symptoms, including eyes, mouth, speech, swallow, and neck (Horisawa et al., 2021). Therefore, unilateral PVP remains a viable treatment option for patients with dystonia. In addition, it has a price advantage amounting to below 20% of the total cost for GPi (or STN) DBS in China. Therefore, PVP can be particularly appropriate for dystonia patients who cannot afford DBS therapy.

Two studies (Fonoff et al., 2012; Dec et al., 2014) indicated STN DBS increased further benefits for patients with dystonia who experienced partial improvement after the initial PVP, suggesting the synergistic effect of bilateral STN on PVP. Therefore, we hypothesized that STN-DBS plus unilateral PVP is an effective alternative for STN plus GPi-DBS after unsatisfactory GPi-DBS outcomes. Through the adoption of this surgical method, we acknowledge its cost-saving advantage,

Abbreviations: GPi, Globus pallidus internus; DBS, deep brain stimulation; STN, subthalamic nucleus; PVP, posteroventral pallidotomy; LFU, last follow-up; BFMDRS, Burke-Fahn-Marsden Dystonia Rating Scale; IPGs, implanted pulse generators; MRI, magnetic resonance imaging; VTA, tissue activated; CT, computed tomography; SCP, superior cerebellar peduncle; DN, dentate nucleus.

as the bilateral electrodes of STN can be connected to the previous IPG. Therefore, in this study, we aimed to investigate whether STN-DBS plus PVP is effective for patients with isolated dystonia who have undergone secondary failure of GPi-DBS.

Materials and methods

Patients

We recruited six patients at the Functional Neurosurgical Center of Shanghai Ruijin hospital from June 2018 to June 2020. The inclusion criteria were (i) diagnosis of isolated dystonia, including (1) dystonia and an otherwise normal neurological examination, (2) no history of other known etiologies of dystonia, (3) normal brain magnetic resonance imaging (MRI), (4) no family history of dystonia, (5) no previous exposure to medications possibly causing acquired dystonia, including levodopa and dopamine agonists, neuroleptics (dopamine receptor blocking drugs), anticonvulsants, and calcium channel blockers, and (6) no history of trauma, dementia (Mini-Mental State Examination score $>$ 26) or other known metabolic and systemic causes; (ii) record of suboptimal bilateral GPi-DBS; (iii) adequate programming without obvious impact; and (iv) accurate location of the electrodes verified by the postoperative MRI (**Supplementary Figure 1**). A blinded independent expert rater assessed the correctness of the GPi lead placement.

All six patients experienced adequate programming strategies. In detail, if symptoms could not be controlled at 4.5 V or if stimulation-induced adverse effects hindered the further increase in voltage, reprogramming was performed using various procedures, including trying different combinations of large- and small-pulse widths and frequencies, the addition of other monopolar contacts, double monopolar stimulation, a bipolar stimulation mode, or interleaving stimulation. However, the results were either ineffective or included reports of adverse effects, encompassing dysarthria, increased muscle tone, gait disorders, paresthesia, and blurred vision. **Table 1** presents the last set of stimulation parameters for GPi.

All six patients were unable to accept staged surgery due to superimposed surgical trauma or increased costs. Post-operative MRI also excluded the DBS lead malposition. Patients 2, 3, 4, and 5 completed the whole exome sequencing and no genetic mutations were found. *DYT1* and *DYT6* genes were routinely tested in patients with dystonia and the results of patients 1 and 6 were negative.

Patient 1 is a 63-year-old man who had a 6-year history of cervical and oromandibular dystonia, featuring difficulty in speech and swallowing before GPi DBS. The disorder began with torticollis, especially when he felt nervous. Three months later, the patient developed spontaneous mouth movements, inarticulacy, and resultant dysphagia. The patient repudiated the history of diabetes, hypertension, infectious diseases, alcohol addiction, smoking, and allergies. Treatment with baclofen and diazepam failed due to their intolerant side effects. From here on, his dysphagia further deteriorated. GPi DBS was performed at the age of 62. Considerable effects were observed in his neck after the operation. The improvements in his mouth and speech reached up to 46.15% in the first 6 months. However, the efficiency decreased later and eventually, recurrence emerged despite the repeated programming. Before the rescue procedures, the patient presented with cranial and cervical dystonia involving the oromandibular muscles, involuntary head rotated and tilted to the right, as well as dysarthria and dysphagia. He also complained of temporomandibular and cervical pain.

Patient 2 developed left torticollis and cervical pain without any known origin at the age of 47. These symptoms significantly improved after treatment with tiapride and baclofen. The medications were eventually suspended due to their side effects. A botulinum toxin injection was attempted 1 year later with considerable, but transient, benefits. Therefore, the patient underwent GPi DBS 2 years after the onset of symptoms. His cervical dystonia improved significantly after the stimulation, with a 66.67% reduction in BFMDRS scores; however, he began to experience foreign body sensations in his eyes, photophobia, and blurred vision 6 months post-surgery. Soon, his eyes started to blink involuntarily, and the frequency of blinking gradually increased. Additionally, the previously relieved cervical dystonia got worse.

Patient 3 suffered from left torticollis at age 37 for an unknown reason. Initially, the twisting was intermittent, occurring 2–3 times a day. The frequency increased within 2 months and was accompanied by neck pain. He tried treatment with baclofen, diazepam, and trihexyphenidyl successively without evident amelioration. At the age of 38, the patient had a botulinum toxin injection, resulting in partial alleviation. However, after three treatments, the efficacy gradually disappeared. He underwent GPi-DBS 2 years after the onset of symptoms (39 years old), and he reported a 50% decrease in BFMDRS scores. The patient was unsatisfied with the effects of this procedure and his symptoms also started to fluctuate. Upon examination prior to the second operation, the patient presented with left torticollis, neck pain, and cervical stiffness.

Patient 4 suffered from neck pain without a known reason. The patient's cervical tilting angle to the right gradually reached 160° at the age of 46. In the beginning, the symptoms occurred occasionally and were relieved by the sensory trick. Two years after onset, the symptoms aggravated with an upregulated frequency and persistent pain. Baclofen, diazepam, and benzhexol hydrochloride were prescribed and a botulinum toxin injection was given. However, the torticollis further deteriorated with the head becoming fixed to the left. There is no history of hypertension, diabetes,

infectious diseases, smoking, alcohol addiction, and allergies in this patient. She received GPi-DBS at the age of 54 and her maximum improvement percentage amounted to 83.3%. However, the pre-operative symptoms reemerged during the 11-month follow-up and a novel symptom of shoulder muscle tension appeared.

TABLE 1 Clinical characteristics and clinical outcomes for each patient.[a]

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Mean ± SD
Age at onset (year)	58	47	37	46	53	45	47.67 ± 7.20
Age at GPi DBS (year)	62	48	39	54	54	60	52.83 ± 8.40
Age at STN DBS plus PVP (year)	63	49	40	56	56	61	54.17 ± 8.47
Gender	M	M	M	F	M	F	
Duration (months)	50	12	25	101	17	300	84.17 ± 110.70
Body distribution	Segmental	Segmental	Focal	Focal	Segmental	Multifocal	NA
Affected regions	Eye, mouth, neck	Eye, neck	Neck	Neck	Eye, mouth	Mouth, limbs, neck	NA
Gene mutation	n.a.	None	None	None	None	n.a.	NA
Failed preoperative medication	Baclofen, diazepam	Trihexyphenidyl, tiapride, botulinum toxin	Baclofen, diazepam, trihexyphenidyl, botulinum toxin	Baclofen, diazepam, benzhexol hydrochloride, botulinum toxin	Botulinum toxin, diazepam	Diazepam, trihexyphenidyl	NA
Classification	Sporadic, isolated	Idiopathic sporadic, isolated	Idiopathic sporadic, isolated	Idiopathic sporadic, isolated	Idiopathic sporadic, isolated	Sporadic, isolated	NA
LFU after GPi DBS (months)	12	18	13	28	23	18	18.67 ± 6.06
LFU after STN DBS plus PVP (months)	24	19	15	13	12	13	16.00 ± 4.65
The last stimulation parameters for GPi (amplitude [V]/frequency [Hz]/pulse width [msec])	Lt: 3.55/160/70 case(+) 9(−); Rt: 3.75/160/70 case(+) 0(−)	Lt: 3.45/160/80 case(+) 8(−)9(−); Rt: 3.35/160/80 case(+) 0(−)1(−)	Lt: 3.0/140/110 case(+) 8(−); Rt: 3.5/140/90 case(+) 0(−)	Lt: 3.9/160/90 case(+) 8(−); Rt: 3.65/160/90 case(+) 0(−)	Lt: 3.75/160/70 case(+) 8(−)9(−); Rt: 2.95/160/90 case(+) 0(−)	Lt: 3.45/160/90 case(+) 8(−); Rt: 3.75/160/90 case(+) 0(−)	NA
Optimal stimulation Parameters for STN (amplitude [V]/frequency [Hz]/pulse width [msec])	Lt: 3.05/145/60 case(+) 10(−); Rt: 3.15/145/60 case(+) 2(−)	Lt: 2.85/130/60 case(+) 1(−); Rt: 2.35/130/60 case(+) 2(−)	Lt: 2.25/135/90 case(+) 10(−); Rt: 3.25/135/90 case(+) 2(−)	Lt: 2.95/145/60 case(+) 10(−); Rt: 2.25/145/60 case(+) 2(−)	Lt: 2.05/145/60 case(+) 3(−); Rt: 2.55/135/60 case(+) 3(−)	Lt: 1.7/170/90 case(+) 9(−); Rt: 2.5/170/90 case(+) 2(−)	NA

[a]None underwent gene test and found no mutation; n.a, did not do gene test; LFU, last follow-up; GPi, globus pallidus internus; STN, subthalamic nucleus; PVP, posteroventral pallidotomy; m/d scores, movement/disability scores; bi, biliteral; uni, unilateral; duration, duration before GPi DBS; NA, not applicable. Description statistics are shown with the mean ± standard deviation.

Patient 5 suffered from progressive blepharospasm first noted at 53 years old, without any related history and evidence of a psychogenic disorder. It was followed by uncontrolled jerking in the inferior face and severe tongue spasms, resulting in inarticulate speech. Scans from brain magnetic resonance imaging (MRI) were normal. Treatment with botulinum toxin, diazepam, and trihexyphenidyl produced little benefit. He accepted GPi-DBS 1 year later. The symptoms in his eyes and mouth were relieved during the first 6 months postoperatively, with a 75% reduction in BFMDRS scores. However, the symptoms recurred and intensified afterward, with durative blepharospasm, constant mouth movements, and a low speaking tone. Upon preoperative assessment, the patient showed severe blepharospasm and oromandibular dystonia.

Patient 6 was admitted to our hospital when she was 60 years old, with a chief complaint of involuntary movement in the mouth and upper limbs. The abnormality was intensified when performing tasks that require fine motor skills, such as writing. There is no family history of any movement disorder and no record of any relevant medication intake. The general practitioner prescribed haloperidol and clonazepam, which brought about transient improvement and eventually followed by deterioration. Before GPi-DBS, there were sustained involuntary actions in both arms and the speech was slurred. GPi-DBS was performed after a complete evaluation. The symptoms took a favorable turn in the first 6 months, with a 54.54% improvement. However, the efficiency decreased 8 months later, and repeated programming could not alleviate the symptoms. Before the alternative surgery was performed, the patient displayed severe involuntary movement in the arms, shoulders, neck, and mouth.

Clinical evaluations

Clinical evaluations were performed at baseline and at 1 month, 6 months, and LFU (12–24 months; see **Table 1**) postoperatively. All patients were assessed with the movement and disability subscales of the BFMDRS and SF-36. The LFU estimation was conducted with the STN bilateral OFF, unilateral ON (opposite side of the PVP), and bilateral ON states. The patients were first examined under STN bilateral ON, and then they were evaluated 12 h after STN unilateral ON. All six patients completed this step. Then, the other side of STN was switched-off for another 12 h. However, patients 2 and 5 were unable to tolerate the abrupt worsening of the dystonia (i.e., could not open their eyes) within 30 min after the bilateral switch-off. Therefore, clinical evaluation was performed immediately, and DBS was reinitiated within 30 min upon request of the patients. The other four patients completed the whole process, although symptom deterioration occurred within the first 30 min after STN was bilaterally switched off. All subjects confirmed reaching their original DBS clinical effect within 3 days of rebooting the bilateral stimulation. A trained rater who was blinded to the group status scored each follow-up according to standardized criteria. A specialist who was not blinded saw the patients regularly in the outpatient clinic to adjust the DBS parameters based on their clinical responses.

Surgical procedures

A Leksell stereotactic frame (Elekta, Stockholm, Sweden) was mounted on the patient's head under local anesthesia prior to obtaining a computed tomography (CT) scan. The fusion image was obtained by merging the images from CT and MRI (1.5 T, General Electric) using the Surgiplan software (Elekta, Stockholm, Sweden) for GPi and STN targeting, as previously described (Lin et al., 2019). Under local anesthesia, the previously implanted GPi-DBS lead was pulled out and PVP was performed. The GPi was located 2–4 mm anterior to the anterior commissure–posterior commissure (AC-PC) line midpoint, 18–22 mm lateral to the AC-PC line, and 2–4 mm below the AC-PC line. A radiofrequency electrode (Radionics) with a 2-mm diameter radiofrequency probe and a 2-mm exposed tip was used for impedance measurement. The tip of the electrode was heated to 70–80°C for 60 s. The length of the lesion was about 5 mm. New Quadripolar DBS electrodes (model 3387, Medtronic) were then implanted into the STN and connected to the previously implanted extension wire and IPG (37612 RC or 37603 SC, Medtronic) under general anesthesia. Postoperative MRI and CT confirmed the precision of PVP and electrode placement (**Figure 1**) and the targeted and actual (post-op imaging-derived) anterior commissure AC-PC coordinates of the STN leads and PVP are listed in **Supplementary Table 1**.

The surgery site for unilateral PVP was decided based on the dystonia distribution. Generally, the contralateral hemisphere to the most affected side by dystonia was chosen as the surgical site. For patients 1, 2, 3, and 4 who all exhibited asymmetrical cervical dystonia, laterocollis. Therefore, the contralateral side to the direction of neck tilting was chosen as the surgical side, which was consistent with another article we reported (Lai et al., 2020). For example, patient 3 presented with left laterocollis and underwent right PVP. Patients 1, 2, and 4 presented with right laterocollis and underwent left PVP. For patient 5, he presented symmetrical midline symptoms. For him, the right PVP was chosen. Unilateral PVP was reported to significantly improved all midline BFMDRS subitems (eyes, mouth, speech/swallow, neck, and trunk) (Horisawa et al., 2021). However, studies have shown that left PVP produced more impairment in verbal fluency than right PVP (Crowe et al., 1998; Junqué et al., 1999). Therefore, for patients only presenting with symmetrical symptoms, the right PVP is preferred. For

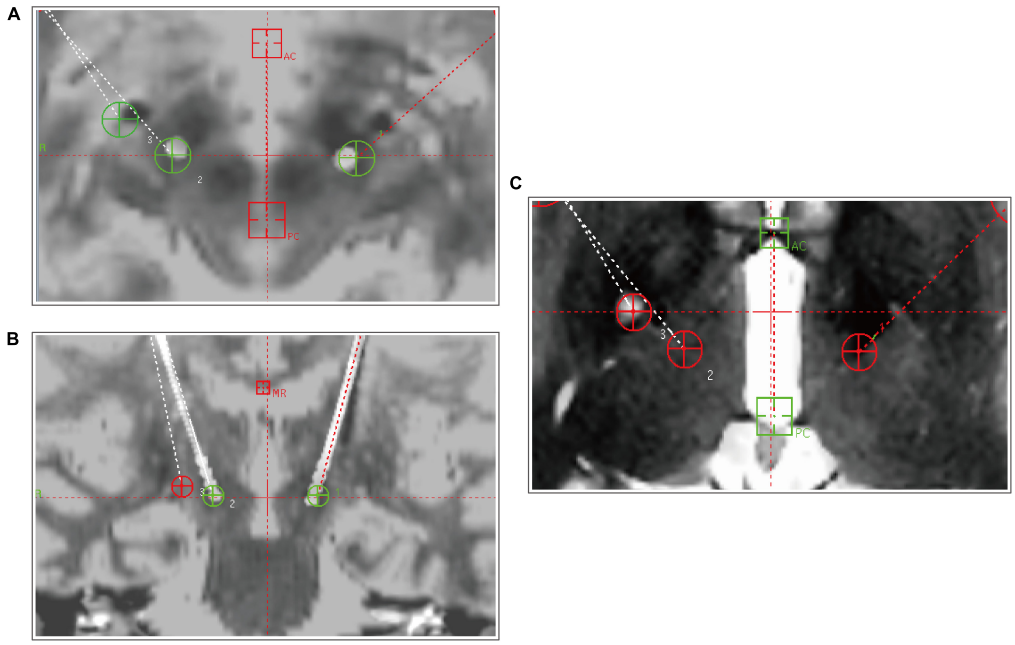

FIGURE 1
Postoperative MR images of patient 5, demonstrating positions of the implanted electrodes in the bilateral STN **(A,B)** and unilateral pallidotomy. The two red orthogonal lines refer to the Cartesian coordinate system in each view, whereas the diagonal lines, with or without green circles, represent the trajectories of the implanted leads. In the center of each view, the two green circles (named 1 and 2) in **(A,B)** show the planned targets, and the one red circle (named 3) in **(C)** shows the position of pallidotomy. AC, anterior commissure; MR, midline reference; PC, posterior commissure.

patient 6, she presented more severe right upper limb symptoms, and left PVP was chosen for her.

Postoperative stimulation parameters and statistical analysis

The patients were discharged from the hospital 1 week after surgery, and stimulation parameters were adjusted in an outpatient setting according to the patient's clinical status at each follow-up postoperatively. All statistical analyses were performed using Graphpad prism 8. The differences in DBS efficacy after each follow-up are analyzed by parametric tests (Student paired-sample *t*-tests) or non-parametric models (paired-sample Wilcoxon signed-rank tests). A *p*-value of < 0.05 was considered statistically significant.

Results

Demographics and clinical data

Table 1 presents the clinical characteristics and preoperative scores of each patient (two females and four males). The age of patients undergoing surgery ranges from 40 to 63 years old.

Outcomes of dystonia

Based on total movement BMFDRS scores, significant amelioration was achieved at 1-month (6.5 ± 7.45; $p = 0.0049$), 6-month (5.67 ± 6.3; $p = 0.0056$), and at LFU (4.67 ± 4.72; $p = 0.0094$) follow-up compared with the baseline (LFU of GPi DBS with on status) (17.33 ± 11.79). The percentage of improvement reached 70.6, 74.67, and 77.05%, respectively (Table 2 and Figure 2). At LFU, a significant difference was found between stimulation bi-OFF and uni-ON (11.08 ± 8.38 vs. 9 ± 8.52, $p = 0.0191$), as well as between stimulation bi-OFF and bi-ON (11.08 ± 8.38 vs. 4.67 ± 4.72, $p = 0.0164$).

The total disability BFMDRS scores reduced significantly at 1-month (2.67 ± 3.88; $p = 0.0313$), 6-month (2.67 ± 3.88, $p = 0.0313$), and 12-month follow-up (2.67 ± 3.88; $p = 0.0313$) compared with baseline (7 ± 4.9), with an improvement of 77.62%, respectively (Table 2).

Assessment of quality of life

STN plus STN DBS remarkably upregulated the quality of life evaluated by SF-36, 1, 6, and 12 months postoperatively (Table 3). Noticeable elevation was discovered in every subscale

TABLE 2 Effect of treatment on BFMDRS movement and disability scales after surgery.[a]

Variable	Patient 1 (m/d)	Patient 2 (m/d)	Patient 3 (m/d)	Patient 4 (m/d)	Patient 5 (m/d)	Patient 6 (m/d)	Movement scores (mean ± SD)	Disability scores (mean ± SD)	Mean improvement, % (movement scores)	Mean improvement, % (disability scores)
pre-GPi	26/15	6/3	8/3	3/2	16/3	22/10	13.5 ± 9.29	6 ± 5.29	/	/
GPi 6m	14/6	2/1	4/2	0.5/0	4/1	10/4	6.08 ± 4.82	2.33 ± 2.25	62.61	63.82
GPi LFU	24/14	12.5/6	6/3	4/2	22/5	35/12	17.33 ± 11.79	7 ± 4.9	-34.26	-30
STN + PVP 1m	10/9	2/0	1/0	1/0	5/1	20/6	6.5 ± 7.45	2.67 ± 3.88	70.6	77.62
STN + PVP 6m	9.5/9	1.5/0	0.5/0	1/0	5/1	16.5/6	5.67 ± 6.3	2.67 ± 3.88	74.67	77.62
STN + PVP LFU STN-bi-off	14.5/10	9.5/3	1.5/0	4/1	12/2	25/6	11.08 ± 8.38	3.67 ± 3.72	42.59	56.43
STN + PVP LFU STN-uni-on	13.5/10	5.5/2	1.5/0	1/0	9/2	23.5/6	9 ± 8.52	3.33 ± 3.93	60.44	67.54
STN + PVP LFU STN-bi-on	9.5/9	1.5/0	0.5/0	1/0	4/1	11.5/6	4.67 ± 4.72	2.67 ± 3.88	77.05	77.62

***P*-value[b]**

Variable	GPi 6m vs. pre-GPi	GPi LFU vs. pre-GPi	GPi LFU vs. STN + PVP 1m	GPi LFU vs. STN + PVP 6m	GPi LFU vs. STN + PVP 12m bi STN-off	GPi LFU vs. STN + PVP 12m uni STN-on	GPi LFU vs. STN + PVP 12m bi STN-on	STN + PVP 12m STN-bi-off vs. 12m STN-uni-on	STN + PVP 12m STN-uni-on vs. 12m STN-bi-on	STN + PVP 12m STN-bi-off vs. 12m STN-bi-on
Movement scores	**0.0172**	0.1676	**0.0049**	**0.0056**	**0.0139**	**0.0034**	**0.0094**	**0.0191**	0.0538	**0.0164**
Disability scores	**0.0313**	0.25	**0.0313**	**0.0313**	**0.0041**	**0.0012**	**0.0313**	0.1747	0.25	0.125

[a]Pre, preoperative. BFMDRS scores in each patient are shown in (m/d). m/d, BFMDRS movement scores/BFMDRS disability scores. Description statistics are shown with the mean ± standard deviation; % improvement in the post-GPi = BFMDRS score (baseline—6 months or LFU)/baseline; % improvement in the post-PVP + STN = BFMDRS score (GPi LFU-each follow-up after PVP + STN)/GPi LFU.

[b]*P*-value for comparisons between each follow-up as analyzed by parametric tests (Student paired-sample *t*-tests) or non-parametric models (paired-sample Wilcoxon signed-rank tests).

The bold values refers to the *p* values below 0.05.

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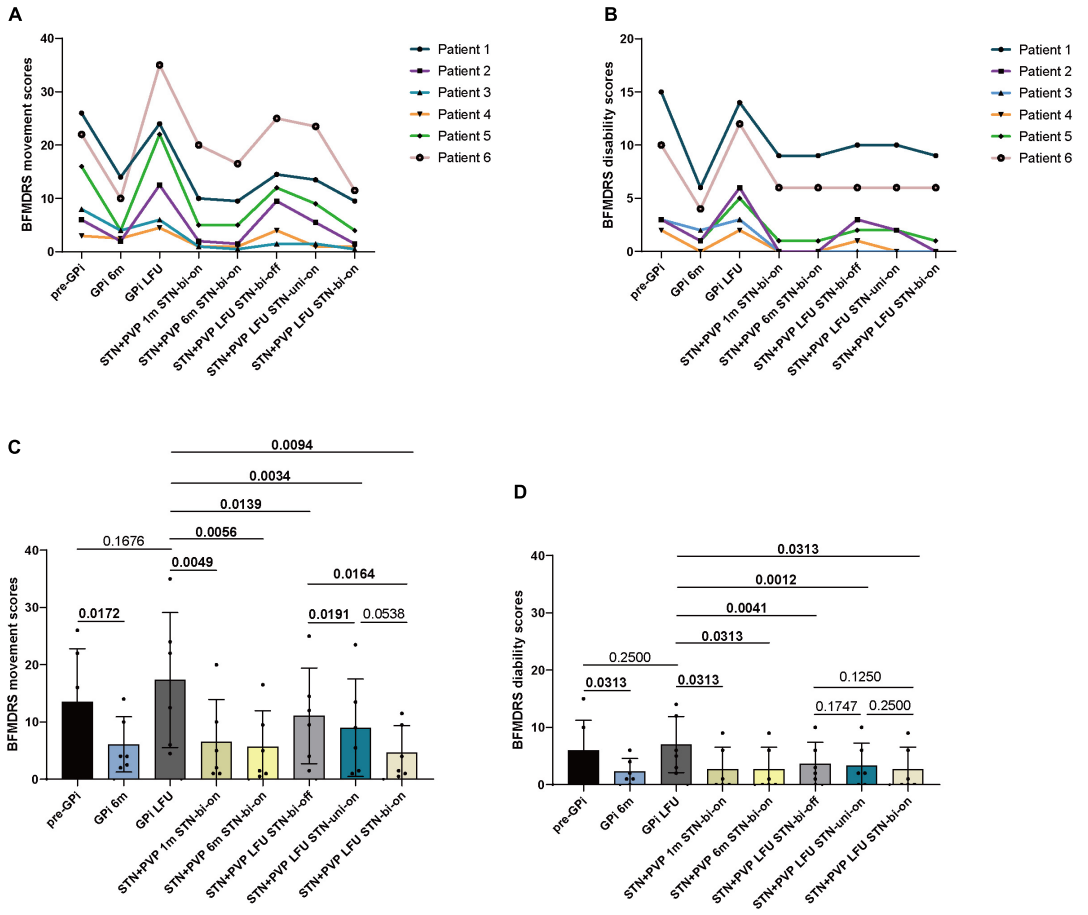

FIGURE 2
Individual BFMDRS movement **(A)** and disability scores **(B)** before bilateral GPi DBS surgery (pre-GPi), at 6 months (GPi 6m) and the last follow-up (LFU) after GPi DBS (GPi LFU), and at 1 month (STN + PVP 1m STN-bi-on), 6 months (STN + PVP 6m STN-bi-on), and LFU post-bilateral STN plus unilateral PVP surgery. The LFU post-bilateral STN plus unilateral PVP was evaluated at three conditions: STN bilateral OFF (STN + PVP LFU STN-bi-off), STN unilateral ON (STN + PVP LFU STN-uni-on), and STN bilateral ON (STN + PVP LFU STN-bi-on). **(C)** Mean BFMDRS movement scores and **(D)** disability scores at each follow-up. LFU, last follow-up; m, month; Pre, pre-operation; PVP, posteroventral pallidotomy; bi, bilateral; uni, unilateral. *P*-values for comparisons between each follow-up are analyzed by parametric tests (Student paired-sample *t*-tests) or non-parametric models (paired-sample Wilcoxon signed-rank tests). A p-value of < 0.05 was considered statistically significant.

of SF-36 except for role physical, especially in general health and mental health, aspects reaching a *p*-value lower than 0.01.

Adverse events

Overall, the surgical procedures were well-tolerated in this population. There were no hardware-related side effects, infections, intracranial hemorrhages, or extension or lead fractures from DBS implantation during the follow-up period. Although patient 2 experienced dysarthria due to stimulation intensities above the therapeutic threshold, it was eliminated immediately after reprogramming. Stimulation-induced paresthesia took place in all six patients but vanished after adjusting the stimulation parameters. Common adverse events associated with STN-DBS in patients with Parkinson's disease, including fatigue and dyskinesia, were not observed in any of our patients.

Discussion

Here, STN-DBS plus unilateral PVP significantly improved overall movement and disability BFMDRS scores by 77.05 and 77.62%, respectively, at the final follow-up (mean 16.00 ± 4.65 months) in patients with previously failed GPi-DBS.

There were few reports available considering rescue strategies for suboptimal DBS in dystonia. Thus, for the literature review, we used the search terms "dystonia" and

"thalamotomy" or "pallidotomy" or "subthalamic nucleus" or "globus pallidus internus" or "lesional surgery" in combination with "failed," "previously undergone," "prior," "suboptimal," or "rescue" in PUBMED and EMBASE databases. All articles in English published before 10 April 2022 were included. The full text was checked to select the studies investigating practice for unsatisfied response to GPi-DBS. Ultimately, four original studies including case reports were identified for further discussion (**Table 4**). Ellis et al. (2008) did a case series with four patients receiving lead replacement (average distance of adjustment: 6.7 mm, bilateral or unilateral) after a less satisfying response to bilateral GPi-DBS. Two patients had their neck dystonia greatly relieved while one had benefits for motor symptoms and the other had mild recovery in speech and swallowing. Similarly, Oyama et al. (2011) reported the inconformity of lead position in one patient whose left GPi lead was 2.4 mm more anterior than the right one indicated by neuroimaging. Thus, the replacement was implemented followed by achieving the desired effect. Aragão et al. (2021) reported a patient with refractory Meige syndrome who was initially stimulated at GPi and achieved satisfactory alleviation after shifting the target to STN. Likewise, Oyama et al. (2011) reported a patient with dystonia received noticeable symptomatic relief after bilateral STN-DBS, which was the rescue procedure 2 years after the unsatisfying bilateral GPi-DBS.

Multiple factors could contribute to insufficient outcomes after GPi-DBS in isolated dystonia. Pauls et al. (2017) analyzed 22 isolated dystonia cases with Gpi-DBS failure and found lead displacement and inappropriate stimulation are the most common causes and thus should be excluded first. In our study, we ruled out these possibilities by verifying lead placement with postoperative MRI (**Supplementary Figure 1**) and sufficient programming. And the considerable improvement generated in the first 6 months (46.15–83.33%) further confirmed the initially accurate placement and suitable stimulating parameters.

Body distribution of dystonia may affect long-term outcomes. In our cohort, the areas involved were mainly cranial-cervical and cranial-facial. It was reported that cranial and cervical dystonia exhibit variant outcomes after GPi-DBS. Limotai et al. (2011) reported a remarkable variation of improvement among six patients with cranio-facial and cranio-cervical dystonia (reduction percentage of 16.6–100% indicated by BFMDRS scores) 12 months after GPi DBS, with two of them having less than 20% amelioration. The investigation from Sensi et al. (2009) showed that the improvement percentage of BFMDRS ranged from 30 to 82% in the long run for patients with segmental dystonia treated with GPi DBS. Martinez-Torres et al. (2009) reported that GPi DBS improved trunk and oropharyngeal dystonia but the benefit was absent for blepharospasm in isolated dystonia. Larger and longer prospective studies with blinded evaluation are needed to explore whether the regions involved are indicators for response to GPi-DBS and the underlying mechanisms.

Another reason worth considering is habituation. The term "habituation," previously known as "tolerance," is referred to as the vanishing of DBS efficacy despite reprogramming that could not be explained by loss of micro-lesional implant effect or disease progression (Fasano and Helmich, 2019; Peters and Tisch, 2021). It is mostly reported in cases of essential tremor cases, but the phenomenon has also been described in dystonia patients receiving GPi DBS (Shah and Jimenez Shahed, 2014). Currently, the underlying mechanism remains unclear. It is well-established that the dysfunction of the cortico-basal ganglia-thalamo-cortical circuit is a crucial contributor to dystonia (Vitek, 2002). Previous studies have shown that GPi-DBS could normalize excessive cortical plasticity and is one of the fundamental factors for its effect (Tisch et al., 2007; Ruge et al., 2011a, Barow et al., 2014). However, it has been suggested that habituation may also be generated from neural reorganization (Dostrovsky and Lozano, 2002; Ruge et al., 2011a, Peters and Tisch, 2021). And theoretically, it should be noted that STN DBS may also induce habituation. Nevertheless, in our cohort, all six patients relapsed within 1 year after GPi DBS; in contrast, no recurrence was reported before our last follow-up (12–24 months). A long-term follow-up is still needed.

Disease progression can also contribute to the decline of DBS efficacy. However, it is difficult to distinguish natural disease progression from habituation (Ruge et al., 2011a, Peters and Tisch, 2021). The emergence of new symptoms may be an indicator of disease deterioration. Therefore, in our study, the novel blepharospasm by patient 2 and the newly emerged shoulder muscle tension shown by patient 5 are possibly derived from disease deterioration.

In our study, the efficiency of PVP alone may be reflected by the status at bilateral STN OFF. While this conclusion must be considered with caution because it is possible that effects generated by STN DBS may not be washed out completely, it remains interesting that the unilateral PVP was highly effective and could rescue the failed bilateral GPi DBS. This may be related to the different mechanisms of action between these two procedures. Since GPi consists of gamma-aminobutyric-acid mediated inhibitory neurons, DBS at this location will lead to neural depolarization and subsequently suppresses abnormally enhanced synchronized oscillatory activity within the motor cortico-basal ganglia network in dystonia (Dostrovsky and Lozano, 2002; Ni et al., 2018). As for PVP, it may correct the irregular neuronal firing in the network by destroying the afferent and/or efferent circuitries (Lozano et al., 1997; Vitek et al., 1999). In addition, as mentioned before, DBS may probably generate habituation. Dystonic disorders are commonly characterized by strengthened plasticity and decreased inhibition in the

TABLE 3 Health-related quality of life data as a function of STN + PVP before surgery and at 1, 6, and LFU months after surgery.[a]

	Score: Mean ± SD						*P*-value [b]				
SF36 subscale	**Pre_GPi**	**GPi_6m**	**GPi LFU**	**STN + PVP 1m STN_bi_on**	**STN + PVP 6m STN_bi_on**	**STN + PVP 12m STN_bi_on**	**GPi_6m vs. Pre_GPi**	**GPi_LFU vs. Pre_GPi**	**STN + PVP 1m vs. GPi LFU**	**STN + PVP 6m vs. GPi LFU**	**STN + PVP 12m vs. GPi LFU**
General health	22 ± 6.8	58 ± 13	20 ± 6.3	47 ± 9.3	68 ± 5.2	69 ± 6.6	0.0006	0.6383	0.0001	<0.0001	<0.0001
Physical function	36 ± 21	77 ± 19	29 ± 22	78 ± 20	82 ± 25	90 ± 22	0.0313	0.5	0.0003	0.0313	0.0313
Role physical	21 ± 40	50 ± 55	21 ± 40	50 ± 55	67 ± 52	67 ± 52	0.5	/	0.5	0.25	0.25
Role emotional	11 ± 17	44 ± 11	11 ± 17	50 ± 28	83 ± 18	89 ± 17	0.0625	/	0.0625	0.0313	0.0313
Social functional	21 ± 19	46 ± 10	21 ± 10	54 ± 19	79 ± 19	79 ± 19	0.0625	>0.9999	0.0313	0.0313	0.0313
Body pain	50 ± 10	70 ± 6	50 ± 12	72 ± 8	77 ± 5.2	77 ± 5.2	0.0625	>0.9999	0.0005	0.0313	0.0313
Vitality	37 ± 12	58 ± 8	34 ± 8	58 ± 7.5	73 ± 9.4	77 ± 11	0.0026	0.5177	<0.0001	0.0002	0.0313
Mental health	35 ± 4.1	62 ± 21	31 ± 13	57 ± 17	65 ± 18	69 ± 19	0.0625	0.625	0.0019	0.0012	0.0012

[a]LFU, last follow-up.

Values are expressed as the mean ± standard deviation. Scores range from 0 to 100, and an increase in score indicates improvement.

[b]*p*-value for every subscale comparison between 1 month and pre-operation, 6 months and 1 month, and LFU and 6 months in each group.

10.3389/fnins.2022.924617

motor cortex (Ridding et al., 1995; Quartarone et al., 2003). The investigation from Ruge and his co-workers suggested that these two parameters were normalized in the primary dystonia at 3- and 6-month follow-up (Ruge et al., 2011b) but showed distinct patterns from healthy controls in the long run (Ruge et al., 2011a). In our cohort, time points when the decay of established stimulation benefits took place were more than 6 months after implantation. Hence, though Ruge's observations might be influenced by the bias of the small sample size and different genetic backgrounds, non-beneficial impacts from continuous stimulation may exist and may partly contribute to the unsatisfied response. Moreover, thalamotomy was indicated effective for failed thalamic DBS (Bahgat et al., 2013; Peters and Tisch, 2021), suggesting a possible disparity of effects between ablation and DBS as well.

The synergistic effect of unilateral PVP plus STN DBS was observed when comparing the benefits with that of bilateral-off, unilateral-on, and bilateral-on status of STN DBS at the last follow-up (**Figure 2**). There is growing evidence that dystonia is the reflection of multi-level network dysfunction (Jinnah et al., 2017). Therefore, stimulating different sites of the circuit spontaneously may generate combinational effects. Schjerling and his co-workers suggested double stimulation at GPi and STN was more effective than stimulating either target alone in dystonia (Schjerling et al., 2013). Two teams (Fonoff et al., 2012; Dec et al., 2014) reported STN DBS could generate further alleviation in patients with dystonia after partial improvement yielded from initial PVP, suggesting the collaborative effect of these strategies. Moreover, Horisawa et al. (2019) performed lesions at contralateral Forel's field H1, the efferent fibers from the Gpi to the thalamus, on 11 patients with dystonia who had undergone unilateral PVP. They proposed the significant improvement observed derived from the congenerous effects of the combined surgeries. It is worth mentioning that Forel's field H1 is located close to the dorsal border of the STN, which is the preferred target of STN in dystonia (Cao et al., 2013; Ostrem et al., 2017). Thus, the combined effect of unilateral PVP plus STN DBS in our study may have a similar mechanism to the unilateral PVP plus contralateral campotomy.

There are few reports exploring the washout time of STN DBS in dystonia. Miocinovic et al. (2018) performed a 90-min for DBS washout and worsen dystonia was observed, but the performance would not drop back to that at baseline. Wagle Shukla et al. (2018) adopted 4–8 h for washout of STN stimulation and a significant worsening of dystonic symptoms was observed. In our study, even though a 12-h washout was used and significant upregulation of BFMDRS scores was observed, insufficient washout could not be excluded. Further exploration of washout time on DBS for dystonia is necessary.

TABLE 4 Reports of rescue procedures after failed DBS or lesion surgery in patients with dystonia.[a]

Author, year		Aragão et al., 2021	Ellis et al., 2008			Oyama et al., 2011		Blomstedt et al., 2016
Diagnosis		Meige syndrome	D	D	D	D	CD	TD	D
Age at onset (years)		65	66	40	8	43	32	25	10
Disease duration[a] (months)		228	7	5	3	12	13	8	732
Last follow-up (months)		24	12	24	6	6	17	15	12
Previous surgery		bi GPi DBS	bi GPi DBS	bi GPi DBS	bi GPi DBS	bi GPi DBS	bi GPi DBS	bi GPi DBS	bi GPI DBS (hardware-infection).
Rescue procedure		bi STN DBS	Replace leads-bi	Replace lead-uni	Replace leads-bi	Replace leads-uni	GPi DBS (L)	Bi STN DBS	Uni Pdt
UDRS	Baseline	NA	NA	NA	NA	NA	11	28	NA
	Before rescue	NA	22	NA	50	6	6	24	NA
	Post rescue	NA	18	NA	46	4	4	8	NA
	Improvement	NA	45.4%	NA	8%	33.3%	33.3%	66.7%	NA
BFMDRS	Baseline	NA	NA	NA	NA	NA	NA	NA	39
	Before rescue	17	NA	NA	NA	NA	NA	NA	2.5
	Post rescue	1.5	NA	NA	NA	NA	NA	NA	5
	Improvement	92.10%	NA	NA	NA	NA	NA	NA	-50%

[a]bi, Bilateral; uni, unilateral; D, Dystonia; CD, cervical dystonia; TD, Torsion dystonia; Pdt, Pallidotomy; UDRS, Unified Dystonia Rating Scale; NA, Not available; Duration, between onset and rescue procedure; improvement, before rescue and last follow-up.

Patient 6 showed upper limb torsion, which was less common in isolated dystonia. After excluding neuropathy abnormalities, such as neurodegeneration, acquired impairment (like intracerebral lesions), metabolism, or other systemic factors, she was finally diagnosed with isolated dystonia (idiopathic or genetic etiology) according to the consensus in 2013 (Albanese et al., 2013). This diagnosis was supported by Bettina Balint and her team who reviewed the cases of idiopathic or genetic isolated dystonia and found that upper limb involvement was a typical clinical manifestation of monogenic dystonia (Balint et al., 2018).

The current report described the effectiveness and safety of bilateral STN-DBS plus unilateral PVP in six patients with isolated dystonia who had previously undergone unsatisfactory GPi-DBS. This rescue procedure was selected for the following reasons: First, it is cost-saving without an additional IPG, compared with bilateral stimulation at both GPi and STN. Second, it is also suitable for patients who prefer not undergoing staged surgery or having two implanted IPGs. In these cases, whether they have financial concerns or not, STN plus PVP is a viable alternative option for them to choose.

There are several limitations to this study. First, the sample size is small and the background is relatively heterogenous since two subjects declined whole exome sequencing. This may lead to a deviation in our results because the response to GPi or STN-DBS may vary depending on certain genetic backgrounds (Aravamuthan et al., 2017). Second, patients 2 and 5 could not tolerate a bilateral STN-off. Therefore, upon request, we switched on STN in advance, which might introduce some bias into the results. Third, owing to the worsening of dystonia in the stimulation "off" state, blinding the participants to stimulation status was not possible. Fourth, the time period for DBS OFF was relatively short and may not achieve a complete washout thus influencing the evaluation of PVP's effect. Fifth, our study could not conclude whether the efficiency of PVP plus STN-DBS is better than the effect achieved by STN-DBS alone due to the persistent effect of PVP. It is more rigorous to conduct a staged surgery that the bilateral STN DBS is first applied and PVP can be considered according to STN-DBS's effect. Our strategy is suitable for patients who are unwilling to undergo two surgeries with superimposed surgical trauma. Future studies should enroll eligible patients to address this issue.

Conclusion

This study confirmed the significant improvement in BFMDRS motor scores (77.05% reduction) during the 16-month follow-up after bilateral STN-DBS plus unilateral PVP in patients with isolated dystonia who experienced secondary failure following GPi-DBS. The bilateral STN-DBS plus unilateral PVP may be an alternative rescue procedure for isolated dystonia. Larger and longer prospective studies with blinded evaluation are needed to elucidate the effect of bilateral STN-DBS plus unilateral PVP on dystonia.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ruijin Hospital Ethics Committee of the Shanghai Jiao Tong University School of Medicine. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

DL, YW, SL, and BS contributed to conception and design. SL, SG, YW, TW, LW, CZ, and YS conducted the acquisition of data. SL, YW, LW, SG, and HL analyzed and interpreted the data. SL, LW, and HL drafted the article. DL, SL, YW, LW, YS, SG, and CZ critically revised the article. DL, SL, YW, CZ, LW, SG, and TW gave administrative, technical, and material support. DL, SL, YW, and LW offered study supervision. All authors reviewed the manuscript for submission and YW approved the final version of the manuscript on behalf of all authors.

Funding

This work was supported by the Shanghai Health and Family Planning Commission research project (201840001), the Shanghai Youth Science and Technology Talents Sailing Project (20YF1426600), the National Natural Science Foundation of China (81870887 and 81771482), the Shanghai Collaborative Innovation Center for Translational Medicine (TM201904), the Shanghai Health and Family Planning (201440504), and innovative research team of high-level local universities in Shanghai.

Acknowledgments

We thank all six patients for participating in this study. We also thank Gabrielle Shall for providing constructive comments during the revision of this manuscript.

Conflict of interest

DL and CZ have received honoraria and travel expenses from the Deep Brain Stimulation industry (Medtronic, PINS, and SceneRay). BS has received research support from PINS and SceneRay (donated devices).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.924617/full#supplementary-material

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frontiers | Frontiers in Neuroscience

TYPE Original Research
PUBLISHED 08 September 2022
DOI 10.3389/fnins.2022.994570

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

RECEIVED 15 July 2022
ACCEPTED 22 August 2022
PUBLISHED 08 September 2022

CITATION
Xie Z, Yan J, Dong S, Ji H and Yuan Y (2022) Phase-locked closed-loop ultrasound stimulation modulates theta and gamma rhythms in the mouse hippocampus. *Front. Neurosci.* 16:994570. doi: 10.3389/fnins.2022.994570

Phase-locked closed-loop ultrasound stimulation modulates theta and gamma rhythms in the mouse hippocampus

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Previous studies have demonstrated that open-loop transcranial ultrasound stimulation (TUS) can modulate theta and gamma rhythms of the local field potentials (LFPs) in the mouse hippocampus; however, the manner in which closed-loop TUS with different pressures based on phase-locking of theta rhythms modulates theta and gamma rhythm remains unclear. In this study, we established a closed-loop TUS system, which can perform closed-loop TUS by predicting the peaks and troughs of the theta rhythm. Comparison of the power, sample entropy and complexity, and phase-amplitude coupling (PAC) between the theta and gamma rhythms under peak and trough stimulation of the theta rhythm revealed the following: (1) the variation in the absolute power of the gamma rhythm and the relative power of the theta rhythm under TUS at 0.6–0.8 MPa differ between peak and trough stimulation; (2) the relationship of the sample entropy of the theta and gamma rhythms with ultrasound pressure depends on peak and trough stimulation; and (3) peak and trough stimulation affect the PAC strength between the theta and gamma rhythm as a function of ultrasound pressure. These results demonstrate that the modulation of the theta and gamma rhythms by ultrasound pressure depends on peak and trough stimulation of the theta rhythm in the mouse hippocampus.

KEYWORDS

closed-loop, ultrasound stimulation, theta rhythm, gamma rhythm, ultrasound pressure

Introduction

Neural oscillatory activity refers to continuous and rhythmic neural activity of neurons in the brain, which plays an important role in the information processing of neural networks (Ward, 2003; Buzsaki and Draguhn, 2004; Uhlhaas and Singer, 2010). Previous studies have found that neural oscillatory activity in specific frequency bands, including theta (4–8 Hz), alpha (9–13 Hz), beta (14–30 Hz), and gamma (above 30 Hz), is associated with learning and memory performance. Theta oscillation (4–8 Hz) plays a key role in learning, spatial encoding, memory, and sniffing movement (Düzel et al., 2010; Fell and Axmacher, 2011; Hsieh et al., 2011). Some researchers have proposed that the theta rhythm is induced by the hippocampal cortical pathway, which subsequently enters the neurons in different cortical areas, and connects the separated neurons through synchronous oscillatory activity for information transmission processing. The establishment of this connection forms the physiological basis for working memory and encoding of new information (Colgin, 2013; Kropff et al., 2021; Nuñez and Buño, 2021). The gamma rhythm represents fast oscillatory activity of neurons and neuron groups, which is mainly generated by the network composed of inhibitory interneurons and can facilitate synaptic transmission and modulate sensory cognitive activities, such as attention and memory tasks (Colgin and Moser, 2010; Buzsáki and Wang, 2012; Mably and Colgin, 2018). Theta and gamma rhythms play an important role in the evaluation of external stimuli, such as optogenetic stimulation, deep brain stimulation, transcranial magnetic stimulation, etc. (Mangia et al., 2014; Noda et al., 2018; Etter et al., 2019).

Low-intensity transcranial ultrasound stimulation (TUS), which has recently emerged as a non-invasive neuromodulation technique, possesses high spatial resolution and the ability to access deep structures of the brain (Bystritsky et al., 2011; Niu et al., 2018; Yu et al., 2020). In the past decade, low-intensity TUS has been widely used in the field of neuromodulation (Jiang et al., 2018; Baek et al., 2020; Yuan et al., 2020). Previous studies have reported the ability of TUS to elicit the encoding of neural information in the cortical and deep brain regions, especially theta and gamma rhythms of local field potentials (LFPs). For example, studies have shown that the relative power in the theta (4–8 Hz) frequency band of the mouse motor cortex under open-loop ultrasound stimulation decreases with the increase in ultrasound pressure at 0–0.5 and 0.5–1 s, the relative power in the gamma (30–45 Hz) band increases with the increase in ultrasound pressure and stimulation duration (Wang et al., 2019).

Ultrasound stimulation of the hippocampus significantly enhances both signal pressure of the gamma band (Tufail et al., 2010) and power pressure in the gamma band in the stimulation area (Yu et al., 2016). Studies have also shown that TUS significantly modulates the phase-amplitude coupling (PAC) strength between the theta and gamma bands in the rat hippocampus, which increases with ultrasound pressure (Yuan et al., 2016a,b). We also found that TUS of the thalamus enhances the amplitude of the theta rhythm of the thalamus and the pressure of the theta rhythm in the motor cortex (Wang et al., 2021). Another study showed that open-loop ultrasound stimulation alters the phase distribution of intrinsic brain activity at the beta frequency, but not at gamma frequency. This modulation is accompanied by changes in the phase rate of the beta and gamma frequencies (Mueller et al., 2014). In conclusion, open-loop ultrasound can significantly modulate theta and gamma rhythms of LFPs in different brain regions including the cortex, hippocampus, and thalamus. Closed-loop ultrasound stimulation better enables perform phase-locked neuromodulation according to the characteristics of the signal compared to open-loop stimulation. In previous studies, we found that peak and trough stimulation of the theta rhythm can enhance the power of the theta rhythm (Yang et al., 2020). However, the manner in which TUS with peak and trough stimulation of the theta rhythm modulates the gamma rhythm and PAC between the theta and gamma rhythms remains unelucidated.

Ultrasound parameters play a key role in ultrasound stimulation. Previous research has proven that modifying the parameters of ultrasound radiation pressure (such as frequency, pressure, duty cycle, etc.) can elicit different ultrasound neuromodulation functions. In open-loop TUS, ultrasound pressure has a significant effect on the stimulation results with respect to the motor response, neural firing, cerebral hemodynamics, etc. For example, the pressure of ultrasound stimulation is correlated with the observed robustness of the motor response with the increase in ultrasound pressure, and the amplitude of the motor-responsive electromyogram signal decreases with the increase in ultrasound pressure (Tufail et al., 2010; Mehiæ et al., 2014). The strength of the calcium response and neural response evoked by ultrasound neuronal stimulation increases with the increase in ultrasound pressure (Qiu et al., 2019; Yoo et al., 2022). Moreover, the current pressure of ultrasound-induced TWIK-related arachidonic acid activated K^+ (TRAAK) channels increases with the surge in ultrasound pressure (Sorum et al., 2021). The number of ultrasound-evoked spikes in I92L-infected neurons is dependent on the peak negative pressure associated with the increase in ultrasound stimulation pressure (Ye et al., 2018), and the coupling strength between neural oscillations and hemodynamics exhibits a linear increase with an increase in ultrasound pressure (Yuan et al., 2021). In conclusion, the modulation effect of open-loop ultrasound stimulation on neural activity depends on ultrasound pressure. However, until now, the manner in which neural firing activity, including the theta and gamma rhythms, varies with ultrasound pressure under phase-locked TUS remains unknown.

Therefore, we conducted this study to obtain answers to the above-mentioned questions, and to this end, established

a closed-loop TUS system that can accurately track the peaks and troughs of the theta rhythm. We recoded the LFPs of the stimulation area under closed-loop TUS. Thereafter, we analyzed the power spectrum, complexity, sample entropy, and PAC strength of the theta and gamma rhythms under peak-to-trough stimulation as a function of ultrasound pressure.

Materials and methods

Animals and groups

Sixteen mice (C57BL/6, male, body weight: 20–25 g, Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd., China) were used in this study. All procedures were conducted in accordance with the relevant regulations of animal ethics and the Ethics Committee of Yanshan University. The mice were housed in standard cages under a light/dark cycle of 12-h/12-h and provided food and water *ad libitum*. The mice were randomly divided into the peak stimulation (8 mice) and trough stimulation groups (8 mice).

Operation

General anesthesia was induced with isoflurane 2% during the procedure. After administering anesthesia in the induction box, the mice were fixed on an adapter (68030, Reward Company, China) and placed on a stereotaxic device (68001, Reward Company, China). The anesthesia mask of the gas anesthesia machine (R540 mobile small animal gas anesthesia machine, Reward Company, China) was placed over the mouse's mouth for real-time anesthesia. The fur covering the animal's skull was shaved, and the skin was cleaned with physiological 0.9% sodium chloride solution. The scalp was incised along the midline of the skull, and the subcutaneous tissue and periosteum were removed in preparation for the experiment. A hole with diameter of 0.5 mm was drilled at the following coordinates relative to the bregma: anteroposterior (AP) = −2 mm, medial-lateral (ML) = 2 mm, and dorsoventral (DV) = −1.5 mm. A tungsten microelectrode (WE50030.1B10, MicroProbe, United States) was used to record the LFP signals. Two holes were drilled in the nasal bone to fix the ground and reference electrodes. During the experiment, all mice were anesthetized using 0.3% isoflurane.

Closed-loop ultrasound stimulation system and ultrasound parameters

The schematic of the TUS procedure is shown in **Figure 1A**. An ultrasound transducer (V301-SU, Olympus, United States) was attached to the mouse skull through a conical collimator filled with a bubble-free ultrasound coupling gel, which was aimed at the CA1 region of the hippocampus; the angle of the collimator to the recording electrode was approximately 45°. In our experiment, the fundamental frequency, stimulation duration, pulsed repetition frequency, and duty cycle within the stimulation, and stimulation interval were 2.25 MHz, 100 ms, 1 kHz, and 30%, and 3 s, respectively (**Figure 1B**). The ultrasound pressure ranged from 0.05 to 0.8 MPa. Ultrasound field distribution under the skull in the xz and xy planes were shown in **Figure 1C**. Reconstruction profiles were placed along the white dotted lines in the xz and xy planes (**Figure 1D–F**). The diameter of the focal area measured at full width at half maximum (FWHM) was ∼1.5 mm.

A computer was used to receive a multi-channel neural signal processor (Apollo, Bio-Signal Technologies, United States) through a universal serial bus connector to record the LFP signal (sampling rate: 30 kHz) from the electrodes. When the neural signal is calculated, the computer issues stimulation instructions according to the calculation results. A control signal generator (DG2052, RIGOL, China) generated the modulation signal, which was first transmitted through a linear RF power amplifier (240L, ENI Inc., United States) and then transmitted to the ultrasound transducer to emit ultrasound waves. The neural signal processor continuously collects LFP signals in real-time during the experiment, and records the ultrasound trigger signal of the signal generator at the same time. All experiments were conducted in an electromagnetic shielding cage to prevent external electromagnetic interference.

The algorithm for predicting the theta rhythm used in this experiment is based on a previous study (Kanta et al., 2019). The algorithm is programmed in MATLAB software. The computer receives the real-time LFP signals and performs downsampling (500 Hz) and filtering (4–8 Hz) with a FIR digital filter (passband frequency range: 4–8 Hz, transition stopband width: 2 Hz, stopband attenuation > 50 dB, sampling rate: 500 Hz, filter order: 826). Subsequently, the amplitude of theta is extracted using the "double threshold detection module" of the theta rhythm signal, followed by application of the Hilbert transform to extract its phase information. An ultrasound stimulation command is issued when the theta amplitude exceeds the threshold and the algorithm detects the selected phase.

Data preprocessing

We obtained the theta (4–8 Hz) and gamma (30–45 Hz) rhythms of the LFP by filtering. A second-order IIR digital filter with a filter constant of 0.995 and a sampling frequency of 500 Hz was used for 50 Hz notch filtering on the LFP signals. The LFP signal was divided into two parts, viz. pre-stimulation (pre-stim) and post- stimulation (post-stim), for data analysis.

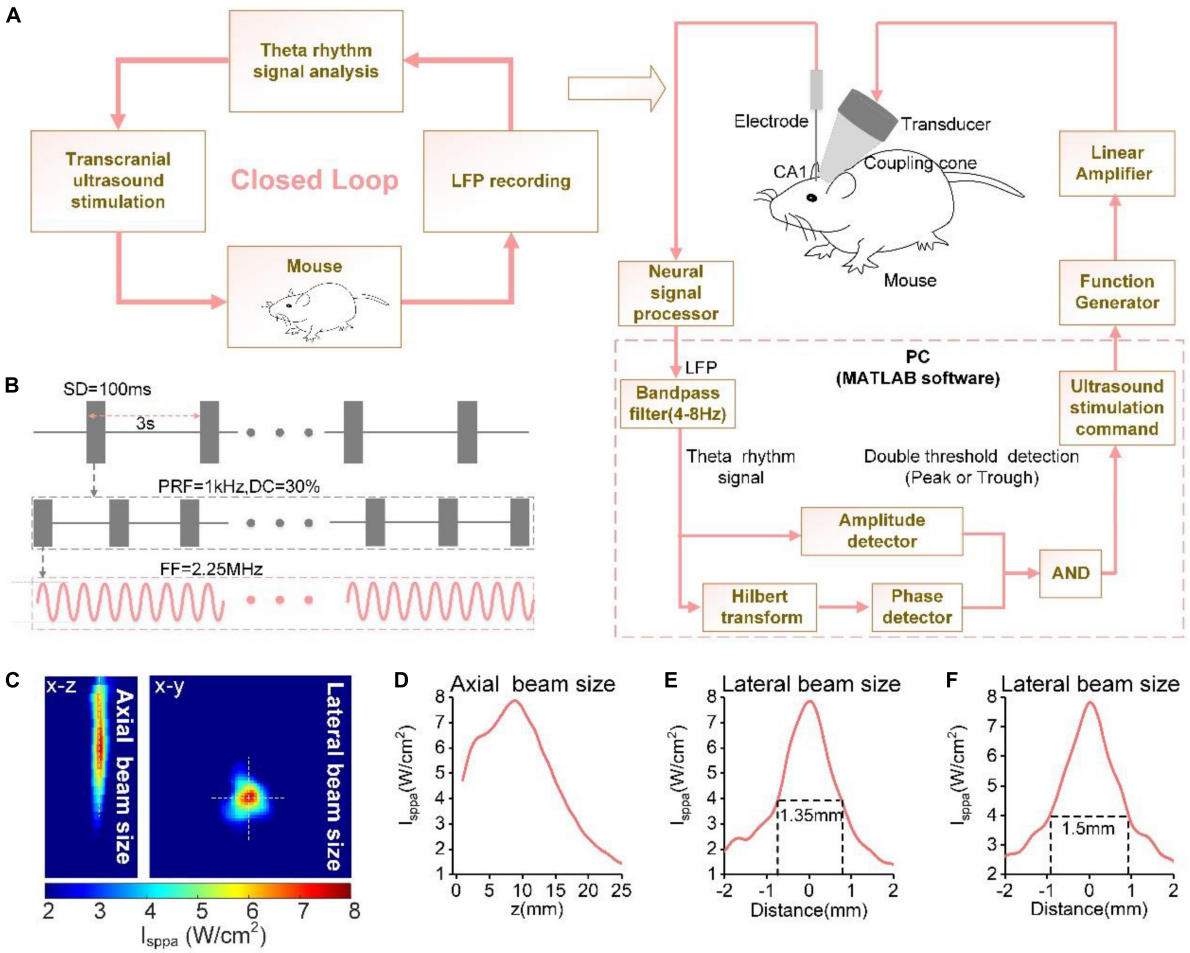

FIGURE 1
(A) The schematic of the phase-locked close-loop ultrasound stimulation system. **(B)** Time sequence of ultrasound stimulation. **(C)** Ultrasound field distribution under the skull in xy plane. The reconstruction profile along the blue dotted lines. Ultrasound field distribution under the skull in the xz and xy planes. **(D–F)** The reconstruction profile along the white dotted lines in **(C)**.

Power spectrum

The LFP signal data were subjected to Welch power spectrum estimation, and the absolute power in the two frequency bands of theta (4–8 Hz) and gamma (30–45 Hz) was calculated. The total absolute power of the frequency bands (4–200 Hz) was obtained by summing the absolute powers of all frequency bands. The relative power of each frequency band was equal to the corresponding absolute power divided by the total absolute power.

Sample entropy and Lempel-Ziv complexity

We calculated the sample entropy and Lempel-Ziv complexity of the theta and gamma rhythms, respectively. Sample entropy is a complex measure and a non-linear analysis method. The higher the sample entropy value, the greater the complexity of the signal time series. This method is especially suitable for analyzing non-stationary and non-linear LFP signals. It is calculated using the following formula:

$$SampEn\,(m, r, N) \;=\; -\,ln\left[\frac{C^{m+1}\,(r)}{C^{m}\,(r)}\right] \qquad (1)$$

where N is the length of the signal, m is the embedding dimension, and r is the threshold size and $m = 2$, $r = 0.25^{*}\mathrm{SD}$, and SD is the signal standard deviation.

Lempel-Ziv complexity is a non-linear analysis method used to characterize the degree of disorder in a time series by measuring the rate at which new patterns emerge. The formula is as follows:

$$LZC \;=\; \frac{c\,(n)\cdot log_{L}\,(n)}{n} \qquad (2)$$

where n is the length of the signal, and L is the number of coarse-grained segments. In this study, $L = 2$, c(n) represents the different substrings constructed by binarizing the original LFP sequence and repeated cascading.

Phase-amplitude coupling

PAC is used to analyze the degree of coupling between the low-frequency phase and the high-frequency amplitude. We used the phase locking value algorithm to calculate the PAC. It is calculated using the following formula:

$$PAC = \left| \frac{1}{N} \sum_{t=1}^{N} e^{i\left(\varphi_{low}(t) - \varphi_{highamp}(t)\right)} \right| \quad (3)$$

where N is the length of the signal, $\varphi_{low}(t)$ is the phase of the low-frequency signal, and $\varphi_{highamp}(t)$ is the phase of the amplitude of the high-frequency signal modulated by the low frequency.

Statistical analysis

The results were analyzed using the Kruskal–Wallis test and Mann-Whitney test. Differences were considered significant at p-values < 0.05. All statistical analyses were performed using the MATLAB software.

Results

Power spectrum of the theta and gamma rhythms evoked by peak and trough stimulation with different ultrasound pressures

First, we analyzed the changes in the power spectrum of the theta and gamma rhythms under peak and trough stimulation with different pressures. **Figure 2A** depicts the LFPs and their corresponding theta rhythm before ultrasound stimulation, with peak stimulation and trough stimulation, respectively. A significant increase was observed in the LFP amplitude as well as the amplitude of the theta rhythm under peak and trough stimulation, which is consistent with our previous results (Yang et al., 2020). We counted the phase of the theta rhythm corresponding to the time point when the closed-loop system sent ultrasound stimulation in the experiment, calculated the probability of occurrence of different stimulation phases, and created the phase distribution histogram of ultrasound stimulation (**Figures 2B,C**). We found that the phase distribution of ultrasound stimulation position in the theta rhythm is concentrated at $\pi/2$ and $-\pi/2$, respectively, which shows that the system can accurately stimulate the peak and trough of the theta rhythm.

We analyzed the absolute power of the theta and gamma rhythms under peak and trough stimulation. As shown in **Figures 3A,B**, the absolute power of the theta and gamma rhythms after TUS was higher than that before peak and trough stimulations. We observed that the absolute power of theta and gamma increased with the increase in ultrasound pressure for both peak and trough stimulations. However, the absolute power in the theta band did not differ from 0.05 to 0.8 MPa between peak and trough stimulations. There was no difference in the gamma rhythm between 0.05 and 0.5 MPa. The absolute power of the gamma rhythm under trough stimulation was higher than that under peak stimulation between 0.6 and 0.8 MPa ($^*p < 0.05$; Kruskal–Wallis test). **Figures 3C,D** show the relative power of the theta and gamma rhythms. We observed that the relative power of the theta rhythm after both peak and trough stimulations was significantly lower than that before stimulation, and decreased with the increase in ultrasound pressure from 0.05 to 0.8 MPa. The relative power of the theta band under trough stimulation was significantly higher than that under peak stimulation between 0.6 and 0.8 MPa ($^*p < 0.05$; Kruskal–Wallis test). We also noticed that the relative power of the gamma rhythm after peak and trough stimulation was mostly lower than that before stimulation, which varied with ultrasound pressure, independent of peak and trough stimulation. Last, the Pearson correlation coefficients of absolute power and relative power of LFP between peak stimulation and trough stimulation were calculated to evaluate their change trend (Absolute power, theta frequency band: 0.86 ± 0.03, gamma frequency band: 0.65 ± 0.06; Relative power, theta frequency band: 0.68 ± 0.07, gamma frequency band: 0.28 ± 0.1). The above results indicate that the absolute power and relative power of LFP have a similar trend with the increase of ultrasound pressure under peak stimulation and trough stimulation. These results demonstrate that the absolute power of the gamma rhythm and the relative power of the theta rhythm under TUS at 0.6–0.8 MPa differ between peak and trough stimulation.

Sample entropy and complexity of the theta and gamma rhythms evoked by peak and trough stimulation with different ultrasound pressures

Subsequently, we analyzed the sample entropy and complexity of the theta and gamma rhythms under peak and trough stimulation with different ultrasound pressures. The results of sample entropy and complexity are shown in **Figures 4A,B**. We observed that the sample entropy of

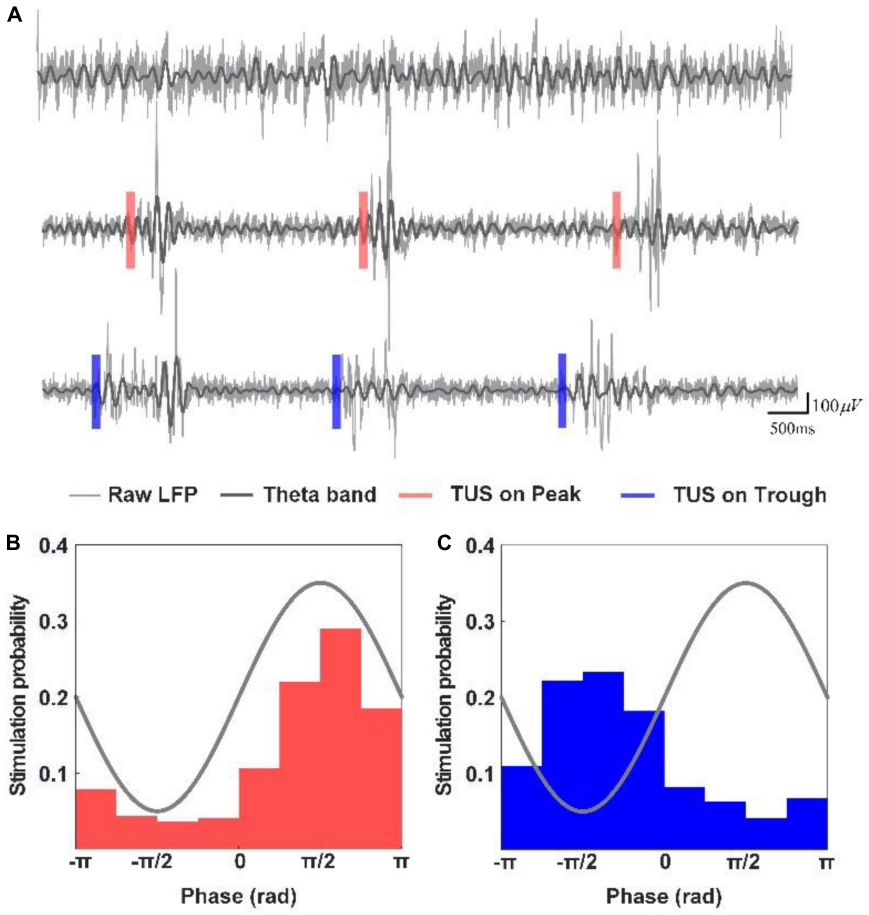

FIGURE 2
(A) LFPs and their corresponding theta rhythm before ultrasound stimulation, with peak stimulation and trough stimulation, respectively. **(B,C)** Phase distribution histogram of ultrasound stimulation.

the theta and gamma rhythms after TUS was lower than that before TUS under both peak and trough stimulations. We also observed that the sample entropy of the theta rhythm increased, and that of the gamma rhythm decreased with the increase in ultrasound pressure under peak and trough stimulations, respectively. However, the sample entropy of the theta rhythm under trough stimulation was higher than that under peak stimulation between 0.6 and 0.8 MPa and the sample entropy of the gamma rhythm under peak stimulation was higher than that under trough stimulation from 0.7 to 0.8 MPa ($^{*}p < 0.05$, $^{**}p < 0.01$; Kruskal–Wallis test). The complexity of the theta and gamma rhythm (Figures 4C,D) after TUS was lower than that before TUS under peak and trough stimulations. Moreover, the complexity of theta and gamma changed with ultrasound pressure, independent of peak and trough stimulation. The above-mentioned results indicate that the variation in sample entropy in the theta and gamma rhythms with ultrasound pressure is dependent on peak and trough stimulation.

Phase-amplitude coupling between the theta and gamma rhythms evoked by peak and trough stimulation with different ultrasound pressures

Finally, we analyzed the PAC strength of the theta and gamma rhythms evoked by peak and trough stimulation with different ultrasound pressures. First, we divided the LFP signals into six segments, viz. −0.15 to 0 s, 0–0.15 s, 0.15–0.3 s, 0.3–0.45 s, 0.45–0.6 s, and 0.6–0.75 s under peak and trough stimulation (Figure 5A). Thereafter, the PAC strengths of the theta and gamma rhythms at different time points were calculated, as shown in Figure 5B. We found that the PAC strength at 0–0.15 s (after TUS) showed a rising trend compared to −0.15 to 0 s (before TUS) under peak and trough stimulation, albeit without statistical significance. The coupling strength increased at 0–0.15 s, decreased at 0.15–0.3 s, and increased again at 0.3–0.45 s. In order to verify whether the value of the coupling strength differed under peak and trough stimulation during these periods

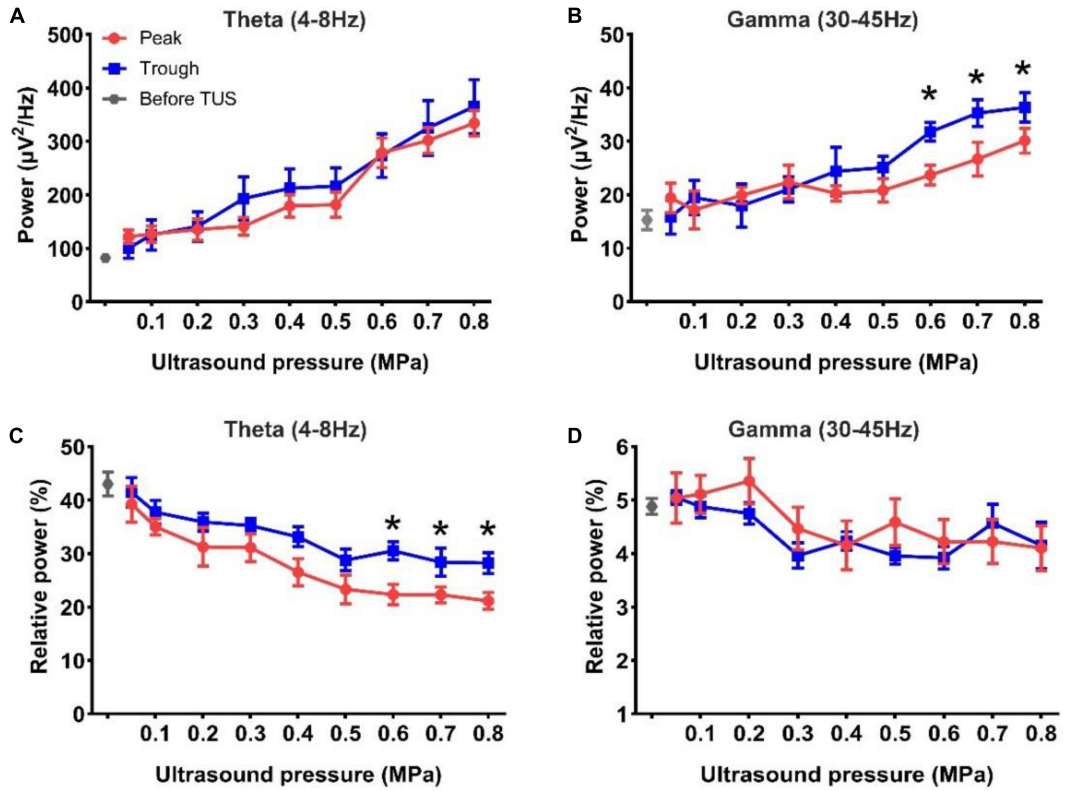

FIGURE 3
(A,B) The absolute power of the theta and gamma rhythms before and after TUS under peak and trough stimulations with different ultrasound pressures, **(A)** theta rhythm, **(B)** gamma rhythm. **(C,D)** The relative power of the theta and gamma rhythms before and after TUS under peak and trough stimulations with different ultrasound pressures, **(C)** theta rhythm, **(D)** gamma rhythm. (mean ± SEM, n = 8 for peak stimulation, n = 8 for trough stimulation, $^{*}p < 0.05$; Kruskal–Wallis test).

(0–0.15, 0.15–0.3, and 0.3–0.45 s), we calculated the relative values of coupling strength at 0–0.15, 0.3–0.45, and 0.15–0.3 s. As shown in Figure 5C, We observed that the relative values of coupling strength at 0–0.15 and 0.3–0.45 s under trough stimulation were higher than those under peak stimulation ($^{*}p < 0.05$, $^{**}p < 0.01$; Mann-Whitney test). We analyzed the change in the coupling strength relative to ultrasonic pressure under peak and trough stimulations (Figure 5D). The coupling strength under trough stimulation was higher than that under peak stimulation between 0.6 and 0.8 MPa ($^{*}p < 0.05$, $^{**}p < 0.01$; Kruskal–Wallis test). These results demonstrate that peak and trough stimulation affect the PAC strength between the theta and gamma rhythms as a function of ultrasound pressure.

Discussion

In this study, we established a closed-loop ultrasound stimulation system based on the judgment of the theta rhythm peaks and troughs. Comparison of the power, sample entropy and complexity and PAC between the theta and gamma rhythms of the LFPs under peak and trough stimulation of the theta rhythm revealed the following: (1) the variation in the absolute power of the gamma rhythm and the relative power of the theta rhythm under TUS at 0.6–0.8 MPa differ between peak and trough stimulation; (2) the relationship between sample entropy of the theta and gamma rhythms and ultrasound pressure depends on peak and trough stimulation; and (3) peak and trough stimulation affect the PAC strength between the theta and gamma rhythms as a function of ultrasound pressure. To the best of our knowledge, this is the first study to demonstrate the changes in the theta and gamma rhythms with ultrasound pressure under peak and trough stimulation of the theta rhythm, which will provide the research basis for the use of ultrasound stimulation for theta- or gamma-related neural activity.

The comparison between the changes in the theta and gamma rhythms based on the stimulation pressure under closed-loop peak and trough stimulation to that under open-loop ultrasound stimulation is of considerable relevance for the following reasons. First, the relative power of the theta rhythm induced by open-loop ultrasound stimulation decreased significantly with the increase in ultrasound pressure, and there were significant differences between different pressures within 1 s after TUS (Wang et al., 2019). During closed-loop peak

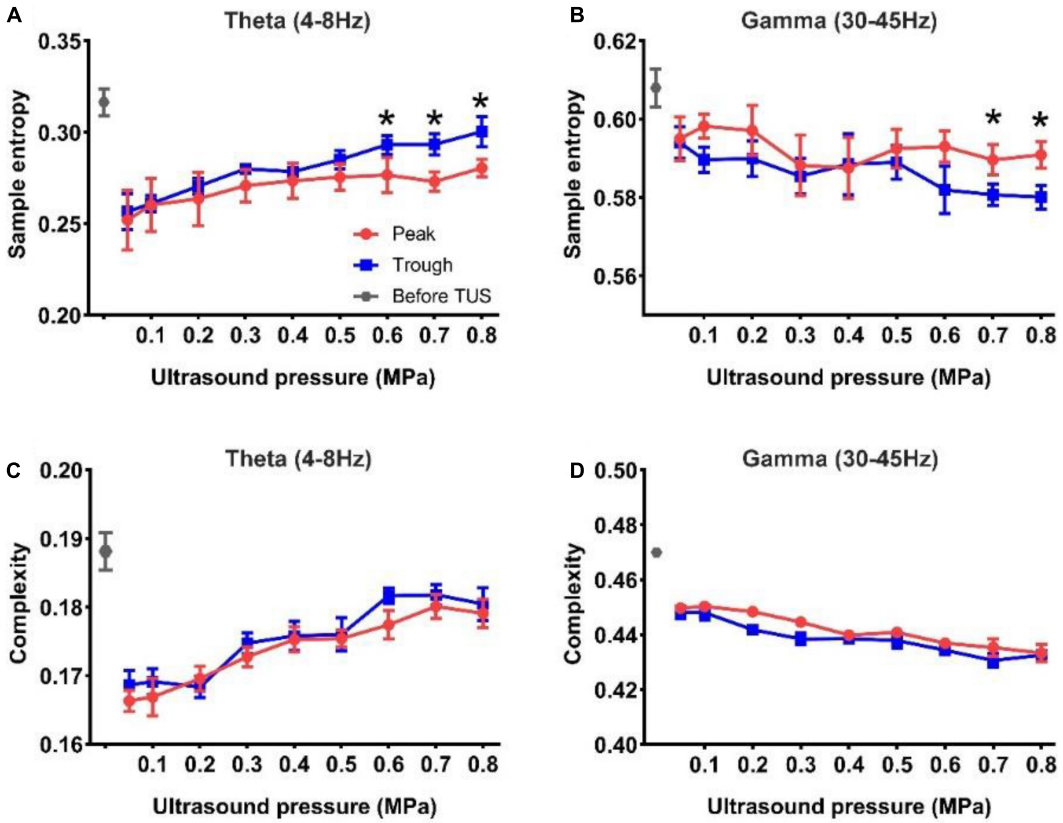

FIGURE 4
(A,B) The sample entropy of the theta and gamma rhythms before and after TUS under peak and trough stimulations with different ultrasound pressures, **(A)** theta rhythm, **(B)** gamma rhythm. **(C,D)** The complexity of the theta and gamma rhythms before and after TUS under peak and trough stimulations with different ultrasound pressures, **(C)** theta rhythm, **(D)** gamma rhythm. (mean ± SEM, $n = 8$ for peak stimulation, $n = 8$ for trough stimulation, $^*p < 0.05$; Kruskal–Wallis test).

and trough stimulation, the relative power of the theta rhythm decreases with the increase in ultrasound pressure, which is consistent with the results of open-loop stimulation. When the ultrasound pressure was between 0.6 and 0.8 MPa, the relative power of the theta rhythm under trough stimulation was higher than that under peak stimulation. Second, in open-loop ultrasound stimulation, the relative power of the gamma rhythm increased significantly with the increase in ultrasound pressure, and there were significant differences between different pressures within 1 s of stimulation (Wang et al., 2019). During closed-loop peak and trough stimulation, the relative power of the gamma band showed a declining trend with the increase in ultrasound pressure after stimulation, but its change relative to ultrasound pressure was independent of peak and trough stimulation. Interestingly, the relative power of the gamma rhythm elicited by closed-loop peak and trough stimulation of the theta rhythm was opposite to that evoked by open-loop stimulation. Third, in open-loop ultrasound stimulation, the mean PAC strength between the theta and gamma bands increased significantly with ultrasound pressure (Yuan et al., 2016b). During closed-loop peak and trough stimulation, the PAC strength of theta and gamma did not change with the increase in the ultrasound pressure under peak stimulation, and increased with ultrasound pressure under trough stimulation. The PAC strength under trough stimulation was higher than that under peak stimulation between 0.6 and 0.8 MPa. We found marked differences in the PAC results of the theta and gamma rhythms between phase-locked closed-loop stimulation and open-loop stimulation. This comparison facilitates the increase in the number of available optional ultrasound parameters and stimulation patterns for the modulation of the theta and gamma rhythms.

In this study, we found that the changes in the theta and gamma rhythms with ultrasound pressure depended on peak and trough stimulation of the theta rhythms, but the underlying reasons were unclear. This observation may be closely related to the potential mechanism of ultrasound stimulation and the neural information contained in the peak and trough of the theta rhythm. Previous studies have shown that cholinergic neurotransmission plays an important role in the generation of theta rhythms in the hippocampus. For example, the modulation of specific receptor agonists,

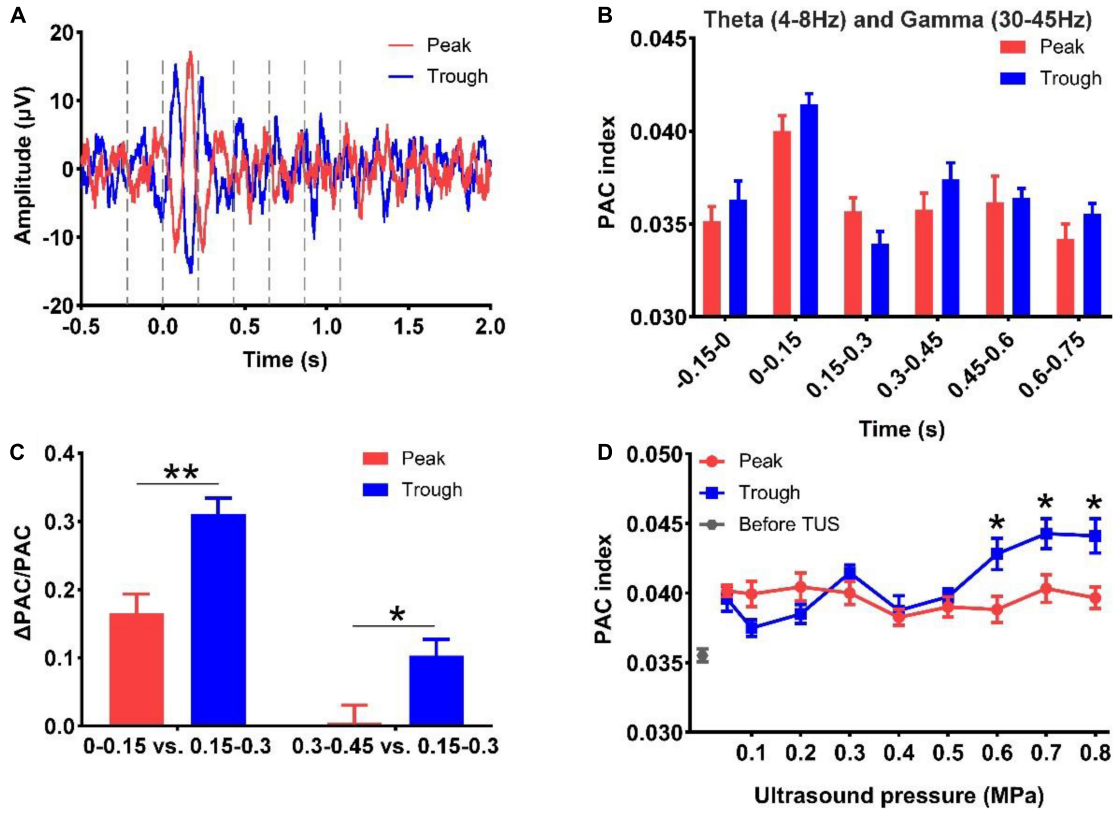

FIGURE 5
(A) LFP signals into six segments, viz. -0.15 to 0 s, 0–0.15 s, 0.15–0.3 s, 0.3–0.45 s, 0.45–0.6 s, and 0.6–0.75 s under peak and trough stimulation. **(B)** Phase-amplitude coupling strengths of the theta and gamma rhythms at different time points. **(C)** The relative values of phase-amplitude coupling strength at 0–0.15 s, 0.3–0.45 s, and 0.15–0.3 s under peak and trough stimulation. (mean ± SEM, n = 8 for peak stimulation, n = 8 for trough stimulation, $^{*}p < 0.05$, $^{**}p < 0.01$; Mann-Whitney test). **(D)** The coupling strength relative to ultrasonic pressure under peak and trough stimulations. (mean ± SEM, n = 8 for peak stimulation, n = 8 for trough stimulation, $^{*}p < 0.05$; Kruskal–Wallis test).

including the metabolic acetylcholine receptor and nicotine acetylcholine receptor agonists, can modulate theta rhythms in the hippocampus, which are determined by the activation of local neural circuits (Sun et al., 2001; Ma et al., 2020; Gao et al., 2021). In addition, the γ-aminobutyric acid type A receptor (GABAaR) also plays a key role in the modulation of the theta rhythm. For example, effective activation of GABAaR in the CA1 region of the rat hippocampus can regulate the theta rhythm (Adams et al., 2020). Gamma rhythms in the hippocampus can be induced by tonic electrical stimulation, agonists of metabotropic glutamate receptors and kainate receptors, and potassium ion solutions. Inhibitory synapses are necessary for the generation of gamma synchronization under sufficient conditions (Whittington et al., 1995; LeBeau et al., 2002; Cardin et al., 2009). Some properties of inhibitory interneuron networks are closely related to the generation of gamma oscillations. In ultrasound stimulation, ultrasound functions as a mechanical wave that can open or close mechanosensitive ion channels of neuronal cell membranes, and depolarize or hyperpolarize neurons, thereby generating neuronal action potentials (Fomenko et al., 2018; Kubanek et al., 2018; Qiu et al., 2019; Kamimura et al., 2020). Moreover, ultrasound can open TRPA1 channels in astrocytes, and Ca^{2+} influx through TRPA1 enables astrocytes to release glutamate through the Best1 channels (Oh et al., 2019). The mechanical pressure exerted by ultrasound signals (acting as mechanical waves) on neurons can significantly affect the activity of potassium-sodium mechanosensitive ion channels, including TREK-1, TREK-2, TRAAK K^{+} channels, and NaV1.5 (Sorum et al., 2021). Studies have also shown that ultrasound stimulation can promote the expression of proteins such as neurotrophic factors. In summary, since the changes in theta and gamma rhythms are affected by some receptor agonists or ion channels, we speculate that ultrasound waves affect the opening of channels through pressure, and subsequently induce changes in the theta and gamma rhythms. Furthermore, the peaks and troughs of the hippocampal theta rhythm are known to reflect differential neural information encoding, and optogenetic stimulation of the peaks and troughs produces different stimulation effects (Siegle and Wilson, 2014). Therefore, we speculate that the

theta and gamma rhythms may be affected by theta rhythms stimulation with changes in ultrasound pressure. These different responses to peak and trough stimulation are closely related to the difference in neural function between the peak and trough of the theta rhythm. We endeavor to perform in-depth research to ascertain the biophysical mechanism underlying the different responses of ultrasound stimulation on peak and trough stimulation of the theta rhythm in our next study.

Several studies have reported that TUS can activate cortical neurons via auditory responses (Guo et al., 2018; Sato et al., 2018; Park et al., 2021). In a subsequent study, Mohammadjavadi et al. (2019) used ultrasound to stimulate deaf knockout mice and demonstrated that direct neural activation was caused by TUS, instead of auditory effects. Recently, Yu et al. (2021) showed that ultrasound can elicit direct responses in the rodent brain, independent of hearing. In our last research, we performed closed-loop ultrasound stimulation experiments, and found that the amplitude changes and dynamic responses on the electromyogram and LFP in normal mice were substantially similar to those in deaf mice, demonstrating that ultrasound induces motor responses and neural responses by stimulating brain tissue rather than indirect auditory effects (Yuan et al., 2022). Therefore, we speculate that the changes in theta and gamma rhythms induced by ultrasound under peak and trough stimulation are not the results of auditory effects.

Previous studies have shown that theta rhythms are related to attention to conditioned stimuli, information processing, visual search, arousal, decision-making, and memory consolidation (Kienitz et al., 2018; Fiebelkorn and Kastner, 2019; Nicolás et al., 2021). The modulation of neuronal firing and neural networks by gamma neural oscillations is closely related to the function of the nervous system. These functions of gamma neural oscillations mainly include sensation and perception, arousal, motor, attention, and memory, etc. Gamma neural oscillations play a key role in sensory feature binding, selective attention, and execution of memory tasks (Magazzini and Singh, 2018; Kanta et al., 2019; McNally et al., 2021). Our study found that the power spectrum, sample entropy and complexity, and PAC strength of the theta and gamma rhythms can be modulated by phase-locked closed-loop ultrasound stimulation. On the basis of the adjustment of these parameters, we speculate that closed-loop ultrasound stimulation based on theta rhythmicity may play a modulatory role in brain functions related to theta and gamma rhythms such as arousal, cognition, attention, memory, etc. Therefore, we can choose the ultrasound stimulation patterns that are beneficial to memory and cognition. Additionally, the theta and gamma rhythms are closely related to epilepsy, Parkinson's disease, Alzheimer's disease, depression, and other neurological or psychiatric diseases. This system can be used to stimulate theta rhythms of different phases to select the appropriate ultrasound stimulation pattern to improve the therapeutic effect.

Conclusion

In conclusion, our study demonstrates that the modulation effect of ultrasound stimulation on the theta and gamma rhythms by different ultrasound pressures depends on peak and trough stimulation of the theta rhythm.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study involving animals was reviewed and approved by the Ethics Committee of Yanshan University.

Author contributions

YY and HJ designed and coordinated the study. ZX, JY, SD, HJ, and YY carried out the experiment and data process and drafted the manuscript. All authors gave final approval for publication.

Funding

This research was supported by the Natural Science Foundation of Hebei Province (No. F2022203050), the National Natural Science Foundation of China (No. 61827811), and the Scientific and Technological Innovation 2030 (No. 2021ZD0204300).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Neuroscience

TYPE Original Research
PUBLISHED 14 September 2022
DOI 10.3389/fnins.2022.1002816

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

RECEIVED 25 July 2022
ACCEPTED 24 August 2022
PUBLISHED 14 September 2022

CITATION
Xu Y, Han L, Wei Y, Mao H and Yu Z (2022) Combined repetitive transcranial magnetic stimulation and medication treatment for depression is associated with serum amyloid a level: Evidence from naturalistic clinical practice. *Front. Neurosci.* 16:1002816. doi: 10.3389/fnins.2022.1002816

Combined repetitive transcranial magnetic stimulation and medication treatment for depression is associated with serum amyloid a level: Evidence from naturalistic clinical practice

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Objective: Repetitive transcranial magnetic stimulation (rTMS) has a positive effect on patients with depressive disorder, while the underpinning molecular mechanism is unknown. Here, we aimed to investigate the effect of rTMS on serum levels of serum amyloid A (SAA) and testosterone in a real-world setting.

Materials and methods: In total, ninety-seven patients with depressive disorder were treated with medicine and rTMS (the rTMS group) while 122 patients were treated using the medicine only (the control group). Plasma levels of SAA (n = 52) and testosterone (n = 37) were measured before and after 2 weeks of treatment, and the treatment effect was evaluated by Hamilton Rating Scale for Depression (HAMD).

Results: The treatment effect revealed by the percentage of decrease in HAMD in the second week was significantly greater in the rTMS group compared with the control group. No significant difference was found in SAA or testosterone levels between the two groups. However, the percentage of changes in SAA (r = −0.492, p = 0.017) in the second week was significantly correlated with the percentage of decrease in HAMD score in the rTMS group, but not in the control group.

Conclusion: Patients with depression benefit more from combined rTMS and medication treatment in this naturalistic study. Changes in SAA level, but not testosterone level, were related to depressive remission after 2 weeks' combined treatment.

KEYWORDS

rTMS, depression, HAMD, SAA, testosterone

Introduction

Depression is a common psychiatric disorder with high lifetime prevalence, affecting up to 15% of the world's population (Moussavi et al., 2007). Depressive disorders, such as major depressive disorder (MDD) and dysthymic disorder, are psychiatric illnesses with devastating personal and social consequences owing to a persistent depressed mood, negative thoughts, and fatigue. The WHO (World Health Organization, 2017) has declared depression to be the leading cause of disability worldwide. Current pharmacologic treatment options show limited effectiveness in countering the disease (Turner et al., 2008; Cipriani et al., 2018), and approximately 30% of patients do not experience sustained symptomatic remission despite multiple treatment attempts (Rush et al., 2006).

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulation technique with broad clinical applications. A significant positive effect of rTMS on adult MDD patients has been demonstrated in several studies (McNamara et al., 2001; Burt et al., 2002; Herrmann and Ebmeier, 2006; O'Reardon et al., 2007). In current clinical practice, the left unilateral dorsolateral prefrontal cortex (DLPFC) 10 Hz stimulation protocol has been approved by the food and drug administration (FDA) for treatment-resistant depression patients. However, a meta-analysis suggested that the efficacy was not robust across studies or participants (Hyde et al., 2022). Full elucidation of the antidepressant mechanism of rTMS may help to explain the heterogeneity, and increase the chance of discovering new therapeutic strategies. A recent review (Luan et al., 2020) summarized the anti-depressant mechanism of rTMS in preclinical studies, namely, anti-inflammatory effects, anti-oxidative stress effects, enhancement of synaptic, and neurogenesis, the increased content of monoamine neurotransmitters, and the reduced activity of the hypothalamic-pituitary-adrenocortical axis. Another review has shown that the rTMS may exert a neuroprotective effect by acting on neuroinflammation in animal models of depression (Yulug et al., 2016). When unclear factor-E2-related factor 2 (Nrt2), which has an anti-inflammatory effect, was silenced, the antidepressant effect produced by the rTMS was abolished (Tian et al., 2020). The mechanism that rTMS effectively reverse despair-like behavior in rats could be related to regulating metabotropic glutamate receptors 5 (mGluR5)/N-Methyl-D-Aspartic acid receptor type 2B (NMDAR2B)-related inflammatory signaling pathways in the anterior agranular insular (Hu et al., 2022).

Several inflammatory markers, namely interleukin-1β (IL-1β), IL-6, and C-reactive protein (CRP), are associated with depression (Howren et al., 2009; Zunszain et al., 2013). Serum amyloid A (SAA), like CRP, is an acute-phase plasma protein, synthesized predominantly by the liver and induced by IL-1β and IL-6 (Moshage et al., 1988; Smith and McDonald, 1992; Eklund et al., 2012). Elevated levels of SAA have been detected in the plasma of patients with clinical depression compared with healthy controls (Wang et al., 2016). Another population-based cohort study has found that patients with depressive disorders had higher plasma SAA concentrations relative to individuals without such disorders (van Dooren et al., 2016). Plasma SAA was closely associated with depression severity across diagnostic boundaries in a naturalistic outpatient psychiatric sample (Bryleva et al., 2017). Serum levels of inflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor (TNF)-α were found to decrease after rTMS intervention (Zhao et al., 2019; Perrin and Pariante, 2020; Liu et al., 2022), which suggested that the antidepressant effect of rTMS may be related to changes in inflammatory (Wang et al., 2022). Besides, partial improvement of cognitive dysfunction by rTMS might be attributable to the reduction of peripheral IL-1β levels (Tateishi et al., 2020). Thus, SAA may be a part of the molecular mechanism of rTMS efficacy.

On the other hand, the association between testosterone and depression has been extensively debated because testosterone is a neuroactive steroid hormone influencing mood (Amiaz and Seidman, 2008). A population-based, longitudinal study showed inverse associations between androgens and depressive symptoms, although the associations were not independent of relevant confounders (Kische et al., 2017). In another longitudinal study on children, the rTMS was effective in remediating testosterone to levels seen in age-matched controls (Bolotova et al., 2017). Besides, gonadal steroids are involved in regulating cortical excitability induced by rTMS (Bonifazi et al., 2004). Exogenous application of testosterone can also modify connectivity between the DLPFC and the amygdala, which is related to emotion regulation (Votinov et al., 2020). Based on these, we speculated that testosterone was also a potential molecular mechanism or an indicator of rTMS efficacy.

In the present study, we aimed to verify the effectiveness of combined rTMS and medication depression therapy in real-world clinical settings, and investigate the effect of rTMS on serum levels of SAA and testosterone in depression patients.

Materials and methods

Participants

This study included inpatients from the Affiliated Mental Health Center and the Hangzhou Seventh People's Hospital, Zhejiang University School of Medicine. All the data were acquired from the Integration Platform for Clinical and Scientific Research on Mental Disorders. In total, 3,091 patients aged between 18 and 65 years, were diagnosed with depressive disorder by two treating psychiatrists according to the international classification of diseases, tenth (ICD-10) revision. Those who completed more than six sessions of rTMS (except for the control group) and finished clinical assessment

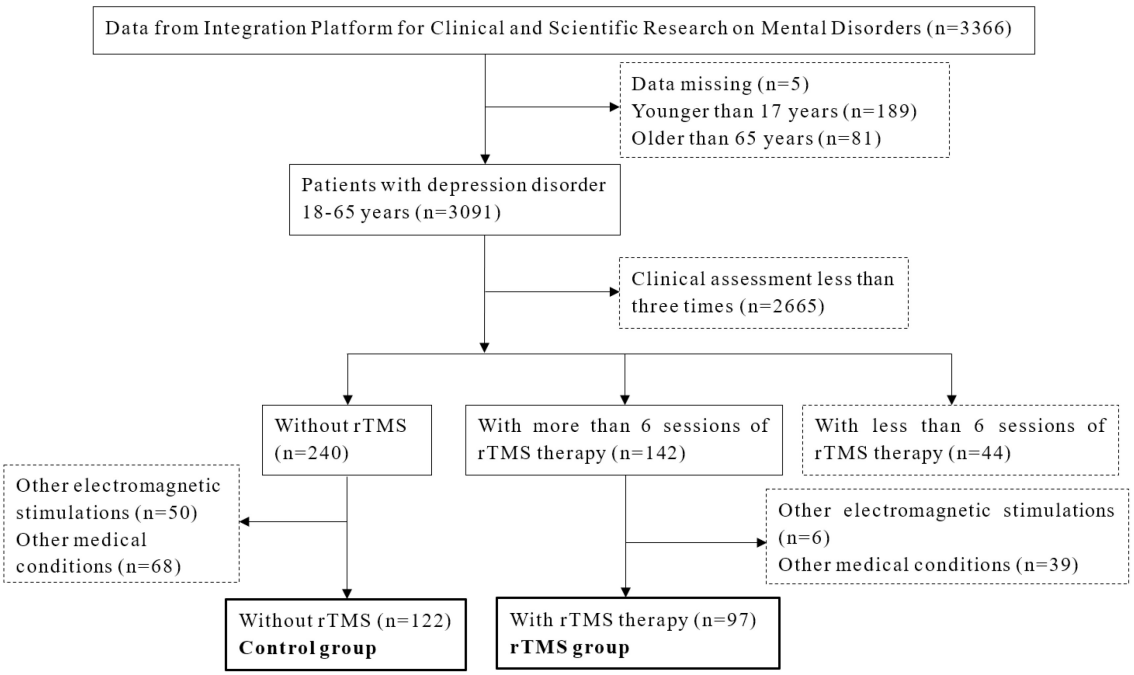

FIGURE 1
Flow diagram of the study.

of the Hamilton Rating Scale for Depression (HAMD) three times would be included in this study. Exclusion criteria were: received other electromagnetic stimulations such as electroconvulsive therapy; depression caused by other severe psychiatric disorders; history of severe somatic diseases and organic diseases of the brain; and having medication other than antidepressants, benzodiazepines/non-benzodiazepines, or low-dose of olanzapine/quetiapine. The flow chart of the study design is shown in Figure 1. A total of 219 patients were enrolled in this study, with 122 in the control group and 97 in the rTMS group. There were 52 patients (23 from the rTMS group and 29 from the control group) who measured SAA and 37 patients (20 from the rTMS group with 16 women, and 17 from the control group with 14 women) who measured testosterone at baseline and second week. The study protocol was approved by the ethics committee of the local hospital. Informed consent was obtained and the study abided by the Declaration of Helsinki principles.

Repetitive transcranial magnetic stimulation treatment

All the rTMS treatment was administered by trained medical doctors. All the patients were seated in a comfortable chair while TMS stimuli were delivered to the left prefrontal cortex (using the 5-cm rule) with a figure-of-eight coil and an OSF-6 magnetic stimulator (Wuhan Aosaifu Medical Technology Co., Ltd., China). The patients received 5 sessions of rTMS treatment per week and the stimulation frequency was 10 Hz with power (intensity) level of 90% of motor threshold (MT). Each session contained 60 rTMS trains with 40 pulses per train and the intertrain interval was 15 s.

Clinical assessment

The primary outcome of the study was the percentage of decrease in scores on the 24-item version of the HAMD. The outcome measure of HAMD was assessed at baseline (before rTMS treatment), first week (5 sessions), and second week (10 sessions). Response to treatment was defined as an over 50% decrease in HAMD. Remission was defined as a HAMD score of less than 8 in the second week.

Blood sampling procedures and analyses

The blood sample was collected between 7:00 and 9:00 a.m. in a fasting state. Analyses of SAA and testosterone were performed on fresh biospecimens on the day of sample collecting. The SAA was analyzed using a particle-enhanced turbidimetric immunoassay (PETIA) and testosterone was analyzed using chemiluminescence analysis (CLIA).

TABLE 1 Demographic, Hamilton Rating Scale for Depression (HAMD) score, serum amyloid A (SAA), and testosterone in the control and the repetitive transcranial magnetic stimulation (rTMS) groups.

	Control group (n = 122)	rTMS group (n = 97)	t/χ^2	P
Age (years) [mean (SD)]	48.3 (11.5)	45.3 (11.8)	1.879	0.062
Age range (years)	18–65	22–65		
Female [n (%)]	91 (74.6)	76 (78.4)	0.516	0.528
Benzodiazepine [n (%)]	120 (98.4)	96 (99.0)	0.148	0.586
Olanzapine/Quetiapine [n (%)]	90 (73.8)	62 (63.9)	2.471	0.077
Baseline assessments				
HAMD [Mean (SD)]	23.5 (6.4)	23.5 (5.6)	0.024	0.981
SAA (n = 52)	7.5 (1.2)	9.3 (9.7)	−0.771	0.444
Testosterone (n = 37)	3.0 (5.6)	2.7 (4.2)	0.156	0.877
First-week assessment				
HAMD [Mean (SD)]	14.5 (4.9)	13.5 (4.4)	1.561	0.120
Decrease in HAMD [Mean (SD)]	9.1 (4.6)	10.1 (4.6)	−1.570	0.118
Percentage of decrease in HAMD [%]	37.6	42.5	−2.088	0.038
Second-week assessment				
HAMD [Mean (SD)]	7.5 (4.0)	6.4 (3.3)	2.098	**0.037**
Decrease in HAMD [Mean (SD)]	16.1 (5.2)	17.1 (5.7)	−1.404	0.162
Percentage of decrease in HAMD [%]	68.2	72.2	−2.112	**0.036**
Remission rate (%)	59.0	66.0	1.113	0.180
Response rate (%)	87.7	87.6	< 0.001	0.573
SAA (n = 52)	8.7 (11.5)	7.0 (5.0)	0.673	0.504
Testosterone (n = 37)	2.9 (5.3)	3.3 (5.8)	−0.184	0.855

Bold values indicates a significant difference at the p = 0.05 level.

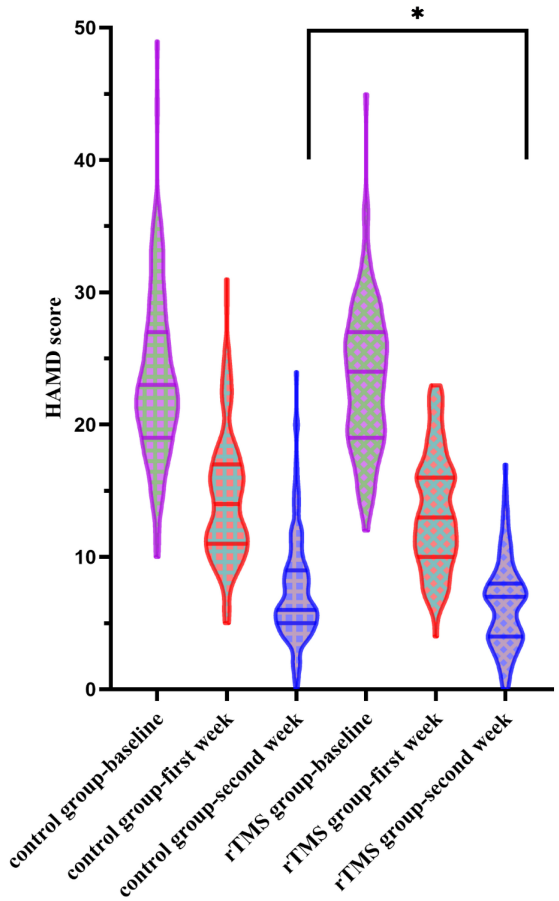

FIGURE 2
Hamilton Rating Scale for Depression (HAMD) scores at the baseline, the first week, and the second week in the control and the repetitive transcranial magnetic stimulation (rTMS) group. *Indicates a significant difference at the p = 0.05 level.

Data analysis

Data were analyzed using standard descriptive statistics in PASW Statistics 18.0 (SPSS Inc., Chicago, IL, United States) statistical software. The control group (n = 122) included patients with medical treatment while the rTMS group (n = 97) included those with joint medicine and rTMS treatments. Chi-square tests were used to investigate differences in men/women between groups. Repeated two-way ANOVA (group * time) was conducted to investigate the HAMD score/percentage of decrease in HAMD/SAA level/testosterone level difference between groups across 2 weeks of measures. Student's t-test were used to investigate differences between groups in the percentage of changes in HAMD score, SAA, and testosterone level. Pearson correlation was used to investigate the relationship between the percentage of changes in SAA, testosterone levels, and HAMD score.

Results

Demographics, Hamilton Rating Scale for Depression score, serum amyloid A, and testosterone statistics

As shown in Table 1, the distributions of sex and age did not differ between the control and the rTMS groups. The percentage of patients who used benzodiazepine/olanzapine/quetiapine did

not differ between the two groups either. The HAMD score at baseline and the first week did not differ between the two groups. However, the rTMS group scored lower than the control group on HAMD in the second week.

Effectiveness of repetitive transcranial magnetic stimulation

When conducting the two-way ANOVA statistic with group factors (rTMS/Control) and time factors (baseline, first week and second week) within HAMD score, there was a significant effect in time [$F_{(2,434)} = 1431.734$, $p < 0.001$] but not in the group [$F_{(1,217)} = 1.506$, $p = 0.221$] and interaction [$F_{(2,434)} = 1.753$, $p = 0.175$] (Figure 2). When conducting the two-way ANOVA statistic with group factors (rTMS/Control) and time factors (first week and second week) within percentage of decrease in HAMD, there were significant effects both in group [$F_{(1,217)} = 5.799$, $p = 0.017$] and time [$F_{(1,217)} = 809.113$, $p < 0.001$], but not in interaction [$F_{(1,217)} = 0.143$, $p = 0.706$], suggesting the effectiveness of rTMS treatment along the time. Further *t*-test showed that the percentage of decrease in HAMD in the rTMS group was significantly greater than in the control group in the first [$t_{(217)} = -2.088$, $p = 0.038$] and second week [$t_{(217)} = -2.112$, $p = 0.036$]. However, the response rate or remission rate of the rTMS group did not differ from the control group in the second week.

No differences in serum amyloid A/testosterone levels between the control and the repetitive transcranial magnetic stimulation groups

When conducting the two-way ANOVA statistic with group factors (rTMS/Control) and time factors (baseline and second week) within SAA level of 52 patients, there was no significant group effect [$F_{(1,51)} = 0.696$, $p = 0.408$], time effect [$F_{(1,51)} = 0.871$, $p = 0.355$], or interaction effect [$F_{(1,51)} = 0.242$, $p = 0.625$] (Figure 3A). The percentage of decrease in SAA level

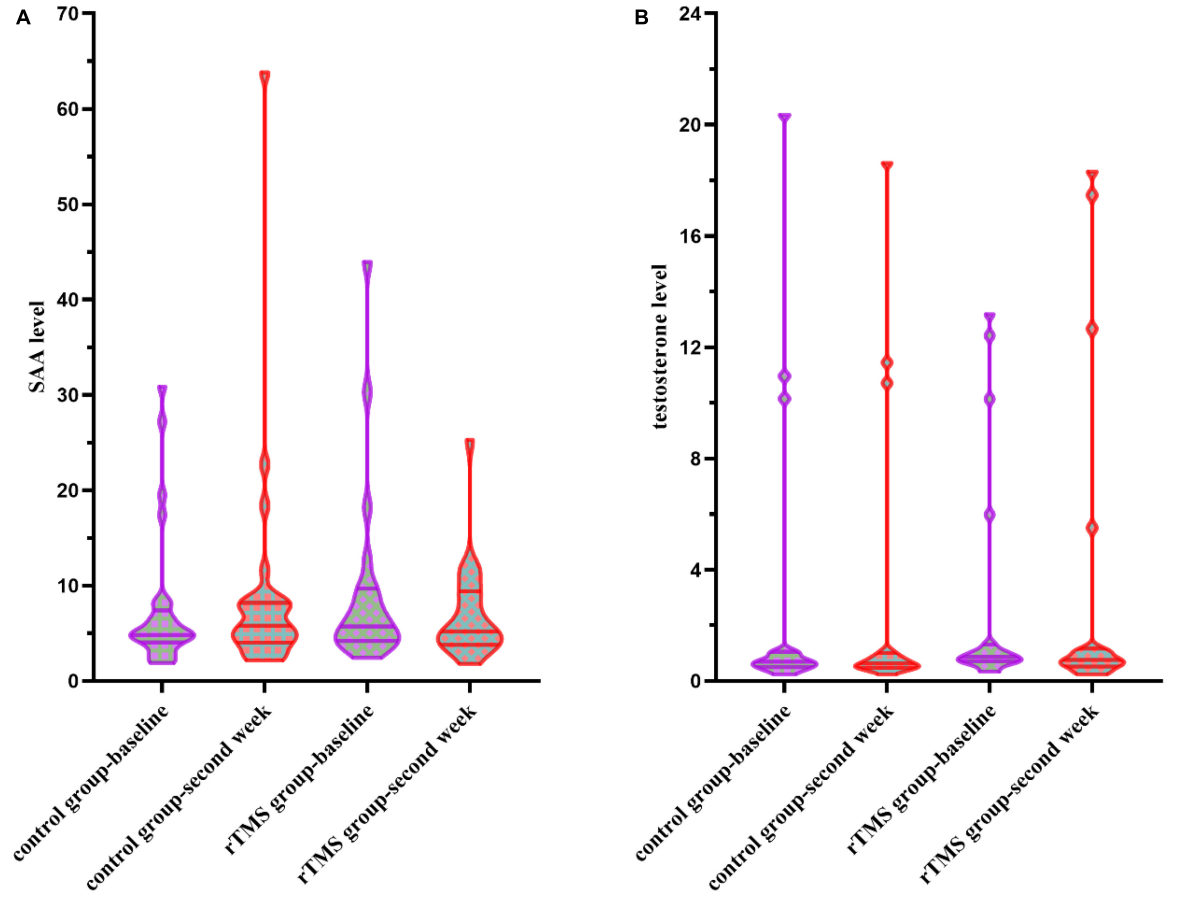

FIGURE 3
(A) Serum amyloid A (SAA) levels at the baseline and the second week in the control group and the rTMS group. **(B)** Testosterone levels at the baseline and the second week in the control group and the rTMS group.

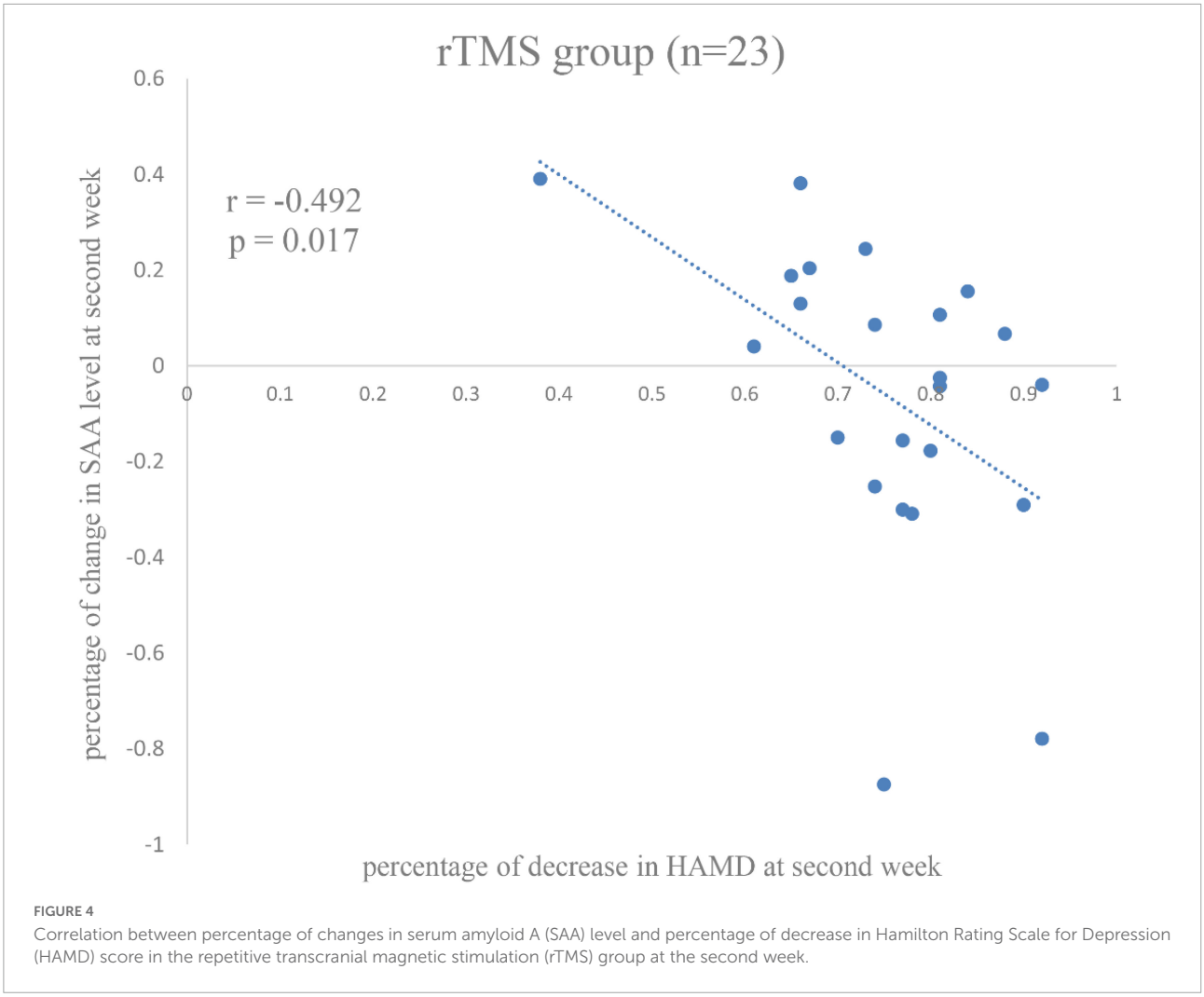

FIGURE 4
Correlation between percentage of changes in serum amyloid A (SAA) level and percentage of decrease in Hamilton Rating Scale for Depression (HAMD) score in the repetitive transcranial magnetic stimulation (rTMS) group at the second week.

[$t_{(50)}$ = 1.550, p = 0.128] did not differ between control and the rTMS group.

When conducting the two-way ANOVA statistic with group factors (rTMS/Control) and time factors (baseline and second week) within testosterone level of 37 patients, there was no significant group effect [$F_{(1,35)}$ = 0.001, p = 0.979], time effect [$F_{(1,35)}$ = 0.791, p = 0.380], or interaction effect [$F_{(1,35)}$ = 1.313, p = 0.260] (Figure 3B). The percentage of changes in testosterone level [$t_{(35)}$ = 0.671, p = 0.507] did not differ between control and the rTMS group.

Relationships between serum amyloid A/testosterone level changes and Hamilton Rating Scale for Depression decrease

The significant relationship was found between percentage of decrease in SAA level and the percentage of decrease in HAMD score in the rTMS group at second week (r = −0.492, p = 0.017) (Figure 4), rather than the control group (r = 0.105, p = 0.579), or among all patients (r = −0.025, p = 0.858).

Notably, no relationship was found between the percentage of change in testosterone level at second week and the percentage of decrease in HAMD score, neither in all patients (r = −0.071, p = 0.675) nor in separate groups (the rTMS group: r = −0.214, p = 0.366; the control group: r = 0.267, p = 0.299).

Discussion

In this study, we found a greater percentage of decrease in HAMD score in the second week when combined with rTMS treatment than medical treatment only in depression patients, and the percentage of decrease in HAMD score was associated with the percentage of changes in SAA level in the second week.

The rTMS could accelerate the onset time of beneficial treatment effects and improve clinical symptoms of depression

(Dai et al., 2022). In a study of depression patients who were administrated with drugs combined with rTMS treatment, the active rTMS group demonstrated a more significant score reduction compared to the sham rTMS group in the second week (Dai et al., 2022). Here, rTMS also showed early effectiveness in the second week. Research has indicated that benzodiazepines (BZD) may impede the response to rTMS (Deppe et al., 2021). Although most patients in this study took BZD during rTMS treatment due to insomnia, rTMS still showed its effectiveness within 2 weeks.

Although no significant result was found on the SAA level during the 2 weeks of combined rTMS treatment, the percentage of decrease in SAA level was related to the percentage of decrease in HAMD score. Changes in inflammatory mediators such as SAA were related to insomnia (Xia et al., 2021), which is a common symptom of depression disorder. In rodents, liver-specific SAA1 overexpressing mice were considered a valuable model to study depression (Jang et al., 2017). The cytokine production of T helper 17 (Th17) cells was regulated by SAA (Lee et al., 2020), and the increase in Th17 production promoted by SAA may induce depressive-like behaviors in mice (Medina-Rodriguez et al., 2020). Thus, a segmented filamentous bacteria (SFB)/autoinducer-2 (AI-2)/SAA1-2/Th17 cell pathway that promoted depressive-like behavior was uncovered (Medina-Rodriguez et al., 2020). The evidence suggested that the SAA level may regulate depressive symptoms. Despite the lack of significant SAA level difference between the two groups, improvement of depressive symptoms in the second week was found associated with SAA level drop. As a matter of interest, this association was only present when rTMS treatment was combined. Consistently, another study in depression model mice found that rTMS reversed the down-regulation of astrocytes and inhibited high levels of IL-6, and IL-1β caused by chronic unpredictable mild stress (CUMS) in the hippocampus and prefrontal cortex (Zuo et al., 2022). Therefore, inhibition was also found on SAA in patients with the depressive disorder who were treated by rTMS combined with medicine, but not by medicine only. Inflammation was suggested to be associated with non-response to psychological therapy (Strawbridge et al., 2020), while it may be an indicator of rTMS therapy. Nevertheless, whether SAA is a state marker or a trait marker is still unclear due to the configuration results (Kling et al., 2007; Dahl et al., 2014). Thus, a follow-up study would be useful to answer this question.

It should be pointed out that no significant difference was found in testosterone levels between the two groups. Besides, no relationship was found between therapeutic effects and changes in testosterone levels either. Although lower testosterone level was associated with depression in men (McIntyre et al., 2006; Westley et al., 2015; Giltay et al., 2017), the results were inconsistent in women. A meta-analysis and Mendelian randomization study show that women with depression do indeed display significantly different serum levels of testosterone, which was most likely a manifestation of the disease itself (Maharjan et al., 2021). The meta-analyses indicate that testosterone appears to have a small antidepressant effect, while they do not provide strong support for the use of testosterone in depressive disorders in general (Dichtel et al., 2020; Dwyer et al., 2020). It is observed that most antidepressants can influence testosterone levels (Pavlidi et al., 2021), but the relationship between testosterone level and depressive symptom remission was not found in patients treated with the medicine. The hypothalamic-pituitary-gonadal (HPG) axis may offer a pathway to explain the impact of rTMS on this outcome (Crewther et al., 2022). Testosterone has been examined using rodent models of rTMS (Hedges et al., 2002, 2003). Nevertheless, these studies on rats failed to find an effect of HF–rTMS on testosterone levels. Therefore, testosterone might not be an indicative factor for rTMS or medicine treatments.

The limitations of this study are intrinsic to those of retrospective research conducted in a naturalistic setting. A sham rTMS group was not used to control for placebo effects. In addition, the present study used an atypical rTMS treatment protocol and did not control for concurrent medications or psychotherapy. The sample size of patients who completed baseline and second-week serological examinations was small, which limited our further analysis. At last, the follow-up time was only 2 weeks, which helped to understand the early onset but not the long-term effectiveness of rTMS therapy.

In conclusion, patients with depression benefit more from combined rTMS treatment with medicine in a naturalistic study. Changes in SAA but not testosterone level were related to depressive remission after 2 weeks of combined treatment. Future research is needed in the form of double-blind, randomized control trials that examines the relationship between SAA level and rTMS depression outcome.

Data availability statement

The datasets generated and analyzed during the current study are not publicly available because permission is needed to access the database of the hospital, but they are available from the corresponding author on reasonable request. Requests to access these datasets should be directed to ZY, yuzhcoo@sina.com.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Affiliated Mental Health Center and Hangzhou Seventh People's Hospital, Zhejiang University School of Medicine. The

patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Funding

This work was supported by the National Important Project on Science and Technique Innovation 2030—"Brain Science and Brain-Inspired Intelligence Technology" (grant number 2021ZD0201905-1) and the Zhejiang Provincial Commonweal Foundation (grant number LGF20H090004).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor HJ declared a shared parent affiliation with the authors at the time of review.

Publisher's note

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TYPE Systematic Review
PUBLISHED 28 December 2022
DOI 10.3389/fnins.2022.1082383

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SPECIALTY SECTION
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

RECEIVED 28 October 2022
ACCEPTED 05 December 2022
PUBLISHED 28 December 2022

CITATION
Wang Y, Xu N, Wang R and Zai W (2022) Systematic review and network meta-analysis of effects of noninvasive brain stimulation on post-stroke cognitive impairment.
Front. Neurosci. 16:1082383.
doi: 10.3389/fnins.2022.1082383

Systematic review and network meta-analysis of effects of noninvasive brain stimulation on post-stroke cognitive impairment

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Objective: To systematically assess the effects of Noninvasive Brain Stimulation (NIBS) on post-stroke cognitive impairment (PSCI) and to compare the efficacy of two different NIBS.

Methods: Computer searches of PubMed, Web of Science, Cochrane Library, Embase, China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (VIP), Chinese Biomedical literature Service System (SinoMed), and Wanfang Database were conducted using a combination of free words and subject terms. The search was conducted from the database creation date to 27 November 2022. The risk of bias in the included literature was assessed using the Cochrane Risk Assessment Scale. The quality of the included literature was assessed using the physiotherapy evidence database (PEDro) scale. A standard meta-analysis of study data for each outcome indicator was performed using RevMan 5.4 software. Network meta-analysis was performed using State 14.0 according to the Bayesian framework.

Results: A total of 18 studies involving 809 patients were included. Meta-analysis shows NIBS significantly improved montreal cognitive assessment (MoCA) scores (standardized mean difference [SMD] = 0.76, 95% confidence interval (CI) 0.49–1.02, $P < 0.05$), mini-mental state examination (MMSE) scores (SMD = 0.72, 95% CI 0.25–1.20, $P < 0.05$), and modified barthel index (MBI) and functional independence measurement (FIM) scores (SMD = 0.33, 95% CI 0.11–0.54, $P < 0.05$) in patients with PSCI. The surface under the cumulative ranking curve (SUCRA) of different NIBS in improving MoCA scores were in the order of transcranial direct current stimulation (tDCS) (SUCRA = 92.4%) and transcranial magnetic stimulation (TMS) (SUCRA = 57.6%). The SUCRA of different NIBS in improving MMSE scores were in the order of tDCS (SUCRA = 81.6%) and TMS (SUCRA = 67.3%). The SUCRA of different NIBS in improving MBI and FIM scores were in the order of tDCS (SUCRA = 78.6%) and TMS (SUCRA = 65.3%).

Conclusion: The available evidence suggests that NIBS improves cognitive impairment. tDCS appeared more effective than TMS for cognitive function and activities of daily living in PSCI patients. Limited by the number of

included studies, more large-sample, multicentre, double-blind, high-quality randomized controlled clinical trials are needed to further confirm this study's results.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, identifier: CRD42022372354.

KEYWORDS

noninvasive brain stimulation (NIBS), transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS), post stroke cognitive impairment (PSCI), systematic review, meta-analysis

Introduction

Stroke is a neurological disorder caused by blood circulation disorder and is the second leading cause of death worldwide, with over 13 million new cases each year (Feigin et al., 2022). Studies have shown that the incidence of post-stroke cognitive impairment (PSCI) is 80.97%, significantly affecting patients' ability to care for themselves and participate in society (Qu et al., 2015; Du et al., 2020; Weaver et al., 2021). Therefore, the rehabilitation of cognitive function in stroke patients is an issue that requires urgent attention. Currently, the treatment for patients with PSCI consists of medication and cognitive rehabilitation training. However, there are problems such as adverse drug reactions, complicated operations, and prolonged treatment periods (Urbanova et al., 2018).

Noninvasive brain stimulation (NIBS) therapy has become a hot topic of research for improving cognitive impairment after stroke (Li H. et al., 2021; Kim et al., 2022). NIBS mainly consists of transcranial electrical stimulation (TES) and transcranial magnetic stimulation (TMS). TES works by placing the positive and negative electrodes on the scalp surface and applying a current of 1–2 milliamps. This current alters the resting potential of the nerve cell membrane, lowers or raises the activation threshold of the neuron, and regulates the neuron's activity (Liu et al., 2018). TES primarily includes transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS). At present, there is more evidence of high-quality clinical studies on tDCS in rehabilitating cognitive impairment after stroke. However, the clinical application of tACS and tRNS is still in its infancy. The tDCS mode of action includes anodal tDCS stimulation alone, cathodal tDCS stimulation alone, and bilateral simultaneous anodal and cathodal tDCS stimulation (Solomons and Shanmugasundaram, 2019; Bhattacharya et al., 2022). TMS works by a coil placed on the scalp to transmit short pulses of current, creating a pulsed magnetic field (Hernandez-Pavon and Harvey, 2019). This magnetic field causes an induced current to form in the cerebral cortex at the site of stimulation, which alters the membrane potential of nerve cells and affects metabolism and associated electrophysiological activity in the brain (Klomjai et al., 2015). TMS stimulation modes primarily includes repetitive TMS (rTMS) and theta burst stimulation (TBS). According to different frequency parameters, rTMS can be divided into high-frequency rTMS (3–20 Hz) and low-frequency rTMS (≤1Hz); TBS can be divided into intermittent TBS (iTBS) and continuous TBS (cTBS) (Smith and Stinear, 2016).

Previous studies have shown that NIBS is important in rehabilitating post-stroke cognitive impairment. Kang et al. (2009) found that anodal tDCS stimulation significantly improved attentional function in patients with PSCI. Smirni et al. (2015) found that cathodal tDCS of the right dorsolateral prefrontal cortex (DLPFC) can improve recognition memory in healthy people. Shaker et al. (2018) treated PSCI patients with bilateral tDCS, and the patients showed significant improvements in attention and logical reasoning. Tsai et al. (2020) found significant improvements in attention and delayed memory after applying 5 Hz rTMS to patients with PSCI. Kim et al. (2018) found that 0.9 Hz rTMS improved cognitive function in stroke patients. However, the sample sizes of individual studies were minor, inclusion criteria and study methods varied, and there was no evidence of a difference in treatment effects between the two NIBS modalities.v This is highly detrimental to developing the clinical practice of NIBS for post-stroke cognitive impairment. Therefore, in this study, the efficacy of different NIBS stimulation techniques was evaluated and ranked according to the pathophysiological basis of PSCI using a network meta-analysis (NMA) to find the optimal neurostimulation protocol for patients with PSCI and to provide an evidence-based basis for clinical treatment decisions.

This systematic evaluation program has completed registration in the PROSPERO database (CRD42022372354).

Materials and methods

Search strategy

Computer searches of PubMed, Web of Science, Cochrane Library, Embase, China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (VIP),

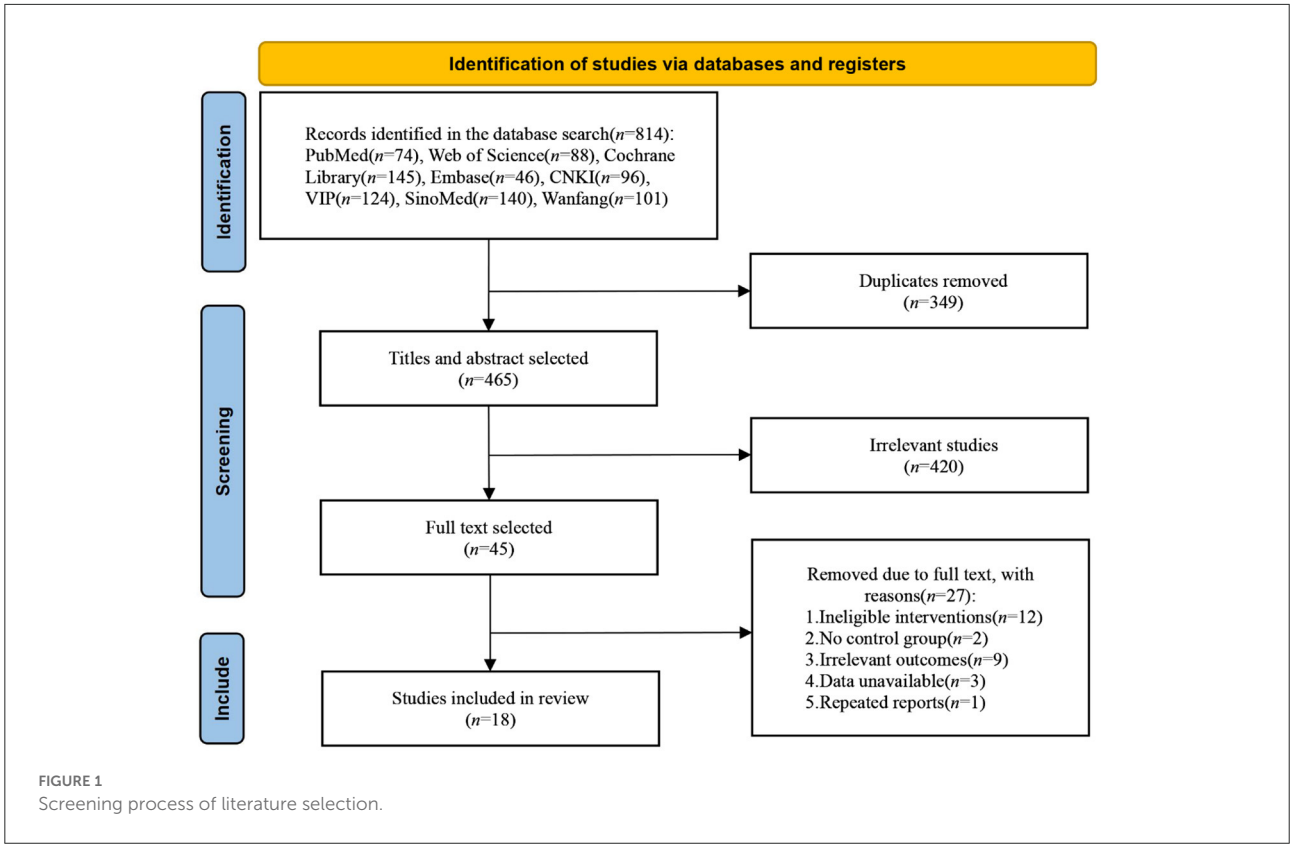

FIGURE 1
Screening process of literature selection.

Chinese Biomedical literature Service System (SinoMed), and Wanfang Database were conducted using a combination of free words and subject terms. The search was conducted from the database creation date to 27 November 2022. The search formula was (stroke OR cerebrovascular OR hemiplegia OR cerebral hemorrhage OR cerebral infarction OR cerebral stroke OR acute stroke) AND (noninvasive brain stimulation OR transcranial electrical stimulation OR transcranial direct current stimulation OR transcranial alternating current stimulation OR transcranial random noise stimulation OR transcranial magnetic stimulation OR repetitive transcranial magnetic stimulation OR theta burst stimulation OR intermittent theta burst stimulation OR continuous theta burst stimulation OR TES OR tDCS OR tACS OR tRNS OR TMS OR rTMS OR TBS OR iTBS OR cTBS) AND (cognitive dysfunction OR cognitive impairment OR cognition disorders) AND (randomized controlled trial OR random OR controlled trials OR RCT). After each of the two researchers (YW, RW) had completed the search independently, the results were cross-checked. In disagreement, the decision was discussed with a third researcher (NX).

Inclusion criteria

(1) Population: patients with a precise clinical diagnosis of hemorrhagic stroke or ischemic stroke, with no restrictions on nationality, gender, age, or educational background, had significant cognitive impairment.
(2) Intervention: NIBS.
(3) Comparison: sham-NIBS.
(4) Outcome: montreal cognitive assessment (MoCA), mini-mental state examination (MMSE), modified barthel index (MBI), and functional independence measurement (FIM).
(5) Study design: randomized controlled trial (RCT).

Exclusion criteria

Animal experiments and repeat studies; interventions other than NIBS and conventional cognitive rehabilitation training were present in the experimental group; unavailability of full text; failure to extract outcome data; non-RCT studies such as self-control and case-control studies.

Data extraction

Export the titles and abstracts of the retrieved documents and use Endnote 20 to eliminate duplicates. An initial screening of the literature was completed by browsing through the titles and abstracts. The literature was downloaded and read carefully to identify literature for inclusion based on inclusion and

TABLE 1 Characteristics of included studies.

Reference	Sample size (E/C)	Gender (male/female)	Age (E/C, year)	Course of disease (E/C, day/week/month)	Intervention	Stimulation	Intervention length	Evaluation	Outcome
Yun et al. (2015)	15/15/15	6/9; 7/8; 7/8	(60.9 ± 12.9)/(58.9 ± 15.0)/(68.5 ± 14.6)	(42.2 ± 31.9)/(38.1 ± 27.0)/(39.5 ± 29.6) d	2.0 mA tDCS	Anode: left fronto-temporal area/anode: right fronto-temporal area	30 min/d, 5 d/wk, 3 wks	Before the intervention; after 3 wks	K-MMSE, K-MBI
Shaker et al. (2018)	20/20	Not described	(54.45 ± 4.68)/(53.05 ± 6.32)	(14.05 ± 1.53)/(16.55 ± 2.78) m	2.0mA tDCS	Anode: left or right DLPFC cathode: contralateral area	30 min/d, 3d/wk, 1 mo	Before the intervention; after 1 mo	FIM
Zeng et al. (2019)	15/15	9/6; 11/4	(56.21 ± 9.11)/(53.14 ± 7.12)	(41.29 ± 10.37)/(43.36 ± 12.17) d	2.0 mA tDCS	Left DLPFC	20 min/d, 5 d/wk, 4 wks	Before the intervention; after 4 wks	MoCA, MMSE
Ai et al. (2021)	14/13/14	11/3; 10/3; 10/4	(61.64 ± 10.33)/(61.36 ± 8.51)/(58.77 ± 9.61)	(7.75 ± 6.66)/(4.77 ± 2.19)/(6.77 ± 5.70) w	2.0 mA tDCS	Anode: left DLPFC cathode: right supraorbital area; tDCS treatment and conventional rehabilitation at the same time/separate tDCS treatment and conventional rehabilitation	30 min/d, 5d/wk, 2wks	Before the intervention; after 2 wks	MoCA, MBI
Liu et al. (2021)	20/20	12/8; 7/13	(63.72 ± 8.41)/(60.06 ± 8.26)	2.5(1, 3)/2.5(2, 4) m	2.0 mA tDCS	Anode: left DLPFC cathode: right DLPFC	20 min/d, 5 d/wk, 4 wks	Before the intervention; after 4 wks	MMSE
Chen et al. (2022)	36/36	18/18; 19/17	(64.01 ± 5.71)/(63.58 ± 5.48)	(37.18 ± 10.52)/(36.74 ± 10.23) d	1.5–2.0 mA tDCS	Anode: C3 or C4 of the primary motor cortex cathode: bilateral supraorbital area	20 min/d, 5 d/wk, 4 wks	Before the intervention; after 4 wks	MoCA, MMSE
Yan et al. (2022)	30/30	16/14; 17/13	(56.07 ± 8.52)/(57.40 ± 7.88)	(39.87 ± 12.67)/(38.90 ± 13.26) d	2.0 mA tDCS	Anode: the affected side of DLPEC	20 min/d, 5 d/wk, 4 wks	Before the intervention; after 4 wks	MoCA
Ko et al. (2022)	12/14	4/8; 8/6	(61.25 ± 12.85)/(57.86 ± 10.04)	Not described	2.0 mA RS-tDCS	Anode: left DLPFC cathode: right supraorbital area	30 min/d, 5 d/wk, 4 wks	Before the intervention; after 4 wks	K-MoCA
Liu et al. (2020)	29/29	10/19; 16/13	(58.55 ± 6.24)/(57.69 ± 7.25)	(8.79 ± 1.84)/(8.62 ± 1.84) m	10 Hz TMS, 90% RMT	Left DLPFC	5 d/wk, 4 wks	Before the intervention; after 4 wks	MMSE

(Continued)

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TABLE 1 (Continued)

Reference	Sample size (E/C)	Gender (male/female)	Age (E/C, year)	Course of disease (E/C, day/week/month)	Intervention	Stimulation	Intervention length	Evaluation	Outcome
Yin et al. (2018)	12/13	11/1; 12/1	(58.58 ± 11.98)/(60.15 ± 10.29)	(59.83 ± 30.59)/(56.15 ± 23.74) d	10 Hz rTMS, 80% RMT	Left DLPFC	20 min/d, 5 d/wk, 4 wks	Before the intervention; after 2 wks; after 4 wks	MoCA, MBI
Ma et al. (2020)	30/30	18/12; 17/13	(58.53 ± 13.63)/(59.20 ± 13.06	(2.47 ± 0.88)/(2.38 ± 0.86) m	10 Hz rTMS, 80% RMT	Left DLPFC	20 min/d, 5 d/wk, 4 wks	Before the intervention; after 4 wks	MoCA
Kim et al. (2010)	6/6/6	4/2; 2/4; 4/2	(53.5 ± 16.9)/(68.3 ± 7.4)/(66.8 ± 17.2)	(241.2 ± 42.5)/(404.4 ± 71.7)/(69.7 ± 39.0) d	10 Hz rTMS, 80% RMT/1Hz rTMS, 80% RMT	Left DLPFC	5 d/wk, 2wks	Before the intervention; after 2 wks	MBI
Zhang and Zou (2019)	30/30	20/10; 18/12	(58.44 ± 16.60)/(55.11 ± 18.03)	(46.83 ± 28.13)/(49.00 ± 37.01) d	5 Hz rTMS, 80% RMT	Left DLPFC	20 min/d, 5 d/wk, 4 wks	Before the intervention; after 4 wks	MoCA, MBI
Li Y. et al. (2020)	14/14	Not described	(65.47 ± 3.68)/(64.53 ± 4.72)	(22.73 ± 8.05)/(19.13 ± 7.95) d	5 Hz rTMS, 100% RMT	Left DLPFC	5 d/wk, 3 wks	Before the intervention; after 3 wks	MoCA, MMSE
Lu et al. (2015)	19/21	12/7; 13/8	(42.5 ± 12.3)/(47.3 ± 11.8)	67 (30, 365)/56 (30, 296) d	1 Hz rTMS, 100% RMT	Right DLPEC	5 d/wk, 4 wks	Before the intervention; after 4 wks	MoCA
Li H. et al. (2021)	33/32	21/12; 19/13	(61.79 ± 5.51)/(59.47 ± 6.7)	(28.64 ± 12.60)/(27.78 ± 11.01) d	1 Hz rTMS, 90% RMT	Contralateral DLPEC	20 min/d, 5 d/wk, 4 wks	Before the intervention; after 4 wks	MoCA, MBI
Zhang et al. (2021)	21/22	15/6; 14/8	(60.67 ± 9.53)/(58.95 ± 7.88)	(51.90 ± 21.90)/49.50 ± 29.39) d	1 Hz rTMS, 90% RMT	Contralateral DLPEC	20 min/d, 5 d/wk, 4 wks	Before the intervention; after 4 wks	MoCA, MMSE
Li W. et al. (2022)	28/30	16/12; 18/12	69.5 (60.0,78.0)/66.0 (53.0,75.0)	25(17,30)/25(18,30) d	iTBS, three continuous pulses at 50 Hz repeated at 5 Hz (2s on, 8s off) for a total of 192 s and 600 pulses	Left DLPFC	5 d/wk, 2wks	Before the intervention; after 2 wks	MMSE

Data presented as mean ± SD or median (interquartile range, IQR); E, experiment group; C, control group; RMT, resting motor threshold.

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TABLE 2 Physiotherapy evidence database scores of the included studies.

Study	1	2	3	4	5	6	7	8	9	10	11	Total	Quality level
Yun et al. (2015)	Yes	1	0	1	1	1	1	1	1	1	1	9/10	High
Shaker et al. (2018)	Yes	1	0	1	1	0	0	1	1	1	1	7/10	High
Zeng et al. (2019)	Yes	1	0	1	1	0	0	1	1	1	1	7/10	High
Ai et al. (2021)	Yes	1	0	1	1	0	1	1	1	1	1	8/10	High
Liu et al. (2021)	Yes	1	0	1	1	0	1	1	1	1	1	8/10	High
Chen et al. (2022)	Yes	1	0	1	1	0	0	1	1	1	1	7/10	High
Yan et al. (2022)	Yes	1	0	1	1	0	0	1	1	1	1	7/10	High
Ko et al. (2022)	Yes	1	0	1	1	1	1	1	1	1	1	9/10	High
Liu et al. (2020)	Yes	1	1	1	1	1	1	1	1	1	1	10/10	High
Yin et al. (2018)	Yes	1	0	1	1	0	1	1	1	1	1	8/10	High
Ma et al. (2020)	Yes	1	0	1	1	0	0	1	1	1	1	7/10	High
Kim et al. (2010)	Yes	1	0	1	1	1	1	1	1	1	1	9/10	High
Zhang and Zou (2019)	Yes	1	0	1	1	0	1	1	1	1	1	8/10	High
Li Y. et al. (2020)	Yes	1	0	1	1	1	1	1	1	1	1	9/10	High
Lu et al. (2015)	Yes	1	0	1	1	1	0	1	1	1	1	8/10	High
Li H. et al. (2021)	Yes	1	0	1	1	0	0	1	1	1	1	7/10	High
Zhang et al. (2021)	Yes	1	0	1	1	0	0	1	1	1	1	7/10	High
Li W. et al. (2022)	Yes	1	0	1	1	1	1	1	1	1	1	9/10	High

exclusion criteria. The above screening process was carried out independently by two researchers (RW, WZ), and the results were cross-checked. In disagreement, the decision was discussed with a third researcher (NX). Data were extracted from the literature, including first author, year, sample size, gender, age, course of disease, intervention, stimulation site, intervention length, evaluation time, and outcome indicators. Data were recorded using an Excel spreadsheet. Outcome data (mean ± standard deviation [SD]) for the final included literature were approximated according to the formulae in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins et al., 2022) to eliminate potential differences in patients at baseline further. The value of the correlation coefficient (Corr) was 0.5.

$$\mathrm{Mean}_{\mathrm{change}} = \mathrm{Mean}_{\mathrm{final}} - \mathrm{Mean}_{\mathrm{baseline}}$$

$$\mathrm{SD}_{\mathrm{change}} = \sqrt{\mathrm{SD}^2_{\mathrm{baseline}} + \mathrm{SD}^2_{\mathrm{final}} - (2 \times \mathrm{Corr} \times \mathrm{SD}_{\mathrm{baseline}} \times \mathrm{SD}_{\mathrm{final}})}$$

Quality assessment

The risk of bias in the included literature was assessed using the Cochrane Risk Assessment Scale. The scale consists of seven components: random sequence generation, allocation concealment, blinding of investigators and subjects, blinded assessment of study results, completeness of outcome data, selective reporting of study results, and other biases. Risk levels were determined using "low risk of bias", "high risk of bias", and "uncertain risk of bias". The quality of the included literature was assessed using the physiotherapy evidence database (PEDro) scale. The scale consists of 11 items: eligibility criteria were specified, random participant, allocation concealed, allocation groups similar at baseline, subject blinding, therapist blinding, assessor blinding, <15% dropout, intention to treat analysis, statistical comparisons between groups, point measures, and variability data. The first item was not scored, and the remaining ten were answered as yes (score = 1) or no (score = 0). A score out of 10 was assigned, with ≥7 being high quality, 5-6 being moderate quality, and ≤4 being low quality. The quality assessment was carried out independently by two researchers (YW, RW), and the results were cross-checked. In disagreement, the decision was discussed with a third researcher (NX).

Statistical analysis

Meta-analysis

A standard meta-analysis of study data for each outcome indicator was performed using RevMan 5.4 software. I^2 statistics and Cochrane's Q test were used to assess heterogeneity

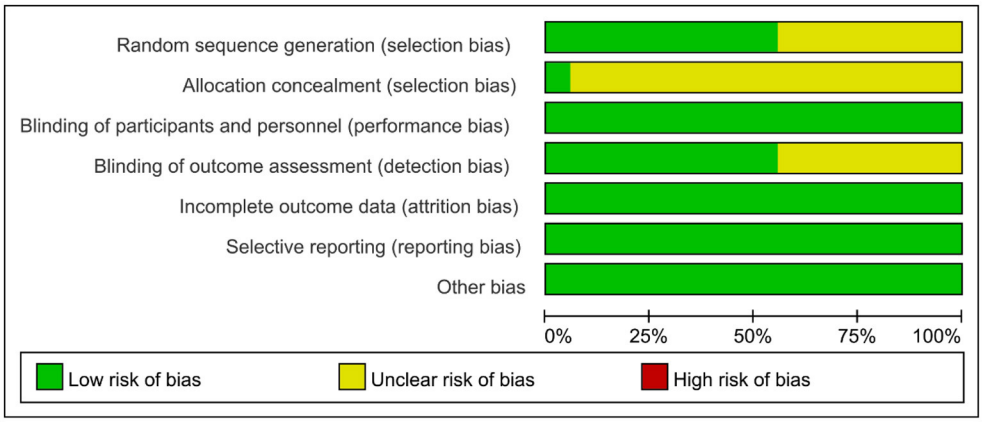

FIGURE 2
Risk assessment of bias.

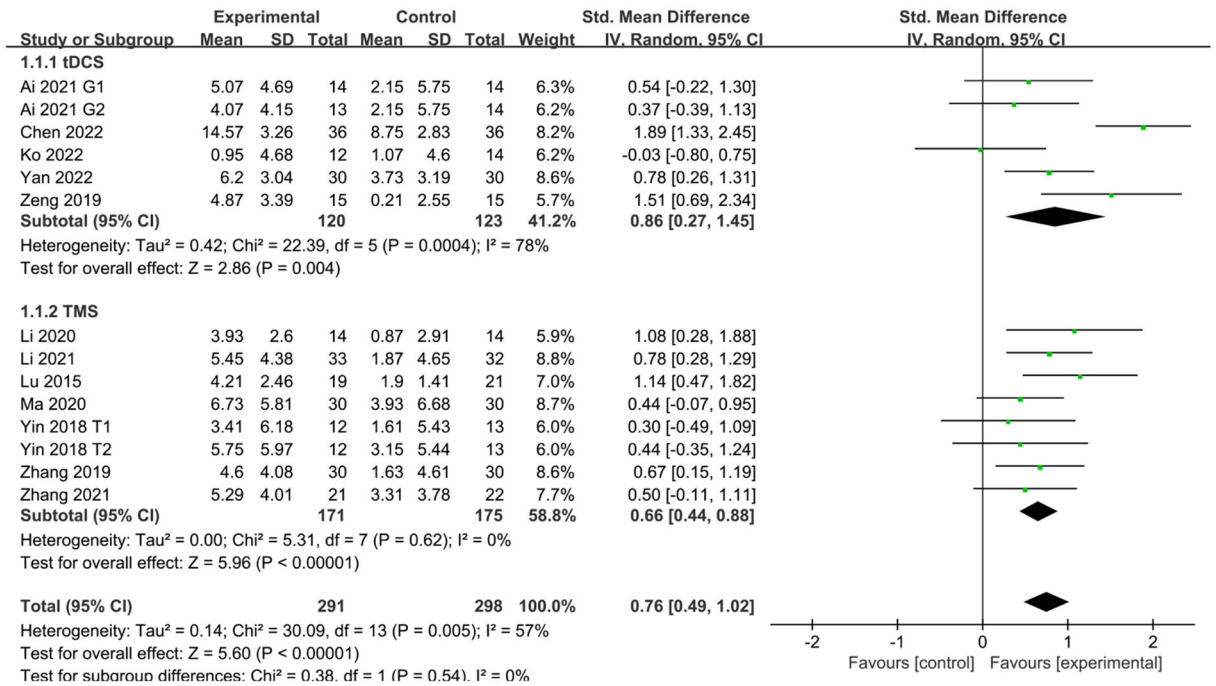

FIGURE 3
Effect of NIBS on MoCA score in PSCI patients.

among included studies. If $I^2 \leq 50\%$ and $P \geq 0.1$, there was considered no significant heterogeneity between the included studies, and the data were analyzed using a fixed effects model. If $I^2 > 50\%$ and $P < 0.1$, significant heterogeneity was considered between the included studies. A random-effects model was used to analyze the data, and subgroup and sensitivity analyses were used to identify sources of heterogeneity. The outcome indicators in this study are continuous variables, and the assessment methods used may differ for each study. The effect sizes were expressed as standardized mean differences (SMD) with 95% confidence intervals (CI). If $P \leq 0.05$, the combined statistic of multiple studies is significant. If $P > 0.05$, multiple studies' combined statistic is insignificant.

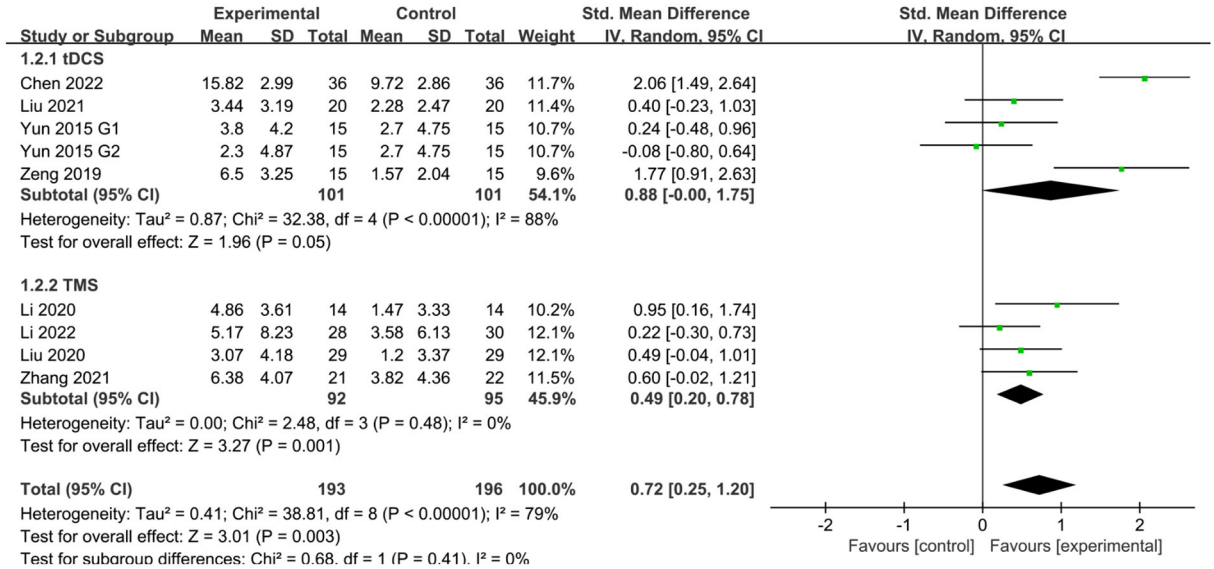

FIGURE 4
Effect of NIBS on MMSE score in PSCI patients.

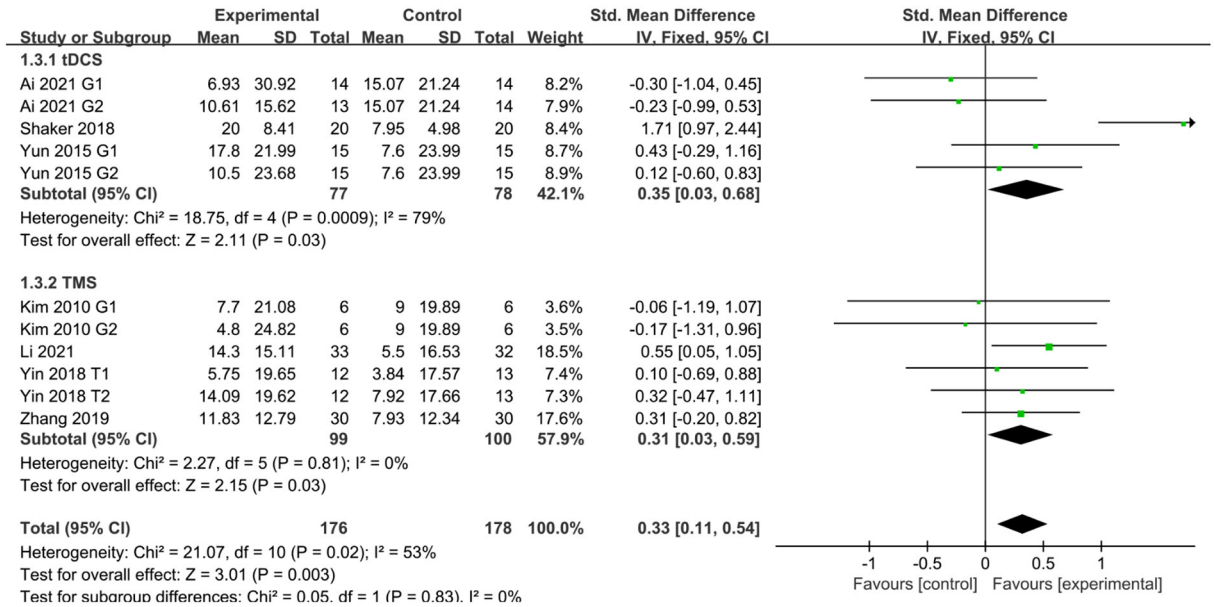

FIGURE 5
Effect of NIBS on MBI and FIM score in PSCI patients.

Network meta-analysis

NMA was performed using the network and mvmeta packages in State 14.0 according to the Bayesian framework. Evidence network plots were drawn presenting direct comparisons or indirect comparisons of relationships between different interventions. The dots in the graph represent interventions, with larger dots indicating more patients using the intervention. A straight line indicates that a direct comparison exists between two interventions. The thickness of the line segment represents the number of studies with direct comparisons. The surface under the cumulative ranking curve (SUCRA) is used to express the ranking probability ($0\% \leq \text{SUCRA} \leq 100\%$). A larger SUCRA for an intervention indicates that the intervention is more effective.

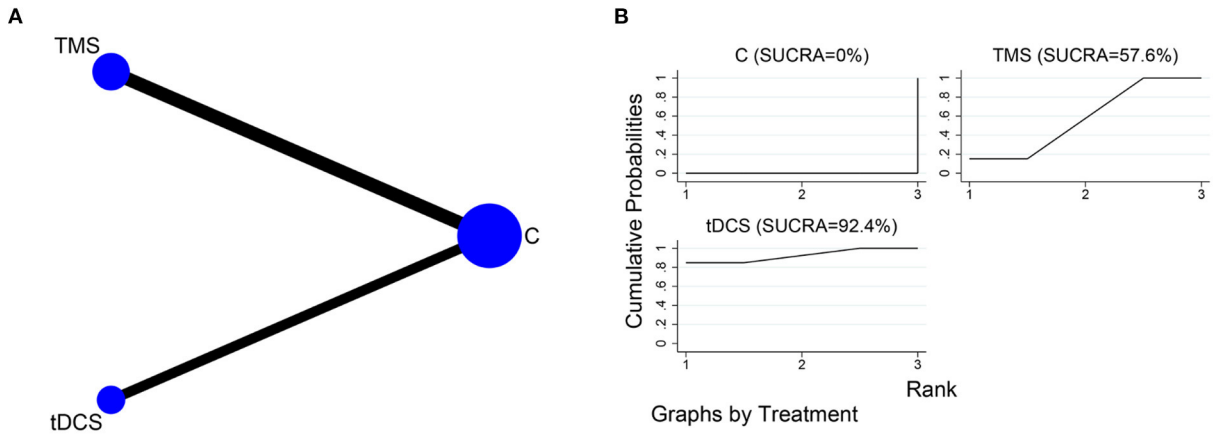

FIGURE 6
NMA of MoCA scores at different NIBS. **(A)** Network plot; **(B)** SUCRA plot.

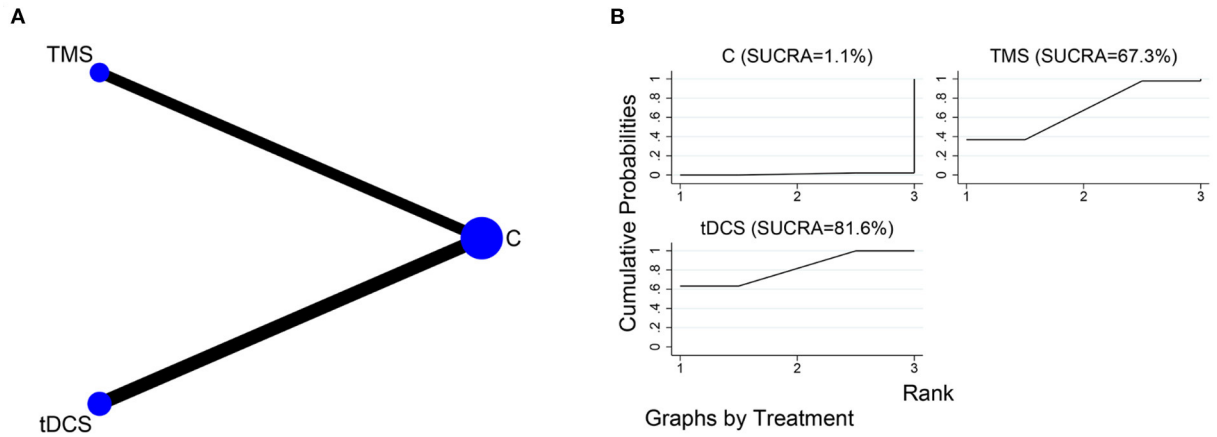

FIGURE 7
NMA of MMSE scores at different NIBS. **(A)** Network plot; **(B)** SUCRA plot.

Additional analyses and small study effects

Subgroup analysis of different outcome indicators based on the timing of the intervention to determine the optimal intervention period. Comparison-adjusted funnel plots for NMA were drawn to determine the presence of small sample effects and publication bias based on symmetry.

Results

Study selection

A total of 814 studies were obtained, including 353 in English and 461 in Chinese. 18 studies (Kim et al., 2010; Lu et al., 2015; Yun et al., 2015; Shaker et al., 2018; Yin et al., 2018; Zeng et al., 2019; Zhang and Zou, 2019; Liu et al., 2020, 2021; Li Y. et al., 2020; Ma et al., 2020; Ai et al., 2021; Li H. et al., 2021; Zhang et al., 2021; Chen et al., 2022; Ko et al., 2022; Li W. et al., 2022; Yan et al., 2022) were finally included, including 9 in English (Kim et al., 2010; Lu et al., 2015; Yun et al., 2015; Shaker et al., 2018; Liu et al., 2020; Li Y. et al., 2020; Li H. et al., 2021; Ko et al., 2022; Li W. et al., 2022) and 9 in Chinese (Yin et al., 2018; Zeng et al., 2019; Zhang and Zou, 2019; Ma et al., 2020; Ai et al., 2021; Liu et al., 2021; Zhang et al., 2021; Chen et al., 2022; Yan et al., 2022). The literature selection process is shown in Figure 1. 809 patients were included in the 18 studies, including 418 in the NIBS group and 391 in the sham stimulation group. The essential characteristics of the included literature are shown in Table 1.

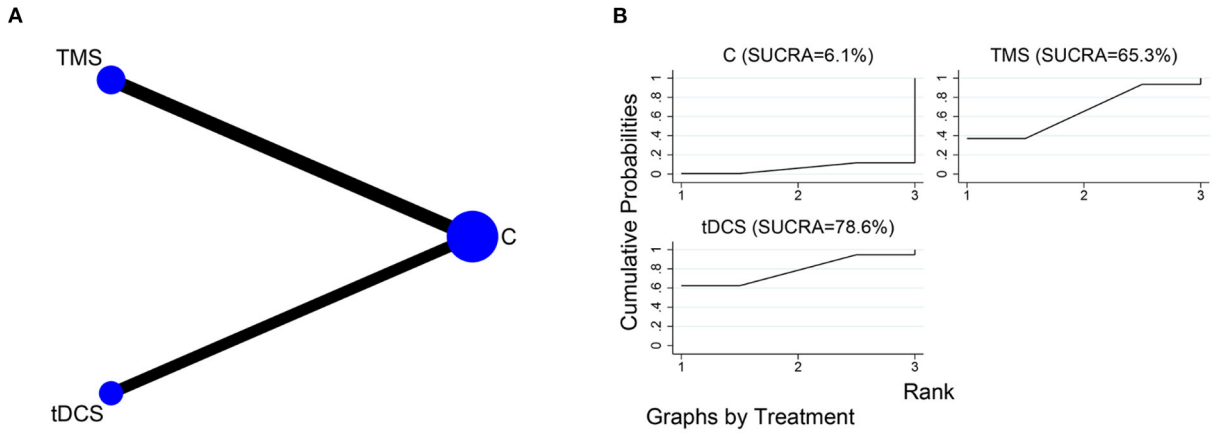

FIGURE 8
NMA of MBI and FIM scores at different NIBS. **(A)** Network plot; **(B)** SUCRA plot.

Quality assessment

Of the 18 studies, eight (Lu et al., 2015; Yin et al., 2018; Zeng et al., 2019; Zhang and Zou, 2019; Ai et al., 2021; Li H. et al., 2021; Liu et al., 2021; Zhang et al., 2021) used the number table method to generate random sequences, two (Liu et al., 2020; Ko et al., 2022) used a computer for randomized assignment. The remaining eight mentioned "random grouping" but did not specify the randomization method. Only one study used sealed opaque envelopes for allocation concealment (Liu et al., 2020). All studies were blinded to treatment participants. Ten studies (Kim et al., 2010; Yun et al., 2015; Yin et al., 2018; Zhang and Zou, 2019; Liu et al., 2020, 2021; Li Y. et al., 2020; Ai et al., 2021; Ko et al., 2022; Li W. et al., 2022) were blinded to assessors. The results of all studies were complete and not selectively reported. The distribution of the risk of bias across studies is shown in Figure 2. The 18 studies were all high quality, with a mean score of 8 (Table 2).

Meta-analysis

Cognitive functions

MoCA

Twelve studies (Lu et al., 2015; Yin et al., 2018; Zeng et al., 2019; Zhang and Zou, 2019; Li Y. et al., 2020; Ma et al., 2020; Ai et al., 2021; Li H. et al., 2021; Zhang et al., 2021; Chen et al., 2022; Ko et al., 2022; Yan et al., 2022) reported MoCA scores in patients with PSCI after treatment. The results showed that MoCA scores were better in the NIBS group than in the sham stimulation group, with a statistically significant difference (SMD = 0.76, 95% CI 0.49–1.02, $P < 0.05$). Subgroup analysis showed that both the tDCS and TMS groups were more effective than the sham stimulation group, with a statistically significant difference (SMD = 0.86, 95% CI 0.27–1.45, $P < 0.05$ and SMD = 0.66, 95% CI 0.44–0.88, $P < 0.05$) (Figure 3).

MMSE

Eight studies (Yun et al., 2015; Zeng et al., 2019; Liu et al., 2020, 2021; Li Y. et al., 2020; Zhang et al., 2021; Chen et al., 2022; Li W. et al., 2022) reported MMSE scores in patients with PSCI after treatment. The results showed that MMSE scores were better in the NIBS group than in the sham stimulation group, with a statistically significant difference (SMD = 0.72, 95% CI 0.25–1.20, $P < 0.05$). Subgroup analysis showed that both the tDCS and TMS groups were more effective than the sham stimulation group, with a statistically significant difference (SMD = 0.88, 95% CI −0.00–1.75, $P = 0.05$ and SMD = 0.49, 95% CI 0.20–0.78, $P < 0.05$) (Figure 4).

Activities of daily living

Seven studies (Kim et al., 2010; Yun et al., 2015; Shaker et al., 2018; Yin et al., 2018; Zhang and Zou, 2019; Ai et al., 2021; Li H. et al., 2021) reported activities of daily living in patients with PSCI after treatment. Six studies (Kim et al., 2010; Yun et al., 2015; Yin et al., 2018; Zhang and Zou, 2019; Ai et al., 2021; Li H. et al., 2021) used MBI to assess patients and one study (Shaker et al., 2018) used FIM to assess patients. The results showed that activities of daily living were better in the NIBS group than in the sham stimulation group, with a statistically significant difference (SMD = 0.33, 95% CI 0.11–0.54, $P < 0.05$). Subgroup analysis showed that both the tDCS and TMS groups were more effective than the sham stimulation group, with a statistically significant difference (SMD = 0.35, 95% CI 0.03–0.68, $P < 0.05$ and SMD = 0.31, 95% CI 0.03–0.59, $P < 0.05$) (Figure 5).

TABLE 3 Subgroup analysis of NIBS on PSCI patients.

Subgroup analysis		Studies	SMD (95% CI)	*P*	X^2	I^2 (%)	Tau^2
MoCA							
Intervention length	<4w	4	0.44 [0.10, 0.79]	0.01	4.05	1%	0.00
	≥4w	9	0.89 [0.57, 1.22]	<0.0001	21.59	63%	0.15
MMSE							
Intervention length	<4w	3	0.29 [−0.08, 0.67]	0.28	3.80	21%	0.03
	≥4w	5	1.04 [0.34, 1.74]	0.003	24.97	84%	0.53
MBI, FIM							
Intervention length	<4w	4	0.01 [−0.30, 0.32]	0.95	2.59	0%	0.00
	≥4w	4	0.69 [0.12, 1.27]	0.02	0.51	72%	0.24

Network meta-analysis

Cognitive functions

MoCA

The network relationships for the different NIBS, using MoCA as the outcome indicator, are shown in Figure 6A. Five of the included studies (Zeng et al., 2019; Ai et al., 2021; Chen et al., 2022; Ko et al., 2022; Yan et al., 2022) had tDCS as the intervention, and seven (Lu et al., 2015; Yin et al., 2018; Zhang and Zou, 2019; Li Y. et al., 2020; Ma et al., 2020; Li H. et al., 2021; Zhang et al., 2021) had TMS as the intervention. The SUCRA of different NIBS in improving MoCA scores were in the order of tDCS (SUCRA = 92.4%) and TMS (SUCRA = 57.6%) (Figure 6B).

MMSE

The network relationships for the different NIBS, using MMSE as the outcome indicator, are shown in Figure 7A. Four of the included studies (Yun et al., 2015; Zeng et al., 2019; Liu et al., 2021; Chen et al., 2022) used tDCS as the intervention, and four (Liu et al., 2020; Li Y. et al., 2020; Zhang et al., 2021; Li W. et al., 2022) used TMS as the intervention. The SUCRA of different NIBS in improving MMSE scores were in the order of tDCS (SUCRA = 81.6%) and TMS (SUCRA = 67.3%) (Figure 7B).

Activities of daily living

The network relationships for the different NIBS, using MBI and FIM as the outcome indicator, are shown in Figure 8A. Three of the included studies (Yun et al., 2015; Shaker et al., 2018; Ai et al., 2021) used tDCS as the intervention, and four (Kim et al., 2010; Yin et al., 2018; Zhang and Zou, 2019; Li H. et al., 2021) used TMS as the intervention. The SUCRA of different NIBS in improving activities of daily living were in the order of tDCS (SUCRA = 78.6%) and TMS (SUCRA = 65.3%) (Figure 8B).

Adverse reaction

Six studies (Lu et al., 2015; Yin et al., 2018; Li Y. et al., 2020; Ai et al., 2021; Liu et al., 2021; Li W. et al., 2022) reported that a few patients experienced transient dizziness, pain, and pins and needles, and sneezing during treatment. The patients could be relieved after rest and did not affect the treatment. No adverse effects were reported in other studies.

Subgroup analysis of outcomes

Subgroup analyses were conducted on the MoCA, MMSE, MBI, and FIM scores according to the length of the intervention. The results are shown in Table 3.

Sensitivity analysis

The meta-analysis results were analyzed for sensitivity using a one-by-one exclusion method, removing one study at a time. The results showed no significant change from the above results, indicating that the meta-analysis results were relatively stable.

Publication bias

A funnel plot analysis of the included literature with MoCA, MMSE, MBI, and FIM as outcome indicators showed that the scatter was generally symmetrical, and the Meta-analysis results were reliable (Figure 9).

Discussion

In the included studies, patients' cognitive function was assessed using MoCA and MMSE, and patient's ability to

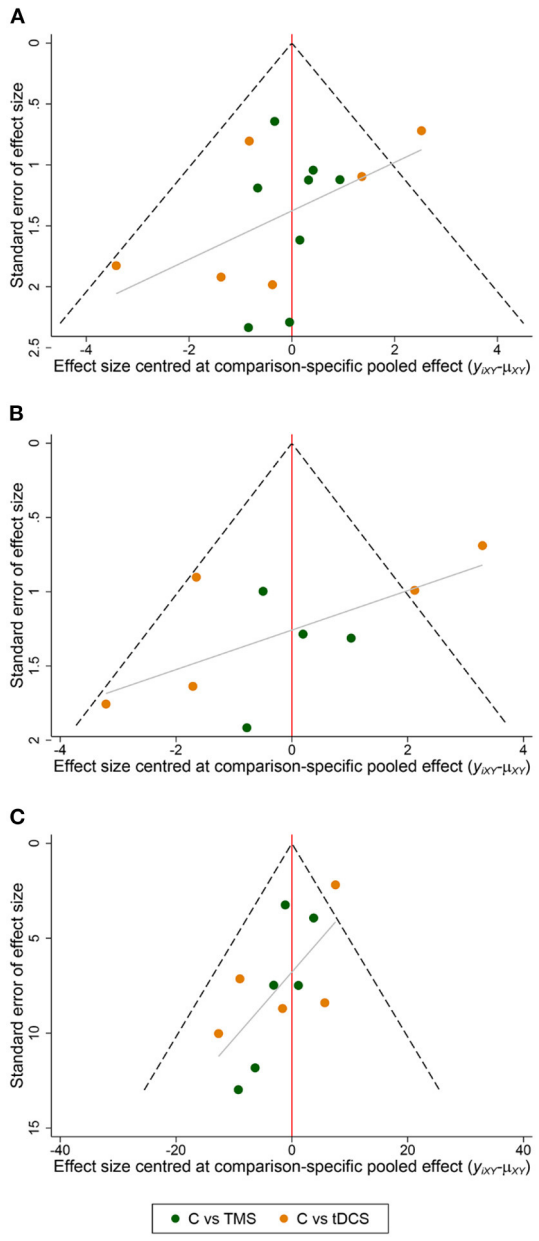

FIGURE 9
Funnel plot of the included studies. **(A)** MoCA; **(B)** MMSE; **(C)** MBI, FIM.

perform activities of daily living was assessed using MBI and FIM. The meta-analysis showed that both tDCS and TMS significantly improved the cognitive function and activities of daily living of PSCI patients compared to the control group. Network meta-analysis showed that tDCS appeared more effective than TMS for cognitive function and activities of daily living in PSCI patients. NIBS stimulation parameters and treatment duration are important factors that also influence efficacy. In the included literature, the parameters of tDCS were mainly 2.0 mA for 20–30 min; the TMS stimulation modality commonly used was rTMS, with low-frequency rTMS mainly at 1 Hz and high-frequency rTMS at 5 and 10 Hz for about 20 min. Better cognitive rehabilitation results were achieved with a total intervention time of NIBS above 4 weeks.

NIBS' current mechanism of action on improving cognitive function in patients with PSCI consists of three main aspects: first, by affecting cortical excitability; second, by improving neuroplasticity; third, by regulating cerebral blood flow. The theory of interhemispheric competition suggests that the mechanism of PSCI is the inability of the affected cerebral hemisphere to form a normal inhibitory effect on the healthy hemisphere, resulting in pathological excitation in the healthy hemisphere (Di Pino et al., 2014). NIBS primarily uses two treatment modalities, excitation of the affected hemisphere and inhibition of the healthy hemisphere (Li L. et al., 2021), thereby facilitating the recovery of cognitive function in patients with PSCI. Kenney-Jung et al. (2019) found that anodal tDCS stimulation increased the frequency of spontaneous firing in neuronal cells and increased cortical excitability; cathodal tDCS stimulation caused hyperpolarization of neuronal cell membranes and decreased cortical excitability. It was shown that high-frequency rTMS stimulation activates many voltage-gated channels, producing a depolarizing effect and increasing cortical excitability; low-frequency rTMS inhibits neuronal activity and reduces cortical excitability (Klomjai et al., 2015; Mikellides et al., 2021).

Stroke causes damage to synaptic signal transmission and synaptic structures. Studies have shown that synaptic damage in the hippocampus is associated with decreased spatial learning and memory function (Xu et al., 2018). Therefore, improving synaptic plasticity is also a meaningful way to treat cognitive impairment (Rolland et al., 2013). NIBS has been shown to improve synaptic plasticity, and this change may be related to both long-term potentiation and long-term depression (Huang et al., 2017; Jones, 2017; Cavaleiro et al., 2020). In addition, Monai et al. (2016) showed that tDCS can modulate synaptic plasticity by altering the concentration of calcium ions in astrocytes. Further studies have shown that tDCS stimulation can affect synaptic plasticity by altering the concentration of γ-aminobutyric acid secreted by astrocytes (Antonenko et al., 2017). Lenz et al. (2016) found that 10 Hz rTMS can affect synaptic excitability in the proximal dendrites of hippocampal CA1 pyramidal neurons. Li et al. (2019) found that 0.5 Hz rTMS increased the density of synaptic ultrastructure in the hippocampal CA1 region.

Cerebral vascular occlusion after stroke leads to tissue infarction and propagation of damage to adjacent cells, creating an ischemic semidark zone between the ischemic site and normal tissue. Reduced local blood flow to brain tissue in the focal and semidark areas leads to ischemic white matter lesions and cognitive impairment (Inaba et al., 2019). It has been demonstrated that NIBS has the effect of modulating cerebral

blood flow, which improves cognitive function. Bragina et al. (2018) found that anodal tDCS induces the dilation of small arteries and modulates capillary blood flow velocity, leading to increased cerebral blood flow. Hara et al. (2017) identified that the degree of decreased cerebral perfusion on the affected side was reduced after patients received high-frequency rTMS; patients receiving low-frequency rTMS had reduced perfusion in the healthy hemisphere, reduced inhibition on the affected side, and increased cerebral blood flow on the affected side.

Limitations

There are some limitations to this study. First, the total number of subjects included in the literature was small. Second, some literature does not hide the order of assignment and assessor blinding, which may lead to a potential risk of bias. Third, age differences in the study population and varying severity of illness may have impacted the rehabilitation outcomes. Fourth, the frequency and periodicity of interventions in the literature varied, which may have biased the study results. Fifthly, some literature had short treatment cycles, and most studies did not have a long-term follow-up after treatment.

Conclusion

In summary, NIBS has shown promising results in improving patients' cognitive function and activities of daily living with PSCI. In the future, more extensive and rigorous double-blind randomized controlled trials are needed to explore the optimal stimulation parameters and intervention cycles for NIBS. The combination of NIBS and brain imaging technology should be enhanced, and in-depth mechanistic studies should be conducted to provide more reliable evidence-based medical evidence for clinical rehabilitation.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

YW designed and wrote this study. NX guided the methodology. RW reviewed the entire manuscript. YW and RW took part in the data selection and extraction. RW and WZ performed the statistical analysis and analyzed the data. All authors contributed to the article and approved the submitted version.

Funding

This study was received support from the Social Science Planning Research Project of Shandong Province (20CZXJ06).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Neuroscience

TYPE Systematic Review
PUBLISHED 23 January 2023
DOI 10.3389/fnins.2022.1081278

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

RECEIVED 27 October 2022
ACCEPTED 31 December 2022
PUBLISHED 23 January 2023

CITATION
Ma H, Zhao K, Jia C, You J, Zhou M, Wang T and Huang C (2023) Effect of transcranial direct current stimulation for patients with disorders of consciousness: A systematic review and meta-analysis.
Front. Neurosci. 16:1081278.
doi: 10.3389/fnins.2022.1081278

Effect of transcranial direct current stimulation for patients with disorders of consciousness: A systematic review and meta-analysis

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Introduction: Transcranial direct current stimulation (tDCS) could potentially facilitate consciousness improvement in patients with disorders of consciousness (DOC). The aim of this study was to investigate the therapeutic efficacy of tDCS on consciousness recovery for patients with DOC.

Methods: Eight databases were systematically searched from their inception to June 2022. Quality of included studies were assessed using PEDro score and Cochrane's risk of bias assessment. All statistical analyses were performed using RevMan software. Seventeen studies with 618 patients were identified eligible for this study, and fifteen studies with sufficient data were pooled in the meta-analysis.

Results: The results of meta-analysis showed a significant effect on increasing GCS scores (MD = 1.73; 95% CI, 1.28–2.18; $P < 0.01$) and CRS-R scores (MD = 1.28; 95% CI = 0.56–2.00; $P < 0.01$) in favor of the real stimulation group as compared to sham. The results of subgroup analysis demonstrated that only more than 20 sessions of stimulation could significantly enhance the improvement of GCS scores and the CRS-R scores. Moreover, the effect of tDCS on CRS-R score improvement was predominant in patients with minimal conscious state (MCS) (MD = 1.84; 95% CI = 0.74–2.93; $P < 0.01$).

Conclusion: Anodal tDCS with sufficient stimulation doses appears to be an effective approach for patients with MCS, in terms of CRS-R scores.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42022336958.

KEYWORDS

disorders of consciousness, transcranial direct current stimulation, meta-analysis, systematic review, coma recovery scale-revised

Introduction

A disorder of consciousness (DOC) is a state of medical condition that inhibit consciousness due to primary or secondary substantial brain injuries (Eapen et al., 2017). Conscious behavior requires two main components: adequate arousal and awareness of content. Disruption of one or both of these components could result in DOC (Bernat, 2006). DOC can be categorized into

different types: coma, in which a patient is in deep state of prolonged consciousness, and fails to respond normally to internal or external stimulations; unresponsive wakefulness syndrome (UWS), which is previously known as vegetative state (VS), where a patient has sleep-wake cycle, but lacks awareness; minimal conscious state (MCS), where the patient has intermittent periods of awareness and wakefulness (Giacino et al., 2018). At a conservative estimate, about 5/100,000 people will enter a prolonged DOC from acute onset and progressive brain damage, and the incidence rate of DOC is growing, as the development of neurocritical care (Wade, 2018). As patients with DOC cannot participate in physical therapy actively, most of them have sever medical complications, including respiratory system disorders, skeletal muscle system disorders, endocrine and metabolic abnormalities, urinary system infection, autonomic nerve disorder, deep vein thrombosis and others, which would hinder the recovery process (Choi et al., 2008; Estraneo et al., 2018). Therefore, DOC patients place great financial strain on medical structures due to prolonged intensive care (Laureys and Schiff, 2012).

A lot of crucial work has been done on the accurate diagnosis of patients with DOC, which can lead to important medical decisions, such as withdrawal of life-sustaining care (Giacino et al., 2014; Boly et al., 2017). Nevertheless, no diagnostic assessment procedure had moderate or strong evidence for use in DOC (Giacino et al., 2018). Although neuroimaging and electrophysiologic procedures, including EMG, EEG, fMRI, and PET, are evolving as potential components of the DOC clinical assessment, there were insufficient evidentiary support to include them in formal diagnostic criteria or routine clinical care (Owen and Coleman, 2008; Schnakers et al., 2008). According to the American congress of rehabilitation medicine, the Coma Recovery Scale-Revised (CRS-R) with high sensitivity ranked the top-rated neurobehavioral rating scale for clinical assessment of patients with DOC (Seel et al., 2010). The CRS-R consists of 23 items comprised of six subscales designed to assess audition, receptive and expressive language, communication ability, visuoperception, motor functions and arousal level, including reflex behaviors and cognitively mediated behaviors (Annen et al., 2019). A CRS-R total score of 10 has 100% specificity for UWS, although also a false negative diagnostic error rate of 22% (Bodien et al., 2016). Therefore, most studies associated to DOC always selected CRS-R as an outcome measure or as a covariate in neuroimaging and neurophysiological analyses (Zhang et al., 2017; Feng et al., 2020). Meanwhile, the Glasgow Coma Scale is another clinical scale used to reliably measure a patient's level of consciousness, which is widely used by neurosurgeons and nurses in more than 80 countries (Teasdale et al., 2014). Despite there are many neuroimaging and neuroelectrophysiological examinations, neurological and behavioral assessment is still the primary approach to determine the DOC progression, because it is generally believed that the higher-level behaviors correspond to higher levels of neurological functioning, as well as the ability to demonstrate lower-level behaviors or the disappearance of pathological behaviors as sign of recovery.

The neural mechanisms of DOC are complex and still unclear (Edlow et al., 2021). The mesocircuit fronto-parietal model supported that frontal cortex, central thalamus, brain stem, striatum and globus pallidus intema play important roles in consciousness processing, which are also intervention targets for DOC (Thibaut et al., 2019b). However, the clinical management of patients with DOC remains challenging, and the therapeutic options for DOC are also limited (Thibaut et al., 2019b). According to the 2018 edition of the Practice Guidelines for consciousness Disorders in the United States, no treatment for DOC has sufficient evidence to prove its absolute effectiveness (Giacino et al., 2018). The therapeutic options include pharmacological and non-pharmacological interventions. For pharmacological interventions, only few and limited evidence supported that patients with prolonged DOC could benefit from amantadine and zolpidem (Giacino et al., 2012; Whyte et al., 2014). Non-pharmacological interventions are always neuromodulation techniques attempting to promote DOC recovery by modulating brain excitability, including invasive and non-invasive brain stimulations (NIBS). Invasive brain stimulation consists of deep brain stimulation (DBS) and vagus nerve stimulation (VNS). NIBS consists of transcranial direct current stimulation (tDCS), repeated transcranial magnetic stimulation (rTMS), transcutaneous VNS and low intensity focused ultrasound pulse. Unfortunately, the therapeutic effects of such neuromodulation techniques are inconsistent and limited (Bourdillon et al., 2019). DBS is an invasive stimulation with severe side effects possibly (Lemaire et al., 2018). Due to the stimulation targets and parameters of DBS are various and methodological limitations, the overall quality of evidence based on the results of previous studies was not high (Bourdillon et al., 2019). VNS is a less invasive stimulation alternative to DBS, but only one case investigated its therapeutic potential in patients with DOC (Corazzol et al., 2017). rTMS is a non-invasive neuromodulation technique which can trigger firing of action potentials, but can induce epilepsy potentially, however, the level of evidence supporting its therapeutic effects of patients with DOC is low (Lefaucheur et al., 2014). tDCS delivers a weak intensity and continuous current to modulate the neural resting state membrane potential polarization, which is widely used in psychiatric mental illness and post stroke dysfunction previously (Palm et al., 2016; Sehm, 2017). Compared with rTMS, tDCS is less possible to induce epilepsy and its therapeutic effects last more than a few minutes which could induce after-effects mediated by synaptic pathways (Kronberg et al., 2017). Moreover, the equipment of tDCS is inexpensive and implemented without site restrictions, which is more convenient to use at bedside or at home than rTMS. Since Thibaut et al. firstly published a sham-controlled randomized study on tDCS for patients with DOC in 2014, more researchers investigated the efficacy of tDCS for patients with DOC, however, due to the various stimulation parameters, the results were conflicting and controversial (Thibaut et al., 2014). A meta-analysis assessing the effects of NIBS in patients with DOC concluded that patients with MCS could benefit from tDCS, but no dose-session effect was found (Feng et al., 2020). The authors stated that additional high-quality studies were required to validate their findings. Some well-designed studies investigating the role of tDCS in patients with DOC were published recently (Chen et al., 2021; Guo et al., 2021; Li et al., 2021; Barra et al., 2022). Consequently, the present systematic review and meta-analysis aimed to integrate new evidence presented in recent years to evaluate the efficacy of tDCS for patients with DOC.

Methods

The present systematic review and meta-analysis were performed and reported in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2020 statement (PRISMA 2020), and *Cochrane Handbook for Systematic Reviews of Interventions* (Cumpston et al., 2019; Page et al., 2021). In addition, the present systematic review was registered in

the International Prospective Register of Systematic Reviews (PROSPERO): CRD42022336958.

Data sources and search strategies

We systematically searched for relevant articles available in both Chinese and English in electronic databases, including MEDLINE (*via* Ovid), Web of Science, Embase (*via* Ovid), CENTRAL (Cochrane library), Physiotherapy Evidence Database (PEDro), Chinese National Knowledge Infrastructure (CNKI), Wanfang Data and Weipu Database from their inception until June 2022. Search terms included key words associated with DOC, MCS, VS, and tDCS. The specific search strategy of all databases used are presented in **Supplementary Digital Content 1**. Furthermore, a manual screening of reference lists of the articles was performed to identify additional relevant studies. No ethical approval or patient consent was required because all analyses were based on previously published studies.

Study selection

Endnote software was used to check for duplicated studies. Two investigators reviewed the studies independently and selected studies based on the predetermined criteria. All potentially relevant articles were retrieved from the databases for the assessment of their full text based on titles and abstracts. Studies that did not meet the inclusion criteria were excluded. Discrepancies between two reviewers were resolved through discussions with a third reviewer until a consensus was reached. The included studies were required to meet the following criteria: (1) studies were RCTs in either parallel or cross-over design published in English or Chinese, (2) studies were recruited adult participants with DOC, (3) intervention treatments were tDCS and sham stimulation as the control, and (4) with regard to outcome measures, studies used CRS-R or GCS as outcome measure for the recovery of DOC. Studies meeting any of these criteria were excluded: (1) studies published in dissertations, conference abstracts, or other types without peer-review; (2) non-randomized controlled trials or outcome measures without GCS or CRS-R scores; (3) studies published in neither English nor Chinese.

Data extraction and quality assessment

Two reviewers independently extracted relevant data onto a pre-developed data extraction sheet, and disagreements were adjudicated by a third reviewer. The data extracted from selected studies included basic information (first author, year of publication), study design, demographic characteristics of patients (sample size, patient diagnosis), details of interventions applied to the experimental and control groups (stimulation protocol, brain target, and stimulation dose), relevant outcome measures.

Eligible articles were scrutinized for methodological quality by two independent reviewers using PEDro scale. The PEDro scale comprises 11 items with a total score ranging from 0 to 10 (except for item 1). The methodological quality of studies scoring 9–10 was considered to be of "excellent" quality, studies scoring 6–8 were considered to be of "good" quality, studies scoring 4–5 were considered to be of "fair" quality, and studies scoring below 4 were considered to be of "poor" quality (Foley et al., 2003). Discrepancies between two reviewers were resolved through discussions with a third reviewer. Additionally, risk of bias assessments were performed using the criteria described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Cumpston et al., 2019). The evaluation entries included the following aspects: random sequence generation, allocation concealment, masking, incomplete outcome data, and selective outcome reporting among others. The included articles were evaluated as "low risk," "high risk," or "unclear risk." Quality assessment was not used as a selection or exclusion criterion.

Data synthesis and analysis

The results of all included studies were pooled using standard meta-analytic methods to estimate the effect of tDCS for the recovery of DOC. Based on the nature of extracted data, we assessed the mean differences (MDs) and 95% confidence intervals (CIs) for continuous outcomes. A P-value < 0.05 (two-sided) was considered statistically significant in the estimation of effects. Statistical heterogeneity was evaluated using chi-square test and I^2 statistic. P-value < 0.05 or I^2 value $> 40\%$ was considered high heterogeneity. A fixed-effects model was used when P-value was > 0.05; otherwise, a random-effects model was used. Sensitivity analyses were performed by excluding each study from the analysis when heterogeneity was detected, and the subgroup analyses were performed based on the different stimulation protocols, stimulation doses or patient diagnoses. Publication bias was not assessed due to the limited number of included studies. All statistical analyses were performed using RevMan software (Version 5.3; Cochrane Collaboration, Copenhagen, Denmark).

Results

Search results

The initial electronic search resulted in a total of 4,579 studies, of which 4,229 unique articles were retrieved after duplicates were removed. After screening the titles, abstracts, and full text of the articles based on the inclusion and exclusion criteria, 17 studies (Thibaut et al., 2014, 2017, 2019a; Estraneo et al., 2017; Huang et al., 2017; Zhang et al., 2017, 2020; Chi et al., 2018; Martens et al., 2018, 2019, 2020; Cavinato et al., 2019; Wu et al., 2019; Chen et al., 2021; Guo et al., 2021; Li et al., 2021; Barra et al., 2022) with a total of 618 participants with DOC were identified as eligible for the systematic review. Two studies did not report enough data for calculating effect size and therefore were excluded from the meta-analysis (Cavinato et al., 2019; Thibaut et al., 2019a). Finally, 15 studies with 580 DOC patients were included in the quantitative synthesis (Thibaut et al., 2014, 2017; Estraneo et al., 2017; Huang et al., 2017; Zhang et al., 2017, 2020; Chi et al., 2018; Martens et al., 2018, 2019, 2020; Wu et al., 2019; Chen et al., 2021; Guo et al., 2021; Li et al., 2021; Barra et al., 2022). The details of the search process are shown in **Figure 1**.

Description of studies

The studies included in this systematic review were published between 2014 and 2022. Five of them were published in Chinese

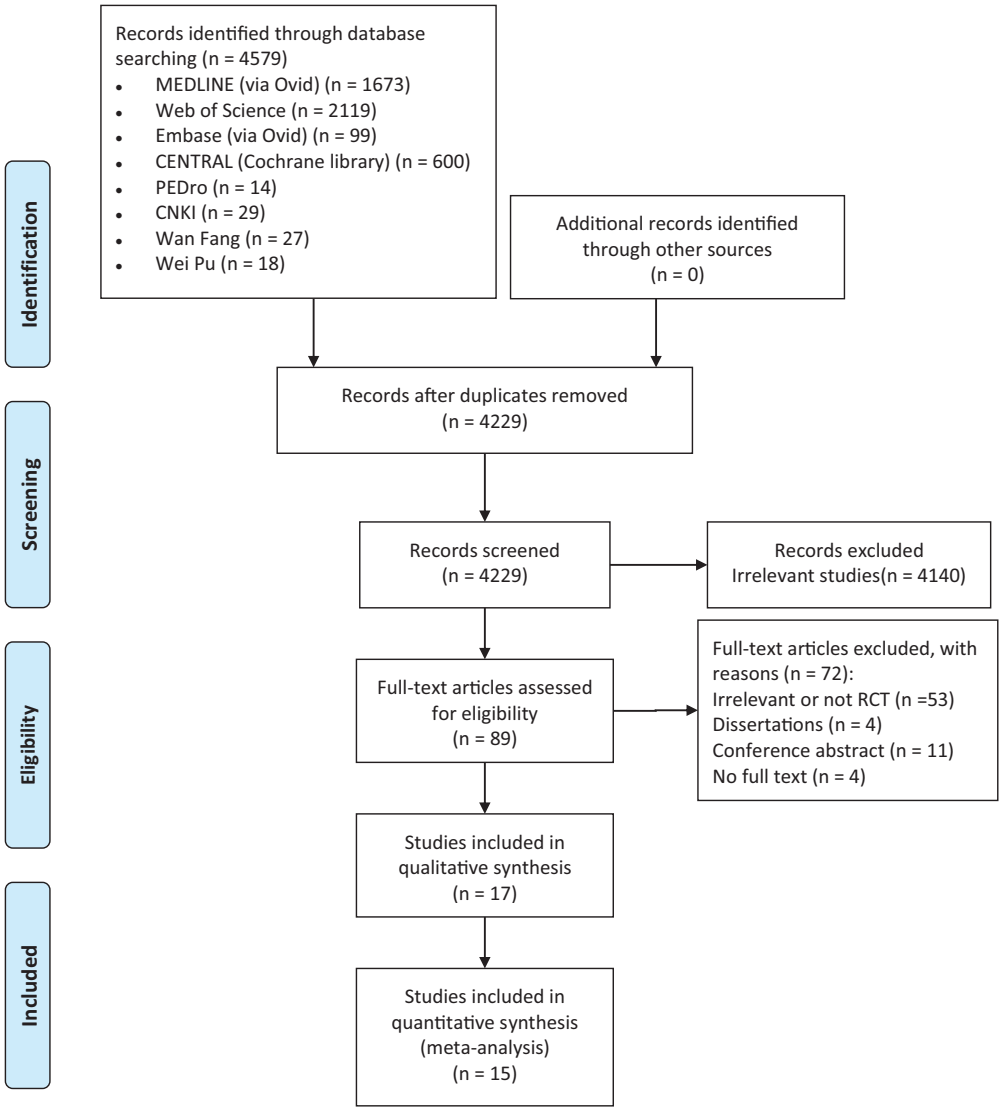

FIGURE 1
PRISMA flow diagram.

(Chi et al., 2018; Zhang et al., 2020; Chen et al., 2021; Guo et al., 2021; Li et al., 2021) and 12 of them were published in English (Thibaut et al., 2014, 2017, 2019a; Estraneo et al., 2017; Huang et al., 2017; Zhang et al., 2017; Martens et al., 2018, 2019, 2020; Cavinato et al., 2019; Wu et al., 2019; Barra et al., 2022). The sample size ranged from 10 to 113 participants. The characteristics of included studies, including study design, patient diagnosis, details of intervention, and outcome measures, were summarized in **Table 1**.

All studies included in the current systematic review and meta-analysis satisfied specific inclusion and exclusion criteria. For study design, seven studies were randomized parallel design (Zhang et al., 2017, 2020; Chi et al., 2018; Wu et al., 2019; Chen et al., 2021; Guo et al., 2021; Li et al., 2021), and ten studies were randomized cross-over design (Thibaut et al., 2014, 2017, 2019a; Estraneo et al., 2017; Huang et al., 2017; Martens et al., 2018, 2019, 2020; Cavinato et al., 2019; Barra et al., 2022). All participants in the selected studies were diagnosed with different degrees of DOC. Nine studies distinguished between MCS and VS/UWS (Thibaut et al., 2014, 2017; Estraneo et al., 2017; Huang et al., 2017; Zhang et al., 2017, 2020; Cavinato et al., 2019; Martens et al., 2019, 2020), while the other eight studies did not (Chi et al., 2018; Martens et al., 2018; Thibaut et al., 2019a; Wu et al., 2019; Chen et al., 2021; Guo et al., 2021; Li et al., 2021; Barra et al., 2022). For intervention strategies, all experimental groups received anodal tDCS targeting F3, except one study with four anodal tDCS targeting F3, F4, CP5, and CP6 (Martens et al., 2020). For stimulation doses, the intervention period ranged from 1 day to 8 weeks. Five studies conducted a single session of tDCS totally (Thibaut et al., 2014, 2019a; Martens et al., 2019, 2020; Barra et al., 2022), and 12 studies conducted five or more sessions of tDCS totally (Estraneo et al., 2017; Huang et al., 2017; Thibaut et al., 2017; Zhang et al., 2017, 2020; Chi et al., 2018; Martens et al., 2018; Cavinato et al., 2019; Wu et al., 2019; Chen et al., 2021; Guo et al., 2021; Li et al., 2021). Outcomes were measured at baseline and at the end of the intervention. 14 studies used CRS-R to evaluate the DOC, four studies used GCS and one study used both scales to evaluate the DOC.

TABLE 1 Characteristics of included studies in this review.

References	Study design	Participants	Intervention	Brain target	Duration	Outcome
Barra et al. (2022)	Double blind, randomized, cross-over	12 DOC	Group 1: 6–10 Hz tPCS with a biphasic current of 2 mA peak to peak Group 2: maximum of 2 mA anodal tDCS Group 3: sham stimulation 5-day washout	Bi-mastoid LDLPFC (F3)	tDCS: 20 min for one session tPCS: 20 min for one session	EEG CRS-R Side effect
Cavinato et al. (2019)	Double blind, randomized, cross-over	24 DOC (12 MCS, 12 UWS)	EG: 2 mA anodal tDCS CG: sham tDCS 10-day washout	LDLPFC (F3)	20 min per session, 1 session per day, 5 days per week for 2 consecutive weeks	EEG CRS-R WNSSP
Chen et al. (2021)	Randomized, parallel group	52 DOC	EG: 2 mA anodal tDCS paired with 50 Hz and 200 μs MNES CG: conventional therapy only	LDLPFC (F3) Right median nerve	tDCS: 20 min per session, 1 session per day, 6 days per week for 4 consecutive weeks MNSE: 30 min per session, 2 sessions per day, 6 days per week for 4 consecutive weeks	GCS GOS DRS BAEP USEP
Chi et al. (2018)	Randomized, parallel group	38 DOC	EG: 2 mA anodal tDCS paired with conventional therapy CG: conventional therapy only	LDLPFC (F3)	20 min per session, 1 session per day, 6 days per week for 20 sessions	BAEP USEP EEG GCS PVS
Estraneo et al. (2017)	Double blind, randomized, cross-over	13 DOC (7 VS, 6 MCS)	EG: 2 mA anodal tDCS CG: sham tDCS 1-week washout	LDLPFC (F3)	20 min per session, 1 session per day for five sessions	CRS-R EEG
Guo et al. (2021)	Randomized, parallel group	113 DOC	EG: 1.4 mA anodal tDCS paired with perceptual level arousal intervention CG: perceptual level arousal intervention only	LDLPFC (F3)	20 min per session, 1 session per day, 6 days per week for 4 consecutive weeks	CRS-R GCS DFS EEG Latency of evoked action potential
Huang et al. (2017)	Double blind, randomized, cross-over	37 MCS	EG: 2 mA anodal tDCS CG: sham tDCS 5-day washout	LDLPFC (F3)	20 min per session, 1 session per day for five sessions	CRS-R
Li et al. (2021)	Randomized, parallel group	102 DOC	Group 1: 2 mA anodal tDCS paired with conventional therapy Group 2: 60 Hz and 250 μs MNES paired with conventional therapy Group 3: tDCS and MNES paired with conventional therapy	LDLPFC (F3) Right median nerve	20 min per session, 1 session per day, 6 days per week for 8 consecutive weeks	Somatosensory evoked potential GCS
Martens et al. (2018)	Double blind, randomized, cross-over	27 DOC	EG: 2 mA anodal tDCS paired with conventional therapy CG: sham tDCS paired with conventional therapy 8-week washout	LDLPFC (F3)	20 min per session, 1 session per day, 5 days per week for 4 consecutive weeks	Adverse events CRS-R

(Continued)

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TABLE 1 (Continued)

References	Study design	Participants	Intervention	Brain target	Duration	Outcome
Martens et al. (2019)	Double blind, randomized, cross-over	10 DOC (4 UWS, 6MCS)	EG: 2 mA anodal tDCS CG: sham tDCS 24-h washout	Primary motor cortex (C3-C4)	20 min for one session	CRS-R
Martens et al. (2020)	Double blind, randomized, cross-over	46 DOC (17 UWS, 23 MCS, 6 EMCS)	EG: tDCS with 4 anodes and 4 cathodes, 1 mA per anode CG: sham tDCS 2–6-day washout	Anodes placed on F3, F4, CP5 and CP6	20 min for one session	CRS-R EEG
Thibaut et al. (2014)	Double blind, randomized, cross-over	25 VS/UWS 30 MCS	EG: 2 mA anodal tDCS paired with conventional therapy CG: sham tDCS paired with conventional therapy 2-days washout	LDLPFC (F3)	20 min a single session	CRS-R
Thibaut et al. (2017)	Double blind, randomized, cross-over	16 MCS	EG: 2 mA anodal tDCS paired with conventional therapy CG: sham tDCS paired with conventional therapy 1-week washout	LDLPFC (F3)	20 min per session, 1 session per day for 5 consecutive days;	CRS-R
Thibaut et al. (2019a)	Double blind, randomized, cross-over	14 DOC	EG: 1 mA anodal tDCS paired with conventional therapy CG: sham tDCS paired with conventional therapy 2-days washout	LDLPFC (F3) RDLPFC (F4)	20 min a single session	MAS CRS-R EEG
Wu et al. (2019)	Randomized, parallel group	15 DOC	Group 1: 2 mA anodal tDCS anode placed over the left DLPFC paired with conventional therapy Group 2: 2 mA anodal tDCS anode placed over the right DLPFC paired with conventional therapy Group 3: sham tDCS paired with conventional therapy	LDLPFC (F3) RDLPFC (F4)	20 min per session, 1 session per day, 10 working days (from Monday to Friday in two consecutive weeks).	CRS-R GOS-E EEG
Zhang et al. (2017)	Double blind, randomized, parallel	26 DOC (11VS, 15MCS)	EG: 2 mA anodal tDCS paired with conventional therapy CG: sham tDCS paired with conventional therapy	LDLPFC (F3)	20 min per session, 2 session per day, 10 consecutive working days (from Monday to Friday).	CRS-R ERP
Zhang et al. (2020)	Double blind, randomized, parallel group	18 MCS	EG: 2 mA anodal tDCS paired with conventional therapy CG: sham tDCS paired with conventional therapy	LDLPFC (F3)	20 min per session, 2 sessions per day for 10 consecutive working days	CRS-R ERP

CG, control group; DIT, diffusion tensor imaging; BAEP, brain stem auditory evoked potential; DRS, disability rating scale; EEG, electroencephalogram; EG, experimental group; EMCS, emerged from minimally conscious state; EMG, electromyography; ERP, event-related potentials; FOUR, full outline of unresponsiveness scale; GCS, Glasgow coma scale; GOS, Glasgow outcome scale; L/RDLPFC, left/right dorsolateral prefrontal cortex; MBI, modified Barthel index; MCS, minimally conscious state; PVS, persistent vegetative state; tDCS, transcranial direct current stimulation; tPCS, transcranial pulsed-current stimulation; USEP, upper limb somatosensory evoked potential; UWS, unresponsive wakefulness syndrome; WNSSP, western neurosensory stimulation profile.

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TABLE 2 PEDro assessment quality results of included studies.

References	Eligibility*	Random allocation	Concealed allocation	Baseline comparability	Blind subjects	Blind therapists	Blind assessors	Adequate follow-up	Intention-to-treat analysis	Between-group comparisons	Point estimates and variability	Total score (0–10)	Quality
Barra et al. (2022)	Yes	1	0	1	1	1	1	1	1	1	1	9	Excellent
Cavinato et al. (2019)	Yes	1	0	1	1	1	1	1	1	1	1	9	Excellent
Chen et al. (2021)	Yes	1	0	1	0	0	0	1	1	1	1	6	Good
Chi et al. (2018)	Yes	1	0	1	0	0	0	1	1	1	1	6	Good
Estraneo et al. (2017)	Yes	1	0	1	1	1	1	1	1	1	0	8	Good
Guo et al. (2021)	Yes	1	0	1	0	0	0	1	1	1	1	6	Good
Huang et al. (2017)	Yes	1	0	1	1	1	1	1	1	1	1	9	Excellent
Li et al. (2021)	YES	1	0	1	0	0	0	1	1	1	1	6	Good
Martens et al. (2018)	Yes	1	0	1	1	1	1	0	1	1	1	8	Good
Martens et al. (2019)	Yes	1	0	1	1	1	0	1	1	1	1	8	Good
Martens et al. (2020)	Yes	1	0	1	1	1	1	1	1	1	1	9	Excellent
Thibaut et al. (2014)	Yes	1	0	1	1	1	1	1	1	1	1	9	Excellent
Thibaut et al. (2017)	Yes	1	0	1	1	1	1	0	1	1	1	8	Good
Thibaut et al. (2019a)	Yes	1	0	1	1	1	1	1	1	1	1	9	Excellent
Wu et al. (2019)	Yes	1	0	1	1	0	1	1	1	1	1	8	Good
Zhang et al. (2017)	Yes	1	0	1	1	0	1	1	1	1	1	8	Good
Zhang et al. (2020)	Yes	1	0	1	1	1	0	1	1	1	1	8	Good

*Eligibility criteria is not included in the scoring of PEDro scale.

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Quality

PEDro scores of the included studies ranged from 6 to 9, with a mean score of 7.88, indicating a high methodological quality of our included studies. The methodological quality of six studies was considered to be of "excellent" quality (Thibaut et al., 2014, 2019a; Huang et al., 2017; Cavinato et al., 2019; Martens et al., 2020; Barra et al., 2022), while that of 11 studies was considered to be of "good" quality (Estraneo et al., 2017; Thibaut et al., 2017; Zhang et al., 2017, 2020; Chi et al., 2018; Martens et al., 2018, 2019; Wu et al., 2019; Chen et al., 2021; Guo et al., 2021; Li et al., 2021). A detailed evaluation of the PEDro scores is presented in **Table 2**. All included studies reported adequately with regard to their random sequence generation and baseline comparability. Unfortunately, no studies satisfied the concealed allocation criteria. Four studies did not satisfy the subject blinding (Chi et al., 2018; Chen et al., 2021; Guo et al., 2021; Li et al., 2021), six studies did not satisfy the therapist blinding (Zhang et al., 2017; Chi et al., 2018; Wu et al., 2019; Chen et al., 2021; Guo et al., 2021; Li et al., 2021), and six studies did not state assessor blinding (Chi et al., 2018; Martens et al., 2019; Zhang et al., 2020; Chen et al., 2021; Guo et al., 2021; Li et al., 2021). Risk of bias assessment of the studies included in the present systematic review and meta-analysis is illustrated in **Figures 2**, **3**.

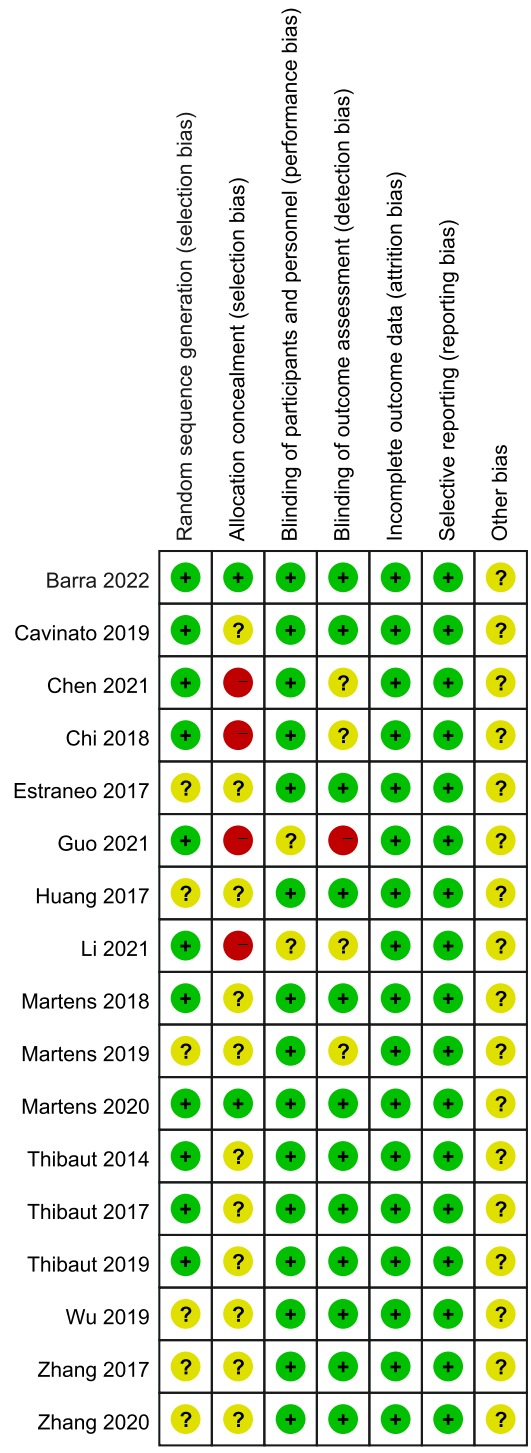

FIGURE 2
Risk of bias summary according to the Cochrane risk of bias tool: "-", "+", and "?" indicate high, low, and unclear risk of bias, respectively.

Effect of intervention

Glasgow coma scale

Four studies reported the GCS scores of patients with DOC. A fixed-effects model was used for the meta-analysis of GCS scores. The results of meta-analysis indicated that GCS increased significantly in favor of the intervention group (*MD* = 1.73; 95% CI, 1.28–2.18; $P < 0.01$; **Figure 4**). On the basis of subgroup analysis for stimulation protocol, two studies used anodal tDCS paired with median nerve electrical stimulation (MNES) and two studies used anodal tDCS, for intervention group. The results of meta-analysis showed that the GCS scores of both stimulation protocols increased significantly when compared to the control group (anodal tDCS paired with MNES: *MD* = 1.34; 95% CI = 0.65–2.03; $P < 0.01$; anodal tDCS: *MD* = 2.01; 95% CI = 1.42–2.61; $P < 0.01$; **Figure 5**). Furthermore, for the subgroup analysis of stimulation doses, on study conducted 20 sessions of stimulation totally (*MD* = 1.90; 95% CI = −0.60–4.40; $P = 0.14$), two studies conducted 24 sessions totally (*MD* = 1.97; 95% CI = 1.40–2.53; $P < 0.01$), and one study conducted 48 sessions of stimulation totally (*MD* = 1.24; 95% CI = 0.46–2.02; $P < 0.01$; **Figure 6**). No heterogeneity was detected among these studies in all above meta-analysis ($I^2 = 0\%$; $P > 0.10$). Publication bias was not assessed due to the limited number of included studies.

Coma recovery scale-revised

Twelve studies reported the CRS-R scores of patients with DOC. A fixed-effects model was used for the meta-analysis of CRS-R scores. The results of meta-analysis indicated that the CRS-R scores increased significantly as a result of tDCS when compared with the control group (*MD* = 1.28; 95% CI = 0.56–2.00; $P < 0.01$; **Figure 7**). Pooled studies were homogenous ($I^2 = 12\%$; $P = 0.33$). Moreover, on the basis of subgroup analysis for patient diagnoses, 11 studies reported the CRS-R scores of patients diagnosed with MCS, and five studies reported the CRS-R scores of patients diagnosed with UWS or VS (*MD* = −0.06; 95% CI = −0.56 to 0.43; $P = 0.80$; **Figure 8**). For patients with MCS, the results showed that the CRS-R scores increased significantly as a result of tDCS when compared with control group (*MD* = 1.65; 95% CI = 0.90–2.40; $P < 0.01$; **Figure 8**). The results of heterogeneity test showed that there was a significant heterogeneity across studies ($I^2 = 48\%$; $P = 0.04$). Therefore, the random-effects model was used for this subgroup data analyses (*MD* = 1.84; 95% CI = 0.74–2.93; $P < 0.01$). Furthermore, for the

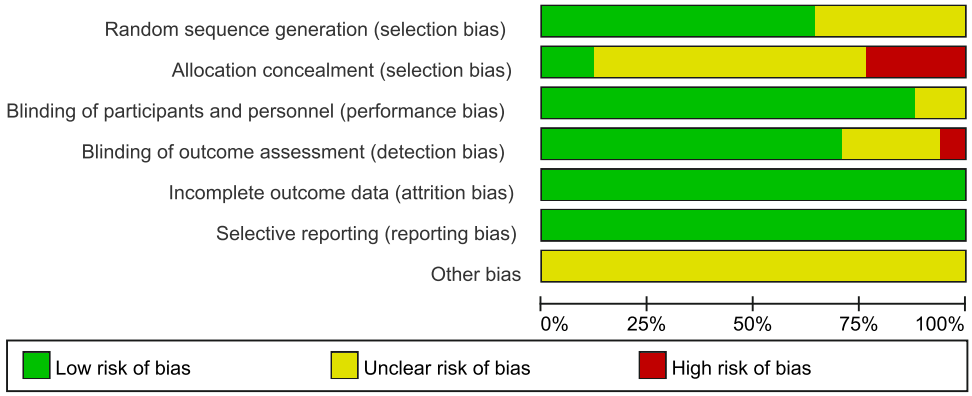

FIGURE 3
Risk of bias graph according to the Cochrane risk of bias tool.

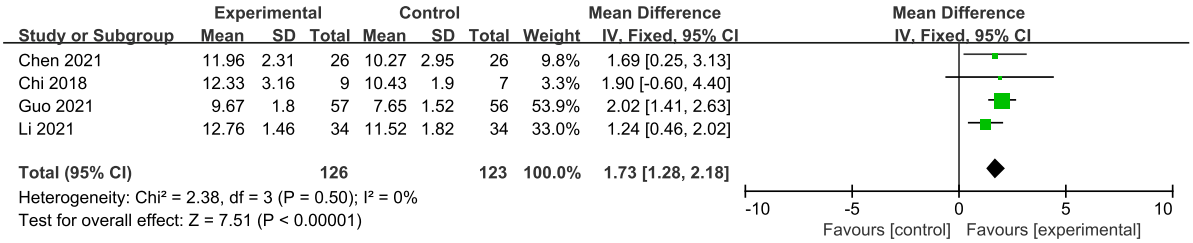

FIGURE 4
Meta-analysis of all studies on GCS scores in patients with DOC.

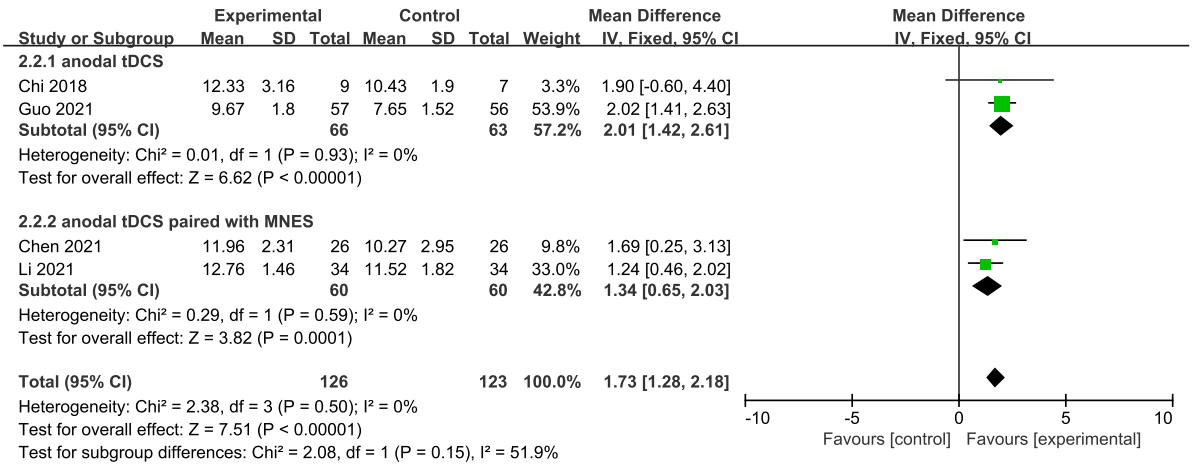

FIGURE 5
Subgroup analysis of stimulation protocol on GCS socres in patients with DOC.

subgroup analysis of the stimulation doses, four studies conducted single session of tDCS (*MD* = 0.79; 95% CI = −0.41 to 1.98; *P* = 0.20; Figure 9), three studies conducted five sessions of tDCS totally (*MD* = 0.77; 95% CI = −0.46 to 2.00; *P* = 0.22; Figure 9), one study conducted ten sessions of tDCS totally (*MD* = 1.80; 95% CI = −3.31 to 6.91; *P* = 0.49; Figure 9). No heterogeneity was detected among these studies in above three subgroup analyses (I^2 = 0%; *P* > 0.05). Moreover, four studies conducted more than 20 sessions of tDCS for patients with DOC (*MD* = 2.54; 95% CI = 1.15–3.92; *P* < 0.01). However, the result of heterogeneity test showed that there was a significant heterogeneity across studies in this subgroup analyses (*P* = 0.12; I^2 = 49%), so the random-effects model was used for this subgroup data analyses (*MD* = 2.71; 95% CI = 0.58–4.84; *P* = 0.01).

Discussion

Patients with DOC face a significant lack of treatment options, especially pharmacological ones, and therefore are unable to participate in active rehabilitation programs, which results in poor

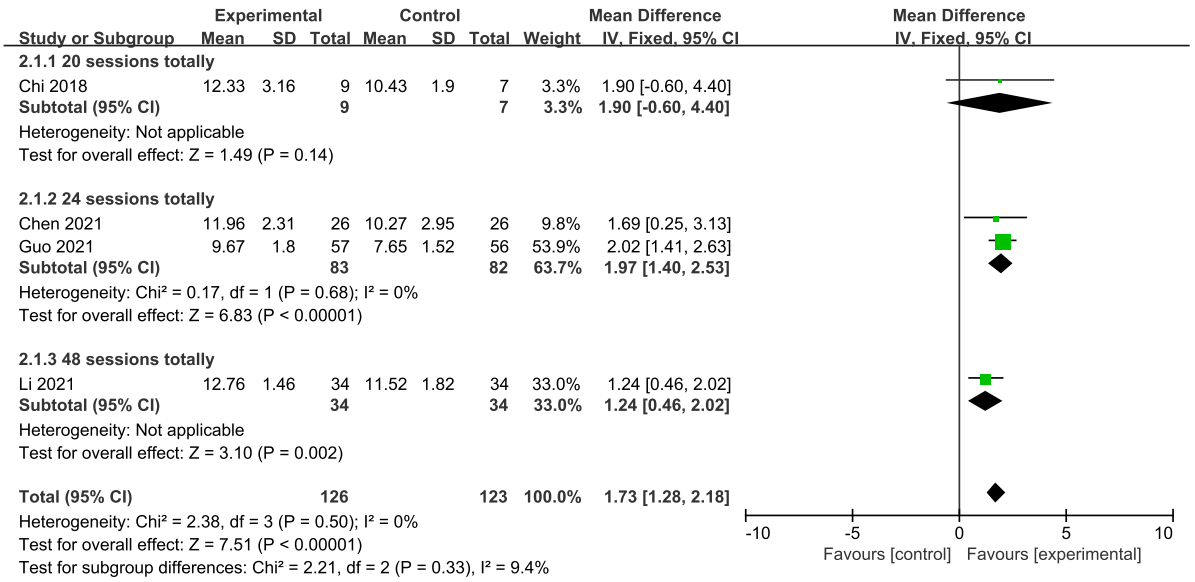

FIGURE 6
Subgroup analysis of stimulation doses on GCS scores in patients with DOC.

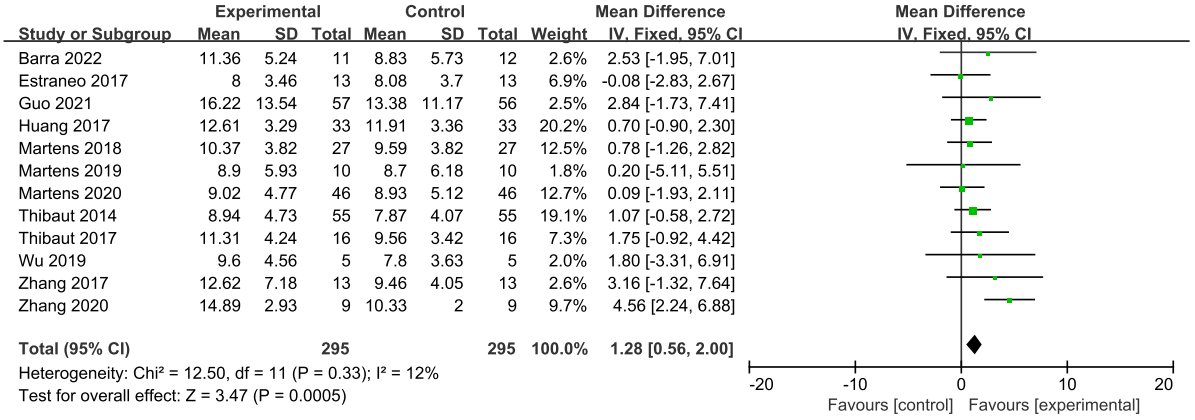

FIGURE 7
Meta-analysis of all studies on CRS-R scores in patients with DOC.

function outcomes. Neuromodulation techniques are alternative options to treat DOC. As a NIBS technique, tDCS can modulate cortical excitability by the direct current, but its therapeutic efficacy, especially behavioral effect, for DOC is not consistent. This systematic review, aimed to investigate the effect of tDCS for patients with DOC, included 17 eligible studies, and 15 studies with 580 DOC patients were included in the quantitative synthesis. The results of our meta-analysis showed that anodal tDCS can effectively enhance the recovery on GCS and CRS-R scores in patients with DOC.

Previous reviews summarized that patients with DOC could benefit from tDCS (Bourdillon et al., 2019; Thibaut et al., 2019b; Zaninotto et al., 2019), though the overall quality of evidence was not strong, which is consistent with our results. A recent systematic review and meta-analysis published by Feng et al. (2020) investigated the effect of NIBS for patients with DOC. The results of this study showed that anodal tDCS could significantly enhance the CRS-R scores in patients with DOC, which is also consistent with the results of our meta-analysis. Feng et al. (2020) stated that there is a lack of correlation between stimulation dose and effect sizes based on meta-regression, due to that behavioral changes may be too subtle to be detected by CRS-R in short-term tDCS. In our meta-analysis, however, we conducted subgroup analysis divided by total stimulation sessions and found that only more than 20 sessions of stimulation significant enhances the improvement of GCS scores and the CRS-R scores. Therefore, behavioral changes of patients with DOC require repetitive tDCS. Moreover, the different diagnosis of patients with DOC may be variously susceptible to tDCS intervention. The results of our meta-analysis showed that patients with DOC diagnosed with MCS were significantly benefit form tDCS on CRS-R scores improvement, while patients diagnosed with UWS or VS did not benefit, which is also in line with Feng's results (Feng et al., 2020). The possible reasons are higher level of under-excitability of the DLPFC and lower capacity for neural plasticity in patients with UWS or VS (Monti, 2012). Bai et al. (2017) found that the global cerebral excitability increased in both MCS and VS patients after tDCS intervention, but the increased excitability of patients with

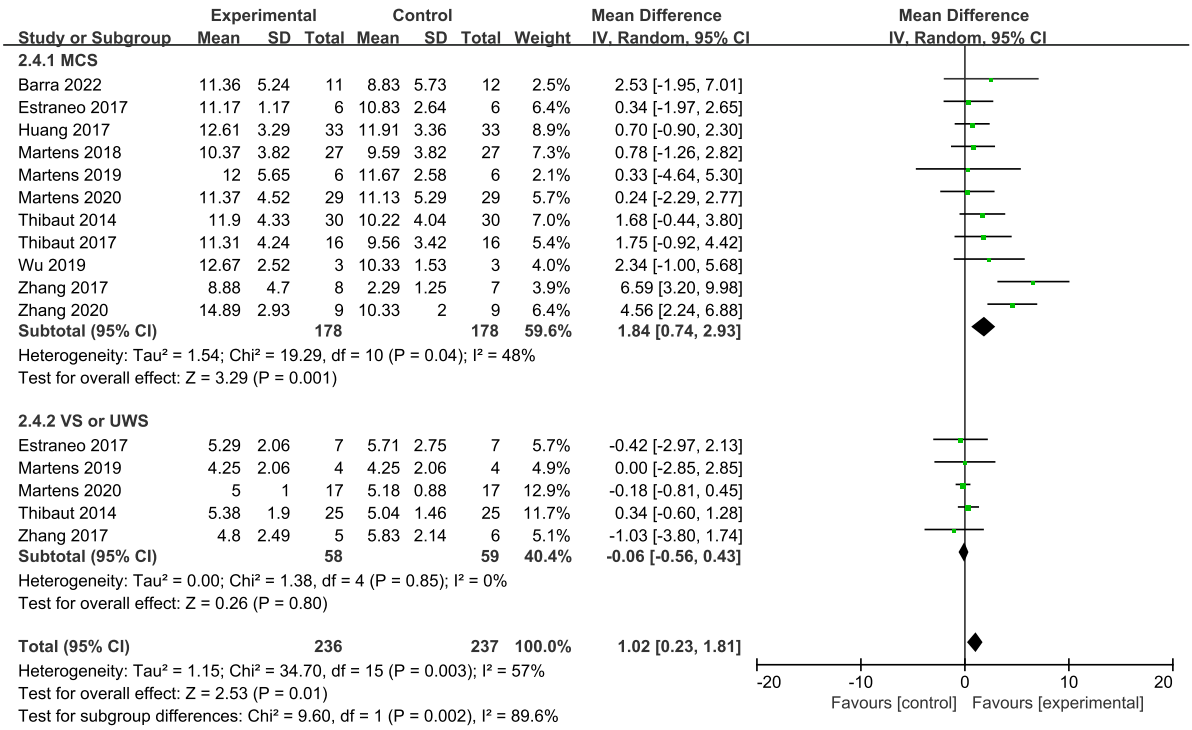

FIGURE 8
Subgroup analysis of patient diagnosis on GCS scores in patients with DOC.

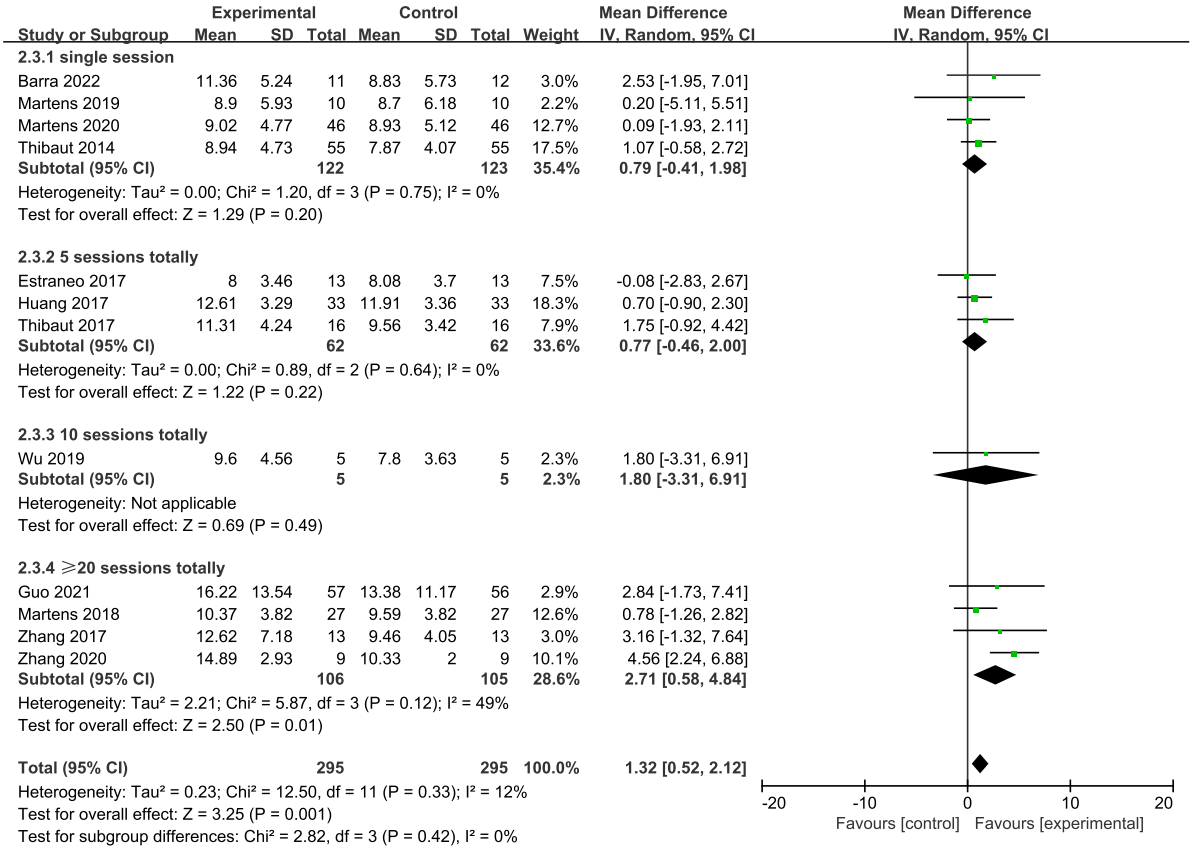

FIGURE 9
Subgroup analysis of stimulation doses on CRS-R scores in patients with DOC.

VS in temporal and spatial domains was less than that of patients with MCS, which can partly explain why the behavioral changes of patients with VS are not as significant as those of patients with MCS.

The stimulation parameters of tDCS for patients with DOC, including electrode positioning, current intensity, stimulation duration, are without uniform standard. The brain targets of tDCS depends on the characteristics of anode electrode for modulating cortical excitability, and brain functional regions related to consciousness. Anodal or cathodal current could facilitate the depolarization or hyperpolarization of cortical neurons, respectively (Nitsche et al., 2003). The consciousness of human consists of two critical components: wakefulness and awareness (Steriade, 1996). Previous researches demonstrated that the wakefulness pathways originated in the brainstem activate awareness network and its thalamocortical network, which is conceptualized as the ascending reticular activating system (Parvizi and Damasio, 2001). Awareness is mediated by the brain cortex, which is superficial and therefore frequently chose as stimulating targets in NIBS researches (Zeman, 2006). The DLPFC is a key brain region to manage the higher cognitive functions which are closely related to awareness, and it is also found that stimulating DLPFC could release the inhibition of the thalamus which can facilitate the wakefulness (Thibaut et al., 2012). That is the reason why most NIBS studies chose DLPFC as brain target to promote consciousness recovery. Another brain target of tDCS is motor cortex, which was proved to be effective in promoting motor recovery for patients with neurological disorders (Lefaucheur et al., 2020). Therefore, some researchers thought behavioral changes measured by CRS-R could be detected by stimulating the motor cortex (Martens et al., 2019). The current intensity of all included studies was 1–2 mA which was thought a safety intensity for tDCS, and therefore no adverse events were reported in all included studies. However, current density is the main indicator to measure the safety of electrical stimulation, but few studies mentioned this concept in their stimulation protocols. It is also regrettable that no trials explored the relationship between stimulus intensity and the therapeutic effect for patients with DOC. The stimulation doses of included studies are various. The cortex excitability can be modulated by single session of tDCS, but no or only transient behavioral effects can be detected (Thibaut et al., 2014). What's more, for the material of electrodes, one study used round rubber electrodes (12 cm^2) (Barra et al., 2022), one study used eight gelled electrodes (3.14 cm^2 Ag/AgCl) (Martens et al., 2020), and the rest studies all used saline-soaked surface sponge electrodes (35 cm^2) (Thibaut et al., 2014, 2017, 2019a; Estraneo et al., 2017; Huang et al., 2017; Zhang et al., 2017, 2020; Chi et al., 2018; Martens et al., 2018, 2019; Cavinato et al., 2019; Wu et al., 2019; Chen et al., 2021; Guo et al., 2021; Li et al., 2021). Although the material of electrodes is related to the definition of tDCS, due to the limited number of studies, it is difficult to evaluate the therapeutic effect of different materials, and no studies has investigated the relationship of tDCS definition and therapeutic effect for patients with DOC. Physiologically, the establishment of the long-lasting after-effects depends on membrane potential changes as well as modulations of N-methyl-D-aspartic acid receptor efficacy, which can induce long-term potentiation and long-term depression-like effect (Cirillo et al., 2017; Kronberg et al., 2017; Kuo et al., 2017). Therefore, repeated tDCS is necessary for the long-term effect of DOC, which is consistent with our findings.

Consequently, based on the evidence provided by our study, tDCS is effective in promote DOC recovery, in terms of GCS scores and CRS-R scores. However, further researches regarding the mechanistic and optimal stimulation parameters of tDCS for DOC should be conducted.

Study limitations

There are some limitations in our systematic review and meta-analysis. Firstly, studies published in languages other than English or Chinese were not included. Secondly, we only evaluated the behavior efficacy of tDCS for patients with DOC, and are unable to quantitatively analyses the neurophysiological changes due to the various methods of neuroimaging and neurophysiological assessments. Thirdly, because of the limited number of eligible studies and various of stimulation protocols, we are unable to recommend the optimal stimulation parameters. Fourthly, our results may be influenced by unavoidable heterogeneity as a result of that most studies did not strictly screen the patients for the onset time and diagnosis of DOC. Finally, outcomes of included studies were measured immediately after intervention without any long-term follow-up.

Conclusion

In conclusion, the results of our studies indicated that anodal tDCS can effectively enhance the improvement in GCS and CRS-R scores in patients with DOC. Anodal tDCS with sufficient stimulation doses appears to facilitate recovery of consciousness for patients with MCS, in terms of CRS-R scores.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

HM, KZ, and CH: concept and idea, project management, and consultation. HM, KZ, and CJ: search design. HM and KZ: writing and data analysis. JY, MZ, and TW: data extraction and quality assessment. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by grants from the 1·3·5 project for disciplines of excellence–Clinical Research Incubation Project, West China Hospital, Sichuan University (2021HXFH063) and the Key Project of the Science and Technology Department in Sichuan Province (2021YJ0184). These funding sources were not involved in the literature review, systematic review, meta-analysis, and writing of the report.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.1081278/full#supplementary-material

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frontiers | Frontiers in Neuroscience

TYPE Original Research
PUBLISHED 07 February 2023
DOI 10.3389/fnins.2023.1100464

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

RECEIVED 16 November 2022
ACCEPTED 16 January 2023
PUBLISHED 07 February 2023

CITATION
Wu X, Wang R, Wu Q, Liao C, Zhang J, Jiao H, Chen B, Wang S and Liu R (2023) The effects of combined high-frequency repetitive transcranial magnetic stimulation and cervical nerve root magnetic stimulation on upper extremity motor recovery following stroke. *Front. Neurosci.* 17:1100464. doi: 10.3389/fnins.2023.1100464

The effects of combined high-frequency repetitive transcranial magnetic stimulation and cervical nerve root magnetic stimulation on upper extremity motor recovery following stroke

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Introduction: Upper limb motor impairments after stroke cause patients partial or total loss of the capability of performing daily living, working, and social activities, which significantly affects the quality of life (QoL) of patients and brings a heavy burden to their families and society. As a non-invasive neuromodulation technique, transcranial magnetic stimulation (TMS) can act not only on the cerebral cortex, but also on peripheral nerves, nerve roots, and muscle tissues. Previous studies have shown that magnetic stimulation on the cerebral cortex and peripheral tissues has a positive effect on the recovery of upper limb motor function after stroke, however, few studies have reported the combination of the two.

Objective: This study was to investigate whether high frequency repetitive transcranial magnetic stimulation (HF-rTMS) combined with cervical nerve root magnetic stimulation more effectively ameliorates upper limb motor function in stroke patients. We hypothesized that the combination of the two can achieve a synergistic effect and further promotes functional recovery.

Methods: Sixty patients with stroke were randomly divided into four groups and received real or sham rTMS stimulation and cervical nerve root magnetic stimulation consecutively before other therapies, once daily over five fractions per week for a total of 15 times. We evaluated the upper limb motor function and activities of daily living of the patients at the time of pre-treatment, post-treatment, and 3-month follow up.

Results: All patients completed study procedures without any adverse effects. The upper limb motor function and activities of daily living improved in patients of each group were improved after treatment (post 1) and 3 months after treatment (post 2). Combination treatment was significantly better than single treatments alone or sham.

Conclusion: Both rTMS and cervical nerve root magnetic stimulation effectively promoted upper limb motor recovery in patients with stroke. The protocol combining the two is more beneficial for motor improvement and patients can easily tolerate it.

Clinical trial registration: https://www.chictr.org.cn/, identifier ChiCTR2100048558.

KEYWORDS

repetitive transcranial magnetic stimulation, stroke, upper limb motor function, rehabilitation, cervical nerve root

1. Introduction

Stroke is a common disease that seriously threatens human health. In recent years, the incidence of stroke has been increasing and gradually showing a younger trend. According to statistics, approximately 85% of stroke survivors have upper extremity dysfunction in the early stage (Naghavi et al., 2017). Although receiving conventional rehabilitation, such as physical therapy (PT), occupational therapy (OT), acupuncture and massage, a considerable number of patients still suffer from varying degrees of upper extremity motor dysfunction (Winstein et al., 2016). In the early stage after stroke, the main symptoms are limb paralysis and sensory disturbance, and in the later stage, limb spasm, pain, decreased coordination and flexibility may occur. The upper limb responsible for the complex, dexterous and coordinated motion, and usually it has slower recovery rate compared to the lower limb (Micera et al., 2020). Upper limb dysfunction caused by stroke make patients complete or partial loss the ability of daily living, which impose a great burden on the family and society, and it has always been the focus and difficulty of rehabilitation. Transcranial magnetic stimulation (TMS) is a non-invasive neuromodulation technique that induces currents in local areas of the cerebral cortex through brief, high-intensity magnetic fields to alter neuronal activity in the cerebral cortex and to promote neuroplasticity (Klomjai et al., 2015). Repetitive TMS (rTMS) refers to multiple TMS pulses given consecutively. Being painless, safe, effective, easy to operate, and simple, TMS has been widely applied in clinics nowadays. TMS can act not only on the cerebral cortex but also on peripheral nerves, nerve roots, and muscle tissue, which is also called peripheral magnetic stimulation (PMS) or functional magnetic stimulation (FMS) (Rossini et al., 2015). Li et al. (2016) found that low-frequency repetitive transcranial magnetic stimulation (LF-rTMS) and high-frequency repetitive transcranial magnetic stimulation (HF-rTMS) can significantly decrease the cortical latency of motor-evoked potentials (MEPs) and central motor conduction time, and improve upper-limb motor function in patients with cerebral infarction. Yang et al. (2021) reported that HF-rTMS (5 Hz) applied over the ipsilesional M1 for 10 days enhances hand functional recovery in subacute adult stroke patient. A randomized controlled trial (Jiang et al., 2022) showed that rPMS of upper limb extensor can improve arm function and muscle strength of stroke patients, and achieve grip strength and elbow flexion and extension. Although rTMS and PMS are both beneficial to improve upper limb motor function and daily living activities of patients with stroke, few studies have reported the efficacy of the combination of the two (Kumru et al., 2017; Gao et al., 2020). Neuromodulation of stroke should focus not only on the cortex but also on the nerve roots, and peripheral nerves. The stimulation of the cortex can top-down modulate neural plasticity, peripheral stimulation can bottom-up provide motor and sensory input, and the combination of the two forms a circuit to more effectively promote the recovery of neurological function after stroke. Based on this, the present study aimed to investigate the effects of the protocol that repeated HF-rTMS combined with cervical nerve root magnetic stimulation and provide a basis for clinical treatment.

2. Materials and methods

2.1. Participant

Sixty patients with upper limb hemiplegia after stroke who met the inclusion criteria and were hospitalized in the Rehabilitation Department of Tangdu Hospital from June 2021 to May 2022 were selected as the study subjects.

We included patients with (1) the first onset, meeting the diagnostic criteria for stroke (Zhang et al., 2020), (2) a unilateral lesion, confirmed by CT or MRI as a hemorrhagic or ischemic lesion in basal ganglia region; (3) the course of the disease is 2 weeks to 6 months, male or female, 30–80 years old; (4) conscious and the vital signs were stable; (5) has upper limb dysfunction and Brunnstrom stage I∼III of the affected upper limb; (6) willing to recover actively and able to cooperate with treatment instructions; (7) no mental abnormality; (8) the patient and/or the patient's family members are authorized to understand and sign the informed consent.

The exclusion criteria were as follows: (1) patients with severe cognitive impairment or mental illness who cannot cooperate with treatment and evaluation; (2) intracranial metal foreign body; (3) history of epilepsy or family history of epilepsy; (4) pacemakers, stents, and cochlear implants; (5) those who cannot tolerate treatment; (6) have serious liver or kidney disease; (7) unable to cooperate with follow-up.

The demographic and clinical characteristics of the patients are summarized in **Table 1**.

2.2. Experimental design

The study was a randomized, double-blind, sham-controlled trial, following the principle of randomization, control, and repeatability. As shown in **Figure 1**, the participants were randomly divided into four groups with 15 patients in each group. Group A, rTMS combined with cervical nerve root magnetic stimulation; Group B, only rTMS; Group C, only cervical nerve root stimulation; Group D, sham stimulation. Treatment was delivered once daily over five fractions per week for a total of 3 weeks. All patients underwent routine medical treatment and rehabilitation therapy during the implementation of treatment protocol. Motor function and daily living ability were evaluated at pre-treatment (baseline), post-treatment (post 1), and 3 months

TABLE 1 Comparison of basic data of patients.

Characteristics	Group A	Group B	Group C	Group D	F/x²/H	p
Cases (*n*)	15	15	15	15		
Age ($\bar{x} \pm s$, years)	54.60 ± 11.16	57.00 ± 10.76	54.87 ± 11.60	55.33 ± 10.30	0.15	0.93
Gender (Male/female, *n*)	10/5	12/3	11/4	15/0	6.49	0.10
BMI ($\bar{x} \pm s$, kg/m^2)	23.76 ± 4.59	24.55 ± 4.63	25.55 ± 2.75	25.40 ± 3.02	0.69	0.56
Type of stroke (hemorrhagic/ischemic, *n*)	7/8	10/5	9/6	7/8	1.82	0.61
Course of disease (day)	38 (28,50)	34 (20,46)	26 (21,59)	32 (21,72)	0.46	0.79
Side of lesion (left/right, *n*)	9/6	12/3	9/6	5/10	6.78	0.08

BMI, body mass index; FMA-UE, Fugl-Meyer Assessment Upper Extremity Scale; WMFT, Wolf Motor Function Test; MBI, Modified Barthel Index.

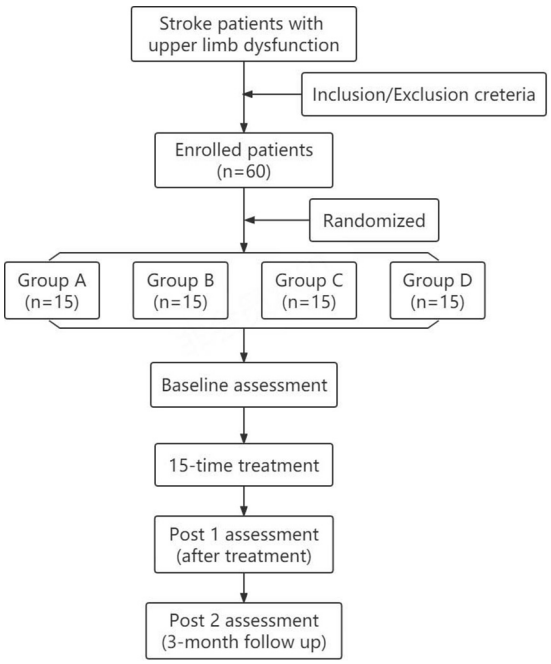

FIGURE 1
Flowchart showing recruitment, group allocation, allocation treatment, follow-up and analysis.

follow-up (post 2). The evaluation was performed by specially trained physicians who were unaware of the grouping and treatments. The person who performed the data analysis was also blinded. Figure 2 shows the schematic diagram of central and peripheral stimulation.

2.3. rTMS protocol

The rTMS protocols used in this study comply with the safety guidelines for rTMS applications (Lefaucheur et al., 2014; Rossi et al., 2021). In this study, all interventions were performed using a transcranial magnetic stimulator (CCY-I, YIRUIDE Medical Equipment Company, Wuhan, China). Brain stimulation was applied to the ipsilateral M1 using a 95 mm focal figure-of-eight coil and magnetic stimulation of the cervical nerve root were performed with a 125 mm circular coil. All participants receiving repetitive transcranial magnetic therapy used magnetic navigation software to exactly localize the optimal stimulation sites for rTMS.

Participants first underwent resting motor threshold (RMT) measurement to determine the intensity of treatment throughout the trial. RMT was defined as the minimum stimulation intensity needed to cause a MEP in the first dorsal interosseous muscle at least five of ten consecutive stimulations.

For group A, patients received rTMS at first and then went on to receive cervical nerve root magnetic stimulation. Repetitive TMS was delivered over the ipsilesional scalp site corresponding to the upper limb area of the primary motor cortex (M1), with the coil tangent to the hotspot. A total of 1,000 pulses of 10 Hz rTMS were applied, with the intensity at 80% of RMT (Kim et al., 2006) for 1 s followed by an inter-stimulus interval of 5 s. The target site of cervical nerve root magnetic stimulation is the cervical thoracic segment (C5-T1) of the hemiplegic side. Subjects received cervical nerve root stimulus protocol for 10 Hz consisting of 100 sequences of 10 pulses each, with 5 s between each sequence, for a total of 1,000 pulses, with a stimulation intensity to be the lowest stimulation intensity that can trigger muscle contraction (Matsumoto et al., 2013).

For group B, 10 Hz rTMS was performed over the ipsilateral M1 at 80% of RMT for a total of 1,000 pulses. Sham stimulation over the cervical nerve root was performed with the coil held at an angle of 90° to the hemiplegic side to reproduce the noise associated with the 10 Hz stimulus.

For group C, sham stimulation at ipsilateral M1 and real stimulation were performed over the cervical nerve root. The protocol of cervical nerve root stimulation is the same as that of group A. Sham stimulation was applied over ipsilateral M1 with the coil placed perpendicularly to the head.

For group D, the participants received sham stimulation at the same sites in the same order as group A. The coil was held at an angle of 90° to the hotspot so that patients could hear the sound but no actual stimulation effect.

2.4. Rehabilitation program

During the implementation of the protocol, all patients received conventional rehabilitation therapy composed of a 30-min of PT and a 30-min of OT, twice per day, five times per week for 3 weeks. PT includes a range of motion (ROM) training, anti-spasm training, muscle strength training, posture control training, balance and coordination training, etc. OT is mainly task-oriented functional training.

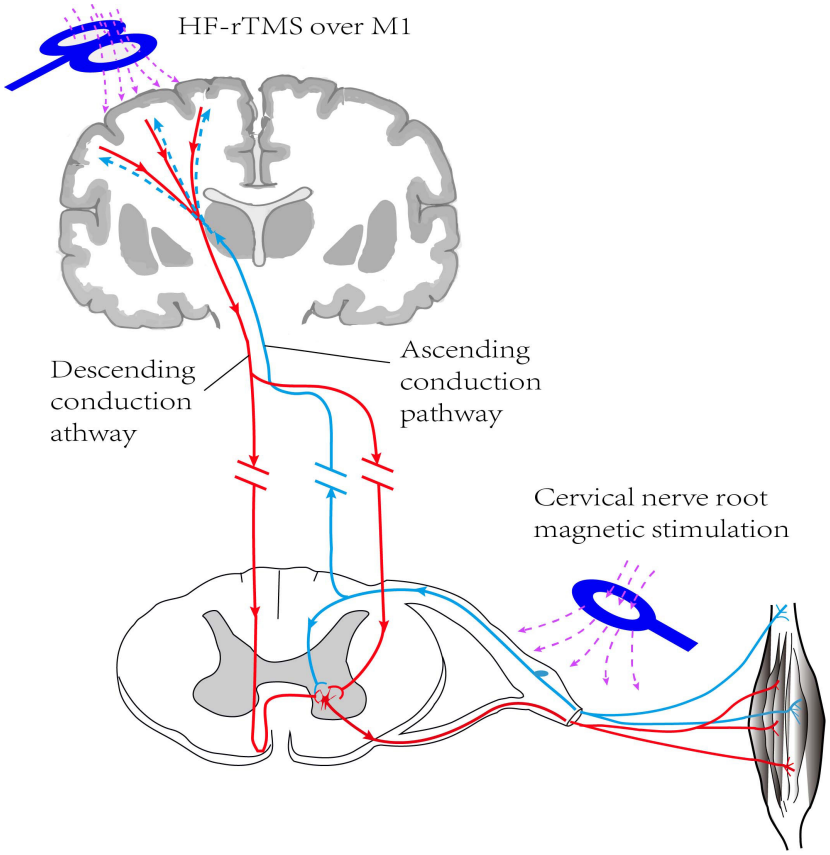

FIGURE 2
Schematic diagram of HF rTMS over ipsilateral M1 combined with magnetic stimulation of cervical nerve root on hemiplegic side (non-simultaneous). HF-TMS, high frequency repetitive transcranial magnetic stimulation; M1, primary motor cortex.

TABLE 2 Fugl-Meyer Assessment of the Upper Extremity (FMA-UE) score for all the groups at different time points ($\bar{x} \pm S$).

	Baseline	Post 1	Post 2	*F*	*p*
Group A ($n = 15$)	14.80 ± 4.38	22.80 ± 6.13*	38.80 ± 3.78*#	212.92	<0.001
Group B ($n = 15$)	15.47 ± 3.48	21.93 ± 4.92*	34.73 ± 5.48*#	137.96	<0.001
Group C ($n = 15$)	15.33 ± 3.99	20.73 ± 4.89*	33.53 ± 5.95*#	112.59	<0.001
Group D ($n = 15$)	14.93 ± 3.94	18.80 ± 4.69*	30.70 ± 5.65*#	71.09	<0.001
F	0.096	1.671	6.927		
P	0.962	0.184	<0.001		

FMA-UE, Fugl-Meyer Assessment Upper Extremity Scale.
*$p < 0.01$, compared with Baseline level.
#$p < 0.01$, compared with Post 1 level.

2.5. Observation indicators

The Fugl-Meyer Assessment of the Upper Extremity (FMA-UE) Scale (Hernandez et al., 2019) and the Wolf Motor Function Test (WMFT) (Bornheim et al., 2020) were used to measure the improvement of the upper limb and hand function in patients. FMA-UE included 10 major events, and 33 minor events, such as voluntary movement, reflex activity, grasp, and coordination, with a total score of 66 points. The higher the score, the better the Upper limb motor function. WMFT consists of 15 events, from 1 to 6 are simple joint movements, and from 7 to 15 are complex functional movements. The lowest score for each task is 0, and the highest score is 5, with a total score of 75. Higher scores represent a better function.

Modified Barthel Index (MBI) (Ohura et al., 2017) was used to measure the patient's activities of daily living (ADL), which include 10 items, such as eating, grooming, dressing, transfer, et. The total score was 100. The scoring standard is as follows: ≤20 were totally dependent in life; 21–40 were classified as severe dysfunction and obvious life dependence; 41–59 were classified as moderate dysfunction and need some help with activities of daily life; ≥60 were classified as mild dysfunction and able to care for themselves in basic ADL; 100 were completely independent.

We use Brunnstrom motor recovery stage of the upper extremity and hand (Ikbali Afsar et al., 2018) to evaluate the motor development of hemiplegic patients. Brunnstrom staging criteria are as follows: stage I, muscle retardation, no reflex; stage II, mild spastic, with

minimal voluntary movement; stage III, spasticity aggravates and can cause cooperative movement at will; stage IV, spasticity begins to decline, and can make a separated movement; stage V, establishment autonomous movement; stage VI, movement is close to normal.

2.6. Statistical analysis

Data conforming to the normal distribution are presented as mean ± standard deviation. Repeated measures analyses of variance (ANOVA) were used for multiple group comparisons. When the ANOVA revealed an interaction between time and intervention method, *post hoc* multiple pairwise comparisons were made with the Bonferroni correction. M(QR) was used for measurement data that did not conform to a normal distribution, the Kruskal–wallis rank sum test was used for inter-group comparison, and the Wilcoxon sign rank sum test was used for intra-group comparison; Classification data and grade data were expressed by constituent ratio or rate, and rank sum test was used for inter-group comparison. A p-value less than 0.05 was considered to be statistically significant.

3. Results

3.1. The outcome of upper extremity motor function and daily living ability

Tables 2–4 shows the FMA-UE, WMFT, and MBI scores for each group at different time points. Before treatment (baseline), there was no significant difference between FMA-UE, WMFT, and MBI scores in each group, which were comparable (all $p > 0.05$).

Repeated measures of two-way ANOVA showed that FMA-UE score and WMFT score were significantly influenced by time ($F = 513.69$, $p < 0.001$ for FMA-UE; $F = 875.86$, $p < 0.001$ for WMFT), treatment ($F = 3.13$, $p = 0.033$ for FMA-UE; $F = 5.09$, $p = 0.003$ for WMFT) and by the time × treatment interaction ($F = 5.19$, $p \leq 0.001$ for FMA-UE; $F = 6.87$, $p \leq 0.001$ for WMFT). For MBI, there was a significant main effect for time ($F = 1205.50$, $p < 0.001$) along with a significant time × treatment interaction effect ($F = 3.2$, $p = 0.006$), but the main effects of the treatment interaction were not significant ($F = 1.95$, $p = 0.132$).

Figure 3 shows the Comparisons of changes in FMA-UE score, WMFT score and MBI score at the time of post-treatment (post 1) and 3-month follow up (post 2). The score of FMA-UE, WMFT, and MBI exhibited significantly improvement at post 1 and post 2. The improvement of group A was significantly better than the other three groups.

At post 1, there were no statistically significant differences in FMA-UE score between each group ($F = 1.671$, $p = 0.184$), but the differences in WMFT score ($F = 6.82$, $p < 0.001$) and MBI score ($F = 3.08$, $p = 0.035$)were significant. At post 2, the differences in FMA score ($F = 6.927$, $p < 0.001$), WMFT score ($F = 8.41$, $p < 0.001$), and MBI score ($F = 3.771$, $p = 0.015$) among those four groups were significant.

Subsequently, we use *post hoc* analysis to compare the four groups at three time points. p-values for the comparisons is showed in Table 5.

At post 1, group A manifested considerable improvement in FMA-UE score ($p = 0.039$), WMFT score ($p < 0.001$) and MBI score ($p = 0.005$) compared with group D. Group B and group C exhibited significant improvement in terms of WMFT score ($p = 0.015$ for group B, $p = 0.023$ for group C), but no significant changes were detected in the FMA-UE score ($p = 0.104$ for group B, $p = 0.312$ for group C) and MBI score ($p = 0.243$ for group B, $p = 0.448$ for group C)

TABLE 3 Wolf Motor Function Test (WMFT) score for all the groups at different time points ($\bar{x} \pm S$).

	Baseline	Post 1	Post 2	*F*	*p*
Group A ($n = 15$)	12.53 ± 3.68	28.67 ± 5.84*	40.07 ± 6.41*#	315.23	<0.001
Group B ($n = 15$)	12.27 ± 3.90	25.00 ± 4.91*	35.93 ± 4.06*#	218.80	<0.001
Group C ($n = 15$)	11.60 ± 4.58	24.67 ± 4.82*	33.27 ± 4.09*#	199.92	<0.001
Group D ($n = 15$)	12.33 ± 4.67	20.40 ± 4.37*	31.87 ± 4.31*#	131.82	<0.001
F	0.138	6.82	8.41		
p	0.937	<0.001	<0.001		

WMFT, Wolf Motor Function Test; *$p < 0.01$, compared with the Baseline level.
#$p < 0.01$, compared with Post 1 level.

TABLE 4 Modified Barthel Index (MBI) score for all the groups at different time points ($\bar{x} \pm S$).

	Baseline	Post 1	Post 2	*F*	*p*
Group A ($n = 15$)	36.13 ± 9.47	58.40 ± 7.31*	87.07 ± 6.69*#	366.45	<0.001
Group B ($n = 15$)	35.33 ± 7.16	53.60 ± 7.73*	83.67 ± 7.42*#	322.61	<0.001
Group C ($n = 15$)	36.67 ± 7.11	52.27 ± 7.21*	81.67 ± 6.80*#	278.26	<0.001
Group D ($n = 15$)	35.87 ± 6.66	50.33 ± 8.09*	78.47 ± 7.76*#	249.26	<0.001
F	0.078	3.08	3.778		
p	0.971	0.035	0.015		

MBI, Modified Barthel Index.
*$p < 0.01$, compared with the Baseline level.
#$p < 0.01$, compared with Post 1 level.

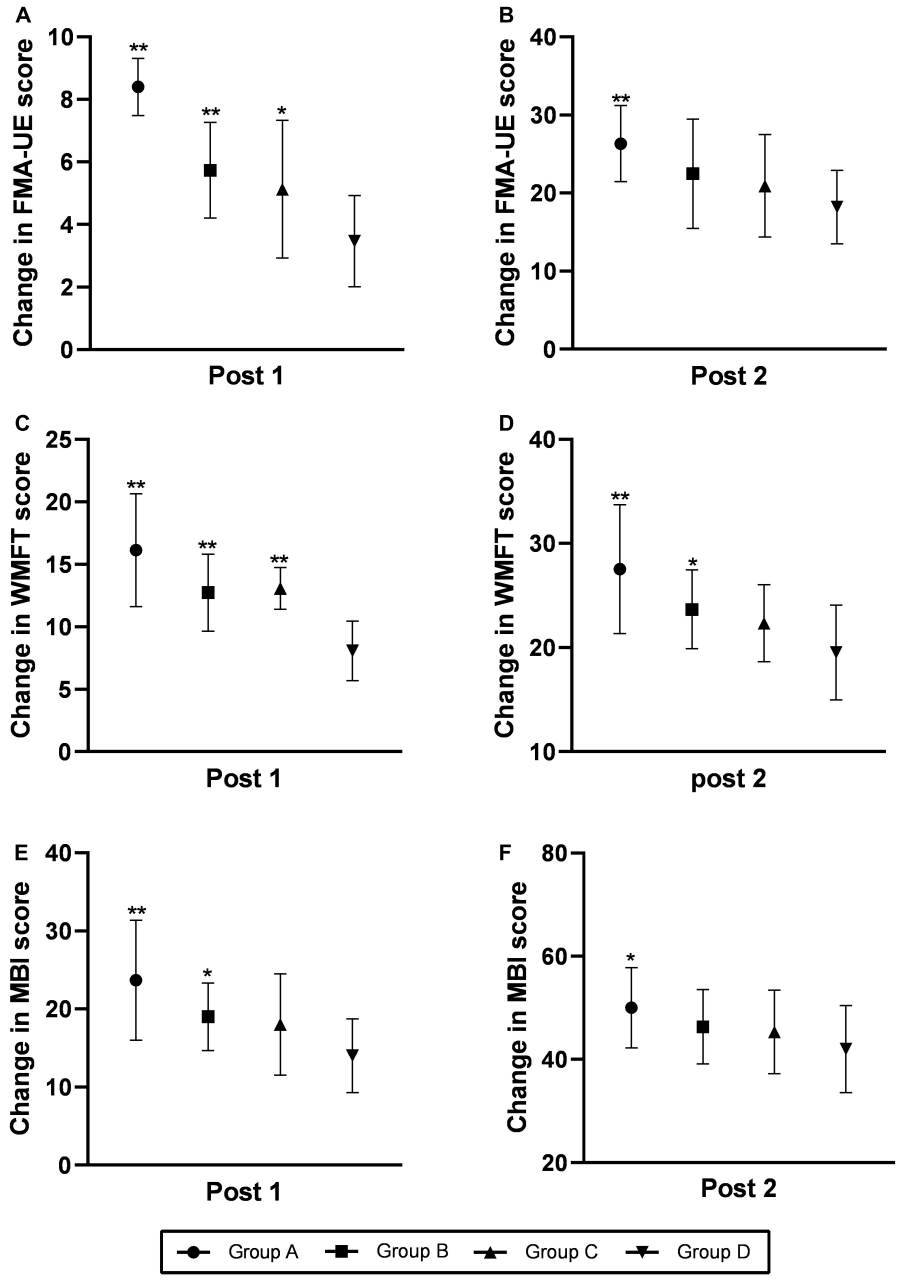

FIGURE 3
Comparison of changes in the FMA-UE **(A,B)**, WMFT **(C,D)**, and MBI **(E,F)** scores at the time of post-treatment (post 1) and the 3-month follow-up (post 2). Changes were calculated as follows: post-intervention value minus baseline value. The data were shown as the mean ± standard deviation values. Comparisons of value changes among the four groups were performed using ANOVA. *$p < 0.05$ and **$p < 0.01$ relative to group D. FMA-UE, Fugl-Meyer Assessment of the Upper Extremity; WMFT, Wolf Motor Function Test; MBI, Modified Barthel Index.

relative to the group D. There was no significant difference in FMA-UE score ($p = 0.529$), WMFT score ($p = 0.856$) and MBI ($p = 0.632$) scores between Group B and Group C.

At post 2, the group A exhibited significant improvements in terms of FMA scores ($p < 0.001$), WMFT score ($p < 0.001$) and MBI score ($p = 0.022$) relative to the group D. Compared with group D, group B manifested considerable improvements in terms of both the FMA-UE score ($p = 0.019$) and the WMFT score ($p = 0.019$), but no change were detected in MBI score ($p = 0.052$). There was no significant difference in FMA-UE score ($p = 0.537$), WMFT score ($p = 0.242$) and MBI score ($p = 0.449$) between group B and group C and between group C and group D.

3.2. Brunnstrom stage

Tables 6, 7 showed the Brunnstrom grading of upper limbs and hands of the four groups of patients at each evaluation time point. Before treatment, there was no significant difference in the motor function grading of the Brunnstrom stage among the four groups ($H = 0.648$, $p = 0.885$ for upper limb; $H = 2.65$, $p = 0.499$ for hand). At post 1 and post 2, the Brunnstrom motor function grades of upper limbs and hands in each group were improved, and the improvement of Group A was better than those of other groups, there is no statistical significant difference among all groups ($p > 0.05$).

TABLE 5 *P*-values for the comparisons of FMA, WMFT, and MBI score in the four groups at different evaluation time points.

	FMA-UE score			WMFT score			MBI score		
	Baseline	Post 1	Post 2	Baseline	Post 1	Post 2	Baseline	Post 1	Post 2
Group A vs. Group B	0.647	0.649	0.040	0.864	0.050	0.018	0.776	0.089	0.200
Group A vs. Group C	0.714	0.280	0.008	0.548	0.033	<0.001	0.850	0.031	0.044
Group A vs. Group D	0.927	0.039	<0.001	0.897	<0.001	<0.001	0.925	0.005	0.022
Group B vs. Group C	0.927	0.529	0.537	0.668	0.856	0.242	0.636	0.632	0.449
Group B vs. Group D	0.714	0.104	0.019	0.966	0.015	0.019	0.850	0.243	0.052
Group C vs. Group D	0.783	0.312	0.078	0.637	0.023	0.227	0.776	0.448	0.228

FMA-UE. Fugl-Meyer Assessment Upper Extremity Scale; WMFT, Wolf Motor Function Test; MBI, Modified Barthel Index.

TABLE 6 Brunnstrom Stage of each group–upper limb.

	Baseline [*n* (%)]			Post 1 [*n* (%)]		Post 2 [*n* (%)]		
	I	II	III	II	III	III	IV	V
Group A (*n* = 15)	5 (33.3)	9 (60.0)	1 (6.7)	2 (13.3)	13 (86.7)	1 (6.7)	9 (60.0)	5 (33.3)
Group B (*n* = 15)	5 (33.3)	10 (66.7)	0 (0)	8 (53.3)	7 (46.7)	3 (20.0)	9 (60.0)	3 (20.0)
Group C (*n* = 15)	4 (26.7)	11 (73.3)	0 (0)	4 (26.7)	11 (73.3)	2 (13.3)	8 (53.3)	5 (33.3)
Group D (*n* = 15)	6 (40.0)	9 (60.0)	0 (0)	8 (53.3)	7 (46.7)	6 (40.0)	8 (53.3)	1 (6.7)
H	0.648			7.622		7.39		
P	0.885			0.055		0.06		

TABLE 7 Brunnstrom Stage of each group–hand.

	Baseline [*n* (%)]		Post 1 [*n* (%)]			Post 2 [*n* (%)]			
	I	II	I	II	III	II	III	IV	V
Group A (*n* = 15)	8 (53.3)	7 (46.7)	1 (6.7)	9 (60.0)	5 (33.3)	1 (6.7)	8 (53.3)	5 (33.3)	1 (6.7)
Group B (*n* = 15)	11 (73.3)	4 (26.7)	3 (20.0)	11 (73.3)	1 (6.7)	2 (13.3)	8 (53.3)	5 (33.3)	0 (0)
Group C (*n* = 15)	10 (66.7)	5 (33.3)	2 (13.3)	9 (60.0)	4 (26.7)	1 (6.7)	7 (46.7)	6 (40.0)	1 (6.7)
Group D (*n* = 15)	12 (80.0)	3 (20.0)	5 (33.3)	9 (60.0)	1 (6.7)	4 (26.7)	9 (60.0)	2 (13.3)	0 (0)
H	2.65		7.15			6.01			
P	0.449		0.067			0.11			

3.3. Safety of the intervention

The four treatments protocol were well tolerated and all patients completed the treatment and follow-up. During the intervention, there was no significant change in vital signs. No patient experienced any adverse reactions, such as epilepsy, pain, or deterioration of the condition.

4. Discussion

The recovery of upper limb motor function has been a difficult issue for rehabilitation after stroke. Even though many stroke patients have undergone systematic rehabilitation treatment, the effect of upper limb function recovery is still poor. The application of rTMS improves the effect of upper limb functional rehabilitation after stroke (Hsu et al., 2012).

In this study, the FMA-UE score, WMFT score and MBI of the four groups were improved after treatment (post 1) and 3 months after treatment (post 2), which means the upper limb activity and the ADL of the patients in each group were improved. The increasement of group A, B, and C was higher than that of group D. At post 1, compared with group D, the increase rates of group A, B, and C were statistically significant ($p < 0.05$). At post 2, only the increasement of group A was significantly different from that of group D ($p < 0.05$). The results of this study showed that both HF-rTMS and cervical nerve root magnetic stimulation can effectively promote the recovery of upper limb function and improve ADL performance in stroke patients. The combination of the two was significantly more effective than either treatment alone or sham, and the therapeutic advantages can last for 3 months.

It is well known that cortical spinal tract (CST) plays a critical role in motor recovery after stroke, specifically fine motor control of upper limb and finger (Sterr et al., 2014; Rondina et al., 2017). Stroke disrupts neural circuit connectivity, which results in long-term neurological disability. RTMS can mediate the recovery of motor function after stroke by inducing cortical reorganization and regulating the excitability of corticospinal tract through neural plasticity (Auriat et al., 2015). RTMS modulates neuroplasticity not only locally below the magnetic coil but also in remote cortical and subcortical regions through functional connectivity of motor network (Cheng et al., 2014).

In this study, we used high-frequency (10 H z) rTMS because it has been reported that HF-rTMS have a more effective impact when compared to LF-rTMS (Sasaki et al., 2013; Caglayan et al., 2019). Kim et al. (2006) study demonstrated HF-rTMS of the affected motor cortex can produce increase in corticospinal excitability, facilitate practice-dependent plasticity and improve the motor learning performance in chronic stroke victims. Hong et al. (2020) shown that 10 Hz rTMS on the ipsilateral M1 can reduce infarct volume and promote functional recovery by inhibiting neurotoxic effects and reversing neuroprotective effects on astrocytes after ischemia/reperfusion injury in rats. Another study (Hong et al., 2022) showed that HF-rTMS could inhibit M1 polarization of microglia after cerebral ischemia-reperfusion injury and alleviate ischemic stroke injury via regulating leT-7B-5P/HMGA2/NF-κB signaling pathway. A systematic review (Vabalaite et al., 2021) showed that HF-rTMS increased impaired upper limb motor function better than sham stimulation in stroke patients. Moslemi Haghighi et al. (2021) also showed that HF-rTMS could effectively improve upper limb function for hemiplegia patients in the subacute stage of stroke. In addition, rTMS can protect nerves, supply nerve nutrition, promote nerve repair and regeneration, and reduce infarct area (van Lieshout et al., 2020).

Peripheral magnetic stimulation (Struppler et al., 2007) induces proprioceptive input of the central nervous system by directly stimulating peripheral sensorimotor afferent nerves or indirectly stimulating mechanical receptors by stimulating the muscle to produce rhythmic contraction and vibration. Gallasch et al. (2015) found that rPMS can induce the activation of sensorimotor networks and the changes of corticomotor excitability. Litvak's experiment (Litvak et al., 2007) proved that magnetic stimulation of muscles or peripheral nerves can help the upper limb motor function recovery of stroke patients by promoting the plasticity change of M1 region and providing sensory input. The study of Chen et al. (2020) proved that PMS can improve the Fugl-Meyer score and Barthel index of upper limbs in stroke patients, and the efficacy is superior to LF-RTMS. Struppler et al. (2007) study on eight patients with mild paralysis after focal cerebral ischemia found that after repeated PMS, the recovery of hand function and the decrease of spasticity were related to the significant increase of neural activation within the superior posterior parietal lobe and the premotor cortex areas. Ke et al. (2022) randomized controlled trial showed that high-frequency (20 Hz) repetitive peripheral magnetic can significantly improve motor function and proximal muscle strength of the upper and lower limbs of patients with intracerebral hemorrhage. An animal experiment (Zheng et al., 2022) found that the nerve root magnetic stimulation enhanced nerve conduction in the injured spinal cord and promoted the recovery of synaptic ultrastructure in the sensorimotor cortex. These results indicate that PMS can regulate cerebral cortex function, improve brain plasticity, and have positive significance in improving muscle tone, limb function, muscle strength, and daily living activities after stroke. In addition, compared with rTMS, PMS was generally safe and tolerable for patients with stroke (Beaulieu and Schneider, 2015).

The present scheme is similar to paired associative stimulus. Paired associative stimulation (PAS) is a stimulation method pairing TMS with peripheral electrical stimulus (Stefan et al., 2002), which first reported by Stefan. PAS has been shown to elicit neural plasticity, enhance nerve conduction and promote function recovery after stroke, which has been widely used in stroke patients as an recovery treatment. In the present study, we modified the PAS protocol, and proposed the paired associative magnetic stimulation (PAMS) (Sun et al., 2022) protocol, which combines PMS and HF-rTMS of the ipsilesional sensorimotor cortex. Compared with peripheral electrical stimulation (PES), PMS has the following advantages. PMS generates a greater proprioceptive inflow through recruitment of muscle and joint afferents (Beaulieu and Schneider, 2015), provide a greater range of depth and less pain, and without removing clothes (Rossini et al., 2015), so it is easier to be implemented in clinical settings. Another main difference is that PAS activates the sensory and motor system simultaneously and induces spike timing dependent plasticity (Brzosko et al., 2019), however, our protocol applying the cortical and peripheral stimulation consecutively.

The protocol in present study is a novel magnetic stimulation protocol, targeting the motor cortex and the spinal nerve roots. On one hand, rTMS activates the cerebral cortex of the ipsilateral side, promotes the downward projection of the corticospinal tract, and may improves the plasticity of the nerve. On the other hand, peripheral nerve stimulation can enhance peripheral sensory and motor input and feedback, and promote the ascending pathway from nerve root to the cortex. Central intervention and peripheral intervention are organically combined to form closed-loop information feedback (Liu et al., 2022), to enhance the plasticity of the brain and the remodeling of neural pathways, promote the recovery of upper limb motor function and improve rehabilitation efficiency. An animal experiment (Gao et al., 2020) showed that PAMS activates the ipsilateral sensorimotor and sensory cortex, and that it upregulates the expression of brain plasticity-related proteins to ultimately change behavior. Kumru et al. (2017) about eleven healthy subjects found that PAMS can increase corticospinal excitability and reduce intracortical inhibition, but the effects were not present when the PMS and LF-rTMS were applied separately. These might be relevant for motor rehabilitation. In addition, the cervical nerve root magnetic stimulation can stimulate the spinothalamic tract up and the peripheral nerve down, to achieve bidirectional regulation of nerve effect. In conclusion, this treatment may be a valuable treatment for stroke patients. However, due to the multiple parameters of TMS and PMS, the combination of different parameters can produce different therapeutic effects, so how formulating the optimal treatment prescription to achieve the best therapeutic effect for patients is worthy of further study. At the same time, the sample size of this study was small and the observation time was short, so the mechanism of action of this scheme could not be further studied through other auxiliary examinations. In the future, we will further expand the sample size and further study the effect of this treatment plan on neurological function recovery combined with functional magnetic resonance and electrophysiological examination, to provide a reference for clinical application.

5. Conclusion

Both 10 Hz rTMS to the ipsilateral M1 and cervical nerve root magnetic stimulation to the hemiplegic side can effectively promote the recovery of upper limb function in patients with stroke. The protocol combining the two is more beneficial for motor improvement and patients can easily tolerate it. This protocol is worthy of clinical application.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Tangdu Hospital of Air Force Military Medical University. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Ethics Committee of Tangdu Hospital of Air Force Military Medical University.

Author contributions

RL and XW contributed to the conception and design of the study. QW, CL, JZ, and HJ carried out the experiments. BC and SW performed the statistical analysis. XW and RW wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 81771469) and Key basic research projects of basic strengthening plan (No. FK2022030029). The funding body had no role in the design of the study, data collection, analysis, and interpretation and in writing the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Neuroscience

TYPE Original Research
PUBLISHED 09 March 2023
DOI 10.3389/fnins.2023.1107116

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

RECEIVED 29 November 2022
ACCEPTED 24 February 2023
PUBLISHED 09 March 2023

CITATION
Guo Z, Qiu R, Qiu H, Lu H and Zhu X (2023) Long-term effects of repeated multitarget high-definition transcranial direct current stimulation combined with cognitive training on response inhibition gains. *Front. Neurosci.* 17:1107116. doi: 10.3389/fnins.2023.1107116

Long-term effects of repeated multitarget high-definition transcranial direct current stimulation combined with cognitive training on response inhibition gains

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Background: Few studies have investigated the effects of repeated sessions of transcranial direct current stimulation (tDCS) combined with concurrent cognitive training on improving response inhibition, and the findings have been heterogeneous in the limited research. This study investigated the long-lasting and transfer effects of 10 consecutive sessions of multitarget anodal HD-tDCS combined with concurrent cognitive training on improving response inhibition compared with multitarget stimulation or training alone.

Methods: Ninety-four healthy university students aged 18–25 were randomly assigned to undergo different interventions, including real stimulation combined with stop-signal task (SST) training, real stimulation, sham stimulation combined with SST training, and sham stimulation. Each intervention lasted 20 min daily for 10 consecutive days, and the stimulation protocol targeted right inferior frontal gyrus (rIFG) and pre-supplementary motor area (pre-SMA) simultaneously with a total current intensity of 2.5 mA. Performance on SST and possible transfer effects to Stroop task, attention network test, and N-back task were measured before and 1 day and 1 month after completing the intervention course.

Results: The main findings showed that the combined protocol and the stimulation alone significantly reduced stop-signal reaction time (SSRT) in the post-intervention and follow-up tests compared to the pre-intervention test. However, training alone only decreased SSRT in the post-test. The sham control exhibited no changes. Subgroup analysis revealed that the combined protocol and the stimulation alone induced a decrease in the SSRT of the low-performance subgroup at the post-test and follow-up test compared with the pre-test. However, only the combined protocol, but not the stimulation alone, improved the SSRT of the high-performance subgroup. The transfer effects were absent.

Conclusion: This study provides supportive evidence for the synergistic effect of the combined protocol, indicating its superiority over the single intervention

method. In addition, the long-term after-effects can persist for up to at least 1 month. Our findings also provide insights into the clinical application and strategy for treating response inhibition deficits.

KEYWORDS

transcranial direct current stimulation, stop-signal task, cognitive training, response inhibition, neuroplasticity, right inferior frontal gyrus, pre-supplementary motor area

1. Introduction

Response inhibition comprises the ability to withhold irrelevant or context-inappropriate responses following changes in the environment so that one can make flexible and goal-directed behavioral responses, which is one of the core components of executive function (Verbruggen and Logan, 2009; Diamond, 2013). It is an essential factor for self-adaptation and self-regulation of the dynamics of actions (Aron, 2007; Sandrini et al., 2020). Response inhibition is closely associated with many other cognitive abilities, such as impulse control, working memory (WM), and cognitive inhibition (Dalley and Robbins, 2017; Zhao et al., 2018; Xu et al., 2020; Weidler et al., 2022). It is commonly impaired in many psychiatric disorders, such as substance use disorder, psychopathy, attention deficit hyperactivity disorder (ADHD), and schizophrenia (Hughes et al., 2012; van Rooij et al., 2015; Kohl et al., 2019; Gillespie et al., 2022).

Due to its great importance, the neural substrates and the approach to enhancing response inhibition have recently received increasing attention. Accumulating evidence has identified a frontal-basal ganglia network engaged in response inhibition, including the right inferior frontal gyrus (rIFG), the pre-supplementary motor area (pre-SMA), and the basal ganglia (Aron and Poldrack, 2006; Duann et al., 2009; Aron et al., 2014; Hannah and Aron, 2021). Transcranial direct current stimulation (tDCS) is a promising and widely used neuromodulatory technique for regulating cortical activity and neuroplasticity and enhancing cognitive function (Nitsche and Paulus, 2001; Pisoni et al., 2018). It is a suitable tool to infer the causality for the links between brain function and corresponding behavioral changes (Filmer et al., 2014; Gbadeyan et al., 2016; Yavari et al., 2018). tDCS is safe, non-invasive, tolerable, and easy-to-operate (Bikson et al., 2016) and has been found to effectively enhance response inhibition *via* anodal stimulation targeting rIFG or pre-SMA (Hsu et al., 2011; Jacobson et al., 2011; Ditye et al., 2012; Kwon and Kwon, 2013b,a; Stramaccia et al., 2015; Sandrini et al., 2020; Fujiyama et al., 2021).

New forms of tDCS emerge as research into the effect of tDCS on enhancing response inhibition progresses. High-definition tDCS (HD-tDCS) is an optimized form of conventional pad-tDCS with high spatial precision and produces more prominent behavioral and neurophysiological effects (Kuo et al., 2013; Sehatpour et al., 2021). Multitarget stimulation refers to simultaneous stimulation with the same polarity on multiple functionally related brain cortices, which can modulate the cortical activity more efficiently and enhance tDCS effects more prominently than conventional single-target stimulation (Hill et al., 2018; Gregoret et al., 2021; Guo et al., 2022a). Given behavioral and neuroimaging evidence, a previous study has shown that multitarget high-definition stimulation of rIFG and pre-SMA is more effective in improving response inhibition compared with the commonly used single-target stimulation on rIFG or pre-SMA alone (Guo et al., 2022a).

Importantly, repeated sessions of tDCS can increase efficacy through cumulative effects, yield long-lasting after-effects and stable changes in brain function, and are tolerated and safe (Nitsche et al., 2008; Cohen Kadosh et al., 2010; Paneri et al., 2016; Turski et al., 2017; Di Rosa et al., 2019; Song et al., 2019). Since cognitive training and tDCS both modulate neuroplasticity, combining tDCS and related cognitive training that involves the same or similar neural network may generate a synergistic and additional effect (Elmasry et al., 2015; Val-Laillet et al., 2015; Allenby et al., 2018; Berryhill and Martin, 2018; Wilkinson et al., 2019; Schneider et al., 2021). This combined approach can affect the trained tasks and be generalized to other untrained cognitive functions (transfer effect), including near and far transfer effects (Filmer et al., 2017a; Berryhill and Martin, 2018; Brem et al., 2018; Forcano et al., 2018; Smits et al., 2021).

However, limited studies focused on whether repeated tDCS combined with concurrent behavioral task training further extends response inhibition performance relative to a single intervention method, and the findings are heterogeneous among these few studies. Some studies have shown that this combination can induce greater response inhibition enhancement or better clinical outcomes (improved abstinence rate of alcohol), with the effects lasting 1 or 2 weeks (Dousset et al., 2021; Dubuson et al., 2021). However, according to some findings, this combination cannot produce additional benefits for response inhibition performance at post-intervention or follow-up sessions (Smits et al., 2021; Westwood et al., 2021; Zhou and Xuan, 2022). Additionally, the near and far transfer effects generated by this combined approach have scarcely been explored and warrant further studies. For instance, a previous study using tDCS together with stop-signal task (SST) training found that non-trained task (implicit association task) showed no evidence of intervention effects (Smits et al., 2021). To date, no researchers have investigated the effect of repeated daily multitarget tDCS (a new stimulation montage) combined with concomitant cognitive training on extending performance improvements of response inhibition. In addition, its long-term after-effects and transfer effects should be examined.

To fill the research gap, we designed this study to investigate the effects of 10 consecutive sessions of multitarget anodal HD-tDCS targeting rIFG and pre-SMA combined with concurrent cognitive training on improving response inhibition compared with 10 repeated sessions of multitarget stimulation or training alone, including long-lasting effects and transfer effects. Based on available research, we hypothesized that (1) the combined approach would extend and enhance performance improvements of response inhibition compared to multitarget stimulation or cognitive training alone, and the improvement effects would persist to follow-up session (i.e., long-term after-effect), (2) multitarget stimulation or cognitive training alone would induce response inhibition improvements compared to sham tDCS, and (3) the transfer effects would be absent. To the best of our knowledge, this study is the first to examine the effects of repeated daily multitarget anodal HD-tDCS combined with concurrent cognitive training on response inhibition, providing a preliminary insight into strategies to enhance response inhibition ability for both psychiatric and non-psychiatric populations.

2. Materials and methods

2.1. Participants

Ninety-four healthy university students were included in this study. Prior to inclusion, the participants were screened to ensure they were ≥18 years of age and unfamiliar with tDCS-related research. They reported no neuropsychiatric disorders or use of psychotropic medication. All the participants (n = 94, mean age = 20.88 ± 1.77 years, range = 18–25 years, 41 males) had a normal or corrected-to-normal vision, no contraindications to tDCS (e.g., metal implants in the head, open wounds in the scalp, a family or personal history of epilepsy), and were right-handed as assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). The participants were also evaluated in hyperactivity/impulsivity and inattention using the Adult ADHD Self-report Scale (ASRS), and only those with scores of <17 in both subscales were included because individuals with a score of ≥17 on either subscale were likely to have ADHD (Kessler et al., 2005; Yeh et al., 2008). The participants were randomly assigned to four groups: (1) real stimulation combined with SST training, n = 24 (stimulation + training group); (2) real stimulation, n = 21 (stimulation group); (3) sham stimulation combined with SST training, n = 24 (sham + training group); and (4) sham stimulation, n = 25 (sham control). Each group underwent intervention separately, without knowing each other. We used G*power 3.1.9.6 to compute a prior sample size with a medium effect size of 0.25, two-tailed α of 0.05, and power (1-β) of 0.80, and a sample of 52 participants was planned (13 per group) (Cohen, 1992; Faul et al., 2007). Written informed consent was obtained from all the participants after the experimental procedure was explained to them. They were free to withdraw from the study at any stage. All the experimental protocols were reviewed and approved by the Tangdu Hospital Ethics Committee, Air Force Medical University, and were performed under the Declaration of Helsinki. After finishing the experiment, the participants received monetary compensation for their time.

2.2. Design and procedure

The current study had a single-blind, randomized, parallel-group, and sham-controlled design. The participants were blind to the intervention conditions and study hypotheses. Before undertaking the experiment, the participants were asked to complete a brief questionnaire to collect their demographic information, the ASRS scores, and assess their eligibility for tDCS. There were 13 sessions in this study: pre-intervention test, 10 intervention sessions, post-intervention test, and a follow-up test after a month. After the pre-test, the participants were randomly assigned to four intervention conditions. Each participant received 10 sessions of corresponding intervention for 20 min per day on 10 consecutive days. The training did not start until a stable holding current was obtained to avoid the confounding effect of current fluctuations (Zhou and Xuan, 2022). Side effects and blinding efficacy were evaluated *via* interviews with the participants after finishing the intervention sessions. All the participants completed the measurements before the intervention (pre-intervention test), the day after the end of the intervention (post-intervention test), and 1 month after intervention (follow-up test). The test contents were identical every time (**Figure 1**), including the Barratt Impulsiveness Scale-Version 11 (BIS-11), SST, color-word Stroop task, N-back task, and attention network test (ANT). The BIS-11 lasted for about 5 min; the test SST, Stroop task, and N-back task each lasted for about 10 min; the ANT lasted for about 16 min. In addition to SST, which assessed response inhibition, other tasks examined the potential transfer effects (near transfer: Stroop task; far transfer: N-back task and ANT). Before each measurement, BIS-11 was used to assess changes in self-reported impulsivity. The tasks were computerized and run on E-prime 3.0 software (Psychology Software Tools, Inc., Sharpsburg, PA, USA). The behavioral tasks were administered in a randomized order (Martin et al., 2013; Dubuson et al., 2021). Before beginning each task, the participants were instructed on how to perform the task; then, a standardized written instruction appeared on the screen.

2.3. High-definition transcranial direct current stimulation

Multitarget HD-tDCS was delivered using an M × N-9 HD-tES Stimulator (Soterix Medical, Inc., New York, NY, USA), following the procedures for HD-tDCS usage specified in a previous study protocol (Villamar et al., 2013). The stimulation procedure in this study used multitarget HD-tDCS on rIFG and pre-SMA from our previous study (Guo et al., 2022a). The electrodes were localized according to the international 10-10 EEG system (Jurcak et al., 2007). Anodes were placed at C2 (1.48 mA) and FT8 (1.02 mA) (a total current intensity 2.5 mA), with return cathodes at Fz (−0.51 mA), C4 (−0.52 mA), P4 (−0.36 mA), FT10 (−0.53 mA), TP8 (−0.17 mA), and FC4 (−0.41 mA) (**Figure 2A**). The electric field and current flow were simulated (**Figures 2B, C** and **Supplementary Figures 1–5**) using HD-explore and HD-Targets software (Soterix Medical, Inc., New York, NY, USA). This simulation method has been widely used in prior studies and proved effective (Shen et al., 2016; Stephens and Berryhill, 2016; Reinhart and Nguyen, 2019). Participants in the sham stimulation

condition underwent the same procedure as the real stimulation condition. The panel of the instrument was not visible to the participants. The current intensity of each electrode was smaller than 1.5 mA, which has been shown to be safe and reliable enough to improve cognitive performance (Villamar et al., 2013; Bikson et al., 2016; Hogeveen et al., 2016; Abellaneda-Perez et al., 2021; Zhou et al., 2021). Real stimulation was applied for 20 min with a ramp-up of 30 s at the beginning and a ramp-down of 30 s at the end. Sham stimulation consisted of a 30 s ramp-up and a 30 s ramp-down at the beginning and end, respectively, with no current during the intervening time, facilitating blinding by mimicking the sensations of real tDCS without actual neurophysiological changes (Di Rosa et al., 2019; Sharma et al., 2021). After stimulation sessions, the participants guessed which kind of stimulation they received (real or sham) and rated the confidence level based on a numeric analog scale ranging from 0 = *absolute guess* to 10 = *absolutely sure*. Additionally, participants completed a side-effect survey to report their dominant sensations (e.g., itching, tingling, burning, metallic taste, no special sensation) during the stimulation, and an 11-point scale was used to evaluate the intensity of sensations they felt, ranging from 0 = *no sensation* to 10 = *strongest sensation imaginable* (Hill et al., 2017).

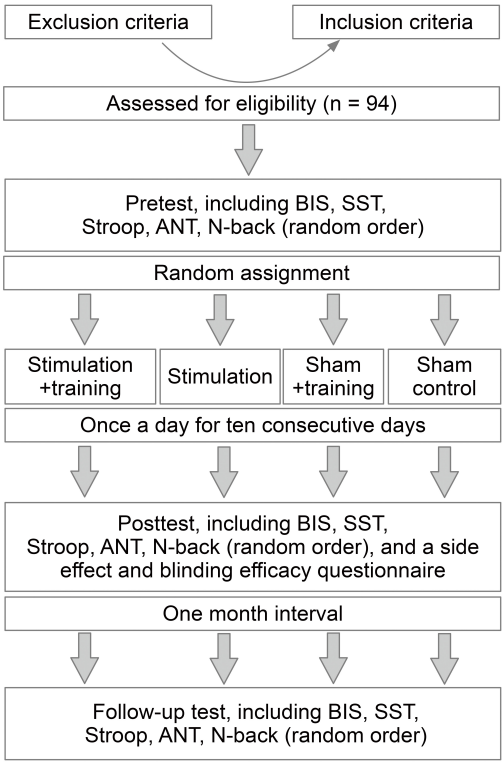

FIGURE 1
Experimental procedure. The study followed a single-blind, randomized, parallel-group, and sham-controlled design. The order of SST, Stroop task, ANT, and N-back task were randomized.

2.4. Tasks and measures

2.4.1. Barratt impulsiveness scale-version 11

Barratt impulsiveness scale-version 11 was employed to evaluate the impulsivity of the participants. It comprises 30 items and can be divided into three dimensions: attentional impulsivity, motor impulsivity, and non-planning impulsivity, with 10 items in each dimension (Patton et al., 1995; Bari and Robbins, 2013). In the current study, we used the revised Chinese version of BIS-11 (Li et al., 2011). It is reliable and has been widely used in previous studies (Ran et al., 2021; Guo et al., 2022b). Each item can be rated from 1 to 5 based on a five-point Likert scale. The dimensional score and total score range from 0 to 100 after being converted, with higher scores indicating higher levels of impulsivity (Li et al., 2011; Ran et al., 2021). The internal consistency of the BIS scale and its three subscales were good in our sample, with the Cronbach's α ranging from 0.70 to 0.91 at an arbitrary test time point.

2.4.2. Stop-signal task

We used SST to evaluate the response inhibition performance (Logan et al., 1984; Verbruggen and Logan, 2008; Verbruggen et al., 2019). The task settings were identical to our previous study (Guo et al., 2022a). In the pre-potent go trials (75% of total trials), the participants were instructed to discriminate the direction of the right arrow or left arrow go signal on the screen by pressing the corresponding key (F for the left arrow and J for the right arrow) on a standard keyboard as quickly and accurately as possible. However, in the stop trials (25% of total trials), a small red square (stop signal) was presented above the arrow after an interval (stop signal delay, SSD), indicating the need to withhold their initiated response. The SSD was dynamically adjusted stepwise (initial SSD = 250 ms, 50-ms step, range = 0–1250 ms) to ensure that each participant had an approximately 50% successful inhibition rate. **Figure 3A** presents the details of the task parameters. We estimated the primary outcome measure using the stop-signal reaction time (SSRT) determined by the integration method (Verbruggen et al., 2019), with shorter SSRT indicating superior response inhibition. SSRT was determined as follows: (1) calculating p(response| stop-signal), which means the probability of response to a stop signal; (2) ranking all RT of go trials from the minimum to the maximum with go omissions assigning the maximum RT (RT distribution); (3) calculating nth RT which corresponds to the p(response| stop-signal)-percentile of the RT distribution; and (4) using nth RT minus mean SSD to calculate SSRT. In addition to SSRT, other SST performance metrics, such as stop accuracy (the probability of inhibiting responses on stop stimulus) and goRT (mean RT on correct go trials), were also assessed.

The SST was not only the test task for all groups but also the training task for the two groups using SST training. The test SST included a practice block of 48 trials and a formal test block of 200 trials (25% stop-signal trials), while the training SST consisted of 48 practice trials and 400 formal trials (30-s rest when finishing 200 trials). The training SST finished within the stimulation duration to guarantee the identical training amount. All the trials were presented at random.

2.4.3. Color-word Stroop task

The participants performed a classical color-word Stroop task at the pre-test, post-test, and follow-up test, which is a measure of cognitive inhibition (Lu et al., 2020a; Parris et al., 2021; Wu et al., 2021b; Zhou and Xuan, 2022). The Stroop task was used

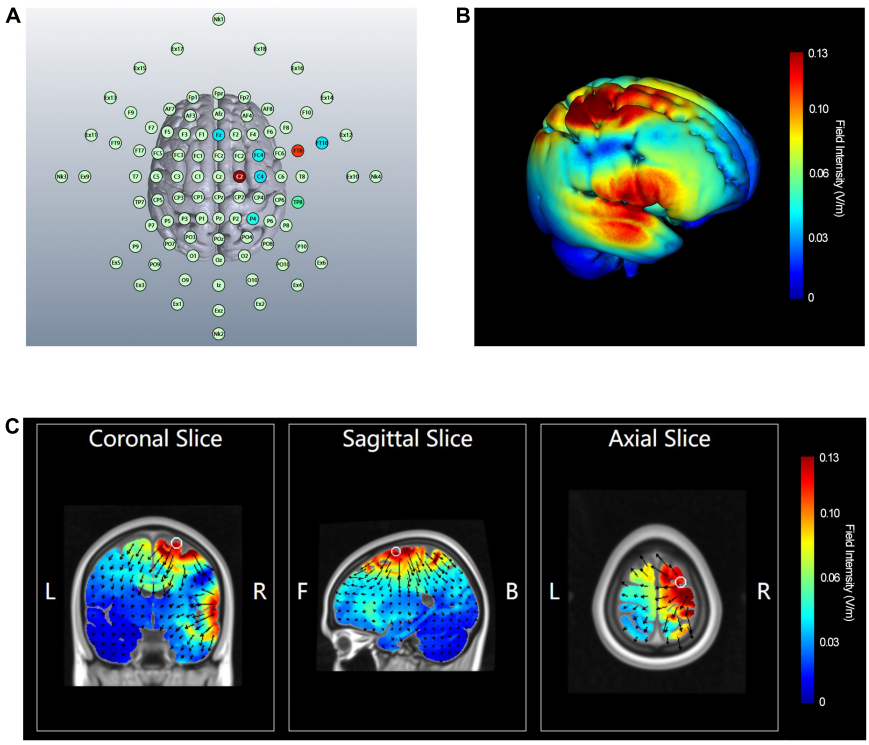

FIGURE 2
Electrode configuration and computational neurostimulation modeling of multitarget HD-tDCS. **(A)** Electrodes configuration. **(B)** A 3D view of the simulated electric field. **(C)** The section view of simulated electric field and current direction. The color bar represents the field intensity. The arrow points in the direction of the current flow, and the length indicates the current flow intensity. L, left; R, right; F, front; B, back.

to explore the near-transfer effect of various interventions on cognitive inhibition. The task included a practice block of 15 trials and two test blocks of 45 trials each, with a 30-s rest between formal experimental blocks. The stimulus was chosen randomly from one of three Chinese characters ("红" for red, "绿" for green, and "黄 for yellow) printed in different colors of ink, either red, green, or yellow (Lu et al., 2020a). The practice block was presented with feedback, and the participants did not proceed to the formal test block until 80% accuracy was achieved. The formal test block had no feedback. Each trial began with a fixation cross (+) at the center of the screen for 300 ms, which was replaced by a Stroop stimulus. The participants were instructed to press "D" for red, "F" for yellow, and "J" for green on the keyboard, according to the color rather than the meaning of the Chinese character, as quickly and accurately as possible. The stimulus interface lasted up to 1500 ms or was terminated with a blank screen (800–1000 ms) immediately after a key-press response (**Figure 3B**). During the congruent trial, the word matched the color (e.g., "红" in red), while in the incongruent trial, the word conflicted with the ink color (e.g., "红" in yellow). In our task, 40% of trials were incongruent, and all the trials were presented randomly (Fu et al., 2019). We adopted the Stroop effect as the primary outcome. It was characterized by a longer reaction time in incongruent conditions compared with color-word congruent conditions and measured by the mean correct RT in incongruent trials, subtracting the mean correct RT in congruent trials. A lower Stroop effect indicated a higher inhibitory performance (Stroop, 1935; Fu et al., 2019; de Boer et al., 2021).

2.4.4. Attention network test

Attentional network test (ANT) is a classic task to study attention ability, which simultaneously measures the efficiency of individual alerting, orienting, and executive control networks involved in attention (Fan et al., 2002; Goldin et al., 2014; Lu et al., 2020b). The ANT was used to measure the transfer effect on attentional function. In our study, the ANT featured identical visual and timing parameters to those previously described (Fan et al., 2002). The target was preceded with one of the four cues, namely no cue, center cue, double cue, and spatial cue, and was flanked on either side by two arrows pointing in the same direction (congruent condition), opposite direction (incongruent condition), or no direction (neutral condition). The participants were asked to identify the direction (left/right) of the targeted arrow in the upper or lower visual hemifield by pressing a corresponding key ("F" for the left arrow, "J" for the right arrow) as quickly and accurately as possible. A session included a 24-trial practice block and two test blocks of 96 trials each (Rinne et al., 2013). The participants did not enter the test block until 60% accuracy of the practice block was achieved. The trials were presented in a random order. There was a 30-s rest between two experimental blocks to avoid mental fatigue in the participants. **Figure 3C** presents more details. Outcome measures included the following: (1) conflict effect = RT (incongruent)–RT (congruent); (2) orienting effect = RT (central cue)–RT (spatial cue); and (3) alerting effect = RT (no cue)–RT (double cue) (Fan et al., 2002). The higher the orienting and alerting effects, the better the attentional processing; the lower the conflict effect, the better the ability to deal with interference.

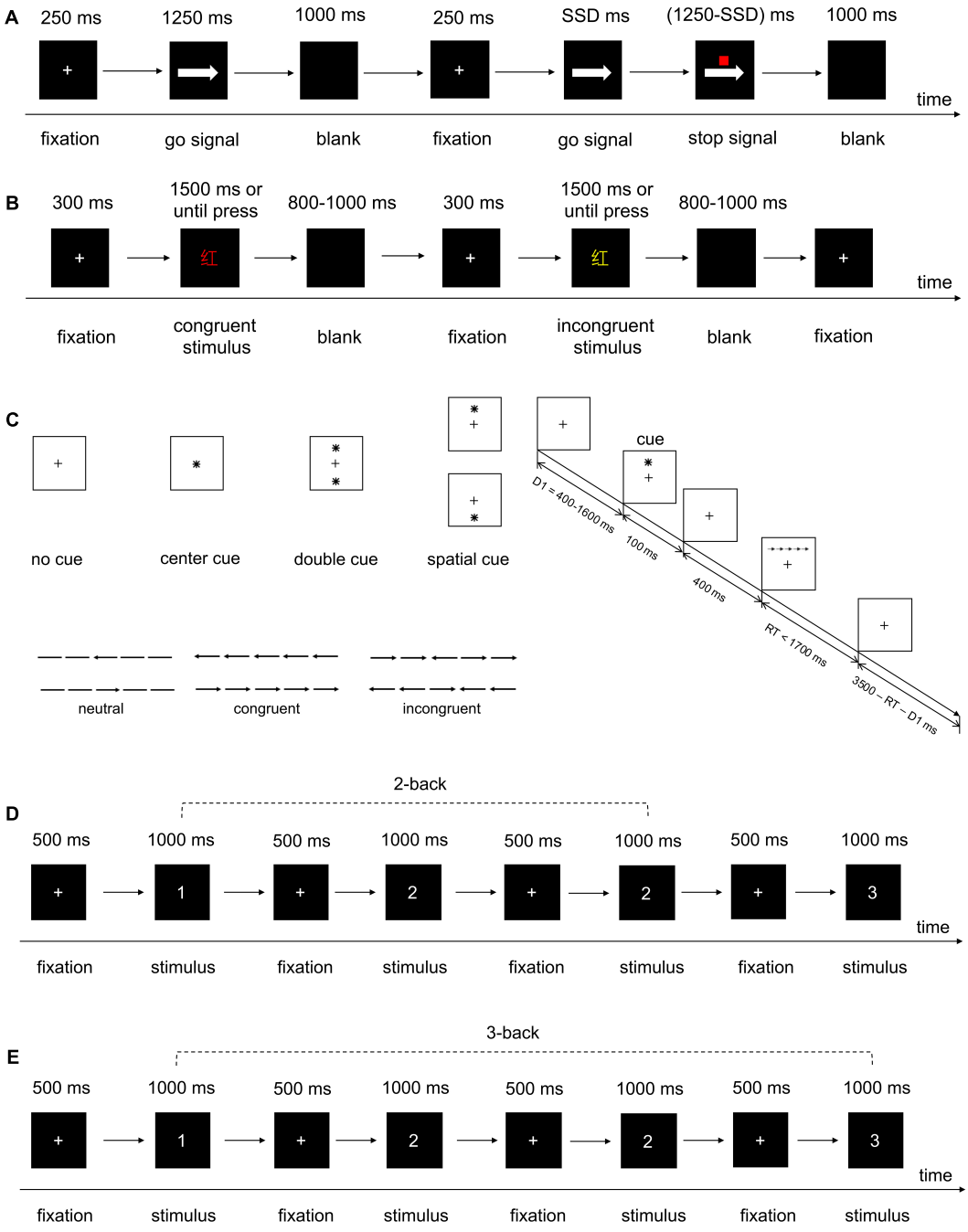

FIGURE 3
Detailed information about procedures of behavioral tasks. **(A)** SST. **(B)** Color-word Stroop task. **(C)** ANT. **(D)** 2-back task. **(E)** 3-back task.

2.4.5. N-back task

To probe the far transfer effect on the WM, we used an N-back task that is widely used to measure WM performance (Owen et al., 2005; Alizadehgoradel et al., 2020; Kaminski et al., 2020). We used a 2-back combined with a 3-back task with two blocks of each kind of task, and the 2-back task was conducted before the 3-back task. A cue appeared before each task block to alert the participants whether the next block was a 2-back or 3-back block. A number stimulus ranging from 1 to 9 appeared on the screen every time, and the participants were instructed to press the "J" key when the targets were identical to the ones presented two numbers before in a 2-back task block or three numbers before in a 3-back task block; otherwise, they pressed "F" in the keyboard. There were 62 trials in a 2-back task block and 63 trials in a 3-back task block, and the participants could have a 30-s rest between blocks. The participants had to finish the practice block before the test block started. **Figures 3D, E** present the details of the time sequence of the trials. The mean RT of correct responses and response accuracy were assessed as a result, and shorter RT and higher accuracy rates indicated better WM performance (Alizadehgoradel et al., 2020; Nejati et al., 2020).

2.5. Data pre-processing

Concerning SST, five participants were excluded from further analyses because they showed (1) stop accuracy <0.25 or >0.75 or (2) SSRT <50 ms (Congdon et al., 2012). After exclusion, the sample for SST analysis consisted of 89 subjects (n = 23, 21, 22, 23 for groups 1 to 4, respectively). Five participants were excluded from the Stroop effect analysis due to RT exceeding ± 3 SD of the mean (Fu et al., 2019). After exclusion, the Stroop task analysis was based on n = 23, 21, 22, 23 for groups 1 to 4, respectively. As for the N-back task, four participants with accuracy or RT exceeding ± 3 SD of the mean were excluded, leaving 90 participants for further analyses (n = 23, 20, 23, 24 for groups 1 to 4, respectively). Concerning ANT, five participants were excluded due to RT deviating >3 SDs of the mean. The final sample for ANT analysis comprised 89 participants (n = 22, 21, 21, 25 for groups 1 to 4, respectively). Notably, the number of participants varied by measure because of data filtering of corresponding behavioral measures, which was common practice in previous studies (Biggs et al., 2015; Dagan et al., 2018).

2.6. Statistical analysis

We used the IBM SPSS statistical package version 26 to conduct data analyses. The normality in the distribution of data was evaluated using the Shapiro-Wilk test, and the homogeneity of variances was confirmed using Levene's test. When necessary, the sphericity assumption was verified by Mauchly's sphericity test, and Greenhouse-Geisser was applied when the sphericity assumption was not met. Categorical variables such as gender and blinding were represented as count or proportion and examined by the chi-squared test. Continuous variables such as accuracy and RT were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to test baseline performance and continuous data that measured once such as demographic variables. If the outcome measures differed at baseline (i.e., pre-test), they were analyzed by creating contrasts (δ values) between the post-test or follow-up test and pre-test to eliminate the interference of baseline, thereby ensuring that any performance changes would be attributable to the intervention. In addition, one-way ANOVA with Bonferroni's-corrected statistical threshold was used to test group differences of $\delta_{post-pre}$ or $\delta_{follow-up-pre}$.

Each behavioral task and its outcome measures and BIS-11 scores were tested using a series of 4 × 3 repeated-measures ANOVA (RM-ANOVA) with group (stimulation + training/combined condition, stimulation, sham + training, sham control) as between-subject factor and time (pre-test, post-test, follow-up test) as within-subject factor. *Post-hoc* tests were performed using Bonferroni's-corrected pairwise comparisons. To further detect the effects of different intervention conditions on improving response inhibition, we conducted a subgroup analysis of SSRT. The participants in each group were separated into high-performance (HP) and low-performance (LP) subgroups based on baseline SSRT *via* a median-split method (Whelan et al., 2012; Schmicker et al., 2021). Subgroup analysis for each condition was performed using a 2 (subgroup: HP and LP) × 3 (time: pre-test, post-test, and follow-up test) RM-ANOVA. To explore possible relationships between SST and other behavioral tasks, we computed correlations of baseline outcome measures (excluding participants according to data filtering criteria of both tasks) using bivariate Pearson's correlation analysis (two-tailed test). For exploring purposes, the statistical threshold of correlation analysis was not corrected. Concerning RM-ANOVAs, the significant interaction term was the focus of this study. The statistical significance level was set at $\alpha = 0.05$. For ANOVAs, partial eta-squared (η_p^2) was calculated as measure of effect sizes.

3. Results

3.1. Demographics and baseline performance

As shown in Table 1, the four groups were matched. There were no significant differences in demographic and basic characteristics between the groups ($ps > 0.05$), including gender distribution, age, years in education, scores of hyperactivity/impulsivity and inattention subscales of ASRS, and sleep duration per night. In addition, one-way ANOVA for scores of BIS-11 and outcome measures of SST, Stroop task, N-back task, and ANT revealed no significant differences in the variables at baseline between the groups ($ps > 0.05$), except for 2-back accuracy, 3-back accuracy, and orienting effect (Table 1).

3.2. HD-tDCS safety, blinding efficacy, and electric field simulation

All the participants tolerated the stimulation well, and only mild side effects (i.e., tingling, burning, itching) were reported. Most of the participants reported tingling sensation, with 19 (79.2%), 15 (71.4%), 21 (87.5%), and 21 (84.0%) subjects in groups 1 to 4, respectively. Moreover, there was no significant difference in the ratings of the intensity of tingling sensations between the four intervention conditions [$F_{(3,72)} = 1.704$, $p = 0.174$, $\eta^2_p = 0.066$]. There were 24 (100%), 19 (90.5%), 23 (95.8%), and 24 (96%) participants in groups 1 to 4, respectively, who believed that they underwent real stimulation. No significant differences were found between the groups in the number of participants reporting real or sham stimulation ($\chi^2 = 2.385$, $p = 0.45$). The confidence level scores were also non-significant when they were compared between the stimulation + training (8.21 ± 1.29), stimulation (7.57 ± 2.40), sham + training (7.38 ± 2.16), and sham control (8.24 ± 1.76) conditions [$F_{(3,90)} = 1.245$, $p = 0.298$, $\eta^2_p = 0.04$]. The electric field modeling showed that the electric field distribution generated by multitarget HD-tDCS was focused around the anodes and the electric field and current flow produced was largely restricted within the ring of return electrodes (Figures 2B, C).

3.3. Stop-signal task

A significant group × time interaction effect on SSRT was observed [$F_{(6,170)} = 2.161$, $p = 0.049$, $\eta^2_p = 0.071$]

TABLE 1 Demographic data, scale scores, and behavioral tasks performance at baseline.

Variable	Stimulation + training	Stimulation	Sham + training	Sham control	F/χ^2	*p*
n	24	21	24	25		
Gender (male/female)	10/14	10/11	10/14	11/14	0.213	0.976
Age (years)	20.83 (1.74)	20.71 (1.95)	20.88 (1.75)	21.08 (1.73)	0.170	0.917
Education (years)	15.42 (1.77)	15.24 (1.79)	15.46 (1.93)	15.64 (1.73)	0.192	0.902
ASRS-inattention	12.00 (2.83)	12.57 (2.01)	13.17 (2.53)	12.24 (2.51)	0.983	0.404
ASRS-hyperactivity/impulsivity	9.33 (2.88)	9.00 (2.92)	9.46 (2.86)	9.64 (3.16)	0.187	0.905
Sleep duration per night (hours)	7.00 (0.83)	6.81 (0.87)	6.75 (0.74)	6.64 (0.57)	0.968	0.412
BIS-11						
Non-planning impulsivity	28.65 (14.52)	30.83 (13.45)	31.88 (13.48)	28.20 (11.78)	0.416	0.742
Motor impulsivity	29.27 (9.22)	32.62 (8.27)	32.50 (8.20)	32.60 (10.29)	0.788	0.504
Attentional impulsivity	31.88 (9.00)	34.76 (9.74)	33.02 (6.84)	29.40 (8.14)	1.644	0.185
SST						
SSRT (ms)	274.73 (28.47)	272.06 (32.82)	277.56 (34.75)	274.03 (36.57)	0.101	0.959
Stop accuracy	0.51 (0.07)	0.51 (0.04)	0.53 (0.06)	0.50 (0.06)	1.337	0.268
GoRT (ms)	565.20 (202.08)	506.71 (152.52)	569.66 (216.79)	497.66 (221.20)	0.796	0.499
Stroop task						
Stroop effect (ms)	114.58 (47.34)	121.60 (67.99)	131.96 (72.02)	100.98 (57.96)	1.005	0.395
ANT						
Orienting effect (ms)	122.74 (27.01)	132.02 (24.90)	107.10 (30.62)	127.16 (32.99)	2.899	0.040
Conflict effect (ms)	51.34 (32.41)	56.25 (20.94)	39.24 (26.98)	50.42 (28.06)	1.438	0.237
Alerting effect (ms)	52.42 (32.14)	54.35 (25.96)	45.88 (25.19)	50.95 (27.54)	0.356	0.785
N-back task						
2-back accuracy	0.82 (0.07)	0.68 (0.22)	0.71 (0.12)	0.70 (0.18)	3.207	0.027
2-back RT (ms)	652.56 (72.61)	657.90 (96.93)	685.37 (65.40)	657.05 (81.27)	0.815	0.489
3-back accuracy	0.73 (0.11)	0.69 (0.12)	0.62 (0.10)	0.71 (0.15)	3.652	0.016
3-back RT (ms)	640.07 (74.14)	660.87 (57.69)	651.52 (97.77)	611.24 (111.20)	1.335	0.268

Values are counts or means (standard deviations). ASRS, adult ADHD self-report scale; BIS-11, Barratt impulsiveness scale-version 11; SST, stop-signal task; SSRT, stop-signal reaction time; GoRT, mean reaction time on correct go trials; ANT, attention network test. The gender distribution was tested by the χ^2 test, and other variables were examined using one-way analysis of variance.

(Figure 4A). The main effects of time and group were also significant (*p*s < 0.05). *Post hoc* analysis with a Bonferroni's-correction showed a significant decrease in SSRT both in the stimulation + training and stimulation alone groups form pre-intervention to post-intervention ($p = 0.005$ and $p < 0.001$, respectively) and from pre-intervention to follow-up test ($p = 0.008$ and $p = 0.003$, respectively). It also revealed a significant decrease in SSRT between pre-intervention and post-intervention in the sham + training group ($p = 0.037$) but not between pre-intervention vs. 1-month follow-up ($p = 0.737$). *Post hoc* analysis showed no significant changes in SSRT in the sham control group (*p*s > 0.999). There were no significant group × time interaction effects for the stop accuracy [$F_{(5.36,152.95)} = 0.387$, $p = 0.869$, $\eta^2_p = 0.013$] and goRT [$F_{(5.42,153.69)} = 0.776$, $p = 0.578$, $\eta^2_p = 0.027$], and the main effects were all non-significant (*p*s > 0.05).

Subgroup analysis showed a significant subgroup × time interaction for SSRT in both stimulation + training [$F_{(2,42)} = 3.538$, $p = 0.038$, $\eta^2_p = 0.144$] and stimulation conditions [$F_{(2,38)} = 5.105$, $p = 0.011$, $\eta^2_p = 0.212$]. The main effects of time and subgroup reached significance in the stimulation + training group (*p*s < 0.05), and the time main effect was significant in the stimulation group [$F_{(2,38)} = 13.182$, $p < 0.001$, $\eta^2_p = 0.41$]. In the combined intervention (stimulation + training) condition, the Bonferroni's-corrected *post hoc* analysis showed significantly decreased SSRT between pre-intervention and follow-up in the HP subgroup ($p = 0.002$), and between pre-test and post-test ($p < 0.001$) and between pre-test and follow-up test in the LP subgroup ($p < 0.001$) (Figure 4B). In the stimulation-alone condition, the SSRT significantly decreased in the post-test ($p < 0.001$) and follow-up test ($p < 0.001$) compared to the pre-test in the LP subgroup but not in the HP subgroup (Figure 4C). For the sham + training and sham control conditions, the interactions of subgroup × time were not significant ($p = 0.214$ and 0.098, respectively). The main effect of the subgroup was significant in the sham + training group [$F_{(1,20)} = 4.568$, $p = 0.045$, $\eta^2_p = 0.186$]. There were no significant main effects in the sham control group (*p*s > 0.05).

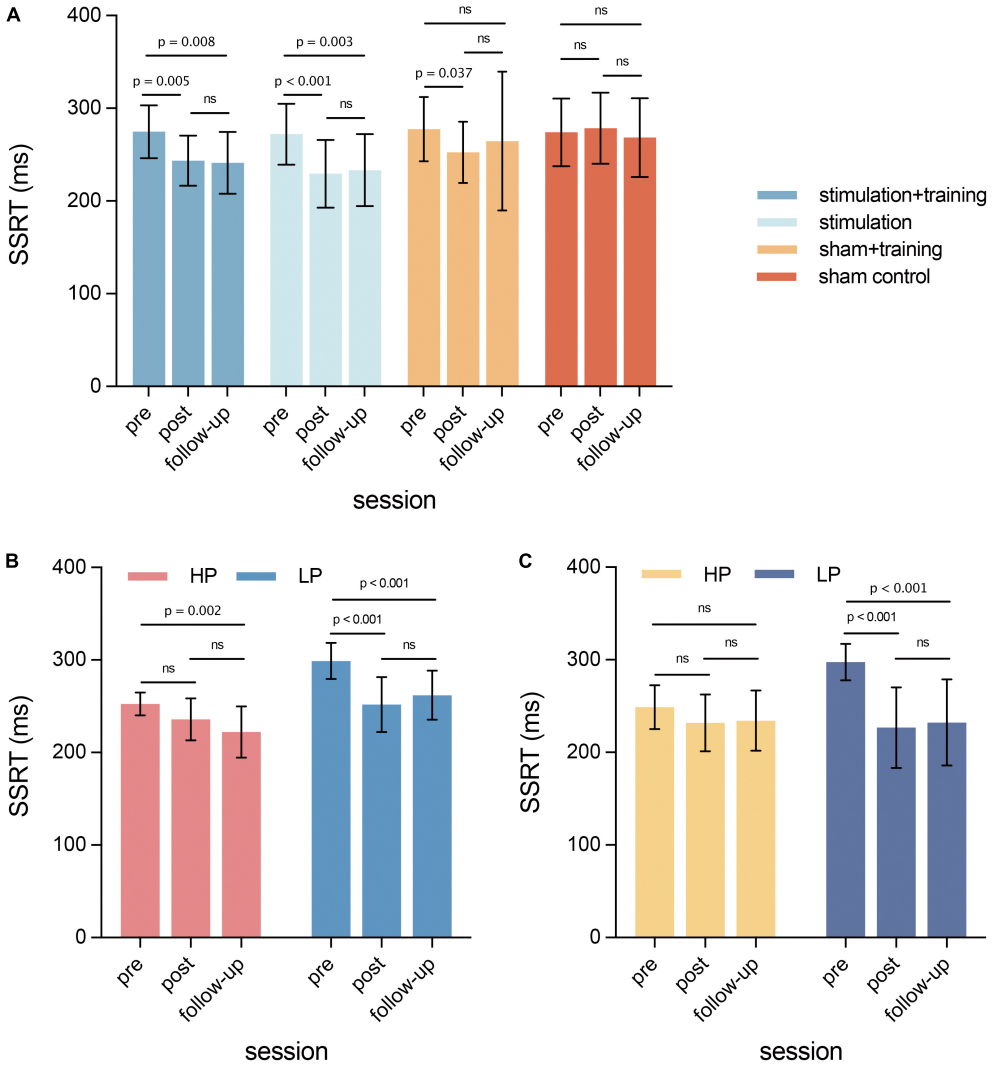

FIGURE 4
The effects of different intervention conditions in relation to the stop-signal task. **(A)** Significant interaction between group and time. **(B)** Subgroup analysis in the stimulation + training group. **(C)** Subgroup analysis in the stimulation group. HP, high performance; LP, low performance. All error bars represent standard deviation.

3.4. Transfer tasks

In the Stroop task, the main effect of time was significant [$F_{(2,170)}$ = 24.085, p < 0.001, η^2_p = 0.221] due to decreased Stroop effect at the post-test (95.73 ± 5.44 ms) and follow-up test (73.36 ± 4.79 ms) compared to the pre-test (117.28 ± 6.53 ms). The interaction effect of group × time and the main effect of the group were not significant (ps > 0.05). In the ANT, one-way ANOVA showed that both the orienting effect δ values were not significant (ps > 0.05). The time effects for conflict [$F_{(1.82,155.04)}$ = 11.705, p < 0.001, η^2_p = 0.121] and alerting [$F_{(2,170)}$ = 4.057, p = 0.019, η^2_p = 0.046] effects were significant but not interaction terms or group effects (ps > 0.05). Concerning the N-back task, the baseline 2-back accuracy significantly differed between the combined intervention and stimulation conditions (Table 1), with the former exhibiting significantly higher accuracy than the latter (p = 0.047). One-way ANOVA showed that the $\delta_{post-pre}$ and $\delta_{follow-up-pre}$ for 2-back accuracy reached significance (p = 0.024 and 0.017, respectively, with corrected α = 0.025), but not 3-back accuracy (ps > 0.05). *Post hoc* analysis revealed that the combined intervention condition exhibited a smaller 2-back accuracy for $\delta_{post-pre}$ (p = 0.022) and $\delta_{follow-up-pre}$ (p = 0.013) compared to the stimulation condition. The main effects of time for 2-back RT and 3-back RT were significant (ps < 0.001) due to the reduction of RT at the post-test and follow-up test compared to the pre-test, but the interaction terms and the group effects were not significant (ps > 0.05).

3.5. Barratt impulsiveness scale-version 11

None of the group × time interactions and main effects of time and group for non-planning impulsivity, motor impulsivity, and attentional impulsivity reached significance (ps > 0.05).

3.6. Correlation analysis

Pearson's correlation analysis showed that SSRT was significantly and negatively associated with 2-back ($r = -0.259$, $p = 0.015$) and 3-back ($r = -0.239$, $p = 0.024$) accuracy but was not correlated with the Stroop effect in the Stroop task or orienting, conflict, alerting effects in ANT ($ps > 0.05$).

4. Discussion

To the best of our knowledge, this randomized, parallel, and sham-controlled study is the first to examine whether repeated daily multitarget HD-tDCS applied to rIFG and pre-SMA, combined with concurrent SST response inhibition training, enhanced the response inhibition improvements. Consistent with the study hypotheses, our main findings showed that the combined protocol could generate a synergistic effect, compared to the single intervention condition, which also improved the response inhibition compared to the sham control. The decreased SSRT suggests improved response inhibition (Verbruggen and Logan, 2008; Verbruggen et al., 2019). According to the current results of SSRT, the combined protocol and the stimulation alone significantly improved response inhibition after the intervention, and the improvement persisted for up to at least 1 month. Given that the training alone only produced post-intervention effects, this condition was inferior to the combined condition and the stimulation alone in the long-term effects. However, the combined condition not only enhanced the LP subgroup performance but also improved the HP subgroup performance at the follow-up session compared to the stimulation-alone condition, which only enhanced the response inhibition of the LP subgroup. According to the compensation hypothesis (Shaw and Hosseini, 2021; Teixeira-Santos et al., 2022), the effects of cognitive enhancement techniques, such as tDCS and cognitive training, depend on baseline performance, and individuals with high baseline performance are difficult to be enhanced because they may already be near the peak level of cognitive ability. Therefore, there is less room for improvement. Conversely, individuals with low baseline performance have more room for improvement and are predisposed to enhancement. Many studies favor the compensation hypothesis (Krebs et al., 2021; Wu et al., 2021a,b; Assecondi et al., 2022). Despite the high baseline performance of the HP subgroup in this study, the combined protocol produced an improved effect at the follow-up session. Overall, the repeated daily HD-tDCS combined with SST training yielded the most significant effects and extended the improvement effects of stimulation or training alone.

The main finding is consistent with numerous previous studies that repeated tDCS accompanied by cognitive training could induce a synergistic effect after the intervention (Filmer et al., 2017b; Dousset et al., 2021; Dubuson et al., 2021; Schneider et al., 2021; Corrêa et al., 2022; Han et al., 2022; Lo et al., 2022; Szymkowicz et al., 2022). Importantly, the response inhibition improvement in this study persisted for up to 1 month following the intervention, consistent with previous studies in which repeated sessions of tDCS combined with concurrent cognitive training could produce after-effects that persisted from 1 week to 1 month (Dousset et al., 2021; Dubuson et al., 2021; Lee and Kim, 2021; Pisano et al., 2022). However, in the two studies involving response inhibition (Dousset et al., 2021; Dubuson et al., 2021), the after-effects lasted for 1 or 2 weeks, which differs from the 1-month after-effects in our study. This inconsistency may be attributed to the duration of intervention in previous studies that used four or five daily sessions of 20 min compared with 10 daily sessions of 20 min in this study. Most previous studies did not focus on the effects of combined condition on response inhibition, and among the few relevant studies, some findings rule out the synergistic effect of tDCS combined with response inhibition training (Smits et al., 2021; Westwood et al., 2021; Zhou and Xuan, 2022). However, our study provides evidence to support the higher efficacy of the combined protocol than commonly used single training or tDCS, providing further support for the limited literature on the efficacy of combined protocol in further improving response inhibition.

Previous studies have proposed that the best effects of tDCS are achieved when the stimulated neural network is already activated or pre-activated (e.g., *via* a behavioral task that involves the same brain region). Simultaneous activation of shared neural networks by both applied tDCS and performing relevant tasks can produce a synergistic effect. In addition, repeated tDCS and cognitive training may interactively facilitate the beneficial effect which occurs through specific neuroplastic changes such as the *N*-methyl-D-aspartate (NMDA)-dependent mechanism (Gilmore et al., 2018; Wilkinson et al., 2019; Breitling et al., 2020; Schneider et al., 2021; Westwood et al., 2021). The SST was widely used to study response inhibition and has been shown to engage the rIFG and pre-SMA (Aron and Poldrack, 2006; Duann et al., 2009; Watanabe et al., 2015; Hannah and Aron, 2021). Based on previous studies, we speculate that the neural mechanisms underlying the synergistic effect in our study may lie in the neural plasticity changes of the shared response inhibition cortices, including rIFG and pre-SMA, which were activated and shaped by the SST training and multitarget HD-tDCS. However, future studies are warranted, including the use of neuroimaging tools such as tDCS-compatible fMRI or magnetic resonance spectroscopy (MRS) to record simultaneous brain activity during the tDCS combined with SST training.

Additionally, we found that the repeated sessions of multitarget stimulation or SST training alone could improve response inhibition compared with the sham control condition, consistent with our hypothesis. The favorable effect of multitarget stimulation over sham control on response inhibition is in line with our previous study (Guo et al., 2022a). It is also similar to published studies indicating that multitarget stimulation exerted more significant effects on motor function than sham control (Dagan et al., 2018). Furthermore, this study explored the long-term effects of the multisession multitarget stimulation and found the improvement persisted for 1 month after intervention, similar to a previous study in which 10 repeated sessions of tDCS over dorsolateral prefrontal cortex (DLPFC) could improve task performance for 1 month after the intervention (Alizadehgoradel et al., 2020). This finding also showed that SST training alone improved response inhibition ability after the intervention. Not surprisingly, training is one of the crucial cognitive enhancers, and several studies have confirmed that SST training plays an important role in facilitating response inhibition (Berkman et al., 2014; Zhou and Xuan, 2022).

We found the good performance of SST was associated with high N-back accuracy at baseline, suggesting a correlation between response inhibition and WM in the mechanism. This is consistent with previous studies that at a behavioral level, response inhibition and WM are correlated (Alderson et al., 2010, 2017; Raiker et al., 2012), and at a functional level, response inhibition and WM both activate the rIFG (McNab et al., 2008). The scores of BIS-11 subscales measuring trait impulsivity showed no changes in this study, which is consistent with a previous study that revealed no variations of BIS-11 under the influence of time and intervention (training combined with either real or sham stimulation) (Gilmore et al., 2018). According to previous studies, personality traits increase in stability during puberty and remain relatively stable after that (Hayes et al., 2017). Therefore, the absence of an intervention effect is probably because the trait impulsivity assessed *via* BIS-11 remained relatively stable in our sample that comprised adults aged 18 years and older.

Although the 2-back accuracy δ values of the stimulation condition were higher than those of the combined protocol, this was attributed to the baseline difference between the two conditions. Since the 2-back accuracy of the combined protocol was significantly higher than the stimulation condition, it had less room for improvement (Shaw and Hosseini, 2021; Teixeira-Santos et al., 2022). Therefore, the difference was unrelated to the interventions. Overall, the transfer effects on the Stroop task, ANT, and N-back task, which measure cognitive inhibition, attentional function, and WM, respectively, showed no group differences attributable to the intervention. A previous study showed that seven daily sessions of SST training positively impacted the Stroop task performance, while the anodal stimulation on pre-SMA combined with SST training did not (Zhou and Xuan, 2022). This is partly consistent with our findings, but some discrepancy exists in that the SST training had no transfer effects in our study. This discrepancy might have arisen from the variations in the number of formal SST training trials; the SST training comprised 400 trials per session in our study, whereas the SST training consisted of 720 trials per session in the previous study. Furthermore, the total number of trials was less in our study (4000 vs. 5,040 trials). Concerning the transfer effect on attention and WM, previous studies have revealed that 10 online (i.e., tDCS concurrent with the task) sessions of tDCS + dual N-back training could produce a transfer effect to an untrained test of attention and WM at follow-up (Martin et al., 2013), or five sessions of multiple-task cognitive training with tDCS could lead to a near-transfer effect of attention gains (Boroda et al., 2020). However, no studies on online tDCS combined with response inhibition training have explored transfer effects on ANT or N-back. Therefore, they cannot be directly compared with our study. The transfer effect should be further considered and investigated.

In this study, to stimulate pre-SMA, we placed central anode at C2. A circuit was formed between the anode and cathodes, which led to current density and electric field existing between the electrodes—between the anode at C2 and the cathodes at Fz and FC4. The detailed simulation (**Supplementary Figures 1–5**) showed that the electric field extended through the anterior portion of Area 6 (Area 6a and 6ma) to the transition of Area 6 and Area 8 (Area i6-8 and s6-8). It cannot be excluded that parts of the motor area were stimulated as well, but fortunately this brain cortex has not been shown to be involved in the response inhibition process, which did not impact the interpretation of the findings in this study. Furthermore, there may be some confusions arising from the anode placement of pre-SMA because some previous studies placed the central anode at Fz to stimulate pre-SMA (Berglund-Barraza et al., 2020; Chiang et al., 2021). This is because there may be some ambiguity in what people are calling "pre-SMA." We see that some places call Area 8 pre-SMA and others call the anterior Area 6 pre-SMA. Here we adopted the latter definition.

The current study has important theoretical and clinical implications. Regarding the theoretical implications, our findings support the synergistic effect of combining tDCS and concurrent cognitive training, indicating better improvement effects than the single intervention method. Moreover, we provided evidence that the combined protocol can be effectively applied in the field of response inhibition enhancement, with the long-lasting after-effects persisting for at least 1 month. Regarding the clinical implication, this study may provide insights into the treatment strategy for the clinical populations with inhibition-deficit-related mental diseases, who need to enhance response inhibition.

Despite these important implications, this study has some limitations. First, this study did not use neuroimaging method; therefore, we cannot infer the neural plasticity changes caused by the intervention. In the future, we plan to study brain functional and structural changes induced by this combined protocol. Second, the long-term after-effects were not investigated thoroughly. We only conducted a 1-month follow-up test, and further long-term effects were unknown, which should be dealt with in future studies. Third, this study focused on only young, healthy adults; therefore, it is not known how generalizable our findings are to other populations, such as the clinical sample, and the applicability of our results to other populations requires replication in other samples. Fourth, the study used a single-blinded design due to experimental constraints, possibly weakening the power of this study. Future studies should use more rigorous experimental designs to minimize potential bias, such as the Rosenthal effect. Fifth, the focality of multitarget anodal HD-tDCS in this study has to be improved. The electric field simulation result showed that the maximal electric field strength achieved underneath the anodes C2 and FT8, which we intended to stimulate pre-SMA and rIFG. However, the anode at C2 may also stimulated right motor cortex. Hence, in this study, the electric field produced by the stimulation protocol covered pre-SMA but the precision and focality were not enough, indicating the multitarget stimulation protocol needs to be improved to increase the focality of stimulation. Finally, due to the inter-individual variations of the cortical anatomy and reactivity to stimulation, the individual MRI data should be collected to improve the spatial localization accuracy and the individualized multitarget stimulation protocol for optimal effectiveness is highlighted, and this personalized application might be developed in the future.

5. Conclusion

The present study is the first to use multitarget stimulation combined with concurrent SST training to explore the enhanced improvement effect of response inhibition of this protocol compared to stimulation or training alone. We found that 10 daily sessions of combined interventions and the stimulation alone

improved response inhibition, and the effects persisted for 1 month. The training alone only caused improved performance after the intervention. Furthermore, the combined protocol could modulate the performance of the individuals with high baseline response inhibition, which was not seen in the stimulation-alone condition. Notwithstanding the absence of transfer effects, it is too early to conclude that there is no transfer effect, and further studies are warranted. Thus, this study provides supportive evidence for the synergistic effect of the combined protocol. In addition, the long-term after-effect can persist for at least 1 month. Our findings also provide insights into the clinical application and strategy for treating response inhibition deficits.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Tangdu Hospital Ethics Committee, Air Force Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZG and XZ: concept and design. ZG, RQ, HQ, and HL: acquisition, analysis, and interpretation of data. ZG: drafting of the manuscript. XZ: obtained funding. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Major Project of Medicine Science and Technology of PLA (AWS17J012).

Acknowledgments

We thank all the individuals who participated in the study. We also thank MogoEdit (https://www.mogoedit.com) for its English editing during the preparation of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1107116/full#supplementary-material

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frontiers | Frontiers in Neuroscience

TYPE Review
PUBLISHED 06 April 2023
DOI 10.3389/fnins.2023.1091925

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

RECEIVED 07 November 2022
ACCEPTED 20 March 2023
PUBLISHED 06 April 2023

CITATION
He Y, Liu S, Chen L, Ke Y and Ming D (2023) Neurophysiological mechanisms of transcranial alternating current stimulation. *Front. Neurosci.* 17:1091925. doi: 10.3389/fnins.2023.1091925

Neurophysiological mechanisms of transcranial alternating current stimulation

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Neuronal oscillations are the primary basis for precise temporal coordination of neuronal processing and are linked to different brain functions. Transcranial alternating current stimulation (tACS) has demonstrated promising potential in improving cognition by entraining neural oscillations. Despite positive findings in recent decades, the results obtained are sometimes rife with variance and replicability problems, and the findings translation to humans is quite challenging. A thorough understanding of the mechanisms underlying tACS is necessitated for accurate interpretation of experimental results. Animal models are useful for understanding tACS mechanisms, optimizing parameter administration, and improving rational design for broad horizons of tACS. Here, we review recent electrophysiological advances in tACS from animal models, as well as discuss some critical issues for results coordination and translation. We hope to provide an overview of neurophysiological mechanisms and recommendations for future consideration to improve its validity, specificity, and reproducibility.

KEYWORDS

transcranial alternating current stimulation, neurophysiological mechanisms, neural entrainment, animal models, translation

1. Introduction

Neural oscillation is a prominent feature of neural activity's temporal dynamics, correlated outcomes in both the health and clinical populations have shaped the core status of brain rhythms in neuroscience over the last decade (Engel et al., 2001; Schnitzler and Gross, 2005). Numerous studies have shown that cognition functions arise from the coordination of neural activity within intra-and inter-regional brain networks, which is dependent on the successful synchronization of various neural oscillations (Siegel et al., 2012).

In the field of brain science, brain stimulation by alternating current (AC) has a long history. AC brain stimulation at lower intensities was first used in 1950s by Anan'Ev and colleagues, which is known as "cranial electrotherapy stimulation" (Anan'Ev et al., 1957). It has also been used to treat tremor, dyskinesia and dyskinesia in this century (Limoge et al., 1999; Vitek, 2008). In 1986, Chan and Nicholson found that alternating electric stimulation can directly modulate brain activity (Chan and Nicholson, 1986). In recent years, transcranial alternating current stimulation (tACS) is gaining popularity as a non-invasive brain modulation for synchronizing electrophysiological rhythms, allowing for the establishment of causal links in the oscillation-cognition relationship (Uhlhaas and Singer, 2006). The conventional tACS stimulation pattern

involves delivering weak sinusoidal currents at commonly constant frequencies through strategically placed electrodes, with appealing properties such as high operability, suitability for sham-controlled studies, and the absence of any serious adverse side effects (Ali et al., 2013; Alexander et al., 2019). Studies in animal models have been conducted to investigate the effect of tACS on neuronal polarization, which underpins the function of specific neurons and the cerebral cortex (Francis et al., 2003; Fujisawa et al., 2004; Deans et al., 2007). In humans, studies place electrodes above a targeted cortical region associated with specific functions, with the assumption that the underlying neuronal activity will be increased or decreased (Kar and Krekelberg, 2014; Riecke et al., 2015; Alekseichuk et al., 2016). Despite these findings, the mechanisms underlying the relatively high frequency-specificity remained unclear, which may limit our understanding of the temporal effect and its potential application for dynamic adaptivity enhancement (Nasr et al., 2022).

Animal models have been widely used to investigate the physiological mechanisms underlying tACS (Fröhlich and McCormick, 2010; Reato et al., 2010; Huang et al., 2021; Asamoah et al., 2022; Krause et al., 2022). In comparison to human interventions, these efforts using invasive approaches such as local field potentials (LFPs) and spiking activity of individual neurons have allowed for the direct evaluation of the effect of tACS in deep structure (Buzsáki et al., 2012). In this review, we summarize the current research on the tACS effect at the mesoscopic and macroscopic levels, as well as its possible neurophysiological mechanisms. We discuss some concerns about tACS efficacy and conclude with some recommendations with the aim of improving its robustness and replicability for future applications. We also propose potential avenues for translation to humans in order to advance our understanding of how tACS works.

2. Neurophysiological mechanism of tACS

2.1. Acute mechanism under subthreshold electric fields

In animal models, several studies have shown that the electric fields used by tACS are weak (~1 V/m), which is lower than the limited strength necessary to affect neuronal activity inside the brain (Vöröslakos et al., 2018; Asamoah et al., 2019). *In-vitro* electric field strengths as low as 0.2 V/m have been discovered to result in synchronous firing in phase with the applied stimulation (Reato et al., 2010); similar physiological effects have also been reported at an effective field value of 0.3 V/m (Deans et al., 2007). Taking in count the endogenous electric fields, Fröhlich and McCormick applied *in-vivo* electric fields at a threshold of 0.5 V/m and demonstrated that multi-unit activity (MUA) was synchronized to LFP oscillations *via* intracranial recordings from anesthetized ferrets (Fröhlich and McCormick, 2010). Subsequent *in-vitro* studies discovered that *in vivo*-like endogenous network activity influences the enhancing effect of tACS on endogenous oscillations (Schmidt et al., 2014).

To directly modulate the neuronal spike and local circuits within the target sites, a voltage gradient equal to or greater than 1 V/m is necessary, which is close to the upper limit as determined by invasive intracranial measurements in animal models (Ozen et al., 2010). The peak intensity of the applied field must be more than 4 V/m to exhibits multiunit neuronal firing (Up state) and exhibits multiunit neuronal (Down state) (Fröhlich and McCormick, 2010). In addition, a higher field intensity of 5–20 V/m is required for the cerebral blood flow alteration (Turner et al., 2021). Furthermore, the strength of the electric field necessary for specific physiological effects needs to be confirmed in both *in-vivo* and *in-vitro* studies, that can have distinct "activation" thresholds.

It is also known that a subthreshold electric field bi-directionally modulates its spontaneous spiking activity *via* resting membrane potential alterations (Deans et al., 2007; Radman et al., 2007). Besides, neurons also encode information in their temporal spiking patterns. Individual action potential temporal codes are reported to carry important information as well, and previous research has found that cortical neurons fire in synchrony with ongoing extracellular potential oscillations and task execution (Mehta et al., 2002; Harris et al., 2003). Understanding how such subthreshold electric fields affect spike timing and neural information processing in the central nervous system is therefore critical.

Several *in-vitro* investigations have been conducted to investigate the consequences of neuronal firing characteristics under subthreshold electric fields. For each 1 V/m of external field, a membrane potential change less than 0.5 mV depolarization of the cell bodies was found (Jefferys et al., 2003), and the degree of depolarization of the axon terminal is far more sensitive than that of the soma (Reato et al., 2010). Since such tiny membrane potential alternation is far below the spike initiation threshold, it is difficult to alter spiking activity in quiescent neurons under common *in-vitro* conditions. However, a 1 mV depolarization of a suprathreshold neuron can raise the firing rate by 6–9 Hz on average according to *in-vivo* investigations (Carandini and Ferster, 2000). The small but widespread depolarization contributes to a network-wide amplification of membrane potential perturbation and leads to the alternation in neuronal spike timing in the sustained networks, which are susceptible to electric fields. These depolarization amplification findings offered a potential network mechanism of tACS under subthreshold electric fields. The modulations of membrane potential and cortical excitability usually depend on the cortical excitation–inhibition balance, although the balance can be inverted by a short-duration, suprathreshold pulse-train (Khatoun et al., 2017). When these network effects are combined, the influences of a periodic AC field are not as simple as scaling frequency power in a given frequency range; they may be represented by sophisticated non-linear dynamics (Bestmann and Walsh, 2017).

In 2018, Liu et al. distinguished five mechanisms to explain the effects of tACS on neuronal and network activity: resonance, rhythmic resonance, temporal biasing of neuronal spikes, entrainment of network patterns, and imposed patterns (see Liu et al., 2018 for details). The authors stated that the physiological effect of tACS is determined by the interaction of endogenous and exogenous oscillations, and the strength of the required tACS field increases from stochastic resonance to the imposed patterns. These ideas support the existence of "response thresholds" for tACS. While these thresholds are possibly different for each specific case, this means that the tACS effectiveness is dependent on the combination of the brain region of interest and stimulation parameters including intensity and frequency. When the tACS frequency matches that of the exogenous field, the subthreshold effect of the exogenous extracellular field followed a frequency-specific resonance pattern and the endogenous oscillation can be successfully entrained (Asamoah et al., 2022). For the resonance

pattern, very weak forces with resonant neuronal properties can modulate the spike timing of target neurons near the firing threshold during each cycle, causing cumulative effects over multiple cycles (Geisler and Goldberg, 1966; Francis et al., 2003; Deans et al., 2007).

Apart from the resonance pattern, tACS at non-preferred frequencies in intrinsic network necessitates stronger periodic stimulation for successful entrainment. For example, a 2 V/m frequency-matched tACS successfully entrained the intrinsic oscillation, and yet a 4 V/m field amplitude was required when frequency was not matched (Fröhlich and McCormick, 2010). An *in-vitro* study examined the correlation between native network activity and applied electric field (Schmidt et al., 2014). The authors found that the endogenous oscillations affect the role of exogenous fields and the main mechanism of tACS is possibly boosting the natural network rather than overriding, which questioned the supposed imposed mode. However, Krause recently discovered a competition mechanism in *in-vivo* studies (Krause et al., 2022). When tACS frequency is far from the endogenous dominant frequency, tACS and endogenous oscillations compete for spike timing control, with the entrainment effect determined by how ongoing oscillations influence neural activity. In specific, entrainment is reduced when neurons are strongly locked to ongoing oscillation, and it is reduced when neurons are strongly locked to ongoing oscillation. The origin spiking activity can be reversed and controlled at higher stimulation intensities.

Mc Laughlin et al. discovered a similar phenomenon, finding that when using 1 mA tACS, entrainment relative to baseline decreased, whereas when using 2 mA tACS, a comparable amount of synchronization to the stimulation waveform at a new phase was imposed (Mc Laughlin et al., 2022). According to the findings, increasing intensity causes neurons to desynchronize and re-train to the new phase. That is, the relative strengths of entrainment to the ongoing physiological oscillation and the tACS-induced electric field influence the entrainment effect. This can be thought of as an example of an imposed mode. In the imposed mode, the applied electric field has to be in opposition to the original endogenous electric field, and this mode necessitates a higher stimulation intensity.

2.2. Lasting mechanism of tACS

Aside from neuronal entrainment, the large-scale impact of tACS is linked to alterations in neuroplasticity (Korai et al., 2021). These alterations appear to be associated with tACS after-effects that remain longer than the stimulation duration (Vossen et al., 2015). However, the effects of tACS on neuroplasticity depend on certain experimental conditions. In a mouse research, 40 Hz-tACS, 20 min per day, had a substantial effect on the long-term enhancement of synaptic transmission in Alzheimer's disease models after 2 weeks (Jeong et al., 2021). The study discovered that changes in protein synthesis, such as brain-derived neurotrophic factor (BDNF), are required for long-term plastic changes. There is an assumption that tACS causes neuroplasticity changes *via* long-term potentiation (LTP) and long-term depression (LTD; Zaehle et al., 2010). However, the direct induction of LTP and LTD in the context of tACS is still unclear. The potential effect of tACS in BDNF changes has been highlighted, as this neurotrophin can boost LTP by increasing synaptic responsiveness to high-frequency stimulation and physically by enhancing dendritic spine and arborization to facilitate synaptic transmission (Figurov et al., 1996; Amaral and Pozzo-Miller, 2007).

Human studies are also being conducted to investigate the role of BDNF-dependent plasticity in the after-effects of tACS. However, this effect now appears to be frequency dependent. According to one study, the Val66Met polymorphism, a single nucleotide polymorphism at codon 66 (Val66Met) in the BDNF gene, modulates the tACS effect in target oscillations under alpha tACS (Riddle et al., 2020). Furthermore, it was discovered that 20 Hz beta-tACS can induce NMDAR-mediated plasticity in the motor cortex and enhance cortical excitability as well as beta oscillations for at least 60 min (Wischnewski et al., 2019). Similarly, a human research discovered that after 20 min of tACS at the individual alpha frequency, the boosted alpha power can last for 70 min, compared to the sham-stimulation group (Kasten et al., 2016). However, the phenomenon was not detected using gamma tACS (Giustiniani et al., 2021). Fifty Hertz gamma-tACS was not successful in inducing an after-effect modulating sport performance in this study on healthy sports participants.

Furthermore, the intracranial electric field has been shown to affect glial cells and neurotransmitters in research on transcranial direct current stimulation (tDCS) (Monai et al., 2016; Gellner et al., 2021). To our knowledge, the effect of tACS on glial cells and neurotransmitters has yet to be investigated. It is still debatable whether after-effects are induced solely by neural plasticity or by a combination of neural plasticity and entrainment.

3. Factors influencing tACS efficacy

3.1. Detection methods

Detection methods play an important role in understanding tACS efficacy. For example, steady-state brain responses can be used to investigate the phase specificity of tACS. Previous research discovered that tACS had a long-lasting phase-specific enhancing or suppressing effect on steady-state brain responses (Fiene et al., 2020; Haslacher et al., 2022; Krause et al., 2022). When compared to spontaneous ongoing activity, tACS is expected to alter the phase of evoked brain activity with more difficulty as the steady-state signals always show dominant phase locking to rhythmic stimulation.

tACS neurophysiological studies typically employ inspection window lengths that correspond to the length of the entire stimulation period (Ozen et al., 2010; Asamoah et al., 2019; Krause et al., 2019). Mc Laughlin et al. discovered that neural entrainment detection is highly dependent on the observation window and epoch length (Mc Laughlin et al., 2022). Long epoch lengths, in particular, can detect entrainment while shorter windows cannot. When data collection time is limited, the researchers suggest that optimizing tACS paradigms to have fewer repetitions, but longer epoch durations will increase the likelihood of detecting an entrainment effect. Moreover, Haslacher et al. discovered a transient enhancement and suppression of oscillatory activity, as well as accomplishing millisecond-precise modulation of oscillations using a closed-loop approach, which provides an idea for reconciling the extensive variability of tACS (Haslacher et al., 2022). This predicts that standardization and refinement of spatio-temporal detection accuracy in detection methods will be beneficial for further investigation of the tACS effect.

3.2. Brain state

Much of the discussion regarding the validity of neural stimulation efficacy is linked to the state of endogenous oscillations (Bradley et al., 2022). tACS was shown to be capable of controlling transitions between different activity states (Kutchko and Fröhlich, 2013). A small periodic input, as stated in the resonance pattern, can cause neuron entrainment at the matched stimulation frequency (Riddle et al., 2022).

The role of endogenous oscillations in tACS effect is embodied in not only the degree to which neurons are entrained but also the phase difference between tACS and endogenous oscillations (Fiene et al., 2020; Haslacher et al., 2022; Krause et al., 2022). Evoked brain potentials have been used to study phase-dependent enhancement and suppression of endogenous oscillations (Fiene et al., 2020). These findings altogether suggested a dynamically adjusted protocol based on the current brain state. Recently, the closed-loop approach which allows for phase-locked to endogenous oscillations to selectively enhance or suppress ongoing activity, has been shown to improve modulation effects and robustness of tACS (Frohlich and Townsend, 2021; Haslacher et al., 2022; Nasr et al., 2022). By online adjustment of stimulation parameters, this brain-state dependent closed-loop protocol is expected to achieve dynamic adjustment and precise modulation.

However, the closed-loop protocol is challenging given the large artifacts caused by simultaneous signal acquisition and stimulation. There are efforts underway to carefully separate stimulation artifacts from physiological signals. Noury et al. proposed a mathematical model for the transfer function based on the amplitude and phase properties of stimulation artifacts (Noury et al., 2016; Noury and Siegel, 2017, 2018). Witkowski et al. used magnetoencephalography (MEG) in conjunction with synthetic aperture magnetometry (SAM) and successfully reconstruct responses during amplitude-modulated tACS (Witkowski et al., 2016). In addition, Ketz et al. found that pausing stimulation for a few seconds allowed for signal reconstruction in electroencephalogram (EEG) when trying to target low-frequency oscillations (Ketz et al., 2018). Later, Haslacher et al. used stimulation artifact source separation (SASS) to separate EEG signals from artifacts (Haslacher et al., 2021, 2022). While strategies for rejecting artifacts in other situations are still being investigated.

The neural entrainment from tACS can be shaped as "Arnold tongues" (Frohlich and Riddle, 2021). As shown in Figure 1A, the inverted triangle shapes the possible entrainment areas under specific stimulation intensities and frequencies and explains the dynamics between endogenous oscillation and tACS field (Ali et al., 2013; Frohlich and Riddle, 2021). The entrainment area is centered on the intrinsic frequency of the stimulated network and radiates to the surrounding bands. Moreover, as the stimulus intensity increases, so does the range of entrainment and with a broader range. Recently, the Arnold tongues were observed in an *in-vivo* study on awake ferrets (Huang et al., 2021). In this study, triangular tongues were demonstrated by the synchronization map between single-units and tACS, implying that particular parameter combinations of tACS give a reasonable approach for mode design.

Most *in-vivo* experiments are conducted in anesthetized animals. Anesthesia, on the other hand, can alter neural dynamics and brain metabolism (Paasonen et al., 2018). Given the change in network structures with awake states, care should be taken when translating or comparing these findings to human studies (Krause et al., 2022). As shown in Figure 1B, the endogenous oscillation can be increased when tACS is precisely matched to the dominant frequency, whereas it can be reduced even with minor frequency detuning. Entrainment was found to be increased when the stimulation intensity exceeded about 66% of the amplitude of the ongoing oscillation. In awake states, endogenous networks may reflect more complicated oscillations, inadvertently strengthening or weakening the effects of externally applied tACS fields (Laufs, 2008; Johnson et al., 2020).

Generally, a detailed state assessment may be required during the stimulation process. During a rodent study, Khatoun et al. checked the anesthesia level by checking the toe-pinch reflex and promptly provided intraperitoneal drug perfusion to ensure a relatively stable oscillation structure during the stimulation process (Khatoun et al., 2017). It is possible that the same stimulation protocols will produce different electrophysiological responses depending on the current state of the brain. Thus, the reproducible results caused by uncontrolled state-dependency phenomenon increase the difficulty of obtaining reliable stimulation outcomes. This can be explained by the aforementioned response thresholds and the entrainment area in varied Arnold tongues under changing endogenous structures.

3.3. Factors influencing entrainment

The specificity of the tACS effect is an important premise for therapeutic applications, as it can provide relative target modulation within neuronal circuits (Kanai et al., 2008; Ali et al., 2013). The field primarily affected the spike of neurons beneath montages based on the expected settings (Figure 1C). Apart from spatial specificity, a frequency-specific pattern was found. As shown in the right column of Figure 1D, entrainment of neurons beneath target region only significantly increased at or around the tACS frequency (Krause et al., 2019). Furthermore, the study revealed spike timing entrainment in a dose-dependent manner (Johnson et al., 2020; Figure 1E). This finding was consistent with the network perspective discussed previously. In addition to the single entrainment within the region of interest, a unique cross-frequency phase-amplitude coupling (PAC) stimulation pattern has emerged (Helfrich et al., 2016; Jones et al., 2020; Grover et al., 2021). tACS can manipulate inter-regional phase synchronization and yield cross-frequency coupling between endogenous and exogenous activity in this manner, providing a unique application for tailored stimulation. Grover et al. recently reviewed the likely mechanisms underlying the tACS effect on PAC and associated therapeutic applications (Grover et al., 2021); they will not be covered in detail here due to space and scope constraints.

One study revealed that cortical excitation varied non-linearly with increasing intensity of 140 Hz tACS (Moliadze et al., 2012). In addition, subsequent research discovered a lower effectiveness in generating membrane polarization at higher stimulation frequency (Deans et al., 2007; Khatoun et al., 2017). These findings appear to point to a mechanism of mutual cancelation of inhibitory and excitatory effects. Considering the high firing frequency determined by repolarization and lower membrane time constant in inhibitory neurons, they may be more sensitive to 140 Hz tACS at lower intensities than excitatory neurons. The effect of tACS on cortical excitation necessitates systematic titration of stimulation parameters, and non-linear modulation deserves careful consideration, given the change in the time required for a neuron to cross the threshold for action potential generation caused by tACS (Radman et al., 2007).

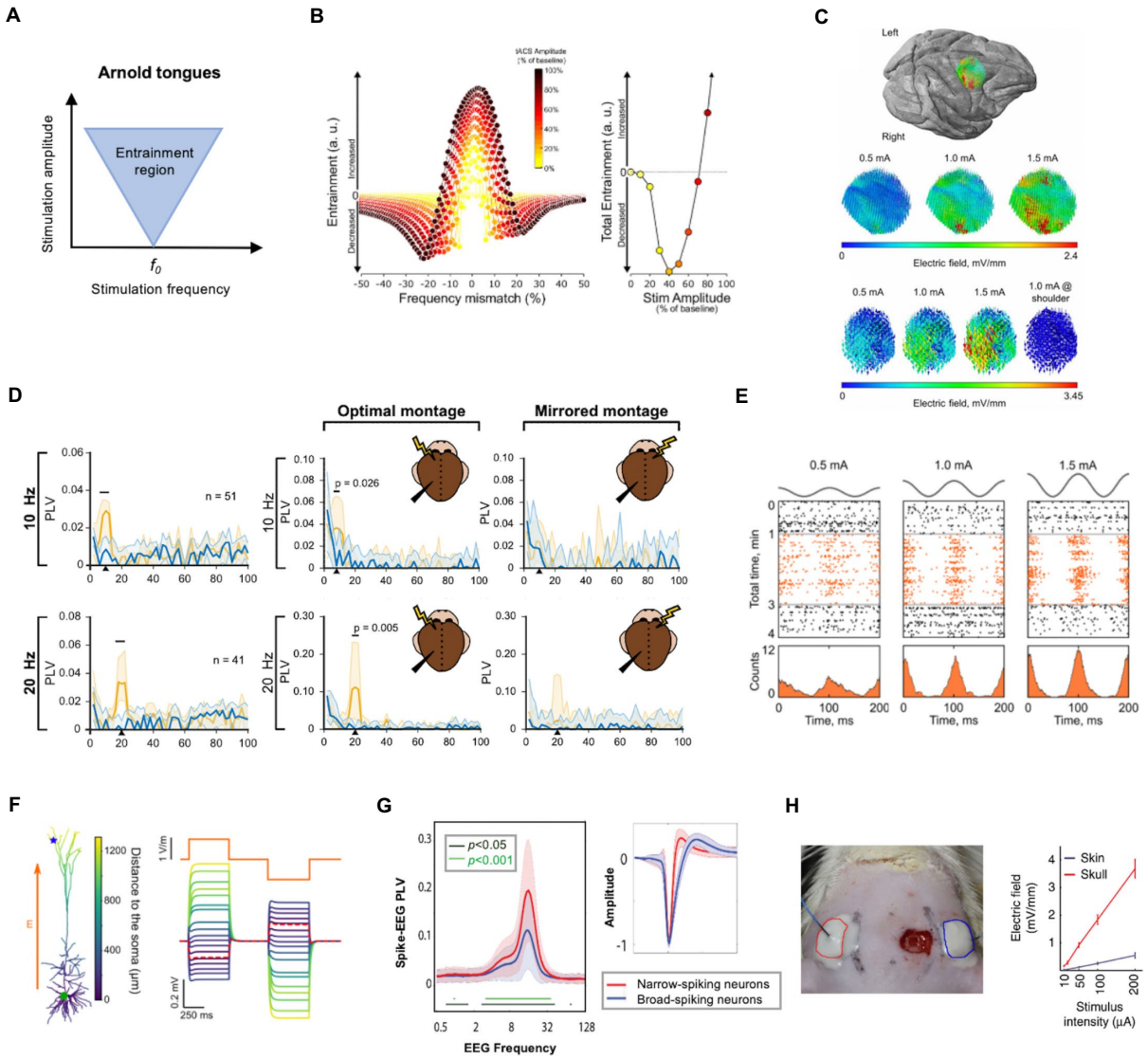

FIGURE 1
Neurophysiological mechanisms and essential factors for tACS. **(A)** Illustration of Arnold tongue. The inverted triangle shapes the possible stimulation amplitude and frequency parameter combinations. **(B)** The entrainment changes at different combinations of frequency mismatch and stimulation intensity (left) and total entrainment are calculated by integrating the left curves within 2Hz bins (right). It is illustrated that enhanced oscillation occurs when the stimulation frequency closely matches the endogenous oscillation; however, even a minor mismatch can result in decreased oscillation when the stimulation amplitude is relatively weak. **(C)** Electrical field intensity distribution in one monkey on the target brain area. The field distributions had the same orientation and relative spatial relationships as the intensity increased linearly from 0.5 to 1.5mA. **(D)** Phase-locking value (PLV) spectra of target neurons during tACS at 10 and 20Hz during sham (blue line) and active tACS duration (orange line). The tACS effects of target neurons are specific to the stimulation frequency (left column) and location (right column). Only around the stimulation frequency did neural entrainment occur at each frequency (horizontal black lines indicate significant bands). Furthermore, when compared to the contralateral side, tACS on the ipsilateral side of signal recording showed significant entrainment. **(E)** tACS-induced entrainment of one representative neuron from an awake monkey at 0.5, 1, and 1.5mA intensities. The spike rate (lower row) and time course (upper row: black dots for pre–/post-stimulation duration, orange dots for stimulation duration) revealed an increase in neuron spikes and clustering to the peak of the sine wave as the intensity increased during the stimulation period, indicating a dose-dependent manner. **(F)** Electric field sensitivity in a passive pyramidal cell model (the blue star represents the apical dendrites, and the green circle represents the soma). The orange line represents the stimulation current. The figure depicts how the induced field differed from neuron morphology. **(G)** The synchrony between network-scale oscillations and interneurons varies according to neuron type. Right: Two types of neurons were identified based on spike characteristics: narrow-spiking (red line) neurons and broad-spiking neurons (blue line). Narrow-spiking neurons had higher PLV than broad-spiking neurons. **(H)** A diagram of transcutaneous stimulation in rodent research (left) and a comparison of the electric field values of transcutaneous and subcutaneous stimulations at the same stimulus intensities (right). The field value is heavily influenced by the stimulation pattern. Subfigures B, C, D, E, F, G and H have been adapted with the authors' and publisher' permission.

According to the current state of cortical excitation-inhibition balance, a weak external electric field is more likely to affect neurons close to the threshold and synchronize their spiking time. Together, these results suggest that frequency-matched oscillatory electrical fields mostly affect the temporal structure of the neural activity without major changes in the overall activity level.

However, increased power after tACS in the target region is widely regarded as evidence of successful neuronal entrainment (Vinck et al.,

2010; Hill et al., 2011). The direction of the applied AC field influences the modulation pattern of neuronal firing, and the entrainment effect is also related to the direction of the electric field. Previous research has confirmed the definitive role of entrained neurons' biophysical properties, such as morphology, phase preference, and orientation (Aspart et al., 2018; Toloza et al., 2018; Tran et al., 2022; Figure 1F).

Neuron type is another factor that may influence tACS responsiveness. An alpha-tACS study in awake head-fixed ferrets revealed that synchrony between field oscillations and single-unit spikes was stronger in narrow-spiking neurons than in broad-spiking neurons, possibly due to stronger endogenous coupling between fast-spiking inhibitory interneurons and alpha oscillation (Huang et al., 2021; Figure 1G). These findings lay the foundation for prominent entrainment of target neurons as well as network-scale oscillations during transcranial stimulation. A tACS study on morphologically realistic neurons also suggested that the applied electrical field may primarily target large pyramidal neurons (Tran et al., 2022). Overall, precise neural entrainment is a significant challenge that limits the replicability of tACS.

4. Concerns about tACS effectiveness

The assumption that tACS of the human brain works similarly to animal experiments is risky. Transcutaneous delivery is the most common application of tACS in humans, whereas animal models are always subcutaneous. Under transcutaneous pattern, only a small portion of the applied current enters the deep brain structure. According to Vöröslakos et al., the electric field on the scalp is significantly stronger than that in the cortex. They demonstrated in this study that nearly 75% of the scalp-applied current was attenuated in the tortuous gyrus of the brain in human cadavers (Vöröslakos et al., 2018). The authors also conducted *in-vivo* experiments on rats, delivering subcutaneous and transcutaneous electric stimulation *via* similarly sized electrodes as in the human samples, and they reported an 80 ± 5% current loss under the transcutaneous condition regardless of current intensity (Figure 1H).

Likewise, Ozen et al. delivered electrical fields to rodents *via* electrodes on the surface of the skull while simultaneously recording intracranial neural activity (Ozen et al., 2010). They observed an increase in the percentage of phase-locked neurons to external stimulation as the intensity increased. The electric fields inside the brain are large enough to modulate brain activity even if the majority of current is lost due to shunting. However, the differences in effective stimulation intensity with various tACS delivery approaches should be evaluated further. Given the massive current shunting, the subcutaneous approach should provide smaller stimulation intensity than the transcutaneous approach.

However, concerns have been raised regarding potential confounders (Raco et al., 2014). The physiological response could be caused by peripheral nerve stimulation or other peripherally mediated effects such as retinal stimulation, and due to the high conductivity of the eyes and a relatively low-resistance pathway, both close and distance montages could induce a current to the retina (Laakso and Hirata, 2013). Indeed, even with a small fraction of the total current, the visual information distribution and processing during stimulation are sufficient to generate subjective sensations. Such sensations are known as phosphenes, and they are a common side effect of stimulation at 10–20 Hz (Kanai et al., 2008; Kar and Krekelberg, 2012). Individual stimulation intensity below the phosphene-threshold is one available method in human studies to avoid that phenomenon (Pogosyan et al., 2009; Feurra et al., 2011). Considering the amount of visual processing influences phosphene perception, stimulating in a brighter environment or with eyes open and administering a visual task during stimulation may be beneficial for weakening phosphene perception (Ahn et al., 2019; Alexander et al., 2019; Frohlich and Riddle, 2021). It is also reported that amplitude-modulated tACS (AM-tACS) showed no phosphene with stimulation intensities of up to 2 mA (Thiele et al., 2021).

Thus, neurons may be entrained by upstream areas rather than by the current directly present in the area of interest. Asamoah et al. recently distinguished the transcranial and transcutaneous mechanisms of tACS in rodents and human volunteers (Asamoah et al., 2019). They conducted four separate experiments by selectively blocking the pathways and found that the tACS directly affect the peripheral nerves while having an indirect effect on motor cortex activity. Combined with the transcranial-only results, which showed that the weak electric field generated by tACS at around 1 V/m can cause significant entrainment in cortical neurons, there are still significant challenges in explaining tACS effects in nonmotor systems.

In response to the above complications, recent studies on non-human primates have provided some support for the efficacy of tACS. Krause et al. created two montages of recording and stimulation sites that produced equivalent stimulation through the retinal area by reflecting an optimal and mirrored pattern (Krause et al., 2019). They found that neural entrainment occurred only at the optimized pattern, not the mirrored one. Because the mirrored montage should produce a similar sensation, the absence of spiking activity modulation in that case rules out the possibility that the effects of neuronal activity using the optimal montage are indirect. Similarly, Johnson et al. conducted a control block by peripherally mediated effects in awake primates (Johnson et al., 2020). When tACS electrodes were attached to the right upper arm, there was no entrainment, and this finding favored the direct effect of tACS on the brain. In line with these findings, Vieira et al. devised a novel experiment in which somatosensory input was blocked by applying a topical anesthetic to the skin surrounding each stimulation site (Vieira et al., 2020).

5. Translation

5.1. Translation across species

Although field values below 1 V/m are considered effective as above mentioned, however, voltage gradients described in animal research cannot be directly compared to human investigations. Disparities in brain volume, anatomy, and skull thickness can all have a significant impact on physiological effects, posing significant challenges to translation. In fact, the field strengths in animal models are several times larger than values reported in human studies. In non-human primates, Krause et al. found that when two macaque monkeys were given 2 mA tACS (4 mA peak-to-peak amplitude) through personalized electrode montage during an arousal and motivational state, the peak electric field strength in the hippocampus and basal ganglia was 0.28 V/m in one monkey and 0.35 V/m in the other (Krause et al., 2019). Moreover, Johnson et al. reported

comparable field strength in awake primates (Johnson et al., 2020). It was discovered to be slightly stronger through invasive measurement, reaching as high as 1.33 V/m during 1.5 mA tACS. These studies took great care in measuring electric fields and ensuring that the amplitudes used were comparable to human studies.

In humans, *in-vivo* intracranial attempts are being made to determine the spatial and temporal distribution of intracranial electric fields induced by tACS, which provide a valuable picture of how the applied alternating current flows in the brain. In 2016, Opitz et al. used stereotactic EEG to measure the spatial distribution of applied electric fields (Opitz et al., 2016). Maximum field strengths in human brains can reach 0.36 V/m in one participant and 0.16 V/m in the other for 1 mA stimulation currents. In 2017, Huang et al. expanded the sample size to 10 humans and provided extensive field estimates of the entire brain in conjunction with calibrated modeling (Huang et al., 2017). They found that the maximal electric field values are around 0.4 V/m and 0.16 V/m in more extended regions under 2 mA scalp current, which is the generally reported maximum stimulation strength in human research. Moreover, Louviot et al. recently demonstrated similar field distributions while investigating the electrical field in deep brain structures using high-density tACS (HD-tACS) (Louviot et al., 2022).

In a comparative study, computational modeling with finite element models (FEM) was applied across studies in animals and humans, and it was discovered that field strength was inversely proportional to head size (Alekseichuk et al., 2019). Compared to rodents, field values in non-human primates with larger head sizes were relatively comparable to humans under matched stimulation conditions, with a value difference of around 1 V/m (Figure 2). This finding allows for a quantifiable scaling metric to enable intuitive comparisons between human and animal models in translational studies.

5.2. Translation across techniques

Another barrier to translation is at the technical level, where results obtained through several methodologies might be difficult to translate directly. Some tACS *in-vitro* studies demonstrated electric field magnitudes of up to 20 V/m, which is 10–20 times greater than the electric field observed in *in-vivo* studies (~1 V/m) (Chan and Nicholson, 1986; Deans et al., 2007). Parallels between these two approaches should be drawn with caution. Computational models are anticipated to offer a thorough understanding of how the applied current diffuses from the stimulation site to the entire brain in this context and to bridge the gap between different levels of observation (Bai et al., 2013; Laakso and Hirata, 2013). The current diffusion process assists us in understanding the mechanism of action of tACS and provides an answer to the question of how tACS could alter brain activity effectively and reproducibly.

The increasingly advanced computational models certainly assist in understanding which regions are most stimulated by certain stimulation patterns, the proportion of stimulation diffusion, and which regions are unaffected by a particular electrode montage. The ideal electrode design is then selected based on the predicted field distribution (Dmochowski et al., 2011; Ruffini et al., 2014). While, more emphasis is suggested to place on the distribution of the electric field under different stimulation conditions, for example, the relative value of the field strength. To achieve a viable tACS application, it has been suggested that the electrode placement accuracy be less than 1 cm (Opitz et al., 2018).

Besides, modeling studies rely heavily on the construction of three-dimensional head models and the determination of conductivity (Saturnino et al., 2019; Louviot et al., 2022). To acquire the electrical field distribution, the head modeling requires *in-vivo* validation. Huang et al. found that a full-head clinical magnetic resonance imaging (MRI) scan, from neck to crown, is required to get reliable findings (Huang et al., 2017). Furthermore, while calibrating using *in-vivo* intracranial recordings, the authors discovered that variances in skull layers or conductivity variations induced by current direction in white matter had no effect on accurate model prediction. However, there are still doubts regarding whether the computational models based on these conductivities can truly give reliable information for improving electrode location and electric field distribution, which must be confirmed in *in-vivo* investigations (Kasinadhuni et al., 2017).

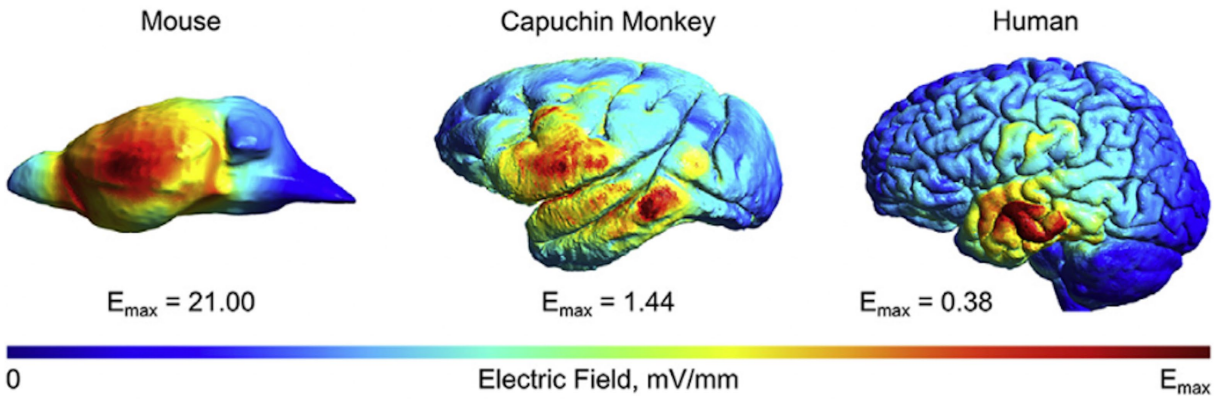

FIGURE 2
Different field values across species. A comparison of the normalized electrical fields induced by transcranial electrical stimulation in mice, monkeys, and humans. The electrode montage was identical across species, and the current intensity was set to the same level. It can be seen that the maximum field strength of the three models decreases exponentially with increasing brain volume. The figure has been adapted with the authors' and publisher' permission.

6. Directions for future studies

For future applications of tACS, a comprehensive knowledge of tACS, including electrophysiological effectiveness and rational experimental design, is required (Figure 3). We summarized various prospective perspectives in the domains of innovative and individual stimulation patterns, with the goal of concluding with suggestions for optimal modulation.

6.1. Novel stimulation protocol

The conventional saline-soaked sponge electrodes of square centimeter scale in human research may lead to more current being shunted, and increases the likelihood of inducing skin sensation (Turi et al., 2014). From this perspective, a focused and small montage is likely to weaken peripherally mediated effects by limiting the shunted current (Khatoun and Mc Lauglin, 2017). Numerous unique tACS stimulation patterns have appeared during the past 10 years, including high definition tACS with muti-montage around the central single electrode and the ring montage made up of a tiny center electrode and an encircling ring electrode (Preisig et al., 2021). These new paradigms encourage field focality, and allow for adequate management of the spatial peak fields around the target area (Saturnino et al., 2017). There is also great effort being put into developing tACS protocols that stimulate target brain regions, such as high-density transcranial alternating current stimulation (HD-tACS) (Helfrich et al., 2014) and temporal interference stimulation (Grossman et al., 2017), to improve the spatial specificity of tACS.

The customizable current waveform is essential for temporal intention given the large parameter space for tACS. For example, random noise, AM-tACS and non-sinusoidal current emerge for target neural rhythm (Terney et al., 2008; Fröhlich and McCormick, 2010; Negahbani et al., 2018). To comprehend the electrophysiological response foundation of novel stimulation modalities, as well as non-neuronal possibilities such as neurotransmitter metabolism, trophic factors, and immune system components, further experiments in animal models are required (Liu et al., 2018).

6.2. Personalized stimulation strategy

Before undertaking human investigations, it is very desirable to expand clinical applications of effective medicines in pre-clinical animal models. To optimize electrode positions for a desired field distribution, computational models could therefore make use of data from animal and human studies (Dmochowski et al., 2011; Sánchez-León et al., 2018). MRI models that are specifically tailored to the particular patient may be useful. Wang et al. observed that with the

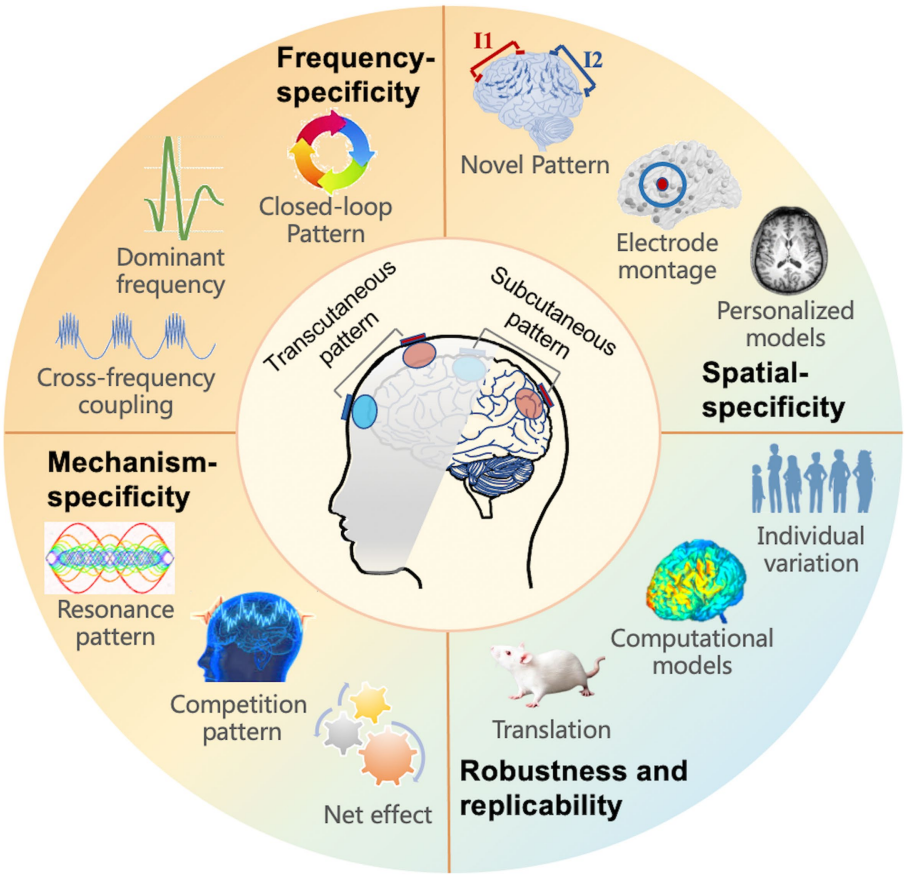

FIGURE 3
Future directions for tACS, including four aspects to take into account: frequency, spatial, mechanism-specificity, as well as robustness and replicability. The subfigure introduces the implicated or prospective study directions.

same tACS intensity, a personalized simulation pattern accurately predicted the electrical field (Wang et al., 2021).

Individual variation in neuroanatomy (scalp, muscle, and skull thickness and scalp-to-cortex distance), cortical excitability, and specific inhibitory and excitatory circuits of local networks, each of which may change susceptibility to an external electric field, is a major factor altering the modulation effect between individuals (McConnell et al., 2001; Krause and Cohen Kadosh, 2014; Kasten et al., 2019). According to studies, the induced electric field and inherent oscillation properties can account for between 54 and 65% of the variability in the tACS effect (Zanto et al., 2021). According to our research, human participants with lower endogenous activity can benefit more from particular tACS, whereas subjects with beginning performance following stimulation showed mild or even negative alterations (Liu et al., 2022).

The peak frequency of the intended endogenous oscillation might be used as the stimulation frequency for each participant as an additional strategy to reduce individual variations because the dominant frequency of endogenous oscillations differs between individuals (Chiang et al., 2011; Ruhnau et al., 2016). Likewise, it is important to take into account the stimulus goal as a consideration. A recent research on a patient with depression used customized intracranial brain stimulation to target a particular circuit and discovered dependable mood improvements (Scangos et al., 2021). This method enables the replication of findings between species and laboratories. Additionally, the psychological condition of patients should be taken into consideration while evaluating the value of tACS, since the way in which stimulation is perceived subjectively can significantly affect how it works. Although this is reasonably simple to detect in people, it is more difficult to notice in animal models, which presents difficulties for efforts at cross-species and cross-laboratories translation.

7. Conclusion

In recent decades, research has demonstrated the important role of neural oscillations in information exchange and transmission between brain networks. Over the last 10 years, tACS has emerged as an indispensable neuromodulation tool for understanding the link between behavior and brain oscillations. tACS has demonstrated a unique role in clinical intervention and improvement of cognitive function. However, the electrophysiological mechanisms of tACS are still unclear, and further exploration and understanding of micro-mechanisms are necessary from animal models. It is worth mentioning that the spatial and temporal targeting is a fundamental stage in the application for the treatment of psychiatric illness. Preclinical experiments are also necessary to enable parameter titrations and customized stimulation techniques. While inconsistent stimulation settings and tactics may be the main cause of inconsistent translation outcomes between laboratories and species, improving this pipeline will be essential for improving the possibility of translating research from animal models to people.

We carefully examined the neurophysiological mechanisms behind tACS in this review. The application of animal models opens up new avenues for human study by enabling the validation and back-translation of human findings, which, in theory, will result in innovative treatment methods. Future research should concentrate on understanding the very complicated mechanisms behind common brain illnesses as well as non-invasive treatment approaches. It is particularly important to take into account the security and protection of study animals, necessitating that experimenters balance animal welfare and create animal models with a high level of translational validity (Homberg et al., 2021).

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

This work was funded by National Natural Science Foundation of China (No. 81925020 and 19JCYBJC29200).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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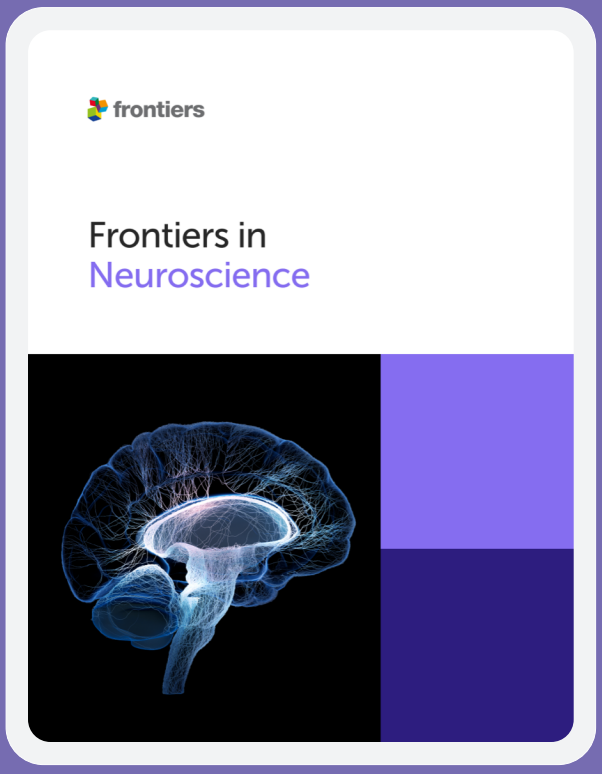

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