Repetitive Transcranial Magnetic Stimulation for Musculoskeletal Pain in Patients with Parkinson's Disease

Clinical Trial Protocol and Statistical Analysis Plan

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LIST OF ABBREVIATIONS

EEG	electroencephalogram
ESS	Epworth Sleeping Scale
FDR	false discovery rate
FC	functional connectivity
fMRI	functional magnetic resonance imaging
fALFF	fractional amplitude of low-frequency fluctuations
HAMA	Hamilton Anxiety Scale
HAMD	Hamilton Depression Scale
ITT	Intention-to-treat
LEDD	levodopa-equivalent daily dose
MDS-UPDRS	MDS–Unified PD Rating Scale
MS	Microstate
MMSE	mini-mental state examination score
MKPPS	Modified King's Parkinson's Disease Pain Scale
MDS	Movement Disorder Society
MSP	Musculoskeletal pain
NRS	numeric rating scale
PD	Parkinson's disease
PDQ-39	Parkinson's Disease Questionnaire-39
PDSS-2	PD Sleep Scale–2
PET	positron emission tomography
M1	primary motor cortex
RCT	randomized controlled trial
rTMS	Repetitive transcranial magnetic stimulation
RMT	resting motor threshold
SCOUP-AUT	Scale for Outcomes in Parkinson's disease for Autonomic Symptoms
sTMS	Single-pulse TMS

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1. Summary

Title	Repetitive Transcranial Magnetic Stimulation for Musculoskeletal Pain in Patients with Parkinson's Disease
Background	Pain is an increasingly recognized non-motor symptom of Parkinson's disease (PD), with significant prevalence and negative impact on the quality of life of patients. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive brain stimulation technique that may be useful for the treatment of various psychiatric and neurological disorders. However, very few placebo-controlled studies have been performed specifically to relieve pain in Parkinson's disease.
Objectives	The overall objective of this study is to test the analgesia of 20 Hz-rTMS delivered to primary motor cortex (M1) in PD patients with chronic musculoskeletal pain. The secondary objective is to evaluate the efficacy and safety of 20 Hz-rTMS delivered to M1 on PD related symptoms, and elucidate its underlying neurophysiological mechanisms through functional magnetic resonance imaging (fMRI) and electroencephalogram (EEG).
Study Design	The study is a blinded endpoint, parallel design, randomized controlled trial planned to enroll sixty-two PD patients with musculoskeletal pain. All participants will be allocated into two arms by randomization in a 2:1 ratio. Based on the anti-parkinsonism medications, active-rTMS will be given to the intervention group and sham-rTMS will be given to the control group. The therapeutic effect of M1-rTMS will be evaluated after 7 sessions of intervention by comparing the magnitude of clinical scales reduction from baseline over a 2-month period. Alongside clinical evaluations, we collected resting-state EEG, TMS-EEG and fMRI data of PD patients before and after treatment at the same time.
Study Subjects	**Inclusion Criteria:** ☑ Idiopathic PD was diagnosed according to the 2015 Movement Disorder Society (MDS) clinical diagnostic criteria

	☑ Hoehn and Yahr stages of I to III. ☑ musculoskeletal pain was detected based on the Ford classification system for pain in PD. Pain duration of at least 3 months, with continuous moderate intensity pain (≥ 3/10 on a 0-10 numerical rating scale) occurring at least three days per week. ☑ stable antiparkinsonian therapy for ≥4 weeks. **Exclusion Criteria：** ☒ Contraindications to rTMS. ☒ unstable ongoing psychiatric disorder, history of substance abuse (alcohol, drugs). ☒ Prior treatment with any form of rTMS. ☒ Mini-mental State Examination scores ≤24. ☒ Other pain conditions, such as apparent osteoarthritis, or rheumatoid arthritis depended on laboratory or imaging findings.
Interventions	The M1 target was defined as the "hand knob" region, which corresponded to the motor cortical representation of the hand, regardless of pain location. The coil was centered over the scalp above the M1 target contralateral to the maximal pain area or positioned left side in case of bilateral pain. **Intervention programs** A MagPro X100 machine (Magventure, Farum) connected to an MCF-B70 figure-of-eight coil delivered TMS during the real stimulation condition. **Control programs** Sham stimulation was delivered using an MCF-P-B65 figure-of-eight coil (Magventure), which produces an auditory percept similar to real TMS without inducing cortical activation.
Outcomes	The outcome is the clinical assessments and relevant brain changes in PD patients with pain following 20 Hz M1-rTMS over the course of 2 months. **<u>Primary outcome</u>：** • Change in the Modified King's Parkinson's Disease Pain Scale (MKPPS)-Domain 1 score.

	• Change in a 0-10 numeric rating scale (NRS). • Proportion of participants who achieved treatment response (defined as a ≥30% reduction in MKPPS Domain 1 score from baseline). **Secondary outcome:** • Change in MDS–Unified PD Rating Scale (MDS-UPDRS) I, II, III, IV. • Change in the 24 items Hamilton Depression Scale (HAMD). • Change in the 14 items Hamilton Anxiety Scale (HAMA). • Change in the Scale for Outcomes in Parkinson's disease for Autonomic Symptoms (SCOUP-AUT). • Change in the PD Sleep Scale–2 (PDSS-2) • Change in the Epworth Sleeping Scale (ESS) • Change in the Parkinson's Disease Questionnaire-39 (PDQ-39) • Changes in time-varying EEG network dynamics • Changes in EEG microstates. • Changes in EEG relative power • Changes in fractional amplitude of low-frequency fluctuations
Sample Size	The sample size was determined as a priori using G*Power3.1 (Faul, Erdf-elder, Buchner& Lang, 2009). To estimate the within-between interaction for repeated measures, we calculated that 52 patients would be required to achieve 80% power in a medium effect size of 0.25 at an alpha risk of 0.01. Considering the attrition rate of 20%, the minimum sample size required for total groups was 62.
Statistical Analysis	The study will be analyzed in accordance with the intention-to-treat (ITT) principle. To ensure comparability between the two groups, between-group comparisons of the characteristics of the study participants will be conducted using Student's t-tests or chi-square tests. Comparisons of each study outcome between groups will be performed using a mixed-effects model to correct for correlation between repeated measures data and potential confounding effects. Confounding variables will include pre-intervention values, and pain location. For comparison of brain metrics between the sham and M1-rTMS groups, independent samples t-tests or Mann-Whitney U tests were employed, as appropriate. Within-group

comparisons before and after rTMS were performed using paired samples t-tests or Wilcoxon signed-rank tests. A false discovery rate (FDR) correction was applied to adjust for multiple comparisons. All statistical tests were two-sided, and *P* value of <0.05 was considered statistically significant. All analyses were performed using SPSS software (version 26; SPSS, Chicago, IL, USA).

2. Background

Parkinson disease (PD) is a complex neurodegenerative disorder that involves multiple systems, which affects up to 2% of the general population older than 65 years. Chronic Pain is among the most frequently reported nonmotor symptoms, with a prevalence ranging from 40% to 85%[1,2]. Musculoskeletal pain (MSP) affects over 80% of patients, occurring both in prodromal phases and during subsequent stages of the disease, imposing a tremendous burden[3]. However, literature reports that only a maximum of 50% of PD patients receive any form of pain therapy[4]. The treatment of pain is often insufficient, partly due to an incomplete interpretation of the underlying neural mechanisms. Currently, no level A evidence-based recommendations exist for treating PD-related pain. Therefore, identifying new safe and effective treatments for pain is imperative and it is also crucial to further supplement the current evidence for the treatment of PD-related pain.

In recent years, new approaches based on non-invasive brain stimulation, particularly repetitive transcranial magnetic stimulation (rTMS), have emerged as therapeutic interventions for various neurological and psychiatric disorders. Level A evidence supports the efficacy of rTMS for neuropathic pain, while level B evidence exists for fibromyalgia[5], and rTMS has also demonstrated clinical efficacy for PD-related symptoms, including freezing of gait[6] and depression[7]. However, few well-designed randomized controlled trials (RCTs) have specifically investigated rTMS for relieving MSK pain in PD, particularly using functional magnetic resonance imaging (fMRI)-guided individualized targeting. The bulk of evidence indicates that optimal rTMS stimulation parameters for inducing analgesia include a frequency of 10-20 Hz, an intensity of 80-120% of the resting motor threshold (RMT), 1000-2000 pulses per session, and 5-10 sessions[5,8]. The primary motor cortex (M1) is the main target for chronic pain treatment with rTMS, as it is considered interconnected with cognitive, affective, and somatosensory networks[9]. When TMS is delivered over M1, a brief

high-intensity magnetic field induces an electrical current in underlying cortical tissue by electromagnetic induction. This local cortical stimulation has been able to exert changes in the functioning of distant brain areas. Furthermore, rTMS has the capacity to induce persistent changes in cortical activity extending beyond the immediate stimulation period[10].

Analgesic effects persist beyond the duration of rTMS sessions, appear correlated with restoration of normal cortical excitability, and depend on modulation of brain regions involved in pain processing, specifically descending antinociceptive systems[11]. Moreover, TMS–positron emission tomography (PET) studies demonstrated that high-frequency motor cortex stimulation increases dopamine release[12]. Concurrent TMS/fMRI research revealed that M1 stimulation evokes activation in key networks including the insular, while modulating domain-general networks such as the default mode network (DMN) and attention network[13].Multiple neurotransmitters are involved in pain processing steps, including gamma-aminobutyric acid (GABA), the major brain inhibitory neurotransmitter[14]. Meta-analyses established significant associations between prefrontal gamma-band oscillations and chronic pain[15]. Previous systematic reviews of molecular changes following rTMS in humans indicate potential increases in brain derived neurotrophic factor (BDNF), GABA, and beta-endorphin[16]. The pathophysiology of pain in PD exhibits considerable heterogeneity, potentially involving dopamine depletion along with dysfunctional pain processing and modulation pathways[1]. Longitudinal neuroimaging comparisons hold significant promise for selecting TMS candidates and monitoring treatment efficacy.

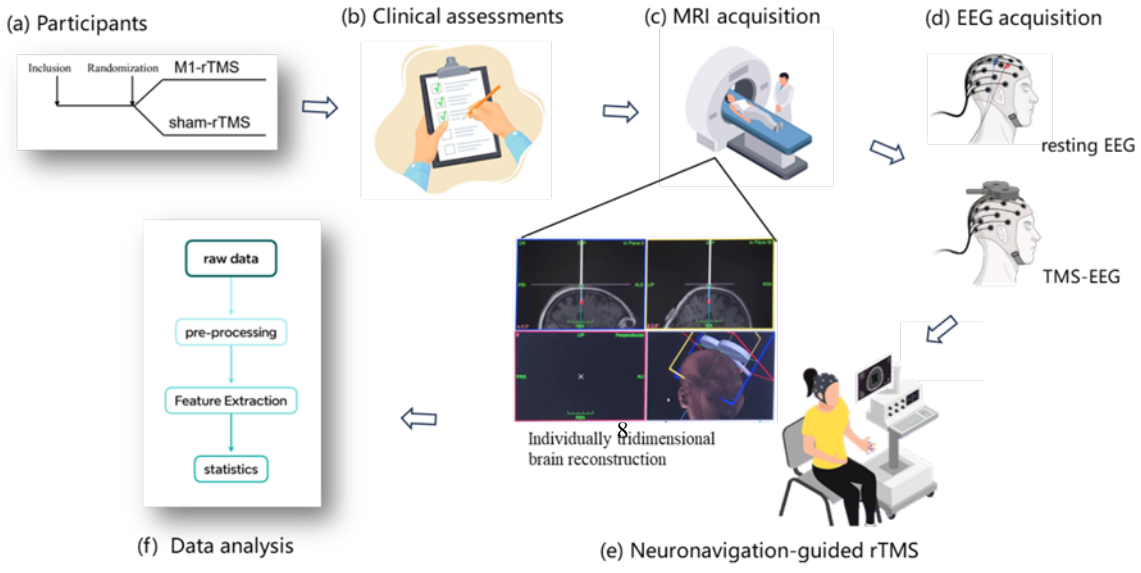
Based on behavioral measures alone, it is impossible to reveal extra-motor areas and entire brain networks reflecting the impact of TMS. Thus, establishing a causal link between the neuromodulation of cortical targets and chronic pain treatments necessitates integrating rTMS techniques with brain imaging and network dynamics analysis. For example, fMRI has assisted in identifying TMS-induced modifications in functional connectivity (FC) and regional activation patterns, mapping pain-related network plasticity. Concurrently, electroencephalography (EEG) has contributed to capture neuromodulatory effects of TMS, such as alterations in oscillatory dynamics and cortical excitability, reflecting transient or sustained neural reorganization[17]. Recent advancements in TMS-compatible electroencephalography (TMS-EEG) enable straightforwardly measuring effective connectivity within cortical circuits, bypassing sensory and motor pathways[18]. Combining

fMRI and EEG, particularly TMS real-time EEG, provides more temporal and spatial information for investigating TMS intervention effects, which helps us to have a deeper understanding of the specific mechanism behind its action.

By a double-blind, sham-controlled and randomized study, we aimed to investigate the structural and functional neurophysiological basis underlying the clinical efficacy of M1-rTMS for pain in PD patients. We expected to confirm that M1-rTMS, but not sham stimulation, significantly alleviates clinical pain and improves overall disease severity. In addition, we planned to integrate multimodal data - including resting state EEG, TMS real-time EEG, and fMRI-related parameters - linking these measures to clinical outcomes. This integration will elucidate how TMS modulates key neural circuits to relieve PD-related MSK pain and produce clinical benefits. We hypothesized that clinical efficacy would be associated with significant changes in cortical functional changes induced by stimulation and pain modulation systems.

3. Study Design

The study is a single-center, blinded-endpoint, parallel-controlled, randomized clinical trial planning to recruit 62 PD patients with MSP from the Movement Disorders Clinic at the Second Affiliated Hospital of Soochow University. All participants will be consecutively recruited and randomly assigned to either the active rTMS group or sham stimulation group with a 2:1ratio **(Figure.1a)**. In addition to the conventional anti-parkinsonism medications, the control group will receive sham-rTMS treatment and the intervention group will receive active-rTMS treatment. To longitudinally assess clinical and physiological measures, participants were assessed across treatment and follow-up phases at four time points: baseline (T0); after completion of all rTMS or sham stimulation sessions on Day 8 (T1); 1 month post-treatment (T2); and 2 months post-treatment (T3) **(Figure.2)**. Assessments involved the



completion of standardized questionnaires, fMRI and EEG acquisition.

Figure.1 Schematic representation of the study

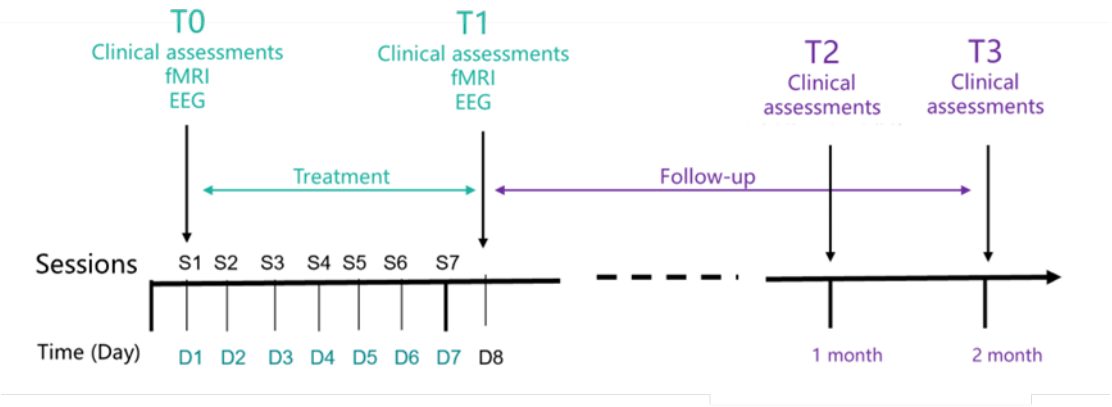


Figure.2 Timeline of procedures. Treatment assessments were performed in the day before (T0) and after completion of all rTMS or sham stimulation sessions (T1). Follow-up assessments occurred at 1 to 2 months post-treatment (T2 and T3, respectively). rTMS therapy was provided on 7 consecutive days.

4. Objectives

The overall objective of this study is to test the efficacy and safety of 7 sessions of 20 Hz-rTMS delivered to primary motor cortex (M1) in PD patients with chronic MSP. The secondary objective is to elucidate its underlying neurophysiological mechanisms through fMRI and EEG.

5. Study Subjects

This study is planned to enroll 62 PD patients with MSP.

5.1 Inclusion criteria:

- ☑ Idiopathic PD was diagnosed according to the 2015 Movement Disorder Society (MDS) clinical diagnostic criteria
- ☑ Hoehn and Yahr stages of I to III.
- ☑ musculoskeletal pain was detected based on the Ford classification system for pain in PD. Pain duration of at least 3 months, with continuous moderate intensity pain (≥ 3/10 on a 0-10 numerical rating scale) occurring at least three days per week.
- ☑ stable antiparkinsonian therapy for ≥4 weeks.

5.2 Exclusion criteria:

- ☒ Contraindications to rTMS.
- ☒ unstable ongoing psychiatric disorder, history of substance abuse (alcohol, drugs).

- ☒ Prior treatment with any form of rTMS.
- ☒ Mini-mental State Examination scores ≤24.
- ☒ Other pain conditions, such as apparent osteoarthritis, or rheumatoid arthritis depended on laboratory or imaging findings.

6. Randomization and Recruitment

Patients will be randomised to receive either active or sham-rTMS according to a 2:1 ratio. The randomization list is generated by the Microsoft Office Excel 2021 (Microsoft, USA) software and kept confidential until eligible study subjects enrolled. After being fully informed about the protocol of this study and signing the informed consent form, the study subjects will receive a free physical and mini-mental state examination score (MMSE) assessment to determine if they meet the eligibility criteria. After providing the enrollment number of the study subjects, the physician will have access to the randomization outcome.

7. Interventions

All participants sat in a comfortable reclining chair and remained as relaxed as possible, protected by earplugs during all procedures. Each participant received 7 sessions of treatment once a day at the same time continuously for 7 days. Anti-parkinsonism drugs remained unchanged throughout the whole study.

Neuronavigation（Northern Digital Inc., Polaris Spectra, Canada）with an optical tracking system (TMS Navigator, Localite GmbH) monitored coil positioning throughout all rTMS sessions. High resolution T1-weighted MRI (1.5 tesla) slices of each participant were integrated to allow individually tridimensional brain reconstruction prior to the first session (Fig.1e). An infrared camera facilitated precise coil positioning over the target area under real-time visualization. The M1 target was defined as the “hand knob” region, which corresponded to the motor cortical representation of the hand, regardless of pain location. The coil was centered over the scalp above the M1 target contralateral to the maximal pain area or positioned left side in case of bilateral pain.

7.1 Interventions for the experimental group

A MagPro X100 machine (Magventure, Farum) connected to an MCF-B70 figure-of-eight coil delivered TMS during the real stimulation condition. The stimulation paradigm consisted of 100 trains of TMS pulses delivered at 20Hz with an11-second intertrain interval, resulting in 2,000 pulses per session. Stimulation intensity was set to 80% of the RMT, determined as

the minimum intensity required to elicit an electromyographic response ⩾50 μV in the first dorsal interosseus muscle of the hand in at least 5 out of 10 trials.

7.2 Interventions for the control group

Sham stimulation was delivered using an MCF-P-B65 figure-of-eight coil (Magventure), which produces an auditory percept similar to real TMS without inducing cortical activation. The stimulation paradigm consisted of 100 trains of TMS pulses delivered at 20Hz with an11-second intertrain interval, resulting in 2,000 pulses per session. Stimulation intensity was set to 80% of the RMT.

8. Data Collection

Data collection is carried out by trained and visited doctors and investigators, and the screening, baseline, and follow-up programs and the schedule for data collection are shown in **Table 1**. Monitoring of intervention effects are collected from study subjects through clinical scores, resting-EEG, TMS-EEG and fMRI. The specific methods are shown below:

8.1 Questionnaire

- Demographic data: age, sex and education.
- Medical history: disease duration, levodopa-equivalent daily dose (LEDD), Primary symptoms (tremor/rigidity/bradykinesia).
- Pain symptoms: pain locations, MKPPS domain 1, NRS.
- PD related symptoms: MDS-UPDRS I, II, III, IV. H-Y stage, HAMA, HAMD, PDSS-2, ESS, SCOPA-AUT, PDQ-39.
- Adverse events: dizziness, headaches, tinnitus, etc.

8.2 Resting-EEG Recording

The EEG recording protocol consisted of a 5-minute resting-state paradigm without any task involvement.

8.3 TMS-EEG Recording

The EEG recording protocol consisted of a 20-minute session comprising approximately 120 TMS trials at an intensity of 120% RMT.

8.4 MRI data acquisition

Resting-state fMRI.

Table 1. Visiting Assessment Schedule.

Data collection	**Screening**	**Stage 1 Treatment**		**Stage 2 Follow-up**	
		baseline	Day 8	1 month	2month

Screening form	×				
MMSE	×				
Informed consent		×			
Demographic data	×	×			
Medical history	×	×			
Pain symptoms	×	×	×	×	×
PD related symptoms		×	×	×	×
Resting-EEG data		×	×		
TMS-EEG data		×	×		
MRI data		×	×		
Adverse events		×	×	×	×

9. Methods of Data Measurement

9.1 Clinical measures

Clinical assessments were performed at 4 time points: T0, T1, T2 and T3 **(Figure.2)**. All participants were evaluated during their “ON” medication states at approximately the same time of day. A face-to-face survey is conducted by a trained and qualified investigator with each participant, using a standardized, self-administered questionnaire to collect demographic information (age, sex, education), medical history (disease duration, LEDD, primary symptoms). Standardized scales are used to assess pain symptoms and PD related symptoms. Specifically, LEDD = (Standard-release levodopa × 1) + (Controlled-release levodopa × 0.75) + (Pergolide × 100) + [(Standard-release levodopa × 1) + (Controlled-release levodopa × 0.75)] × 0.33 (with concurrent Entacapone) + (Piribedil × 1) + (Pramipexole × 100) + (Selegiline × 10) + (Rasagiline × 100) + (Amantadine × 1). Pain intensity was assessed over the past 24 hours using a 0-10 numeric rating scale (NRS), where 0 indicating no pain and 10 indicating maximal pain. The Modified KING’S PD Pain Scale (MKPPS), adapted for suitability in a Chinese population and incorporating Ford’s pain subtypes based on the original KPPS, comprises 16 items divided into 5 separate domains. MKPPS Domain 1 was specifically used to evaluate MSK pain and divided into 5 items (neck, back, upper and lower extremities, buttock) according to location. Each item was scored by multiplying severity (0-3) by frequency (0-4), resulting in a total possible score range from 0 to 60. The scales used to

assess motor symptoms are parts III (ranging from 0 to 132) and IV (ranging from 0 to 24) of the MDS–Unified PD Rating Scale (MDS-UPDRS), with higher scores indicating more severe symptoms. The scales used to assess non-motor symptoms included parts I (ranging from 0 to 52) and II (ranging from 0 to 52) of the MDS-UPDRS, with higher scores indicating more severe symptoms. The depression score (ranging from 0 to 76 with higher scores indicating more severe depression) from the 24 items Hamilton Depression Scale (HAMD). The anxiety score (ranging from 0 to 60 with higher scores indicating more severe anxiety) from the 14 items Hamilton Anxiety Scale (HAMA). The Scale for Outcomes in Parkinson's disease for Autonomic Symptoms (SCOUP-AUT, ranging from 0 to 67, with higher scores indicating higher autonomic nervous system dysfunction). The sleep problem index (from 0 to 68 with higher scores indicating more severe sleep problem) from the PD Sleep Scale–2 (PDSS-2). The daytime sleepiness will be assessed by the Epworth Sleeping Scale (ESS), which has a score range of 0–24, with higher scores indicating more severe symptoms. We will also assess change in quality of life from the Parkinson's Disease Questionnaire-39 (PDQ-39), ranging from 0 to 156 with higher scores indicating more serious influence.

9.2 Resting-EEG Recording

For each patient, resting-EEG data were collected at T0 and T1 **(Figure.2)**. Participants were asked to wash and brush their hair. During EEG recordings, participants sat alone in a quiet room and were requested to minimize body movements, mostly with their eyes closed. EEG data were recorded using a TMS-compatible EEG amplifier (Neuracle, NSH0128, Changzhou, China) and a cap (Greentek, Wuhan, China) equipped with 64 TMS-compatible coated electrodes arranged according to the international 10/20 system. Skin/electrode impedance was maintained below 10KΩ. The resting-EEG recording protocol consisted of a 5-minute resting-state paradigm without any task involvement **(Figure.1d)**.

9.3 TMS-EEG Recording

For each patient, TMS-EEG data were collected at T0 and T1 **(Figure.2)**. Single-pulse TMS (sTMS) was delivered to M1 during EEG recording by means of a figure-of-eight coil oriented to elicit a posterolateral-anteromedial current flow in the brain. The interval of each sTMS was set at 4 seconds to prevent habituation with repeated stimulation. The EEG signal was bandpass filtered (0.1-100Hz) and digitized at 16 kHz. Participants wore inserted earplugs to avoid signals contamination from the TMS discharge click. Additionally, a 0.5 mm

foam pad was attached to the TMS coil to minimize electrode movement and bone-conducted auditory artifacts. The TMS-EEG recording protocol consisted of a 20-minute session comprising approximately 120 TMS trials at an intensity of 120% RMT **(Figure.1d)**.

9.4 MRI data acquisition

MRI data were acquired at baseline and post-treatment using a 3.0-Tesla scanner (Siemens, Prisma, Germany) equipped with a 64-channel head coil **(Figure.1c)**. During scanning, participants were instructed to remain still, keep their eyes closed without falling asleep, and think of nothing in particular. Tight yet comfortable foam padding and earplugs were used to minimize head motion and reduce scanner noise. Resting-state fMRI was scanned using a gradient-recalled-echo planar imaging sequence with the following parameters: repetition time (TR)/echo time (TE) = 1240/32 milliseconds, flip angle (FA) = 67°, matrix size = 86×86, field of view (FOV) = 215 mm×215 mm, slice thickness/gap =2.5/0 mm, 57 slices, 300 volumes and an acquisition time of 384 s. High-resolution brain T1-weighted (T1w) MRI was obtained using a sagittal 3D magnetization-prepared rapid gradient echo (MPRAGE) sequence: TR/TE = 2300/2.34 milliseconds, FA = 8°, matrix = 256×256, FOV = 256 mm×256 mm, slice thickness/gap =1.0/0 mm, voxel size = 1×1×1 mm^3, 240 volumes and an acquisition time of 234 s.

10. Outcomes

This study is designed to investigate the structural and functional neurophysiological basis underlying the clinical efficacy of M1-rTMS for pain in PD patients. The outcomes include the changes in clinical scores and relevant brain metrics.

Primary outcome:

- Change in the MKPPS-Domain 1 score.
- Change in NRS.
- Proportion of participants who achieved treatment response (defined as a ≥30% reduction in MKPPS Domain 1 score from baseline).

Secondary outcome:

- Change in MDS-UPDRS I, II, III, IV.
- Change in HAMD.
- Change in HAMA.
- Change in SCOUP-AUT.

- Change in PDSS-2.
- Change in ESS.
- Change in PDQ-39.
- Changes in time-varying EEG network dynamics
- Changes in EEG microstates.
- Changes in EEG relative power
- Changes in fractional amplitude of low-frequency fluctuations

11. Assessment of Outcomes

In this study, outcomes are assessed using standardized, internationally accepted measures. Blindness (the follow-up assessors are unaware of the grouping of study subjects) is used in the outcome assessment process.

12. Statistical Power and Sample Size

The sample size was determined as a priori using G*Power3.1 (Faul, Erdf-elder, Buchner& Lang, 2009). To estimate the within-between interaction for repeated measures, we calculated that 52 patients would be required to achieve 80% power in a medium effect size of 0.25 at an alpha risk of 0.01. Considering the attrition rate of 20%, the minimum sample size required for total groups was 62.

13. Data management

All data are entered into the electronic database in the department of neurology, the second affiliated hospital of Soochow University by trained and qualified doctors or investigators. The data entered are reviewed for a final data check and quality control.

14. Statistical Analysis Plan

14.1 clinical data analysis

Intention to treat (ITT) analyses will be conducted, in which study outcomes will be compared between participants according to their randomization assignment, regardless of their actual adherence to the intervention. To compare the two groups for comparability, between-group comparisons of the characteristics of the study participants will be performed using Student's t-tests or chi-square tests as appropriate. A generalized mixed-effects model for repeated measures was applied to estimate rTMS effects on clinical outcomes with time expressed as a continuous variable, group (M1-and sham-rTMS), group by time interaction as fixed effects, baseline values and pain location as covariates, and subjects as random effects. All tests will

be two-sided, with a *P* value of <0.05 suggesting a statistical significance, and all statistical analyses will be performed using SPSS software (version 26; SPSS, Chicago, IL, USA).

14.2 Time-varying network analysis of TMS-EEG

TMS stimulates were utilized as stimulus labels, which time points corresponding to each label were set as time'0'. Then, the data corresponding to 0.5s before and 1s after the markers were extracted (a total of 1.5s for each segment). Eight-rate down-sampled segmentation was applied to reduce calculation load[19]. To examine dynamic information processing during TMS-evoked disturbances, an adaptive directed transfer function (ADTF) value was used to construct time-varying network[20]. Then a time-varying multivariate adaptive autoregressive (tv-MVAAR) model and the ADTF were calculated. The tv-MVAAR model coefficients were estimated by the Kalman filter algorithm, with the order of the model automatically determined by the Akaike Information Criterion[21].

14.3 Electroencephalography Preprocessing

EEG preprocessing was performed offline using the EEGLAB toolbox (Delorme & Makeig, 2004) implemented in MATLAB R2013b (The MathWorks Inc., Natick, Massachusetts, United States). Raw data were down-sampled to 512 Hz, re-referenced to the average of residual channels, bandpass filtered between 1 and 80 Hz, and a 50 Hz notch filter was applied. Independent component analysis (ICA) was used to remove components representing eye movements and muscle artifacts. Subsequently, all data sets were visually inspected, and remaining bad intervals were marked for rejection. Finally, EEG recordings of 3 minute were segmented for further analysis.

In order to detect EEG power in different regions, the brain of 64-channel cap was divided into four regions **(Figure 3)**: frontal (F, FPz FP1 FP2 AF7 AF3 AF4 AF8 F7 F5 F3 F1 Fz F2 F4 F6 F8), central (C, FC5 FC3 FC1 FCz FC2 FC4 FC6 C5 C3 C1 Cz C2 C4 C6 CP5 CP3 CP1 CP2 CP4 CP6), temporal (T, FT7 T7 TP7 FT8 T8 TP8), and posterior (P, P7 P5 P3 Pz P4 P6 P8 PO7 PO5 PO3 POz PO4 PO6 PO8 O1 Oz O2).

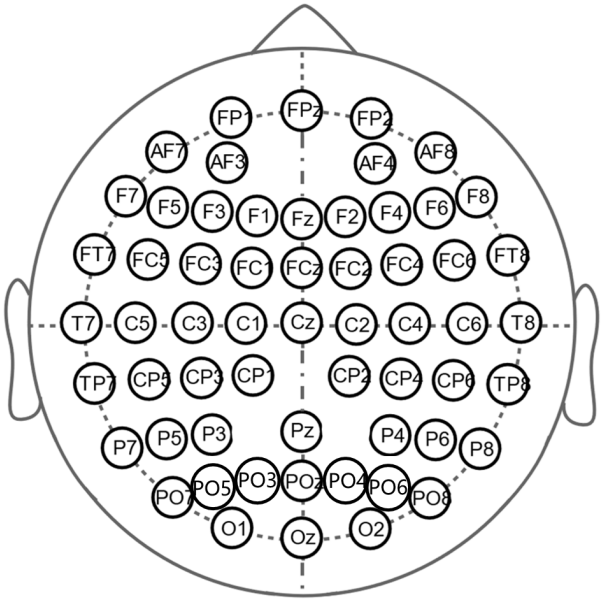


Figure.3 EEG electrode positions on the brain.

14.4 Microstate analysis

Microstate (MS) analysis was performed using CARTOOL software[22] in three stages: individual-level clustering, group-level clustering, and backfitting. For each participant, EEG topographies at global field power (GFP) peaks, representing timepoints of the highest signal-to-noise ratio, were extracted and clustered with a modified k-means clustering, yielding a variable number of individual-level cluster topographies. Next, individual cluster topographies were concatenated and clustered at the group level. As an increasing number of studies was now using a data-driven approach, CARTOOL implemented meta-criterion was used as guidance to determine the optimal number of microstates. The original momentary maps from both groups were separately clustered into five MS classes. Topographies were visually inspected and compared with those reported in the literature. The first 4 topographies closely resembled the well-known "canonical" MS (class A to D). MS class A exhibited an upper left-bottom right orientation, class B an upper right-bottom left orientation, class C an anterior-posterior orientation, and class D a trend of increasing gradually from the front to the center to the maximum. The fifth topography, labeled MS class E, consistently demonstrated a left lateralized maximum as reported in more recent studies[23-25]. In order to compare the differences of groups, the following microstate parameters for each MS segmentation class were calculated: a) Mean Duration: Average time (milliseconds) of microstate segments; b) Time Coverage: Percentage of total time occupied by a microstate; c) Segment Count Density:

Number of times that a microstate recurs across all analysis epochs.

14.5 Relative power

The pre-processed data were segmented into consecutive, non-overlapping 2 s epochs. For each EEG epoch, we computed the fast Fourier transform (FFT) algorithm to convert to frequency domain. The power values were obtained for following five frequency bands: delta (1-4Hz), theta (4-8Hz), alpha(8-13Hz), beta(13-30Hz), gamma(30-80Hz). Relative power of each band of power from 1 to 80 Hz was averaged in each region and calculated by

$$RP(f1, f2) = \frac{P(f1, f2)}{P(1,80)}$$

where $P(.)$ indicates the power, $RP(.)$ indicates the relative power, and $f1, f2$ indicate the low and high frequency, respectively.

These EEG power values were compared using non-parametric Mann-Whitney U tests. The significance level of $P < 0.05$ was corrected by a false discovery rate (FDR) for multiple comparisons.

14.6 fMRI data pre-processing and fractional amplitude of low-frequency fluctuations analysis

All resting-state fMRI data were preprocessed using the Data Processing & Analysis for Brain Imaging (DPABI, http://rfmri.org/dpabi), a toolbox developed based on MATLAB. Briefly, the first 10 volumes of each scan were discarded to allow for magnetization equilibrium and participant adaptation. The remaining images were corrected for differences in slice acquisition timing, followed by realignment to adjust for head motion. Functional images were then normalized to the standard Montreal Neurological Institute (MNI) space and resampled to an isotropic voxel size of 3 × 3 × 3 mm^3. To reduce non-neuronal confounds, nuisance signals including the Friston 24 head motion parameters, white matter, and cerebrospinal fluid were regressed out.

To characterize regional spontaneous brain activity, the fractional amplitude of low-frequency fluctuations (fALFF) was computed. Specifically, the amplitude of low-frequency fluctuations (ALFF) was derived by transforming each voxel's time series into the frequency domain using a fast Fourier transform and calculating the square root of the power within the 0.01–0.1 Hz band. fALFF was then obtained by dividing ALFF by the total amplitude across the entire frequency range, thereby improving specificity by reducing nonspecific physiological noise.

To enhance the normality of the data distribution, voxel-wise z-score standardization was applied to the resulting fALFF maps before statistical analysis.

15. Quality Control

Quality control will be conducted by a team of investigators, key research staff, and project inspectors. Strict quality control will be implemented at every step of the study including project preparation, training, screening, intervention, and data collection. A manual of procedures will be developed to detail the standardized approaches used in the study, such as participant recruitment, intervention, doctor training (protocol-based treatment, health coaching, and follow-up), patient education (medication adherence), and other procedures of the study. All study personnel will be required to participate in a study training session prior to the initiation of any study procedures. The quality control team will review the study data regularly to ensure that all phases of the project are strictly implemented according to the study protocol and the authenticity, completeness, accuracy, and reliability of the research data.

15.1 Preparation phase

A manual of procedures will include detailed descriptions of all trial procedures and will be used for training purposes and as a reference for all study investigators and staff. Standard forms, devices, and procedures in the field for rTMS treatment and other data collection procedures will be standardized. Furthermore, standard event definitions and event validation procedures will be used. The project will purchase the devices used in the study which pass the national quality inspection. The study protocol, manual of procedures, study forms, training materials, and other written materials will be prepared centrally.

15.2 Screening and recruitment phase

- To conduct targeted health education sessions for potential participants and their families/caregivers, ensuring they fully understand the impact of musculoskeletal pain on quality of life in Parkinson’s disease. The goal is to build awareness that pain is a common but manageable non-motor symptom, and to encourage participation of all eligible individuals.
- Enrolled patients will receive training to ensure they are able to accurately complete and submit required study materials using provided digital tools.

15.3 Implementation stage

- Each enrolled patient will undergo daily rTMS sessions over 7 consecutive days, administered by certified TMS technicians. Stimulation parameters will follow the study protocol and be documented per session.
- It is required that all EEG, MRI, and clinical scale data be completed for each patient before the first TMS session and within 24 hours after the final session. All responsible personnel are expected to coordinate proactively and upload data promptly to ensure the smooth progress of the study.
- Dedicated follow-up appointments to reduce missed appointments.
- Outcomes are assessed by applying a blinded method (the assessor is unaware of the grouping of study subjects).

16. Security Monitoring

16.1 Adverse events and reports

The intervention program in this study is more feasible. Possible adverse events include dizziness, headaches and tinnitus, which have been monitored at treatment and follow-up, and other adverse events not listed are reported and registered by the study subjects and their families.

16.2 Data security

After reviewing and confirming that the database created is correct, the data are locked by the principal investigator, the sponsor, and the statistical analyst. No further changes are made to the data files after locking. Problems identified after data locking should be followed strictly by the process of unlocking and re-locking the data. The Data Safety Committee and Ethics Committee are responsible for monitoring.

17. Research Organization

The study is conducted jointly by the Department of Neurology, Clinical Neurophysiology and Radiology, The Second Affiliated Hospital of Soochow University, forming a multidisciplinary and multi-departmental collaborative team of researchers (**Figure.4**). Decision-making, protocol design, overall organization, management and coordination of the study are carried out by the study steering committee. Screening, recruitment and intervention management of the study subjects are implemented by the intervention team. Blinded assessment of the outcome is implemented by the follow-up team. Data quality control is implemented by the quality control team. Resting-EEG, TMS-EEG and MRI data acquisition are implemented by the Department of Clinical Neurophysiology and Radiology, respectively,

and statistical analysis and reporting of the results is carried out by the Data Analysis Centre. Statistical analysis and reporting of results are carried out by the Data Analysis Centre, and the Data Safety Management Committee supervised the entire implementation of the project. Adopting a management model of overall coordination and division of labor and responsibility, and implementing a job responsibility system.

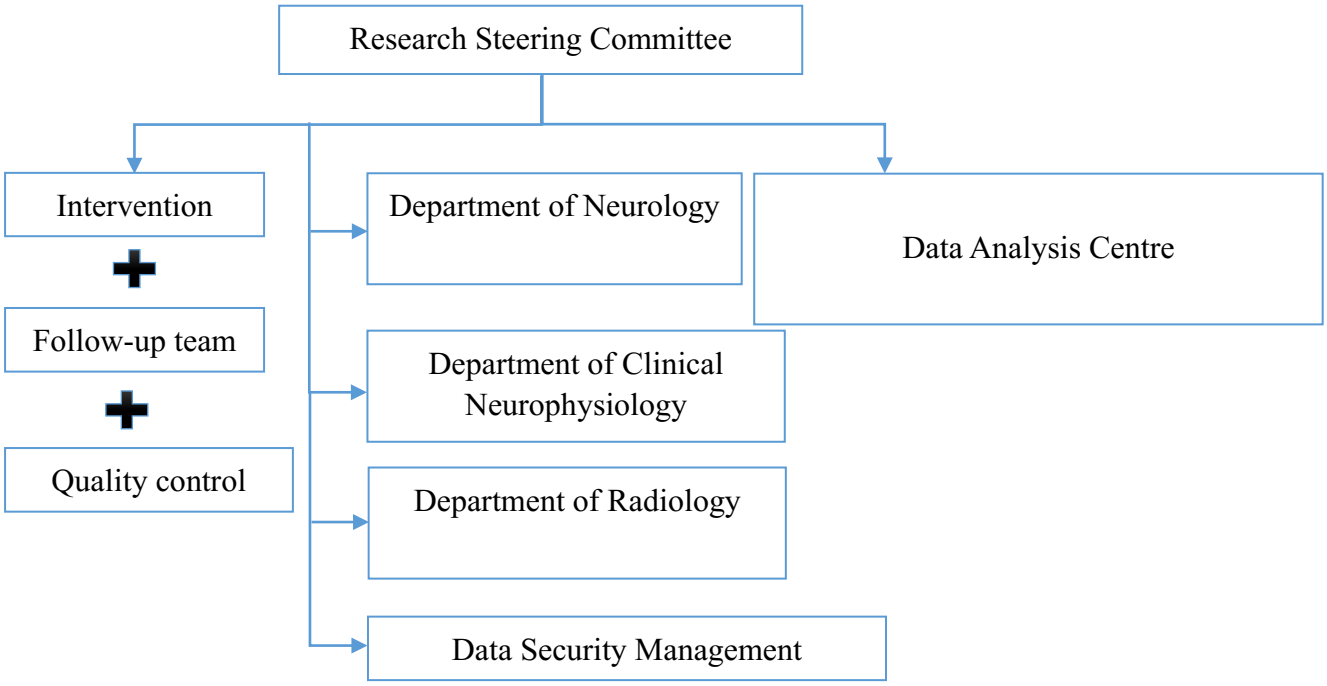


Figure 5. Study Organization Structure.

18. Ethical considerations

It is the responsibility of the principal investigator to ensure that this trial will be conducted in full compliance with the Declaration of Helsinki and the Chinese Code for Quality Management of Clinical Trials, as well as other relevant regulations. The study protocol was reviewed by the Institutional Review Board of the Second Affiliated Hospital of Soochow University, and all study subjects and their guardians signed an informed consent form.

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