Schizophrenia Research: Cognition 39 (2025) 100339

Contents lists available at ScienceDirect

Schizophrenia Research: Cognition

journal homepage: www.elsevier.com/locate/scog

The efficacy and safety of dual-target rTMS over dorsolateral prefrontal cortex (DLPFC) and cerebellum in the treatment of negative symptoms in first-episode schizophrenia: Protocol for a multicenter, randomized, double-blind, sham-controlled study

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ARTICLE INFO

Keywords:
Schizophrenia
Negative symptoms
Repetitive transcranial magnetic stimulation
Cerebellum
Dorsolateral prefrontal cortex
Randomized controlled trial

ABSTRACT

Background and objective: The dorsolateral prefrontal cortex (DLPFC) - cerebellum circuit has been implicated in the pathogenesis of negative symptoms of schizophrenia (SZ). Both areas are considered separate targets for repetitive transcranial magnetic stimulation (rTMS) treatment, showing potential for improving negative symptoms. However, there is still a lack of research that targets both DLPFC and cerebellum simultaneously. In this study, we will explore the efficacy and safety of dual-target rTMS based on the DLPFC-cerebellum circuit in the treatment of negative symptoms in SZ.

Methods: The study is a multicenter randomized, double-blind, and sham-controlled trial. First-episode schizophrenia is treated with adjunctive 1 Hz rTMS to the right DLPFC and intermittent theta burst stimulation (iTBS) to the cerebellum delivered sequentially in 20 sessions (active group) or a sham condition (sham group) along with antipsychotics. Clinical symptoms are assessed using the Positive and Negative Symptom Scale (PANSS) at baseline (T0), at the middle of the TMS intervention (after 10 sessions, T1), at the end of the intervention (after 20 sessions, T2), and at a 4-week follow-up after the intervention concludes (T3). Subjects will undergo magnetic resonance imaging (MRI) scans twice: once at baseline (T0) and again at the end of TMS intervention (T2). Comparisons of improvements in negative symptoms are conducted between the active and sham groups. Alterations in functional connectivity (FC) are also compared between both groups. Pearson or Spearman correlation analysis is performed to estimate the relationship between FC alteration and clinical symptom remission (PANSS negative subscale reduction scores and response rates, etc) depending on whether the data follows a normal distribution. In addition, potential neuroimaging biomarkers based on MRI associated with TMS treatment will be explored.

Discussion: Positive results from this double-blind, sham-controlled, randomized study may optimize the TMS treatment strategy for SZ, particularly in managing negative symptoms. Clinicians can select TMS with increased confidence as a safe adjunctive treatment option. Furthermore, the findings of this trial may offer preliminary insights into the potential neuroimaging therapeutic mechanisms of TMS interventions targeting the prefrontal-cerebellar circuit.

Trial registration: ClinicalTrials.gov NCT04853485
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https://doi.org/10.1016/j.scog.2024.100339
Received 15 April 2024; Received in revised form 27 October 2024; Accepted 25 November 2024

1. Introduction

Schizophrenia (SZ) is a devastating psychiatric disorder affecting millions of people worldwide. It presents as a combination of positive symptoms (*e.g.*, hallucinations and delusions), negative symptoms (*e.g.*, avolition, alogia, and expressive deficits), and cognitive dysfunction (*e.g.*, deficits in memory and executive function) (Schultz and Andreasen, 1999; Faden and Citrome, 2023). Negative symptoms have been regarded as core symptoms of SZ (Galderisi et al., 2018), which are common throughout the course of the illness (Howes et al., 2023) and are perhaps the most disabling feature of SZ (McKechanie et al., 2016). They are also strongly associated with low daily functioning and poor long-term prognosis (Strauss et al., 2010; Fenton and McGlashan, 1994; Leucht et al., 2017). Yet, currently available pharmacological and psychosocial treatments do not lead to any significant improvement in this core component of SZ (Strauss et al., 2020). Therefore, the identification and development of other efficacious treatment options for negative symptoms, such as non-pharmacotherapies, are a priority.

With advances in positron emission tomography (PET) and MRI, previous studies have identified several brain regions and neural circuits associated with negative symptoms. The dorsolateral prefrontal cortex (DLPFC) is frequently linked to these negative symptoms based on prior imaging research (Galderisi and Kaiser, 2023). Additionally, several studies have indicated that reduced activation of the ventrolateral prefrontal cortex (VLPFC) (Goghari et al., 2010) and the ventral striatum (Radua et al., 2015) contributes to the pathophysiology of negative symptoms. Other brain regions, including the anterior cingulate cortex (ACC), dorsal striatum (DS), orbitofrontal cortex (OFC), amygdala (AMY), and the basal ganglia, have also been implicated in the pathogenesis of negative symptoms (Bègue et al., 2020). Notably, recent research has demonstrated that alterations in the functional connectivity of cerebellar networks and frontal brain circuits are strongly associated with the negative symptoms of SZ (Choi et al., 2023; Feng et al., 2022; Brady Jr. et al., 2019).

With the discovery of neural circuits and networks associated with negative symptoms, physical therapies such as repetitive transcranial magnetic stimulation (rTMS) have emerged as significant research focal points in the treatment strategies for SZ in recent decades. This is particularly true for their effectiveness in improving negative symptoms and cognitive function, owing to their advantages in neuromodulation that specifically target these neural circuits. So far, researchers have conducted numerous studies on the efficacy and mechanisms of various modes of TMS, including low-frequency rTMS ($\leq$1 Hz), high-frequency rTMS (>1 Hz), intermittent theta burst stimulation (iTBS), continuous theta burst stimulation (cTBS), unspecified theta burst stimulation, and deep TMS, in alleviating negative symptoms. Some studies have shown some encouraging results (Bation et al., 2021; Kumar et al., 2020; Prikryl et al., 2007; Zhuo et al., 2019; Fitzgerald et al., 2008), while others have reported negative outcomes (Rosa et al., 2007; Novák et al., 2006; Rabany et al., 2014). A recent systematic review and meta-analysis by Rasmus Lorentzen et al (Lorentzen et al., 2022) included randomized controlled trials (RCT) studies and identified the left DLPFC was the primary stimulation target in the majority of these trials. This meta-analysis also reported a statistically significant superiority of rTMS (SMD = 0.41, 95 % CI: 0.26; 0.56, p-value < 0.001) (Lorentzen et al., 2022) on negative symptoms compared to sham-controls. Furthermore, TMS targeting the left DLPFC with a stimulation frequency > 1 Hz was found to be the most effective, despite substantial heterogeneity and a high risk of bias among the included studies (Lorentzen et al., 2022). The findings of previous studies have not demonstrated satisfactory efficacy of TMS in treating negative symptoms. Variations in targeted brain regions or networks for intervention, as well as differences in TMS parameters such as stimulation intensity, the number of sessions, and the number of pulses per session, may contribute to the lack of encouraging results observed in some prior studies. The optimal TMS parameters have yet to be established, as proposed by Rasmus Lorentzen et al. (Lorentzen et al., 2022)

Recently, Jessica P. Y. Hua et al. reviewed studies on cerebellar stimulation in SZ and identified post-cerebellar modulation in SZ, as evidenced by the alleviation of certain clinical symptoms, primarily negative and depressive symptoms, along with increased frontal-cerebellar connectivity (Hua et al., 2022). This systematic review suggested that cerebellar stimulation is a promising intervention for individuals with SZ, particularly concerning negative symptoms. To achieve a therapeutic effect in a significantly shorter duration and to enhance participant tolerance among compared to conventional rTMS, iTBS has been favored in recent studies (Poorganji et al., 2023). Notably, since the FDA approved Stanford Accelerated Intelligent Neuromodulation Therapy (SAINT or SNTFDA) in 2022, the iTBS protocol has also been more encouraged to explore the therapeutic effects of negative symptoms in SZ. By integrating neuroimaging data, TMS can enhance the precision of target localization, which theoretically should improve the therapeutic outcomes. Indeed, when combined with functional magnetic resonance imaging (fMRI), several trials have demonstrated that TMS can modify behavior through the regulation of neural networks, leading to an improved therapeutic effect on negative symptoms (Brady Jr. et al., 2019; Fox et al., 2014; McClintock et al., 2011; Pascual-Leone et al., 2011; Shafi et al., 2012; Basavaraju et al., 2021). The study conducted by Roscoe O. Brady Jr. et al. observed that negative symptoms were alleviated as functional connectivity (FC) changed through the iTBS protocol targeting the cerebellar vermis (Brady Jr. et al., 2019). Subsequently, Rakshathi Basavaraju's team performed a RCT trial to validate the improvement of negative symptoms using a similar iTBS approach targeting the cerebellum. Although the clinical effect was not significantly better than that of a sham control, a change in FC between the cerebellum and the prefrontal network was observed. However, this imaging change was not correlated with symptom improvement (Basavaraju et al., 2021). These studies prompted researchers to further investigate the treatment of negative symptoms through the prefrontal-cerebellar network. Consequently, well-designed research is still necessary to validate the effects of the prefrontal-cerebellar network on negative symptoms and to identify more optimized parameter settings, such as a longer duration of intervention, as suggested by Rakshathi Basavarajua (Basavaraju et al., 2021). In this study, we employed a similar iTBS pattern targeting the cerebellum, as utilized in previous trials (Brady Jr. et al., 2019; Basavaraju et al., 2021).

Previous literature has reported that a dual-target intervention using a transcranial alternating current stimulation (tACS) protocol, which simultaneously targets the prefrontal and temporal regions, improved cognition in older adults. This suggests that dual-target stimulation may play a significant role in reshaping neural networks and functional connections between different brain regions. Furthermore, it also provides insights into strategies for neuro-regulatory interventions, such as target selection for psychiatric disorders (Reinhart and Nguyen, 2019). Therefore, we introduce an additional target: the right DLPFC, which has a strong relationship with cerebellum, to facilitate dual-target stimulation (Brady Jr. et al., 2019). Additionally, we have increased the number of TMS sessions from 10 to 20, as used in prior studies (Brady Jr. et al., 2019; Basavaraju et al., 2021), to more effectively address negative symptoms. To ensure the successful completion of 20 rTMS sessions, we will recruit individuals with first-episode schizophrenia (FES) and disease duration of <5 years, thereby minimizing the influence of chronic illness and complex treatment regimens. We hypothesize that the dual-target intervention of TMS targeting the prefrontal-cerebellar network may mitigate negative symptoms more effectively than the sham TMS. Additionally, we hypothesize that improvements in negative symptoms may be associated with alterations in FC related to this network.

2. Materials and methods

This study is a multicenter, randomized, double-blind, and sham

stimulus-controlled clinical study. The samples will be recruited from two centers: Shanghai Mental Health Center (SMHC) as the lead unit and Suzhou Guangji Hospital (SZGJ) as the participating unit. The trial has been registered at http://www.clinicaltrials.gov (NCT04853485). The study protocol is approved by the Ethics Committee of the SMHC and SZGJ and will be conducted in accordance with local regulations and the principles outlined in the Declaration of Helsinki. All participants will be required to provide written informed consent.

2.1. Sample size calculation

For patients with early-stage mental disorders (including first onset), drug intervention is currently effective in 50 % of patients, with a reduction rate of psychiatric symptoms reaching 50 %. Assuming that the intervention involving the cerebellar-right frontal lobe can increase the effectiveness to 80 %, with a type I error rate of 0.05, the confidence level of the intervention experiment is 0.8. The estimated sample size for each group is as follows:

$$n1 = n2 = \frac{\left(z_{\alpha/2}+z_{\beta}\right)^2(p_1(1-p_1)+p_2(1-p_2))}{\varepsilon^2} = \frac{(1.96+0.84)^2+(0.5(1-0.5)+0.8(1-0.8))}{(0.8-0.5)^2} =$$

36RTMS intervention for one course, based on past experience, the dropout rate is around 20 % to 25 %. Calculated at 25 %, 48 cases need to be enrolled in each group. Therefore, we consider 50 cases in each group, which could essentially verify the hypothesis.

2.2. Types of outcomes

2.2.1. Primary outcomes

The primary outcomes of this study include the reduction scores on the PANSS negative subscale and the clinical response rates at various intervention time points: mid-term (after 10 sessions), at the conclusion of the intervention (after 20 sessions), and at a 4-week follow-up. The reduction score is defined as the relative change, calculated as ($PANSS_{baseline}$-$PANSS_{10\text{-}session/20\text{-}session/4W\text{-}followup}$)/ $PANSS_{baseline}$ × 100 %, in the PANSS subscale compared with the baseline. Clinical response is defined as a reduction of 50 % or more in the PANSS score.

2.2.2. Secondary outcomes

Tolerance of TMS treatment: The dropout rate is used to assess the tolerance of TMS treatment. The number of patients who initially enrolled, dropped out, and completed the study will be assessed to estimate the dropout rate. The dropout is defined as a participant who is unable to complete the entire research process for any reason.

Reduction scores and clinical response rates for the PANSS positive subscale, the general psychopathology subscale, and the total score are evaluated at various intervention time points: mid-term (after 10 sessions), at the end of the intervention (after 20 sessions), and at a 4-week follow-up after the intervention's conclusion. These assessments are considered secondary outcomes. The definition and calculation method of clinical response rate are similar to those of primary outcomes.

Changes in both the MCCB score and FC within the right DLPFC-cerebellar network, as well as between the right DLPFC or cerebellum and other areas of the brain, will be evaluated from baseline (T0) to the conclusion of the 20-session TMS intervention (T2) as secondary outcomes.

2.3. Participant recruitment

Participants aged 14 to 45 years who meet the diagnostic criteria for SZ as outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) will be independently evaluated by two clinical physicians. The diagnosis will be confirmed using the Structured Clinical Interview for DSM Disorders (SCID). These patients will be recruited from the inpatient departments of two medical centers.

The inclusion criteria include: (1) being diagnosed with SZ according to the DSM-5 diagnostic criteria, (2) being willing to receive TMS therapy, and providing signed informed consent, (3)14–45 years old, (4) having an IQ ≥ 70, (5) be experiencing their first episode of psychosis without achieving full remission, (6) having a positive and negative syndrome scale (PANSS) score ≥ 55, and (7) may receive second-generation antipsychotic drugs other than clozapine during the study. The specific type and dosage of drugs are determined by the clinical attending physician. Patients will be excluded if they have: (1) obvious impulsive or negative tendencies towards dangerous behaviors, (2) a history of manic or severe depressive episodes, (3) drug or alcohol dependence diagnosed with DSM-5 within the past three months, (4) sensorimotor disorders, neurological diseases, or other physical diseases, (5) an inability to give informed consent or contraindications to MRI, (6) rTMS treatment contraindications such as metal implants.

Withdrawal criteria include: (1) when researchers determine that the experiment cannot continue due to adverse events or abnormal laboratory test values; (2) when subjects voluntarily withdraw from the trial; (3) in cases of obvious protocol violation (including poor compliance as determined by the researcher); (4) if the subject becomes pregnant during the experiment; (5) when other researchers believe that continuing the experiment is challenging.

2.4. Allocation and comparison methods

This study adopts a randomized, double-blind, and sham-stimulus -controlled clinical trial. Participants are randomly divided into either an active stimulus or a sham stimulus group at a 1:1 ratio in two centers. The randomization method involves a computer software- generated random sequence of numbers by (block randomization) along with corresponding serial numbers. All numbers are categorized into the active group (TMS true stimulus group) and Sham group (TMS pseudo stimulus group). The allocation of the enrolled subjects is concealed. Subjects who meet the conditions and voluntarily participate in the trial will be assigned to either the active or sham group based on their random numbers. Each subject in the active or sham group requires specific intervention targeting the imaging coordinates of the right DLPFC and cerebellum based on their structural MRI.

The implementation of random grouping, as well as the verification and storage of grouping information, is carried out by specialists. Grouping information should not be disclosed to personnel other than those involved in implementing TMS intervention treatment. Treatment personnel also need to keep the grouping information confidential. Clinical symptom evaluators, patients, and MRI data analysts are kept blind to the treatment grouping. True and sham TMS are administered in the same treatment room to prevent patients from guessing their grouping assignment. Patients in different groups are scheduled to complete treatment at different time slots.

2.5. Clinical and safety assessments

Demographic information, such as age, gender, years of education, marital status, occupation, history of traumatic brain injury, growth and development history, and family history, will be collected for all participants at the time of enrollment. Clinical symptoms, including negative symptoms, positive symptoms, general psychopathology ， and total score, will be assessed using the Positive and Negative Symptoms Scale (PANSS) (Kay and Opler, 1987). All the clinical assessments will be conducted four times for each participant: at baseline (T0), mid-term after 10 sessions (T1), at the end of TMS intervention after 20 sessions (T2), and at a 4-week follow-up after the completion of TMS (T3).

MRI scans and neurocognitive function tests will be conducted separately at baseline (T0) and at the end of the TMS intervention (T2). The Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Cognitive Consensus Battery (MCCB) will be employed to evaluate the neurocognitive and social cognitive functioning across various domains, including the speed of processing (SoP), attention/vigilance (AV), working memory (WM), verbal learning (Vrbl

Lrng), visual learning (Vis Lrng), reasoning and problem-solving (PRS), and social cognition (SC). Intelligence assessment will be performed using some subtests (vocabulary and figure-piecing subtests) from the Chinese version of the Wechsler Adult Intelligence Scale (WAIS).

An adverse reaction record table for rTMS intervention has been designed for the experiment. Patients will be evaluated after each rTMS intervention based on a score of 0–9, including symptoms such as headache, weakness, dizziness, discomfort in the eyes/nose, discomfort in the jaw joint/teeth or facial discomfort, and drowsiness.

2.6. Individualized and precise rTMS intervention

This study utilizes a dual-targeted precise rTMS intervention plan guided by personalized MRI images to deliver accurate treatment. The intervention targets include the cerebellum and the right DLPFC, with specific coordinates identified as the optimal target for group intervention. T1-weighted structural MRI is obtained from each participant before the initiation of rTMS. The cerebellar vermis is identified using coordinates from the Talairach coordinate system (0, -82, -30) (Schmahmann et al., 1999), and the right DLPFC is determined by coordinates from the Montreal Neuroimaging Institute (MNI) coordinate system (36, 24, 30) (Brady Jr. et al., 2019). The intervention sequence for these two targets starts with the right DLPFC, followed immediately by the cerebellum. RTMS is delivered as iTBS over the cerebellum and as 1 Hz over the right DLPFC. RTMS is administered using YRD CCY-I (YIRUIDE) system at SZGJ and the Magventure Pro system at SMHC respectively, both employing a figure-of-8 coil based on the existing magnetic stimulation equipment at the two centers. Dosing is at 100 % of the resting motor threshold (RMT) over the right DLPFC and 120 % of RMT over the cerebellum. The RMT is defined as the lowest intensity that produces a motor evoked potential (MEP) of 50 μV, peak-to-peak, in five out of ten trials in the relaxed abductor pollicis brevis (APB) (Daskalakis et al., 2008). The detailed steps for measuring RMT can be found in our previous literature (Tang et al., 2014). ITBS is administered in 20 trains of 2 s on and 8 s off cycle, each containing 3-pulse bursts at 50 Hz, delivered at theta frequency (every 200 ms). In total, 600 pulses are delivered over 3 min and 12 s. On the other hand, 1 Hz rTMS is administered in trains of 60 pulses, totaling 12 trains, 720 pulses in a session, with a 30-second interval between trains. The total stimulation time is 17 min and 30 s. Participants will undergo a total of 20 sessions, which are administered over 4 weeks.

Precise positioning of rTMS coil: Utilizing the infrared navigation positioning system, the procedure involves the following basic steps: calibrating the subject's head, establishing a correlation with their MRI image, using the nasal root and bilateral ear processes as reference points, conducting preliminary registration, and then sampling the scalp for accurate surface registration. Determine the intervention targets of the cerebral cortex and scalp surface (or intervention pathway), select rTMS intervention magnetic heads, calibrate them, and finally locate the scalp surface targets and intervention routes under the guidance of the navigation system. Accurately position and orient the rTMS magnetic heads on the scalp surface. For the sham group, the localization procedure is identical to that of the active group; however, the magnetic coil was rotated 90 degrees to create a false stimulation effect.

MRI scanning: MRI scans are conducted at the Radiology Department of SZGJ and SMHC. The Siemens 3 T Verio MRI system and a 32-channel head coil are used for MRI acquisition. The scanning sequence includes structural imaging (T1) and resting-state fMRI.

Structural image (T1): Using an MP-RAGE sequence with TR = 2300 ms, TE = 2.96 ms, a flip angle of 9 degrees, FoV = 256 mm × 256 mm, voxel size of 1 mm × 1 mm × 1 mm, continuous scanning of 192 slices in the sagittal position, and a scanning duration of approximately 5 min.

Resting fMRI: Using a multi-band parallel acquisition sequence with TR of 2500 ms, TE of 30 ms, a flip angle of 90 degrees, FoV of 224 mm × 224 mm, 149 time points, voxel size of 2 mm × 2 mm × 2 mm, continuous axial scanning of 68 layers, and a scanning duration of approximately 6 min.

The patients underwent MRI scanning twice, once at baseline (T0) and again at the end of TMS intervention after 20 sessions (T2). Patients' initial MRI scans were obtained within 24 h prior to the first rTMS session, and the final MRI scan was collected 24–48 h after the last session. Participants are required to keep their eyes open and refrain from engaging in any specific thinking during the scanning period.

The TMS protocol adhered to all safety guidelines and recommendations endorsed by the International Federation for Clinical Neurophysiology (Rossi et al., 2009).

2.7. Statistical methodology

2.7.1. Clinical and demographic parameters

Clinical and demographic variables are compared between the two groups using an independent *t*-test for continuous variables or a chi-square test for categorical variables at baseline (T0). We adopt standard intention-to-treat (ITT) analysis. To compare the effects of rTMS on clinical symptoms between the active and sham groups at various time points following the intervention (T0, T1, T2, T3), a linear mixed-effects model was developed. In constructing the model, the group (active, sham), time (T0, T1, T2, T3), and their interaction effects were included as fixed effects, with subject ID treated as a random effect. Age, gender, and years of education were incorporated as covariates for control (Formula: Clinical symptom ~ Time * Group + Age + Gender + Education). Significance was determined using a threshold of $p < 0.05$ (two-sided). All statistical analyses were conducted using SPSS version 22.0 software (www.spss.com/statistics) and R.

The flow diagram of the trial is depicted in Fig. 1.

2.7.2. fMRI data processing and analysis

(1) fMRI data preprocessing

The data preprocessing pipeline, including head movement correction, alignment, standardization, smoothing, and other steps, is briefly described as follows. Firstly, the initial 10 time points are removed to achieve signal equilibrium and allow subject to adapt to the scanning noise. Secondly, we conduct slice-timing correction, realignment correction, normalization, and resampling to 3 × 3 × 3 mm^3 are conducted. Thirdly, the nuisance covariates, including 24 motion parameters, white matter (WM), cerebrospinal fluid (CSF) signals, and linear trending, are regressed out. Subsequently, temporal scrubbing and temporal filtering (0.01–0.1 Hz) are performed. Finally, the data is smoothed (FWHM = 6 mm). Differences due to head motion (frame-wise displacement, FD) are assessed using repeated-measures ANOVA and post-hoc analyses. SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12) and the CONN toolkit (functional connectivity toolbox, http://www.nitrc.org/projects/conn/) are used for preprocessing fMRI data. Two key steps are included in preprocessing. Firstly, the ART (artificial time points) method is used to detect artifacts such as head movement and physiological signals; the second method involves using the CompCor method to eliminate the influence of overall noise. The brain region is segmented using the Harvard Oxford template, dividing the cortical structure is divided into 91 ROIs, the subcortical structure into 15 ROIs, and the cerebellum into 26 ROIs based on the AAL template, totaling 132 ROIs. At the same time, a brain template based on MNI coordinates (264 spherical ROIs) obtained by Power et al., which is based on resting-state FC features, will also be utilized. This template has been used in the classification of depression subtypes, as demonstrated by Drysdale (Drysdale et al., 2017).

(2) FC data processing

a) **ROI Correlation Matrix:** Extract the entire time series signal of each ROI, and calculate the correlation coefficient between the two ROI

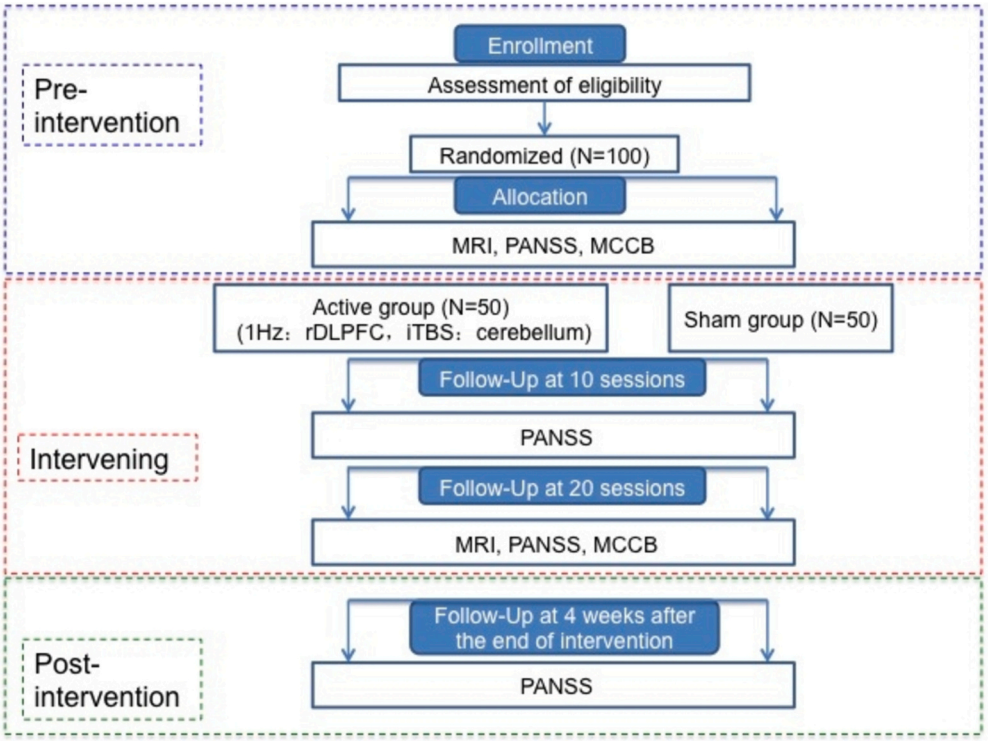

Fig. 1. Flowchart of trial.

time series. The magnitude of the correlation coefficient reflects the strength of the FC between the two ROIs. The above calculation is performed on the whole brain ROI, which can establish the correlation matrix of the whole-brain ROI. The static FC calculation is completed using the CONN software package.

b) **Seed-driven Correlation Analysis:** Taking bilateral DLPFC and cerebellum as seed regions. BOLD signal time series from various subpoints are extracted, Pearson correlation coefficients are calculated for every pair of ROI time series, and Fisher transformation is applied to convert them to *Z*-value. This transformation reflects the FC between ROIs.

(3) Comparison of FC between two groups

The interaction effect of group by time is estimated to investigate whether two treatment methods result in different alterations in whole-brain FC. Multiple comparisons correction is performed for the above analyses based on whole-brain FC maps using a height threshold (min z N 3.1) for individual voxel and a cluster size based on Gaussian Random Field theory, which corresponds to a cluster-level corrected $P = 0.05/3$ due to 3 seeds. Based on the above analyses, regions of FC with significant interaction effects are extracted for post-hoc analysis. Two paired *t*-tests are used to compare the longitudinal changes between baseline (T0) and the end of the rTMS intervention (20 sessions, T2) for each group. Two sample t-tests are performed to compare the differences between the two patient groups at baseline (T0). If the baseline FC shows significant differences between the two patient groups, the FC at pre-treatment is controlled as a covariate in the following repeated measures analysis of variance (ANOVA). Differences between two groups are also investigated using independent sample t-tests.

2.7.3. Correlation between altered FC and clinical symptoms

The average FC alterations that showed significant interaction effects are extracted as the subtraction of coefficients between T0 and T2. Pearson or Spearman correlation analysis is conducted to estimate the relationship between each FC alteration and clinical variables of symptom remission (PANSS negative reductive scores and response rates) for the active and sham groups individually, depending on whether the data follows a normal distribution.

2.7.4. FC as a biomarker for predicting the effect of rTMS

We will divide patients of the active or sham group into two subgroups based on the treatment outcome, respectively: refractory or non-refractory subgroups, with a 50 % reduction rate of PANSS negative subscale score as the cutoff value (AR *vs.* ANR: refractory group for active group *vs.* non-refractory for active group; SR *vs.* SNR: refractory group for sham group *vs.* non-refractory for sham group).

Repeated measures two-way ANOVA is conducted with the between-subject factor (outcome: AR *vs.* ANR for the active group; SR *vs.* SNR for the sham group) and the within-subject factor (time: T0 *vs.* T1 or T0 *vs.* T2) within the active group or sham group separately. The interaction effect of outcome and time is used to investigate the specific changes observed among the responders in the active or sham group. Two post-hoc paired *t*-tests are separately performed in the AR and ANR groups, or in the SR and SNR groups, to detect the longitudinal changes of FC after treatment.

3. Discussion

The present study is a randomized, double-blind, and sham-controlled trial designed to explore the efficacy and tolerance of dual-target TMS with 1 Hz over the DLPFC and iTBS over the cerebellum as an add-on treatment for negative symptoms in a multicenter sample of patients with FES.

Given the significant impact of negative symptoms on long-term function and the clinical challenge of treating thesesymptoms (Strauss et al., 2010; Fenton and McGlashan, 1994; Leucht et al., 2017), the use of safe and potentially effective non-invasive brain stimulation techniques such as rTMS to alleviate negative symptoms in the early stages of SZ may enhance the disorder's outcomes. In previous studies, applications focusing on negative symptoms have shown promising results. However, its efficacy requires significant improvement, with an urgent

need to optimize targeting and precision, especially in response to the prominent complaints of patients.

Previous research has indicated that the cerebellum (Andreasen et al., 1998; Barch, 2014)and prefrontal cortex (Hill et al., 2004; Potkin et al., 2002; Wolkin et al., 1992)play a role in the pathogenesis of SZ, including negative symptoms. There have also been many studies on neural regulation (Hyde et al., 2022; Aleman et al., 2018) such as rTMS (Wang et al., 2017), exploring these two brain regions. It has been found that targeting the cerebellum or the prefrontal cortex, specifically DLPFC, is more common with some positive results observed (Lorentzen et al., 2022). While previous studies, including a few RCTs have been conducted to test the efficacy of rTMS on negative symptoms in SZ, most of them were limited by the lack of assessment of maintenance effects or small sample size (Brady Jr. et al., 2019; Bation et al., 2021; Lorentzen et al., 2022). Additionally, previous studies have always assessed the impact of interventions on these two areas separately. Here, we will propose a new fronto-cerebellar rTMS protocol to assess, for the first time, the influence of stimulating these two cortical areas simultaneously on negative symptoms in a larger sample of early-stage psychiatric patients.

In addition, this study will allow us to identify and assess the value of neuroimaging measures such as FC for predicting and elucidating improvements in negative symptoms after rTMS. Structural or functional abnormalities based on MRI, such as FC, cortical thickness, and surface area, have been found to be associated with negative symptoms, cognitive function, long-term functional outcomes, and clinical outcomes (Cattarinussi et al., 2023; Zhu et al., 2022). This makes these aforementioned indices promising neurobiological markers for negative symptoms. The detection of predictive and interpretive biomarkers of clinical response to rTMS is an expanding field with the potential to enhance therapeutic strategies for patients with SZ, while advancing our understanding of the neural substrate underlying the effects on negative symptoms of rTMS. The successful implementation of this project will promote optimizing the targets and enhancing the precision of rTMS treatment. The results of this study will simplify and optimize the screening of participants receiving rTMS treatment, thereby enhancing treatment efficacy and reducing costs through precision medicine regimens.

In general, the current project, if successful, will upgrade the use of rTMS in psychiatry, especially in addressing negative symptoms, to a significantly higher level. On the other hand, the clinical efficacy of rTMS using the current protocol will be utilized to validate the biotypes of FC in early psychosis. The biotypes will be determined using an existing independent dataset, which includes 650 cases of resting MRI (comprising 400 patients in the prodromal phase, 100 patients with the first episode, and 150 controls). This datasets may serve as a biomarker to predict the outcome of rTMS in the future. Additionally, the successful implementation of this project will help to preliminarily reveal the potential neuroimaging mechanisms of this rTMS intervention model in improving clinical symptoms, especially negative symptoms.

4. Limitation

Our research has certain limitations. The primary concern is that the use of medication in this study has not been controlled, which could inevitably confound the results. The optimal design, whether in terms of dosage or type of medication, is to use rTMS alone without the combination of drugs. However, this poses significant difficulties in clinical practice for patients with SZ experiencing acute episodes. In the future, screening drug-naïve patients with lower aggression and certain insight, who are only receiving rTMS therapy, could allow for the observation of the pure effect and mechanism of rTMS. The follow-up observation period after rTMS intervention is relatively short, which may not be conducive to observing the longest duration of sustained effects of rTMS.

Ethics and dissemination

The study protocol is approved by the Ethics Committee of the Shanghai Mental Health Center and Suzhou Guangji Hospital. The results will be submitted for publication in a peer-reviewed journal. In the event of any changes to the protocol during the conduct of the study, details of the changes, including the date of each amendment, description of the change and the rationale, will be indicated in the reporting of the study results.

Role of funding sources

This research was supported by the National Natural Science Foundation of China (NSFC82151314); Clinical Research Plan of SHDC (SHDC12022113); Suzhou Municipal Science and Technology Bureau (SKJY2021142).

CRediT authorship contribution statement

Junjie Wang: Writing – original draft, Methodology, Investigation, Funding acquisition. **Yanyan Wei:** Writing – review & editing, Investigation, Data curation. **Qiang Hu:** Writing – review & editing. **Yingying Tang:** Writing – review & editing, Supervision, Methodology. **Hongliang Zhu:** Writing – review & editing, Investigation. **Jijun Wang:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors report no conflicts of interests.

Acknowledgements

The authors would like to thank all the subjects for participation in this study.

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