NON-INVASIVE BRAIN STIMULATION IN THE STUDY AND MODULATION OF METAPLASTICITY IN NEUROLOGICAL DISORDERS

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EDITED BY: Mariagiovanna Cantone, Giuseppe Lanza, Federico Ranieri, George M. Opie and Carmen Terranova

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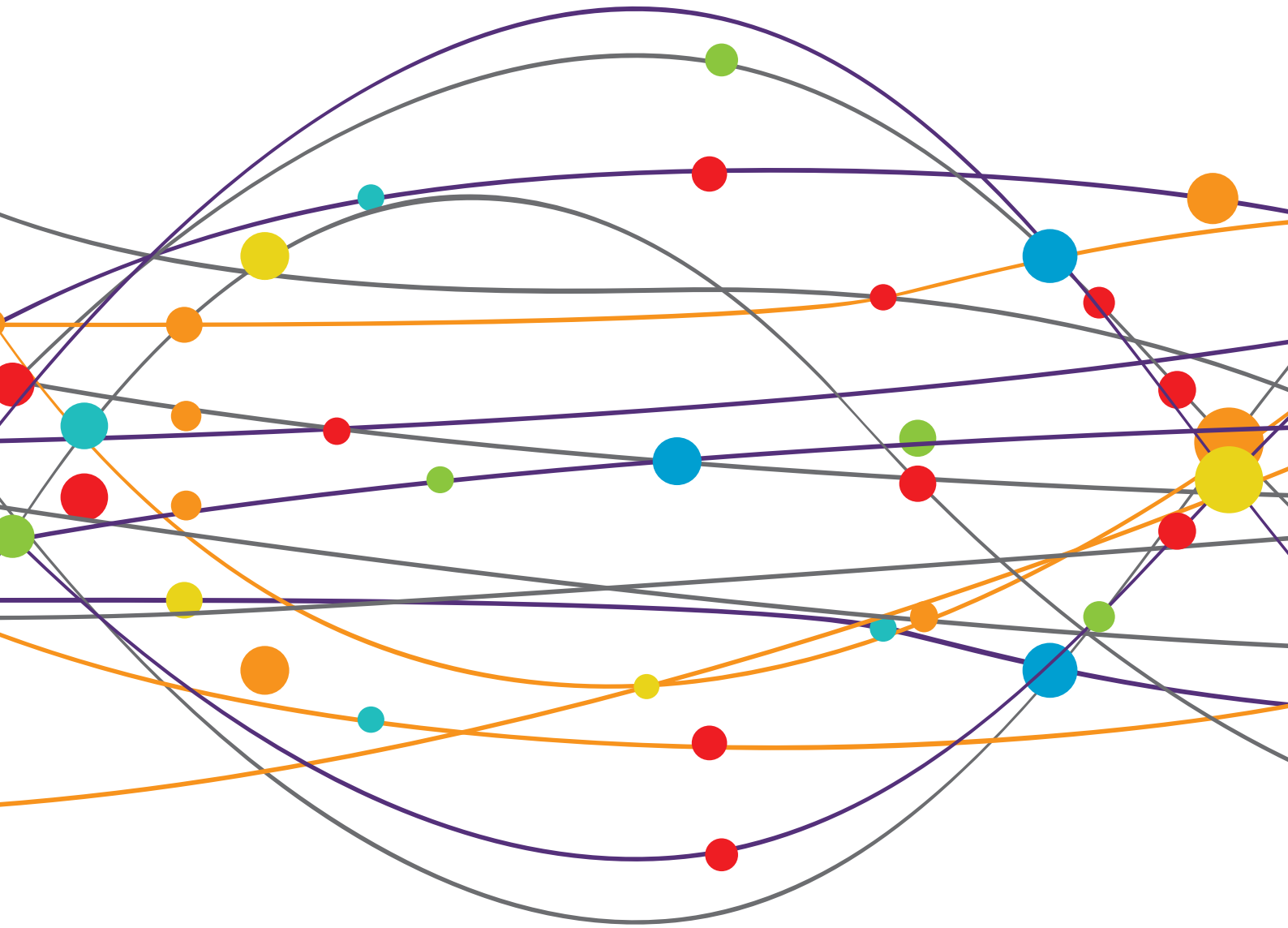

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NON-INVASIVE BRAIN STIMULATION IN THE STUDY AND MODULATION OF METAPLASTICITY IN NEUROLOGICAL DISORDERS

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EDITORIAL
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Editorial: Non-invasive Brain Stimulation in the Study and Modulation of Metaplasticity in Neurological Disorders

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Keywords: transcrancial magnetic stimulation, direct current stimulation, neuroplasicity, stroke rehabilitation, depression treatment

Editorial on the Research Topic

Non-invasive Brain Stimulation in the Study and Modulation of Metaplasticity in Neurological Disorders

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This Research Topic, which consists of 8 articles by a total of >40 authors, addresses different aspects of metaplasticity in acquired neurological and psychiatric disorders. Metaplasticity refers to the activity-dependent modulation of synaptic plasticity. This pivotal determinant of learning, memory, and other functions represents a higher order of synaptic plasticity that acts on the threshold for modifying synaptic strength (1). However, our understanding of the cellular and molecular mechanisms underlying distinct forms of synaptic plasticity, including metaplasticity, remains limited. Moreover, impaired synaptic plasticity, the so-called "maladaptive plasticity," has been associated with the pathogenesis and trajectory of several brain diseases, including contributions to the dysfunctional remodeling of underlying neural networks (2–5).

Given its role in regulating synaptic plasticity, alterations to metaplastic mechanisms are likely to represent an important element of many neurological disorders. Until relatively recently, though, investigation of these processes was limited to invasive techniques in animal models. However, the development of non-invasive brain stimulation techniques (NIBS) has meant that it is now possible to induce and modulate metaplasticity in human subjects. Excitingly, there is a rapidly growing constellation of novel interventions that have been developed using NIBS, many of which are showing promise as therapeutic tools for treating neurological and neuropsychiatric disorders, despite our still limited understanding of the contribution made by metaplasticity. In support of this, the study by Thomson and Sack reviewed studies utilizing transcranial magnetic stimulation (TMS, a form of NIBS involving magnetic pulses applied over the scalp) to study and modulate metaplasticity, with specific interest in clinical applications. In particular, they focused on the use of repetitive TMS (rTMS) with intermittent theta burst stimulation (iTBS) and continuous TBS, as these are two of the most known and applied stimulation paradigms within research and clinical settings. After reviewing the relevant literature, the authors concluded that there is indeed a great potential to develop metaplasticity-based treatments to induce or restore a desired level of synaptic plasticity. They further identified accelerated iTBS at longer intervals (60 min) as being of particular

interest, as it seems to maximize metaplasticity effects and clinical outcomes.

While TMS was the original NIBS technique to be used for the investigation of metaplasticity, the more recently developed transcranial direct (tDCS) and alternating (tACS) current stimulation, both of which involve low intensity electrical stimulation to the scalp, have also become widely applied within this field. In the study by Korai et al., the neurobiological mechanisms underlying the after-effects of tDCS and tACS was therefore reviewed. The authors discuss that, in contrast to TMS, these forms of NIBS do not produce action potentials in affected tissues. Instead, they modulate membrane potential within a sub-threshold range, and this leads to consequent changes in synaptic transmission. The role of meta-plasticity in mediating these effects is further discussed by the authors. In particular, the way in which synaptic efficacy is effectively modulated only when concurrent neuronal discharge take place (6, 7). This opens new insights on rehabilitation protocols based on concomitant NIBS and training-induced neuronal activation.

Although applied broadly across many clinical domains, there has been a preponderance of NIBS-based research in the area of stroke. In particular, the development of interventions able to promote functionally beneficial patterns of brain activity in stroke patients has been common, and this approach likely involves metaplastic mechanisms. In an alternative take on this goal, the study by Hamaguchi et al. instead aimed to identify if it is possible to predict participants that will benefit from a combination of NIBS and occupational therapy (OT) (i.e., "responders") based on pre-treatment functional scores. In 1,254 patients with upper extremity post-stroke paralysis, the authors therefore assessed if the response to low frequency (i.e., 1 Hz) rTMS applied to the contralesional primary motor cortex (M1) immediately prior to OT could be predicted by pretreatment Fugl-Meyer Motor Assessment (FMA) scores of the upper limb. The intervention showed a facilitation of muscle movements by the rTMS-modulation of M1 excitability. Moreover, the probability of being non-responders was 59.2% when the initial FMA score was 48.9, whereas when the initial score was 38.8 the incidence of responders and hyper-responders was 45.5 and 16.0%, respectively. Notably, ∼45% of the patients with FMA scores from 30 to 40 before treatment improved, and even >25% of those with more severe initial values. Overall, these results suggest that pretreatment assessment can estimate the possibility of a patient's recovery in the chronic phase, with relevant implications for therapists and patient's compliance and cooperation.

Using a slightly different approach that nonetheless highlights the utility of combining NIBS with functional interventions in stroke patients, the study by Zhong et al. tested how the site of stimulation influences recovery from dysphagia in subacute stroke patients. Specifically, the benefit of 5 Hz-rTMS combined with standard sensory-motor rehabilitation of dysphagia was compared when applying stimulation to the M1 and cerebellum of both affected and unaffected hemispheres. They reported that, relative to a non-stimulated control group, 2-weeks of combined stimulation and training resulted in improved recovery, and this was consistent across all sites of stimulation. This implies that rTMS may have stimulated the training-induced plasticity involved in swallowing control, possibly by acting on different circuits, although the specific pathomechanisms need to be clarified.

While a large amount of the literature utilizing NIBS in stroke has been focused on improving motor symptoms, the interesting study by Fray et al. instead evaluated the use of intense rTMS to treat post-stroke depression (PSD). In six subacute stroke patients, high-frequency (20 Hz) rTMS was applied over the left dorsolateral prefrontal cortex (DLPFC) during five sessions per day and over 4 consecutive days (20 sessions in total). At the end of the procedure and after 3 months, scores of depression significantly decreased, without any procedure-related adverse event. The authors concluded that, despite the small sample size of this pilot study, intense rTMS may be a safe and effective alternative or adjunctive therapy for PSD patients.

In further support of the cognitive benefits that are achievable when applying NIBS in the clinic, the elegant study by Sumiyoshi et al. determined whether tDCS improves semantic memory in schizophrenia patients, assessed using text-mining analyses of category fluency data. Indeed, semantic memory deficits have been previously reported in schizophrenia and associated with negative symptoms and quality of life. In 28 schizophrenia patients, cognitive assessment was carried out at baseline and 1 month after tDCS, which was performed twice per day for five consecutive days, with the anode electrode over the left DLPFC and cathode electrode over the right supraorbital area. After multi-session tDCS, the authors observed a normalization of semantic associations. The left prefrontal region is assumed to be related to the ability of tDCS to improve the organization of information and retrieval of clustered words, thus supporting the role of neuromodulation in improving cognitive functions in psychiatric disorders.

The third review within this edition also serves to demonstrate the cognitive benefits that can be derived from utilizing NIBS as an adjunctive therapy within a clinical population. Accordingly, the mini-review by Suarez-Garcia et al. sought to characterize the current evidence supporting the use of tDCS for treating cognitive impairment in Parkinson's disease (PD). A systematic review was used to identify 8 studies, the data from which was subsequently entered in to a meta-analysis. Although the results of this analysis were limited by the low number of studies and the heterogeneity of stimulation protocols and clinical features, they nonetheless identified strong benefits to executive functions in patients. In particular, anodal tDCS appears to improve problem solving and planning, verbal fluency, and cognitive flexibility.

Finally, an example of metaplastic modulation in clinical practice has been described in the case reported by Serrano-Castro et al. Despite an invasive neurostimulation approach, they opened the way to a customized neuroplastic-guided rehabilitation protocol, which allowed a previously inoperable tumor to be successfully removed and subsequently help treat the patient's refractory epilepsy.

In conclusion, this Research Topic includes a number of remarkable advances that further our understanding of the complex phenomena underlying metaplasticity, demonstrate how aberrant metaplasticity can contribute to

pathophysiology, and show that modifying metaplasticity with NIBS can be an effective avenue for treating network disorders of the brain. Translationally, this will encourage future clinical and neurophysiological studies and open novel therapeutic perspectives in this fascinating topic.

AUTHOR CONTRIBUTIONS

MC, FR, and CT draft the manuscript. MC, GL, and CT conduct the analysis of data. GL and GO revise the manuscript critically for important intellectual content. All the authors approve the version of the manuscript to be published.

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CASE REPORT
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Neuroplasticity and Epilepsy Surgery in Brain Eloquent Areas: Case Report

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Introduction: Neuronal plasticity includes changes in any component of the central nervous system in response to intrinsic or extrinsic stimuli. Brain functions that depend on the epileptogenic cortex pose a challenge in epilepsy surgery because many patients are excluded from pre-surgical evaluation for fear of the possible sequelae. Some of these patients may be rescued by enhancing neuronal plasticity with brain neuromodulation techniques.

Case Report: We describe a 6-year-old child with refractory focal motor seizures symptomatic to a neuroepithelial dysembryoblastic tumor in the left temporo-parietal region. He underwent limited resection of the lesion in order to avoid sequelae in his language function. A functional study at age of 17 years revealed an overlap of Wernicke's area with the tumor and areas of incipient language reorganization in the contralateral hemisphere. An invasive neuromodulation procedure was designed to enhance neuroplasticity. After craniotomy, he underwent language training and simultaneous electrical inhibition of language using an electrode grid placed over the lesion. The intensity of the language inhibitory stimulus was increased every day to force the use of accessory language areas in the right hemisphere by neuroplasticity.

Results: The language of the patient improved for six consecutive days until he was able to speak and understand while undergoing maximum electrical inhibition. The tumor was resected using a cortical mapping guide.

Discussion: Application of direct cortical stimulation techniques and language pre-habilitation before epilepsy surgery can be useful to rescue patients excluded from resective surgery, especially young patients with long-term lesions.

Keywords: language prehabilitation, epilepsy surgery, eloquent area surgery, neuropshycological measures, Boston test, language functional MRI

INTRODUCTION

In 1894, Santiago Ramón y Cajal was the first to apply the term "plasticity" to the central nervous system at the International Medical Congress held in Rome (1), where he described dynamism or adaptation related to structural neuronal changes in response to external stimuli. Neuronal plasticity is now considered to refer to changes in any component of the central nervous system produced by intrinsic or extrinsic stimuli (2).

Knowledge of neuronal plasticity has expanded over recent decades, through the application of non-invasive electrical or magnetic stimulation procedures to complement conventional cognitive rehabilitation techniques after acquired brain damage (3–6). The main challenges are the evanescence of induced changes due to the distance between application and brain tissue and the interposition of the skull. These limitations may be overcome by using more invasive techniques, such as cortical stimulation mapping (CSM). CSM has long been used to identify eloquent areas in the presurgical study and to demarcate epileptogenic sites. CSM has also confirmed the plastic potential of brains in childhood and adolescence (7, 8). There has been abundant research on the application of CSM in animal models of neuronal plasticity modification (9–11). Functional magnetic resonance imaging (fMRI) studies of humans have also shown that long-term lesions in eloquent areas can permanently modify functional circuits by innate plasticity processes (6, 12, 13).

The prognosis of patients undergoing brain neurosurgery is influenced by the extent of resection, which is limited by the presence of brain functions dependent on the cerebral cortex. This causes many patients to be excluded from functional epilepsy surgery. Some of these patients might be rescued for the only curative treatment currently available if brain neuromodulation techniques could develop their neuronal plasticity. The number of patients who could benefit from such techniques is probably high given that the prevalence of active epilepsy in the world is 6.38/1,000 people (95% CI 5.57–7.30) (14), and of these, ∼20–40% behave as refractory to medical treatments (15). Although there are no reliable data in the literature on the percentage of these patients with lesions in eloquent areas, it is known that this is a major clinical problem that has forced the development of various therapeutic strategies in these patients (16).

We report a case in which the neuronal plasticity of language was induced before epilepsy surgery.

CASE REPORT

We describe the case of a right-handed 6-years-old child with focal motor seizures of the right lower limb and sudden aphasia, without awareness impairment secondary to a space-occupying lesion in the left temporoparietal region. He underwent partial resection of the lesion, which was limited by the need to avoid sequelae in his language function. The pathological study reported WHO grade I neuroepithelial dysembryoplastic tumor (Ki-67 cell proliferation index $< 1\%$). After the surgery, the patient continued with daily epileptic seizures refractory to medical treatment.

At the age of 17 years, a follow-up neuroimaging study showed an increase in the volume of the lesion, and an fMRI scan revealed an overlap of the area of Wernicke with the tumor and areas of incipient functional language reorganization in the homologous contralateral hemisphere.

Since the beginning of his illness, the patient has undergone multiple drug regimens, including oxcarbazepine, valproic acid, lamotrigine, eslicarbazepine acetate, lacosamide clobazam, and brivaracetam, in different rational combinations, without achieving the goal of freedom from seizures.

Methods

An invasive neuromodulation procedure was designed to enhance neuroplasticity.

Step 1: First awake intraoperative CSM: As the preoperative fMRI showed some transferred language areas to the right hemisphere, a first CSM was performed intraoperatively to confidently assess whether there was or not residual and functional language located over or nearby the tumor. Awake CSM followed left parietotemporal-wide craniectomy over the lesion. Phase-reversal of N20 was first tested. Once Rolandic sulcus was accurately showed up, the motor strip was stimulated while performing electrocorticography, with a monopolar handheld stimulating probe rectangular, monophasic, anodal multipulse ($N = 7$ ISI $= 4$ [250 Hz]) stimulus, with a duration of 0.2–0.5 ms and up to 25 mA of intensity using a 16-channel neurophysiological intraoperative monitoring device (Protektor by Xtelk®). With the motor threshold, we started the language direct cortical stimulation mapping using the Penfield technique with a handheld bipolar probe with 5 mm between the tips of the probe (biphasic starting positive, at 60 Hz, duration of 1 ms) with an intensity between 2 and 20 mA during 4 sg ($N =$ 240 stimuli) using a cortical stimulator (Nimbus®), while the patient was performing motor language tasks: counting numbers days of the week; comprehension tasks: pictures descriptions, and repetition, reading, and witting tasks. As we found, there was some residual language just over the tumor that should be resected. To minimize language deficits, we decided to continue the procedure of prehabilitation and proceed with the placement of 20 subdural grid electrodes.

Figure 1 depicts the location of functional areas of language; electrode 17 is located on the sensitive area (Wernicke's) and electrode 4 on the motor area (Broca).

Step 2: Language Prehabilitation: 1 day after this surgery, in the patient's room, we performed a cortical stimulation through the 20 subdural grid electrodes, detecting the electrodes that were over Broca and Wernicke areas. Once the target electrodes were identified, we connected them (electrodes 4 and 17) to an external stimulator (Medtronic 3625, Medtronic Ibérica SA) to perform continuous electrical stimulation. The stimulation of these electrodes generated language dysfunction. The parameters used were 130 Hz, 1 ms, and intensity up to 10 V, which was increased daily in steps of 2 V to reach the limit of language function inhibition.

This stimulation was continuously active for 6 days, with increases or changes between the stimulating electrodes when

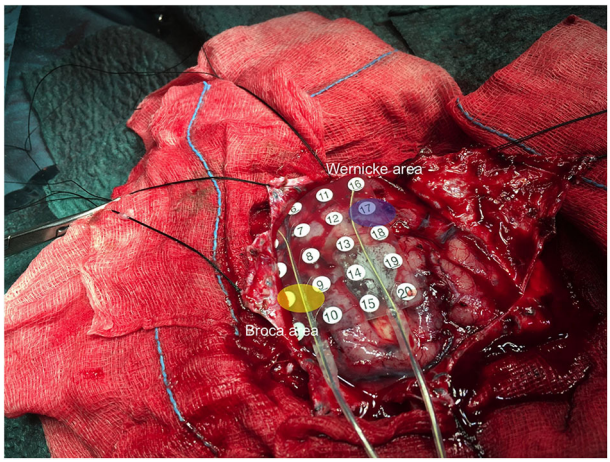

FIGURE 1 | Surgical image during CSM, indicating the sensitive and motor language eloquent areas identified.

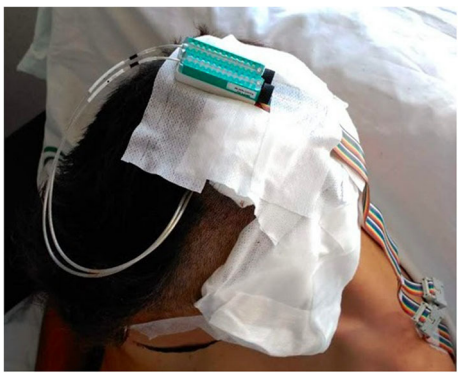

FIGURE 2 | Detail of the position of the external stimulator during step 2.

necessary to reach the inhibition of the language again, as a habituation phenomenon was present (**Figure 2**).

During this procedure, every day, after adjustment of the intensity of the continuous electrical stimulation, during at least 6 h a day, the neuropsychologist performed an intensive work on the specific deficits of the patient using material from the Spanish version of Psycholinguistic Assessments of Language Processing in Aphasia (PALPA) for the training of spontaneous language and denomination (17) and material from the Barcelona test for the training of understanding, denomination, repetition, reading, and writing (18).

Step 3. Second Awake Cortical Mapping and Definitive Surgery: On the 7th day after the first awake craniotomy, the definitive surgery was done. The second CSM revealed that there was no residual language over the tumor that was completely resected with no further functional deficits.

At 1 month before surgery and again at 3 months post-surgery, the patient underwent a neuropsychological evaluation of language and an fMRI with language paradigms.

The Boston Naming Test is one of the most widely used visual confrontation naming tests to evaluate the lexical and semantic system in aphasic patients (19). We used this test for language evaluation, as it is a widely used test in the preoperative evaluation of epileptic patients and with which our group has extensive experience.

A 3.0 Teslas Philips Intera® MRI (release 2.6) system was used for blood-oxygen-level-dependent (BOLD) signal fMRI acquisition. The scanning session included one T1 structural image for precise anatomical localization of language areas and T2-weighted fast field echo, echo planar imaging (repetition time 3,000 ms, echo time 35 ms, field-of-view 230 mm, and matrix size 80/128 r). Auditory and block design fMRI paradigms (verbal fluency, semantic decision, verb generation, and passive story listening) were performed to determine the eloquent areas of language Broca and Wernicke.

Ethics

The patient and his parent signed informed consent in the hospital. The study was conducted following the principles of the Declaration of Helsinki (20), with Spanish regulations on biomedical research and with European personal data protection regulations. It was approved (code 0698-N-20) by the institutional ethical committee of our hospital (Comité de Ética de la Investigación provincial de Málaga).

RESULTS

Prehabilitation Procedure

The patient improved his linguistic ability for 6 consecutive days after the start of language prehabilitation. On the day before the second surgery, he was able to speak and understand without major deficits despite the application of maximum electrical inhibition to the Wernicke area of the left hemisphere. The tumor was then completely resected with cortical mapping in the awake patient.

Outcome

The patient has been seizure-free for more than 1 year after the surgery and has returned to his usual academic and social activities. He is currently receiving brivaracetam and eslicarbazepine acetate in descending doses.

Neuropsychological Evaluations

Neuropsychological language evaluations in our patient showed a progressive deterioration over the 2 years preceding surgical intervention in listening, fluency, denomination, and writing. More severe impairment was observed in some categories explored by the Boston test, including those related to category denomination and especially, written vocabulary and narrative writing, which deteriorated from normal results for his age at 2 years presurgery to very low scores at 1 month presurgery (**Table 1**).

These deficits were recovered after the language prehabilitation process and tumor resection. The most important recoveries were observed in areas of auditory understanding, denomination, basic vocabulary, and narrative writing.

TABLE 1 | Results of the regulated neuropsychological evaluation at 2 years before surgery, 1 month before surgery, and 3 months after surgery.

		Boston test				
		2 years before surgery (Percentile)	1 month before surgery (Percentile)	Language evolution previous to surgery	3 months after surgery (Percentile)	Language Evolution after surgery
Auditive understanding	Word discrimination	60	40	↓	50	↑
	Orders	100	70	↓	100	↑↑
	Complex Ideation material	60	40	↓	70	↑↑
Fluency	Phrase length	100	70	↓	100	↓
	Melodic line	100	70	↓	60	↓
	Grammatical form	100	70	↓	70	=
Recitation		100	100	=	100	=
Repetition	Words	100	100	=	100	=
	Sentences	100	100	=	100	=
Denomination	Naming response	100	70	↓	100	↑↑
	Boston vocabulary test	40	60		70	↑
	Category Denomination	100	50	↓↓	100	↑↑
Reading	Match writing types	100	100	=	100	=
	Match numbers	100	100	=	100	=
	Match drawing-word	40	30	↓	40	↑
	Reading words aloud	100	100	=	100	=
	Reading sentences aloud	100	100	=	100	=
	Understanding sentences spoken aloud	100	100	=	100	=
	Understanding sentences and paragraphs spoken aloud	100	60	↓	100	↑↑
Writing	Shape	100	100	=	100	=
	Choice of letters	100	100	=	100	=
	Motor facility	100	40	↓↓	50	↑
	Basic vocabulary	100	30	↓↓↓	100	↓↓↓
	Regular phonetics	100	100	=	100	=
	Common irregular words	100	100	=	100	=
	Written designation of drawings	100	100	=	100	=
	Narrative writing	100	40	↓↓	80	↑↑

Percentile values. Red shading: <50 percentile. Orange shading: 50–90 percentile range. Green shading: >90 percentile. ↓↓↓, very important worsening; ↓↓, important worsening; ↓, worsening; =, no changes; ↑, moderate improvement; ↑↑, significant improvement; ↑↑↑, very important improvement.

From a practical viewpoint, this improvement opened up the possibility of the patient returning to his normal academic life after surgery.

fRMI

When comparing fMRI before and after the tumor resection and the stimulation protocol, postsurgical images show decreased activity in the left hemisphere areas and greater activation in the right temporal areas, including the right homologous area of the left Wernicke's. This suggests neuroplasticity in these right eloquent auditory and language areas and could explain the improvements in language comprehension in this patient (see **Figures 3**, **4**).

DISCUSSION

To our best knowledge, we present the first report of modulation of cerebral plasticity in a patient undergoing epilepsy surgery in language-eloquent areas. In 2016, Rivera et al. (21) described a series of five patients with WHO grade II or III glial lesions in language-eloquent areas who underwent a similar procedure, reporting that it induced an acceleration of neuroplasticity

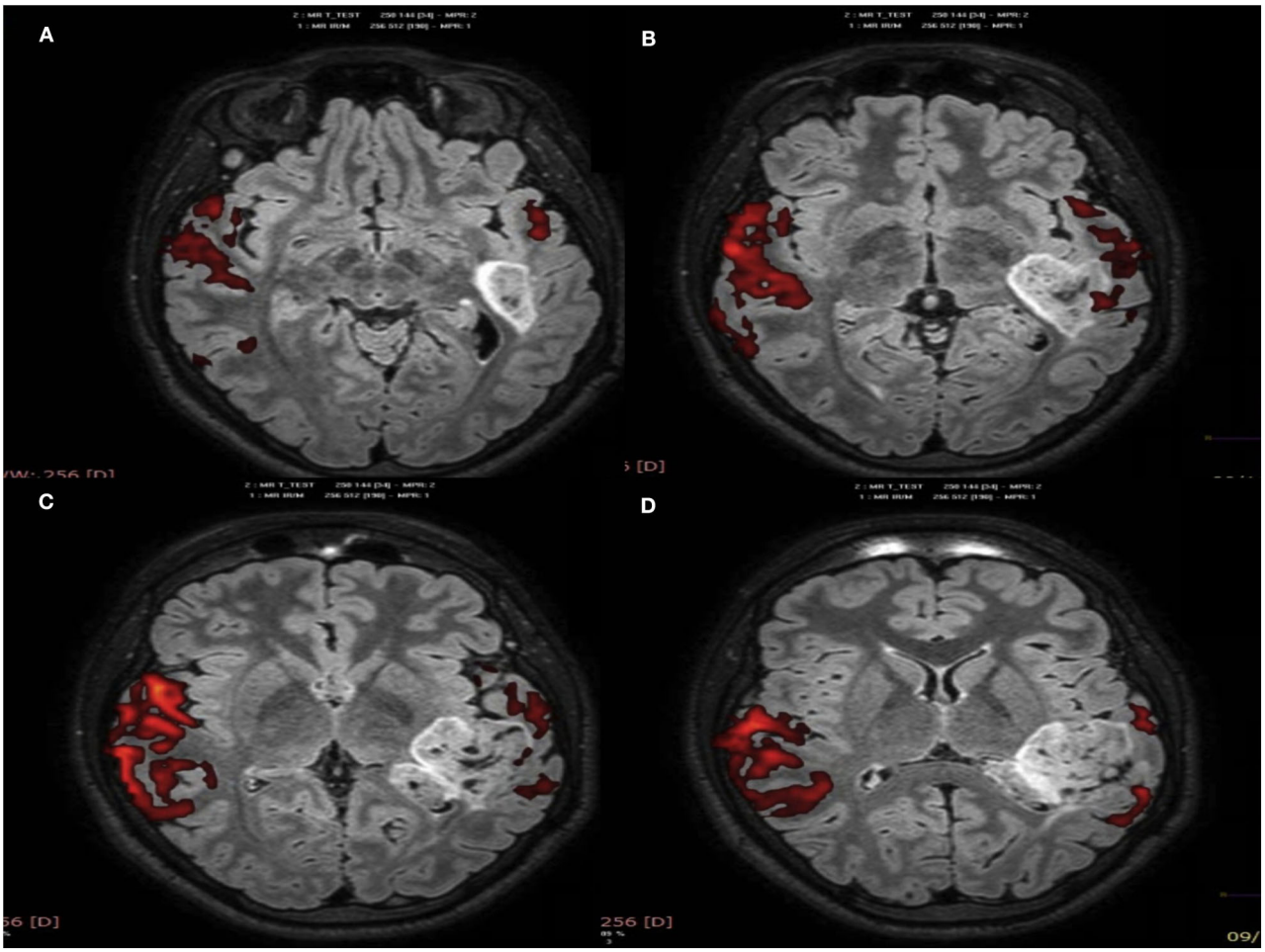

FIGURE 3 | Presurgical fMRI, story passive listening paradigm **(A–D)**: axial brain planes show, in red color, the activation of left temporoparietal areas corresponding to Wernicke's, associative language, and the auditory areas within and surrounding the lesion. Greater activation is shown in the right temporoparietal hemisphere (homologous areas), probably due to neuroplasticity.

processes. They were older than the present patient, and their lesions were more recent, circumstances that do not favor neuroplasticity. Besides his younger age, our patient had a very long-term lesion, and an intrinsic neuroplasticity process was already underway (see **Figure 3**). Chronic lesions in the eloquent cortex are known to cause neuroplasticity that results in the cortical reorganization of functional areas (22–24). In this way, patients can develop language-eloquent areas in other parts of the brain, usually in contralateral homologous areas.

We consider that the improvement found between 1 month presurgery and 2 months post-surgery in our patient indicates the implementation of the incipient functional areas of language developed by neuroplasticity in the right hemisphere over the years, similar to previous observations in patients with long-standing lesions in eloquent areas (23, 24).

We propose that functional inhibition of the Wernicke area of the left hemisphere, together with the simultaneous intensive language training, enabled this process. In this line, good outcomes have previously been described for the rehabilitation of stroke sequelae through the inhibition of functional areas and the simultaneous rehabilitation of damaged areas (25, 26).

These results suggest that the prehabilitation of language with this type of procedure can help in the implementation of areas developed by intrinsic neuroplasticity in patients with long-term lesions in language-eloquent areas. The prehabilitation process is probably not capable of transferring functions, as claimed by Rivera et al., but it can implement an area previously developed by intrinsic neuroplasticity.

Many molecular adjustments have been found and may constitute the substrate of neuroplasticity changes induced by electrical neuromodulation. According to recent molecular studies, direct current stimulation produces significant changes in neurotrophic factors, especially on brain-derived neurotrophic factor (BDNF). Thus, variations in BDNF secretion correlated

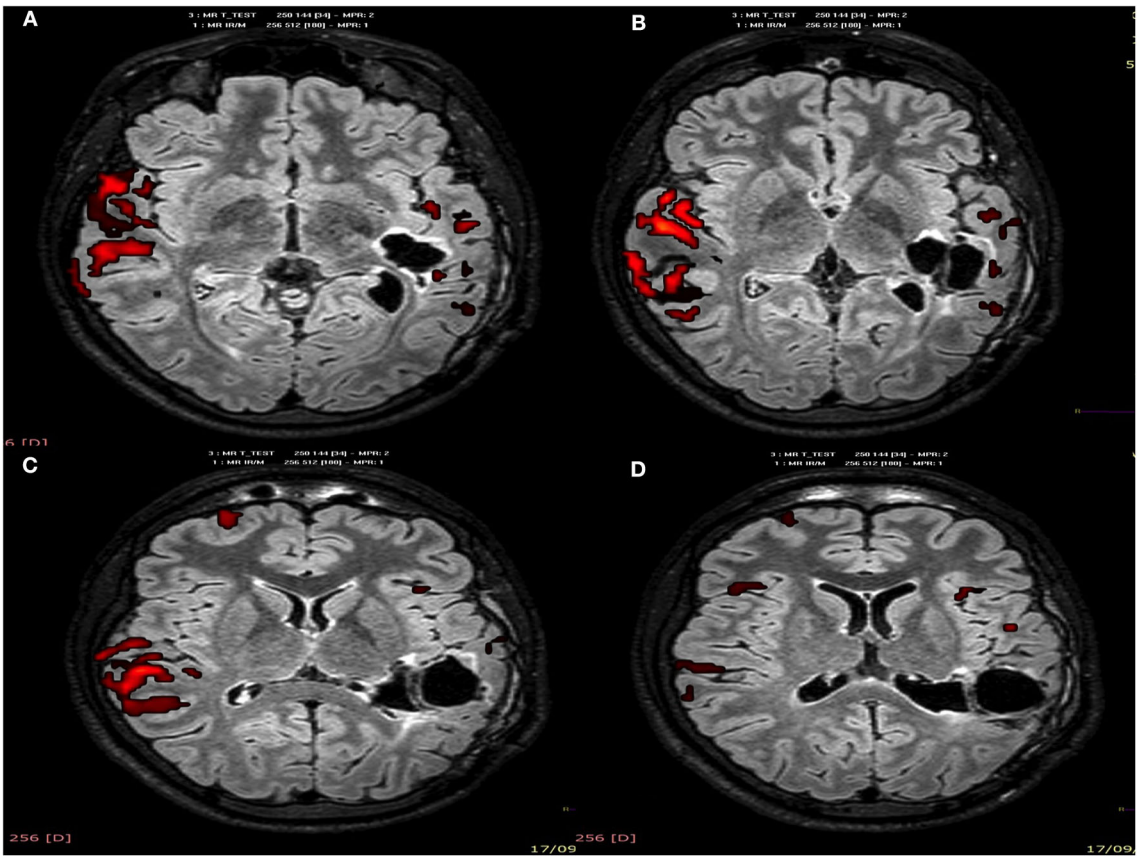

FIGURE 4 | Postsurgical fMRI, story passive listening paradigm **(A–D)**: axial brain planes show, in red color, a decrease activation after surgery of the left temporoparietal areas corresponding to Wernicke's, associative language, and the auditory areas. Greater activation is shown in the right temporoparietal hemisphere (homologous areas), probably due to neuroplasticity.

to recovery after direct stimulation in preoperative treatment of pain control and Parkinson's disease (27, 28). Similarly, elevated nerve growth factor (NGF) serum levels in patients with depression have been suggested as adaptive neuroplasticity and associated with cognitive improvement after direct current stimulation (29). Recent works in experimental models have demonstrated that direct current stimulation in the CA1 region of rat hippocampus mediates elevated levels of BDNF in the hippocampus and priming of N-methyl-D-aspartate receptor-dependent long-term potentiation, eliciting metaplastic aftereffects on hippocampal synaptic plasticity. Induced enhancement of long-term potentiation was completely blocked with an antagonist of TrkB, demonstrating the role of BDNF/TrkB signaling in these effects (30). More recent, circulatory microRNAs (miRNAs) have also been involved in neuronal plasticity response in neuropathological conditions, and they may represent a fine-tuning mechanism able to integrate multiple inputs and outputs. In this sense, a very recent analysis from serum profiles and exosomal miRNAs showed genetic pathways involved in neuronal cell proliferation and differentiation significantly enriched with miRNA targets and identified epilepsy-induced peripheral downregulation of miR-15a-5p, miR-34a, miR-106b-5p, and miR-146 (31). Furthermore, electric stimulation of the ventral hippocampal commissure delays the development of epilepsy in a rat model and produces a highly specific regulation of a set of miRNAs implicated in the shape of dendritic spines (32).

The most important study weakness is that it addresses an isolated case, limiting the conclusions that can be drawn. Nevertheless, it opens the way for investigation of an application that could have a major impact on patients with refractory epilepsy who experience a progressive deterioration but cannot currently access epilepsy surgery. On the other hand, there is no established protocol for prehabilitation, and it is possible that outcomes could be improved by applying different parameters.

In conclusion, direct cortical stimulation techniques and simultaneous language prehabilitation may be a useful approach in epilepsy surgery, especially in young patients with long-term lesions who have demonstrated the beginning of function remodeling through intrinsic neuroplasticity.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethics Committees of our hospital (Comité de Ética de la Investigación provincial de Málaga, code 0698-N-20). Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of this case report, including any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

PS-C, BR-L, VF-S, LM-B, GE-T, and GI-B contributed in the conception and design of the procedure. PS-C, BR-L, VF-S, LM-B, GI-B, PC-G, MA-C, MV-D, EC-A, and MP-P participated in the implementation of the procedure. PS-C wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Novel TMS for Stroke and Depression (NoTSAD): Accelerated Repetitive Transcranial Magnetic Stimulation as a Safe and Effective Treatment for Post-stroke Depression

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Background: Post-stroke depression (PSD) affects up to 50% of stroke survivors, reducing quality of life, and increasing adverse outcomes. Conventional therapies to treat PSD may not be effective for some patients. Repetitive transcranial magnetic stimulation (rTMS) is well-established as an effective treatment for Major Depressive Disorder (MDD) and some small trials have shown that rTMS may be effective for chronic PSD; however, no trials have evaluated an accelerated rTMS protocol in a subacute stroke population. We hypothesized that an accelerated rTMS protocol will be a safe and viable option to treat PSD symptoms.

Methods: Patients ($N = 6$) with radiographic evidence of ischemic stroke within the last 2 weeks to 6 months with Hamilton Depression Rating Scale (HAMD-17) scores >7 were recruited for an open label study using an accelerated rTMS protocol as follows: High-frequency (20-Hz) rTMS at 110% resting motor threshold (RMT) was applied to the left dorsolateral prefrontal cortex (DLPFC) during five sessions per day over four consecutive days for a total of 20 sessions. Safety assessment and adverse events were documented based on the patients' responses following each day of stimulation. Before and after the 4-days neurostimulation protocol, outcome measures were obtained for the HAMD, modified Rankin Scale (mRS), functional independence measures (FIM), and National Institutes of Health Stroke Scales (NIHSS). These same measures were obtained at 3-months follow up.

Results: HAMD significantly decreased (Wilcoxon $p = 0.03$) from M = 15.5 (2.81)−4.17 (0.98) following rTMS, a difference which persisted at the 3-months follow-up ($p = 0.03$). No statistically significant difference in FIM, mRS, or NIHSS were observed. No significant adverse events related to the treatment were observed and patients tolerated the stimulation protocol well overall.

Conclusions: This pilot study indicates that an accelerated rTMS protocol is a safe and viable option, and may be an effective alternative or adjunctive therapy for patients suffering from PSD. Future randomized, controlled studies are needed to confirm these preliminary findings.

Clinical Trial Registration: https://clinicaltrials.gov/ct2/show/NCT04093843.

Keywords: post-stroke depression, transcranial magnetic stimulation, stroke recovery, neurostimulation, ischemic stroke, neurorehabilitation, accelerated TMS

INTRODUCTION

The interplay between depression and cerebrovascular disease is complex and clinically important. Post-stroke depression (PSD) is the most common neuropsychological complication of stroke, with a prevalence of ∼33% (1) in stroke survivors. PSD adversely influences outcomes by reducing quality of life, increasing caregiver burden, and increasing early mortality as much as ten-fold (2–4). As acute stroke interventions continue to improve, stroke survivorship and associated morbidity will also increase, making the need to explore innovative treatments for PSD even more urgent.

Despite the significant clinical burden of PSD, there are limited treatment options to prevent or reduce its severity. Psychotherapy and pharmacotherapy are well-established as treatments of choice in major depression, however a subset of patients do not respond to either of these first-line therapies (5). Selective Serotonin Reuptake Inhibitor (SSRI) use has been associated with increased risk of hemorrhagic complications as well as increased risk of falls in the elderly, while other studies have shown that SSRIs are actually associated with increased risk for stroke, myocardial infarction, and all-cause mortality (6). A recent meta-analysis for stroke patients concluded that antidepressants did not significantly improve patients' general recovery, achieved varied response rates, and were not tolerated due to adverse effects (7). Compliance, communication problems, and lack of access to psychiatric care are further challenges to treating PSD.

Repetitive transcranial magnetic stimulation (rTMS) may represent an effective treatment option that mitigates the issues associated with the standard PSD interventions. The FDA approved rTMS for patients with Major Depressive Disorder (MDD) in 2008 (8). The typical rTMS protocol that has been used effectively for major depression is 5 days per week for 4–6 weeks. Conventional rTMS paradigms have been studied in the PSD population, and many studies including a meta-analysis have shown that conventional rTMS is likely effective for chronic, refractory PSD (9, 10). However, these conventional paradigms may be inconvenient for patients with limited transportation access and may limit compliancy of patients. Therefore, an accelerated protocol which minimizes the number of days needed to complete the full treatment may be more accessible to patients and may increase compliancy. While there have been some accelerated rTMS paradigms that have been designed to treat conditions such as alcohol withdrawal and treatment-resistant depression (11–14), similar accelerated protocols have not been studied in patients suffering from PSD. Applying accelerated rTMS to the PSD population comes with unique and complex factors. For example, the theoretical risk of seizure using an accelerated protocol may be higher, and this risk may increase even further in patients in the acute to subacute stroke period. Therefore, it is important to study the safety of an accelerated protocol in this population. In addition, the period immediately following cerebrovascular ischemia potentially represents a biologically unique phase amenable to intervention given that both neuroplasticity as well as recurrent stroke risk are highest during this time (15, 16).

There is a clear medical need to further address the impact of rTMS for PSD and to optimize stimulation parameters. We hypothesized that an accelerated 4-days rTMS protocol would be a safe and viable method for treating PSD and would help ameliorate depressive symptoms.

METHODS

This prospective open label study was approved by our Institutional Review Board (IRB # 1804090922) and the Food and Drug Administration granted this study an Investigational Device Exemption (IDE) Number: G180102. The raw data supporting the conclusions of this article will be made available upon request, without undue reservation.

Participants

All patients admitted to the inpatient stroke service at our tertiary comprehensive stroke center are routinely screened for depression. Patients were screened for depression with the Hamilton Depression Rating Scale (HAMD-17). Study patients were identified either during their acute hospitalization or their follow up clinic visit. Patients who met the inclusion criteria and were otherwise free from the exclusion criteria were eligible to enroll (**Table 1**). Patients were eligible if the stimulation protocol could be applied between 2 weeks to 6 months following their acute stroke. Between November 2018 and March 2019, 62 of the 98 screened patients fulfilled the inclusion criteria. Although 62 patients were eligible, several patients had logistical issues unique to their own family or social situation and were unable to participate. Six patients were successfully enrolled and completed the stimulation protocol.

Stimulation

Neurostimulation was performed using the Neurostar system 2.0 figure of eight coil (Neuronetics, Malvern, PA). Prior to

TABLE 1 | List of inclusion and exclusion criteria.

Inclusion criteria
1. Aged 22-85 years old
2. Radiographic evidence of ischemic stroke
3. Stroke within 2 weeks to 6 months
4. HAMD score ≥ 8
Exclusion criteria
1. Metallic objects or neurostimulators implanted intracranially
2. Stroke in the area of stimulation (L DLPFC)
3. Known history of epilepsy or seizure disorder
4. A woman who is pregnant or breastfeeding
5. History of psychiatric hospitalization unrelated to current PSD
6. Current suicidal ideation or MINI suicide scale > 8
7. ASRM score > 6
8. Current illicit drug use
9. History of head trauma resulting in loss of memory > 5 min or requiring hospitalization
10. Evidence of hemorrhage in the brain at the time of study
11. Clinically significant EKG abnormalities including QTC prolongation > 450 msec in men or > 480 msec in women
12. Any other mental or physical conditions that are inappropriate for study participation at the PI's discretion

stimulation sessions, patients that were successfully enrolled had additional survey tools administered for baseline assessments in the following categories: modified Rankin Scale (mRS) to assess level of independence, Functional Independence Measures (FIM) to assess quality of independent lifestyle, and HAMD to assess level of depression. Patients were also assessed with the National Institutes of Health (NIH) Stroke Scale to determine physical disabilities resulting from their stroke. All functional scales were performed by trained study personnel and the same rater for each patient was used to minimize variability and inter-rater bias. Vital signs including an electrocardiogram (EKG) were performed before and after each stimulation session. Patients were surveyed about adverse events following each stimulation day.

On the first day, patients underwent a mapping procedure to determine the patient's individualized and optimal Resting Motor Threshold (RMT) over the left motor cortex. The RMT was defined as the minimum stimulation intensity required for visual muscle twitch of the right abductor pollicis brevis (APB) muscle in five out of 10 consecutive single pulse stimulations. After establishing RMT, the coil was moved 5.5 cm anteriorly to the patient's left dorsolateral prefrontal cortex (DLPFC). Patients underwent repeat mapping if necessary. The NeuroStar system has a method for saving each patient's measurements in the system to ensure that the coil is positioned in the same place for each new session. Earplugs were used to prevent any hearing injury. All mapping and treatment sessions were performed by TMS-certified nurses and physicians at our Behavioral Medicine facility where emergency equipment was readily available.

Patients sat in the NeuroStar system chair for all treatment sessions, which has mechanisms to keep the patient properly positioned for mapping and stimulation sessions. The treatment protocol was adapted from other accelerated rTMS protocols in the literature for other indications (11, 12). The protocol included high frequency (20 Hz) rTMS applied over the left DLPFC at 110% RMT for five sessions per day, over four consecutive days for a total of 20 sessions. Forty trains of two second duration were applied with a 12 second intertrain interval for a total of 1,560 pulses per session. Patients were given the opportunity to rest for 10–15 min in between sessions. The treatment sessions lasted for about an hour and a half each day. Variations on the accelerated paradigm we used in this study using different frequencies and different trains may be possible to test in future studies.

At the end of the 4 days of stimulation, patients were once again surveyed with the HAMD, mRS, and FIM. Post-treatment NIH was also performed. The patients were also surveyed at the end of each stimulation day as well as at the end of all 4 days regarding any adverse events they may have experienced. These same measures were once again repeated at the patient's 3-months follow-up.

The primary outcome of this study was safety and viability as defined as the successful recruitment and treatment of participants using the outlined accelerated protocol with no significant adverse effects observed. The secondary outcome was any effect on depressive symptoms as measured by the HAMD. We defined a meaningful response as remission of depression to non-depressed range (HAMD < 8) or at least a 50% reduction in overall score.

Statistical Analysis

All analyses were conducted in SAS 9.4. Categorical variables are described with frequencies and valid percentages, continuous variables with means and standard deviations. Alpha was set to 0.05 unless otherwise noted. Differences were explored using Wilcoxon signed rank tests on the differences between pre- and post- for continuous variables. Symmetry tests and McNemar's exact tests were run on the ordinal and binary outcome data. Finally, associations were examined between continuous data using Pearson correlations, and with categorical data using Wilcoxon two-sample tests with two-sided t-approximation.

RESULTS

Demographically, five of the study participants were male and the average age was 66.33 (range 57–71). Stroke etiology included two large artery atherosclerosis (LAA), one small vessel disease (SVD), two cardioembolic (CE), and one embolic source of unknown significance (ESUS). Half of the patients were taking SSRIs at the time of the study (**Table 2**).

No significant adverse events related to the treatment were observed. All participants tolerated the stimulation well. One subject described a headache that was milder than his usual chronic headaches and another subject experienced transient facial sensitivity ipsilateral to the coil at the beginning of the first day of stimulation. Neither of these observations were rated as bothersome by the participants and both were self-limited.

HAMD significantly decreased (Wilcoxon $p = 0.03$) from M $= 15.5$ (2.81) to 4.17 (0.98) following rTMS, a difference which persisted at the 3-months follow-up ($p = 0.03$). There was no statistically significant difference in FIM, mRS, or NIH (**Table 3**).

In terms of number of patients going from "depressed" (HAMD ≥ 8) to "non-depressed" (HAMD < 8), four participants

(66.67%) had moderate depression (HAMD 14–18) and 2 (33.33%) had severe depression (19–22) at baseline. At post-assessment, all scores dropped below the cut-off for non-depressed. At 3-months follow-up, 5 of 6 patients remained non-depressed, and one patient scored eight at the lowest end of mild depression (**Figure 1**).

DISCUSSION

Our results demonstrate that the use of an accelerated rTMS protocol in patients with PSD during the subacute period following stroke is a safe and viable option for stroke patients. None of the participating patients reported any significant adverse effects. This high degree of tolerability is similar to the previous published experience with accelerated protocols (11–18). All treated patients experienced a significant improvement in depressive symptoms, with a remission rate of 100% directly following TMS. Remission status persisted in five of the six patients at 3-months follow-up, with one patient scoring borderline mild depressed but still maintaining a 47% reduction in her depression score from baseline.

There have been a few other small studies that have looked at rTMS for chronic PSD (9, 19–21) as well as a recent meta-analysis of 22 randomized controlled trials comparing active rTMS stimulation to sham stimulation (10). These trials indicated that rTMS is an effective tool to treat chronic PSD. Other forms of non-invasive brain stimulation such as electroconvulsive therapy (ECT) and transcranial direct current stimulation have limited data for the treatment of PSD. ECT is largely regarded as the most powerful tool to treat severe depression, however, it is limited by side effects of amnesia (22, 23). Within the PSD population, these findings with ECT are echoed with respective response and remission rates of 60 and 50% (24). In spite of this, rTMS is still the best at controlling frequency and location of stimulation, which offers certain advantages (23). Our data demonstrates that an accelerated version of rTMS may be an effective treatment for PSD as well.

The mechanism underlying rTMS efficacy is still largely unknown. It is hypothesized that low frequency TMS stimulates inhibitory neurons while high frequency TMS stimulates excitatory projection neurons, thus mimicking neuroplasticity through long-term potentiation (23). Thus we chose high-frequency stimulation of the left DLPFC given that this area is associated with depression. However, the translation of cortical excitation to clinical response with rTMS is incompletely characterized (25). Therapeutic benefit is likely achieved through multiple mechanisms enhancing neuroplasticity, increasing available concentrations of critical neurotransmitters, and reinforcing emotionally positive connectivity networks

TABLE 2 | Baseline characteristics of the six participants.

Variable	Mean or N	SD or %
Age		
Years	66.33	4.97
Gender		
Male	5	83.33%
HLD*		
Yes	5	83.33%
DM†		
Yes	2	33.33%
AF‡		
Yes	1	16.67%
Tobacco		
Yes	3	50.00%
SSRI§		
Yes	3	50.00%
Family history		
Yes	1	16.67%

*HLD, hiperlipidemia.
†DM, diabetes mellitus.
‡AF, atrial fibrillation.
§SSRI, serotonin selective reuptake inhibitor.

TABLE 3 | Participant outcome measures ($N = 6$).

Variable	*N*	Pre mean (SD)	Post mean (SD)	3 month Mean (SD)	Diff (pre to post)	*p*-value*	Diff (pre to 3 month)	*p*-value*
HAMD	6	15.50 (2.81)	4.17 (0.98)	3.50 (2.66)	11.33 (2.94)	0.03	12.00 (3.63)	0.03
FIM	6	115.33 (8.12)	122.17 (6.97)	–	−6.83 (4.17)	0.063	–	–
NIHSS	6	1.83 (2.99)	1.00 (1.67)	–	0.83 (2.04)	1.00	–	–
Variable	**Category**	**Pre *N***	**(%)**	**Post *N***	**(%)**	***p*-value****		
mRS						0.80		
	0	1	(16.67%)	2	(33.33%)			
	1	4	(66.67%)	3	(50.00%)			
	2	1	(16.67%)	1	(16.67%)			
NIHSS < 4	Yes	4	(66.67%)	5	(83.33%)	0.32		

*Wilcoxon signed rank test.
**Symmetry test, McNemar's exact test.

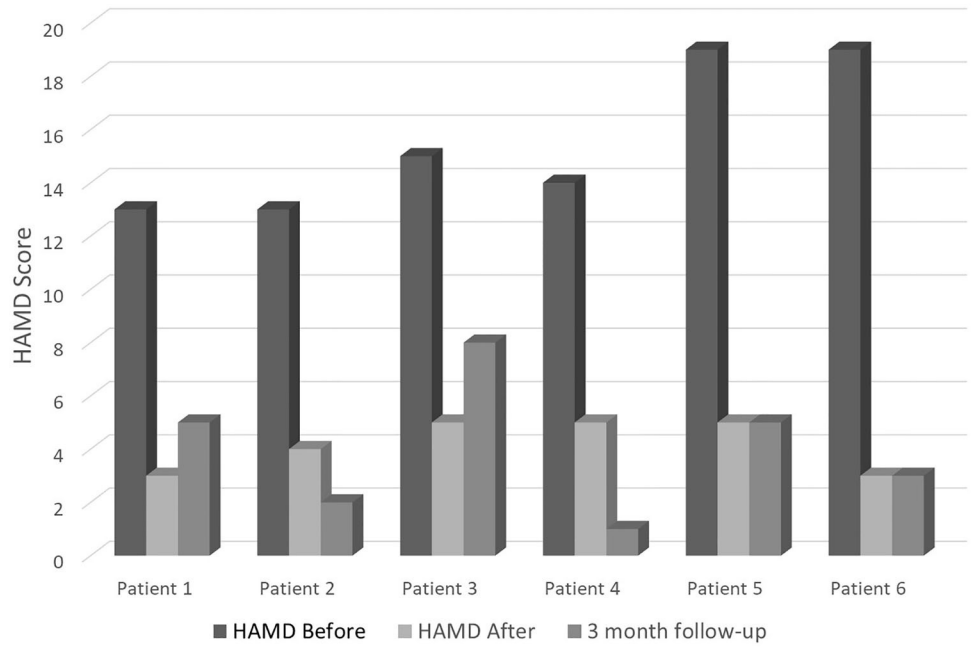

FIGURE 1 | HAMD scores in our six patients before the TMS sessions (baseline), immediately after completing the full 4-days neurostimulation protocol, and at their 3-months follow-up appointment.

while diminishing connectivity in emotionally negative loops (26–28). Low levels of peripheral and central brain derived neurotropic factor (BDNF) have been observed in depressed individuals as well as those who develop PSD (29–33). Glutamate is emerging as another biomarker for treatment response with increased radiolabeled activity in the DLPFC following stimulation (34). rTMS treatment has also increased dopamine concentrations (35–37), and increased activity within mood networks on functional imaging (38). Exactly how rTMS exerts its influence, however, remains a critical question. Understanding its underlying mechanism will potentially increase our understanding of PSD itself and help identify therapeutic targets.

The novelty of this rTMS paradigm is the accelerated protocol as well as the stimulation in the acute to subacute stroke period. Similar accelerated protocols have been used in other populations (12, 13, 17, 18) (treatment resistant depression and alcohol withdrawal craving) and there have also been studies conducted of rTMS in the acute stroke setting for complications unrelated to depression (39–42); however, a similar paradigm has not yet been employed in a PSD population. A major barrier of current rTMS protocols is the 4–6 weeks timeline before clinical benefit is achieved, so an accelerated protocol is an important potential solution to this problem. The accelerated protocol that was used in this study enabled patients to receive 20 total stimulation sessions, which is the typical minimum number of sessions that patients receive in a conventional rTMS protocol (20 sessions spread out over 4 weeks, receiving one session per day Monday through Friday). Condensing these 20 sessions into four consecutive days allowed patients to participate who otherwise may have faced logistical challenges to obtaining this treatment.

Although this study was underpowered to demonstrate efficacy, the significant remission rate is promising. Larger, randomized studies are needed to confirm these results. There are several limitations in this study. The open label design of this study allows for patients to know they are receiving active stimulation, and the placebo effect could very well have influenced the robust improvement in depression following rTMS. It is important to conduct future trials with a control group and appropriate blinding to truly determine if the rTMS itself is causing a meaningful response in depressive symptoms. Another major limitation is the small sample size. The patients enrolled in the study all had high functional levels according to their FIM, NIHSS, and mRS scores, which may indicate a self-selection bias. It is unclear if patients with a higher functional status were more interested in the study, if these patients were more likely to be aware of their depressive symptoms and want to participate for this reason, or if these patients were more capable of driving themselves to the appointments and thus more willing to participate. In addition, the fact that such a small proportion of eligible patients ultimately enrolled in this study underscores the complexities of treating this patient population and the explicit barriers to enrollment deserve dedicated further study. Regardless, a larger sample size

with a group representative of the whole spectrum of post-stroke functionality would allow the results to be applicable to a broader population. In addition, half of our patients were already taking an anti-depressant at the time of enrollment. We chose not to exclude patients on SSRIs since the main goal of this study was to first establish safety and tolerability of using accelerated rTMS in this population, however we did ensure that all patients continued concurrent pharmacologic treatment throughout the duration of the study. Future studies would benefit from excluding patients on SSRIs, and larger studies would also benefit from comparing patients receiving rTMS alone vs. rTMS plus SSRIs to determine if there is a synergistic effect in this population. Similar to major depression, some studies have shown synergism between rTMS and pharmacologic therapy as opposed to either alone (43). However, a meta-analysis of all rTMS in PSD trials published found an rTMS effect size greater among those not on any pharmacologic treatment (0.96) compared to combination therapy (0.51) (22). Future studies may also benefit from the use of neuronavigation to confirm coil position as well as EEG compatible TMS to assess for subclinical seizure activity in a population with a theoretically increased risk of seizure (44). Given the subjective nature of depressive symptom reporting and known placebo effect among depressed patient populations, it is imperative to confirm our findings in larger, randomized studies with a sham stimulation arm as a control group.

CONCLUSION

Our results indicate that accelerated rTMS is a safe and viable treatment option for PSD in the subacute stroke population. Depressive symptoms significantly improved in all treated patients. Confirming these results in larger randomized settings has the potential to establish accelerated rTMS as a potent therapy for PSD. Further studies regarding mechanism of action, subgroups particularly responsive to the treatment, and durability of rTMS for PSD are warranted. We are currently conducting a larger randomized controlled study in efforts to answer these questions.

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 West Virginia University Institutional Review Board (IRB). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JF was responsible for the conceptual design of this study, writing of the first draft, all major revisions, patient recruitment, data collection, and data analysis. AA was responsible for writing the first draft, major revisions, collaboration of the experimental design, patient recruitment, data collection, and data analysis. CL was responsible for the statistical analysis and revisions to the manuscript. UN was responsible for revisions to the manuscript and contribution to conceptual design. All authors approved the final manuscript.

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Prediction of Motor Recovery in the Upper Extremity for Repetitive Transcranial Magnetic Stimulation and Occupational Therapy Goal Setting in Patients With Chronic Stroke: A Retrospective Analysis of Prospectively Collected Data

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Recovery from motor paralysis is facilitated by affected patients' recognition of the need for and practice of their own exercise goals. Neurorehabilitation has been proposed and used for the treatment of motor paralysis in stroke, and its effect has been verified. If an expected score for the neurorehabilitation effect can be calculated using the Fugl-Meyer Motor Assessment (FMA), a global assessment index, before neurorehabilitation, such a score will be useful for optimizing the treatment application criteria and for setting a goal to enhance the treatment effect. Therefore, this study verified whether the responsiveness to a treatment method, the NovEl intervention using repetitive transcranial magnetic stimulation and occupational therapy (NEURO), in patients with post-stroke upper extremity (UE) motor paralysis could be predicted by the pretreatment FMA score. No control group was established in this study for NEURO treatment. To analyze the recovery of the motor function in the UE, delta-FMA was calculated from the pre- and post-FMA scores obtained during NEURO treatment. The probability of three levels of treatment responsiveness was evaluated in association with delta-FMA score (<5, $5\leq$ delta-FMA <10, and ≥ 10 as non-responders; responders; and hyper-responders, respectively) according to the reported minimal clinically important difference (MCID). The association of the initial FMA scores with post-FMA scores, from the status of the treatment responsiveness, was determined by multinomial logistic regression analysis. Finally, 1,254 patients with stroke, stratified by FMA scores were analyzed. About 45% of the patients who had FMA scores ranging from 30 to 40 before treatment showed improvement over the MCID by NEURO treatment (odds ratio = 0.93, 95% CI = 0.92–0.95). Furthermore, more than 25% of the patients with more severe initial values, ranging from 26 to 30, improved beyond the MCID calculated in the acute phase

(odds ratio = 0.87, 95% CI = 0.85–0.89). These results suggest that the evaluated motor function score of the UE before NEURO treatment can be used to estimate the possibility of a patient recovering beyond MCID in the chronic phase. This study provided clinical data to estimate the effect of NEURO treatment by the pretreatment FMA-UE score.

Keywords: transcranial magnetic stimulation, occupational therapy, stroke, motor paralysis, prediction

INTRODUCTION

Motor paralysis due to the aftereffects of stroke impairs the activities of daily living (ADL) and quality of life (QOL) of patients; it also affects their individual or social activities (1, 2). In particular, motor paralysis of the upper extremity has a large impact on ADL (3). Recovery from motor paralysis is facilitated by patients recognizing the need for and practicing their own exercise goals (4). The type of goals that patients set are related to their goal satisfaction scores, with impairment-based goals being rated significantly higher than activity-based and participation-based goals (5). It is known that patients' level of knowledge of their rehabilitation goals leads to effective treatment results (6). Thus, clinicians and patients are active partners in setting goals within stroke rehabilitation (5). In previous studies, some prognosis prediction systems were developed for motor paralysis (7–9), and they have been used to set goals for rehabilitation in patients with stroke.

Neurorehabilitation has been proposed and used for the treatment of motor paralysis in stroke, and its effect has been verified (10–14). One of the treatment methods, the NovEl intervention Using Repetitive transcranial magnetic stimulation and Occupational therapy (NEURO), facilitates peripheral muscle movement by controlling the excitability of the motor cortices by repetitive transcranial magnetic stimulation (rTMS). It also promotes peripheral muscle exercise and practice, for the active use of the paralyzed upper extremity (15, 16). NEURO's efficacy has been proved in a randomized controlled study (17). To date, many patients have been treated by using NEURO; however, the prediction regarding whether patients' recovery from motor paralysis after treatments can be predicted before treatment, has not been verified. If the Fugl-Meyer Motor Assessment (FMA) score before treatment can be used to predict NEURO treatment response, the score can be used as an effective goal for rehabilitation, by patients and therapists.

The minimal clinically important difference (MCID) of motor paralysis in the upper extremity has been investigated (18–20). If the expected value of an effect exceeding MCID can be calculated using FMA score measured before NEURO treatment, such a value will be useful for optimizing the treatment application criteria and setting a goal to enhance the treatment effect. For that purpose, it is sufficient to retroactively analyze the band of the FMA score before NEURO for a patient who is significantly improved. Therefore, this study verified whether the responsiveness of NEURO treatment for patients with post-stroke upper extremity motor paralysis could be predicted by the pre-treatment FMA score.

Abbreviations: ADL, activities of daily living; AIC, Akaike Information Criterion; FMA, Fugl-Meyer Motor Assessment; HF, high frequency; LF, low frequency; MCID, minimal clinically important difference; NEURO, NovEl intervention Using Repetitive transcranial magnetic stimulation and Occupational therapy; OT, occupational therapy; QOL, quality of life; rTMS, repetitive transcranial magnetic stimulation; SD, standard deviation; TMS, transcranial magnetic stimulation; UE, upper extremity.

METHODS

Participants

This is a multi-institutional open-label study without control patients. In January 2019, we surveyed the medical records of all patients with post-stroke muscle paralysis who had been admitted to six participating institutions (Jikei University Hospital, Jikei Third Hospital, Tokyo General Hospital, Kyoto Ohara Memorial Hospital, Nishi-Hiroshima Rehabilitation Hospital, Shimizu Hospital) between March 2010 and December 2018 for NEURO. For patients who had been treated with NEURO, the inclusion criteria were based on the TMS guidelines (21, 22) as follows: (1) upper limb hemiparesis categorized as cerebral infarction or cerebral hemorrhage; (2) age >20 years; (3) ≥4 months since stroke; (4) history of a single stroke only (no bilateral cerebrovascular lesions); (5) no cognitive deficits (a Mini Mental State Examination score ≥26); (6) no active physical or mental illness requiring medical management; (7) no history of convulsion for ≥1 year; (8) no intracranial metal clips or intracardiac pacemaker; and (9) no history of neurolytic nerve block (phenol or botulinum toxin) to the affected upper limb.

To verify if the upper extremity function was maintained after NEURO, patients were excluded: (1) if they did not have at least one FMA score before and after treatment, (2) if they had an initial FMA for upper extremity (FMA-UE) score <26/66, with severe motor impairment (15, 23), and those with a diagnosis of subarachnoid hemorrhage were excluded.

NEURO and Occupational Therapy (OT) Sessions

OT was provided in addition to conducting NEURO sessions; therapy was planned to suit the needs of each patient. All the patients were hospitalized for 15 days to receive rTMS (15) and OT (24). During hospitalization, each patient received a 40-min rTMS session and an OT session every day, except on Sundays and the day of admission/discharge. All OT sessions were started within 10 min of rTMS.

Focal 1 Hz rTMS was applied to the contralesional hemisphere over the primary motor area, as described in previous studies (15, 23). A 70-mm figure–8 coil, attached to a MagPro R100 stimulator (MagVenture Company, Farum, Denmark) was used

for rTMS application; for this, 2,400 pulses lasting for 40 min were applied. The stimulation intensity, set to 90% of the resting motor threshold for the first dorsal interosseous muscle on the unaffected side, was defined as the lowest intensity of the stimulation that could activate the motor-evoked potentials (MEP) of the muscle.

OT was performed twice daily, 6 days a week (excluding Sundays), and involved 60-min individual training sessions. The main goal of the OT sessions was to help the patients avoid focusing on the functional training and to encourage them to use their affected upper limbs again in daily activities. Treatment strategy included: (1) daily physical activities (e.g., eating), which included repetitive movements of the arm during flexion and extension; (2) individualized functional training tasks, which enabled the patients to improve on their movements, such as washing their hands and grasping small items with their paralyzed fingers; (3) elements involved in gross motor function, fine motor function, and multitasking; (4) clear demonstrations of the position of the upper limb to draw attention to this position during training; (5) staged interventions; (6) ADLs and unsupervised training tasks that could be continued after discharge; and (7) the provision of action feedback by passive intervention with verbal instructions.

Sample Size Calculation for Analysis

Based on multivariate linear regression (*F*-tests), an effect size f^2 of 0.03, power (1—β) of 0.95, α of 0.05, and 6 explained predictors, the minimum sample size of each group was 674 patients (derived using G*Power 3.1) (25). Furthermore, with an expected dropout rate of 30%, we planned to recruit in total a minimum of 963 patients with stroke treated with NEURO. To examine whether detectable logistical separations in upper extremity motor function owing to NEURO could occur, about 1,000 patients with stroke were included in the analysis.

Outcomes

The primary outcome was the FMA score. To predict the responsiveness to NEURO treatment from the initial score of FMA-UE, FMA scores (before and after treatment), age, sex, diagnosis (cerebral infarction or intracerebral hemorrhage), the dominant hand, and the time it took to recover motor function after the onset of stroke were investigated (**Figure 1**).

Clinical Evaluation of the Motor Function

The motor function of the affected upper extremity was evaluated on both the day of the admission and discharge using FMA score. The FMA was devised in 1975 (26), and is a global assessment index used to quantitatively evaluate the recovery of post-stroke hemiparetic limbs. The FMA has high interrater and test-retest reliability, as described previously (27). The FMA is a performance-based quantitative measure made up of 33 items used to evaluate the upper limb motor function. Each item is rated on a 3-point ordinal scale (0 = cannot perform, 1 = can perform partially, and 2 = can perform fully), with a maximum score of 66 points. The severity of paralysis according to the FMA score is distributed as follows: ≤25, 26–45, and 46–66 for severe, moderate, and mild paralysis, respectively (28–30). The MCID of FMA for the upper extremity in a population of patients with stroke is 4–10 points in the acute or subacute phase (19, 20), and 5 points in the chronic phase (31).

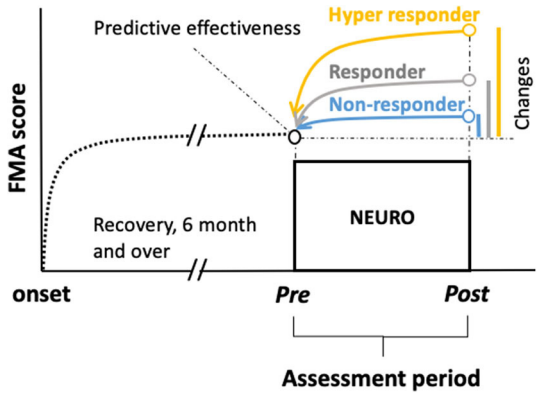

FIGURE 1 | Chart showing schemes of retrospective prediction of the motor recovery of the upper extremities to determine the goals before treatment in patients with chronic stroke undergoing NEURO. To examine the hypothesis that being a responder, non-responder, or hyper-responder resulted in NEURO treatment can be discriminated using multinomial logistic regression to determine the association of FMA score between initial and delta scores in patients with post-stroke hemiparesis. Delta FMA-UE scores were calculated by subtracting the post- from the pre-NEURO score. The black dotted line drawn from the onset indicates the recovery curve from the acute to the chronic phase. The blue, gray, and yellow lines indicate the non-responders, responders, and hyper-responders of NEURO, respectively, regarding the recovery of motor function of the upper extremity. FMA-UE, Fugl-Meyer assessment of upper extremity; NEURO, NovEl intervention Using Repetitive transcranial magnetic stimulation and Occupational therapy.

Statistical Analyses

To analyze the recovery of the motor function in the upper extremity, delta-FMA was calculated from the pre- and post-FMA scores obtained during NEURO treatment. In this study, the probability of the three levels of treatment responsiveness was evaluated in association with the delta-FMA score (<5, 5≤ delta-FMA <10, and ≥10 as non-responders; responders; and hyper-responders, respectively) according to previous studies (19, 20, 31). The association of the initial FMA scores with post-FMA scores, from the status of the treatment responsiveness, was determined by multinomial logistic regression analysis. The principle of multinomial logistic regression analysis requires that the probability (*p*) of the three levels (non-responders, responders, and hyper-responders) of the dependent variable, delta-FMA score, be fitted. The probability for the non-responders was the reference level; then the regression models were developed as follows:

$$\mathbf{g}\left(\mathbf{x}_{\mathbf{nonresponders}}\right) = \frac{\mathbf{1}}{\mathbf{1}+\mathbf{e}^{\mathbf{f}\left(\mathbf{x}_{\mathbf{responders}}\right)}+\mathbf{e}^{\mathbf{f}\left(\mathbf{x}_{\mathbf{hyper-responders}}\right)}} \quad (1:\text{non}-\text{responders})$$

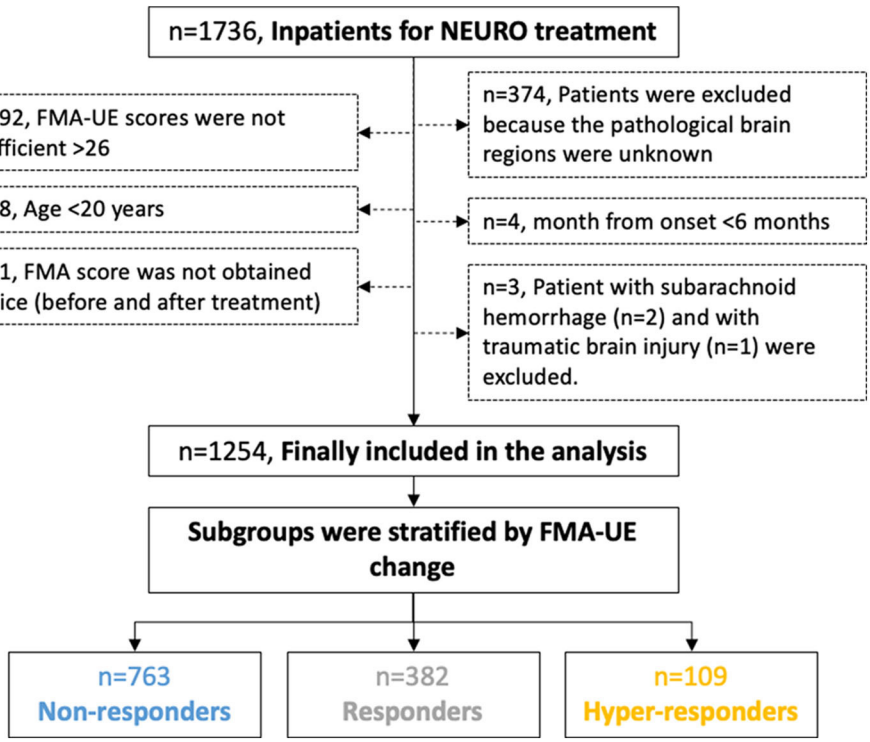

FIGURE 2 | Flow chart of the study design protocol for the data acquisition and the selection of the participants for the analysis. The participants were divided into three groups by the reported MCID of FMA-UE. MCID, minimal clinically important difference; FMA-UE, Fugl-Meyer assessment of upper extremity; NEURO, NovEl intervention Using Repetitive transcranial magnetic stimulation and Occupational therapy.

$$f\left(\mathrm{x}_{\text{responders}}\right) = \text{intercept}_{\text{responders}\mid\text{nonresponders}} + \beta_{\text{responders}\mid\text{nonresponders}}\,\mathrm{x_i}$$

$$f\left(\mathrm{x}_{\text{hyper-responders}}\right) = \text{intercept}_{\text{hyper-responders}\mid\text{nonresponders}} + \beta_{\text{hyper-responders}\mid\text{nonresponders}}\,\mathrm{x_i}$$

$$\mathbf{g}\left(\mathbf{x}_{\text{responders}}\right) = \frac{\mathbf{e}^{\mathbf{f}(\mathbf{x}_{\text{responders}})}}{\mathbf{1} + \mathbf{e}^{\mathbf{f}(\mathbf{x}_{\text{responders}})} + \mathbf{e}^{\mathbf{f}(\mathbf{x}_{\text{hyper-responders}})}} \quad (2:\text{responders})$$

$$\mathbf{g}\left(\mathbf{x}_{\text{hyper-responders}}\right) = \frac{\mathbf{e}^{\mathbf{f}(\mathbf{x}_{\text{hyper-responders}})}}{\mathbf{1} + \mathbf{e}^{\mathbf{f}(\mathbf{x}_{\text{responders}})} + \mathbf{e}^{\mathbf{f}(\mathbf{x}_{\text{hyper-responders}})}} \quad (3:\text{hyper}-\text{responders})$$

where $\mathbf{x_i}$, the initial-FMA-UE score, was the explanatory variable, $\beta_\mathbf{i}$ and $\mathbf{intercept_i}$ is the partial regression coefficient in each group, and **e** is Napier's constant. Therefore, for the multilevel responses, the cumulative probability was calculated at each level to generate a simple regression coefficient. The covariates influencing the recovery of the upper limb motor paralysis after treatment were: (1) age, (2) gender, (3) time from stroke onset to NEURO initiation, and 4) the dominant hand. To identify the model, the Akaike Information Criterion (AIC) was used (32). Applicability of the predictive model was assessed using McFadden's coefficient of determination, R^2, between the initial score and the delta-FMA scores for all 1,254 patients (33). All statistical analyses were performed using R 3.6.0 software (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Figure 2 shows the flow chart of the study design and patients selection based on the diagnosis. The median age and interquartile range of all patients were 63 and 56–70 years respectively. **Table 1** summarizes the clinical characteristics of the patients; the distributions of the characteristics were comparable across groups. Right-handed patients accounted for 95%, which is approximately equal to the same proportion for all Japanese. There were about twice as many males as females.

The multinomial logistic regression model fitted showed statistically significant valid logistic probability between delta- and the initial FMA score, adjusted for covariates, age, sex, time from onset, diagnosis, and dominant hand (McFadden's $R^2 = 0.103$, AIC = 1,999, $\chi^2 = 227$, $p < 0.001$) (**Table 2**). Time-series plots of the FMA scores are shown in **Figure 3.** The logistic curves discriminating between the probability of being responders ($5 \leq$ delta-FMA <10) from non-responders (delta-FMA <5) showed a significant model fit ($z = 5.31$; $p < 0.001$; odds ratio = 15.5, 95% Cl = 5.7–42.9). Similarly, hyper-responders (delta-FMA ≥ 10) and non-responders (delta-FMA <5) were differentiated according to the initial-FMA score ($z = 6.38$; $p < 0.001$; odds ratio = 166.8, 95% Cl = 34.6–803.5).

TABLE 1 | Patient characteristics among groups at baseline.

Characteristic	Non-responders	Responders	Hyper-responders
Participants (n)	763 (61%)	382 (26%)	109 (13%)
Age (years)	63 (56–70)	63 (55–70)	64 (56–69)
Sex (n)			
Female	247 (32%)	123 (32%)	41 (38%)
Male	516 (68%)	259 (68%)	68 (62%)
Paralysis side (n)			
Left	315 (41%)	173 (45%)	55 (50%)
Right	448 (59%)	209 (55%)	54 (50%)
Dominant hand (n)			
Left	35 (5%)	23 (6%)	5 (5%)
Right	728 (95%)	359 (94%)	104 (95%)
Diagnosis			
CI	387 (51%)	194 (51%)	51 (47%)
ICH	376 (49%)	188 (49%)	58 (53%)
Time from onset (months)	41 (23–74)	41 (24–75)	37 (21–58)
FMA-UE (in charge)	54 (46–60)	47 (39–52)	40 (33–45)

Values are n (%) or median (interquartile range). CI, cerebral infarction; ICH, intracranial hemorrhage; FMA-UE, Fugl-Meyer Assessment score.

TABLE 2 | Model coefficients of treatment responsiveness and initial FMA-UE score.

Separated responsiveness	Predictor	Estimate	95% CI	*z*	*p*
Responders|Non-responders	Intercept	2.75	1.73, 3.76	5.31	<0.001
	Initial FMA-UE	−0.07	−0.08, −0.06	−10.01	<0.001
	Age	−0.00	−0.01, 0.01	−0.17	0.863
	Sex	0.02	−0.25, 0.30	0.16	0.871
	Month from onset	−0.00	−0.00, 0.00	−0.25	0.806
	Diagnosis	0.04	−0.22, 0.30	0.30	0.764
	Handedness	0.35	−0.21, 0.92	1.22	0.224
Hyper-responders|Non-responders	Intercept	5.11	3.54, 6.69	6.38	<0.001
	Initial FMA-UE	−0.14	−0.16, −0.12	−11.64	<0.001
	Age	−0.01	−0.03, 0.01	−1.03	0.302
	Sex	−0.24	−0.69, 0.22	−1.02	0.306
	Month from onset	−0.00	−0.01, −0.00	−0.74	0.458
	Diagnosis	0.28	−0.16, 0.72	1.25	0.213
	Handedness	0.18	−0.85, 1.2	0.34	0.729

FMA-UE, motor function score of upper extremity by Fugl-Meyer Assessment; N-R, non-responders; R, responders; H-R, hyper-responders.

According to the multinomial logistic regression models, the probability of being a non-responders was 59.2% when the initial FMA score was 48.9. Similarly, when the initial FMA score was 38.8, the incidence of responders and hyper-responders was 45.5 and 16.0%, respectively (**Table 3**).

DISCUSSION

Recently, the maximum recovery state of motor function of the upper extremity in patients with stroke hemiparesis, including spontaneous recovery, has been estimated, based on the measured acute phase value (7, 34–36). Subsequent studies have also shown that NEURO treatment may restore motor function in the upper extremities during the chronic phase (15, 17). In this study, motor function of the upper extremities, based on values measured prior to NEURO treatment, was used to estimate post-treatment recovery rates based on previously reported acute and chronic MCID levels (19, 20, 31). The results of this study showed that about 45% of patients in the chronic stage who had FMA scores ranging from 30 to 40 before treatment showed improvement over the MCID by NEURO treatment. Furthermore, more than 25% of the patients with more severe initial values ranging from 26 to 30 improved beyond the MCID calculated in the acute phase. These results suggest that the evaluated motor function scores of the upper extremities before NEURO treatment can be used to estimate the occurrence of patients recovering beyond MCID among the patients in the chronic phase.

It is known that the effect of rehabilitation is enhanced when patients recognize the need to achieve their own goals and actively engage in pursuing them (37, 38). In addition, patients who practice self-efficacy affect the recovery of the upper limb motor function (4). Patients' recognition of the need to have their own behavioral goals and practice upper limb exercises display enhanced performance (4). Therefore, prediction of the treatment effect on the patient is important for the therapist and can facilitate patients' consent and cooperation with the treatment (39). To judge from the results of this study, the extent of recovery by NEURO treatment can be predicted, to some extent, from the patients' pre-treatment upper extremity functional evaluation, and this is useful information for the attending physician to provide the patient.

In this NEURO treatment, low frequency (LF)-rTMS was used. Ferbert et al. discovered that stimulation of the contralateral motor cortex immediately after stimulation of the motor area reduces the potential of stimulation of the contralateral hemisphere to evoke finger muscles (40). Moreover, Wards et al. reported that in the case of unilateral brain injury, the activity of the contralateral hemisphere was increased, and hyperactivity of the non-lesional hemisphere excessively induced the interhemispheric inhibition on the lesional side (41). In other words, unbalanced excitement of the cerebrum on the non-lesioned hemisphere adversely affects functional improvement. Since nervous activity is suppressed by LF-rTMS, the activity of the non-lesional hemisphere can be suppressed by applying LF-rTMS to the motor cortex of the non-lesional hemisphere (42), and suppression of interhemispheric inhibition of the non-lesional hemisphere indirectly increases the activity of the lesional side (43). On the other hand, high-frequency (HF)-rTMS evokes nervous activity and stimulates the motor cortex of the lesional hemisphere to enhance activity at the lesional site directly (44). Intensive upper-limb exercises are performed immediately after rTMS while the neurological activity of patients with stroke is adjusted, thus facilitating motor function (10, 45). The stimulation method corresponding to the effects of the neuromodulation in patients with various levels of disability will hopefully

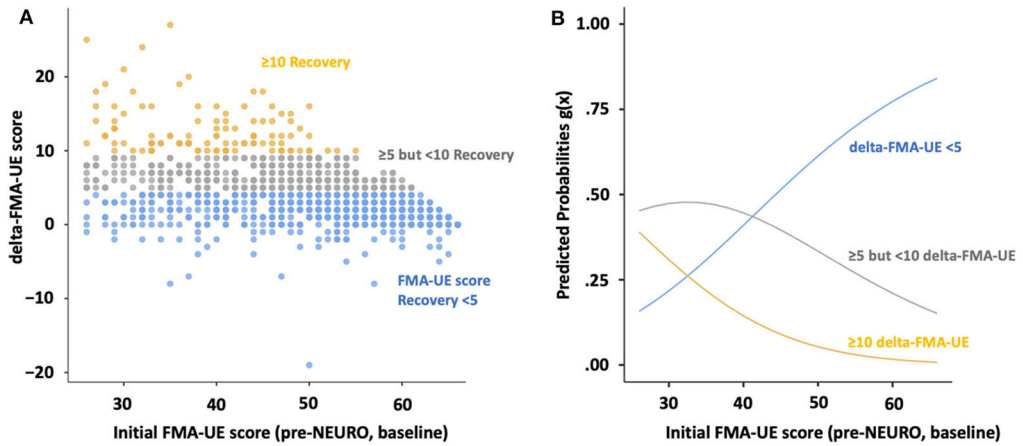

FIGURE 3 | Scatterplots and multinomial logistic probability plots showing the association between level of agreement for initial- and delta FMA score. (A) Initial FMA-UE score plots and histogram of FMA-UE score change for the upper extremities are divided by recovery, according to MCIDs. (B) The logistic curves were discriminated by the probability of being non-responders (delta-FMA-UE score <5 points, blue line), responders (5 ≤ delta-FMA-UE, gray line <10 delta-FMA-UE), and hyper-responders (delta-FMA-UE, yellow line ≥10). FMA: Fugl–Meyer assessment; NEURO, NovEl intervention Using Repetitive transcranial magnetic stimulation and Occupational therapy; MCID, minimal clinically important difference.

TABLE 3 | Estimated marginal means of Fugl-Meyer Assessment score in upper extremity, compared with responsiveness of treatment.

Initial FMA-UE	Responsiveness	Probability	SE	95% Confidence Interval	
				Lower	Upper
38.8⁻	N-R	0.384	0.036	0.307	0.462
	R	0.455	0.039	0.372	0.539
	H-R	0.160	0.034	0.087	0.234
48.9μ	N-R	0.592	0.035	0.518	0.667
	R	0.347	0.033	0.277	0.419
	H-R	0.060	0.016	0.027	0.093
59.0⁺	N-R	0.760	0.030	0.695	0.825
	R	0.221	0.029	0.158	0.284
	H-R	0.019	0.006	0.005	0.032

CI, cerebral infarction; ICH, intracerebral hemorrhage, N-R, nonresponders; R, responders; H-R, hyper-responders. –, mean – 1SD; μ, mean; +, mean + 1SD; FMA-UE, Fugl-Meyer Assessment score; SD, standard deviation; SE, standard error.

be of use in the clinical setting after further validation of its effectiveness.

In this study, recovery from motor paralysis in the upper extremities with NEURO treatment tended to occur more frequently in patients with moderate paralysis. In the chronic phase of stroke, the most widely accepted explanation for the efficacy of the 1-Hz stimulation of the unaffected hemisphere is the reduction in the abnormally high transcallosal inhibition toward the affected hemisphere (46, 47). In the acute phase, Wang et al. reported that HF-rTMS and exercise therapy could improve motor recovery at about a 10 FMA-UE score in patients with severe hemiplegic stroke (48). Similarly, Watanabe *et al.* reported that patients in the acute phase had reduced muscle spasticity and recovery of motor function with rTMS (49). Even when motor paralysis was severe, improvement of motor function in the upper extremities was observed by adjusting the excitability of the motor cortex in this study. In addition, the FMA-UE assesses the patients, post-stroke, per the sequential recovery stages (26). The FMA items are hierarchically organized from synergistic to voluntary movements. Synergistic movements exhibit abnormally stereotyped behavior that does not allow the combination of different movement patterns. For example, an attempt to raise the arm results in elbow flexion, shoulder abduction, and internal rotation. The flexor and extensor synergy components were tested before the movements combining the synergies with the movements out of synergy. Ya-yun et al. reported that the increase in FMA-UE score reflects the improvement of the proximal upper extremity movement (50). It is considered that the rTMS treatment improved the FMA score, and the patients with more severe motor paralysis had improved proximal upper limb movements. Schambra *et al.* reported that there was no difference in FME-UE score recovery with or without MEP in patients in the acute phase, but there was less improvement in patients with high FMA scores than in those with low FMA scores, and FMA recovery curves plateaued below the reported normal levels for both the arm and hand (51). The lower response of patients with high motor function compared to moderately paretic patients in our study might be because the treatment-recovery values were low in patients with high motor function. Furthermore, Veldema et al. reported that in patients with stroke, severe hand dysfunction was associated with a strong suppression of the ipsilesional cortico-spinal excitability and a shift in excitability toward the contralesional hemisphere (52). In the same study, mild hand movement impairment was associated with a shift in cortico-spinal excitability toward the ipsilesional hemisphere. Therefore, ipsilesional HF-rTMS may be effective in mild paralysis. As the upper extremities become more active, patients may be willing to actively use it. The results of this study clinically suggested that even in more affected moderate cases of motor paralysis in the chronic phase, the effect of

rehabilitation can be obtained in about 20% of patients, as in the acute phase.

Clinically (although not shown by the data in this study) and frequently, after the treatment there are highly psychologically satisfied patients, because they could use their own extremities and hands due to decreased finger clawing and because objects could be held by the paralyzed hands, even if the FMA score did not significantly change. Therefore, clinicians are required to explain to the patients how much they can improve and motivate them to participate in the treatment. To this end, further research should be conducted on the relationship between patients' motor function and their level of satisfaction, as well as the evaluation of gross and fine movement improvements.

There were some limitations to this study. Although the study did not include treatment data other than for NEURO, the patients included in the analysis may have received other treatments simultaneously, such as exercise therapy or OT. In addition, since the upper extremities are often used in ADL, the amount of functional recovery of the upper extremities is generally increased. The effects of the difference on non-NEURO treatments can be identified by comparing the recovery prediction accuracy between a non-NEURO-treated group and others treated with NEURO. There were more than 1,200 subjects in this study, and performing the stratified analysis described above requires larger samples.

CONCLUSION

This study provided clinical data to estimate the effect of NEURO treatment by pre-treatment FMA-UE score. Further verification is required regarding the need for both the patients and therapists to undergo rehabilitation with the goal of recovery before and after treatment, which has a favorable effect on treatment outcomes.

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 ethics committee of the Tokyo Jikei University School of Medicine and included an opt-out consent method (No. 20-041-5231). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

THam: analysis of data, data interpretation, and writing of the manuscript. NY: data interpretation and revisions. THad: data interpretation and revisions. MA: conception/design of the study, acquisition and analysis of data, data interpretation, writing of the manuscript, and revisions. All authors approved the submitted version and have agreed both to be personally accountable for the authors' contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, have been appropriately investigated, resolved, and the resolution documented in the literature.

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OPINION
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How to Design Optimal Accelerated rTMS Protocols Capable of Promoting Therapeutically Beneficial Metaplasticity

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INTRODUCTION

Our brain is comprised of billions of neurons, which can connect via synapses that rely on electrical signaling and the release of chemical messengers to communicate and propagate signals through neural networks. By forming such networks, neurons are capable of monitoring previous firing activity, and using this information to adapt subsequent firing rate. This so-called activity-dependent plasticity is critical for the encoding of new information, and the tuning of (low activity) connections (1–3). The physiological mechanisms of synaptic plasticity have largely been attributed to Long-Term Potentiation (LTP) (4, 5), and Long-Term Depression (LTD) (6–8), which result from molecular processes such as receptor trafficking or synaptic scaling (3). Both LTP and LTD are induced by postsynaptic NMDA receptor activation, which lead to an influx of calcium into the postsynaptic dendrites (8–10). This triggers a complex series of intracellular signaling cascades, resulting in synaptic modifications such as AMPA receptor trafficking (11, 12). The pattern of stimuli delivered to the post synapse determines whether LTP or LTD will occur; low frequency stimulation induces LTD, whereas high frequency stimulation induces LTP (8, 13). These processes underlie much of our knowledge on the molecular mechanisms of learning and memory.

However, if the principles of Hebbian synaptic plasticity (LTP, LTD) alone were to drive the strengthening and weakening of synaptic connections, activity would, over time, be driven toward destabilization. This is because continuously firing synapses could only become stronger (driven to saturation) and unused synapses quiescent (until completely lost) (14). Consider a synapse that is strengthened by LTP; meaning the presynaptic neuron becomes more effective at depolarizing the postsynaptic neuron. With each continued stimulation, the postsynaptic neuron will be more easily depolarized, in a positive feedback loop, resulting in a hyperexcitable postsynaptic neuron. Over time, not only will the original presynaptic connection be strengthened, but other unrelated presynaptic inputs could cause a depolarization of the hyperexcitable postsynaptic neuron, resulting in unregulated synaptic transmission (15). Therefore, other mechanisms must exist, which regulate synaptic plasticity on a global network level to maintain stability of synapses and maintain specificity of neural activity (16, 17).

Metaplasticity refers to any change in the direction or degree of synaptic plasticity (ex. LTP, LTD) based on prior neural activity (18). While both synaptic and metaplasticity are dependent on previous neural activity, metaplasticity does not *directly* alter the efficacy of synaptic transmission

(as LTP/LTD), but it adjusts the neurons' ability to induce LTP/LTD with *subsequent* neural activity. Metaplasticity in some sense can be considered as the plasticity of synaptic plasticity, e.g., maintaining the dynamic nature of a neuron's firing threshold, when this neuron reaches a certain firing rate (16, 18, 19). Metaplasticity works through similar synaptic modifications as LTP/LTD, such as NMDA receptor activation and modification (20), and changes in calcium signaling triggering complex signaling cascades (18). Metaplastic modifications, for example at NMDA receptors, can occur either at specific synapses or across the whole neuron, and on time scales from minutes to weeks (19). Depending on the temporal pattern and strength of previous neural activity, metaplastic mechanisms can be additive; for example promoting increased synaptic strengthening through repeated excitatory (LTP-inducing) stimulation. Metaplasticity can also be stabilizing; for example acting against subsequent synaptic strengthening when repeating excitatory (LTP-inducing) stimulation (19, 21). This stabilizing form of metaplasticity is often referred to as *homeostatic* metaplasticity, as it specifically regulates the dynamic threshold of synaptic plasticity to maintain equilibrium, or homeostasis (16, 17). We hypothesize, based on research from human and animal studies, that the timing between excitatory stimulations are what differentiate between promoting additive or homeostatic metaplasticity.

We focus on the role of metaplasticity in Transcranial Magnetic Stimulation (TMS). We describe the recent use of accelerated (repeated) stimulation protocols, both in research and clinical applications, and the molecular mechanisms required to promote either homeostatic or additive metaplastic effects. Finally, we showcase the therapeutic potential of accelerated stimulation, and hypothesize that increasing the currently practiced stimulation intervals may be more efficacious in promoting additive metaplastic effects in various clinical applications of rTMS in rehabilitation, neurology, psychiatry, and cognitive decline.

METAPLASTICITY IN TMS

TMS is a widespread and increasingly popular non-invasive brain stimulation technique, where electromagnetic pulses allow stimulation to pass non-invasively through the skull (22). When pulses are applied in a certain pattern, as repetitive TMS (rTMS), protocols can have lasting excitatory or inhibitory effects (23–25). Two commonly used stimulation protocols are *intermittent* Theta Burst Stimulation (iTBS), requiring only 3 min of stimulation time, resulting in a lasting increase of cortical excitability, and *continuous* Theta Burst Stimulation (cTBS), requiring only 40 s of stimulation for a lasting decrease in cortical excitability (26). The after effects of these protocols have been shown for up to 1 h following stimulation (26, 27).

While iTBS is normally an excitatory protocol, causing an increase in cortical excitability of the stimulated brain region, it has been shown that when applied twice in quick succession iTBS effects switch from excitatory to inhibitory (28). Conversely, when cTBS (an inhibitory protocol) is applied for double the normal duration, its effects switch from inhibitory to excitatory (28). Several studies have reported similar effects of repeating iTBS or cTBS stimulation protocols, with the timing between protocols being an important factor in the magnitude and direction of aftereffects (19, 29, 30). For example, using a "priming" iTBS protocol which does not induce plasticity, followed by a "test" iTBS protocol has shown that short intervals of 5 min between priming and test resulted in homeostatic-like changes in excitability, i.e., an opposite effect. Interestingly, longer breaks of 15 min resulted in an increase in MEP amplitude after the test iTBS (30). However, 15 min between priming and test iTBS/cTBS has also been shown to induce in homeostatic-like metaplastic effects (29). While the timing between repeated TBS sessions is clearly important, the optimal interval is less clear. 15 min between iTBS sessions has been shown to promote both homeostatic (29) and MEP enhancement after the second iTBS (30), while 10 min between priming and test iTBS has shown enhancement of MEP amplitude (31), but 5 and 20 min between iTBS sessions did not (32). Therefore, when 2 iTBS sessions are repeated with short (<30 min) between, conflicting effects on MEP amplitude have been reported.

"Accelerated" protocols, which consist of multiple stimulation sessions on a single day, have recently been introduced for the treatment of depression (33–37). Due to their short duration, the TBS protocols, in particular iTBS, have been promising candidates for accelerated protocols (38). Also, a large trial recently found that iTBS was not-inferior to the classical 10 Hz rTMS protocol, confirming the clinical potential of this shorter stimulation protocol to treat depression (39). Indeed, several studies have shown additional benefits for accelerated iTBS protocols in the treatment of severe, treatment resistant depression (40, 41). In the clinic, an interval of 15 min is often used between iTBS sessions, with these sessions repeated up to 5 times on a single treatment day (40, 41).

We recently conducted a study investigating the effects of accelerated iTBS over motor cortex, consisting of 5 repeated iTBS sessions in a single day. iTBS with 8- or 15-min time interval between sessions were delivered to healthy participants in a fully within subject design; where participants received 4 different conditions (accelerated iTBS with 8-min intervals, accelerated iTBS with 15-min intervals, single iTBS and sham) (42). We compared change in Motor Evoked Potential (MEP) amplitude up to 90 min following stimulation, across the stimulation conditions.

We found that there was no difference in the effects of accelerated iTBS on MEP amplitude, also when compared to sham stimulation, and thus no additive metaplasticity induced by five stimulation sessions applied successively in 8- or 15-min intervals. We argue that such intervals between iTBS protocols are likely too short to avoid processes of homeostatic plasticity. With only 8 or 15 min between sessions, homeostatic mechanisms may be working against additive metaplastic effects to maintain network stability and therefore result in a net effect of no change in excitability following these accelerated protocols (42).

TIMING-DEPENDENT METAPLASTICITY

In agreement with this notion, animal studies in rats, and rat hippocampal slices have shown that a sufficiently long pause between excitatory stimulation sessions was necessary for additive (LTP) plasticity effects to occur (43–45). This may have to do with the time required for metaplasticity mechanisms, for example synapse strengthening with AMPA receptor trafficking (15).

It has been well-established in animal studies, that a single round of TBS (a 4-pulse burst at 100 Hz, repeated at 5 Hz for 10 bursts) is effective at inducing LTP in CA1 hippocampal pyramidal neurons (46, 47). TBS has since then been used extensively to reliably induce LTP *in vitro* (48). Interestingly, repeating this single TBS protocol with a time interval of >40 min, was capable of almost doubling the potentiation compared to the first TBS alone (43). This additional potentiation is thought to work through strengthening the smaller synapses which weren't strengthened by the first TBS protocol (43). This may have to do with the number of AMPA receptors; smaller synapses contain fewer AMPA receptors and therefore don't generate a response to trigger a depolarization following a single TBS (43). Several other studies have provided evidence for increased potentiation by spaced TBS, however the magnitude and duration of the effects depended on a series of factors such as rat strain, rat age, and the time interval. In adult Wistar rats, adult Long-Evans (LE) rats, and young LE rats, 4 h was required between TBS to induce additional potentiation (44, 45). However, in young Sprague Dawley (SD) rats, a single TBS repeated at 1-h intervals could induce further potentiation, following up to 3 repeated TBS stimulations (4 did not produce additional potentiation) (43, 45). These different studies used different stimulation intensities; Frey et al. (44) found that reducing stimulation intensity in the second stimulation was effective for promoting potentiation 4-h later, while Cao and Harris (45) and Kramár et al. (43) kept stimulation intensities constant. However, these studies consistently show that additional potentiation following repeated TBS in animal slices is possible. Enhanced, additive LTP-like plasticity may be promoted when repeating TBS with 50–60 min between sessions (43, 45). After 3 TBS protocols, spaced 60 min apart, potentiation had been raised to 150% baseline, which is about three times higher than if just one protocol was given (43, 48). This suggests that 3 TBS protocols repeated at 60 min-intervals may be effective at promoting maximal, additive metaplasticity effects (**Figure 1A**). If there is less time between TBS protocols, for example 10 min, homeostatic metaplasticity mechanisms may dominate, promoting a stabilizing rather than additive plasticity response (**Figure 1B**).

DISCUSSION

Activity-dependent metaplasticity is considered to be *homeostatic* if the first stimulation protocol alters the threshold for subsequent LTP/LTD in the opposite direction, thereby stabilizing (network) brain activity (49). Interestingly, this reversal of aftereffects has been shown specifically when stimulation protocols were given with a short (0–5 min) interval (28, 30), providing support for homeostatic metaplasticity mechanisms in rTMS protocols (19). While homeostatic metaplasticity mechanisms are important for stabilizing network activity, they can be counteractive when promoting plasticity effects through rTMS. In fact, when applying rTMS protocols, the explicit goal is not stabilization but promotion of additive, increased plasticity effects.

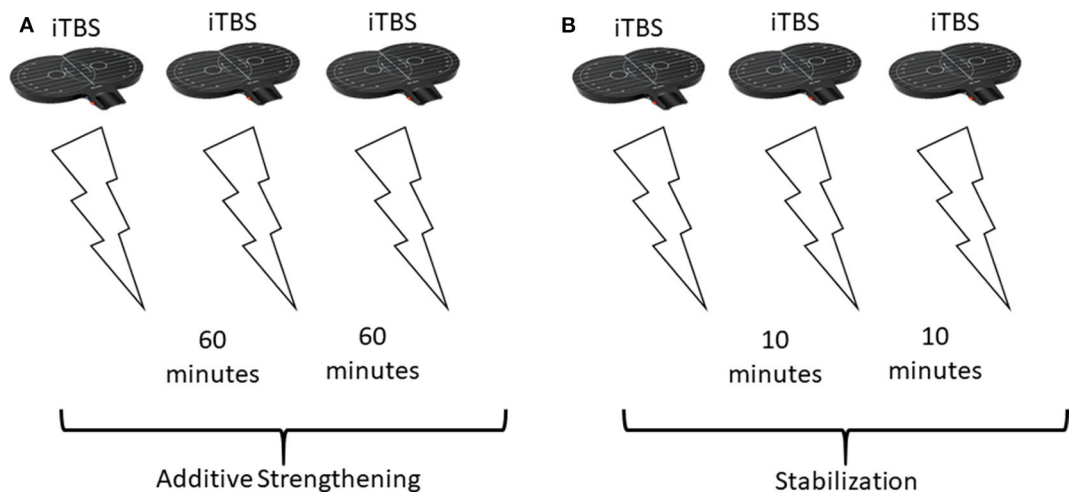

FIGURE 1 | Theoretical stimulation setup and effects in response to different spacings between repeated stimulations. **(A)** Repeating excitatory (iTBS) stimulation 3 times, with 60 min between sessions, promotes additive strengthening of stimulated synapses. Overall, the repeated stimulation increases potentiation [this has been shown in animals using a different TBS protocol (43, 45)]. **(B)** Repeating the same 3 iTBS stimulations, but with only 10 min between sessions results in stabilization (homeostatic metaplasticity) and no change in overall plasticity.

Animal studies have shown that timing is important in the molecular mechanisms underlying metaplasticity. While there is overlap between the mechanisms of additive and homeostatic metaplasticity, there are temporal differences which may differentiate between both principles at the molecular level. Based on evidence form animal models, leaving 60 min between excitatory stimulation protocols may promote additive rather than homeostatic metaplastic effects in accelerated TMS treatment protocols.

Clinical Implications

If longer intervals between iTBS sessions are capable of promoting additive metaplasticity, as has been shown in animal studies (43) as well as improving clinical outcomes in the treatment of depression (50), longer spaced intervals between iTBS sessions will likely be beneficial for other therapeutic applications of iTBS. iTBS is increasingly being used as a treatment in a range of clinical applications such as rehabilitation, as well as neurological and psychiatric disorders. For example, to promote motor recovery after stroke (51), for managing spasticity associated with Multiple Sclerosis (MS) (52), and decreasing obsessive symptomatology associated with Obsessive Compulsive Disorder (OCD) (53), just to name a few. These protocols all must adhere to the established safety guidelines (54), and recommendations for clinical TMS use (55, 56). These include total pulse number, interval between TBS session, intensity of stimulation, and cumulative weekly applications (54). Accelerated iTBS has been successfully and safely used in the treatment of depression (38, 40, 41), with patients receiving a total of 32,400 pulses at 110% resting motor threshold, over 20 sessions (5 sessions per day, 15 min between sessions) in 4 days (41). Therefore, while following the established safety guidelines is the upmost priority, and local health authorities should always approve each stimulation protocol (54), delivering three iTBS sessions on a single day with 1 h between sessions should theoretically be safe and tolerable for most patients.

rTMS is also used as a treatment for the cognitive decline associated with neurodegenerative disorders such as dementia, and Alzheimer's Disease (AD) (57–61). However, there are ethical implications of using rTMS for cognitive enhancement, in particular in healthy participants (62). It is important to maintain the consensus ethical requirements that (1) participants/patients provide informed consent, (2) the benefit of the research outweigh the risks, and (3) there is equal distribution of burdens and benefits across patients (this is violated if a particular group of patients with different economic, physical or social conditions) (54).

Importantly, the here described principles of additive and homeostatic metaplasticity not only apply to the here discussed accelerated TMS treatments and the question of optimal time interval between its repeated stimulation sessions, but likewise can be used to explain and optimize other forms of plasticity-inducing TMS protocols such as Paired Associated-Stimulation (PAS) or paired-coil TMS (pcTMS).

In humans, neural excitability and synaptic plasticity can be probed by TMS to peripheral nerves and motor cortex (63, 64). In such a transcortical loop, timings of afferent (muscle/nerve to brain), cortical, and efferent (brain to muscle) responses can be used to quantify central motor excitability (63). For example, delivering a conditioning TMS pulse to an afferent tract (ex. the wrist), followed (10–48 ms) by stimulation of the efferent tract (motor cortex), will alter Motor Evoked Potentials (MEP's) measured from thumb flexor muscles (63). It has been shown that wrist stimulation 20–22 msec preceding motor cortex stimulation elicits a facilitated MEP, with a latency of about 1 ms, compared to MEPs given without the conditioning wrist stimulation (63). Repeating this afferent (wrist) efferent (motor cortex) stimulation, in Paired Associated Stimulation (PAS), can induce lasting effects on motor cortex excitability (64, 65), providing evidence for synaptic plasticity. Interestingly, evidence of homeostatic and additive metaplastic responses have also been recorded using PAS stimulation (66, 67). When two LTP-inducing PAS protocols were separated by 30 min, a decrease in MEP amplitude was measured, indicating a homeostatic (stabilizing) metaplastic responses (66). Similarly, LTD-inducing PAS immediately preceding a motor-learning task facilitated motor-learning (67), again providing support for homeostatic plasticity mechanisms dominating at early time points following stimulation.

Additionally, the effects of brain stimulation are not only localized to the site of stimulation, but can also spread to different areas through complex cortical networks. Similarly to PAS, this has been shown using paired-coil TMS (pcTMS), where multiple coils are used to probe different cortical areas and assess connectivity (68, 69). For example, a single TMS pulse to motor cortex can cause a depression of the MEP measured following a subsequent (6–30 ms) TMS pulse to contralateral motor cortex (70). Therefore, TMS can also be used to assess connectivity between brain areas (68). In other words, TMS stimulation can propagate to different cortical regions, having both local and remote effects on (meta) plasticity. This has valuable clinical implications, where inducing plasticity effects in a cortical network are important (69). In stroke patients for example, localized damage can disrupt connectivity and can have functional consequences (69), therefore stimulation effects should promote network plasticity, rather than localized plasticity. Similarly, in the treatment of depression, superficial stimulation uses cortical connectivity to influence deeper cortical structures, resulting in improvement of clinical symptoms (71, 72). Therefore, it is important to use TMS to strengthen connectivity, and to promote additive, metaplastic changes also on the network activity level.

With the increasing and widespread application of rTMS protocols in the clinic, it is important to optimize protocols to maximize their effects, while remaining within established safety and ethical guidelines for use in the clinic (54, 56). Single iTBS has proven promising, but accelerated iTBS at longer time intervals (60 min) between sessions could maximize clinical outcomes through additive metaplasticity, preventing homeostatic metaplasticity from stabilizing stimulation effects. Clinical efficacy of PAS and pcTMS protocols may be similarly increased by optimizing the timing between stimulations according to these principles of metaplasticity.

AUTHOR CONTRIBUTIONS

AS: conceptualization, writing-review and editing, supervision, and funding acquisition. AT: investigation, writing-original draft preparation, and visualization. All authors contributed to the article and approved the submitted version.

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Transcranial Direct Current Stimulation to Enhance Cognitive Impairment in Parkinson's Disease: A Systematic Review and Meta-Analysis

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Cognitive deficits are increasingly being recognized as a common trait in Parkinson's disease (PD). Recently, transcranial direct current stimulation (tDCS) has been shown to exert positive effects as an adjunctive therapy on motor and non-motor symptoms in PD. This systematic review and meta-analysis aims to provide an overview of reported evidence on the efficacy of tDCS interventions in the treatment of cognitive impairments in PD. A systematic literature review was conducted to examine articles that were published in the past 10 years and that study the effects of tDCS on cognitive deficits in PD patients. The PubMed, Scopus and Scielo databases were searched. Eight tDCS studies involving 168 participants were included for the analysis. Our meta-analysis results showed that anodal tDCS (atDCS) had various levels or no evidence of effectiveness. In the pre-post stimulation analysis, a strong effect was reported for executive functions (pre-post: $g = 1.51$, $Z = 2.41$, $p = 0.016$); non-significant effects were reported for visuospatial skills (pre-post: $g = 0.27$, $Z = 0.69$, $p = 0.490$); attention (pre-post: $g = 0.02$, $Z = 0.08$, $p = 0.934$), memory (pre-post: $g = 0.01$, $Z = 0.03$, $p = 0.972$) and language (pre-post: $g = 0.07$, $Z = 0.21$, $p = 0.832$). However, in the pre-follow-up stimulation analysis, the duration of the effect was not clear. This study highlights the potential effectiveness of atDCS to improve cognitive performance in PD patients but failed to establish a cause-effect relationship between tDCS intervention and cognitive improvement in PD. Future directions and recommendations for methodological improvements are outlined.

Keywords: Parkinson's disease, transcraneal electric stimulation, neuroplasticity, executive functions, cognition

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INTRODUCTION

There is growing interest in the potential efficacy of transcranial direct current stimulation (tDCS) for treating neurodegenerative conditions such as Parkinson's disease (PD). Previous systematic reviews on PD have supported the efficacy of tDCS for improving motor functions, including balance, gait, and bradykinesia (1–5). However, evidence is not clear regarding its efficacy for PD patients' cognitive symptoms.

Cognitive impairment is frequent in PD, though it can be heterogeneous in its presentation and progression, as it varies regarding clinical features, severity, and progression to dementia. It has been suggested that interventions for cognitive symptoms may be essential in preventing and delaying the onset of cognitive decline and Parkinson's disease dementia (PDD) (6, 7). Approximately 25% of PD patients have mild cognitive impairment (MCI) and an increased risk of developing PDD (8). Most commonly, reported cognitive disorders in PD include executive deficits (9), visuospatial impairments (10), memory deficits (11), action verb, and action conceptualization impairments (12, 13). These can be progressive and make patients more vulnerable to the onset of affective symptoms, behavioral disorders, and other neuropsychiatric symptoms (14).

tDCS is a non-invasive brain stimulation technique modulating cortical activity that acts by inducing a low-frequency electric current (15), usually between 1 and 2 milliamps (mA), to activate the potential of the resting neuronal membrane (16, 17). The current transmission modifies the membrane's polarity (18), producing a facilitating effect when the positive electric current (anodal) is administered or hyperpolarization when the negative electric current (cathodal) is administered (19).

Given the increasing use of tDCS in neurodegenerative diseases such as PD, the present study aimed to systematically review and analyze studies evaluating the effects of tDCS on PD patients' cognitive alterations.

MATERIALS AND METHODS

A systematic literature search was conducted for articles on the effect of tDCS interventions on PD patients' cognitive symptoms. PubMed, Scopus, and Scielo databases were searched for articles published between 2000 and 2020, without language restrictions, combining the following terms: "tDCS," "transcranial direct current stimulation," "non-invasive brain stimulation," and "Parkinson's disease." We also conducted cross-reference searches of original articles and reviews to identify additional studies that could not be retrieved from electronic databases.

Inclusion Criteria

This study follows the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (20).

Eligibility Criteria

We used the following PICOT criteria (population, intervention, comparison outcome, and study type) to define eligibility criteria (see **Supplementary Material**):

- Population: PD and MCI PD patients without dementia diagnosed following UKBB criteria in levodopa on/off stage;
- Intervention: studies evaluating tDCS effects on cognitive functions;
- Comparison outcome: scores obtained on cognitive measures and standard deviation/error.
- Study type: randomized studies with double/single-blind design.

Studies in which data from pre-defined outcomes could not be extracted were excluded (see **Figure 1**). The following studies were also excluded: (a) animal studies, (b) studies combining tDCS and transcranial magnetic stimulation (21), (c) case studies (22), and (d) non-cortical stimulation studies (23, 24).

Data Analysis

Several meta-analyses of tDCS vs. sham on cognitive processing was performed following the procedures outlined by Borenstein et al. (25). Interventions' effect sizes were estimated through mean, standard deviation, and sample size. When it was not possible to extract the data, a web calculator was used (26). Because Cohen's "d" overestimates the effect size with small samples, Hedges' "g" was used to correct this bias (27), discriminating between small (0–0.20), medium (0.50–0.80), and large (>0.80) effect sizes (28). Additionally, a random effects approach was used, given its usefulness when there are different designs and response variables. For each analysis, a z-test was performed to derive a summary p-value. Lau et al.'s (29) study was excluded since needed data for effect-size calculation could not be extracted, while a social cognition meta-analysis could not be performed because Adenzato et al.'s (30) study was the only one to provide such measure.

The cognitive domains were defined according to the characteristics of each study as follows:

- Report of an index or subscale.
- If there were several tasks associated with the same domain, the tasks most used in clinical practice and research were included.
- In the case of a single task/subtest, its effect size was used as the index of the domain to which it was associated.

Meta-analyses were performed at two time points: (a) pre-stimulation to post-stimulation, and (b) pre-stimulation to follow-up. Additionally, as many studies combined tDCS with cognitive training (CT), task scores in interventions that combined stimulation with standard (non-tailored) CT were preferred over task scores in tDCS-only interventions.

Outcome Variables

As primary outcomes we considered: (1) Measures of executive functions: Problem-solving strategies: The Stockings of Cambridge (SOC) subtest of CANTAB (31); Task-Switching: the Wisconsin Card Sorting Test (WCST), the Trail Making Test B (TMT-B) (32, 33); working memory: the Three-back letter task (34), Visual working memory (VWM), the change detection task (29), the working memory test (WM) (33); inhibition: Stroop Test (Color-word interference) (31–33); verbal and phonological fluency: the Verbal Fluency Test (32), the Controlled Oral Word Association Task (COWAT) (31) and tasks of semantic and phonological fluency (35); (2) Measures of visual attention: the TMT-A (32), the number-letter sequence (LNS) (31); (3) Measures of memory: the Hopkins Verbal Learning Test-Revised (HVLT-R) Immediate recall test, the Paragraph Recall Test (31); (4) Measures of visuospatial skills: the Line Orientation Judgment Test (JLO) and Hooper's Visual Organization Test (HVOT) (31); (5) Measures of language: the Boston Naming

FIGURE 1 | Meta-analyses performed in different cognitive domains for two time points showing both each study effect size and their relative weight within the summary effect size. Effect sizes are expressed in Hedges' g, and the forest plots represent the weight of the studies by the size of the squares, their effect size by their position relative to the x-axis and Hedges' g 95% CI by the squares' lateral bars.

Test-Short Form (BNT), the similarity test (31); (6) Measures of theory of mind: the Reading the Mind in the Eyes task, the Attribution of Intentions (AI) task (30); (8) Measures of procedural learning: Probabilistic Classification Learning (PCL) (33); (9) Measures of the inhibition of emotional response: the emotional go/no-go paradigm (29).

TABLE 1 | Effect of transcranial direct current stimulation on cognition in Parkinson's disease.

Study	Cognitive abilities	Test	Total sample (*n*)	Mean age	Evolution of diagnosis	On/off state	Stimulation parameters					Results
							Active electrode	Reference electrode	Intensity (mA)	Duration (min)	Number of sessions	
Adenzato et al. (30)	Theory of mind (ToM)	Reading the mind in the eyes (RME) task Attribution of intentions (AI) task	(*n* = 20) atDCS (*n* = 20) stDCS	65.6 (8.4)	N/R (MCI)	N/R	MFC (FPz)	Between Inion and Oz	1.5	6	1 atDCS session 1 stDCS session	atDCS over the MFC enhances ToM in patients with PD-MCI.
Biundo et al. (36)	Cognitive functions	MoCA, RBANS Tot., list learning, story learning, complex figure copy, orientation line, naming, semantic fluency, digit span, written coding test, list recall, list recognition, story recall, figure recall	(*n* = 24) (*n* = 12) atDCS (n = 12) stDCS	69.1_7.6	N/R (MCI)	N/R	L-DLPFC	Contralateral supraorbital region	2	20	4 sessions	atDCS over the PFC increased performance in immediate memory skills (story learning test) enhancing declarative and long term memory consolidation.
Boggio et al. (34)	Working memory	Three-back letter working memory paradigm	(*n* = 18) (*n* = 9) atDCS 2mA (*n* = 9) atDCS 1mA	45	Experiment 1 13.7 (8.2) Experiment 2 12.7 (8.1)	OFF	L-DLPFC M1	Contralateral right orbit	Different intensities 1–2	20	2 sessions	2mA of atDCS of the LDLPFC may improve working memory. Beneficial effect on working memory in PD patients depends on the intensity and site of stimulation.
Brandão et al. (32)	Speed processing, executive function, working memory, attention, verbal fluency, inhibitory control	Trail Making Test (TMT), Verbal Fluency test, Stroop test,Timed Up and Go test and video gait analysis.	(*n* = 20) (*n* =10) atDCS (*n* = 10) stDCS	64.45 ± 8.98	7.80 ± 5.32	N/R	L-DLPFC	Right orbital frontal cortex (Fp2)	2	20	1 session	After a single session of tDCS over the DLPFC there is improvements on cognitive tests. Cognitive areas improved the performance in the Stroop test and in the Verbal Fluency.
Doruk et al. (33)	Cognitive functions, depressive symptoms and motor functions	Trail making tests A and B (TMTA and B), Wisconsin card sorting test (WCST), probabilistic classification learning (PCL), working memory test (WM) and stroop test.	(*n* = 18) (*n* =5) atDCS R-DLPFC (*n* = 6) atDCS L-DLPFC (*n* = 7) stDCS	40_71	S/R	ON	L-DLPFC R-DLPFC	Right supraorbital region	2	20	10 sessions	Active stimulation over RDLPFC and LDLPFC resulted in prolonged improvements on executive function (TMT-B test).
Lau et al. (29)	Working memory	Visual working memory task and emotional go/no-go paradigm	(*n* = 10)	56–78	7.8 ± 3.6	ON	L-DLPFC	Contralateral (right) supraorbital area	2	20	1 atDCS session 1 stDCS session	Single-session of atDCS over the L-DLPFC did not significantly improve cognitive tasks in PD
Lawrence et al. (31)	Cognitive function and functional outcomes	Tockings of Cambridge (SOC) subtest from CANTAB and the controlled oral word association task (COWAT), letter-number sequencing (LNS) and the stroop (color-word interference) test, Hopkins verbal learning test-revised (HVLT-R) immediate recall subtest (20) and the paragraph recall test, judgment of line orientation (JLO) test and the Hooper visual organization test (HVOT), y Boston naming test-short form (BNT) and the similarities test.	(*n* = 42) SCT (*n* = 7) TCT (*n* = 7) tDCS (*n* = 7) SCT + tDCS (*n* = 7) TCT + tDCS (*n* = 7) Control (*n* = 7)	SCT: 68.14 (8.69) TCT: 65.57 (5.20) tDCS: 72 6.45 SCT + tDCS: 63.57 (15.68) TCT + tDCS: 67.43 (6.37) Control: 72.29 (6.21)	SCT: 5.29 TCT: 5.79 tDCS: 5.50 SCT + tDCS: 6.79 TCT + tDCS: 4.43 Control: 5.36	ON	L-DLPFC	Above the left eye	1.5	20	4 sessions	The intervention groups demonstrated variable statistically significant improvements across executive function, attention/working memory, memory, language, activities of daily living, and quality of life.

(Continued)

TABLE 1 | Continued

Study	Cognitive abilities	Test	Total sample (*n*)	Mean age	Evolution of diagnosis	On/off state	Stimulation parameters				Results
							Active electrode	Reference electrode	Intensity (mA)	Duration (min)	Number of sessions	
Pereira et al. (35)	Phonemic and semantic fluency	Phonemic and semantic fluency tasks	(*n* = 16)	61.5_9.9	S/R	N/R	L-DLPFC L-TPC	Right supraorbital area	2	20	1 session	Functional connectivity in verbal fluency and deactivation task-related networks was significantly more enhanced by tDCS to DLPFC than to TPC. atDCS over l_DLPC increased performance on the phonemic fluency task.

L-DLPFC, Left dorsolateral prefrontal cortex; R-DLPFC, Right dorsolateral prefrontal cortex; M1, Primary motor cortex; L-TPC, Left temporo-parietal cortex; MFC, Medial Frontal Cortex; MCI, Mild Cognitive Impairment; SCT, Standard Cognitive Training; TCT, Tailored Cognitive Training; tDCS, Transcranial direct current stimulation; atDCS, Anodal transcranial direct current stimulation; ctDCS, Cathodal transcranial direct current stimulation; stDCS, Sham transcranial direct current stimulation; N/R, Not reported.

RESULTS

From the initial 248 search results, 32 relevant publications were identified from databases. Of these, eight articles met the inclusion criteria (**see Supplementary Material**). The participants' mean age in these studies was 64.2 ± 3.1 years (min 61–max 69). With a total of 168 subjects, the average size of the groups was 21 (10 min and 42 max). The average disease duration and the L-dopa effect were not reported in all the studies.

Overall, 87.5% of the studies reported better cognitive performance after atDCS (see **Table 1**). Boggio et al. (34) administered 1 and 2 mA atDCS in the left motor cortex (anodal L-M1) or in the left prefrontal dorsolateral cortex (L-DLPFC) with the cathode located in the contralateral supraorbital area (SOAC). They reported high accuracy on the WM, with 2 mA over the L-DLPFC.

Pereira et al. (35) used 2 mA atDCS in the L-DLPFC and left temporoparietal cortex (L-TPC) and cathode in the SOAC. The results showed improvement in phonological verbal fluency after atDCS over L-DLPFC compared to the L-TPC. Additionally, fMRI verified an increase in functional connectivity between the frontal, parietal, and fusiform areas.

Doruk et al. (33) administered 2 mA in the R-DLPFC and L-DLPFC in 18 subjects with PD and located the cathode in the SOAC. The study reports improvement in the TMT-B after bilateral atDCS in the DLPFC.

Biundo et al. (36) used atDCS in the L-DLPFC with 2 mA and placed the cathode in the SOAC in 24 subjects with PD with mild cognitive impairment (MCI-PD). The researchers reported increased immediate memory skills and long-term consolidation of declarative memory.

Lawrence et al. (31) applied atDCS with 1.5 mA in the L-DLPFC and placed the cathode over the left eye in 42 subjects with MCI-PD. The authors implemented various intervention schemes combined with atDCS to assess the impact on cognitive and functional performance. Evidence suggests improvement in executive function, attention/WM, memory, language, daily living activities, and quality of life compared to the control group when combining CT and atDCS.

Adenzato et al. (30) administered 1.5 mA atDCS to the medial frontal cortex (MFC) and placed the cathode between the Inion and Sickle in 20 MCI-PD patients. The authors report a significant correlation between the reaction time (RT) of the Attribution of Intentions (AI) task and the Frontal Assessment Battery (FAB) score and the effect of interference in time and Stroop error. Findings are limited to improvement in RT; no significant improvement in response precision was observed. Researchers suggest that atDCS in MFCs improves deficits in the Theory of Mind (ToM) in MCI-PD.

Brandão et al. (32) investigated the effect of atDCS on executive functions, verbal fluency, and inhibitory control in 20 subjects with PD when administering 2 mA for 20 min in the L-DLPFC. The cathode was placed in the SOAC. The study reports improvement in the performance of cognitive tests STROOP—inhibition and interference—and verbal fluency in the group that received atDCS. The authors do not report a significant difference in the TMT-B or motor measurements.

Lau et al. (29) applied 2 mA to the L-DLPFC in 10 subjects with PD without cognitive compromise, locating the cathode in SOAC. The researchers evaluated VWM and emotional inhibitory control using experimental paradigms. The study suggests that performing a single session of atDCS is insufficient to generate significant VWM and emotional inhibition processes in subjects with PD. However, the authors also highlighted the small sample size.

We ran 2 meta-analyses per cognitive domain: (a) one analyzing the pre-post stimulation period and (b) one analyzing the pre-follow-up stimulation period. Regarding executive functions, the results showed large effects of improvement in performance in the pre-post period and small and non-significant effects in the pre-follow up [pre-post: $g = 1.51$, 95% CI = (0.28, 2.74), $Z = 2.41$, $p = 0.016$; pre-follow up: $g = -0.15$, 95% CI = (−0.75, 0.45), $Z = -0.50$, $p = 0.619$], see **Figure 1**, analysis 1.1 y 1.2. In memory, there was a medium effect for the pre-follow-up period of improvement in cognitive performance, although it was not significant, while for the other period, the effect was small and non-significant [pre-post: $g = 0.01$, 95% CI = (−0.60, 0.63), $Z = 0.03$, $p = 0.972$; pre-follow-up: $g = 0.46$, 95% CI = (−0.24, 1.15), $Z = 1.28$, $p = 0.199$] (**Figure 1**, analysis 1.3 y 1.4). The analyses in visuospatial skills showed medium effects with improvement in the pre-post and decrease in performance in the pre-follow up, although neither was significant [pre-post: $g = 0.27$, 95% CI = (−0.50, 1.04), $Z = 0.69$, $p = 0.490$; pre-follow up: $g = -0.25$, 95% CI = (−0.98, 0.49), $Z = -0.66$, $p = 0.511$], **Figure 1**, **analysis 1.5 y 1.6**. In language, a small and non-significant effect was observed for both time points [pre-post: $g = 0.07$, 95% CI = (−0.55, 0.68), $Z = 0.21$, $p = 0.832$; pre-follow up: $g = 0.09$, 95% CI = (−1.48, 1.65), $Z = 0.11$, $p = 0.915$], **Figure 1**, analysis 1.7 y 1.8. Finally, for visual attention, a small and non-significant effect was observed [pre-post: $g = 0.02$, 95% CI = (−0.35, 0.38), $Z = 0.08$, $p = 0.934$], see **Figure 1**, analysis 1.9.

DISCUSSION

This systematic review has highlighted that there are a limited number of studies examining the effects of tDCS on cognitive outcome measures in PD. The few studies available, suggest that atDCS has a positive effect mainly in executive functions. In this regard, studies have shown better performance in problem-solving tests (31), verbal fluency (35, 36), cognitive flexibility (33), planning, and WM (33, 34). Additionally, two studies highlight greater precision and retention of information in memory tests and procedural learning (35, 36). The meta-analysis converges, highlighting positive effects on executive performance; however, these analyses are small (2–5 studies) and subject to considerable variability, so they should only be taken as exploratory. Similarly, while most results were non-significant, uncertainty around the point estimates was underscored by the wide confidence intervals calculated, further stretching the need for studies to clarify and improve the effect-sizes estimations. Interestingly, variations in the detected effects may arise depending on the time point chosen for assessment, i.e., an effect may remain or disappear in the follow-up, or even appear in the follow-up after not having been detected in the post-treatment measure, which would suggest that some effects are only detected after potential learning effects, masking those that could be attributed to tDCS, have vanished. These findings suggest both the need to control for practice effects and to perform at least one follow-up assessment. Consequently, it is important to fine-tune and standardize the time points for follow-up assessments.

Only one study focused and reported positive effect on electrical activity and functional connectivity circuits in PD (35). It could be speculated that, due to action mechanisms and diffuse effects of tDCS, when applied in frontal areas, this technique increases the electrical activity and functional connectivity of cortico-striatal and thalamocortical circuits (37) affected in PD (38). However, it would be hasty to make this statement without clarity on some methodological aspects and more evidence to support this hypothesis.

Although most studies have used atDCS in the L-DLPFC, some studies do not clarify the neuroanatomical coordinate system used to locate the anode. Thus, it is suggested that future studies verify the correct electrodes' position through mathematical simulation of the electric fields generated by the assembly (39). Moreover, there is variability in current intensity (1–2 mA) and the period of exposure to tDCS, which prevents identifying if effects hold over time. Performing a current stimulation process for a few seconds can generate changes in cortical excitability. However, these are insufficient to consider them significant. Indeed, when stimulation is prolonged or repetitive, effects can last for hours (16, 40) and even days (19). The most widely used stimulation parameters to establish the use of tDCS in PD are 6–20 min per session, and no more than twice per day (41).

Our review and meta-analysis suggest that tDCS has been shown to exert positive effects as an adjunctive therapy on non-motor symptoms in PD. It is not sufficiently evidenced to establish a cause-effect relationship between tDCS intervention, cognitive improvement, electrical activity modulation and functional connectivity increase in PD. Thus, it is essential to (a) explore the potential of tDCS to ameliorate another kind of cognitive symptom reported in PD, such as action verb processing impairment (12, 13, 42–45); to date, there is no evidence about it, and it is feasible to stimulate networks involving cortico-cortical fibers and cortico-subcortical circuits (37) primarily affected in PD (43). It is also essential to (b) perform longitudinal studies to determine whether changes in cognition persist over time. Limited number of sessions and periodicity of the process currently impedes testing whether the effect is transitory and experimentally relevant or if it could go beyond therapeutic and clinical applicability.

Limitations and Suggestions for Further Research

Several factors limit interpretations of these studies' results and the understanding of tDCS effects on cognitive impairments in PD patients. As mentioned by Borenstein et al. (25), including studies with independent and related groups in the same meta-analysis introduces a source of error to be considered. However,

the decision was made due to the limited number of studies; therefore, results should be taken carefully and in an exploratory way. An heterogeneity analysis was not conducted since, as reported previously, for such small analyses this type of test has low statistical power (46, 47).

The lack of standardization of the outcome measures used to assess changes in cognitive performance in different domains, has led to a considerable variability in the analyses performed. This should be addressed in the future by establishing a set of measures that can sensibly evaluate tDCS-related changes. Although results are promising and tDCS is positioning itself as a new adjuvant therapy in PD treatment, sample groups are small and heterogeneous; therefore, it is necessary to conduct studies with larger cohorts. Likewise, it is recommended to combine (a) intervention schemes involving pharmacological treatment and physical and CT programs to determine under what conditions the modulating effect of tDCS is enhanced, and (b) further research should employ neurophysiology measurements to characterize and explore the potential cause-effect relationship between tDCS intervention, cognitive improvement, and neural correlates -as connectivity signatures- in PD.

CONCLUSION

This systematic review and meta-analysis highlight potential effectiveness of atDCS to improve executive (including inhibition of prepotent responses, shifting mental sets, monitoring and regulating performance, goal maintenance, planning, working memory, and cognitive flexibility) and mnemonic performance in PD patients but failed to establish a cause-effect relationship between tDCS intervention and cognitive enhancement in PD.

Considering the potential value of this safe and low-cost technique, it is imperative that well-designed, high-quality, and sufficiently powered randomized studies assess the efficacy of tDCS to treat cognitive impairments in PD and draw new pathways to include it in clinical practice. Evidence from the effects of tDCS on cognitive symptoms in PD patients is sparse, and we suggest that further research is required.

AUTHOR CONTRIBUTIONS

DS-G, JG-C, MZ, and JC developed the review concept. DS-G, JG-C, and JC drafted the manuscript. MZ provided critical revisions. DS-G and JG-C performed the data collection, analysis, and interpretation under the supervision of JC and MZ. All authors approved the final version of the manuscript for submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.597955/full#supplementary-material

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Facilitative Effects of Transcranial Direct Current Stimulation on Semantic Memory Examined by Text-Mining Analysis in Patients With Schizophrenia

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Background: Beneficial effects of transcranial direct current stimulation (tDCS) are relevant to cognition and functional capacity, in addition to psychiatric symptoms in patients with schizophrenia. However, whether tDCS would improve higher-order cognition, e.g., semantic memory organization, has remained unclear. Recently, text-mining analyses have been shown to reveal semantic memory. The purpose of the current study was to determine whether tDCS would improve semantic memory, as evaluated by text-mining analyses of category fluency data, in patients with schizophrenia.

Methods: Twenty-eight patients entered the study. Cognitive assessment including the category fluency task was conducted at baseline (before tDCS treatment) and 1 month after t administration of tDCS (2 mA × 20 min, twice per day) for 5 days, according to our previous study. The category fluency data were also obtained from 335 healthy control subjects. The verbal outputs (i.e., animal names) from the category fluency task were submitted to singular valued decomposition (SVD) analysis. Semantic memory structures were estimated by calculating inter-item cosines (i.e., similarities) among animal names frequently produced in the category fluency task. Data were analyzed longitudinally and cross-sectionally to compare the semantic structure within the patient group (i.e., baseline vs. follow-up) and between groups (patients vs. healthy controls). In the former, semantic associations for frequent items were compared in the form of cosine profiles, while in the latter, the difference in the magnitude of the correlations for inter-item cosines between healthy controls and patients (baseline, follow-up) was examined.

Results: Cosine profiles in the patient group became more cluster-based (i.e., pet, carnivores, and herbivores) at follow-up compared to those at baseline, yielding higher cosines within subcategories. The correlational coefficient of inter-item cosines

between healthy controls and patients was significantly greater at follow-up compared to baseline; semantic associations in patients approached the normality status after multi-session tDCS.

Conclusions: To our knowledge, this is the first study to demonstrate the facilitative effect of tDCS on semantic memory organization in patients with schizophrenia. Text-mining analysis was indicated to effectively evaluate semantic memory structures in patients with psychiatric disorders.

Keywords: schizophrenia, tDCS, semantic memory, category fluency, text-mining analysis

INTRODUCTION

Several domains of cognitive function, specifically, verbal fluency, working memory, and processing speed, are impaired in patients with schizophrenia (1, 2). The cognitive decline compared to healthy adults is in a range of 0.5–2.5 SD (3, 4), hindering functional recovery (5).

Cognitive profiles specific to schizophrenia have been evaluated comprehensively by cognitive batteries, including the Brief Assessment of Cognition in Schizophrenia [BACS; Keefe et al. (6)] and MATRICS Consensus Cognitive Battery [MCCB; Nuechterlein and Green (7)]. Most subtests in these neuropsychological batteries are designed to evaluate executive aspects of cognition (i.e., attention, processing speed, and visual/verbal working memory). Therefore, additional methods are required to assess higher-order cognitive functions, such as semantic memory.

Semantic memory represents a long-term storage of information (8, 9), and semantic structure is defined based on its cohesiveness, i.e., semantic association between items (10). Typically, the semantic structure is represented in the form of clusters, spatial constellations, or networks.

Previous studies have demonstrated aberrant structures of semantic memory in patients with schizophrenia (11–15). Importantly, the disturbance of semantic memory is related with negative symptoms (e.g., alogia) (15) and quality of life (16). These observations indicate the need for the development of effective methods to assess semantic memory in patients with schizophrenia.

Semantic memory is estimated by using data from several cognitive tasks. Specifically, the category fluency task has been used in the study of schizophrenia (11–15). In this task, subjects are instructed to freely recall as many items in a given category (e.g., animal) as possible in a designated time (typically 1 min.). The task is not demanding, and is included in major neurocognitive test batteries, e.g., the MCCB and BACS.

The recent application of text-mining techniques to data from the category fluency task provides objective indices of semantic structures in clinical subjects. For example, network analysis found several parameters, i.e., diameter, average shortest path, and network density, which effectively identify cognitive impairment (17). For the same purpose, latent semantic analysis [LSA; Landauer and Dumais (18)] and singular value decomposition analysis [SVD; Sung et al. (19)] have also been used (19–21). Generally, these methods use a cosine value and vector length to evaluate semantic memory structure (19, 20, 22). The former represents cohesiveness while the latter indicates unusualness of items composing semantic memory. Assuming that disorganization of semantic memory is one of the intermediate cognitive phenotypes of schizophrenia, Nicodemus et al. (20) examined candidate genes related with semantic memory formation by using LSA of category fluency data. They found that average vector length of items was associated with DISC1 in men with schizophrenia. Meanwhile, Sung et al. (19) and Sumiyoshi et al. (21) used SVD analysis, and reported cosine profiles of patients with schizophrenia were deviated from those of healthy controls, revealing unusual structure of semantic memory.

To ameliorate cognitive impairments in schizophrenia, pharmacological, psychosocial, and neuromodulatory approaches have been attempted. Specifically, some types of brain stimulation, particularly non-invasive methods, e.g., transcranial magnetic stimulation and transcranial direct current stimulation (tDCS) have been drawing attention (23, 24). tDCS modulates neural activities in the brain with weak electrical currents (23, 24). The beneficial effects of tDCS are relevant to cognition as well as psychiatric symptoms, functional capacity, and depression in patients with schizophrenia (25, 26).

Although evidence has been accumulated regarding the efficacy of tDCS on cognitive impairment of schizophrenia (26), only a few studies have been conducted to determine whether tDCS would improve higher-order cognition. For example, Vannorsdall et al. (27) reported that tDCS facilitated retrieval of semantically related words in healthy adults. Also, the facilitative effect of tDCS has been found to be more pronounced in category, rather than letter fluency performance (28). These observations suggest that the cognitive enhancement with tDCS is not limited to attention and executive functions, but is also beneficial for a higher level cognitive function, e.g., organization of semantic memory. Thus, it was hypothesized that tDCS would be effective to improve semantic memory structure in patients with schizophrenia.

The aim of the current study was to determine whether tDCS would improve semantic structure, as evaluated by text-mining analyses of category fluency data, in patients with schizophrenia. For this purpose, data were analyzed to compare the semantic structure longitudinally (within the patient group: data at baseline vs. those after tDCS administration) and

cross-sectionally (between groups: patients vs. healthy controls), as demonstrated in **Figure 1D**.

METHODS

Participants

A total of 28 participants were inpatients ($n = 22$) or outpatients ($n = 6$) treated at National Center Hospital, National Center of Neurology and Psychiatry (25). They met DSM-5 criteria for schizophrenia. Patients with alcohol or substance disorder, traumatic brain injury, or epilepsy were excluded. The patients received antipsychotic drugs (25), which were not changed throughout the sessions. Healthy volunteers ($N = 335$) were recruited from the community through local advertisements at Osaka University as participants in a general cognitive assessment (29, 30). They were evaluated using the non-patient version of the Structured Clinical Interview for DSM-IV (SCID) to exclude individuals who had current or past contact with psychiatric services or had received psychiatric medication (31, 32). Data was extracted from our previous study of the effect of tDCS on cognitive function in patients with schizophrenia (25), and from text-mining study using healthy adults (21).

This study was approved by Ethical Committee of National Center of Neurology and Psychiatry, Research Ethics Committee of Fukushima University, and Ethical Committee of Osaka University. The procedures were conducted according to the Declaration of Helsinki and all subjects gave written informed consents.

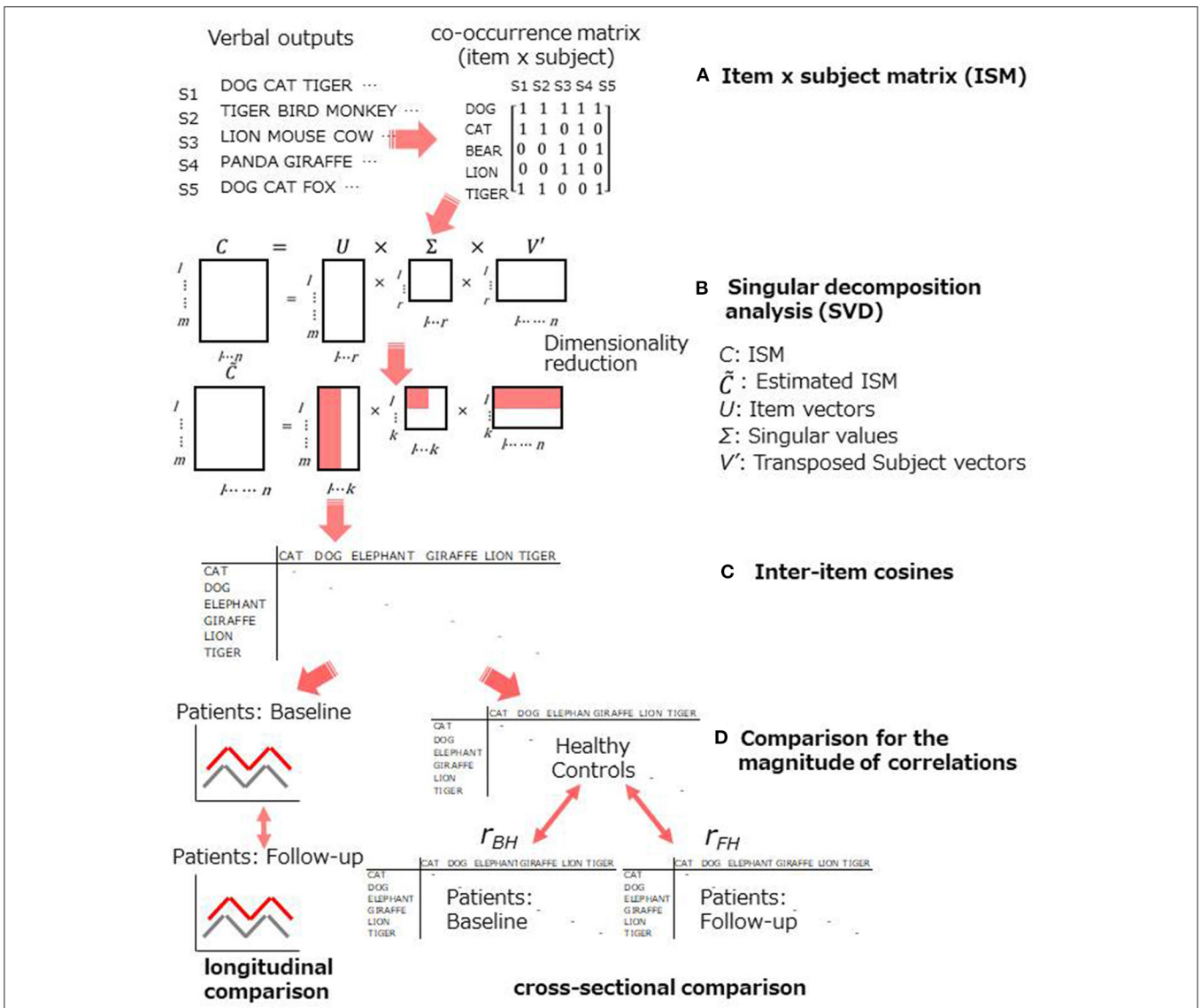

FIGURE 1 | Schematic representation for the statistical procedure. **(A,B)**: an item x subject matrix was produced to submit to SVD analysis, **(C)**: cosine values were used to evaluate the semantic memory structure, **(D)**: improvement was assessed within a group and between groups.

Intervention

tDCS was administered according to a method previously reported (33) in line with a previous study of tDCS on cognition in patients with Schizophrenia (34). Participants underwent 10 active tDCS sessions in 5 consecutive days, twice per day. On each day, tDCS intervention was performed approximately at 10 a.m. and 2 p.m. Patients received no additional behavioral treatment or therapeutic adjustment other than tDCS.

Possible adverse effects related to tDCS, including itching, tingling, headache, burning sensation and discomfort, were monitored using semi-structured checklist (35) after each intervention.

A Soterix Medical 1 × 1 Transcranial Direct Current Low-Intensity Stimulator Model 1,300 A was used for the tDCS through two 35 cm^2 electrodes. We usually soaked 4 ml of saline per side (8 ml into each sponge). For each session, direct current of 2 mA for 20 min was applied. The tDCS montage comprised placement of the anode over the left dorsolateral prefrontal cortex (DLPFC) and the cathode over the right supraorbital area (corresponding to F3 and FP2, according to the International 10–20 electroencephalography system).

Assessment for Cognition and Psychiatric Symptoms

Cognitive function was assessed at baseline and 1-month after the last tDCS administration using the BACS. Verbal outputs of the category fluency task were obtained from the BACS. Category fluency is a free recall task, asking subjects to produce as many animal names as possible in 1 min. According to the normative method (36), errors (i.e., repetitions, proper nouns, and intrusions [e.g., *APPLE* for an animal cue]) were removed from the analysis. Premorbid intelligence was estimated at baseline using the Japanese version of the Adult Reading Test [JART, Matsuoka et al. (37)]. As for healthy controls, category fluency task and the JART were conducted in a general cognitive assessment (29, 30).

Psychiatric symptoms were assessed at baseline and follow-up using the Positive and Negative Syndrome Scale [PANSS; Kay et al. (38)].

Statistical Analysis

Demographic variables and category fluency scores were compared between patients and healthy controls using *t*-test. Comparisons between baseline and follow-up in patients were conducted based on our previous report (25). Inequality of variance between the groups was examined using Levene test. Welch method was applied if inequality was significant.

To evaluate the semantic structure, SVD analysis was conducted for verbal outputs of the category fluency task. **Figure 1** demonstrates schematic representation of the procedure. First, an item x subject matrix (ISM) was created. Rows of the ISM contained animal items (e.g., *DOG CAT*, etc.), while columns contained subjects, and each cell contained a co-occurrence of items (**Figure 1A**). Then, SVD analysis was applied to the matrices obtained from patients and healthy controls (**Figure 1B**). SVD is a general matrix factorization technique based on eigenvalue decomposition [for further information, see Supplementary Materials in Sung et al. (19, 22, 39)]. Each row (i.e., item) is treated as a vector in the space produced by SVD.

A key component of the structure of semantic memory is cosine values in reduced (i.e., higher) dimensions (**Figure 1C**). A cosine close to 1.0 indicates that two items are highly similar (two words frequently co-occur across subjects).

To assess the improvement on semantic memory structure, cosines between the highly frequent items were contrasted longitudinally and cross-sectionally. In the former, cosine profiles of the 6 most frequent items were produced for patients at baseline and at follow-up and compared (**Figure 1D**, left). As for the latter, the improvement was evaluated as follows: (1) inter-item cosines were obtained between the 6 most frequent items; (2) Pearson's correlational coefficients for those cosines were calculated between healthy controls and patients at baseline (r_{BH}) and follow-up (r_{FH}); (3) The difference in the magnitude of the two correlational coefficients were tested by the Meng's method (40) (**Figure 1D**, right). The method was employed because the healthy control group was used as a "reference," and therefore, it was "overlapped" in testing the magnitude of the difference. The significance level was set for $p < 0.05$ with one-tailed (i.e., $r_{BH} < r_{FH}$), hypothesizing that the tDCS treatment could improve higher, as well as lower, level of cognition.

R version 3.2.2 (41) and its LSA package (42) were used for conducting SVD analysis and producing inter-item cosines. For testing correlations, R based software cocor (43) was used. Other statistical analyses were conducted by SPSS ver. 22.

RESULTS

Demographic and Cognitive Variables

Table 1 presents demographic and clinical variables at baseline and category fluency performance. Inequality of variances was significant only in Estimated premorbid IQ ($F = 12.22$, $p < 0.001$) to which Welch method was applied. Healthy controls were significantly younger, more educated, and showed higher premorbid IQ compared to patients. The former group also produced more words in the category fluency task.

SVD Analysis

Table 2 presents 20 items most frequently produced by patients and healthy controls. Out of them, 12 items, i.e., BEAR, BIRD, CAT, DOG, ELEPHANT, GIRAFFE, LION, MONKEY, MOUSE, PANDA, RABBIT, TIGER, were chosen for SVD analysis. They commonly appeared at baseline and follow-up, with the frequency more than 10 (**Table 2**, in bold).

There are no definite rules for choosing an appropriate number of singular values (dimensions) for the dimensionality reduction (44). Therefore, a six-dimensional solution (6D) was used where the sum of the singular values reached 70% to the entire sum. Accordingly, inter-item cosines were calculated in the 6D space.

TABLE 1 | Characteristics of praticipants[a].

Variables	Healthy controls $N = 335$		Patients $N = 28$		x^2/t	df	p
	M	SD	M	SD			
M/F	154/181		16/12		1.295	1	0.255
Age (year)	35.8	11.9	40.9	9.8	−2.205	361	0.028
Education (year)	15.2	2.2	13.8	1.7	3.164	361	0.002
Estimated premorbid IQ (JART[b])	109.3	12.2	99.6	12.0	3.262	29	0.003
Category fluency (Baseline)	20.9	4.5	16.4	5.1	5.071	361	0.000
Category fluency (Follow-up[c])			16.9	5.5	4.475	361	0.000
Age at onset (year)	–		23.6	6.7			
Duration of illness (year)	–		17.4	9.9			
Neuroleptics (CPZ)	–		889.0	587.2			
PANSS[d] Positive syndrome	–		15.7	5.7			
PANSS Negative syndrome	–		14.9	8.0			
PANSS General psychopathology	–		32.0	8.1			

[a] *Demographic variables and PANSS are baseline scores. For the follow-up PANSS scores, see Narita et al. (25) for details.*
[b] *JART, Japanese Adult Reading Test.*
[c] *Scores at Baseline and Follow-up were not statistically different (t = 0.56, df = 27 p = 0.58). See Narita et al. (25) for details.*
[d] *PANSS, the Positive and Negative Syndrome Scale.*

TABLE 2 | Frequencies of animal items.

Rank	Healthy controls ($N = 335$)		Patients ($N = 28$)			
			Base		Follow-up	
1	**DOG**	309	**DOG**	24	**CAT**	24
2	**CAT**	305	**LION**	23	**DOG**	24
3	**LION**	250	**CAT**	22	**LION**	23
4	**GIRAFFE**	244	**ELEPHANT**	21	**ELEPHANT**	19
5	**TIGER**	239	**GIRAFFE**	21	**MONKEY**	15
6	**ELEPHANT**	235	**MOUSE**	17	**TIGER**	15
7	**MONKEY**	234	**TIGER**	17	**BIRD**	13
8	HORSE	171	HORSE	13	**GIRAFFE**	12
9	SHEEP	163	**MONKEY**	13	**BEAR**	11
10	COW	155	**BEAR**	10	GORILLA	11
11	**MOUSE**	152	**BIRD**	10	**MOUSE**	11
12	**RABBIT**	148	**PANDA**	10	**PANDA**	11
13	HIPPOPOTAMUS	143	**RABBIT**	10	**RABBIT**	11
14	**BEAR**	122	RACOON_DOG	8	COW	9
15	RHINOCEROS	116	SHEEP	8	SHEEP	9
16	**BIRD**	115	HAMSTER	7	HIPPOTAMUSE	8
17	**PANDA**	110	LEOPARD	7	HORSE	8
18	CHEETAH	102	RHINOCEROS	7	CHEETA	7
19	SNAKE	102	SPARROW	7	CHIMPANZEE	7
20	ZEBRA	102	ZEBRA	7	RACOON_DOG	7

Cosine Profiles

Each line represents 6D cosine values between one of the top 6 items (e.g., CAT) and the other most frequent 12 items (**Table 2**, in bold). Overall, cosine values uniformly fluctuated at baseline (**Figure 2**, top) indicating the lack of distinct clusters (i.e., subcategories). The profiles became more cluster-based at follow-up, yielding a higher cosine within a pair (e.g., CAT-DOG) but lower cosines between pairs (e.g., [CAT-DOG]-[GIRAFFE-ELEPHANT], **Figure 2**, the bottom) as conceptually shown in **Figure 2**, right.

Difference in Magnitude of Correlations

The top six items in healthy controls (DOG, CAT, ELEPAHANT, GIRAFFE, LION, and TIGER, **Table 2**) were used for the comparison between r_{BH} and r_{FH} to examine how semantic memory in patients became close to that in healthy controls. **Table 3** summarizes correlational coefficients and the difference of the magnitude of correlations. The correlation was considerably higher in follow-up ($r_{FH} = 0.75$) than baseline ($r_{BH} = 0.41$), and the difference was significant ($z = -1.90$, $p = 0.03$, 95% CI $= -1.06$, 0.02). **Figure 3** schematically illustrates the cognitive process of the result. For example, LION is more easily and quickly accessed than other items (e.g., ELEPHANT or CAT) when TIGER is recalled.

DISCUSSION

Multi-session tDCS was found to improve semantic memory organization, as evaluated by text-mining analyses of category fluency data, in patients with schizophrenia. The longitudinal comparison of cosine profiles suggests that the semantic association among typical items (animal names) was more cluster-based, as in healthy adults (21) at follow-up compared to baseline (**Figure 2**). Also, the correlation of cosine values between healthy controls and patients was greater at follow-up than at baseline, indicating that semantic structures of patients approached the normality status after administration of tDCS (**Figure 3**). Probably, patients at follow-up recalled animal names in a similar manner as did healthy people, referring to subcategory (i.e., pet, carnivorous, herbivorous

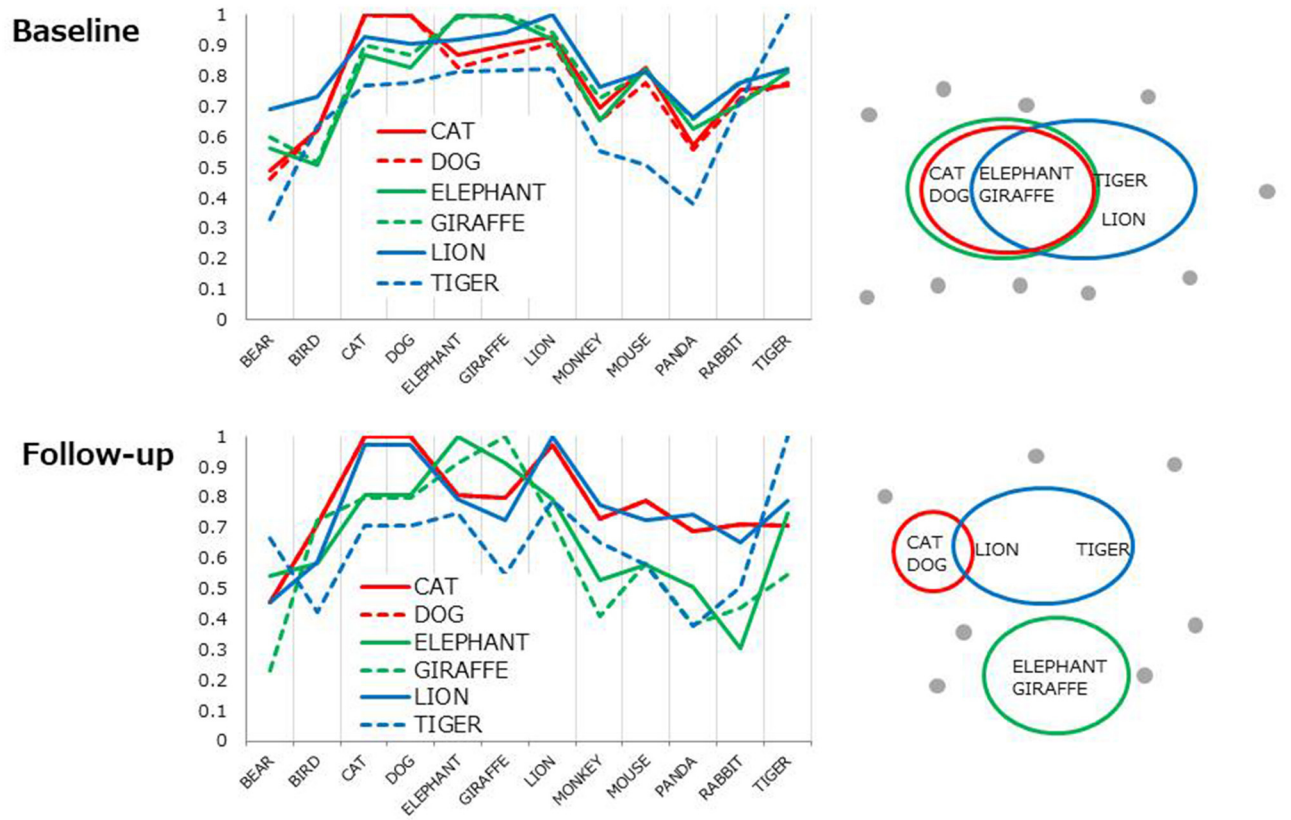

FIGURE 2 | Cosine profiles for the most frequent six items. Each line represents 6D cosine values between one of the six items and the other frequent 12 items. The profile patterns are conceptually illustrated as from of clusters (right).

items, **Figure 3**) to access items more easily and quickly. Associational memory of this kind would be important in real world settings where meaningful conversations and discourses are taking place. Furthermore, it is possible that impairment of associating information in semantic memory may negatively affect competent linguistic behaviors. In fact, adults who later developed psychosis were found to produce discourses similar to those of children, with presentations of repetitions and a limited scope of vocabulary (45). Likewise, schizophrenia patients with severe formal thought disorder exhibited utterances that are syntactically less complex (e.g., reduction of embedded or dependent clauses) compared to those of first-degree relatives or healthy adults (46). Difficulties in associating information in semantic memory may underlie such restricted linguistic behavior in patients with schizophrenia.

There are several hypotheses to explain deterioration of semantic memory structure in patients with psychiatric conditions [(19), for review]. Some assume structural distortions of memory (47) while others claim poor memory activation (19). In both cases, associational retrieval of stored information would be compromised. Although the current study did not directly address this issue, it is worth pursuing the basis for the impairment to understand higher-order cognition in schizophrenia in further studies.

TABLE 3 | Tests for differences in correlational coefficients[a].

	SCZ Baseline	SCZ Follow-up	Healthy controls
SCZ Baseline	–	0.68	0.41
SCZ Follow-up		–	0.75*[b]
Healthy controls			–

[a] *Sample size: SCZ = 28; HC = 335.*
[b]* $r_{FH} = 0.75 > r_{BH} = 0.41$, $z = -1.90$, $p = 0.028$ *(one-tailed), 95% CI = −1.06, 0.02.*

The number of word outputs itself in the category fluency task was not increased significantly after administration of tDCS (**Table 1**). This may be partly due to the relatively short duration assessment span (1 month). Possibly, patients tended to repeat a limited variety of items. In fact, type token ratios(TTR), a measure of variety of words, showed only a slight increase in follow-up (baseline: TTR = 0.26, follow-up: TTR = 0.27). Despite, co-occurrences of typical items came to closer to those in healthy adults, as was indicated by the significantly higher correlation in follow-up than baseline (**Table 3**).

Previous studies support our results with providing the neurophysiological substrate. The left prefrontal region is

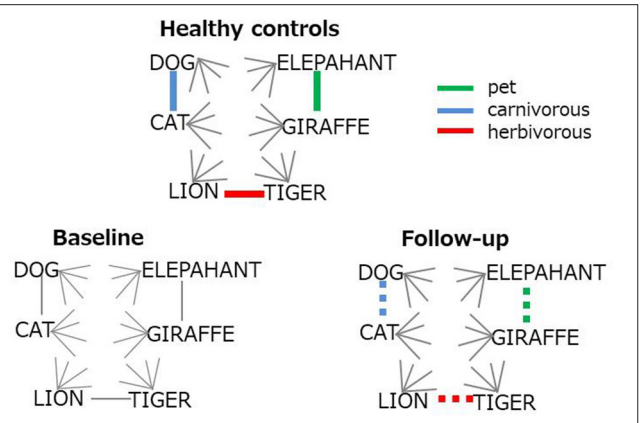

FIGURE 3 | Schematic representation for the improvement on semantic structure. Colored lines represent strong (bold) or restored (dotted) association. Gray lines indicates weak inter-item connection.

assumed to be related to the ability of tDCS to improve organizing of information. For example, a previous study (27) found tDCS over the left DLPFC facilitated retrieval of clustered words. A functional imaging study also found that activation in the left frontal region was correlated with categorical clustering in the recall of a verbal learning task (48). These findings are in accord with our result indicating improvement of semantic association in patients with schizophrenia after tDCS treatment over the left prefrontal region.

Although the number of words in the category fluency task was not significantly changed after administration of tDCS, letter fluency was found to be improved in our previous study with the same protocol (25). Meta-analysis results indicate that tDCS over the left ventral inferior frontal gyrus (49) or the left prefrontal cortex (50) increased the number of words produced in the category fluency task.

Results of the current study based on SVD analysis of the category fluency task may add to the usefulness of text-mining analysis in psychiatry, as has been discussed (51–53). Possibly, novel computational linguistic techniques herein reported, i.e., SVD, LSA, and network analysis may contribute to the advance of the National Institute of Mental Health's Research Domain Criteria (RDoC) initiative (54). For example, these techniques may help evaluate the language or declarative memory construct in the RDoC (53).

Several limitations should be mentioned. First, the current study used the data obtained in a previous one-armed open label study (25, 33) that did not adopt sham comparisons. Second, sample size was considerably larger in healthy controls compared to patients. Inequality happened because the former was used as a reference group to estimate normative semantic structure, requiring relatively large sample size. Finally, healthy control subjects were younger, more educated, and in a higher intellectual status compared with patients. However, this demographic bias may not have affected the comparisons of semantic memory structures, because the knowledge about animals is acquired in the early stage of the development (55). Furthermore, the primitive structures, e.g., clustering, are already present in early childhood (56–58); basic semantic structures should be relatively invariant across ages and educational backgrounds.

In conclusion, the current study demonstrated the facilitative effect of tDCS on semantic memory organization in patients with schizophrenia. Semantic associations in these patients approached the normality status after multi-session tDCS. Text-mining analysis was indicated to effectively evaluate semantic memory structures in patients with psychiatric disorders.

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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of National Center of Neurology and Psychiatry, Research Ethics Committee of Fukushima University, and Ethical Committee of Osaka University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CS and TS designed the study in collaboration with ZN and RH. ZN, TI, YY, KS, YH, and AS collected and prepared the data. CS conducted the analyses and wrote the initial draft. TS, ZN, and RH critically revised the draft for important intellectual contents. All authors contributed to the manuscript writing.

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REVIEW
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Neurobiological After-Effects of Low Intensity Transcranial Electric Stimulation of the Human Nervous System: From Basic Mechanisms to Metaplasticity

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Non-invasive low-intensity transcranial electrical stimulation (tES) of the brain is an evolving field that has brought remarkable attention in the past few decades for its ability to directly modulate specific brain functions. Neurobiological after-effects of tES seems to be related to changes in neuronal and synaptic excitability and plasticity, however mechanisms are still far from being elucidated. We aim to review recent results from *in vitro* and *in vivo* studies that highlight molecular and cellular mechanisms of transcranial direct (tDCS) and alternating (tACS) current stimulation. Changes in membrane potential and neural synchronization explain the ongoing and short-lasting effects of tES, while changes induced in existing proteins and new protein synthesis is required for long-lasting plastic changes (LTP/LTD). Glial cells, for decades supporting elements, are now considered constitutive part of the synapse and might contribute to the mechanisms of synaptic plasticity. This review brings into focus the neurobiological mechanisms and after-effects of tDCS and tACS from *in vitro* and *in vivo* studies, in both animals and humans, highlighting possible pathways for the development of targeted therapeutic applications.

Keywords: transcranial direct current stimulation, transcranial alternating current stimulation, neurobiological after-effects, synaptic plasiticty, non-invasive brain stimulation

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INTRODUCTION

In the last two decades, therapeutic efficacy of non-invasive transcranial brain stimulation techniques through low-intensity electrical fields has been demonstrated by a number of works and clinical trials providing promising results for many neurological disorders, including stroke (1) and epilepsy (2, 3), movement disorders/Parkinson's (PD) (4) and Alzheimer's (AD) (5, 6). Due to non-invasiveness and transient side effects (7), transcranial electrical stimulation (tES) has found progressively a wide field of applications. Moreover, acquisition of recent experimental data has extended our knowledge of the cellular and molecular mechanisms involved in the after-effects of tES, thus supporting its therapeutic potential for brain disorders based on impaired synaptic plasticity (2).

The basic principle of tES is very simple and based on the negative (anodal) and positive (cathodal) currents and their flow into the brain (8). However, neurobiological mechanisms and after-effects are not yet fully understood. Experimental evidence has demonstrated that weak low-intensity ES (at an intensity lower than that needed for triggering action potentials) induces polarity-specific changes in spontaneous and evoked neuronal activity (9, 10): anodal polarization increases neuronal activity, whereas cathodal polarization decreases it (11–14). Accordingly, transcranial direct current stimulation (tDCS) has been shown to induce long-lasting and polarity-specific changes of human motor cortex excitability (15–17) related to modifications of synaptic efficacy similar to those underlying long-term potentiation (LTP) and long-term depression (LTD) of synaptic activity (18, 19). Studies of the effects of direct current stimulation (DCS) in slices of mouse primary motor cortex have shown that anodal DCS, in the absence of simultaneous synaptic activation, does not induce LTP/LTD like changes but it can modulate LTP induction (20). In contrast, by coupling DCS with low frequency stimulation (at 0.1 Hz), a long-lasting polarity- (anodal DCS) and frequency- specific LTP is obtained, mainly depending on N-methyl-D- aspartate (NMDA) receptor activation and secretion of brain-derived neurotrophic factor (BDNF) (21). In summary, these studies highlight the complex nature of tDCS effects, characterized by the capability of inducing and modulating LTP/LTD. However, while the immediate effects of tES can be explained by changes in transmembrane potential influencing neuronal firing, it is plausible that the long-term after-effects are likely due to modifications of intracellular calcium dynamics and mechanisms of synaptic plasticity, based on LTP/LTD processes (18, 22, 23) and/or induction of metaplasticity, the activity-dependent physiological changes that modulate neural plasticity (24). Anodal tDCS, for example, induces neurotrophic BDNF-mediated priming after-effects on synaptic plasticity and memory, making synapses susceptible to LTP induction in the rat hippocampus (25).

This work aims to comprehensively summarize the neurobiological mechanisms of tES and discuss future clinical applications. In particular, we first analyzed the technical aspects of electrical stimulation techniques, and then the neurobiological after-effects of tES on the constituents of the synaptic structure, distinguishing those on membrane polarity, neural transmission, synaptic plasticity, neuronal network and connectivity, and finally the effects on glial cells and neuroinflammation.

We believe that understanding the basis of the modulatory effect of tES would be particularly relevant for its clinical application in humans, where it could be used to shape the plastic properties of the brain.

TECHNICAL ASPECTS: TRANSCRANIAL CURRENT AND MAGNETIC STIMULATION

According to whether direct or alternating current is applied to the brain, the method is referred to as either transcranial direct current stimulation (tDCS) or transcranial alternating current stimulation (tACS). Both techniques produce effects on cortical excitability outlasting the stimulation, up to 3 h with tDCS (26) and up to 1 h with high-frequency tACS (27–29). TDCS acts in a polarity-dependent fashion, with anodal stimulation increasing and cathodal stimulation decreasing neuronal excitability, whereas tACS consists in the application of a sinusoidal waveform current that alternates between the anode and the cathode (*switching polarity*) and modulates the power of oscillatory rhythms in a frequency-dependent manner by synchronizing or desynchronizing neuronal networks (30). For example, in studies that coupled transcranial magnetic stimulation (TMS) with ES, tACS was found to synchronize cortical networks bursting at frequencies higher than 300 Hz (31).

The association between the type of stimulation and neural response depends on many physical properties including the electrode type, length, strength, and frequency of stimulation (32). Low-intensity, constant, or non-constant currents are used for tDCS and delivered in rectangular or sinusoidal waves with pulses of unidirectional current, whilst non-constant current is used for tACS (33). TDCS flows into the brain from a battery-powered generator through a couple of sponge electrodes, with one or both the electrodes fixed over the scalp. It has been demonstrated that current density (i.e., current intensity/electrode size), duration, polarity, and location of stimulating electrodes have important implications in the modulatory outcome of stimulation (34). Generally, tDCS does not involve synaptic effects but polarity changes of the membrane resting potential, does not induce neuronal firing but rather modulates spontaneous neuronal network activity, polarizing brain tissue (35–37). The two types of stimulation, anodal and cathodal, do not contrast each other in terms of after-effects and modulation of their intensity dramatically produces different results. Generally, the cortical excitability is increased by anodal tDCS while it is decreased by the cathodal tDCS over the same area (site specificity).

TACS is a non-constant current which alternates its pulses with the opposite amplitude (38, 39). Despite site specificity, its effects are not site limited as tACS influences other areas of the brain through interneuronal circuits (33) and directly interferes with ongoing brain oscillations (40). TACS shares the same setup of tDCS: it is applied between electrodes placed over the target scalp sites, with intensity in the same range of 1–2 mA. The physiological bases of tACS are less explored than tDCS. The main biophysical (electric field strength and spatial distribution) and polarizing properties of tDCS should also apply to tACS, with the main difference that the polarity (i.e., the direction of current flow) changes of 180° during each cycle of the sinusoidal waveform of tACS and that the maximum current flow is present only at the peak of the alternating current.

The advantage of tACS is that it allows the manipulation of amplitude, frequency, and coherence of intrinsic neuronal oscillations (41, 42). In addition, the effects of tACS could be translated into whole larger brain-network activity through five different neuronal mechanisms (43, 44): (1) *stochastic resonance*, consisting in the stochastic response of tACS-affected neurons to be either polarized or hyperpolarized; (2) *rhythm resonance*,

Abbreviations: ***tES***, transcranial electrical stimulation.

synchronizing tACS frequency with the endogenous oscillations; (3) *temporal biasing of spikes*, a synergistically excitation of the same groups of neurons during each cycle of stimulation; (4) *network entrainment* of an endogenous irregular neuronal activity that necessitates an external current with sufficiently stronger amplitude; (5) *imposed pattern*, tACS overcomes endogenous regular oscillations and introduce a new oscillation. These mechanisms attribute the large-scale effects of tACS to two synergistic phenomena: entrainment and neuroplasticity, respectively. The first takes place when an external rhythmic system affects another one, forcing it to follow its own oscillating frequency and phase; the second, through LTP/LTD phenomena, elicits offline tACS effects by increasing or decreasing neural synchronization, as confirmed by many studies (29, 45–47).

TACS has diverse modes of administration in terms of frequency: the beta (20 Hz), alpha (10–12 Hz), and gamma range (40 Hz), each producing diverse neurobiological effects for modulation of different bands of neural oscillations (42). The effects of alpha and gamma stimulation have been studied on attention with gamma stimulation demonstrating to facilitate endogenous attention (48).

Experimental and clinical applications of transcranial magnetic stimulation (TMS) is widely and progressively increased over the past two decades. In particular, several repetitive TMS (rTMS) protocols have been proved to modulate brain functions (from the molecular to the network scale) and human behavior (49, 50). For example, application of simple rTMS to a target cortical area for several minutes induces after-effects in a frequency- dependent manner (low frequency, $\leq$1 Hz, reduces cortical excitability whereas high-frequency, $>$5 Hz, does the opposite) (51) while theta-burst stimulation (TBS), a patterned protocol, induces longer-lasting effects with shorter application time (continuous TBS has primarily an inhibitory effect on corticospinal excitability, while intermittent TBS has an excitatory effect) (52).

TMS shares fundamental similarities with tES as both share neurobiological modulations at similar levels and involve rapid changes in magnetic fields (53). While TMS requires passing of current through coils to generate a magnetic field that in turn generates an electric field and a current density, in tES the electric field and the current density are proportional to injected current (54).

TABLE 1 | tES after-effects on membrane polarity.

References/Study	Methodology tES	Targets	Main results
Nitsche and Paulus (15); Liebetanz (19); Stagg and Nitsche (55)	tDCS	Pre/post synaptic Na^+ and Ca^{2+} channels	tDCS generates action potential via Na^+ and Ca^{2+} channels by increasing presynaptic release of excitatory transmitters and Ca^{2+} influx
Zaghi et al. (33); Bikson et al. (56)	tDCS	Hippocampal neurons	Somatic polarization was obtained with electric field parallel to somato-dendritic axis in hippocampal neurons
Bikson et al. (56); Arlotti et al. (57); Rahman et al. (58); Pelletier and Cicchetti (32); Seo and Jun (59)	tDCS - aDCS - cDCS	Structural components of neurons	Components at the cathode depolarize while those at the anode hyperpolarize
Francis et al. (60); Deans et al. (61); Reato et al. (62)	tACS	Neuronal resonance	tACS can induce cumulative effects over multiple cycles that can shift in spike timing.
Bindman et al. (11); Bikson et al. (56); Antal and Herrmann (63)	tDCS - aDCS - cDCS	Transmembrane potentials	Constant electric field shifts neuronal transmembrane potential to less negative in cDCS and more negative in aDCS which makes it more prone to generate action potential.

***tES**, transcranial electrical stimulation; **tDCS**, transcranial direct current stimulation; **tACS**, transcranial alternating current stimulation; **a/c tDCS**, anodal/cathodal transcranial direct current stimulation.*

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NEUROBIOLOGICAL AFTER-EFFECTS OF CURRENT STIMULATION OF CENTRAL NERVOUS SYSTEM

Effects on Membrane Polarity

Table 1 summarizes the results of the studies that analyzed the effects of tES on membrane polarity. Evidence has demonstrated that tDCS can modify neuronal membrane polarity and therefore the action potential generation (15, 19, 55) through activation of voltage-gated pre and postsynaptic Na^+ and Ca^{2+} channels thus causing increased presynaptic release of excitatory neurotransmitters and postsynaptic calcium influx, respectively (15). Moderate but prolonged intracellular Ca^{2+} increase causes LTD while short but large Ca^{2+} increase causes LTP (64).

The polarity-dependent effect of tDCS is strictly dependent on the orientation of axons and dendrites (33). Specifically, when the effect of polarity was studied *in vitro* on hippocampal neurons (56), somatic polarization was obtained with the electric field parallel to the somato-dendritic axis, while an effect on afferents without somatic polarization was produced by the electric field perpendicular to the apical-dendritic axis. Moreover, the structural components of the cell at the cathode depolarize while the elements facing the anode are subject to hyperpolarization (32, 56–59). On the other hand, tACS, matching resonant neuronal properties, can induce cumulative effects over multiple cycles that may cause shift in spike timing (60–62).

However, these biophysical properties might produce complex modulatory effects when tES is applied to circuits of the human brain with no uniform spatial orientations. Based on experimental studies (11, 56), the applied constant electric field shifts the transmembrane potential of neurons toward less negative (anodal stimulation) or more negative values (cathodal

stimulation), thus increasing or decreasing the likelihood of generation of action potentials (63), thus influencing both spontaneous and evoked neuronal firing.

Effects on Neural Transmissions

Many studies have shown that tACS interferes with several neurotransmitter systems. The balance between cholinergic and adrenergic system after administration of reserpine (an anti-adrenergic drug that irreversibly blocks the H^+-coupled vesicular monoamine transporters—VMAT) and physostigmine (a parasympathomimetic reversible cholinesterase inhibitor) occurred much faster while applying tACS: it was observed that the quantity of presynaptic vesicles first declined, then increased after 5 min and then returned to baseline levels after tACS (65). Evidence suggested that this type of stimulation might modulate the serotoninergic raphe nuclei, the noradrenergic locus coeruleus, the cholinergic latero-dorsal tegmental, and pedunculopontine nuclei in the brainstem (66). Additionally, tACS was found to modulate the levels of endorphins into the cerebrospinal fluid (67) and naloxone, a pure opioid antagonist, was reported to reduce tACS analgesic effects (67), prompting to hypothesize a tACS-induced modulation of the neurotransmitters' release.

The blockage of serotonin reuptake increases LTP in the motor cortex by anodal tDCS and shifts LTD to LTP after cathodal tDCS (68). In addition, anodal tDCS was demonstrated to reduce γ-aminobutyric acid (GABA) concentration in the stimulated cerebral cortex while cathodal tDCS impaired glutamatergic neuronal activity and reduced GABA concentration (2, 69). Authors argue that these protocols might be used therapeutically to reduce the imbalance between excitatory and inhibitory transmitters (70, 71). These results were also confirmed in humans by magnetic resonance spectroscopy (MRS) studies examining the effects of tDCS on the hand area of the primary motor cortex. Accordingly, authors reported that anodal tDCS causes GABA decrease while cathodal tDCS decreases both the levels of glutamate and GABA (70). Upon administration of GABA antagonists, anodal tDCS produces delayed but enhanced excitability increase in cortical or subcortical areas (72). See **Table 2** for a summary of the studies that analyzed the effects of tES on neural transmissions.

TABLE 2 | tES after-effects on neural transmission.

References/Study	Methodology tES	Targets	Main results
Kirsch and Nichols (65)	tACS	Cholinergic and adrenergic neural transmission	After administration of reserpine and physostigmine and administration of tACS, the quantity of presynaptic vesicles declines and then increased
Nitsche et al. (68)	tDCS - aDCS - cDCS	Motor cortex	Blockage of serotonin reuptake increases LTP via aDCS and shifts LTD to LTP after cDCS
Stagg et al. (70); Nitsche et al. (72)	tDCS - aDCS - cDCS	GABA and glutamate in cortical and subcortical areas	aDCS reduces GABA while cDCS reduces both glutamate and GABA. With GABA antagonists, aDCS produced enhanced excitability in cortical and subcortical areas

***tES**, transcranial electrical stimulation; **tDCS**, transcranial direct current stimulation; **tACS**, transcranial alternating current stimulation; **a/c tDCS**, anodal/cathodal transcranial direct current stimulation; **LTP**, long-term potentiation; **LTD**, long-term depression; **GABA**, gamma amino butirric acid.*

Effects on Synaptic Plasticity

Experimental and human studies suggest that the after-effects of tES might originate from persistent modifications of synaptic efficacy similar to those underlying LTP and LTD of synaptic activity (18, 19, 73). Synaptic plasticity usually involves short- and long-term modifications of existing synapses (formation, removal, and remodeling of synapses and dendritic spines) that in turn modify the activity of brain networks in which they are interposed (50). Mechanisms of synaptic plasticity occur at different levels, from ultrastructural to synapse: calcium dynamics, neurotransmitter release, proteins (receptors, transporters, and ion channels) and gene expression (74). **Table 3** summarizes the main results of the studies that analyzed the tES after-effects on synaptic plasticity.

Experimental evidence using a high frequency pre-synaptic stimulation protocol has showed a polarity-specificity of tDCS in the modulation of LTP induction, with anodal stimulation increasing and cathodal stimulation decreasing the amount of LTP (20). These data suggest that tDCS alone is not capable of changing synaptic strength (i.e., inducing LTP), but rather that tDCS changes the propensity of the synapse to undergo LTP. Accordingly, in the study by Fritsch and colleagues, LTP was obtained after a conditioning anodal tDCS protocol but only in the presence of concomitant synaptic activation by presynaptic inputs (21).

Neurotrophins (BDNF, NGF, NT-3, and NT-4/5) are a large family of complex proteins that regulate several functions, including neuronal survival, differentiation, synaptic function, and plasticity but also neuronal death through interaction with two types of receptors, the tyrosine kinase receptors (TrkA, TrkB, and TrkC) and the common p75NTR receptor (82). Most of neurotrophins, including BDNF, is secreted in an immature form and then converted into the mature, active form by a complex fine-regulated system of proteases (83–85). With this premise, it has been demonstrated that tDCS might increase BDNF concentration when combined with presynaptic stimulation (21) inducing LTP via BDNF/TrkB signaling (25). TrkB stimulation by BDNF also promotes long-lasting synaptic potentiation and late phase LTP requires the conversion of pro-BDNF into mature BDNF in the hippocampus (21). Moreover, enhanced LTP in animals undergoing continuous tDCS can be reduced by TrkB antagonist (86) and anodal tDCS enhances hippocampal LTP and memory via chromatin remodeling of the Bdnf gene regulatory

TABLE 3 | tES after-effects on synaptic plasticity.

References/Study	Methodology tES	Targets	Main results
Ranieri et al. (20)	tDCS - cDCS - aDCS	Neuronal LTP	aDCS increased LTP while cDCS decreased LTP
Fritsch et al. (21); Yu et al. (25)	tDCS	BDNF/TrkB signaling	tDCS increases BDNF concentration which induces LTP. TrkB stimulation by BDNF promotes late phase LTP
Lanté et al. (75); Luscher and Malenka (76)	tDCS	NMDA/AMPA receptors	High frequency stimulation induced LTP in active NMDA receptors, expression of AMPA receptors in postsynaptic neuron and Ca^{2+} rise. Low frequency stimulation induces small rise in Ca^{2+} and presynaptic internalization of AMPA by phosphatase activation and LTD generation
Mycielska and Djamgoz (77); McCaig et al. (78)	tDCS	Cellular migration	tDCS modified the speed and direction of cell migration by shifting intracellular Ca^{2+} and modifying expression of EGFR due to electrostatic effects
Monte-Silva et al. (79); Kuo et al. (80)	tDCS - cDCS - aDCS	L-DOPA induced plastic changes	Anodal L-DOPA suppressed plasticity induced by atDCS while prolonged the reduction of excitability by cDCS
Hurley and Machado (6)	tDCS	Neuronal polarity	When synaptic activity is preconditioned by tDCS, continuous tDCS after interval will modulate polarity
Carvalho et al. (81)	tDCS - aDCS - cDCS	Working memory	Continuous aDCS facilitates performance and cDCS enhances working memory
Zaehle et al. (45)	tACS	Rhythmic patterns and natural pattern	tACS modulates neural synchronization by increasing or decreasing it and induces LTP and LTD

***tES**, transcranial electrical stimulation; **tDCS**, transcranial direct current stimulation; **tACS**, transcranial alternating current stimulation; **a/c tDCS**, anodal/cathodal transcranial direct current stimulation; **LTP**, long-term potentiation; **LTD**, long-term depression; **BDNF**, brain-derived neurotrophic factor; **TrkB**, tyrosine kinase receptor B; NMDA, N-methyl-D- aspartate; **AMPA**, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.*

sequence, increasing the expression of this gene (87). In addition, through TrkB/Fyn signaling, BDNF induces a phosphorylation-dependent enhancement of NMDA receptor activity that further enhances effects of tDCS on LTP (88, 89).

The most prominent phenomena mediating LTP/LTD are the functional state of the synapse, Ca^{2+} signals and activity of NMDA glutamate receptors (74) (**Figure 1**). High-frequency current stimulation, in fact, induces LTP only in active synapses, which express active/open NMDA receptors, rapid expression of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the postsynaptic neuron, and fast intracellular Ca^{2+} increase (90). In contrast, low-frequency, long-lasting stimulation induces small and slow rise in Ca^{2+} concentration, presynaptic internalization of AMPA receptors by phosphatase activation (that reduces glutamate sensitivity), and LTD generation (75, 76).

Studies have showed the tDCS induces changes in the direction and speed of cell migration which may be related to the shift of intracellular Ca^{2+} (77, 78) and to changes in the expression of the epidermal growth factor receptors' (EGFR) due to electrostatic effects of tDCS, ultimately contributing to long-term modulation (78).

The effects of tES on synaptic plasticity are also modulated by concomitant administration of drugs acting on neural transmissions. The dopaminergic, cholinergic, serotonergic systems all affect tDCS-induced plasticity (91) in a dose-dependent manner. For example, low dose administration of the D2/D3 agonist ropinirole abolishes plasticity (91), medium dosed ropinirole reestablishes facilitatory and inhibitory plasticity, whilst high dosage decreases facilitatory plasticity (92). Administration of low dosage or high dosage of anodal L-DOPA suppressed the plasticity induced by tDCS (79), however L-DOPA prolonged the reduction of excitability induced by cathodal tDCS (80).

Induction of plasticity through tES, however, might also arise from simultaneous stimulation of the different components of the neural circuit, from the excitatory/inhibitory synapses to different brain networks, therefore, as a result, it is important to consider the main excitatory (LTP-like) or inhibitory (LTD-like) effect of the brain stimulation. Early LTP/LTD modifications usually last for 30–60 min after induction and reflect post-transcriptional modifications of pre-existing proteins, such as protein phosphorylation, in contrast late LTP/LTD could last hours, days, and even months and require genes and proteins expression (e.g., glutamate NMDA and metabotropic receptors) (50).

In order to shed light on the pathways leading to the synthesis of new proteins, attention has been focused on the group of immediate early genes (IEGs), that are rapidly induced following neuronal activation and are thought to be involved in the maintenance of LTP (93, 94). Among IEGs, zif268 is likely to be specifically related to LTP, since it is expressed under virtually all LTP-inducing situations and shows a high correlation with the duration of LTP (95). After application of

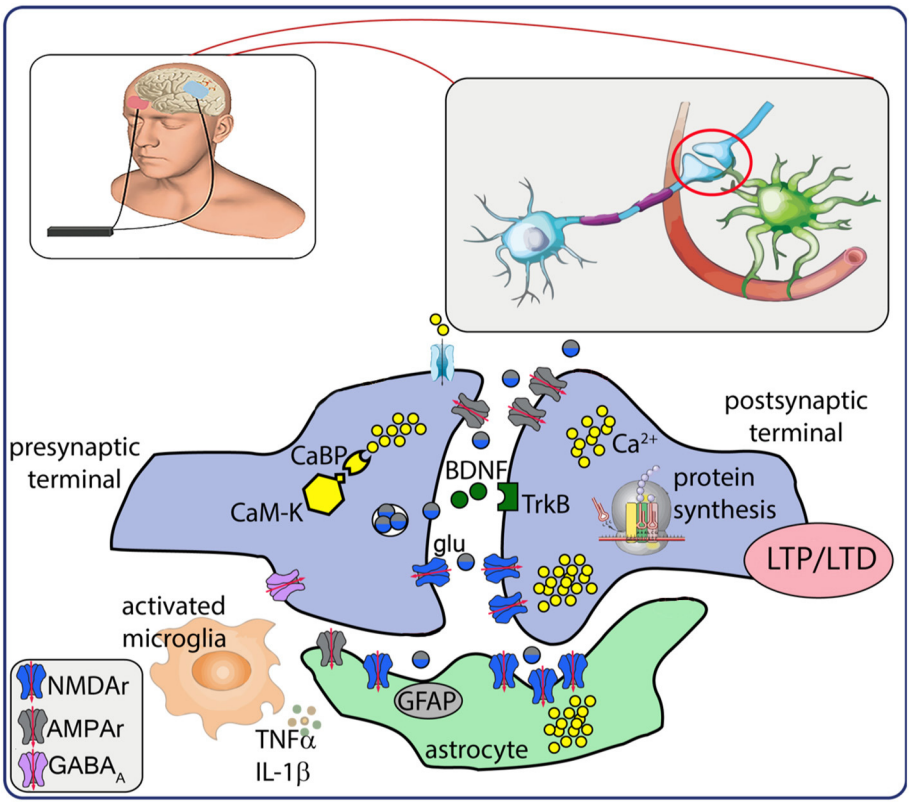

FIGURE 1 | Schematic representation of neurobiological after-effects of transcranial electrical stimulation (tES). tES induces intracellular Ca^{2+} increase and activation of Ca^{2+}-dependent enzymes (CaM-K). Presynaptic mechanisms result in glutamate release that activates AMPA/NMDA receptors, modulates BDNF release and interaction with TrkB receptor, responsible for a cascade of intracellular events that lead to *de novo* protein synthesis. Electrical stimulation also modulates activation of astrocytes and neuroinflammatory response. Altogether, these mechanisms may underlie the establishment of LTP/LTD. **CaBP**, Ca^{2+} binding proteins; **CaM-K**, Ca^{2+} kinases; **glu**, glutamate; **BDNF**, brain-derived neurotrophic factor; **TrkB**, tyrosine kinase receptor B; **LTP/LTD**, long term potentiation/depression; **GFAP**, glial fibrillary acidic protein; **TNFα**, tumor necrosis factor α; **IL-1β**, interleukin 1β; **NMDAr**, N-methyl-D- aspartate receptor; **AMPAr**, alpha-amino-3-hydroxy-5-methyl-4-isoxazolep ropionic acid receptor; **$GABA_A$**, gamma amino butirric acid A receptor.

both anodal and cathodal DCS to hippocampal rat brain slices, zif268 expression was increased, pointing to a possible initial role of zif268 in a cascade of activation of other downstream target genes (20).

Abnormally high activity and hyperexcitability of some subcortical pathways, as in the case of after stroke or during central nervous system (CNS) development, may respond to tES that modulates homeostatic plasticity of the hyperexcitable tissue (96–99). The hyperexcitability is maintained because neurons receive deficient inputs and, in order to compensate, increase excitatory synaptic strength and intrinsic excitability (100, 101).

In addition, metaplastic changes are observed with the administration of tES (6). The term metaplasticity refers to a higher order form of plasticity and reflects the activity-dependent physiological changes that modulate neural plasticity (102). The history of synaptic or cellular activity influences the direction and degree of synaptic plasticity, favoring or inhibiting plasticity induction, synaptic stabilization, and homeostatic regulation of cellular activity (103). Therefore, metaplasticity acts to avoid excessive synaptic strengthening or weakening, to maintain a relatively stable equilibrium of the neural activity in space and time (homeostatic synaptic plasticity), adjusting the balance between synaptic input and neuronal firing, and to prolong the time-window for associative interactions between neural events (associative plasticity) (6). Basically, any recent neural synaptic activity will affect the ongoing activity. For example, if synaptic activity is preconditioned by applying tDCS, the application of continuous tDCS after an interval will modulate polarity which will affect performance (6). Continuous anodal tDCS has shown to facilitate performances while consecutive sessions of cathodal tDCS have shown to enhance working memory (81). Preconditioning neural networks may induce synaptic homeostatic changes that seems to be related to compensatory upregulation at post-synaptic membrane receptors due to inhibition (104, 105). This has been called as the "rebound effect" where neurons are more excitable due to initial downregulation induced by cathodal tDCS and reversed by conditioning cathodal tDCS (13).

Aberrant plasticity induced by non-invasive brain stimulation techniques has been demonstrated in many neurological and neuropsychiatric disorders including PD (106–108), dystonia (109, 110), multiple sclerosis (111), ischemic stroke (112), migraine (113), AD (114), schizophrenia (115–117), and drug addiction (103, 118).

Regarding tACS, both online and offline effects have reported to generate entertainment and neuroplasticity (45). Entertainment is where external rhythmic pattern imposes itself on the intrinsic natural pattern. Neuroplastic changes have been reported via LTP and LTD as tACS modulates neural synchronization by increasing or decreasing it (45). In summary, tES-induced mechanisms of synaptic plasticity cover different aspects of the neurobiology and neurophysiology of CNS, ranging from gene and protein expression, modulation of neurotrophins activity, and neural transmission and, finally, metaplasticity.

Effects on Neuronal Networks and Connectivity

Polarization of the brain tissue can extend beyond the area under the electrodes (119–121) and it may have a functional effect also on distant interconnected neural networks (122, 123). Anodal tDCS of the premotor cortex, for example, increases the excitability of the ipsilateral motor cortex (124) and stimulation of the primary motor cortex has inhibitory effects on contralateral motor areas (125). EEG studies support these findings, showing that stimulation of frontal areas induces all-brain synchronous changes of the oscillatory activity (126, 127). Altered prefrontal oscillations and brain synchronization have been reported by magnetoencephalography (MEG) and EEG study in AD, showing functional disconnection between prefrontal cortex and hippocampus and changes of network connectivity (128–130).

Functional connectivity of cortical networks increased within motor, premotor, and somatosensory areas after anodal tDCS, inducing significant intra and interhemispheric connectivity changes, as revealed by analysis of EEG frequency bands (131).

Brain areas interact mutually creating a complex network that underlie higher brain functions and neural synchronization represents an essential system to coordinate cortico-cortical and cortico-subcortical areas (132, 133). A combined tDCS-fMRI study revealed that after active stimulation functional connectivity showed an increased synchrony in the anti-correlated network (that includes DLPFC) and reduced in the default mode network (DMN) components, thus suggesting a functional reconfiguration of intrinsic brain networks after tDCS (134). This could represent a putative mechanism for tDCS-induced improvement of cognitive functions (134). In addition, using fMRI, anodal tDCS was also shown to modulate functional connectivity of cortical (70), cortico-striatal and thalamo-cortical motor pathway (135). To better grasp the precision of tES, stochastic resonance should be underlined. The concept of stochastic resonance attempts to highlight the importance of wide range of affects due to TES. The electric field can be considered as noise and when added to non-linear systems may enhance or disrupt the state of signal and the noise introduced (136–138). Since the after-effects are not focal but global, the dynamic interactions will modulate not only particular group of neurons but also induce global effects thus affecting neurons near their discharge threshold, thus facilitating or inhibiting a subthreshold signal which will produce two different polarized after effects (138).

TABLE 4 | tES after-effects on neuronal networks and connectivity.

References/Study	Methodology tES	Targets	Main results
Boros et al. (124); Vines et al. (125)	tDCS - aDCS	Motor cortex	aDCS of premotor cortex increases the excitability in ipsilateral motor cortex. Stimulation of primary motor cortex has inhibitory effect on contralateral motor area
Polanía et al. (131)	tDCS	Motor/premotor/ somatosensory areas	Functional connectivity of cortical networks increased with aDCS with intra/interhemispheric connectivity changes
Peña-Gómez et al. (134)	tDCS	Default mode network and DLPFC	tDCS increased synchrony in anti-correlated network and reduced in default mode network
Stagg et al. (55)	tDCS	Cortical/cortico-striatal/thalamo-cortical motor pathways	tDCS modulates functional connectivity of cortical, cortico-striatal and thalamo-cortical motor pathways
Fertonani and Miniussi (138)	tACS/tDCS	–	tES induces stochastic resonance which affects neuronal groups and induces wide range of global effects by facilitating or inhibiting a subthreshold signal

***tES**, transcranial electrical stimulation; **tDCS**, transcranial direct current stimulation; **tACS**, transcranial alternating current stimulation; **a/c tDCS**, anodal/cathodal transcranial direct current stimulation; **DLPFC**, dorsolateral prefrontal cortex.*

See **Table 4** for a summary of the main tES studies and results on neuronal networks and connectivity.

Effects on Glial Cells and Neuroinflammation

The relevance of glial biology cannot be neglected to understand the complexity of the CNS and the comprehensive mechanisms and effects of tES. The significance is clinically appealing as glial cells create a wide neuro-glial network for rapid inter-cellular long-range signaling (73) and are early affected in many CNS disorders. Although the glial cells have attracted limited interest for decades, it is only recently that studies have focused on their role in maintaining synaptic homeostasis and modulating synaptic plasticity in health and disease (139). Astrocytes and microglial cells are in close proximity with synapses as they directly modulate synapse formation and

elimination (140). The loss of integrity of these supportive cells is the trigger of neurodegenerative disorders (141–143). Initially it was believed that AD was consequentially due to Aβ oligomers and fibrils that accumulate and inflammation. However, now it has been demonstrated that glial cells drive the synaptic loss in AD (144–147). In addition, glial mediated synapse formation may impair synaptic turnover and homeostasis which disrupts synaptic plasticity. Reactive gliosis is a process of hypertrophy and proliferation of glial cells in response to an insult such infection/trauma/neurodegenerative disorders (140, 148). This is proceeded by release of chemokines, cytokines and neurotrophic factors that have both neuroprotective (M2-like microglia) and neuroinflammatory effect (M1-like microglia) (84). This leads to a simultaneous process of neural damage and synaptic loss with tissue remodeling and phagocytosis.

To our best knowledge, there are no reports regarding the activity of tACS on glial cells. Significant after-effects of tDCS on glial cells function and plasticity are reported by several groups in the last years (see **Table 5**). This is supported by the fact that astrocytes possess voltage-gated channels and transporters that are sensitive to changes of membrane potential (152, 153). Administration of tDCS has shown to cause a surge in Ca^{2+} in cortical astrocytes that is correlated to an overexpression of the glutamate NMDA receptor (154). Evidence suggests that tES modulates the activity of microglia cells but also the neuroinflammatory response, triggering both pro-inflammatory and anti-inflammatory reaction (149). Cathodal and anodal tDCS produce microglial activation as indicated by the increase of Iba-1, an immunostaining marker of activated microglia (150). High voltage anodal and cathodal tDCS was demonstrated to trigger an inflammatory response in the microglial cell line BV2, showing increase of cyclooxygenase 2 (COX-2) expression, leukocyte transmigration through blood brain barrier (32, 149). On the other hand, there was decrease of tumor necrosis factor-alpha (TNF-α) in rat hippocampus after anodal tDCS of parietal cortex (151). Modulation of the neuroinflammatory reaction is relevant because microglia activation can be beneficial as well as detrimental for neural tissue depending on the time of activation. This is clinically relevant in the case of ischemic stroke, because tDCS can activate innate immune response and attract neural stem cells. *In vitro* experiments suggest that cathodal tDCS, delivered for 5 days, can induce cell proliferation and attract neural crest stem cells (149), forming a reservoir of neurotrophic factors which improved functional recovery. In addition, tDCS has also been shown to influence astrocytes by aligning them perpendicular to the electrical field in both *vitro* and *in vivo* (155–157).

Due to the remarkable connectivity of astrocytes and their pivotal role in neuronal connectivity, non-invasive brain modulation may have profound neurobiological effects (158).

TABLE 5 | tES after-effects on glial cells and inflammation.

References/Study	Methodology tES	Targets	Main results
Rueger et al. (149)	DCS	Microglial cells	tES produces both proinflammatory and anti-inflammatory reactions
Pikhovych et al. (150)	tDCS - cDCS - aDCS	Microglial cells and Iba-1	cDCS and aDCS cause microglial activation with increase in Iba-1 markers
Rueger et al. (149); Pelletier and Cicchetti (32)	High voltage DCS - cDCS - aDCS	Microglial cell BV2	High voltage aDCS and cDCS induces activation of microglial cells BV2 with increased expression of COX-2 (cyclooxygenase 2) and leukocyte transmigration
Spezia Adachi et al. (151)	DCS - aDCS	Hippocampal neurons	aDCS of parietal cortex decreased tumor necrosis factor alfa (TNF-α) in the rat hippocampus
Rueger et al. (149)	DCS	Neural crest stem cells	5-day cDCS induced cell proliferation and attracted neural stem cells

***tES**, transcranial electrical stimulation; **DCS**, direct current stimulation; **a/c DCS**, anodal/cathodal direct current stimulation.*

POTENTIAL CLINICAL APPLICATIONS OF CURRENT STIMULATION

Efficacy of tES in the clinical setting has been supported by many experimental works and clinical reports that has demonstrated a long-lasting efficacy in many neurological and psychiatric conditions (5). Despite neurobiological mechanisms have not been yet fully understood, it is supposed that tES-induced modulation of cortical excitability through changes in cell firing rate could pave the way for future therapeutic applications (159).

Application of tACS in the clinical setting is very limited and largely implemented in the psychiatric settings (160, 161). Accordingly, tACS was shown to successfully manipulate auditory hallucinations in schizophrenia by decoupling interhemispheric connectivity and, when administered to schizophrenic patients to the left dorsolateral prefrontal cortex and posterior parietal region in theta frequency (6 Hz), improved working memory tasks (162). Moreover, 40 Hz tACS induced improvement/remission of symptoms in major depression (163) and obsessive compulsive disorder (164) by modulation of EEG-gamma frequency bands. Enhancement of gamma band power connectivity by tACS was also effective in patients with AD and mild cognitive impairment (165, 166).

Experimental and clinical research with tDCS has been widely explored for its ability to suppress neuronal hyperexcitability or by enhancing inhibition (167). While cathodal tDCS reduces cortical excitability due to neuronal hyperpolarization, anodal tDCS causes an increase in cortical excitability and promotes neuronal depolarization (168). These neurobiological effects might be the substrate to counteract the temporoparietal hypoactivity (atrophy, reduced metabolic rate, and perfusion)

reported in AD, suggesting an innovative therapeutic strategy (169).

In an experimental rat model of stroke, tDCS induced a dramatic increase in spine density of cortical neurons at the site of infarct, indicating that it may promote neural plasticity after stroke (170). Accordingly, tDCS was found to down-regulate the elevated hemichannel pannexin-1 mRNA expression after brain ischemia (thus reducing membrane permeability), but also increase the expression of MAP-2 and GAP-43 proteins, allowing axons to regrow at the infarcted site through the glial scar and redevelop their functions (171). Interestingly, tDCS performed within 3 days after stroke did not improve motor function, in contrast when performed 7–14 days after stroke resulted in more pronounced motor function improvement, thus identifying an optimal time-window for tDCS therapy after stroke (171).

In patients with multiple sclerosis (MS) that received tDCS, MRI detected (1) increased cerebral metabolic rate of oxygen ($CMRO_2$), an indicator of the overall brain/neural activity, and (2) a reduced neuronal reactivity (172).

Seizures are described as a result of an increased excitability and inefficient inhibitory control in foci with altered neuronal homeostasis (72, 173, 174). In the recent years, many works have reported the efficacy of tES in the treatment of drug-resistant seizures. Authors observed an enhanced neuronal plasticity and synaptic reorganization after tES (100). For example, it has been reported that temporal lobe epilepsy responded to tES of hippocampus (101) and low frequency tACS applied over the epileptic foci might reduce interictal and ictal activities in epileptics (175). Moreover, experimental evidence in a rat model of focal epilepsy demonstrated that cathodal tDCS has an anticonvulsant effect through increase of the localized seizure threshold that outlasted the stimulation (176). Similar results were confirmed on a refractory pediatric epileptic patient with focal cortical dysplasia who was treated with cathodal tDCS and experienced marked reduction in the frequency of seizures (177). Along with this, cathodal tDCS was reported to prevent the loss of GABAergic inhibition, which provokes seizures after pentylenetetrazol administration, thus proposing a new antiepileptic mechanism (178). These results, therefore, have posed the basis to the clinical combination of the cathodal tDCS with GABA-agonist antiepileptic drugs (AEDs), such as benzodiazepines, valproic acid, felbamate, topiramate, and barbiturates, in order to increase the antiepileptic stimulation effect.

Application of tDCS is not limited to the cerebral cortex and its disorders but also for the modulation of the excitability in the cerebellum and spinal cord. Since pharmacological approaches to treat cerebellar diseases are still lacking, tES might represent a new potential therapeutic approach that is yet to be explored. The mechanisms behind the neurophysiological effects of tDCS applied over cerebellum have not been extensively researched as compared to cerebral cortex. However, it could be inferred that ionic gradient shifts, cellular activation and inhibition, modulation of neurotransmission may occur in the same way (179). Evidence suggests that cerebellar cathodal tDCS decreases the inhibitory tone of cerebellum on primary motor cortex while anodal tDCS increases it, likely through a specific modulation of dentate-thalamo-cortical connections (21). TDCS also modulates cerebellum-dependent motor learning: anodal tDCS improved the performance in a locomotor adaptation task (180). Mechanisms need to be further explored, however it has been hypothesized that anodal tDCS may broaden the availability of Purkinje cells for learning or increase the dynamic range of these cells, whereas cathodal tDCS may reduce the excitability of Purkinje cells (181). The effects of tDCS on cerebello-motor connectivity were studied in 20 patients with ataxia with administration of cerebello-spinal tDCS (179). Improvement in ataxia was reported and was associated with restoration of motor cortex excitability and cerebellar-brain inhibition.

Application of spinal tDCS is very limited but the preliminary results are extremely interesting. It has been reported that spinal anodal tDCS reduces the amplitude of laser evoked potentials of stimulated Aδ fibers (182) and increases cortico-spinal excitability in a polarity-independent manner (183). While spinal anodal tDCS inhibits the ascending pathways and enhances the reflex circuitry, the spinal cathodal tDCS enhances the activity of ascending pathways and suppresses the reflex circuitry in humans (181). Since there is involvement of the ascending and descending pathways, the glutamatergic, GABAergic and glycinergic systems should be involved in modulating the spinal plasticity (181). The effects of this kind of stimulation can vary in response to several factors including intensity, polarity and direction (184) but also through modulation of the voltage-gated Ca^{2+}channels in the spinal motor neuron dendrites (185). Altogether, these preliminary results demonstrate the ability to modulate spinal plasticity with electrical current stimulation, paving the way for new therapeutic strategies in neurological disorders with impaired spinal excitability.

CHALLENGES AND FUTURE DIRECTIONS

To date, despite the undisputed role of tES in experimental settings in humans as a tool to "switch on/off" specific brain regions that are supposed to be involved in several higher brain functions, its translation into clinical settings is still far to be reached due to the difficulty in producing clinically significant effects in the majority of subjects/patients. This is largely due to the lack of a full comprehension of both the neurobiological bases of tES and the specific neuropathological mechanisms of disease. There are still few data on the possible clinical efficacy of prolonged/repeated protocols of stimulation that might produce persistent changes in synaptic efficacy that cannot be achieved by a short-lasting intervention. In this context, successful trials of prolonged tES protocols could eventually be translated into invasive implants of cortical electrodes for chronic stimulation. Finally, tDCS shows lack of selectivity that might influence different cortical circuits and produce side effects that counteract the effects responsible for the therapeutic action. Therefore, optimizing protocols, electrode size and intensity of stimulation should help to overcome these technical limitations that impedes a tailored approach to the patient and disease.

AUTHOR CONTRIBUTIONS

SK: acquisition, analysis, and interpretation of data for the work and drafting the manuscript. FR: supervising and editing the manuscript and final approval of the manuscript to be submitted. VD: conception and design of the work, supervising and editing the manuscript, and final approval of the manuscript to be submitted. MP: critical supervision, manuscript editing, and final approval of the draft to be submitted. GC: conception and design of the work, analysis and interpretation of data, revising the manuscript, and final approval of the manuscript to be submitted. All authors contributed to the article and approved the submitted version.

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Repetitive Transcranial Magnetic Stimulation at Different Sites for Dysphagia After Stroke: A Randomized, Observer-Blind Clinical Trial

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Background: The clinical efficacy of repetitive transcranial magnetic stimulation (rTMS) protocols on patients with poststroke dysphagia is still unclear.

Objective: This trial aimed to explore and analyze the effectiveness of 5 Hz rTMS on the unaffected hemisphere, affected hemisphere, and cerebellum in stroke patients with dysphagia.

Methods: This observer-blind and randomized controlled trial included a total of 147 patients with stroke. Patients were divided into four treatment groups: the unaffected hemispheric group, the affected hemispheric group, the cerebellum group and the control group. Each group received traditional dysphagia treatment 5 days a week for 2 weeks. All recruited patients except for those in the control group underwent 10 consecutive rTMS sessions for 2 weeks. For the affected hemispheric group and unaffected hemispheric group, 5 Hz rTMS was applied to the affected mylohyoid cortical region or to the unaffected mylohyoid cortical region. For the cerebellum group, 5 Hz rTMS was applied to the mylohyoid cortical representation of the cerebellum (4.3 cm lateral and 2.4 cm below the inion). The Fiberoptic Endoscopic Dysphagia Severity Scale (FEDSS), Penetration/Aspiration Scale (PAS), Gugging Swallowing Screen (GUSS), and Standardized Swallowing Assessment (SSA) were used to evaluate clinical swallowing function before the intervention (baseline), immediately after the intervention and 2 weeks after the intervention.

Results: There were significant time and intervention interaction effects on the FEDSS, PAS, SSA, and GUSS scores ($p < 0.05$). In a direct comparison of the swallowing parameters of the four groups, the changes in FEDSS, PAS, SSA, and GUSS scores showed a significantly greater improvement in the unaffected hemispheric group, the affected hemispheric group and cerebellum group than in the control group ($p < 0.05$).

Conclusions: Whether stimulating the unaffected hemisphere or the affected hemisphere, 5 Hz high-frequency rTMS on mylohyoid cortical tissue might have a positive

effect on poststroke patients with dysphagia. In addition, cerebellar rTMS is a safe method that represents a potential treatment for poststroke dysphagia, and more clinical trials are needed to develop this technique further.

Clinical Trial Registration: chictr.org.cn, identifier: ChiCTR2000032255.

Keywords: repetitive transcranial magnetic stimulation, dysphagia, stroke, cerebellum, mylohyoid cortical

INTRODUCTION

Dysphagia, affecting 27–64% of stroke patients, is one of the most common poststroke sequelae (1) and is often associated with malnutrition, pneumonia, and dehydration (2). Conventional therapies for dysphagia include postural interventions, swallowing maneuvers, and exercises. Even though the above treatments have been widely applied in clinical practice, there is not enough clinical evidence to prove their efficacy (3–5). Recently, non-invasive cortical stimulation, a new strategy, has been used as a way of promoting neurologic rehabilitation after stroke. For example, transcranial magnetic stimulation is considered a well-tolerated technique that can modulate cortical excitability (6, 7). Moreover, repetitive transcranial magnetic stimulation (rTMS) of the motor cortex area related to swallowing directly induces the excitability of swallowing muscles regulated by corticobulbar projections (8), thereby enhancing swallowing function (9, 10). In patients with dysphagia after stroke, the application of 3 Hz (11) and 10 Hz (12) rTMS on the ipsilateral motor cortex represented by the esophageal or mylohyoid cortex showed significant improvement compared with sham stimulation. Meanwhile, both 1 Hz (13) and 5 Hz (9) rTMS on the contralateral motor cortex represented by the pharyngeal or mylohyoid cortex showed improved swallowing function. According to reports, rTMS showed different efficacies when patients with dysphagia were subjected to different stimulation parameters, such as intensity, frequency, and stimulation position.

It is controversial to stimulate either the ipsilesional or contralesional hemisphere. Previous systematic studies have shown different outcomes regarding the efficacy of non-invasive brain stimulation (NIBS) according to its stimulating point. Specifically, a review reported that no differences were found dependent on the stimulation site (14), whereas another study discovered that contralesional stimulation is better than ipsilesional stimulation (15). The latter study applied a combination of 5 Hz rTMS with pharyngeal electrical stimulation on the contralesional hemisphere (16). In conclusion, previous reviews reported different results because of the various stimulation applications, and it was relatively difficult to confirm whether the effect of contralesional rTMS was better than ipsilesional rTMS in regard to improving swallowing function.

Cerebellar neurostimulation has been considered an unexplored method and a prelude of treatment for dysphagia by modulating swallowing pathways. It has been shown that the cerebellum can be strongly activated during swallowing exercise (17), and stimulation of the cerebellum in the hemispheres or midline can induce different pharyngeal electromyography responses. For example, Sasegbon et al. (18) demonstrated that rTMS on the cerebellar vermis had inhibitory effects on pharyngeal motor cortical activity and swallowing behavior. Vasant et al. (19) demonstrated that hemispheric cerebellar rTMS increases cortical pharyngeal motor evoked potential (PMEP) amplitudes. Using the advantages of neuronavigation and comparing the latency and amplitude of pharyngeal motor evoked potentials, the authors confirmed the best position to obtain these responses, which was 4.3 cm lateral and 2.4 cm below the inion (19). Recently, some studies (20, 21) have explored the possibility of rTMS on cerebellar tissue in the treatment of dysphagia.

Therefore, this prospective, randomized, observer-blind clinical study focused on the effectiveness and safety of rTMS in stroke patients with dysphagia. Outcomes after stimulation of the unaffected side, the affected side and the cerebellum were compared to determine which area of stimulation is more beneficial for the recovery of patients with dysphagia to guide clinical work in the future.

MATERIALS AND METHODS

Subjects

One hundred fifty-five poststroke patients suffering from dysphagia were included from April 2020 to April 2021. All of the patients were hospitalized to the Department of Rehabilitation Medicine, Yue Bei People's Hospital, Guangdong Province, China. The inclusion criteria were as follows: (1) subacute stroke <3 months diagnosed by imaging tests, including computed tomography (CT) or magnetic resonance imaging (MRI), hemorrhagic stroke or unilateral ischemia; (2) dysphagia confirmed by fiberoptic endoscopic evaluation of swallowing (FEES); and (3) no prior dysphagia rehabilitation. The exclusion criteria included history of any other neurogenic disease, epilepsy, tumor; severe cognitive impairment or aphasia; and contraindication to electrical or magnetic stimulation. All patients provided written informed consent before inclusion. The trial protocol was approved by the Ethics Committee of Yue Bei People's Hospital, and this clinical study was carried out and reported according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (22). Details of trial protocol registration can be seen in chictr.org.cn (chictr.org.cn Identifier: ChiCTR2000032255).

A total of 155 poststroke patients with dysphagia were recruited before assessment for eligibility, and 147 were included after exclusion.

One hundred forty-seven patients were divided into four groups: the unaffected hemispheric group, affected hemispheric

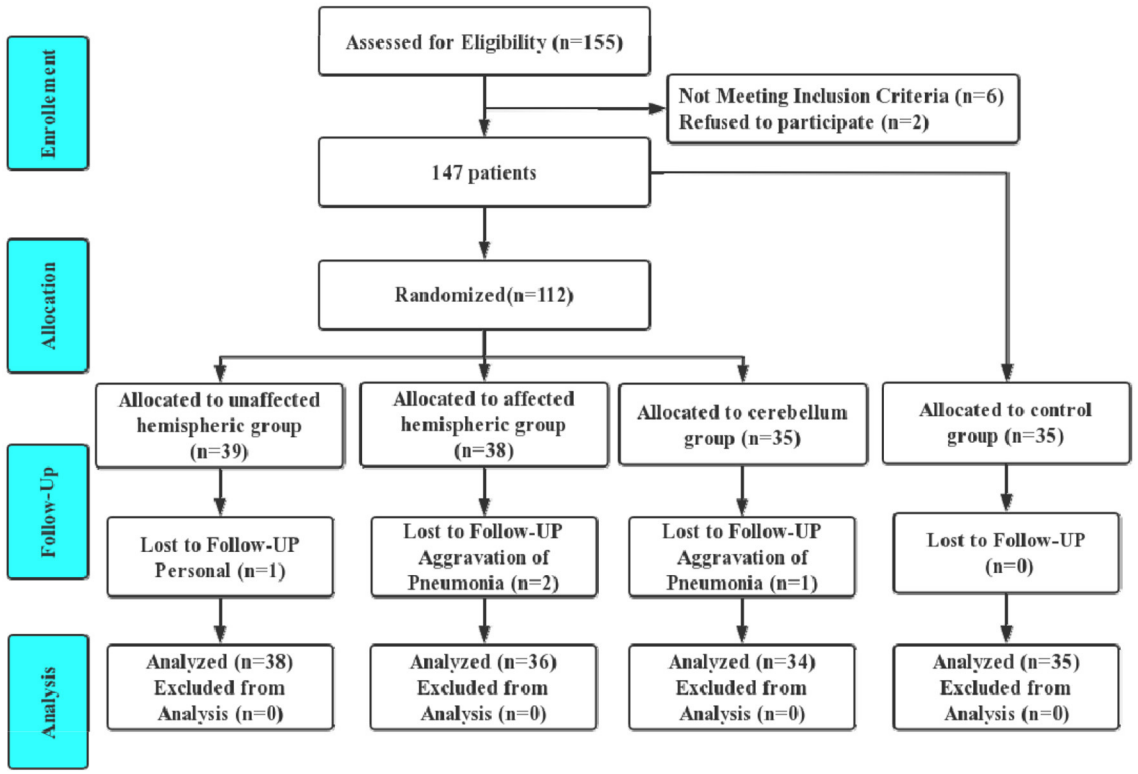

FIGURE 1 | Participant flow diagram.

group, cerebellum group and control group. Four included patients withdrew from the trial. One patient in the unaffected hemispheric group withdrew for a personal reason not relevant to the trial. Two patients in the affected hemispheric group and one in the cerebellum group quit the study due to exacerbated pneumonia. Consequently, 143 patients completed the trial (**Figure 1**).

Experimental Design

This study was an observer-blind and random controlled trial. Patients were randomly divided into three groups by the random number table method. A sealed opaque envelope was opened at patient enrollment to determine whether the patient was to be assigned to the unaffected hemispheric, affected hemispheric or cerebellum group. These three groups of patients received 10 consecutive rTMS sessions for 2 weeks. For the affected hemispheric group and unaffected hemispheric group, 5 Hz rTMS was applied to the affected mylohyoid cortical region (**Figure 2A**) or to the unaffected mylohyoid cortical region (**Figure 2B**). For the cerebellum group, 5 Hz rTMS was applied to the mylohyoid cortical representation of the cerebellum (4.3 cm to lateral and 2.4 cm below the inion) (**Figure 2C**) (19). These three groups of patients received the same amount of traditional dysphagia treatment for 30 min daily after the intervention, such as thermal tactile stimulation, vocal cord exercises, Shaker exercises, Masako maneuvers, oropharyngeal muscle strengthening exercises, and tongue retraction exercises.

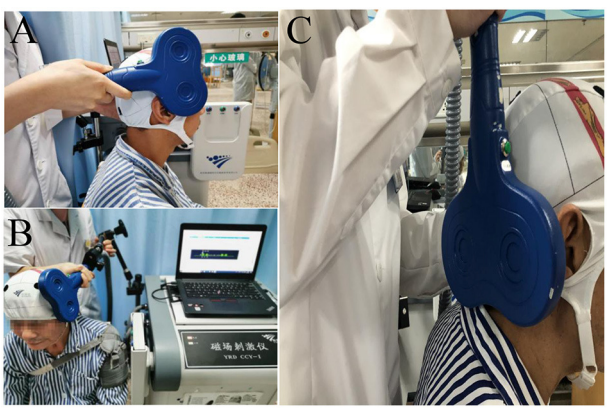

FIGURE 2 | (A) For the affected hemispheric group, 5 Hz rTMS was applied at the affected mylohyoid cortical region. **(B)** For the unaffected hemispheric group, 5 Hz rTMS was applied at the unaffected mylohyoid cortical region. **(C)** For the cerebellum group, 5 Hz rTMS was applied at the mylohyoid cortical representation of the cerebellum (4.3 cm lateral and 2.4 cm below the inion).

These exercises were conducted 5 days a week for 10 days with the guidance of an experienced physical therapist. Meanwhile, patients treated with rTMS were compared with a population of 35 post-stroke patients (control group) suffering from dysphagia who did not receive rTMS. The 35 post-stroke patients only

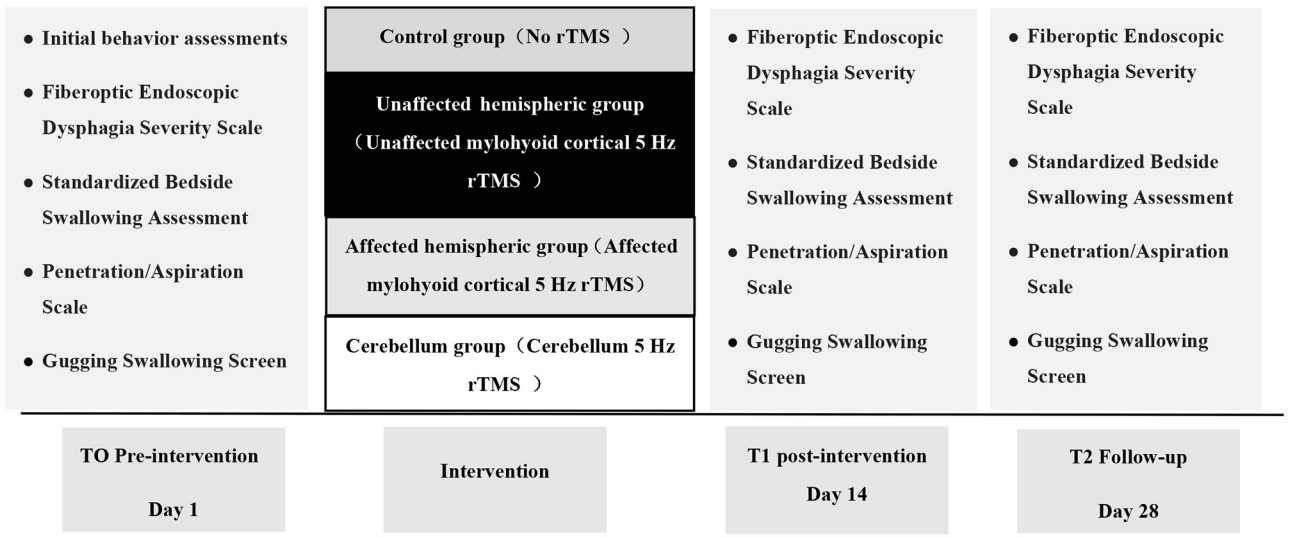

FIGURE 3 | Experimental design.

received traditional dysphagia treatment 5 days a week for 2 weeks.

Determination of the Resting Motor Threshold (RMT)

Unaffected Hemispheric Group and Affected Hemispheric Group

Each patient in the affected hemispheric group and unaffected hemispheric group was seated in a quiet environment and relaxed state. Electromyography (EMG) data representing oral swallowing musculature from mylohyoid muscles were detected using the same methods as Hamdy et al. (23). MagPro CCY-I stimulator (purchased from YIRUIDE Company, Wuhan, China) was used for magnetic stimulations with a 9 cm outer diameter figure-eight coil.

Cortical excitability on both hemispheres separately of each patient, including the motor evoked potential (MEP) and resting motor threshold (rMT) were measured using single-pulse TMS. The coil was moved around in an area within 2–4 cm anteriorly and 4–6 cm laterally of the vertex of the cranium to locate the mylohyoid cortical region of the hemisphere to obtain the maximum MEP recording (23). The maximum MEP recording location was regarded as the "hot spot," representing magnetic stimulation delivered to the area. Single-pulse TMS was then delivered to the "hot spot" with a 2% reduction in the output of the stimulator. The definition of the rMT is that in 10 consecutive trials of mylohyoid muscles, five trials can induce the minimum stimulus intensity of MEP $> 50\ \mu V$. The "hot spot" was defined as an unaffected symmetrical hemisphere if MEPs were absent when the stroke-affected hemisphere was stimulated.

The Cerebellum Group

In previous studies, it has been identified that rTMS stimulation is effective regardless of which side of the cerebellum is stimulated (19, 24). For the cerebellum group, the coil was fixed at the mylohyoid cortical representation of the cerebellum (4.3 cm to lateral and 2.4 cm below the inion) (19). The rMT was determined by the rMT of the mylohyoid cortical area of the unaffected hemisphere.

Repetitive Transcranial Magnetic Stimulation Application

The same parameters of stimulation were used for each intervention group. For each patient, 20 min rMT intensity with 5 Hz at 110% was applied at the "hot spot" area, which would last for 10 days with a total of 1,800 pulses per day. The protocols of rTMS applied in this study were strictly followed by the clinical safety guidelines for rTMS applications (25).

Outcome Measurements

All included participants were assessed at three different times: baseline (before the treatment), 2 weeks (after the treatment), and follow-up (2 weeks after the treatment) (see **Figure 3**). The primary outcome included the FEDSS scale; secondary outcomes involved assessments of the other dysphagia rating scales, such as the SSA scale, PAS scale, and GUSS scale.

Fiberoptic Endoscopic Dysphagia Severity Scale (FEDSS)

All included patients required FEES. First, the secretion status of patients was measured, and then the patient received standard volumes of semiliquid diet, such as soft solid food, liquids, or puree. Stroke-related dysphagia was divided into a six-point FEDSS with 1 score for the best and 6 scores for the worst based on different consistencies of diet observed in the endoscopic examination and the risk of saliva penetration or aspiration (26).

TABLE 1 | The demographic and clinical characteristics of the included patients.

	Unaffected *N* = 38	Affected *N* = 36	Cerebellum *N* = 34	Control *N* = 35	*P*
Sex (F:M)	10: 28	8: 28	14: 20	17: 18	0.063
Age (years)	64.47 ± 13.95	64.67 ± 10.87	63.18 ± 9.92	62.34 ± 11.54	0.814
Type of stroke (Hemorrhage: Ischemia)	18: 20	12: 24	10: 24	14: 21	0.411
Affected hemisphere (Right: Left: infratentorial)	10: 20: 8	10: 14: 12	6: 12: 16	5: 15: 15	0.265
Duration of onset of stroke (days)	30 (15–60)	18 (14–60)	20 (14.25–30)	25 (15–30)	0.433
BADL	28.95 ± 21.91	26.94 ± 22.62	21.47 ± 23.08	23.71± 20.66	0.489
MMSE	13.84 ± 6.71	17.43 ± 8.35	15.02 ± 6.43	14.60 ± 7.57	0.182
EAT-10	17.70 ± 8.72	17.84 ± 10.09	18.84 ± 6.76	18.89± 8.64	0.890
NRS 2002	3 (2–4)	2.5 (2–4)	3.25 (2.75–3.44)	3 (2–4)	0.412
WST	4 (3–5)	4 (3–5)	4 (4–5)	4 (4–5)	0.089
FEDSS	3.68 ± 0.93	3.69 ± 1.19	4.06 ± 0.95	4.06 ± 0.76	0.168
PAS	5.47 ± 1.64	5.19 ± 1.79	5.91 ± 1.38	5.46 ± 1.54	0.311
SSA	27.79 ± 4.83	27.61 ± 4.99	27.56 ± 4.35	27.71 ± 3.50	0.996
GUSS	6.42 ± 5.52	5.72 ± 4.77	5.59 ± 4.77	5.60 ± 4.91	0.874

Data are described as the mean ± SD or median (interquartile range). FEDSS, Fiberoptic Endoscopic Dysphagia Severity Scale; MMSE, Mini-Mental State Examination; PAS, Penetration/Aspiration Scale; WST, Water Swallow Test; SSA, Standardized Swallowing Assessment; BADL, Basic Activities of Daily Living; GUSS, Gugging Swallowing Screen.

Standardized Bedside Swallowing Assessment (SSA)

The SSA consists of three parts. One section comprises eight indicators, including the responsiveness level, breathing, sound intensity, lip closure, control of trunk and head, voluntary cough and pharyngeal reflex. It is scored vary from 8 to 23 points. In the second section, the patients swallowed 5 mL water three times, and at the same time, salivary management and laryngeal movement were assessed. Repetitive swallowing, stridor, choking, and vocal quality were also evaluated, with a score range of 5–11 points. Once patients completed the first two parts of the assessment, they underwent the third part that entailed swallowing 60 mL water; this activity was scored from 5 to 12 points. The total SSA score varied from 18 to 46 points, and higher scores indicated worse swallowing function (27, 28).

Penetration/Aspiration Scale (PAS)

Dysphagia severity was scored by an 8-point scale named the Penetration/Aspiration Scale (PAS). This scale was widely conducted for semiquantitative assessment of the degree of penetration and aspiration of endoscopic or radiological measurements, with higher scores indicating more severe impairment (29).

Gugging Swallowing Screen (GUSS)

The GUSS is a validated reliable screening test for swallowing with a maximum score of 20. This tool consists of two parts: five indirect questions were used to measure the swallow function of the patient, and four direct questions were conducted to assess the physical condition of patients when ingesting liquid, semisolid and solid food. A higher score suggested a milder condition of dysphagia, but a lower score suggested a more serious dysphagia condition. Fourteen points were deemed passing scores for swallowing, and patients who scored <14 points were regarded as having a high likelihood of aspiration (30).

Statistical Analysis

In this study, statistical analyses were conducted with SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Two-way analysis of variance (ANOVA) was used for continuous data among multigroup comparisons (normal distribution), and the chi-squared test was performed for categorical data. To assess the effect of the interaction between intervention and time, repeated measure analysis of variance (ANOVA) was used, in which time was used as a within-subject factor and intervention as a between-subject factor. *Post-hoc* analysis was performed using Bonferroni correction. A Greenhouse-Geisser correction was performed to correct the non-sphericity of the data. A $P < 0.05$ was considered significantly different.

RESULTS

One hundred forty-seven subjects were randomized into four groups. The average ages in the unaffected hemisphere group, the affected hemisphere group, the cerebellum group and the control group were 64.47 ± 13.95 years (28 males and 10 females), 64.67 ± 10.87 years (28 males and 8 females), 63.18 ± 9.92 years (20 males and 14 females), and 62.34 ± 11.54 years (18 males and 17 females), respectively. There were no significant differences between the groups at baseline in clinical and demographic characteristics, Basic Activities of Daily Living (BADL) score, Mini-Mental State Examination (MMSE) score, Eating Assessment Tool-10 (EAT-10) score, Nutrition Risk Screening-2002 (NRS2002) score, Water Swallow Test (WST) score, FEDSS score, PAS score, SSA score, or GUSS score (**Table 1**).

Compared with baseline, the FEDSS and PAS scores of all groups improved at 4 weeks. The FEDSS scores were significantly

TABLE 2 | Clinical rating scales (FEDSS, PAS, SSA, and GUSS) for the four groups at each time.

	Unaffected	Affected	Cerebellum	Control	*P*-value
FEDSS					
Baseline	3.68 ± 0.93	3.69 ± 1.19	4.06 ± 0.95	4.06 ± 0.76	0.168
2 weeks	3.05 ± 1.16	3.06 ± 1.12	3.59 ± 1.21	3.77 ± 0.81	0.008
4 weeks	2.53 ± 1.45	2.50 ± 1.32	2.76 ± 1.54	3.66 ± 1.11	0.001
PAS					
Baseline	5.47 ± 1.64	5.19 ± 1.79	5.91 ± 1.38	5.46 ± 1.54	0.311
2 weeks	4.03 ± 1.82	4.03 ± 2.16	4.41 ± 2.20	5.23 ± 1.17	0.024
4 weeks	3.37 ± 2.17	3.53 ± 2.26	3.59 ± 2.56	5.00 ± 1.28	0.005
SSA					
Baseline	27.79 ± 4.83	27.61 ± 4.99	27.56 ± 4.35	27.71 ± 3.50	0.996
2 weeks	23.92 ± 4.57	22.86 ± 4.32	23.79 ± 3.83	26.03 ± 3.49	0.012
4 weeks	21.66 ± 4.58	21.11± 3.66	21.79 ± 2.78	24.46 ± 3.27	0.001
GUSS					
Baseline	6.42 ± 5.52	5.72 ± 4.77	5.59 ± 4.77	5.60 ± 4.91	0.874
2 weeks	10.37 ± 6.28	8.78 ± 5.14	9.41 ± 6.57	6.23 ± 4.26	0.017
4 weeks	11.37 ± 6.72	10.94 ± 6.38	11.24 ± 7.32	6.94 ± 3.95	0.008

	Unaffected vs. affected (*P*-value)	Unaffected vs. cerebellum (*P*-value)	Affected vs. cerebellum (*P*-value)	Unaffected vs. control (*P*-value)	Affected vs. control (*P*-value)	Cerebellum vs. control (*P*-value)
FEDSS						
Baseline	1.000	0.631	0.718	0.625	0.712	1.000
2 weeks	1.000	0.232	0.254	0.033	0.038	1.000
4 weeks	1.000	1.000	1.000	0.003	0.003	0.044
PAS						
Baseline	1.000	1.000	0.375	1.000	1.000	1.000
2 weeks	1.000	1.000	1.000	0.043	0.048	0.442
4 weeks	1.000	1.000	1.000	0.008	0.024	0.039
SSA						
Baseline	1.000	1.000	1.000	1.000	1.000	1.000
2 weeks	1.000	1.000	1.000	0.176	0.008	0.148
4 weeks	1.000	1.000	1.000	0.008	0.001	0.018
GUSS						
Baseline	1.000	1.000	1.000	1.000	1.000	1.000
2 weeks	1.000	1.000	1.000	0.013	0.354	0.123
4 weeks	1.000	1.000	1.000	0.017	0.046	0.029

FEDSS, Fiberoptic Endoscopic Dysphagia Severity Scale; GUSS, Gugging Swallowing Screen. SSA, Standardized Swallowing Assessment; PAS, Penetration/Aspiration Scale.

different at 2 weeks ($P = 0.008$) and 4 weeks ($P = 0.001$). Similarly, there was a significant difference in PAS scores at 2 weeks ($P = 0.024$) and 4 weeks ($P = 0.005$) (**Table 2**). **Figures 4A,B** showed FEDSS and PAS scores at each time point in the four groups.

After 2 weeks of rTMS treatment, the improvement of dysphagia in the unaffected hemisphere group, the affected hemisphere group and the cerebellum group was significantly better than that in the control group. For the FEDSS, repeated measure analysis of variance showed a significant main effect of assessment time point ($F = 86.106$, $df = 1.724$, $P < 0.001$) and a significant time–group interaction ($F = 3.889$, $df = 5.173$, $P = 0.002$) (**Table 2**; **Figure 4A**).

The SSA and GUSS scores of all patients improved during the follow-up. There were significant differences in SSA scores at 2 weeks ($P = 0.012$) and 4 weeks ($P = 0.001$) (**Table 2**; **Figure 4C**). Similarly, at 2 weeks ($P = 0.017$) and 4 weeks ($P = 0.008$), the GUSS scores were significantly different. Repeated measure analysis of variance showed a significant main effect of the assessment time point ($F = 87.728$, $df = 1.416$, $P < 0.001$) and a significant interaction (time-group) for the GUSS ($F = 5.122$, $df = 4.372$, $P < 0.001$; **Figure 4D**).

Three participants (one unaffected and two affected) suffered transient headache. No participants developed seizures during or after therapy.

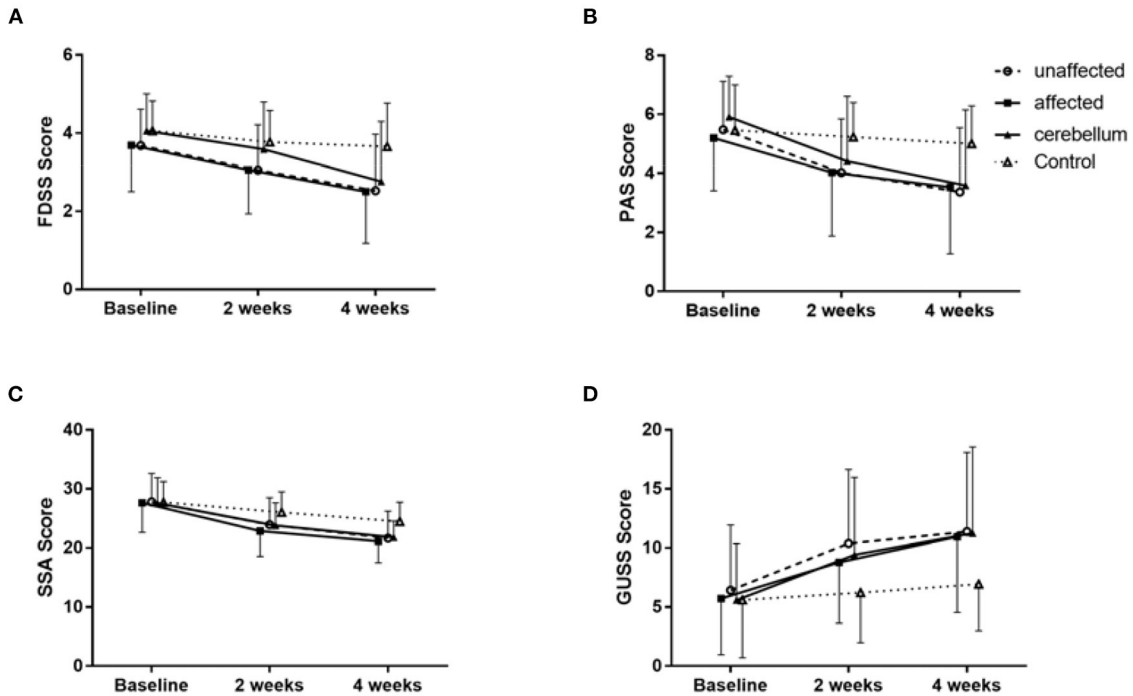

FIGURE 4 | Changes in the mean rating scores of FEDSS **(A)**, PAS **(B)**, SSA **(C)**, and GUSS **(D)** at the three evaluation points in the four groups of patients. Data are described as the mean ± SD. Each group showed significant improvement separately.

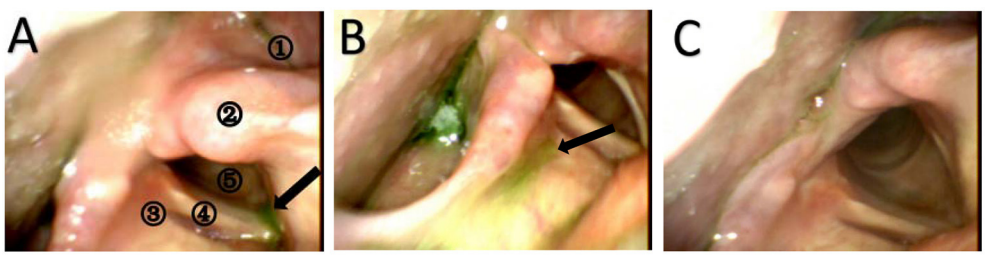

FIGURE 5 | Still images from the FEES examination of a 66-year-old man with dysphagia at three different times. **(A)** FEES examination before the treatment (baseline). The black arrow represents aspirated puree in the subglottis. The patient does not try to cough and clear the material. Therefore, the FEDSS score is 5 points, and the PAS score is 8 points. A1 = pyriform sinus, A2 = arytenoid, A3 = laryngeal vestibule, A4 = vocal fold, A5 = subglottic. **(B)** FEES examination after the treatment (2 weeks). Puree is attached to the laryngeal vestibule, and the patient tries to cough but cannot clear it. The FEDSS score was 4 points, and the PAS score was 3 points. **(C)** FEES examination at the time of follow-up. Food is not inhaled into the laryngeal vestibule or subglottis. The FEDSS score and PAS score were both 1 point.

DISCUSSION

Our study compared the effects of dysphagia intervention based on the stimulation site: the affected mylohyoid cortical area, unaffected mylohyoid cortical area and cerebellum. This study revealed large effect sizes for swallow scores (FEDSS, PAS, SSA, and GUSS) after the end of intervention in the unaffected hemispheric group, the affected hemispheric group and the cerebellum group compared to the control group. These results suggest that rTMS stimulation of the affected hemisphere, unaffected hemisphere and cerebellum was useful in improving swallowing function in patients with dysphagia after stroke. Nevertheless, the effects among these sites were not significantly different. **Figure 5** shows the changes in FEDSS and PAS scores in a patient treated with rTMS.

The mechanism of rTMS is not fully understood. Some previous studies (13, 31) were based on the hypothesis that the balance of activity between the hemispheres of the brain is perturbed after stroke, leading to impaired neurological function. Neurophysiologically, this interhemispheric imbalance is considered to be caused by altered transcallosal inhibition, with an abnormal increase in excitability in the contralesional hemisphere inhibiting the ipsilesional hemisphere. Therefore, in some previous studies (13, 31), rTMS has been used to restore the balance between the hemispheres of the brain to improve functional outcomes. In recent years, studies have confirmed that

the projection of swallowing function in the human cerebral cortex is bilateral, with a dominant hemisphere that controls swallowing in patients with dysphagia (32, 33). High-frequency stimulation promotes cortical excitability, while low-frequency stimulation lowers excitability (34). rTMS can directly affect the cerebral cortex, effectively adjust the excitability of the cerebral cortex, reconstruct the central nervous system, form neural pathways, regulate swallowing centers, and improve swallowing function. Regarding the effects of cerebellar targeted rTMS, it is potentially interpreted that rTMS activates the cerebellar cortex, resulting in subsequent stimulation of dentate nuclei in each individual cerebellar hemisphere (24) because the functions of the cerebellum, which serves as a sensor and motor regulated organ, are predominantly suppressive (35). Hence, rTMS over the cerebellar cortices may lead to a decrease in inhibitory outflow and an increase in cortical activity. In this study, 5 Hz rTMS stimulation of the affected hemisphere, unaffected hemisphere and cerebellum may have facilitated swallowing function by improving cortical excitability of the mylohyoid cortex.

Previous studies have shown different outcomes in which various stimulation parameters of rTMS could improve the function of dysphagia in patients after stroke. For example, Park et al. (9) showed that high-frequency (5 Hz) rTMS application on the contralesional pharyngeal motor cortex was beneficial for poststroke dysphagic patients. Khedr et al. (11) proved that rTMS with 3 Hz high frequency at the lesional pharyngeal motor cortex resulted in significant improvement in dysphagia compared to a sham-stimulated group. These studies indicate that contralesional and lesional pharyngeal motor rTMS stimulation are both beneficial for reducing poststroke dysphagia. This is consistent with our research showing that rTMS stimulation at a high frequency in the unaffected hemisphere and affected hemisphere could significantly promote dysphagia recovery compared with the control group. The recovery of swallowing function may be related to changes in cortical excitability and neuroplasticity. Increases in cortical excitability by application of 5 Hz rTMS may increase stimulation to the motor neurons in the corticobulbar and corticospinal tracts, which enhances the synaptic innervations that project to the mylohyoid muscles, improves the movement of mylohyoid muscle, and promotes the recovery of swallowing function. Further neuroimaging tests or neurophysiologic evaluation are needed to delineate the underlying neuromechanism. Overall, our study and previous studies indicate that high-frequency rTMS stimulation of mylohyoid cortical tissue benefits poststroke dysphagia.

Recently, a growing number of studies have explored the possibility of rTMS on cerebellar tissue in the treatment of dysphagia. Some studies (19, 36) have shown that hemispheric cerebellar rTMS can increase cortical PMEP amplitudes. Vasant et al. (20) found that active cerebellar rTMS can increase PMEP amplitude, and their results indicated that cerebellar rTMS is a safe method that represents a potential treatment for poststroke dysphagia. Sasegbon et al. (24) demonstrated that high-frequency rTMS on the cerebellum could reverse the disruptive effects of a "virtual lesion." These findings provide evidence for the development of cerebellar rTMS as a treatment for dysphagia after stroke. Our findings showed that rTMS stimulation at a high frequency in the cerebellum could significantly promote dysphagia recovery compared with the control group. However, one study (37) showed that, compared with unilateral stimulation, bilateral cerebellar rTMS has a greater promotion effect on corticobulbar motor pathways to the pharynx and may be a more effective clinical therapy. Another study (19) found that 10 Hz rTMS seems to be the best frequency to promote excitement of the pharyngeal motor cortex. At present, the optimal stimulation parameters of rTMS on cerebellar tissue are still uncertain. More clinical trials are needed in the future to further improve the technology.

Recent studies show that compared to unilateral stimulation, bilateral pharyngeal stimulation with 10 Hz rTMS stimulation on "hot spots" has more positive outcomes in both acute and chronic stroke patients (38, 39). However, these trials did not compare the effects of ipsilesional and contralesional rTMS. Furthermore, they did not compare the effects of cerebellar rTMS to cerebral hemispheric rTMS. To the best of our knowledge, our study was the first to directly compare the therapeutic impact of high-frequency rTMS applications on the unaffected hemisphere, affected hemisphere and cerebellum to evaluate the effects on swallowing function applications in stroke patients. Our findings show no difference, based on FEDSS, PAS, SSA, and GUSS outcomes, among the affected hemisphere, unaffected hemisphere and cerebellum. Similarly, there was no statistically significant difference between the groups in the subgroup analysis of a meta-analysis according to intervention site (ipsilesional vs. contralesional site stimulation) (14). However, another meta-analysis reported that contralesional stimulation is better than ipsilesional stimulation (15). The meta-analysis involved interventions that included non-invasive brain stimulation, either rTMS or tDCS. The pooled effect showed high heterogeneity concerning dysphagia evaluations, population, stroke etiology, clinical characteristics of stroke, and intervention time after stroke onset. Therefore, more rigorously designed original studies are necessary to identify the effects of different stimulation sites.

This study may possess the following limitations. First, the difference in swallowing function rehabilitation by stroke type was not analyzed. We were not able to perform cerebellar subgroup analysis according to affected, unaffected and cerebellar stroke lesions on account of the insufficient number of patients with infratentorial stroke lesions. Second, the effect of rTMS in our study was evaluated based on the clinical severity and fiberoptic endoscopic dysphagia severity scale and not on neurophysiologic evaluation, such as MEP amplitude and latency of rTMS. Finally, the effect of rTMS on brain plasticity was not evaluated by neuroimaging tests or neurophysiologic evaluation in our study. In the future, the combination of neuroimaging studies and neurophysiology would be beneficial in exploring the potential mechanism of rTMS in the recovery of dysphagia.

CONCLUSIONS

The present study suggested that 5 Hz rTMS in the affected hemisphere, unaffected hemisphere and cerebellum for 10

days improves swallowing function in poststroke dysphagia patients. However, no difference among the affected hemisphere, unaffected hemisphere and cerebellum was observed. Therefore, regardless of whether the unaffected hemisphere or the affected hemisphere is stimulated, 5 Hz high-frequency rTMS on mylohyoid cortical tissue might have a positive effect on patients with poststroke dysphagia. In addition, cerebellar rTMS is a safe method that represents a potential treatment for poststroke dysphagia, and more clinical trials are needed to further improve this technique.

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 Ethics Committee of Yue Bei People's Hospital. 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

HL contributed to the conception of the study, supervised the clinical trial, and performed manuscript writing and editing. LZ, JW, and JR performed data analyses and manuscript writing and editing. PW and YZ contributed to the conception and design of the study. FL and YP performed data collection. All authors have agreed with the submitted version of the manuscript.

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