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Effect of intermittent theta burst stimulation combined with acoustic startle priming motor training on upper limb motor function and neural plasticity in stroke individuals: study protocol for a randomised controlled proof-of-concept trial

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ABSTRACT

Introduction Stroke is a major cause of acquired disability globally, yet the neural mechanisms driving motor recovery post-stroke remain elusive. Recent research has underscored the growing significance of subcortical pathways in neural plasticity and motor control. Among these, the cortico-reticulospinal tract (CRST) has gained attention in rehabilitation due to its unique ascending and descending structural features as well as its cellular properties which position it as an excellent candidate to compensate for inadequate motor control post-stroke. However, the optimal strategies to harness the CRST for motor recovery remain unknown. Non-invasive modulation of the CRST presents a promising though challenging, therapeutic opportunity. Acoustic startle priming (ASP) training and intermittent theta burst stimulation (iTBS) are emerging as potential methods to regulate CRST function. This study aims to investigate the feasibility of segmentally modulating the cortico-reticular and reticulospinal tracts through ASP and iTBS while evaluating the resulting therapeutic effects.

Methods and analysis This is a randomised, blinded interventional trial with three parallel groups. A total of 36 eligible participants will be randomly assigned to one of three groups: (1) iTBS+ASP group, (2) iTBS+non-ASP group, (3) sham iTBS+ASP group. The trial comprises four phases: baseline assessment, post-first intervention assessment, assessment after 3 weeks of intervention and a 4-week follow-up. The primary outcomes are the changes in the Fugl-Meyer Assessment-Upper Extremity and Modified Ashworth Scale after the 3-week intervention. Secondary outcomes include neurophysiological metrics and neuroimaging results from diffusion tensor imaging and resting-state functional MRI.

Ethics and dissemination The trial is registered with the Chinese Clinical Trial Registry (Registration No. ChiCTR2400085220) and Medical Ethics Committee of Tongji Hospital, affiliated with Tongji Medical College, Huazhong University of Science and Technology (Registration No.TJ-IRB20231109). It will be conducted in the Departments of Rehabilitation Medicine and Radiology at Tongji Hospital in Wuhan, China. The findings will be disseminated through peer-reviewed journal publications and presentations at scientific conferences.

Trial registration number ChiCTR2400085220.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Multimodal imaging comprehensively reflects the structural and functional changes in the cortico-reticulospinal tract.
⇒ By combining sternocleidomastoid muscle activation detection and intermittent theta burst stimulation-induced motor-evoked potentials, the effectiveness of central-peripheral closed-loop interventions is comprehensively assessed.
⇒ Transcranial magnetic stimulation (TMS) is incompatible with MRI systems, making it difficult to monitor brain activity changes during stimulation.
⇒ During MRI scans, patients may become accustomed to the noise, reducing the effectiveness of auditory startle initiation.

INTRODUCTION

Stroke is the leading cause of disability in adults, affecting over 1 million individuals annually in Europe. About two-thirds of patients who had a stroke experience persistent upper limb motor dysfunction and nearly 64% are unable to walk independently.[1–5] Alarmingly, less than 20% can return to their pre-stroke lifestyles, resulting in a substantial economic burden on society.[6] Upper extremity (UE) motor dysfunction following a stroke encompasses a range of impairments, including loss

of voluntary motion, impaired motor control and/or intrusive muscle co-activations, all of which hinder activities of daily living such as grooming or feeding.[7 8] Notably, nearly 60% of individuals with severe or complete upper limb impairment remain incapable of performing any movement up to 6 months after stroke.[9] Consequently, optimising strategies to enhance upper limb functional recovery has become a central focus within the realm of rehabilitation.[10–13]

The extrapyramidal system works in conjunction with the pyramidal system to regulate human movement, with the cortical-reticulospinal tract (CRST) playing a crucial role in descending motion control. This tract consists of two essential components: the cortico-reticular tract (CRT) and the reticulo-spinal tract (RST).[14] Previous research revealed that CRT primarily originates from the primary motor cortex (PMC) and the supplementary motor area.[15] However, recent imaging studies have extended its connections to the frontal and parietal cortex.[16] It has significant cortical overlap with corticalspinal tract (CST).[16] It passes through the anterior and posterior limbs of the internal capsule, partially crosses in the midbrain, and subsequently converges on the pontomedullary reticular formation on both sides.[15] Within the brain, the contribution of RST to descending motor control ranks second only to the CST.[17] Previous studies have demonstrated that the RST modulates the excitability of γ motor neurons in peripheral muscles and primarily participates in maintaining postural stability, gait and control of proximal joint movements.[14 18 19]

The CST, originating from the PMC, is regarded as the most important locomotion-related pathway in the human brain. The integrity of the CST after injury has become a key indicator for judging the prognosis of various types of central nervous system injuries.[20–22] At present, most relevant studies are limited to observing CST-related functions and regulatory networks, neglecting the detection of changes in the function of CRST located in the extrapyramidal system. Some preliminary evidence suggests that the CRST plays an important role in motor recovery after stroke through structural and functional compensation and is closely associated with the occurrence and progression of post-stroke spasticity.[23 24] Some imaging protocols have been well established to track CRT and observe synchronous structural changes during motor recovery after stroke.[25 26] Resting-state functional MRI (fMRI)[27] and diffusion tensor imaging (DTI)[28 29] as non-invasive and non-radiating tools for studying brain function can reveal functional and structural changes in RST-related tissues after treatment or stimulation. Verifying the effectiveness of novel interventions based on these tools appears more feasible than ever before.

Although the compensatory role of the CRST after stroke has been widely recognised due to its origin in the brainstem level, current research methods make it challenging to precisely detect its activity.[19] In addition to imaging, two methods are currently relied on to observe RST activation.[30 31] One method involves using the StartReact effect (SE) to reflect RST facilitation, as indicated by specific activation characteristics of the sternocleidomastoid muscle (SCM) before motor initiation. The other method uses transcranial magnetic stimulation (TMS) to induce motor-evoked potentials (MEPs) in ipsilateral muscles, serving as a reflection of the RST state.[32 33] However, the conditions for inducing ipsilateral MEP are quite harsh, requiring more than 80% of the target muscle's maximum contraction as a prerequisite and are not common in normal people.[34 35] In terms of operability, it is more practical to use the SE effect, represented by SCM activation, to indirectly assess RST facilitation.

Meanwhile, our preliminary research on SE has revealed that the early activation of the SCM, indicative of RST facilitation, occurs simultaneously with the additional activation of the dorsolateral prefrontal cortex (DLPFC) in healthy individuals.[36] This may be attributed to a higher-level regulatory centre for RST facilitation proposed by Chen *et al*.[37] The study of Chen *et al* on SE revealed the presence of 'alertness' effect from RST to the brain cortex which may further shape the motor cortex according to task goals. Moreover, as high as 67.1% direct connectivity from brainstem reticular formation to the lateral cerebral cortex was observed in the fMRI model constructed by Sung and Kwon.[38] These pathways, classified as the ascending reticular activating system, have been shown to play an important role in establishing alertness, arousal and consciousness.[39] Therefore, it is reasonable to speculate that selective regulation of this region can affect the status of the corticoreticular pathway. Thus, facilitating stimulation targeting the DLPFC may potentially regulate CRST status via the above connections. As part of the fronto-parietal network, DLPFC is involved in the advanced regulation of human motor functions. An fMRI study has further confirmed that its excitability is directly related to the recovery of motor functions after stroke.[40] Therefore, the regulation of DLPFC may not be limited to CRT itself and is likely to produce other effects such as improving mood and promoting motor cognition and working memory.[41–43]

The concept of designing central-peripheral closed-loop interventions for post-stroke movement disorders has gained consensus in the field of rehabilitation and has achieved remarkable results.[44 45] The integration of non-invasive brain stimulations with physical training is currently a focal point in post-stroke motor rehabilitation. Compared with isolated motor training or central regulation technique, its advantage lies in better utilisation of the time window of cortical facilitation, thereby maximising the functional outcomes from motor training.[46] Intermittent theta burst stimulation (iTBS) is a non-invasive brain stimulation paradigm that has developed and matured over the past decade. It can induce long-term facilitation of cortical excitability and bring therapeutic benefits for post-stroke subjects.[47 48] iTBS has demonstrated promising therapeutic benefits for various neuropsychiatric disorders, making it a highly promising

treatment approach.[49] Although the exact neural remodelling mechanisms are not yet clear,[50] facilitated regulation of CRT is highly possible with iTBS from the perspective of central intervention. Considering that the CRT primarily governs motor control on the ipsilateral side,[51] we proposed to target the contralesional DLPFC of the unaffected hemisphere in stroke subjects as the target of iTBS intervention to facilitate CRT.

In addition, as proposed earlier regarding acoustic startle priming (ASP), using a sound (>110 decibels (dB)) as the start signal of a task can trigger the early initiation of prepared movement with an extremely short latency and enhance anticipated muscle control. This phenomenon is believed to result from the rapid transmission of subcortical motion commands, mainly through RST.[52] Our recent research on SE has revealed that using the startling cue as a task initiation command can elicit increased motor output, thereby reducing the reaction time of motor responses in patients who had a stroke and improving anticipatory muscle regulation.[53] The facilitation role of this startle-reaching paradigm on RST has been well established. However, whether this transient facilitation performance can accumulate and ultimately promote RST neuroplasticity to improve RST motor output remains uncertain. Thus, it could serve as a peripheral component and contribute to regulation targeting CRST.

We hypothesised that using iTBS and ASP tasks to facilitate CRT and RST respectively would complete a closed-loop training, enhancing the function of the entire cortico-reticulospinal system and promoting upper limb motor recovery after stroke. In this study, we aim to segmentally verify the feasibility of regulating CRT and RST facilitation and observe the resulting therapeutic effects. This study will provide imaging evidence for the specific compensatory mechanisms and characteristics of the CRST loop in stroke subjects and contribute to the development of novel interventions based on CRST facilitation after stroke.

METHODS

Study design

This study will be a blinded interventional trial with three parallel groups. The study will be conducted in accordance with the Declaration of Helsinki and follows the Consolidated Standards of Reporting Trials statement for randomised trials, as well as the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT). The trial has been registered with the Chinese Clinical Trial Registry (Registration No. ChiCTR2400085220) and will be carried out in the Departments of Rehabilitation Medicine and Radiology at Tongji Hospital in Wuhan, China. An overview flowchart of the study is shown in figure 1, while the SPIRIT table for enrolment, interventions and assessments is presented in table 1. A total of 36 eligible subjects will be randomly allocated into three equally sized groups: (1) iTBS+ASP group, (2) iTBS+non-ASP group, (3) sham iTBS+ASP group.[33]

Participants

Inclusion criteria include

(1) Age between 18 and 70 years, with both males and females eligible. (2) Onset of the first stroke within the past 3 months, with clear clinical symptoms and radiological diagnosis. (3) Severe upper limb motor impairment, with a Fugl-Meyer Assessment-Upper Extremity (FMA-UE) score between 0 and 35. (4) Stable vital signs for at least 48 hours. (5) Cognitive capacity to follow instructions, with a Mini-Mental State Examination score of≥22. (6) Tolerance to sudden auditory startling stimuli of 114 dB. (7) Willingness to provide informed consent.

Exclusion criteria include

(1) A history of multiple strokes or bilateral strokes. (2) Severe upper/lower limb spasticity, with a Modified Ashworth Scale (MAS) grade of 3 or higher, or other conditions affecting upper and lower limb function, such as joint muscle contractures, severe frozen shoulder, surgical history, rheumatic diseases. (3) Severe cognitive impairment, inability to effectively communicate with medical personnel.[54] (4) Other conditions deemed unsuitable for participation by the researcher, which may lead to adverse consequences, such as severe hypertension, coronary heart disease, claustrophobia.

Randomisation and blinding

Doctors will screen eligible patients, provide a detailed explanation of the trial, and obtain their consent to sign the informed consent form. Subsequently, demographic information, medical history and medication details will be collected. We will randomise all individuals into one of the three groups in a 1:1:1 ratio. Independent researchers will use consecutively numbered sealed and opaque envelopes to conceal the allocation. In this trial, participants, assessors and data analysts will be blinded to the allocation. However, due to the nature of the interventions, which involves targeting treatment sites in the cerebral cortex, the interveners cannot be blinded. Please refer to table 1 for details of the treatment and outcome assessment plan.

Procedure

The study will comprise four phases: baseline assessment, assessment after the first intervention, assessment after a 3-week intervention and a 4-week follow-up. During the baseline phase, demographic information, disease details as well as motor function and spasticity of the hemiplegic upper limb will be collected. All participants will then undergo a 3-week conventional rehabilitation intervention, encompassing physical therapy, occupational therapy and necessary speech and swallowing training. This rehabilitation programme will be administered for 4–6 hours per day, 5 days a week. The intensity of treatment will be appropriately adjusted within the patient's tolerance range based on the actual performance. The

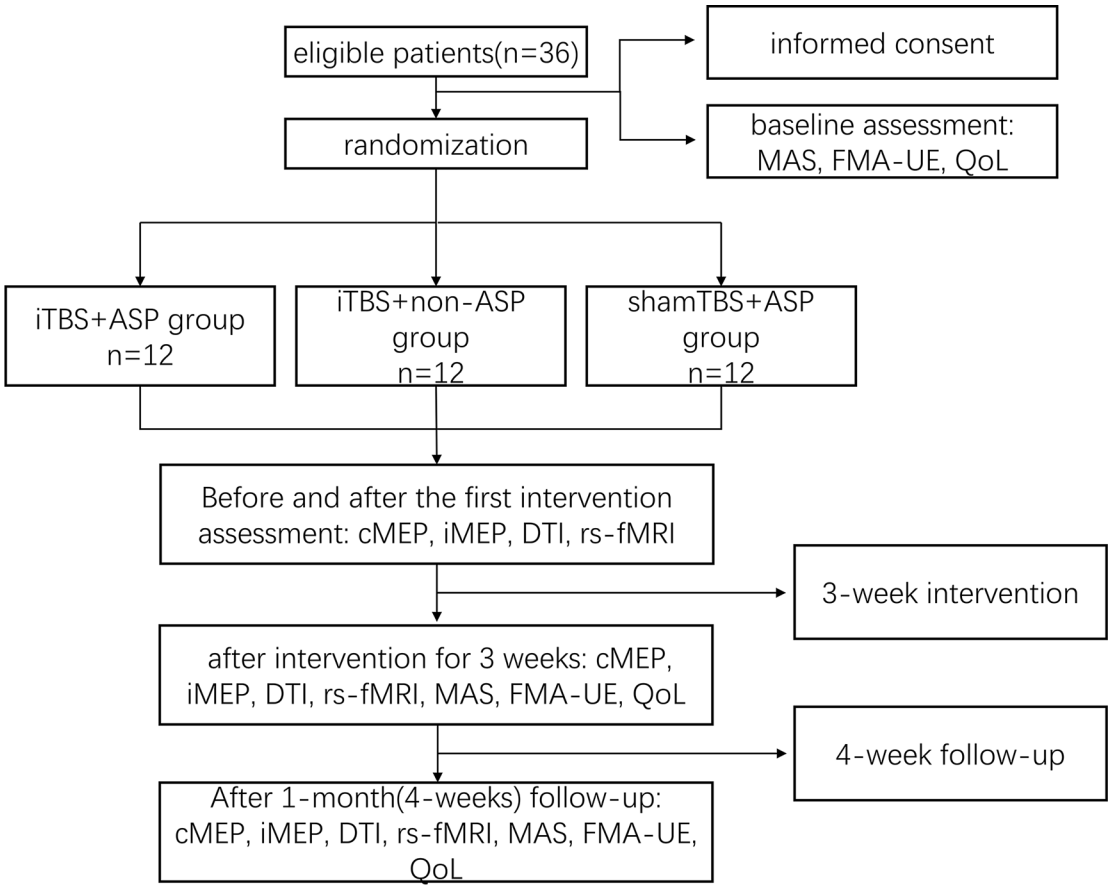

Figure 1 Flowchart of the protocol. ASP, acoustic startle priming; cMEP, contralateral motor evoked potentials; DTI, diffusion tensor imaging; FMA-UE, Fugl-Meyer Assessment-Upper Extremity; iMEP, ipsilateral motor evoked potentials; iTBS, intermittent theta burst stimulation; MAS, Modified Ashworth Scale; QoL, Quality of Life; rs-fMRI, resting-state functional MRI.

iTBS, sham iTBS, ASP or non-ASP training involved in this study will be provided before the daily rehabilitation intervention, 5 days per week for a total of 3 weeks. A single session of iTBS and ASP training lasts approximately 190 s and 30 min, respectively.

Resting-state MRI scans will be conducted before and immediately after the subject's first intervention, 3 weeks after the intervention and during the follow-up at week 7. Meanwhile, DTI scans will also be conducted before and after the 3-week intervention to observe structural changes. Moreover, neuroelectrophysiological tests on CST and CRST will be performed simultaneously with the three imaging scans. MEPs of the biceps brachii and first interosseous muscle ipsilateral and contralateral to the lesion will be collected by a senior neurologist. In addition, clinical assessments via Fugl-Meyer motor function assessment of upper extremity (FMA-UE) and MAS will be performed at baseline, after a 3-week intervention and 1-month follow-up. Figure 1 illustrates the flowchart of this study.

Navigation and iTBS stimulation

A TMS-navigation system (Localite software and NDI Polaris Vicra P6) will be used to locate the therapeutic targets, record data and deliver the stimulation. MRI data for each patient will be collected and imported into the navigation system to build a personalised brain model. Participants will be asked to sit in a comfortable chair with adhesive surface electrodes placed over the muscle belly and tendon of the first dorsal interosseous (FDI). The cortical site inducing the maximal MEP in the FDI muscle at resting will be determined as the hotspot. TMS Motor Threshold Assessment Tool (MTAT V.2.1) (http://www.clinicalresearcher. org/software.html) will be employed to determine the active motor threshold (AMT). TMS will be delivered using a figure-of-eight-shaped coil (MCF-B65, MagVenture MagPro X100, Denmark), with the stimulus intensity set to 80% of the contralesional hemisphere's AMT.

The iTBS paradigm involves a burst-pulse consisting of three pulses at 50 Hz, with each train delivered burst-pulse at a frequency of 5 Hz over a duration of 2 s, comprising 10 bursts-pulse separated by an 8 s interval. Each session encompasses 20 trains, yielding a total of 600 pulses and extending over a period of 190 s.[50] The iTBS intervention will target the contralesional DLPFC of the unaffected hemisphere. The sham iTBS group will undergo iTBS under identical parameters (intensity, duration and location) as the active iTBS targeting the DLPFC in the unaffected hemisphere; however, the coil will be rotated 90 degrees perpendicular to the scalp to minimise the induced current.

Table 1 Treatment and outcome assessment plan

Content of data collection		Baseline	Before and after the first treatment	Intervention	Post-intervention assessment	Follow-up
Time point		−1 week	1 day	0–3 week	3 week	7 week
Inclusion and exclusion criteria		X				
Informed consent		X				
Basic information		X				
Intervention	iTBS+ASP group			X		
	iTBS+non-ASP group			X		
	shamTBS+ASP group			X		
Scale assessment	FMA-UE	X			X	X
	MAS	X			X	X
	QoL	X			X	X
Neural pathway status assessment	cMEP		X/X		X	X
	iMEP		X/X		X	X
Feasibility	Retention				X	
	Adherence				X	
	Tolerability				X	
	Safety				X	
Neuroimage	Rs-fMRI		X/X		X	X
	DTI		X		X	X
	3D-T1		X		X	X

ASP, acoustic startle priming; cMEP, contralateral motor-evoked potentials; DTI, diffusion tensor imaging; FMA-UE, Fugl-Meyer Assessment-Upper Extremity; iMEP, ipsilateral motor-evoked potentials; iTBS, intermittent theta burst stimulation; MAS, Modified Ashworth Scale; QoL, Quality of Life; Rs-fMRI, resting-state functional MRI.

ASP motor training

Participants will undergo ASP training immediately after each iTBS or sham-iTBS intervention. The ASP training task for the upper limb is developed based on our previous study on startle-reaching tasks.[53] Meanwhile, the difficulty of the training tasks is appropriately reduced to accommodate the majority of post-stroke patients who do not have fine hand movements. Participants will wear a headset (Sennheiser HD25-I; Wedemark, Germany) and sit approximately 1.2 metres in front of a 24-inch electronic screen. A height-adjustable table will be placed on the anterolateral side of the affected upper limb, approximately 80% of the length of the unilateral arm from the acromion. The table height will be adjusted to shoulder level, allowing the participant to comfortably place their palm or forearm on the table. Red, blue and yellow square paper cards (size: 15 cm×15 cm) will be arranged side by side on the table from left to right, with approximately 10 cm spacing between each card, to serve as targets for touching. Subjects will be asked to reach the card of the corresponding colour according to the task-initiating cue in the headphones.

A single training session lasts approximately 30 min and comprises 40–60 trials. Each subject is required to repeat the reaching task at least 40 times during a single training session, with each trial lasting about 18 s. In the first 5 s, the screen in front of the subject will display the corresponding task, such as touching the red square. At the fifth second, the system emits three consecutive beeps (80 dB, 1000 Hz, 500 ms) through the earphones to remind the subject to prepare for the current task. Meanwhile, the prompt text on the screen disappears to avoid excessive visual interference. Within the subsequent time window of 7.5–8 s, the system randomly issues a task startup cue, including an 80 dB beep (1000 Hz, 40 ms) and a 114 dB white noise (1000 Hz, 40 ms). In each training session, half of the task initiation sounds were set to 114 dB white noise to elicit sufficient SE. The order in which these Go cues with white noises appear will be randomly assigned to the 40–60 trials to avoid prediction. Participants are instructed to complete the current task as soon as they hear the Go cue. In the next 10 s, subjects are asked to complete the task and return to the initial resting state. All subjects are asked to complete these tasks as quickly and accurately as possible. In addition, subjects will take a 1-min break after completing 10 consecutive trials. The trials are designed and executed using the Psychtoolbox-3 toolkit within MATLAB (2017b, MathWorks, Natick, Massachusetts, USA). The training process is shown in figure 2.

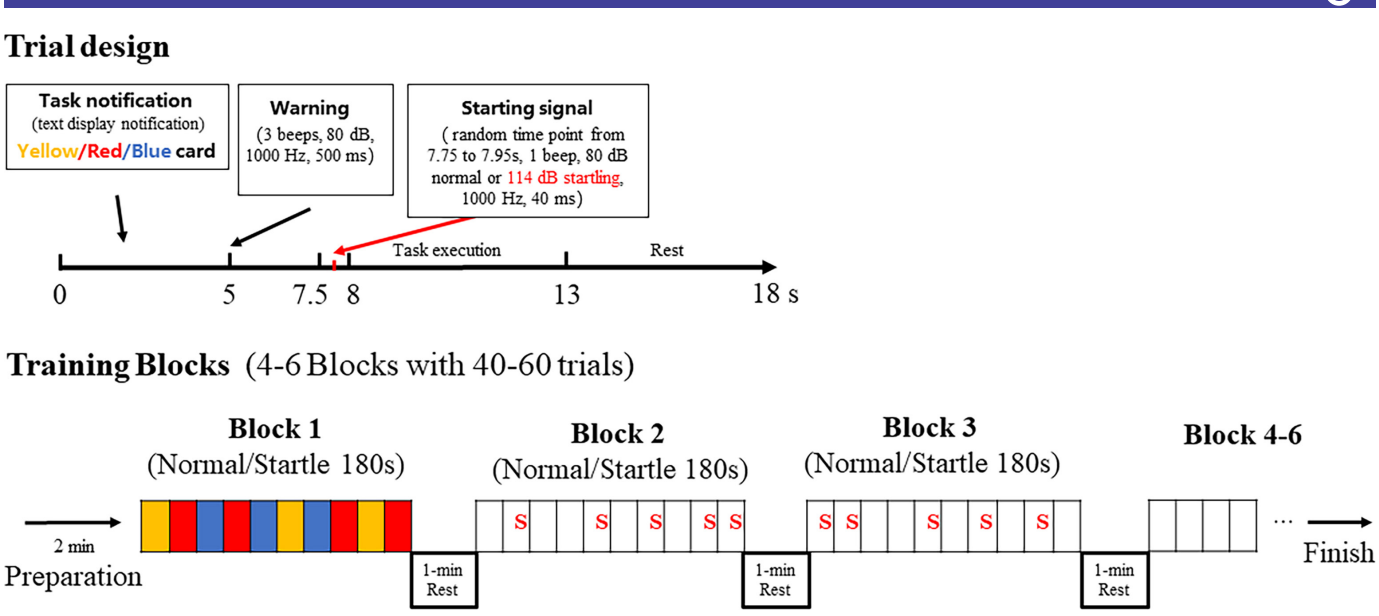

Figure 2 Acoustic startle priming motor training process.

Tolerability and safety

The trial will be halted immediately if a participant experiences any discomfort or pain of moderate severity or higher (Numeric Pain Rating Scale>3/10). If symptoms disappear after appropriate rest, no further action will be necessary. However, persistent symptoms will be considered severe adverse events and require medical intervention. Any other adverse events will also be classified as severe adverse reactions, prompting immediate termination of the trial with appropriate medical intervention. In the event of adverse events, participants' basic information and potential causes will be meticulously documented. Severe adverse events will be reported in accordance with the regulations of the Hospital Medical Quality and Safety Management Committee.

Outcome measurements

The primary outcomes of the study are the changes in FMA-UE and MAS after the 3-week intervention. Both measures will be used to assess improvements in movement capability and alterations in flexor muscle tone of the hemiplegic upper limb. Secondary outcomes encompass the findings from neurophysiological examinations as well as neuroimaging assessments involving DTI and resting-state fMRI. Specifically, the delay and amplitude of ipsilateral and contralateral MEPs will reflect the state changes of CST and RST before and after intervention. Moreover, resting-state fMRI and DTI will reflect the intervention outcomes in terms of alterations in brain functional network connectivity and remodelling of pathway structures, respectively.

The feasibility of this clinical trial will be assessed through a set of predefined metrics focusing on retention, adherence and safety. Retention will be measured as the percentage of participants who complete the full intervention without dropping out. A retention rate of 80% or higher will be considered indicative of feasibility. Adherence to the intervention protocol will be evaluated by tracking participants' compliance with the scheduled iTBS and ASP sessions, specifically the frequency and duration of each task. Feasibility will be demonstrated if at least 80% of participants complete more than 80% of the prescribed sessions. Tolerability will be assessed using a postintervention questionnaire which will evaluate participants' reports of discomfort or adverse events related to the interventions. The intervention will be considered feasible if fewer than 10% of participants report significant adverse effects, such as intolerable discomfort or excessive fatigue. Finally, safety will be monitored by recording the number and type of adverse events (eg, headaches, dizziness, increased spasticity) that occur during or after the intervention. A serious adverse event rate below 5% will serve as a threshold for feasibility in terms of safety.

Clinical assessments

Fugl-Meyer Assessment of Upper Extremity

We will also assess the mean change in the FMA-UE scale. This assessment, focusing on the UE, upper arm and wrist/hand, evaluates motion, coordination and reflexes. Each item on the FMA-UE scale is scored on a 3-point scale (0, 1, 2), with 0 representing the worst performance and 2 the best. The total score ranges from 0 to 66, with higher scores indicating better motor function. The FMA-UE scale is widely recognised for its convenience, effectiveness and reliability in measuring upper limb motor recovery post-stroke.[55]

The Modified Ashworth Scale

MAS is a commonly employed tool for assessing muscle spasticity by measuring resistance encountered during

passive stretching of soft tissues.[56] The MAS has a 6-point scale (ranging from 0 to 4, respectively 0, 1, 1, 2, 3, 4) with a score of 0 representing no spasticity and a score of 4 representing severe spasticity. An experienced clinician will use MAS to evaluate the muscle tone of the elbow flexor, elbow extensor and wrist flexor and extensor on the hemiplegic side of the patient and record them, respectively.

Quality of Life measurement

Quality of life (QoL) is defined as people's perception of their place in the context of their environment.[57] In this study, we use the short form-36 (SF-36) QoL tool with eight domain questions which mainly include: general health, physical functioning, pain, role limitation due to physical problems, social functioning, vitality, role limitations due to emotional problems and mental health.[58] The QoL will be assessed at the patient's baseline status on admission and on completion of 3 weeks of treatment.

Those clinical assessments will be performed at baseline, after 3 weeks of treatment, and at the 7th-week follow-up.

Neurophysiological examinations

Neural pathway function will be evaluated before and after the initial intervention, at 3 weeks postintervention and during a 1-month follow-up period. TMS will be employed to detect contralateral/ipsilateral MEPs (c/iMEPs) corresponding to the biceps brachii and FDI muscles on both the unaffected and affected sides.[31] All participants will be seated with their elbows positioned at a 90° angle and their arms will be secured.[59] Following skin preparation with alcohol, electrodes will be bilaterally placed on the biceps brachii and FDI muscles. Muscle cEMP (contralateral MEPs, motor evoked potentials on the contralateral side of the TMS stimulation site) will be recorded using the described equipment and methods to localise muscle cEMP hotspots and apply stimulation to the hotspot area.[59 60] To evoke iMEPs, a pulsed stimulus will be fired at the hotspot at 80% of the maximal stimulator output,[61] a level known to consistently evoke iMEPs in the proximal muscles of healthy adults.[62]

Neuroimaging: DTI and rest state fMRI

DTI and resting-state fMRI assessments will be evaluated at baseline and after the 3-week treatment. These assessments will be conducted as follows. All participants will undergo scanning using a 3.0 T MRI scanner (Magnetom Skyra, Erlangen, Germany) equipped with a 32-channel head and neck coil to acquire 3D T1-weighted, DTI and resting-state fMRI images before and after therapy. For DTI, diffusion-weighted gradients will be applied along 30 non-collinear directions along with two non-diffusion-weighted volumes. Scanning parameters will include matrix size of 256×256, field of view of 256×256 mm^2, TR (Repetition time) of 7800 ms, TE (Echo time) of 82 ms, flip angle of 90°, number of averages of 1, b value of 1000 s/ mm2, slice thickness of 2 mm and voxel size of 1×1 × 2 mm^3. For resting-state fMRI, data will be collected over 240 time points. Scanning parameters will include a matrix size of 104×104, field of view of 240×240 mm^2, TR of 2000 ms, TE of 35 ms, flip angle of 52°, slice thickness of 2.5 mm and voxel size of 2.3×2.3 × 2.5 mm^3. DTI data processing will be conducted using FMRIB's Diffusion Toolbox (FMRIB Software Library V.6.0, FSL, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL) following the steps below:

(1) Registration: FSL tools will be used to obtain the transformation matrix and correct eddy current distortions in the DTI data sets. Subsequently, each subject's raw diffusion-weighted images will be linearly aligned to a non-diffusion-weighted image (b0) with the removal of non-brain tissues using a brain extraction tool. (2) Local fitting: the extracted brain will be used for diffusion tensors. (3) Normalisation: the fitted FA images will be registered to standard space and the obtained matrix will be applied to the registration of other parameter maps. (4) Estimation of diffusion tensor: the diffusion tensor will be estimated at each voxel to obtain the fractional anisotropy (FA) images. We will select the region of interest (ROI) method to measure tract integrity. The red nucleus (RN) is an appropriate part to draw ROI. We will draw ROI on the posterior limb of the internal capsule and the anterior pons on a color-coded map for CST and on the RN for RST individually. In each ROI, we will extract FA, mean diffusivity, radial diffusivity and axial diffusivity. Fibre tracking of the CST and RST will be performed for each patient.

Resting state-fMRI data processing will be carried out using DPABI_V7.0_230110 (Data Processing and Analysis for Resting-State Brain Imaging,) as follows: (1) Remove the initial 10 time points with unstable signals. (2) Perform slice timing correction to ensure that all voxels within each volume are acquired at the same time. (3) Conduct realignment to correct for slight head motion between different volumes. (4) Perform normalisation to register the brains of different subjects to a standard space atlas for subsequent statistical analysis. (5) Smoothing and detrending as needed to enhance data reliability. (6) Filtering to remove interference from non-cortical brain activity signals. (7) Calculate different metrics based on specific requirements.[63]

Sample size calculation

Sample size calculation was performed using the software G*Power V.3.1, with the upper limb Fugl-Meyer score as the primary outcome measure. Reference was made to the study by Zhang *et al*[64] which yielded an effect size of d=0.6. After transformation, the effect size for the analysis of variance analysis in this study was determined as F=0.3. A within–between interaction correlation of 0.5 was set to detect a medium effect size of Cohen F=0.3. The calculation results showed that a total of 27 samples (9 in each group) will be required to detect differences at a power (1-β) of 0.8 and a significance level (α) of 0.05 for the Cohen F=0.3 effect size. Considering a 20% potential

dropout rate, a total of 36 patients who had a stroke was needed in the study.

Data monitoring and management

The Data Monitoring Committee will oversee safety and data monitoring and provide recommendations for test design adjustments without any conflicts of interest. The Rehabilitation Department of Tongji Hospital will ensure the quality of informed consent, recruit eligible participants, implement intervention measures and manage data. Designated personnel will be responsible for collecting Case Report Forms (CRFs) as well as data transmission and analysis. Investigators and the principal investigator will be responsible for retaining all records, while the data centre will retain anonymised CRF data. Electronic data will be stored on password-protected computers and all paper data will be stored in secure filing cabinets.

Statistical analysis

The modelling and statistical analysis of fMRI data will be conducted using the statistic module of the DPABI_V7.0 software package in MATLAB (R2022b), along with the SPM software package. Statistical analyses of clinical data and DTI indicators will be conducted using the SPSS software. For normally distributed continuous data, t-tests will be employed, while non-normally distributed data will be analysed using the Mann-Whitney U test. Count data will undergo statistical analysis using the Pearson χ^2 test.

Ethics and dissemination

The trial is registered with the Chinese Clinical Trial Registry (Registration No. ChiCTR2400085220) and Medical Ethics Committee of Tongji Hospital, affiliated with Tongji Medical College, Huazhong University of Science and Technology (Registration No.TJ-IRB20231109). It will be conducted in the Departments of Rehabilitation Medicine and Radiology at Tongji Hospital in Wuhan, China. The findings will be disseminated through peer-reviewed journal publications and presentations at scientific conferences.

DISCUSSION

The restoration of motor and sensory functions in patients who had a stroke relies on normalising the network configuration of the bilateral sensorimotor cortex and structurally recovering fibre conduction bundles.[65] Three primary recovery pathways for the CRST after injury are identified: recovery along the original CRST pathway,[66 67] reorganisation around the lesion area[68] and recovery through the corpus callosum.[69 70] Following a stroke, CRT, with its dispersed projections, can be partially preserved, compensating for interruptions in motor pathways and restoring certain motor capabilities. Some preliminary evidence currently suggests that ascending and descending systems originating from the reticular nucleus may play an important role in motor recovery after stroke. Based on this, we designed this proof-of-concept study to better verify the previous hypothesis and explore the feasibility, safety and effectiveness of this new approach.

To the best of our knowledge, this is the first clinical trial to combine ASP (Acoustic startle priming) motor training with iTBS. The SE is thought to result in the transmission of information via the CRST[52] and existing research suggests that stimulation targeting the DLPFC may modulate the CRST.[36 37] Current research indicates that iTBS can modulate changes in grey and white matter microstructure,[71] facilitate the completion of transfer tasks and enhance excitability and functional connectivity in specific cortical regions,[71] thus promoting gait and balance recovery in patients who had a stroke.[47 72 73] Is the combination of central-peripheral closed-loop intervention more effective than single training for upper limb recovery in patients who had a stroke? What are the specific effects of promoting CRST recovery? In this study, we propose to promote both the RST and CRT of patients through the combination of exercise training and non-invasive transcranial stimulation—namely, ASP motor training and iTBS, respectively—to facilitate motor recovery. We plan to use multiple detection methods to comprehensively reflect the structural and functional changes of CRST. We intend to monitor the immediate and long-term effects of training-induced changes in brain activity using resting-state fMRI and detect structural changes in CRST after training using DTI. Additionally, by combining the detection of SCM activation and iTBS-induced MEPs, we aim to comprehensively evaluate the effectiveness of central-peripheral closed-loop intervention.

The study protocol has certain limitations. First, due to the incompatibility between TMS and MRI systems, the immediate effects of TMS on subjects may not be very pronounced, making it challenging to monitor changes in brain activity during the stimulation process. Second, because of adaptation to noise during baseline MRI scans, patients may experience a decrease in the effectiveness of auditory startle initiation. To mitigate this, we will attempt to minimise patient adaptation to noise by selecting better soundproofing equipment and adjusting the timing of baseline MRI scans.

In conclusion, we hope to further confirm and revise our current hypothesis through this proof-of-concept study and provide a theoretical basis for future central-peripheral closed-loop rehabilitation strategies based on CRST regulation. At the same time, the imaging and functional indicators obtained in this study can provide partial support for the safety, effectiveness and efficacy of this clinical practice plan.

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