CLINICAL PROTOCOL

Transcranial Focused Ultrasound Neuromodulation for Post-Stroke Motor Dysfunction

Sponsoring Institution: Taizhou People’s Hospital

Principal Investigator / Department: Zhao Gaonian / Department of Rehabilitation Medicine

Study Period: March 2026 – March 2028

Version: 2.0

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Protocol Synopsis

Project Title	Clinical Protocol for Transcranial Focused Ultrasound Neuromodulation in Post-Stroke Motor Dysfunction
Study Objectives	This study aims to evaluate how transcranial focused ultrasound (tFUS) technology promotes the remodeling of damaged neural networks and functional recovery by activating or inhibiting neural activity in specific brain regions, integrating electroencephalography (EEG) and magnetic resonance imaging (MRI) signal analyses. The primary goals are to improve motor ability and cognitive function, reduce long-term disability, and enhance quality of life. Additionally, this study seeks to optimize stimulation parameters to achieve maximal rehabilitative outcomes and to elucidate the potential mechanisms underlying neuroprotection and functional reconstruction following stroke.
Study Design	This study is a randomized, controlled, single-center trial.
Sample Size	60 participants
Participant Selection	Inclusion Criteria: (1) Patients with a confirmed imaging diagnosis of first-ever ischemic stroke; (2) Unilateral limb motor involvement; (3) Modified Ashworth Scale (MAS) grade ≤3 for the upper extremity, Brunnstrom stage II–V, and upper extremity Fugl-Meyer Assessment (FMA-UE) score between 15 and 60 (inclusive); (4) Age 18–75 years, onset within 21 days to 6 months, in the subacute or recovery phase; (5) Stable vital signs, no consciousness impairment, informed consent provided, ability to cooperate with clinical assessments, and approval obtained from the institutional ethics committee. Exclusion Criteria: (1) Contraindications to MRI; (2) Infarction involving the thalamus; (3) Risk of intracranial hemorrhage; (4) Presence of intracranial metallic foreign bodies, cardiac pacemakers, or cochlear implants; (5) Unstable medical condition, severe cognitive impairment or psychiatric disorders rendering the patient unable to comprehend the study or cooperate with assessments; (6) Conditions affecting limb motor function, including fractures, joint contractures, or severe limb spasticity; (7) Current use of medications that alter cortical excitability.
Treatment Protocol	Neuromodulation Group: Multimodal neuromodulation targeting specific neural regions in addition to conventional therapy. Control Group: Sham stimulation in addition to conventional therapy.
Efficacy Assessment	Primary Outcome Measures: Fugl-Meyer Assessment score; neuroimaging data (cranial CT/MRI); EEG measurements of resting-state and task-state spectral power and functional connectivity; laboratory parameters (complete blood count, CRP, IL-6, S100β, BDNF). Secondary Outcome Measures: Brunnstrom staging, Modified Ashworth Scale grading, muscle strength, and patient comfort assessment. Safety Assessment Indicators: Complete blood count, blood biochemistry, coagulation function.
Statistical Methods	Key outcome measures will be compared between the neuromodulation group and the control group before and after intervention using independent samples t-tests (two groups) or analysis of variance (multiple groups) to detect statistically significant intergroup differences. Non-parametric tests will be applied to data not conforming to a normal distribution.
Study Duration	March 2026 – March 2028

1. Background and Rationale

According to the World Health Organization (WHO), stroke is the second leading cause of death globally [1]. Approximately 15 million individuals worldwide experience a stroke annually, ranking second only to heart disease, and stroke remains one of the most prevalent causes of long-term disability. Ischemic stroke (IS) constitutes the most common subtype, accounting for approximately 85% of all stroke cases [2]. Among all stroke survivors, over 60% experience varying degrees of functional impairment within three months following the event. The American Stroke Association (ASA) has reported [3] that approximately one-third of stroke patients develop severe speech impairment post-stroke, while one in two patients sustains some degree of hand function loss. Stroke patients frequently contend with chronic functional deficits, including motor difficulties, speech impairment, and cognitive dysfunction, all of which profoundly compromise quality of life. These patients typically require prolonged rehabilitation and nursing care, imposing substantial financial burden on families and exerting considerable pressure on healthcare systems.

Conventional pharmacological and rehabilitative interventions for stroke may yield limited efficacy, particularly in patients with severe cerebral injury. Pharmacological therapies such as anticoagulants carry inherent hemorrhagic risks, while the effectiveness of traditional rehabilitation approaches may be negligible in patients with severe functional deficits, often requiring protracted recovery periods. In recent years, neuromodulation technologies have demonstrated significant potential in the investigation and treatment of neurological disorders including stroke [4]. Given that ischemic stroke involves complex neural network dysfunction, neuromodulation technologies, as an emerging therapeutic modality, offer novel avenues for overcoming the limitations of conventional approaches. By activating or inhibiting neural activity in specific brain regions, neuromodulation may promote neural repair, restructure functional networks, and enhance motor and cognitive capabilities. Evidence indicates that deep brain stimulation (DBS) [5] and transcranial magnetic stimulation (TMS) [6] can facilitate the restoration of post-stroke functional deficits by augmenting inter-regional connectivity or reactivating compromised neural pathways.

Nevertheless, prevailing mainstream neuromodulation modalities continue to exhibit substantial limitations that preclude the adequate fulfillment of clinical demands in ischemic stroke management. Specifically, deep brain stimulation (DBS), while demonstrating reliable modulatory efficacy, constitutes an invasive procedure requiring surgical implantation of electrodes, entailing risks of infection, hemorrhage, and other surgery-related complications, as well as the potential for irreversible tissue damage. Moreover, the prohibitive surgical costs and complex postoperative maintenance restrict its applicability in patients with mild symptoms or advanced age. Transcranial magnetic stimulation (TMS), although noninvasive, suffers from limited spatial resolution, rendering it incapable of precisely targeting specific neural pathways in deep brain structures. This limitation predisposes the technique to nonspecific stimulation of surrounding normal brain tissue, resulting in variable modulatory outcomes. Furthermore, its constrained stimulation depth yields suboptimal therapeutic effects in ischemic stroke patients with deeply situated lesions.

In this context, transcranial focused ultrasound (tFUS), as an emerging noninvasive neuromodulation technology, has exhibited distinctive technical advantages [7], presenting novel possibilities for breakthroughs in ischemic stroke treatment [8]. Compared with conventional neuromodulation modalities, tFUS possesses exceptionally high spatial

resolution, enabling precise focusing on lesion sites within deep brain regions and targeted modulation of specific neural networks, thereby effectively circumventing interference with surrounding normal brain tissue. Simultaneously, tFUS requires no craniotomy, is entirely noninvasive, significantly reduces the risk of complications, improves patient treatment compliance, and offers adjustable stimulation depth applicable to ischemic stroke patients with varying lesion depths. Additionally, tFUS can modulate blood-brain barrier permeability [9], facilitating the delivery of neurorepair-related pharmacological agents to lesion sites and achieving synergistic effects between neuromodulation and pharmacotherapy.

Therefore, conducting research on tFUS neuromodulation in ischemic stroke patients is of considerable importance. Such research not only addresses the technological shortcomings of existing neuromodulation therapies by providing safer, more precise, and more effective treatment strategies for ischemic stroke patients, but also further elucidates the intrinsic mechanisms of neural repair following ischemic stroke, thereby advancing both theoretical innovation and clinical translation in the field of stroke neuromodulation [10].

2. Study Objectives

2.1 Primary Objective: This study aims to evaluate how tFUS technology promotes the remodeling of damaged neural networks and functional recovery by activating or inhibiting neural activity in specific brain regions, integrating EEG and MRI signal analyses. The primary goals are to improve motor ability and cognitive function, reduce long-term disability, and enhance quality of life. Additionally, this study seeks to optimize stimulation parameters to achieve maximal rehabilitative outcomes and to elucidate the potential mechanisms underlying neuroprotection and functional reconstruction following stroke.

2.2 Secondary Objective: To explore novel therapeutic protocols for upper limb rehabilitation following stroke.

3. Study Overview

3.1 Study Design

This study is a randomized, controlled, single-center trial.

3.2 Sample Size

Participants will be recruited from inpatients of the Department of Rehabilitation Medicine. Based on preliminary literature review and the current inpatient volume of our department, the provisional sample size is set at n = 60, with 30 participants allocated to each group.

3.3 Study Duration

March 2026 – March 2028

3.4 Participant Selection

Inclusion Criteria: (1) Patients with a confirmed imaging diagnosis of first-ever ischemic stroke; (2) Unilateral limb motor involvement; (3) Modified Ashworth Scale (MAS) grade ≤3 for the upper extremity, Brunnstrom stage II–V, and upper extremity Fugl-Meyer Assessment (FMA-UE) score between 15 and 60 (inclusive); (4) Age 18–75 years, onset within 21 days

to 6 months, in the subacute or recovery phase; (5) Stable vital signs, no consciousness impairment, informed consent provided, ability to cooperate with clinical assessments, and approval obtained from the institutional ethics committee.

Exclusion Criteria: (1) Contraindications to MRI; (2) Infarction involving the thalamus; (3) Risk of intracranial hemorrhage; (4) Presence of intracranial metallic foreign bodies, cardiac pacemakers, or cochlear implants; (5) Unstable medical condition, severe cognitive impairment or psychiatric disorders rendering the patient unable to comprehend the study or cooperate with assessments; (6) Conditions affecting limb motor function, including fractures, joint contractures, or severe limb spasticity; (7) Current use of medications that alter cortical excitability.

Withdrawal Criteria: (1) Health risks: Occurrence of serious health events such as acute cardiovascular events or other severe illnesses; (2) Non-compliance: Failure to adhere to the treatment protocol or follow-up requirements, compromising data reliability; (3) Personal reasons: Voluntary withdrawal from the study due to personal circumstances; (4) Severe adverse reactions: Occurrence of unmanageable treatment-related discomfort, such as extreme pain or allergic reactions.

3.5 Study Procedures

3.5.1 Study Preparation

Project Initiation: Submission of materials to the Clinical Research Center; project initiation following scientific review approval.

Ethical Approval: Submission of materials to the Ethics Committee for review. Ethical approval to be obtained to ensure compliance with ethical standards.

Resource Preparation: Preparation of neuromodulation equipment and associated technologies. Recruitment and training of research personnel to ensure familiarity with study procedures and operational protocols.

3.5.2 Participant Recruitment

Recruitment announcements will be published to disseminate information regarding the study objectives and eligibility criteria.

3.5.3 Informed Consent

Written informed consent will be obtained from all eligible participants prior to enrollment.

3.5.4 Screening Phase

Medical history documentation will be collected, including sex, age, medical record number, history of present illness, and past medical history. The following examinations will be completed: blood biochemistry, complete blood count, coagulation function, and neuroimaging (cranial CT/MRI). Baseline clinical assessments will be performed, including the Fugl-Meyer Assessment, Brunnstrom staging, and Modified Ashworth Scale grading.

3.5.5 Group Allocation

A total of 60 patients with hemiplegic upper limb dysfunction during the recovery phase of stroke, undergoing inpatient rehabilitation at the Department of Rehabilitation Medicine, Taizhou People’s Hospital, will be randomly allocated into a neuromodulation group and a

control group (n = 30 per group) using a random number table. All participants in both groups will receive conventional pharmacological therapy (including antiplatelet aggregation agents, neurotrophic agents, circulatory improvement agents, plaque stabilizers, antihyperglycemic/antihypertensive agents, and supportive therapy) and standard rehabilitation interventions (physical therapy and occupational therapy). The neuromodulation group will receive one week of transcranial focused ultrasound stimulation in addition to conventional treatment. The control group will undergo the same assessment and procedural steps as the experimental group but will receive sham stimulation.

3.6 Neuromodulation Intervention

The transcranial focused ultrasound stimulation device employed in this study is the NeuroFUS DPX-500, equipped with a 4-element transducer array and the BrainSight neuronavigation system. Individualized modeling and target segmentation are performed using high-resolution structural MRI and CT images acquired at baseline. Target planning and neuronavigation are conducted based on intracranial acoustic field simulation. The total stimulation duration is 12 minutes, comprising 480 pulse trains with 0.5 seconds on and 1 second off, yielding a duty cycle of 20%. The intensity at the intracranial target site (after skull attenuation) is uniformly set at 8 W/cm².

Experiments will be conducted in a soundproofed, electromagnetically shielded room free from excessive illumination or reflective surfaces. The facility is equipped with provisions for prompt hair washing and EEG cap cleaning, and is situated within a 10-minute walk to the MRI suite.

The experimental protocol spans one week and comprises three principal phases: pre-test, neuromodulation, and post-test. The pre-test includes cranial MRI acquisition, clinical scale assessment, and EEG data collection. Neuromodulation sessions are administered once daily for a total of five sessions per week, with EEG recordings obtained before, during, and after each stimulation session. The post-test consists of cranial MRI acquisition identical to the pre-test protocol, conducted within two days of completion of all neuromodulation sessions.

The pre-test serves as a baseline measurement and primarily includes neuroimaging acquisition and resting-state/task-state EEG measurements. Neuroimaging acquisition encompasses cranial CT (the most recent CT obtained proximal to onset per admission protocol) and cranial MRI acquired using study-specific sequences, including T1-weighted, T2-weighted, resting-state functional MRI, and diffusion tensor imaging. Pre-test data are also utilized for individualized neuronavigation and acoustic field modeling simulation. This involves deep brain nuclei segmentation based on individual structural images, recording of target coordinates via the neuronavigation system, cranial acoustic parameter analysis using CT and structural images, planning of individualized neuromodulation incidence positions and parameter sequences by setting a uniform in situ target intensity, and concurrent safety testing and thermal simulation.

Five neuromodulation sessions are administered in total. Sessions 2 and 5 involve simultaneous EEG-coupled ultrasound neuromodulation, while Sessions 1, 3, and 4 consist of ultrasound neuromodulation alone. During EEG-coupled sessions, EEG signals are acquired before, during, and after ultrasound stimulation; during standalone sessions, only ultrasound stimulation is delivered without EEG acquisition. Stimulation parameters are maintained at consistent control levels across participants. Based on each individual’s

cranial acoustic parameters, the pre-attenuation stimulation intensity is back-calculated to ensure uniform in situ intensity at the target. The spatial-peak temporal-average intensity at the target is maintained below the diagnostic ultrasound safety threshold (720 mW/cm²). The ultrasound stimulation duration is 12 minutes using a 20% duty cycle pulse sequence.

The post-test cranial MRI protocol is identical to the pre-test and is performed within two days of completion of all neuromodulation sessions.

3.7 Control Group Intervention

The control group receives sham stimulation during the neuromodulation phase. All procedural steps are identical to those of the experimental group, including neuronavigation positioning, application of coupling gel, and all other preparatory procedures; however, ultrasound stimulation is not activated. Participants in the control group wear bone-conduction headphones to simulate and counteract acoustic confounding. Group allocation information is blinded to participants.

3.8 Efficacy Assessment

Primary Outcome Measures: Post-treatment (immediate and 2-week follow-up) upper extremity Fugl-Meyer Assessment score, Brunnstrom staging, and Modified Ashworth Scale grading; neuroimaging data (analysis of ALFF, fALFF, ReHo, FC, and FA indices using functional MRI and diffusion tensor imaging); EEG measurements of resting-state and task-state spectral power and functional connectivity.

Secondary Outcome Measures: Blood biochemistry, complete blood count, C-reactive protein (CRP), interleukin-6 (IL-6), S100β protein, and brain-derived neurotrophic factor (BDNF).

4. Risks and Benefits

4.1 Potential Risks and Mitigation Measures: Abnormal reactions to ultrasound stimulation and potential induction of seizures. Adverse symptoms such as dizziness and headache will be promptly managed. Short-term administration of analgesic agents and antiepileptic medications will be employed as indicated.

4.2 Potential Benefits and Compensation for Participants:

Direct Benefits: Promotion of hemiplegic upper limb functional recovery and enhancement of activities of daily living capacity.

Societal Benefits: It is anticipated that information obtained from this study may benefit the participant or future patients with similar conditions.

5. Adverse Event Monitoring

5.1 Adverse Events (AEs): An adverse event is defined as any unfavorable medical occurrence, unanticipated illness or injury, or unexpected clinical sign (including abnormal laboratory findings) in a participant or other individual, regardless of whether it is considered related to the study intervention or treatment strategy under investigation.

5.2 Serious Adverse Events (SAEs): Serious adverse events are defined as events

occurring during the clinical trial that necessitate hospitalization, prolong existing hospitalization, result in disability, impair work capacity, are life-threatening or fatal, or cause congenital anomalies.

6. Ethical Considerations

6.1 The clinical study shall be conducted only after the trial protocol has been approved by the Ethics Committee prior to the commencement of the study. The investigator is required to promptly report all changes occurring in the course of the research to the Ethics Committee (including modifications to the protocol and/or informed consent form), and shall not implement such changes without prior approval from the Ethics Committee, unless the changes are necessary to eliminate an apparent and immediate risk to participants. In such circumstances, the Ethics Committee shall be notified.

6.2 Prior to enrollment of each participant, the investigator bears the responsibility of thoroughly informing the participant or their legal guardian of the study's objectives, procedures, potential risks, and information regarding alternative treatments, and of obtaining a signed written informed consent form. Participants shall be informed of their right to withdraw from the study at any time. Informed consent forms shall be retained as clinical research documentation for inspection. Throughout the study, the privacy of participants and the confidentiality of their data will be protected.

7. Data Confidentiality

Results obtained from this study may be published in medical journals; however, patient information shall be maintained in strict confidence in accordance with applicable legal requirements. Personal information of patients shall not be disclosed unless mandated by relevant laws and regulations. When necessary, governmental regulatory authorities, the institutional ethics committee, and their designated personnel may access patient records in accordance with applicable regulations.

8. Anticipated Timeline and Completion Date

The total duration of this project is two years, from March 2026 to March 2028. The planned schedule is as follows:

March 2026 – May 2026: Study preparation.

June 2026 – October 2026: Randomized group allocation of enrolled stroke patients, intervention implementation, and collection of relevant outcome measures.

November 2026 – August 2027: Continued case recruitment, statistical analysis of accumulated data, and objective evaluation of outcome measures across groups.

September 2027 – December 2027: Participation in provincial and national rehabilitation conferences, with preliminary findings presented for peer review and scholarly exchange.

January 2028 – February 2028: Continuation of clinical data collection, synthesis of completed research, comprehensive data analysis, and evaluation of study outcomes.

March 2028: Project completion, manuscript preparation, and publication.

9. Research Team

Name	Title / Specialty	Responsibilities
Zhao Gaonian	Chief Physician	Quality Control
Mei Haifeng	Chief Physician	Data Analysis
Yu Xiaorong	Associate Chief Physician	Case Recruitment
Jin Jing	Chief Physician	Data Analysis
Jiang Su	Associate Chief Physician	Manuscript Preparation
Xiao Shen	Attending Physician	Case Recruitment
Wang Wenjun	Attending Physician	Case Recruitment
Lu Zhaohui	Attending Physician	Case Recruitment
Cao Yuan	Attending Physician	Case Recruitment
Liu Wei	Attending Physician	Rehabilitation Assessment
Xu Xinxuan	Senior Therapist	Rehabilitation Assessment

10. References

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