rTMS Combined with Motor Training for the Treatment of Upper Limb Motor Dysfunction in Stroke Patients

Protocol Number: Ruijin Hospital [Approval No. (2023) 63-3],

Yangzhi Hospital [(2025) 050], and Changhai Hospital

[CHEC2025-343]

National Clinical Trial (NCT) Identified Number: NCT06752499

Principal Investigators: Qing Xie, MD, MS

Funded by: Shanghai Government Grant (2021SHZDZX)

Version Number: v5.0

May 7, 2025

Table of Contents

STATEMENT OF COMPLIANCE

This trial will be conducted in accordance with the Declaration of Helsinki (1975, as revised in 2008) and with the ethical standards of the relevant national and institutional committees on human experimentation. The principal investigator will ensure that no deviations or amendments to the study protocol are made without the prior consent of the sponsor and written approval from the central ethics committees of the participating hospitals, unless such changes are necessary to eliminate immediate risks to the participants. All personnel involved in the study have completed certified training in the protection of human subjects and in the procedures related to the study's interventions and assessments. The study protocol, informed consent forms, recruitment materials, and all participant-related documents will be submitted to the ethics committees of relative Hospitals for review and approval. Approval of both the protocol and the consent forms must be obtained before enrolling any participant. Any amendments to the protocol will require review and approval by the ethics committees of relative Hospitals before implementation. All revisions to the consent forms must also receive approval from the same ethics committees, which will determine whether re-consent is required from participants who previously provided consent using earlier versions of the form.

1. PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: rTMS Combined with Motor Training for the Treatment of Upper Limb Motor Dysfunction in Stroke Patients

Study Description：This is a multicenter, non-randomized, double-blind, controlled trial. Stroke patients were enrolled according to predefined inclusion criteria. During specific motor tasks, transcranial magnetic stimulation (TMS) was delivered synchronously to evaluate whether task-synchronized stimulation could enhance upper limb motor recovery after two weeks of treatment. Electromyographic (EMG) signals from the upper limb and shoulder were recorded in real time to trigger, terminate, or adjust stimulation parameters. Structural and functional MRI were acquired before and after the intervention, and scalp EEG was recorded during stimulation. Individualized electromagnetic field simulations were performed to estimate target stimulation intensity and guide coil placement. Brain-state and functional connectivity analyses were used to identify quantitative relationships between cortical activity and motor dysfunction, supporting optimization of stimulation parameters. This protocol aimed to achieve personalized, closed-loop neuromodulation through task-synchronized TMS to promote upper limb motor recovery after stroke.

Objectives： **Primary Objectives:** To evaluate whether there is a sustained long-term overall treatment effect on the Fugl-Meyer Assessment for Upper Extremity (FM-UE) — a standardized measure of motor impairment — among the three treatment groups (concurrent rTMS, non-concurrent rTMS, and sham) at day 105 (±5 days) from baseline (day 0). Additional functional assessments include the Wolf Motor Function Test (WMFT) and Action Research Arm Test (ARAT) for motor performance, as well as the Modified Barthel Index (MBI) for activities of daily living and the Pittsburgh Sleep Quality Index (PSQI) for sleep quality. Further evaluations will be conducted at day 15 (±2 days) and day 45 (±5 days) from baseline (day 0).

Secondary Objectives: To confirm that the proposed intervention is safe (no significant differences in adverse event rates across groups), well tolerated (no withdrawals due to intervention-related causes), and feasible in a multicenter setting (≥80% of participants completing the treatment protocol).

Exploratory Objectives: To explore changes in cortical activation patterns, brain network connectivity, and EEG-derived

	components before and after task-synchronized stimulation, and to assess the potential of these measures as candidate biomarkers for future phase II/III confirmatory studies. In addition, to examine whether structural and functional alterations within the descending motor pathways are directly associated with behavioral motor improvement.
Study Population：	Sixty stroke patients who meet the inclusion and exclusion criteria will be enrolled and assigned to three groups. There are no restrictions regarding gender, age (20-80 years), or geographical distribution.
Research Sites:	Department of Rehabilitation Medicine, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine; Shanghai Yangzhi Rehabilitation Hospital (Shanghai Sunshine Rehabilitation Center); Changhai Hospital, Shanghai, China.
Study Intervention:	This study adopted a non-randomized, controlled, double-blind trial design. Eligible participants were allocated into three groups: the online (concurrent) stimulation group, offline (non-concurrent) stimulation group, and sham stimulation group. Each participant received TMS intervention five days per week, with resting motor threthold (RMT) assessments conducted once per week. The entire intervention period lasted for one month or until hospital discharge. All participants underwent EEG recording and both structural and functional MRI scanning to enable individualized electric field modeling and functional connectivity analysis. These multimodal data were used to identify target cortical regions associated with upper-limb motor performance, and to determine optimized stimulation parameters for each individual. The intervention protocols differed across the three groups as follows: 1. Concurrent (Online) stimulation group: TMS parameters were continuously adjusted based on baseline data and ongoing treatment responses to achieve individualized modulation. During therapy, TMS pulses were delivered synchronously with active upper-limb motor tasks, triggered by real-time electromyographic (EMG-ACC) feedback at the onset of voluntary movement and terminated immediately once the movement ceased or task completion was detected. This setup ensured state-dependent, task-synchronized stimulation. 2. Non-concurrent (Offline) stimulation group: TMS stimulation was administered prior to task execution. After

completion of the stimulation session, participants performed the same motor tasks without concurrent stimulation.

3. Sham stimulation group:

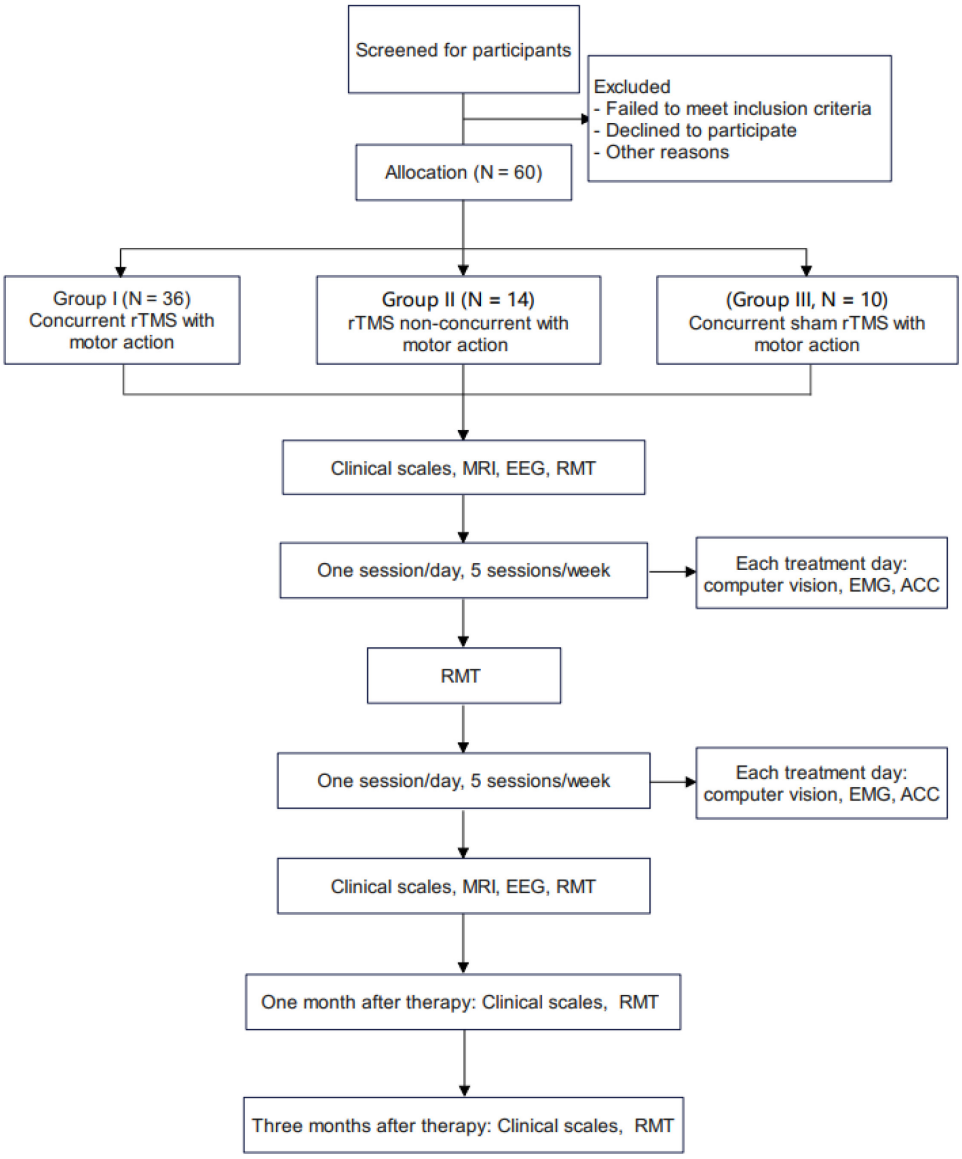
Participants received sham TMS concurrently with the same task paradigm used in the online stimulation group. The procedure and timing were identical, except that the magnetic field was effectively blocked, ensuring blinding to stimulation conditions.

Study duration: 36 months

Subject duration: Approximately 4 months

1.2 SCHEMA

Please Refer to Section 1.3, Schedule of Activities, for details



1.3 SCHEDULE OF ACTIVITIES (SOA)

Event	Baseline	Treatment Sessions (1-10)	Follow Up 1	Follow Up 2	Follow Up 3	End of Study
	Day -6 to 1	Day 1- 14	Day 15 ± 2	Day 45 ± 5	Day 105 ± 5	
Inclusion/ Exclusion	√					
Informed Consent	√					
Demographics	√					
Medical and Social History	√					
Medication Checklist	√					
Vital signs	√					
MEP assessment	√	√	√	√	√	
Allocation	√					
MRI	√		√			
EEG	√		√	√	√	
rTMS therapy		√				
Motor task training		√				
EMG		√				
Video		√				
Fugl-Meyer Assessment– Upper Extremity Scale (FM-UE)	√		√	√	√	
Wolf Motor Function Test (WMFT)	√		√	√	√	
ARAT	√		√	√	√	
MBI	√		√	√	√	
PSQI	√		√	√	√	
End of study						√

2 INTRODUCTION

2.1 RESEARCH SIGNIFICANCE

Cerebral stroke, also known as "stroke" or cerebrovascular accident (CVA), is a disease characterized by brain tissue damage resulting from the sudden rupture of cerebral blood vessels or vascular blockage that prevents blood flow to the brain. It includes both ischemic and hemorrhagic strokes. If not treated promptly, a stroke can cause irreversible harm. With the aging of the population in China, the prevalence and mortality rates of cerebral stroke have been gradually increasing. It has now become the third leading cause of death, following only malignant tumors and heart disease. Additionally, due to damage to the nervous system, patients may experience various

functional impairments after their condition stabilizes, such as motor disorders, speech disorders, and swallowing disorders, with a disability rate as high as 70%. This significantly impacts patients' quality of life and increases the economic and psychological burden on both patients and their families. For neurological dysfunction following a stroke, drug treatments are often of limited efficacy, making comprehensive rehabilitation therapy generally necessary to improve various functions and the overall quality of life for patients.

Non-invasive neuromodulation techniques, such as transcranial magnetic stimulation (TMS), have also been used in the rehabilitation of stroke patients. By inducing changes in bilateral hemispheric neural networks and related cortical-subcortical excitability, TMS can promote functional recovery in post-stroke patients and is considered a feasible intervention. However, due to variations in the location of neurological damage and individual differences among patients, consistent conclusions have not been reached across different studies, which has limited the widespread clinical adoption of TMS.

In summary, this study implements individualized, transcranial magnetic stimulation based on the actual condition of each patient with post-stroke functional impairment. The stimulation is concurrent with personalized task training to achieve synergistic effects and explore improvements in patients' motor dysfunction.

In terms of theoretical research, an in-depth study of individualized rTMS stimulation and understanding the effects of different stimulation sites on patients will contribute to the future promotion and application of TMS and enhance the understanding of brain nerve damage. The clinical adoption of this technology offers enhanced rehabilitation alternatives for a substantial population of stroke survivors and contributes to alleviating the healthcare burden.

2.2 RESEARCH BACKGROUND

1. **Post-Stroke Rehabilitation**

According to the standards of the World Health Organization (WHO), a cerebral stroke is a clinical sign of rapidly developed focal (and sometimes global) cerebral

dysfunction, lasting more than 24 hours (unless it leads to death), with no apparent cause other than a vascular origin[1]. Cerebral stroke is caused by an interruption of the blood supply to the brain due to either vascular occlusion (ischemic stroke, approximately 82%) or vessel rupture (hemorrhagic stroke, approximately 14%) [2]. Both types can lead to a sudden interruption of oxygen and nutrient supply, potentially resulting in permanent brain tissue damage. According to survey data from the American Heart Association (2021), over 50% of stroke survivors may develop various functional impairments that lead to disability, such as motor, speech, and swallowing disorders [3], even patients with milder conditions may still experience impairments in their daily living and social functioning[4,5]. Traditional rehabilitation training is an effective method for improving neurological function after a stroke. Nevertheless, a considerable proportion of stroke patients still experience neurological impairments even after rehabilitation. Therefore, integrating non-invasive neuromodulation techniques with conventional rehabilitation therapy has become an emerging approach in post-stroke recovery.

2. **The Application of TMS in Stroke Rehabilitation**

While considerable progress has been made in the diagnosis, prevention, and treatment of stroke, more research is needed on targeted rehabilitation. Among the available approaches, Transcranial Magnetic Stimulation (TMS) is an emerging technology in the field of post-stroke rehabilitation[6]. Transcranial Magnetic Stimulation (TMS) is a safe and non-invasive technique for modulating the central nervous system. Based on the principle of Faraday's electromagnetic induction, when a highly charged capacitor rapidly discharges a current of several thousand amperes through a coil, it instantaneously generates a magnetic field with a strength of up to 2 Tesla. This magnetic field penetrates extracerebral tissues (such as the scalp, skull, meninges, and cerebrospinal fluid) and decays exponentially with distance. It induces an inverse electric current in the underlying cortex, which can lead to neuronal depolarization and activate cortical neuronal networks, thereby modulating cerebral

cortical excitability[7]. Consequently, TMS offers significant therapeutic potential for various psychiatric and neurological disorders arising from cortical hypoexcitability or hyperexcitability, such as stroke [8]. According to the traditional view, the two cerebral hemispheres inhibit each other via the corpus callosum in a balanced, competitive relationship, where each side exerts an equal inhibitory influence on the other. Following a stroke in one hemisphere, the excitability of the affected side decreases, leading to a reduction in its inhibition of the unaffected hemisphere. Consequently, the unaffected side becomes hyperexcitable and exerts stronger inhibition on the affected hemisphere, resulting in an interhemispheric imbalance[9]. However, the residual neural tissue retains neuroplasticity, which can compensate for lost functions. This suggests that modulating interhemispheric balance—by either upregulating excitability in the affected hemisphere or downregulating it in the unaffected hemisphere—may promote functional recovery after stroke[10,11]，this process of modulating cortical excitability can be achieved using TMS. However, a study suggested that the degree of interhemispheric inhibition varies depending on the extent of brain injury[12]. Therefore, each stroke patient should receive personalized magnetic stimulation therapy tailored to their specific brain injury condition.

Currently, repetitive Transcranial Magnetic Stimulation (rTMS) is a commonly used rehabilitation approach for stroke patients. rTMS delivers magnetic pulses at a fixed stimulation frequency in continuous trains. It primarily employs two treatment modalities: **Low-frequency rTMS** (stimulation frequency ≤1 Hz, most commonly 1 Hz) is applied to the contralesional (unaffected) hemisphere. This induces long-term depression (LTD) of synaptic transmission, reducing cortical excitability in the unaffected hemisphere. Consequently, this disinhibition leads to increased activity in the affected hemisphere. **High-frequency rTMS** (stimulation frequency ≥3 Hz, most commonly 5 Hz) is applied to the ipsilesional (affected) hemisphere. This induces long-term potentiation (LTP) of synaptic transmission, directly increasing cortical excitability in the affected hemisphere and thereby enhancing its activity[13-15]. Numerous studies have demonstrated that repetitive Transcranial Magnetic Stimulation (rTMS)

can modulate neural networks in both cerebral hemispheres and regulate cortical-subcortical excitability, thereby harnessing neuroplasticity to promote the recovery of neurological function in stroke patients [16-18].

Currently, another magnetic stimulation technique—Theta Burst Stimulation (TBS)—is also being applied in the rehabilitation of stroke patients. As a specific pattern of TMS, TBS has demonstrated more rapid and sustained effects compared to conventional rTMS [19], Moreover, TBS is exceptionally well-tolerated in the majority of patients and is associated with minimal side effects. Compared to conventional rTMS, TBS requires fewer pulses and lower stimulation intensity to induce relatively longer-lasting effects in the cerebral cortex, while simultaneously avoiding coil overheating [16,20,21]. TBS primarily employs two stimulation paradigms: intermittent theta burst stimulation (iTBS) and continuous theta burst stimulation (cTBS). iTBS delivers a 2-second stimulus train every 10 seconds (with an 8-second interval between trains), which can induce long-term potentiation (LTP) effects, enhancing cortical excitability for up to 30 minutes. In contrast, cTBS involves continuous, uninterrupted stimulus trains that reduce cortical excitability through long-term depression (LTD) effects, lasting up to 60 minutes, with its peak effect observed as early as 5 minutes after stimulation [7, 22]. Due to its ability to induce significant and sustained effects with very brief stimulation durations, TBS demonstrates considerable potential for clinical application. However, optimal neural targets and treatment parameters still require further refinement.

3. Overview of Relevant Clinical Studies

In clinical practice, the application of TMS in the functional rehabilitation of stroke patients remains under exploration. Significant heterogeneity exists across different studies, and there is currently no unified standard for stimulation parameters and intervention duration. The following section will summarize research from the past

decade on the use of TMS to improve motor, speech, and swallowing functions in stroke patients.

(1) TMS for Improving Motor Function in Stroke Patients

Timing of Intervention

Van Lieshout et al. compared the effects of different TMS intervention timings on upper limb function or activity. They categorized the intervention periods into four types: acute to early sub-acute (<1 month), early sub-acute (1-3 months), late sub-acute (3-6 months), and chronic (>6 months). Their analysis of 38 clinical studies involving a total of 1074 stroke patients showed that the application of TMS within the first month post-stroke yielded significant effects, but no clear benefits were found in the other phases [23]. Similarly, a meta-analysis by Zhang et al. found that the therapeutic effect of TMS diminished with delayed intervention after stroke. This may be related to the temporal dynamics of cortical-muscle interactions during stroke recovery, which often stabilize after the chronic phase, leading to less pronounced intervention effects [24].

Stimulation Sites and Parameters

Most current studies select the primary motor cortex (M1) as the stimulation site for treatment. Beyond the cortex, the lesioned hemisphere and the healthy hemisphere are also among the stimulation targets. The choice of hemisphere is often paired with specific parameters. High-frequency rTMS or iTBS is generally applied to the affected hemisphere, while low-frequency rTMS or cTBS is applied to the unaffected hemisphere; this approach has been introduced into clinical practice.

Apart from the stimulation site, parameters such as TMS stimulation frequency, intensity, and duration can also impose certain limitations on the treatment efficacy. Stimulation frequency is considered a primary determinant of the direction and magnitude of changes in cortical excitability. Studies have found that stimulation at 1-10 Hz is beneficial for motor recovery, particularly 1 Hz stimulation, although changes

in motor evoked potentials are more pronounced after high-frequency stimulation [25]. This finding is inconsistent with previous meta-analyses, which suggested that low-frequency rTMS has a stronger effect on recovery via the unaffected hemisphere than high-frequency stimulation. On the other hand, iTBS is typically applied to the lesioned hemisphere, and cTBS to the non-lesioned hemisphere. iTBS is currently considered beneficial for motor function recovery, whereas the effectiveness of cTBS requires further investigation [26]. Other stimulation parameters, such as the number of pulses, the patient's motor threshold, and the number of stimulation sites, have not been found to significantly impact treatment outcomes.

Upper Limb Functional Recovery

The assessment of TMS-induced improvement in upper limb function generally considers four aspects: finger dexterity, hand strength, functional dexterity, and body function level. The degree of improvement across these four aspects ranks as follows: finger dexterity > hand strength > functional dexterity > body function level [18]. Both short-term studies and long-term follow-ups have found that low-frequency TMS improves patients' upper limb function. Based on this, it can be inferred that low-frequency TMS promotes the recovery of motor function in stroke patients, and this effect can be maintained in the long term [27].

(2) TMS for Improving Speech Function in Stroke Patients

Timing of Intervention

Substantial evidence indicates that TMS can promote speech recovery across all phases following a stroke, including the acute, sub-acute, and chronic stages [28-30].

Stimulation Sites and Parameters

Based on the lateralization of speech function, where the left hemisphere is the dominant hemisphere for speech ability in most individuals, stimulation protocols that have been relatively well-researued and demonstrate potential for post-stroke speech recovery involve applying high-frequency rTMS to left hemisphere speech areas (e.g., Broca's area, Wernicke's area, motor cortex) or applying low-frequency rTMS to

homologous right hemisphere frontal speech areas. Reported speech improvements are diverse, encompassing articulation, speech coherence, verbal fluency, and speech rate [30-33].

In recent years, numerous studies have also explored the use of TBS for speech rehabilitation in stroke patients [34, 35]. A commonly used TBS paradigm employs bursts of three 50 Hz pulses, repeated every 200 ms. This consists of a 2-second stimulation train with an 8-second inter-train interval, delivered at an intensity of 80% of the Active Motor Threshold (AMT). Typically, iTBS is applied to left hemisphere speech areas, while cTBS is applied to the homologous right hemisphere areas [36, 37].

(3) TMS for Improving Swallowing Function in Stroke Patients

Timing of Intervention

Multiple studies have shown that TMS can improve swallowing function in patients with unilateral acute stroke, with therapeutic effects lasting for several months [38]. However, for chronic stroke patients, some studies indicate no significant difference in swallowing function before and after TMS treatment [39]. Whether TMS can enhance swallowing function in chronic stroke patients with dysphagia, its long-term effects (particularly beyond 3 months), and the assessment of brain plasticity changes over time should be further validated in future studies.

Stimulation Sites and Parameters

rTMS stimulation sites vary and primarily include the following:

Low-frequency stimulation of the unaffected hemisphere or high-frequency stimulation of the affected hemisphere: Low-frequency stimulation of the unaffected hemisphere and high-frequency stimulation of the affected hemisphere are currently common in clinical practice. Compared to sham stimulation, applying 3 Hz [38] or 10 Hz [40] rTMS to the cortical areas corresponding to the esophagus or mylohyoid muscle in the

affected hemisphere significantly improved patients' swallowing function. On the other hand, applying 1 Hz rTMS to the cortical areas corresponding to the pharynx or mylohyoid muscle in the unaffected hemisphere also resulted in improved swallowing function [41, 42]. One review found that stimulation of the unaffected hemisphere produced significantly better effects compared to stimulation of the affected hemisphere [43], while another review reported no significant differences between different stimulation sites [44]. Overall, whether to stimulate the affected or unaffected hemisphere remains a contentious issue.

Bilateral high-frequency stimulation: Bilateral high-frequency rTMS has also been shown to enhance swallowing ability in stroke patients [45] and accelerate the timeline of swallowing recovery [46]. This may be because in subacute stroke patients with dysphagia, cortical excitability in the unaffected hemisphere also decreases due to early disruption of the swallowing network, providing a rationale for bilateral high-frequency rTMS to improve post-stroke swallowing function [47].

Cerebellar stimulation: The cerebellum is active during swallowing and is believed to modulate the orderly coordination of swallowing muscles [48, 49]. Cerebellar rTMS has recently become a hotspot in the field of neuromodulation due to its relatively easy targeting and lower risk of inducing seizures. Numerous studies have shown that cerebellar TMS can evoke pharyngeal motor evoked potentials; pulses transmitted by the cerebellum can induce post motor-evoked potential (PMEP) responses in the cortex, thereby promoting rapid improvement in swallowing behavior [50, 51].

4. Commonalities and Challenges in Current Research

A consistent conclusion from research over the past decade is that early intervention is beneficial, and the duration of treatment also influences the final functional outcome [52]. Regarding the choice of stimulation protocol, the interhemispheric inhibition imbalance theory is generally followed: excitatory TMS is applied to the affected hemisphere, and inhibitory TMS to the unaffected hemisphere. Both TMS used alone and TMS combined with conventional rehabilitation therapy

have shown significant improvements in various brain functions in stroke patients, including motor, speech, and swallowing functions. However, due to differences in the location of neurological damage and individual patient variability, stimulation parameters vary considerably across studies, making it difficult to draw consistent conclusions. Therefore, effective application of TMS requires personalized selection of excitatory or inhibitory interventions based on each patient's specific stroke severity, timing, and location.

Although previous research has yielded substantial results, several challenges remain. First, there is a lack of personalized stimulation control parameters. Second, closed-loop stimulation regulation technology is underdeveloped. Third, real-time synchronization of TMS with rehabilitation tasks is difficult to achieve. Based on this research background, our study implements personalized, closed-loop transcranial magnetic stimulation tailored to the specific condition of each patient with post-stroke dysfunction. Stimulation is paired with individualized task training to achieve synchronized effects and explore improvements in motor dysfunction and other brain functions.

2.3 REFERENCES

[1] HATANO S. Experience from a multicentre stroke register: a preliminary report [J]. Bulletin of the World Health Organization, 1976, 54(5): 541-53.

[2] FEIGIN V L M D, LAWES C M M F, BENNETT D A P, et al. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review [J]. Lancet neurology, 2009, 8(4): 355-69.

[3] VIRANI S S, ALONSO A, APARICIO H J, et al. Heart Disease and Stroke Statistics-2021 Update A Report from the American Heart Association [J]. Circulation (New York, NY), 2021, 143(8): e254-e743.

[4] FEIGIN V L, BARKER-COLLO S, PARAG V, et al. Auckland Stroke Outcomes Study: Part 1: Gender, stroke types, ethnicity, and functional outcomes 5 years poststroke [J]. Neurology, 2010, 75(18): 1597-607.

[5] DIONíSIO A, DUARTE I C, PATRíCIO M, et al. Transcranial Magnetic Stimulation as an Intervention Tool to Recover from Language, Swallowing and Attentional Deficits after Stroke: A Systematic Review [J]. Cerebrovascular diseases (Basel, Switzerland), 2018, 46(3-4): 176-83.

[6] HACHINSKI V, DONNAN G A, GORELICK P B, et al. Stroke: Working toward a prioritized world agenda [J]. Stroke (1970), 2010, 41(6): 1084-99.

[7] HUANG Y-Z, EDWARDS M J, ROUNIS E, et al. Theta Burst Stimulation of the Human Motor Cortex [J]. Neuron (Cambridge, Mass), 2005, 45(2): 201-6.

[8] ROTHWELL J C, RIDDING M C. Is there a future for therapeutic use of transcranial magnetic stimulation? [J]. Nature reviews Neuroscience, 2007, 8(1): 559-67.

[9] NOWAK D A, GREFKES C, AMELI M, et al. Interhemispheric Competition After Stroke: Brain Stimulation to Enhance Recovery of Function of the Affected Hand [Z]. Los Angeles, CA; SAGE Publications. 2009: 641-56.10.1177/1545968309336661

[10] MURASE N, DUQUE J, MAZZOCCHIO R, et al. Influence of interhemispheric interactions on motor function in chronic stroke [J]. Annals of neurology, 2004, 55(3): 400-9.

[11] CICINELLI P, PASQUALETTI P, ZACCAGNINI M, et al. Interhemispheric Asymmetries of Motor Cortex Excitability in the Postacute Stroke Stage: A Paired-Pulse Transcranial Magnetic Stimulation Study [J]. Stroke (1970), 2003, 34(11): 2653-8.

[12] LIN Y-L, POTTER-BAKER K A, CUNNINGHAM D A, et al. Stratifying chronic stroke patients based on the influence of contralesional motor cortices: An inter-hemispheric inhibition study [J]. Clinical Neurophysiology, 2020, 131(10): 2516-25.

[13] CHEN R, CLASSEN J, GERLOFF C, et al. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation [J]. Neurology, 1997, 48(5): 1398-403.

[14] PASCUAL-LEONE A, VALLS-SOLé J, WASSERMANN E M, et al. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex [J]. Brain (London, England : 1878), 1994, 117(4): 847-58.

[15] HOUDAYER E, DEGARDIN A, CASSIM F, et al. The effects of low- and high-frequency repetitive TMS on the input/output properties of the human corticospinal pathway [J]. Experimental brain research, 2008, 187(2): 207-17.

[16] GRAEF P, DADALT M L R, RODRIGUéS D A M D S, et al. Transcranial magnetic stimulation combined with upper-limb training for improving function after stroke: A systematic review and meta-analysis [J]. Journal of the neurological sciences, 2016, 369: 149-58.

[17] DIONíSIO A, DUARTE I C, PATRíCIO M, et al. The Use of Repetitive Transcranial Magnetic Stimulation for Stroke Rehabilitation: A Systematic Review [J]. Journal of stroke and cerebrovascular diseases, 2018, 27(1): 1-31.

[18] ZHANG L, XING G, SHUAI S, et al. Low-Frequency Repetitive Transcranial Magnetic Stimulation for Stroke-Induced Upper Limb Motor Deficit: A Meta-Analysis [J]. Journal of neural transplantation & plasticity, 2017, 2017: 2758097-12.

[19] LI C T, HUANG Y Z, BAI Y M, et al. Critical role of glutamatergic and GABAergic neurotransmission in the central mechanisms of theta-burst stimulation [J]. Human brain mapping, 2019, 40(6): 2001-9.

[20] DIEKHOFF-KREBS S, POOL E-M, SARFELD A-S, et al. Interindividual differences in motor network connectivity and behavioral response to iTBS in stroke patients [J]. NeuroImage clinical, 2017, 15: 559-71.

[21] KIM J-H, HOWON U, DEPARTMENT OF OCCUPATIONAL T. Effects of a virtual reality video game exercise program on upper extremity function and daily living activities in stroke patients [J]. Journal of physical therapy science, 2018, 30(12): 1408-11.

[22] CHUNG S W, HILL A T, ROGASCH N C, et al. Use of theta-burst stimulation in changing excitability of motor cortex: A systematic review and meta-analysis [J]. Neuroscience and biobehavioral reviews, 2016, 63: 43-64.

[23] VAN LIESHOUT E C C, VAN DER WORP H B, VISSER-MEILY J M A, et al. Timing of Repetitive Transcranial Magnetic Stimulation Onset for Upper Limb Function After Stroke : A Systematic Review and Meta-Analysis [J]. Frontiers in neurology, 2019, 10: 1269-.

[24] ZHANG L, XING G, FAN Y, et al. Short- and Long-term Effects of Repetitive Transcranial Magnetic Stimulation on Upper Limb Motor Function after Stroke: a Systematic Review and Meta-Analysis [J]. Clinical rehabilitation, 2017, 31(9): 1137-53.

[25] XIANG H, SUN J, TANG X, et al. The effect and optimal parameters of repetitive transcranial magnetic stimulation on motor recovery in stroke patients: a systematic review and meta-analysis of randomized controlled trials [J]. Clinical rehabilitation, 2019, 33(5): 847-64.

[26] ACKERLEY S J, STINEAR C M, BARBER P A, et al. Combining theta burst stimulation with training after subcortical stroke [J]. Stroke (1970), 2010, 41(7): 1568-72.

[27] KHEDR E M, ETRABY A E, HEMEDA M, et al. Long-term effect of repetitive transcranial magnetic stimulation on motor function recovery after acute ischemic stroke [J]. Acta neurologica Scandinavica, 2010, 121(1): 30-7.

[28] HAGHIGHI M, MAZDEH M, RANJBAR N, et al. Further Evidence of the Positive Influence of Repetitive Transcranial Magnetic Stimulation on Speech and Language in Patients with Aphasia after Stroke: Results from a Double-Blind Intervention with Sham Condition [J]. Neuropsychobiology, 2018, 75(4): 185-92.

[29] HU X-Y, ZHANG T, RAJAH G B, et al. Effects of different frequencies of repetitive transcranial magnetic stimulation in stroke patients with non-fluent aphasia: a randomized, sham-controlled study [J]. Neurological research (New York), 2018, 40(6): 459-65.

[30] RUBI-FESSEN I M, HARTMANN A M D, HUBER W P, et al. Add-on Effects of Repetitive Transcranial Magnetic Stimulation on Subacute Aphasia Therapy: Enhanced Improvement of Functional Communication and Basic Linguistic Skills. A Randomized Controlled Study [J]. Archives of physical medicine and rehabilitation, 2015, 96(11): 1935-44.e2.

[31] ABO M, KAKUDA W, WATANABE M, et al. Effectiveness of low-frequency rTMS and intensive speech therapy in poststroke patients with aphasia: A pilot study based on evaluation by fMRI in relation to type of aphasia [J]. European neurology, 2012, 68(4): 199-208.

[32] NAESER M A P, MARTIN P I B S, HO M P, et al. Transcranial Magnetic Stimulation and Aphasia Rehabilitation [J]. Archives of physical medicine and rehabilitation, 2012, 93(1): S26-S34.

[33] WEIDUSCHAT N, THIEL A, RUBI-FESSEN I, et al. Effects of repetitive transcranial magnetic stimulation in aphasic stroke: A randomized controlled pilot study [J]. Stroke (1970), 2011, 42(2): 409-15.

[34] GRIFFIS J C, NENERT R, ALLENDORFER J B, et al. Interhemispheric Plasticity following Intermittent Theta Burst Stimulation in Chronic Poststroke Aphasia [J]. Journal of neural transplantation & plasticity, 2016, 2016: 4796906-16.

[35] VUKSANOVIC J, JELIC M B, MILANOVIC S D, et al. Improvement of language functions in a chronic non-fluent post-stroke aphasic patient following bilateral sequential theta burst magnetic stimulation [J]. Neurocase, 2015, 21(2): 244-50.

[36] SZAFLARSKI J P, VANNEST J, WU S W, et al. Excitatory repetitive transcranial magnetic stimulation induces improvements in chronic post-stroke aphasia [J]. Medical science monitor, 2011, 17(3): CR132-9.

[37] GEORGIOU A, KONSTANTINOU N, PHINIKETTOS I, et al. Neuronavigated theta burst stimulation for chronic aphasia: two exploratory case studies [J]. Clinical linguistics & phonetics, 2019, 33(6): 532-46.

[38] KHEDR E M, ABO-ELFETOH N, ROTHWELL J C. Treatment of post-stroke dysphagia with repetitive transcranial magnetic stimulation [J]. Acta neurologica Scandinavica, 2009, 119(3): 155-61.

[39] CHENG I K Y, CHAN K M K, WONG C-S, et al. Neuronavigated high-frequency repetitive transcranial magnetic stimulation for chronic post-stroke dysphagia: A randomized controlled study [J]. Journal of rehabilitation medicine, 2017, 49(6): 475-81.

[40] LEE J H, KIM S B, LEE K W, et al. Effect of repetitive transcranial magnetic stimulation according to the stimulation site in stroke patients with dysphagia [J]. Annals of rehabilitation medicine, 2015, 39(3): 432-9.

[41] KIM L, CHUN M H, KIM B R, et al. Effect of Repetitive Transcranial Magnetic Stimulation on Patients with Brain Injury and Dysphagia [J]. Annals of rehabilitation medicine, 2011, 35(6): 765-71.

[42] VERIN E, LEROI A M. Poststroke Dysphagia Rehabilitation by Repetitive Transcranial Magnetic Stimulation: A Noncontrolled Pilot Study [J]. Dysphagia, 2008, 24(2): 204-10.

[43] PISEGNA J M, KANEOKA A, PEARSON W G, et al. Effects of non-invasive brain stimulation on post-stroke dysphagia: A systematic review and meta-analysis of randomized controlled trials [J]. Clinical neurophysiology, 2015, 127(1): 956-68.

[44] YANG S N, PYUN S-B, KIM H J, et al. Effectiveness of Non-invasive Brain Stimulation in Dysphagia Subsequent to Stroke: A Systemic Review and Meta-analysis [J]. Dysphagia, 2015, 30(4): 383-91.

[45] KHEDR E M, ABO-ELFETOH N. Therapeutic role of rTMS on recovery of dysphagia in patients with lateral medullary syndrome and brainstem infarction [J]. Journal of neurology, neurosurgery and psychiatry, 2010, 81(5): 495-9.

[46] PARK E, KIM M S, CHANG W H, et al. Effects of Bilateral Repetitive Transcranial Magnetic Stimulation on Post-Stroke Dysphagia [J]. Brain stimulation, 2016, 10(1): 75-82.

[47] TEISMANN I K, SUNTRUP S, WARNECKE T, et al. Cortical swallowing processing in early subacute stroke [J]. BMC neurology, 2011, 11(1): 34-.

[48] SASEGBON A, HAMDY S. The anatomy and physiology of normal and abnormal swallowing in oropharyngeal dysphagia [J]. Neurogastroenterology and motility, 2017, 29(11): e13100-n/a.

[49] SUZUKI M, ASADA Y, ITO J, et al. Activation of cerebellum and basal ganglia on volitional swallowing detected by functional magnetic resonance imaging [J]. Dysphagia, 2003, 18(2): 71-7.

[50] JAYASEKERAN V, ROTHWELL J, HAMDY S. Non-invasive magnetic stimulation of the human cerebellum facilitates cortico-bulbar projections in the swallowing motor system [J]. Neurogastroenterology and motility, 2011, 23(9): 831-e341.

[51] VASANT D H, SASEGBON A, MICHOU E, et al. Rapid improvement in brain and swallowing behavior induced by cerebellar repetitive transcranial magnetic stimulation in poststroke dysphagia: A single patient case-controlled study [J]. Neurogastroenterology and motility, 2019, 31(7): e13609-n/a.

[52] LEFAUCHEUR J P. Stroke recovery can be enhanced by using repetitive transcranial magnetic stimulation (rTMS) [J]. Neurophysiologie clinique, 2006, 36(3): 105-15.

2.4 ANTICIPATED RESEARCH OUTCOMES

Obtaining a sufficient sample size and collecting extensive data, personalized magnetic stimulation concurrent with specific tasks can effectively improve upper limb motor dysfunction in patients, ultimately enhancing the quality of life for stroke survivors. By integrating electric field simulation, brain state analysis, and functional connectivity, key brain regions involved in each type of neurological dysfunction can be identified. This will provide clearer guidance for future transcranial magnetic stimulation interventions targeting brain dysfunction in stroke patients.

2.5 RISK/BENEFIT ASSESSMENT

Criteria for Determining Research Success:

1. No serious side effects or adverse reactions occur in the subjects during the trial.
2. No serious quality issues arise with the experimental product during the trial.
3. Subjects demonstrate improved motor function following the intervention.

Safeguards Against Research Failure:

1. Investigators will strictly adhere to all standards outlined in the study protocol and enroll eligible participants.
2. Prior to trial initiation, investigators will receive training from the intervention provider's technical staff, familiarize themselves with the product manual and study protocol, and master the product's performance, operation methods, and precautions.
3. Investigators will diligently follow the Standard Operating Procedure (SOP) for the informed consent process, specifically informing subjects to promptly report any

discomfort to the physician. Simultaneously, investigators will closely monitor subjects for any adverse reactions or unexpected side effects, providing appropriate treatment and follow-up observation in a timely manner.

4. Investigators will promptly and accurately record all original data from subjects and conduct objective, truthful assessments.
5. The intervention provider will guarantee the supply of qualified experimental products, and the project sponsor will appoint qualified monitors to oversee the trial conduct.

2.5.1 KNOWN POTENTIAL RISKS

Medical Risks:

No serious adverse events caused by TMS have been reported to date. However, studies indicate that some transient and mild discomfort may occur following TMS, including headache, fatigue, drowsiness, neck pain, and anxiety.

Research-Related Risks:

1. The study involves patients aged 20 to 80, with the majority belonging to the middle-aged and elderly population. This demographic may have comorbidities such as hypertension or heart disease, which could correspondingly increase the overall research risk.
2. Participation in this study may require a greater investment of time and effort from patients compared to standard medical care. This includes, for example, weekly assessments, additional therapy sessions, and longer stays at the hospital.

2.5.2 KNOWN POTENTIAL BENEFITS

1. The interventions and assessments in this study are provided free of charge. Through regular one-on-one evaluations and follow-ups, patients can gain a more comprehensive understanding of their rehabilitation progress.
2. Participation in this clinical trial may lead to accelerated improvements in motor functions, as well as a reduction in psychological distress. These potential benefits may not be as readily achievable through conventional treatment alone.

3. By participating, patients will contribute to the advancement of medical science, ultimately helping many others in the future.

2.5.3 POTENTIAL RISK/BENEFIT ASSESSMENT

Measures to minimize risks for participants in this study include:

(1) Professional clinicians will continuously assess participants' physical and mental condition throughout the study. Any adverse events will be promptly addressed, which may include intervention or trial termination.

(2) The autonomy of participants will be fully respected. Should a participant decide to withdraw from the study, the trial will be immediately discontinued upon their request.

(3) All personal information will be kept strictly confidential, used solely for research analysis, and participants' privacy rights will be protected.

By participating in this study, patients will receive professional medical evaluations and personalized therapeutic interventions aimed at enhancing motor function. Throughout the study implementation, clinicians will continuously monitor participants' physical status, and researchers will rigorously protect the confidentiality of personal data, thereby minimizing participant risk. In conclusion, the risk-benefit balance supports conducting this study in the stroke patient population.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine whether there is an initial overall treatment effect among 3 groups: Concurrent rTMS (online) Non-concurrent rTMS (offline) Sham rTMS (sham) Efficacy is measured at day 105 after the initial of the intervention.	FM-UE scale	FM-UE scale is the primary outcome and a measure of motor impairment
	WMFT time score	WMFT is a secondary outcome and a measure of functional motor activity
	ARAT score	ARAT is a secondary outcome and a measure of functional motor activity
	MBI score	MBI is a secondary outcome and a measure of the quality of life
	PSQI score	PSQI is a secondary outcome and a measure of the quality of sleep
Secondary		
To confirm that the proposed intervention is safe, tolerable, and feasible to administer in a multi-site trial setting	Rate of adverse events	Safety needs to be monitored in a phase I+ II study
	Feasibility	How many subjects withdrew from the study as a result of adverse effects?
	Treatment completion rate	80% of treatment completion rate across sites without unexplained/unresolved variability is a measure of feasibility.
Tertiary/Exploratory		
To examine whether wCST-LL (structural assessment of integrity of descending motor tract) is correlated with changes in FM-UE scale, and evaluate the utility of these measures as biomarkers for subject selection criteria in the future confirmatory Phase II and III study.	sMRI (T1 and T2, Diffusion tensor imaging MRI)	Functional and structural aspects of motor tracts and the interaction between motor regions change after intervention. This change can be captured by comparing TMS and MRI measures before and after treatment
To examine changes in functional connectivity between different regions related to motor circuits.	fMRI	fMRI provides a functional measure of changes in functional connectivity within motor-related circuits and serves as an indicator that correlates with improvements in behavioral scales.
The objective is to identify EEG-based biomarkers and determine whether they exhibit a correlation with the behavioral scales.	EEG	Resting-state EEG can be used to test for changes in band power. For task-state EEG with TMS-EEG co-registration, changes in the N100

		component of the Event-Related Potential (ERP) can be examined. In addition, other EEG components should also be analyzed to determine if they exhibit similar alterations.
To investigate whether there is an improvement in the upper limb motor trajectory of patients.	Videos	The patient's motor trajectory can be measured by analyzing the trajectory graph generated during motor training. Additionally, both the time and the variance of the movement trajectory can be calculated.
or MEPs (functional assessment of integrity of descending motor tract)	MEPs	MEPs is a functional measure integrity of descending motor tract using TMS that has shown promise in predicting motor outcomes, but two of them have not been collected and compared in the same study.
To investigate whether short-term or long-term improvements occur in the descending motor pathways.	EMG	It is a metric designed to compute changes in EMG amplitude, thereby assessing muscular alterations over both the short and long term.

4 STUDY DESIGN

4.1 Overall Design

Hypotheses

Based on preliminary investigations, this study hypothesizes that:

Transcranial magnetic stimulation synchronized with specific tasks can significantly enhance the activation of targeted neural circuits, thereby effectively promoting functional recovery in stroke patients. By integrating personalized electric field simulation, brain activity state analysis, and functional connectivity analysis, linear relationships between specific brain regions and behavior can be identified. This allows for the reverse-engineering of an optimized stimulation protocol to enable closed-loop modulation of motor function recovery in stroke patients.

Compared to stroke patients receiving sham stimulation or conventional rehabilitation, those receiving personalized stimulation protocols designed using electric field simulation based on their neuroimaging data, with TMS synchronized to specific tasks for closed-loop

modulation of upper limb motor function, will demonstrate significantly improved rehabilitation outcomes.

Compared to non-personalized online TMS stimulation (using a positioning cap for target localization), online personalized TMS stimulation (synchronizing specific tasks with precise TMS targeting) will lead to greater improvement in motor function, particularly in patients without Motor Evoked Potentials (MEPs).

Study Design

This study employs a non-randomized, multiple control group trial design. A total of 60 participants meeting the inclusion criteria will be recruited and randomly assigned to one of three groups: an Online Stimulation group, an Offline Stimulation group, and an Online Sham Stimulation group.

In the Online Stimulation Group, the TMS parameters will be continuously adjusted based on electric field simulations informed by the patient's baseline data and electromyography (EMG) changes during treatment, enabling personalized intervention. Furthermore, TMS stimulation will be triggered by EMG feedback at the self-initiated start of a specific task and will stop immediately and simultaneously upon task cessation or completion via EMG feedback, achieving real-time synchronization.

In the Offline Stimulation Group, patients will receive TMS stimulation first, followed by the completion of the specific task.

In the Sham Stimulation Group, patients will receive sham TMS stimulation concurrent with the specific task. Similar to the online group, the sham stimulation will be triggered and stopped by EMG feedback corresponding to task initiation and completion.

The Online Stimulation Group will undergo MRI scans at baseline (Week 0, day 0) and after the 2-week intervention period (Week2, day 15). Clinical assessments will be conducted weekly after the intervention (W1-day 6, W2-day 15).

Intervention Methods

The intervention period for all three groups will last 2 weeks.

Online Stimulation Group: TMS parameters will be personalized through continuous adjustment based on electric field simulations using the patient's baseline and treatment-phase data changes. TMS stimulation will be synchronized with specific tasks, triggered

and terminated in real-time via EMG feedback upon task initiation and completion. Interventions will occur 5 days per week for 2 weeks. Upper limb motor ability will be assessed once per week for 2 weeks.

Offline Stimulation Group: TMS stimulation will be administered before the task begins; the task will be performed after the stimulation is complete. Interventions will occur 5 days per week for 2 weeks.

Sham Stimulation Group: Patients will receive sham TMS stimulation concurrent with the specific motor task, identical in procedure to the Online Stimulation group except for the sham nature of the TMS. Interventions will occur 5 days per week for 2 weeks.

During each session, patients will sit in a comfortable, adjustable recliner with a headrest. The chair will be adjusted to a suitable angle, maintaining a comfortable semi-recumbent position with minimal head and neck movement. EMG measurement equipment will be fitted; patients with upper limb motor impairment will wear the device on their affected arm.

Patients will be instructed to minimize head movement during treatment. Those with upper limb motor impairment will place the affected limb on an adjustable tabletop in front of them to perform motor tasks, while the unaffected limb will rest at their side.

TMS will be delivered using a MagPro device with a 90-mm figure-of-eight coil. All TMS interventions and assessments will be conducted in a quiet room by a trained researcher, with another researcher responsible for evaluations.

MRI Scanning Sequences

gre_scout

t1_gre_fsp_3d_sag_iso1

t2_mx3d_flair_sag_iso1

epi_dti_dir64_PA_1.5mm

epi_dti_b0_AP

epi_bold_mb6_iso2.4mm_950 / Multi-echo BOLD

epi_bold_b0fieldmap

_____Active_____

epi_bold_mb6_iso2.4mm_950

epi_bold_b0fieldmap

Note regarding the Multi-echo BOLD sequence: This sequence was independently developed by United Imaging Healthcare (Shanghai, China) and has not yet been officially released to the market. It acquires multiple echo signals (TE ≥ 3) in a single excitation, which can reduce signal loss in areas with short T2* values, better identify noise signals, and enhance the reliability of brain function analysis results.

4.2 Definition of Study Endpoints

The primary study endpoint is defined as the 3-month follow-up time point after stroke patients complete the 2-week TMS treatment regimen. This endpoint is established to assess whether the therapeutic effects observed immediately post-treatment are sustained in the longer term.

4.3 Sample Size Determination

We first conducted a single-center, randomized, double-blind, controlled trial at Ruijin Hospital. Sample size was estimated using PASS 2021 (two-sided α=0.025), assuming an effect size of 1.5 in FM-UE between concurrent and sham rTMS, requiring 27 participants (9 per group, three groups) to achieve 80% power. With an anticipated 10% dropout, the target enrollment was 30. However, stratification by MEP status resulted in insufficient subgroup sizes. To assess the generalizability of individualized concurrent rTMS in MEP-negative participants, the trial will be expanded to a multicenter study including Yangzhi and Changhai Hospitals, where MEP-negative patients will be preferentially enrolled. Ultimately, 36 participants will be enrolled in Group I , 14 in Group II , and 10 in Group III .

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Each subject must meet all of the following criteria to participate in this study:

1. The patient is first diagnosed with stroke through neurological examination, CT or MRI scan; and
2. The vital signs are stable and there is a certain degree of upper limb motor dysfunction; and
3. The age is between 20 and 80 years old; and
4. The cognitive ability is not significantly affected and the patient can cooperate with

various examinations and assessments, with a MMSE score ≥ 20 points; and
5. There are no serious complications (such as pneumonia, heart failure, urinary tract infection or malnutrition); and
6. There is no pathological condition that is a contraindication for TMS in the medical history (for example, patients with metal in the brain, such as aneurysm clips, patients with a cardiac pacemaker, pregnant women, or those with a history of epileptic seizures); and
7. The patient or guardian agrees to sign the informed consent form.

5.2 EXCLUSION CRITERIA

Each subject who meets any of the following criteria will be excluded from the study:
1. patients with severe heart, lung, liver, kidney disease, malignant tumors, and a history of mental illness;
2. a history of seizures or substance misuse;
3. Be inability to complete motor training tasks due to visual, auditory, cognitive, or speech impairments;
4. presence of metal implants, pacemakers, cranial defects, etc., making TMS or MRI impossible;
5. motor disorders caused by any other degenerative or neurological diseases.

5.3 SCREEN FAILURES

Screen failure is defined as a subject that signs a consent form but not allocated. The primary reason for screen failure is mainly due to inclusion/exclusion criteria. Minimal information includes demographics, details of screen failure, and which eligibility criteria were not met.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

We plan to recruit 60 stroke subjects from approximately 3 sites over a 3-year period. This section focuses on potential challenges related to insufficient subject recruitment or retention, the measures we have implemented, and other quality control challenges that may arise during the trial.

(1) Detailed Pre-submission Epidemiological Assessment, Feasibility Survey, and Site Selection:

We have given full consideration to the motivation of potential centers to participate in the trial. We will ultimately select approximately 3 sites that meet the trial requirements and have responded positively to our surveys. In the initial phase, the trial will commence at one site, with an estimated recruitment rate of 1 to 2 subjects per month. Recruitment will later expand to 2 additional sites to ensure the successful enrollment of 60 patients within the 3-year recruitment period.

(2) Prudent Budgetary Considerations:

A study coordinator will be dedicated to the initial screening of potential subjects at the trial's outset. In accordance with the number of interventions in this study, subjects will receive adequate subsidies for transportation and parking expenses to alleviate their financial burden, thereby enhancing both recruitment and retention rates.

(3) Strict Monitoring of Subject Recruitment and Retention:

We will employ a risk-based, centralized monitoring mechanism to quickly identify sites deviating from the protocol. We will work closely with each clinical research center to promptly address any issues that may arise during subject recruitment and retention. Centers that fail to recruit subjects as planned or experience a higher-than-expected dropout rate after recruitment will have their trial participation suspended until effective corrective actions are developed and implemented. If the issues cannot be resolved, the site will be removed from the candidate list and replaced by a new site that meets the trial requirements.

(4) Multi-layered Quality Control Process:

First, necessary on-site workshops will be conducted for investigators and team members at each research site, followed by annual online certification and re-certification for primary outcome assessors.

Second, if needed, co-investigators will conduct selected site visits to ensure strict adherence to the TMS-synchronized motor task protocol.

Third, the assessment process for primary efficacy outcomes will be video-recorded for potential third-party evaluation.

Finally, blinding procedures will be strictly enforced to ensure the independence of subjects and outcome assessors, and to maintain blinding regarding treatment allocation.

(5) Sharing of Best Practices:

We will plan for "share and learn" sessions on the topic of patient recruitment and quality control within enrolling sites as well as with other trials. We will promote effective subject recruitment and quality control strategies throughout the study period.

Specific Recruitment Plans:

Subjects will be identified in inpatient hospital settings, outpatient clinics, or research subject databases.Clinicians involved in the subject's clinical care and research staff will initially identify potential subjects, assess their eligibility against the inclusion criteria, and then introduce the study. If the subject is interested, more detailed information will be provided. After enrollment, the study coordinator will use approved communication channels (e.g., phone, postal address) to regularly remind subjects of their scheduled appointment dates, times, and locations.

At Yangzhi Rehabilitation Hospital and Changhai Hospital, priority for recruitment will be given to stroke patients without MEPs (Motor Evoked Potentials). This aims to more effectively target the non-MEP population for testing the efficacy and safety of the personalized targeting method.

Potential subjects interested in participating will undergo a pre-screening interview conducted by a clinician or researcher via telephone or telemedicine/video software. This process takes approximately 10-15 minutes and involves initial screening based on the following questions:

1. Are you between 20 and 80 years old?
2. Did your stroke occur more than 14 days ago?
3. Were you clinically diagnosed with a stroke via CT or MRI?
4. Can you perform at least minimal movement or lifting of the affected upper limb?
5. Can you understand Mandarin and communicate, even with some difficulty?
6. Can you commit to 10 study sessions (5 days/week for 2 weeks, ~30-50 minutes each)?

7. Do you have any metal implants (including shrapnel injuries) or electronic devices in your body?
8. Can you provide a list of all medications you are currently taking?
If a subject answers "yes" to all 8 questions, they will be invited to participate, undergo a baseline assessment, provide informed consent, and undergo further confirmation of eligibility. We will recommend using our pre-trained study protocol for screening to ensure standardization and consistency of the screening questions. Conducting pre-screening before the formal face-to-face baseline visit aims to effectively reduce screen failures due to ineligibility.

5.5 SUBJECT RECRUITMENT

Eligible participants for this study will be recruited from the inpatient populations of the Rehabilitation Medicine Departments at Shanghai Ruijin Hospital, Shanghai Yangzhi Rehabilitation Hospital, and Shanghai Changhai Hospital. Primary recruitment will be conducted through physician referrals.

5.6 SUBJECT ALLOCATION METHOD

This pilot study adopts a non-randomized allocated design. Participants will be allocated using a convenience allocation method, where assignment to either the intervention or control group is based on the order of enrollment. Recruitment priority will be given to fulfilling the sample size requirements of the intervention group.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The rTMS treatment was delivered using two different TMS stimulators: a YRD NS 5000 (YRD Inc., Wuhan, China) equipped with a 90-mm figure-of-eight coil in Ruijin and Yangzhi Hospitals, and a MagPro X100 (MagVenture, Farum, Denmark) equipped with a 90-mm figure-of-eight coil in Changhai Hospital. The participants were subjected to a two-week treatment with rTMS (total 750 pulses each session, single pulse duration 340 μs ± 20 μs, 5 sessions for 2 weeks). Pulsing protocol: 3 blocks/day with 2-min intervals, 10 x 25 pulses (at 5Hz) per block with 10-s intervals. Stimulation intensity was set at 80% of RMT. For MEP-negative participants, stimulation was delivered at 80% of maximum stimulator output, as previously described.

Repetitive Transcranial Magnetic Stimulation (rTMS): rTMS will be delivered to study subjects using an rTMS coil. The stimulation target is the ipsilesional (affected hemisphere) primary motor cortex (M1), at a high frequency of 5 Hz. Each stimulation train lasts 5

seconds, followed by an inter-train interval of 10 seconds. This pattern is repeated 10 times to form one block. A total of 3 blocks will be administered per session, resulting in 750 total pulses. A rest interval of 2 minutes will be provided between each block.

Task Training: The training protocol consists of a baseline phase and an intervention phase. The baseline phase comprises 10 blocks of tasks. Each block involves 10 movement training trials. Different motor paradigms will be designed based on the patient's specific level of motor impairment, including tasks such as pulling small balls (of varying sizes and shapes), raising and lowering the arm, and moving the arm forward/backward or left/right.

This is followed by the intervention phase, involving 3 blocks of task training. For the concurrent rTMS or sham rTMS group, these 3 blocks are performed. Each block consists of 10 movement training trials. Each trial lasts 5 seconds, followed by a 10-second rest interval. A 2-minute rest interval is provided between each block.

rTMS applied concurrently with motor action：During the 2-week treatment period (1 session/day, 5 days/week), all patients in each treatment group received the following procedures in each session: First, the patient was positioned semi-reclined with neck and head support to minimize motion artifacts. Second, surface EMG (eight channels) and accelerometer sensors were affixed to the wrist of the affected arm. Third, the patient was asked to perform a ball-pulling action for 10 repetitions (with 10-sec inter-trial intervals) and for the maximal altitude of the arm achievable by patient. This provided the baseline motor capability for each session. Fourth, for patients in Group I (concurrent rTMS) and III (sham rTMS), 2-min after baseline motor capability test, the patient performed 3 blocks of 10 ball-pulling trials (~5 sec per trial, 10-sec inter-trial interval). During the onset of each pulling action, rTMS was triggered automatically by accelerometer signal and the rTMS was terminated at the end of the pulling trial. A 2-min rest was allowed between blocks. For sham stimulation, the TMS coil was placed perpendicular to the scalp tangential plane (i.e., rotated 90°), which has been shown to significantly reduce effective cortical stimulation while preserving auditory and somatosensory artifacts (Rossi et al., 2009; Duecker & Sack, 2015; Lisanby, et al., 2001). For patients in Group II (non-concurrent rTMS): After completing the baseline motor capability test, the patient first received 3 blocks of rTMS alone (10 pulses/block, 5-sec duration, 10-sec intervals) without pulling trials, followed by 3 blocks of pulling trials similar to that for Group I and III patients in the absence of rTMS. For a minority of patients that could not perform the ball-pulling task, we used other easier tasks such as raising the arm, horizontally moving the arm, or reaching and grasping objects of different shapes.

TMS synchronization protocol: Arm acceleration was synchronously recorded using the LanMao M8 device(manufactured by Quanlan Company located in Shanghai, China) during the online task. The start and end of arm movements were determined by applying thresholds to the mean and variance of acceleration values within a fixed time window. Upon detecting the start or end of a movement, level signals were sent to the TMS device to initiate or terminate stimulation, ensuring synchronization between the online task and rTMS.

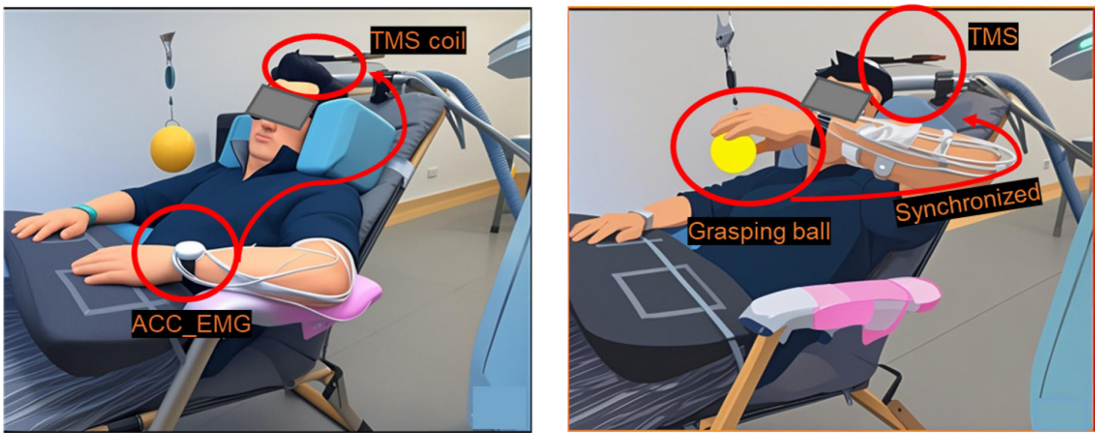


Fig.1 Experimental setup for transcranial magnetic stimulation (TMS) applied concurrently with motor action.

Left Panel: The participant is positioned in a reclining chair, wearing a virtual reality headset. A TMS coil is placed over the relevant cortical area. On the arm, devices for ACC (Accelerometer) and EMG (Electromyography) are attached to monitor movement and muscle activity, respectively. Right Panel: The participant performs a grasping ball task. The TMS stimulation and the grasping action are synchronized, as indicated. The experimental setup remains consistent with the left panel, with the TMS coil still in place and the participant engaged in the motor task.

6.1.2 DOSING AND ADMININSTRATION

Subjects will be allocated to the concurrent rTMS, non-concurrent rTMS, or sham rTMS group. The investigator, therapist, and the subject will be blinded to the group assignment. All subjects will be prepared identically.

For subjects enrolled from Ruijin Hospital, a simple randomization method will be used for allocation. For subjects enrolled from Yangzhi Rehabilitation Hospital and Changhai Hospital, priority will be given to recruiting patients without MEPs (Motor Evoked Potentials) to test the efficacy of the personalized, precisely targeted intervention approach.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

To ensure that a novel stimulation paradigm can ultimately benefit patients, it is first necessary to demonstrate its safety and efficacy, followed by obtaining the appropriate approval from the relevant regulatory authorities. Clinical trials serve as the critical pathway for translating these innovative technologies to subjects and providing the evaluation data necessary for regulatory approval.

In clinical research, these products are still in the experimental stage; consequently, the manner of their use differs from that of established methods in routine clinical practice. After all, the safety and efficacy of using non-routine protocols outside of standard clinical care are not fully validated, which may pose potential risks to subjects. Furthermore, failure to properly manage research materials could not only compromise the reliability of the trial data but also lead to the complete termination of the study.

Therefore, both the sponsor and the investigator share responsibility for the management

of the device. Should any issues arise, both parties will be held accountable.

Accountability:
1. The site investigator is responsible for the investigational products/device accountability at the trial site.
2. The site investigator product/device should be stored in accordance with the applicable regulatory requirements.
3. The site investigator should ensure that the investigational product/device are used only in accordance with the approved protocol.

6.2.2 PRODUCT STORAGE AND STABILITY

The device shall be accessible only to authorized members of the study team. The equipment, including both the TMS and the ACC-EMG devices, must be inspected before and after each treatment session. Each study site will be equipped with two sets of ACC-EMG devices for this purpose: one set for active use and the other serving as a backup. In the event of any issues, they must be immediately reported to the site's principal investigator, and the manufacturer shall be notified promptly.

6.2.3 PREPARATION

The ACC-EMG devices will be directly distributed to each participating hospital to complete system preparation and testing. On-site staff will participate in necessary on-site training workshops to master the operation of the synchronized rTMS system.

6.3 MEASURES TO MINIMIZE BIAS: ALLOCATION AND BLINDING

At Ruijin Hospital, participants were randomly assigned to the three rTMS treatment groups (I, concurrent; II, non-concurrent; III, sham) using simple randomization. In contrast, Yangzhi and Changhai Hospitals preferentially enrolled patients that did not exhibit MEP (MEP-negative) into the Group I to specifically assess the generalizability of individualized concurrent rTMS in individuals with more severe corticospinal tract damage. This study employed a double-blind design: both participants and outcome assessors were blind to which group the patients were assigned. Recruitment and baseline assessments were conducted by attending physicians at each site. To prevent assessment bias, therapists were prohibited from evaluating the clinical outcomes of their own patients.

6.4 STUDY INTERVENTION COMPLIANCE

Compliance with study treatment will be centrally monitored.

7 STUDY INTERVENTION DISCONTINUATION AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Patients will complete routine assessment forms (covering sleep, anxiety, depression, etc.) before and after each intervention session.

Intervention may be discontinued for the following reasons:

(1) The patient voluntarily withdraws for personal reasons.

(2) The patient experiences headaches, other new symptoms, or worsening of pre-existing symptoms (e.g., insomnia).

(3) The patient exhibits a persistent decline in motor function following the intervention.

The reason for intervention discontinuation from the study will be recorded on the Case Report Form (CRF). The subject should continue to be followed and have all study assessments.

7.2 SUBJECT WITHDRAWAL FROM THE STUDY

The investigator may discontinue or withdraw a subject under the following circumstances:

(1) The subject demonstrates clear unwillingness to cooperate with the TMS intervention.

(2) Adverse reactions occur during TMS, or structural MRI reveals abnormalities deeming participation unsuitable.

(3) Disease progression renders the patient unsuitable for TMS.

(4) The patient fails to achieve the intervention frequency of 5 sessions per week for any reason.

(5) New exclusion criteria emerge during the study, and the subject meets these new criteria.

Reasons for the discontinuation/withdrawal of any subject will be documented in the Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention will be replaced. Subjects who sign the informed consent form, are randomized, receive the study intervention, and are subsequently withdrawn will not be replaced.

7.3 LOST TO FOLLOW-UP

If a subject is unable to complete the follow-up procedures specified by the study during the follow-up period, the reason for this loss to follow-up will be carefully documented. Concurrently, the patient's clinical status will be monitored for a period to ensure their safety after exiting the study.

1. **Rigorous Subject Screening:** During the screening phase, potential subjects'

personal and environmental factors will be preliminarily assessed to select those with good anticipated compliance.

2. **Comprehensive Subject Education on Compliance:** Before signing the informed consent form, subjects will be thoroughly informed. Investigators will use various methods to educate subjects about the study's purpose, the importance of adhering to the protocol, and the potential consequences of non-compliance.
3. **Rational Scheduling of Trial Procedures:** The trial workflow will be arranged to minimize patient waiting time at the hospital and prevent frustration. If extended waiting is unavoidable, patients will be accommodated in a relatively quiet environment with their privacy protected.
4. **Proactive Management of Adverse Events (AEs):** During the trial, subjects will be informed about potential adverse reactions and the corresponding management measures. This aims to prevent subject dropout due to mild or expected AEs.
5. **Enhanced Investigator Training:** Investigators will receive comprehensive training to ensure a full understanding of the clinical trial, familiarity with the protocol, and the ability to accurately and appropriately address patient inquiries. Building a strong investigator-patient relationship is key to fostering patient confidence in the trial.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Primary Objectives (Efficacy Outcomes): To determine whether there is an overall persistent intervention effect among 3 groups (concurrent rTMS, non-concurrent rTMS, and sham rTMS) at day 105 after initiation of the intervention in the **Fugl-Meyer Upper-Extremity (FM-UE) scale**, a measure of motor impairment. FM-UE scale consists of a 33-item assessment which provides a global assessment of UE motor impairment. A rater observes 30 voluntary UE motions and 14 voluntary LE motions, 6 tendon tap responses, and provides an ordinal rating (2=near normal ability/response, 1=partial ability, 0=unable to perform/no response). FM-UE scale is a proven scale with excellent intra-rater reliability (0.99), inter-rater reliability (0.99), test-retest reliability (0.94 –0.99), and internal consistency (0.97).

Secondary efficacy outcome measures include the Wolf-Motor-Function-Test (WMFT) or The Action Research Arm Test (ARAT), a measure of functional motor activity, the Modified Barthel Index (MBI), a measure of the quality of life, and Pittsburgh sleep quality index (PSQI), a measure of the quality of sleep at day 105 after initiation of the intervention.

These efficacy outcomes will be collected at the baseline, day 15, day 45 and day 105.

Wolf-Motor-Function-Test (WMFT): WMFT quantifies upper extremity (UE) motor ability through timed and functional tasks. It is a 17-item assessment providing a measure of UE functional ability. In this test, participants are timed as they complete 17 tasks that involve movements and interactions with objects (e.g., placing hand on table, picking up a soda can). These activities begin with those involving only shoulder movements and progress to those requiring distal control of fine motor movements. In addition, subject's movement will be assessed for meeting "Essential Elements" (specific elements) that must be accomplished in order for the task to be deemed complete" vs. "Desired Elements" which are other qualitative elements that should be included in the task but are not necessary for completion" (please refer to details in the Outcome Assessments MOP).

The Action Research Arm Test (ARAT): The ARAT is used to assess upper limb motor function and serves as a valuable supplement to the FMA-UE. The ARAT score comprises four subscales: grasp (6 tasks, score 18), grip (4 tasks, score 12), pinch (6 tasks, score 18), and gross movement (3 tasks, score 9). The total ARAT score is the sum of the scores from the four subscales. It has demonstrated good validity and sensitivity to treatment-related gains post-stroke.

Modified Barthel Index (MBI): The MBI is used to assess activities of daily living, measuring changes in the patient's quality of life. It consists of 10 items: bowel and bladder care, feeding, grooming, bathing, dressing, toileting, ambulation, transfers, and stair climbing. The total score ranges from 0 to 100. It has been proven to be a valid, responsive, and reliable measure for assessing self-care activities in stroke patients.

Pittsburgh Sleep Quality Index (PSQI): The PSQI was developed in 1989 by Dr. Buysse and colleagues at the University of Pittsburgh, USA. It is suitable for assessing sleep quality in individuals with sleep disorders, mental disorders, and also in the general population. It consists of 18 items grouped into 7 components. Each component is scored on a 0-3 scale. The global PSQI score is the sum of the component scores, ranging from 0 to 21; a higher score indicates poorer sleep quality. It takes subjects approximately 5-10 minutes to complete.

8.1.2 EXPLORATORY AIMS MEASURES

8.1.2.1 IMAGING MEASURES

We will examine changes in functional connectivity (FC) between brain regions associated with motor circuits. FC is primarily derived from fMRI data and involves analyzing the changes in functional connections between two or more brain regions.

MRI Data Acquisition

Data were acquired on a UIH uMR890 3T scanner (United Imaging, Shanghai, China) at the Ruijin Hospital using a UIH 48-channel head coil. High-resolution T1w images were obtained using an GRE-FSP sequence with the following parameters: time repetition (TR): 7.5ms; time echo (TE): 3 ms; inversion time: 750 ms; flip angle (FA): 8°; field of view (FOV): 256 mm; slice thickness: 0.8 mm; plane resolution: 0.5mm x 0.5mm.

High-resolution T2w images were obtained using an FSE-MX sequence with the following parameters: TR: 6500 ms; TE: 413.56 ms; inversion time: 1938 ms; FA: 54°; FOV: 256 mm; slice thickness: 0.8 mm; plane resolution: 0.67mm x 0.67mm.

Multi-echo, multi-band resting-state fMRI scans were collected using a T2*weighted echo-planar sequence covering the full brain (TR: 2056 ms; TE1: 14.9 ms, TE2: 40.4 ms, TE3: 65.9 ms, TE4: 91.4 ms, and TE5: 116.9 ms; FOV: 230 mm; FA: 68°; slice thickness: 2.4 mm; plane resolution: 2.4mm x 2.4mm; slice number: 78; AP phase encoding direction; multi-band acceleration factor: 6) with 450 volumes acquired per scan for a total acquisition time of 15 min and 25 s. This sequence was provided by UIH and was evaluated by the relevant institutions (see Supplementary).

Single-echo, multi-band resting-state and task-state fMRI scans were collected using a T2*-weighted echo-planar sequences covering the full brain (TR: 914 ms; TE: 31 ms; FOV: 230 mm; FA: 68°; slice thickness: 2.4 mm; plane resolution: 2.4mm x 2.4mm; slice number: 78; AP phase encoding direction; multi-band acceleration factor: 6) with 700 volumes acquired per scan for a total acquisition time of 10 min and 40 s.

Diffusion-weighted image (DWI) scan was collected using a 2D echo-planar imaging sequence with the following parameters: TR: 3859 ms; TE: 70 ms; FOV: 210 mm; FA: 90°; slice thickness: 1.5 mm; plane resolution: 1.5mm x 1.5mm; PA phase encoding direction; b: 1000 s/mm2; 64 directions with 2 b0 images, with b0 images acquired in AP phase encoding directions for subsequent DWI distortion correction.

Task fMRI design

During scanning, patients performed a grip task using their impaired hand and healthy hand: grip at 0.25 Hz for 24 seconds, followed by a rest period of 20 seconds. This is repeated for three rounds with the left hand, then three rounds with the right hand, followed by four rounds with the left hand and four rounds with the right hand (alternating with 20 s rest). A total of 7 blocks each hand, and 14 rest blocks were performed.

8.1.2.2 EEG MEASURES

We will primarily measure both resting-state EEG and task-state EEG. The resting-state EEG recording involves a 5-minute session with eyes closed and a 5-minute session with eyes open. The task-state EEG protocol includes 80 single-pulse TMS deliveries to the affected hemisphere of M1 and 80 single-pulse TMS deliveries to the unaffected hemisphere of M1, administered at a frequency of 0.25 Hz and an intensity of 100% of the Resting Motor Threshold (RMT).

For the resting-state EEG, the analysis will focus on changes in band power before and after the intervention. For the task-state EEG, the analysis will concentrate on changes in the amplitude of various components of the TMS-evoked potential (TEP) before and after the intervention.

With referenced to previous studies, TMS-EEG recording was carried out using a TMS-compatible EEG equipment (BrainAmp, BrainProducts GmbH, Munich, Germany) with 64 Ag/AgCl electrodes mounted according to the international 10–10 system. The raw signals were online-referenced to FCz, grounded to AFz, digitized at a sampling rate of 5 kHz, and online-filtered below 2 kHz. The impedance between the scalp and the electrodes was maintained below 10 kΩ to optimize the signal-to-noise ratio. During recording, 80 TMS pulses were applied to the M1, with intertrial interval 0.4 Hz. The stimulation intensity for impaired hemisphere is 100% output of TMS, and for healthy hemisphere is 100% of the RMT (resting motor threthold). RMT was defined as the minimum intensity (measured as the % of the maximal stimulator output) that could elicit peak-to-peak MEP amplitudes higher than 50 μV in at least five out of 10 trials. Because the ipsilesional MEPs of some patients were not tested, we will locate the ipsilesional M1 using target deduced by EF simulations. To suppress auditory-evoked potentials produced while the coil discharged, all participants will wear an inserted earphone, and white noise will be played (7). The volume of the noise will be as loud as it could be for all patients. To minimize TMS-decay artifacts, a thin piece of foam (3 mm thick) will be placed underneath the coil to prevent direct contact with the electrodes[7], and the direction of lead wires near the coil will be rearranged so that they can be perpendicular to the coil. To reduce eye movements, participants will be required to gaze at a black cross with a white background, approximately 2 m away.

8.1.2.3 MEP MEASURES

We determined the Resting Motor Threshold (RMT) through MEP measurements at baseline, day 6, day 15, day 45, and day 105. This was primarily done to compare changes in cortical excitability of both the affected and unaffected hemispheres before and after the intervention.The resting motor threshold (rMT) is defined as the lowest TMS stimulus intensity capable of eliciting a recordable motor evoked potential (MEP) from the abductor

pollicis brevis muscle, with a minimum amplitude of 50 μV (peak-to-peak). It reflects the overall excitability of the motor system, encompassing both cortical and spinal levels. For analytical purposes, this parameter will initially be categorized as a binary variable (Present = 1 or Absent = 0). Present = 1 indicates that a recordable MEP is obtained, whereas Absent = 0 indicates the absence of a measurable MEP. The presence of MEPs in stroke patients typically signifies partial preservation of the corticospinal tract (CST). Conversely, the absence of MEPs on the affected limb, despite stimulation of the lesional hemisphere, suggests severe CST disruption. rMT will also be measured from the non-paretic hemisphere, serving as a reference for inter-hemispheric comparison. Based on prior studies, approximately 20% of participants are expected to lack recordable MEPs. If fewer than 10% of patients fall into the "Absent" category, rendering the binary analysis underpowered, an asymmetry index will be used instead, calculated as:

$$\text{Asymmetry Index} = \frac{|\text{rMT}_{\text{affected}} - \text{rMT}_{\text{unaffected}}|}{\text{rMT}_{\text{affected}}}$$

For participants without measurable MEPs, the rMT will be assigned a value of 100% maximum stimulator output (MSO). For those with measurable responses, the rMT—defined as the %MSO required to elicit a 50 μV MEP using the Parameter Estimation by Sequential Testing (PEST) algorithm—will be compared between baseline and post-therapy sessions.

8.1.2.4 EMG MEASURES

During the intervention period, electromyographic (EMG) activity from eight muscles [including Deltoid (Del), Biceps brachii (Bic), Triceps brachii (Tri), Brachioradialis (Brv), Extensor carpi radialis longus (ECRL), Extensor carpi ulnaris (ECU), Extensor digitorum (ED), and Flexor carpi radialis (FCR)] will be measured daily, and EMG will be recorded throughout the entire intervention session. This data will be used to observe the short-term and long-term effects of rTMS.

Acquired EMG data from 8 muscles using the LanMao M8 setup (Quanlan, Shanghai, China). The 8 muscles are: 1. Deltoid muscle 2. Biceps brachii 3. Triceps brachii 4. Brachioradialis 5. Extensor carpi radialis 6. Extensor carpi ulnaris 7. Extensor digitorum 8. Flexor carpi ulnaris. Sample size of EMG raw data was 90 Hz.

The skin surface is cleaned with alcohol before attaching the electromyography electrodes to the respective muscles to collect EMG data. The Online/sham group consists of baseline, task1, task2, and task3. The offline group consists of baseline, TMS1, TMS2, TMS3, as well as task1, task2, and task3.

8.1.2.5 CV MEASURES

During the intervention period, video recordings of the patients will be made daily to measure their motor trajectories. These recordings will similarly be used to assess both

the short-term and long-term effects of rTMS by comparing data collected before and after the intervention period. Based on previous studies , we utilized a depth camera (Kinect, produced by Microsoft Corporation, United States) to identify the facial features and limb parts of patients, including fingers. Programs were designed using Python to track the process from when patients raised their hands to when they grasped the ball, pulled it down, and then put the ball back to its original position. The time taken for this process was calculated, which was regarded as the time for patients to complete one task. Meanwhile, the motion trajectories of the fingers on the affected side of the patients were drawn.

8.2 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

A rTMS questionnaire will be used in each intervention session to assess the common adverse events or tolerability issues associated with its use. Adverse events, including serious adverse events will be collected during the intervention period. Only serious adverse events or clinically related (possibly or definitely) adverse events will be collected after intervention period. Please refer to AE reporting section for details.

8.2.1 DEFINITION OF ADVERSE EVENTS (AE)

An adverse event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation subject administered study intervention and that does not necessarily have a causal relationship with this intervention. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational intervention, whether or not considered related to the investigational intervention. Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject.

8.2.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A SAE is any untoward medical occurrence that:

1. results in death;
2. is life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
3. requires inpatient hospitalization or causes prolongation of existing hospitalization;
4. results in persistent or significant disability/incapacity;
5. is a congenital anomaly/birth defect;
6. is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above. Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

The definition of SAE excludes the following hospitalizations:

1. A visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event);
2. Elective surgery, planned prior to signing consent;
3. Admissions as per protocol for a planned medical/surgical procedure;
4. Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy);
5. Medical/surgical admission other than to remedy ill health and planned prior to entry into the study (appropriate documentation is required in these cases);
6. Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

8.2.3 CLASSIFICATION OF AN ADVERSE EVENT

8.2.3.1 SEVERITY OF EVENT

The severity of AEs will be reported using the grading system outlined in the NCI Common Terminology Criteria for Adverse Events Version 4.03 (CTCAE). The CTCAE provides a grading (severity) scale for each AE term and AEs are listed alphabetically within categories based on anatomy or pathophysiology. The CTCAE displays Grades 1-5 with unique clinical descriptions of severity for each AE based on this general guidance:

The complete definitions of these grades are:
Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated AE.
Gradeb2:Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).
Grade 3:Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
Grade 4: Life-threatening consequences; urgent intervention indicated.
Grade 5: Death related to AE.

8.2.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the subject based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.
1. Unrelated: The temporal relationship between treatment exposure and the adverse event is unreasonable or incompatible and/or adverse event is clearly due to extraneous causes
(e.g., underlying disease, environment);
2. Unlikely: Must have both of the following 2 conditions, but may have reasonable or only tenuous temporal relationship to intervention:

Could readily have been produced by the subject’s clinical state, or environmental or other interventions.

 Does not follow known pattern of response to intervention.

3. Reasonable Possibility: Must have at least 2 of the following 3 conditions:

 Has a reasonable temporal relationship to intervention.

 Could not readily have been produced by the subject’s clinical state or environmental or other interventions.

 Follows a known pattern of response to intervention.

4. Definitely: Must have all 3 of the following conditions:

 Has a reasonable temporal relationship to intervention.

 Could not possibly have been produced by the subject’s clinical state or have been due to environmental or other interventions

 Follows a known pattern of response to intervention.

8.2.3.3 EXPECTEDNESS

In general, expected adverse reactions are AEs that are known to occur for the study intervention being studied and should be collected in a standard, systematic format using a grading scale based on functional assessment or magnitude of reaction. Identify the source of the reference safety information used to determine the expectedness of the AE (e.g., IB, approved labeling). Expectedness is assessed based on the awareness of AEs previously observed, not on the basis of what might be anticipated from the properties of the study intervention. An AE will be considered

unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.2.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

During the assessment and examination processes, subjects may experience clinical adverse events. In the event of any adverse event (including significant adverse events), the following details must be meticulously documented in the Case Report Form (CRF): time of onset, clinical manifestations, management procedures, duration, outcome, and relationship to any relevant examinations.

For any Serious Adverse Event (SAE), a Serious Adverse Event Report Form must be completed and submitted to the Sponsor, the Ethics Committee, and the relevant health administrative authorities within 24 hours.

8.2.5 ADVERSE EVENT REPORTING

NOT APPLIED

8.2.6 SERIOUS ADVERSE EVENT REPORTING

All adverse reactions observed in study subjects must be reported immediately. Prior to the study's commencement, subjects will be informed of potential psychological discomfort associated with the assessments, as well as possible harms related to the examinations and TMS interventions. They will be advised on corresponding management procedures and instructed to promptly report any adverse reactions to the investigator.

In the event of a Serious Adverse Event (SAE), the investigator must immediately report to the department head and, based on the nature of the event, promptly notify the Subject Injury and Emergency Incident Response Team. The SAE must be reported to the

Sponsor, the Ethics Committee, and the relevant health administrative authorities within 24 hours.

Necessary diagnostic and therapeutic measures shall be determined based on the patient's condition, and clinical trial observation for the affected subject must be terminated immediately. The investigator is required to document the subject's symptoms, signs, laboratory findings, the time of injury onset, duration, severity, management measures, and course in the Case Report Form (CRF). A Serious Adverse Event Report Form must be completed, signed, and dated.

The investigator must conduct follow-up on the subject's injury until the condition resolves or stabilizes, providing necessary management and treatment during this period to fully ensure the subject's safety. The entire follow-up process and treatment outcomes must be documented in detail.

9 STATISTICAL CONSIDERATIONS

9.1 PRIMARY AND SECONDARY EFFICACY ENDPOINT(S)

- Primary Efficacy Endpoint(s):

The primary null hypothesis is that there is no difference in the least-squares mean estimate of the change in FM-UE scale on Day 105 after the initiation of the 2-week intervention among the three groups.

H_0: $Y_{concurrent,105d} = Y_{non\text{-}concurrent,\ 105d} = Y_{sham,\ 105d}$

H_A: The means are not all equal

- Secondary Efficacy Endpoint(s):

The secondary null hypothesis is that there is no difference in the least-squares mean estimate of the change in Wolf-Motor Functional Test (WMFT) time score, Action Research Arm Test (ARAT) score, Modified Barthel Index (MBI) score, Pittsburgh Sleep Quality Index (PSQI) score, and Resting Motor Threshold (RMT).

9.2 SAMPLE SIZE DETERMINATION

We initially conducted a single-center, randomized, double-blind, controlled trial at Ruijin Hospital. Sample size estimation was performed using PASS 2021 software, with a two-sided α level of 0.025, designed to test the significance in the outcome differeneces between concurrent rTMS group and non-concurrent rTMS group or sham rTMS group. Based on the data from the preliminary study, we anticipated an effect size of 1.5 in Fugl-Meyer Assessment of the Upper Extremity (FMA-UE) scores between the concurrent rTMS and sham groups. Under these assumptions, a minimum of 18 participants (9 per group) was required to achieve 80% power. An equal sample size of 9 was also planned for the non-concurrent group. Considering the low dropout rate among in-patients (~10%), we aimed to recruit 10 participants per group, totaling 30 patients. However, following stratification by motor evoked potential (MEP) status, the sample size in each subgroup became insufficient. To further evaluate the generalizability of individualized concurrent rTMS in MEP-negative patients, the trial was expanded to a multicenter study. Two

additional hospitals (Yangzhi Hospital and Changhai Hospital) were included, with a focus on prioritizing enrollment of MEP-negative patients for individualized stimulation. To compare the effects of individualized versus non-individualized stimulation across all rTMS conditions, each group included both subtypes. Ultimately, we anticipate enrolling 30 subjects from Ruijin Hospital, with 15 subjects each from Yangzhi Rehabilitation Hospital and Changhai Hospital.

9.3 POPULATIONS FOR ANALYSES

- Modified-full-analysis set (mFAS) population

All analyses will be conducted under the mFAS principle. The mFAS included all enrolled participants who initiated the intervention and had baseline and at least one post-baseline assessment.

- Per-protocol (PP) population

Sensitivity of the primary findings will be assessed using a per-protocol sample a per-protocol sample, defined as participants who completed ten treatment sessions, completed the FM-UE at all three post-treatment visits, did not experience recurrent clinical stroke or other prespecified illness affecting upper extremity motor function during the study period, and did not have any eligibility violations. The intention of the per-protocol analysis was to assess the maximum possible treatment effect achievable. The intent of this analysis sample is to assess the maximum possible treatment effect achievable with concurrent rTMS.

9.4 STATISTICAL ANALYSES

Details of the statistical analyses are provided in the Statistical Analysis Plan.

9.4.1 GENERAL APPROACH

The goal of this proposed phase-1 clinical trial is assessing whether high frequency repetitive transcranial magnetic stimulation (rTMS), when applied concurrent with motor training will evoke a motor impairment reduction as measured by the Fugl-Meyer Upper Extremity (FM-UE) scale immediately after the 14 day intervention and at the time of 1 and 3 months follow-up when compared to non-concurrent rTMS or sham rTMS. The central hypothesis is that rTMS, a non-invasive brain stimulation technique, can enhance current post-stroke motor recovery by inducing plasticity and potentiating the brain to be receptive to proven rehabilitation techniques (i.e., concurrent motor training). However, it is not known whether medium-size pulses of concurrent rTMS is sufficient or whether a higher pulses may evoke a better response while maintaining a similar safety and tolerability profile.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The change in FM-UE Scale in each treatment arm will be modeled using generalized linear mixed effects repeated measures model, where the dependent variable is the change from baseline in FM-UE to post-intervention visit; the model is adjusted for intervention arm, baseline FM-UE, time from stroke, visit and affected hemisphere; and the primary outcome is the fitted estimate at day 105. Model assumptions, including normality, will be assessed and appropriate transformations will be used in keeping with best practices and conventional approaches in the literature. If the primary null hypothesis is rejected, all pairwise secondary hypotheses will be tested using a Tukey type multiple comparisons correction.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Aim 1 will also test the secondary null hypotheses that there is no difference in the least-squares mean estimate of the change in Wolf-Motor Functional Test (WMFT) time score, Action Research Arm Test (ARAT), Modified Barthel Index (MBI) and Pittsburgh Sleep Quality Index (PSQI) at day 105. The procedure described above will be repeated for each of the secondary endpoints with the type-I error rate adjusted using a Tukey or Bonferroni type gate-keeping procedure to sequentially test the hierarchical null hypotheses. That is, if the primary null hypothesis (i.e., no difference in the change in FM-UE Scale between the three groups) is rejected, the secondary null hypothesis of no difference with respect to MBI score will be assessed; if that null hypothesis is also rejected, then the null hypothesis of no difference in the WMFT time score or ARAT score or PSQI score will be tested.

9.4.4 SAFETY ANALYSES

Safety: Analysis Method

Researchers at each participating center will regularly compile all reported adverse events (AEs), including serious adverse events (SAEs), if any. All clinical safety endpoints and SAEs will be summarized according to the event codes specified in the Adverse Event Case Report Form (AE CRF), including the frequency of occurrence, number of affected participants, severity, and relationship to the study intervention.

Clinically significant adverse events include::

- Severe headache
- Second-degree skin burn
- Clinical seizure

The proportion of subjects experiencing each of these events will be provided in the closed report by intervention arm with two-sided 95% CIs and unadjusted relative risks. Based on the Phase I dose-escalation study, no subjects experienced any of the clinically important adverse events. Fisher's exact tests will be used to assess intervention group differences in the rates of clinically important adverse events.

Tolerability: Analysis Method

Tolerability will be primarily assessed by recording the number of patients who discontinue trial participation due to adverse reactions. Differences in tolerability among the three groups will be evaluated using the Kruskal-Wallis test at a type I error rate of 10%.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

The characteristic of subjects at baseline will be tabulated ordered by 3 groups. p value is based on ANOVA if the variable is continuous and is based on Chi-squared test if the variable is categorical.

9.4.6 SUB-GROUP ANALYSES

In pre-specified subgroup analyses, the primary analysis will be repeated but include an interaction effect between sex/gender and intervention arm. Differential effects of rTMS by sex/gender are not anticipated, and therefore the trial is not powered specifically for these subgroup analyses, but these subgroup analyses will allow any unexpected large variations to be identified and regardless of outcome will assist with planning a Phase II and III trial which will account for differences if identified here.

Additional analyses are specified in the statistical analysis plan (SAP).

9.4.7 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual subject data will not be listed or tabulate by measure and time point. Only summarized data by dose group and time points and measures will be presented.

9.4.8 EXPLORATORY DATA ANALYSIS

Multi-echo fMRI preprocessing and analysis

Multi-echo fMRI data will be preprocessed using an established pipeline (Lynch et al.) to perform cortical surface generation, data denoising, and vertex-wise functional network mapping. The InfoMap algorithm's graph density will be tuned (5% to 0.001%) to ensure reliable network identification on the healthy hemisphere, following established practices (Gordon et al.). Manual Freesurfer corrections will be applied when extensive lesions preclude automated surface generation.

Task fMRI preprocessing and analysis

Preprocessing: Functional images will be preprocessed using SPM12. Steps will include realignment, slice-time correction, and normalization to MNI space (incorporating a lesion mask for stroke patients). Data will then be smoothed (8mm FWHM) and high-pass filtered (1/100 Hz). Statistical Modeling: First-level analysis will employ a general linear model. Grip events for each condition will be modeled as separate covariates convolved with a canonical HRF. Individual contrast maps will be generated for subsequent group-level analysis.

DTI data preprocessing and analysis

Diffusion-weighted imaging (DWI) data will be preprocessed using the DESIGNER pipeline in MRtrix3, including denoising, distortion correction, and eddy current correction. Preprocessed DWI will be aligned to T1-weighted and MNI space in a single interpolation step. Fiber orientation distributions will be estimated using constrained spherical deconvolution. Whole-brain probabilistic tractography will be performed using Dipy's Local Tracking module, with streamlines seeded five times per white matter voxel. A group-level structural connectivity matrix will be constructed using the Desikan–Killiany atlas. For personalized targeting, resting-state fMRI, task-fMRI, and DTI results will be integrated to identify motor regions exhibiting functional activation and residual structural connectivity. Electric field simulations will subsequently be used to derive optimized coil placement.

EEG data prepossessing and analysis

Electroencephalography (EEG) data will be preprocessed offline using EEGLAB, TESA, and FieldTrip toolboxes. Continuous EEG will be recorded from 64 scalp sites according to the 10-20 system, with a bandpass filter of 0.1–500 Hz and a sampling rate of 10 kHz. Vertical and horizontal electrooculogram will be simultaneously recorded for ocular artifact detection. TMS-evoked potentials will be epoched from -100 to 1000 ms relative to TMS onset. Artifact-contaminated epochs will be rejected, with retained trials ranging between 450-500 per condition. Five TEP peaks will be analyzed: P30, N45, P65, N100, and P180. Global mean field power will be computed across all electrodes. For cases exhibiting atypical TEP morphology without clear peaks, qualitative analysis will be performed.

EMG data analysis

EMG data will be processed and analyzed using custom Python scripts. Raw signals will be band-pass filtered to remove noise and motion artifacts. For muscle activation quantification, the root mean square (RMS) will be calculated from the filtered data across entire trials. Additionally, integrated EMG (iEMG) will be computed as the area under the rectified EMG signal curve for each trial. Both RMS and iEMG values will be used in statistical models to compare muscle activity patterns across experimental conditions and

assessment timepoints..

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

Informed consent must be obtained before a subject agrees to participate in the study and is maintained throughout the entire research process. The informed consent form, approved by the Ethics Committee, should be read by the subject. The investigator will explain the study procedures and answer any questions raised by the subject, informing them of potential risks and their rights. Subjects may discuss participation with their family or guardian before agreeing to take part. The investigator must inform subjects that participation is voluntary and that they may withdraw from the study at any time. A copy of the informed consent form can be provided to the subject for their records. The rights and welfare of the subjects will be protected, and it will be emphasized that the quality of their medical care will not be affected should they decline to participate.

10.2 PRIVACY PROTECTION

Original records and related forms containing subject data will be accessible only to the principal investigator. These documents will be protected through encryption and secure storage measures to ensure confidentiality.

10.3 COLLECTION AND USE OF SPECIMENS AND DATA

No biological specimens will be collected. All collected patient information will be stored in an encrypted format and will not be accessible to anyone other than the authorized research personnel.

10.4 QUALITY CONTROL AND QUALITY ASSURANCE

All pharmaceuticals and materials used in the clinical study must adhere to quality control standards. To ensure the authenticity of the clinical study records and verify compliance with the study protocol, authorized personnel retain the right to audit the clinical study. Participants will be informed about the audit process; however, patient privacy and data will be strictly protected. All investigators will receive standardized training from physicians within the Rehabilitation Medicine Department of Ruijin Hospital and must strictly follow the study protocol requirements. Throughout the study, the principal investigator will conduct regular reviews of the study progress and quality, overseeing and coordinating the entire process to ensure protocol adherence, maintain timelines, and guarantee quality. Source data verification will be performed to ensure consistency with the original research protocol.

10.5 DATA HANDLING AND RECORD RETENTION

10.5.1 DATA COLLECTION AND MANAGEMENT

Investigators must accurately, thoroughly, and diligently record all items in the Case Report Form (CRF) in accordance with its completion guidelines, ensuring the content is authentic and reliable. All observations and findings in the study must be verified to guarantee that all conclusions are derived from the original data. Completed CRFs, after review by the principal investigator, shall be stored securely in a designated data storage cabinet within the research department under the responsibility of an assigned person. All related processes shall be documented. The archiving of study records, data processing, and relevant test results shall be managed by designated personnel. Subject codes shall be uniformly assigned by the project leader. Demographic data, clinical assessments, and treatment details in the CRFs shall be truthfully recorded by the research physicians and must not be altered arbitrarily. Any necessary corrections shall be made strictly according to the CRF completion guidelines, accompanied by the signature (or initials) and date of the person making the change. All CRFs, after being verified and signed by the research staff, shall be sent to the Rehabilitation Medicine Department of Shanghai Ruijin Hospital, which is responsible for their storage, ensuring the authenticity, integrity, and confidentiality of the clinical trial data. The completed CRFs, following review by the principal investigator, shall be retrieved for secure stored. Data will be entered into the SPSS statistical software for evaluation.

10.5.2 RETENTION OF RESEARCH DATA

In accordance with ICH/GCP guidelines, the investigator/institution shall retain all Case Report Forms (CRFs), all original records supporting the data collected from each subject, and all essential documents specified in Section 8 of ICH/GCP (Essential Documents for the Conduct of a Clinical Trial), along with all other study documents required by relevant regulations. The investigator/institution shall implement measures to prevent the accidental or premature destruction of these documents.

The essential clinical trial documents must be retained for at least 2 years after the formal discontinuation of this clinical trial. The coordinating group is obligated to inform the investigator/institution when retention of these documents is no longer required.

Should the responsible investigator retire, relocate, or for any other reason become unable to continue custodianship of these research records, the custody must be transferred to a person willing to assume this responsibility. The coordinating group must be notified in writing of the new custodian's name and address. Under no circumstances shall the investigator transfer or dispose of any study documents without prior written approval from the coordinating group.

The investigator must allow the coordinating group or relevant regulatory authorities access to these files for review upon request, in relation to this study.

10.6 PUBLICATION AND DATA SHARING AGREEMENT

All materials, results, and associated intellectual property derived from this study are the property of the Principal Investigator. The Principal Investigator may utilize these materials in various forms, such as submission to governmental drug regulatory authorities or disclosure to other researchers.

Concurrently, researchers may use the data obtained from this study for scientific purposes individually. However, prior to any publication, they must consult with the Sponsor and obtain written consent from the Principal Investigator. The Principal Investigator acknowledges the researchers' right to publish the study findings upon its completion.

Nevertheless, researchers must submit a draft manuscript or abstract of the article to the Principal Investigator at least 30 days prior to submission for publication, to allow for review and obtain the Principal Investigator's approval of the final version to be submitted. The Principal Investigator will review the article in a timely manner and shall not unreasonably withhold approval.

In the event of a disagreement regarding the content of a proposed publication between the Principal Investigator and other researchers, the concerned parties shall engage in discussions to reach a resolution satisfactory to all.

10.7 CONFLICT OF INTEREST STATEMENT

All data and materials pertaining to this study are the property of the Principal Investigator. Any manuscript intended for publication must be reviewed by the Principal Investigator and must conform to the pre-approved publication plan. Any individual or institution wishing to publish articles related to this research must obtain written consent from the Principal Investigator prior to submission.

10.8 ABBREVIATIONS

ADL	Activities of Daily Living
AE	Adverse Event
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center

eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FM-UE	Fugl-Meyer Upper Extremity
IADL	Instrumental Activities of Daily Living
ITT	Intention-To-Treat
MEP	Motor evoked potential
MOP	Manual of Procedures
NCT	National Clinical Trial
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TMS	Transcranial magnetic stimulation
UP	Unanticipated Problem
WMFT	Wolf Motor Function Test
ARAT	Action Research Arm Test
MBI	Modified Barthel Index
PSQI	Pittsburgh Sleep Quality Index

RMT	Resting Motor Threshold
EMG	Electromyography
CV	Computer Vision
EEG	Electroencephalography
MRI	Magnetic Resonance Imaging
RMS	Root Mean Square
iEMG	integrated Electromyography

10.9 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
3.0	2024/12/03	Version and Date Update on Title Page	
3.0	2024/12/03	The intervention duration was shortened from four weeks to two weeks.	Due to constraints on patient hospitalization length, the intervention period was reduced from the originally planned four weeks to two weeks.
3.0	2024/12/03	The sample size was revised from the originally planned 111 subjects to 81 patients.	The sample size was re-estimated based on preliminary data, which indicated that a smaller cohort would be sufficient to achieve the desired statistical power.
3.0	2024/12/03	The Pittsburgh Sleep Quality Index (PSQI) was added to the assessment protocol.	Based on previous research indicating that transcranial magnetic stimulation can influence sleep architecture, the Pittsburgh Sleep Quality Index (PSQI) has been incorporated into the assessment protocol. This addition will enable a more comprehensive evaluation of the overall benefits and potential side effects of the intervention.
5.0	2025/05/07	Version and Date Update on Title Page	
5.0	2025/05/07	Repetitive Transcranial Magnetic Stimulation applied concurrently with Motor Training for Upper Limb Motor Dysfunction in Stroke Patients	To prevent duplication with existing records in the registration system, the study title has been revised in both the research protocol and informed consent forms. The core content and methodology of the project remain unchanged.
5.0	2025/05/07	The sample size has been adjusted from 81 patients in the single-center pilot study	This study was initially designed as a single-center, randomized, double-blind, controlled trial conducted at Ruijin Hospital. Sample size

		to 60 patients in the multi-center formal trial.	estimation was performed using PASS 2021 software based on preliminary data, which indicated an effect size of 1.5 in the Fugl-Meyer Assessment of the Upper Extremity (FMA-UE) between concurrent rTMS and sham groups. With a two-sided α of 0.025 and 80% power, a minimum of 18 participants (9 per group) was required. To account for an estimated 10% dropout rate, we planned to enroll 10 participants per group, totaling 30 patients. Following stratification by motor evoked potential (MEP) status, subgroup sample sizes were deemed insufficient. To enhance the generalizability of findings—particularly regarding individualized concurrent rTMS in MEP-negative patients—the study was expanded to a multicenter trial. Two additional sites were included: Yangzhi Rehabilitation Hospital and Changhai Hospital. Enrollment at these centers will prioritize MEP-negative patients for individualized stimulation. To enable comparison between individualized and non-individualized stimulation across rTMS conditions, both subtypes are represented in each group.The final anticipated enrollment is 30 subjects from Ruijin Hospital, with 15 subjects each from Yangzhi Rehabilitation Hospital and Changhai Hospital. Total 60 subjects.
5.0	2025/05/07	The study will be expanded from a single-center to a multi-center trial with the addition of two new clinical sites: Department of Rehabilitation Medicine, Ruijin Hospital Affiliated with	This revision increases the total number of study sites to three, enhancing the geographical representation and patient recruitment capacity of the trial.

		Shanghai Jiao Tong University School of Medicine; Shanghai Yangzhi Rehabilitation Hospital (Shanghai Sunshine Rehabilitation Center); Shanghai Changhai Hospital	
5.0	2025/05/07	The study design was changed from a randomized controlled trial to a non-randomized controlled trial.	This study will utilize a non-randomized controlled trial design. To ensure adequate enrollment in the experimental group, priority for allocation to this group will be given to participants recruited from the newly added clinical sites.
5.0	2025/05/07	The Wolf Motor Function Test (WMFT) has been incorporated as an additional outcome measure in the study protocol.	Protocol Modification for Shanghai Yangzhi Rehabilitation Hospital Site Due to the unavailability of the Action Research Arm Test (ARAT) kit at the Shanghai Yangzhi Rehabilitation Hospital satellite center, the Wolf Motor Function Test (WMFT) will be substituted as the secondary outcome measure at this specific site. This adjustment ensures standardized assessment capabilities across all participating centers while maintaining methodological consistency within each site.