Research Protocol

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A study on precise cerebral localization and network mechanisms of repetitive transcranial magnetic stimulation in treating chronic ankle instability based on high-resolution functional magnetic resonance imaging

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1. Research Background

Chronic ankle instability (CAI) is a prevalent sports injury encountered in clinical rehabilitation settings. Approximately 60% of individuals have experienced ankle sprains. Even with rehabilitation, nearly half of these patients develop recurrent injuries and progress to CAI. Although the direct treatment costs for CAI are relatively low, the indirect costs associated with subsequent rehabilitation and time loss prove to be substantial. Notably, current treatment strategies for CAI patients, including therapeutic exercises and surgical interventions, can effectively restore ankle joint stability. However, for a non-life-threatening condition that is often overlooked, surgery is rarely the first-line option for patients. Even with therapeutic exercises, managing ankle dysfunction and achieving functional recovery still requires a prolonged duration. Consequently, elucidating the pathological mechanisms of CAI and identifying its contributing factors are critical for developing targeted interventions and preventing long-term symptomatic progression.

Abnormal central adaptive alterations may predispose CAI individuals to injury-prone movement patterns. The neurophysiological mechanisms underlying these central nervous system abnormalities remain poorly understood in rehabilitation medicine, warranting urgent investigation. Current consensus posits that CAI-related functional deficits involve not only peripheral systems like ligamentous and muscular components but also exhibit potential neural control dysregulation within central pathways. Emerging evidence demonstrates moderate negative correlations between ankle disability indices and motor cortical excitability of the peroneus longus muscle in CAI populations, accompanied by bilateral elevation of resting motor thresholds. These findings suggest that cortical excitability modulations directly influence ankle functional capacity, with unilateral injury potentially inducing bilateral cortical excitability alterations. Furthermore, compared to healthy controls, CAI patients exhibit diminished tibialis anterior cortical excitability during single-leg stance, correlating with impaired postural control performance. While conventional stability-focused rehabilitation protocols can improve biomechanical parameters, CAI patients demonstrate functional deterioration when executing superimposed motor-cognitive tasks, likely attributable to aberrant cortical activation patterns that precipitate maladaptive movement strategies and reinjury risks. Therefore, integrating cortical excitability modulation and

enhancing central nervous system plasticity should be prioritized as novel therapeutic paradigms in CAI rehabilitation.

Repetitive transcranial magnetic stimulation (rTMS), a safe non-invasive neuromodulation technique, employs high-intensity magnetic pulses through induction coils to regulate cortical excitability, and has gained increasing application in sports injury rehabilitation in recent years. Over the past three decades, blood-oxygen-level-dependent functional magnetic resonance imaging (fMRI) has provided unprecedented opportunities for non-invasive investigation of human brain functional localization and large-scale neural network interactions. Recent discoveries concerning the geometric characteristics and integrative functions of the sensorimotor cortex have challenged traditional textbook concepts of somatotopic organization within this brain region. Parti cularly noteworthy is the development and clinical implementation of Chinese domestically innovated high-end MRI systems with proprietary intellectual property, which has created novel prospects for elucidating multi-modal and multi-scale fine-grained neural architecture. Consequently, utilizing fMRI to validate CAI-specific neural signatures in critical cortical regions, combined with mechanistic validation of rTMS-induced cortical modulation effects on ankle biomechanical adaptations, holds transformative potential for advancing neurorehabilitation protocols in chronic ankle instability management.

2. Aim of this study

This study aims to investigate the therapeutic efficacy of rTMS targeting key brain regions in sports rehabilitation for CAI populations and to evaluate its impact on the activation levels of ankle-stabilizing muscles. Focusing on athletes with CAI, the study integrates rTMS into conventional ankle stability training protocols. A comparative analysis of post-intervention differences in ankle functional scores, muscle activation patterns of ankle stabilizers, and cerebral cortex excitability between the two groups will clarify the superior efficacy of key brain region-targeted rTMS within ankle stability training protocols.

3. Methods and design

3.1 Study setting and design

(1) Study Design

This study is a single-center randomized single-blind parallel-controlled trial.

(2) Generation of Allocation Sequence

The randomization sequence for subject grouping will be generated by research members uninvolved in trial operations and assessments using the SPSS 22.0 statistical software program.

(3) 3.3 Allocation Concealment

Allocation concealment will be implemented by using the sealed envelope method. An opaque envelope containing the randomized grouping scheme will be prepared, and envelopes will be sequentially opened according to the enrollment order to determine subject allocation.

(4) 3.4 Allocation Implementation

The allocation sequence will be generated by personnel not participating in the experiment. Research staff will recruit subjects, and the personnel responsible for generating the allocation sequence will assign the interventions to the subjects based on the allocation sequence.

(5) Blinding

Subjects will be blinded following intervention allocation. This study will employ a randomized single-blind parallel-controlled design. Real rTMS will involve 10 minutes of magnetic stimulation delivered to the target brain region, while sham stimulation will mimick the sensory experience of real stimulation without inducing physiological effects. Unblinding can be performed by researchers at study conclusion or if a subject requires trial discontinuation due to severe adverse reactions.

(6) Sample Size Calculation

This study aims to compare the therapeutic efficacy of two interventions for chronic ankle instability, with the Cumberland Ankle Instability Tool (CAIT) as the primary outcome. Given the limited population size of athletes and anticipated heterogeneous data variance, sample size estimation was performed using the "Two-Sample T-Tests Allowing Unequal Variance" module in PASS 15 software. Based on existing literature, the control group was assumed to have a mean ± standard deviation of 20 ± 4, while the treatment group was projected to demonstrate 24 ± 4. With a power of 0.8 and α= 0.05 (two-tailed), the calculation indicated a

minimum requirement of 17 subjects per group. To account for potential attrition, each group will ultimately enroll 20 subjects.

3.2 Participant recruitment

3.2.1 Inclusion Criteria

(1) Age 18-60 years.

(2) History of at least one significant ankle sprain within the past 12 months, accompanied by inflammatory response (including pain resulting in at least one day of restricted physical activity).

(3) The most recent ankle sprain must have occurred more than 3 months prior to enrollment.

(4) Self-reported symptoms of ankle instability, defined by one or more of the following: At least two episodes of "giving way" in the affected ankle within the past 6 months; Recurrent sprains (≥2 documented sprains in the same ankle); Subjective perception of instability confirmed by any one of the following validated instruments: Ankle Instability Instrument (AII): ≥5 affirmative responses; Cumberland Ankle Instability Tool (CAIT): Score <24; Identification of Functional Ankle Instability (IdFAI): Score >11.

(5) Foot and Ankle Ability Measure (FAAM): Activities of Daily Living (ADL) subscale <90%; FAAM Sports subscale <80%; Foot and Ankle Outcome Score (FAOS): Scores <75% in ≥3 subcategories.

3.2.2 Exclusion Criteria

(1) Chronic ankle instability caused by bilateral ankle sprains;

(2) History of lower extremity musculoskeletal or neurological surgery;

(3) History of lower extremity fractures;

(4) Acute musculoskeletal injuries in other lower extremity joints within 3 months that compromised joint integrity and function, resulting in ≥1 day of interrupted daily or sports activities;

(5) Concomitant severe systemic diseases (cardiac, pulmonary, hepatic, renal, etc.);

(6) Severe psychiatric disorders or cognitive impairments;

(7) Presence of metal implants or inability to tolerate MRI examinations.

(8) Patient refusal to participate.

3.2.3 Withdrawal Criteria

(1) Presence of cardiovascular diseases;

(2) Signs of neurological impairment;

(3) Central nervous system lesions;

(4) Psychiatric or psychological disorders;

(5) Diagnosis of malignant tumors or rheumatoid arthritis;

(6) Current pregnancy or lactation;

(7) Severe scalp trauma, extensive/open craniocerebral injuries contraindicating coil placement or posing infection risks;

(8) Failure to obtain informed consent or concurrent enrollment in other clinical trials.

3.2.4 Study Termination Criteria

Development of adverse events or medical conditions contraindicating continued participation; Voluntary withdrawal by the participant; Protocol non-compliance substantially compromising trial outcome assessments.

3.3 Treatment Protocols

Ankle Stability Training + Sham rTMS Group: Participants underwent once-daily ankle stability training combined with sham rTMS intervention. The ankle stability training protocol comprised three modalities: neuromuscular training, balance training, and proprioceptive training. Specific exercises included single-leg stance, balance pad training, heel raises, resistive ankle inversion/eversion exercises, MOBO board balance exercises, alphabet tracing (toe-writing), lateral walking, heel walking, toe walking, and jump and landing control drills. The training progressed from stable to unstable surfaces, starting at low intensity and gradually increasing in difficulty and intensity. The training was conducted three times per week for a total of four weeks. Before each session of ankle stability training, participants received sham rTMS intervention. The sham stimulation mimicked the sensory experience of real rTMS but did not produce any physiological effects.

Ankle Stability Training + rTMS Group: Participants underwent rTMS intervention prior to daily ankle stability training. The interventional targets were the key cortical brain regions identified in preliminary studies, with stimulation applied to the contralateral cortical areas corresponding to the affected ankle joint. Parameters included a double-cone

coil, 10 Hz frequency, 2-second stimulation duration, 8-second inter-train intervals, 60 repetitions, totaling 1,200 pulses per session, with each session lasting 10 minutes. Ankle stability training commenced within 10 minutes post-rTMS, following the same duration and intensity as the Ankle Stability Training Group + Sham rTMS Group. The treatment was conducted three times per week for a total of 4 weeks.

3.4 Outcomes

3.4.1 Primary parameter

Cumberland Ankle Instability Tool Score (CAIT): A self-reported questionnaire designed to assess functional ankle instability. It consists of 9 items with a total score ranging from 0 to 30, where higher scores indicate better ankle stability.

3.4.2 Secondary parameters

(1) Karlsson-Peterson Ankle Function Score (KPAFS)

The KPAFS is a scoring system used to evaluate ankle dysfunction. It consists of 8 items: pain, swelling, instability, stiffness, stair climbing, running, work activities, and support. Each item is scored based on the patient's condition, with a total possible score of 100. Higher scores indicate better ankle function.

(2) American Orthopedic Foot and Ankle Society Score (AOFAS)

The AOFAS is a widely used assessment tool for evaluating functional outcomes in foot and ankle disorders. It includes domains such as pain, function, gait, range of motion, stability, and foot alignment. The total score ranges from 0 to 100, with higher scores reflecting better foot and ankle function.

(3) Surface Electromyography (sEMG) of Ankle-Stabilizing Muscles

Based on existing literature, the following muscles are selected for sEMG testing: tibialis anterior, gastrocnemius, and peroneus longus. sEMG measurements are conducted during two conditions: single-leg stance and the modified Star Excursion Balance Test (mSEBT). The following parameters are recorded:

a) **Time-domain analysis:** Root Mean Square (RMS), Integrated Electromyography (iEMG), and Averaged Electromyography (AEM).

b) **Frequency-domain analysis:** Mean Power Frequency (MPF) and Median Frequency (MF).

(4) Task-Based fMRI Data Acquisition and Analysis

Functional MRI (fMRI) data will be acquired at baseline and after a 4-week intervention using a 5.0-T scanner (uMR Jupiter, United-Imaging) with a 48-channel head coil, using identical protocols at both time points. Resting-state fMRI (rs-fMRI) will be acquired while participants remain awake with eyes open, using an echo-planar imaging (EPI) sequence (repetition time [TR] = 1600 ms, echo time [TE] = 28.5 ms, flip angle = 60°, field of view = 211×211 mm, matrix = 132×132, 1.6 mm slice thickness, 85 slices). Task-based fMRI (tb-fMRI) will employ an isometric ankle dorsiflexion paradigm using a block design of alternating 20-second task and rest blocks, with the affected ankle mechanically stabilized. Following a pre-scan maximum contraction calibration (Borg CR-10 scale), participants will execute maximal contractions guided by visual cues, reporting their subjective effort post-block to monitor compliance. The tb-fMRI images will use identical EPI parameters, except for a TR of 2000 ms. All fMRI data will be preprocessed using SPM (spm_25.01.02), encompassing slice-timing and motion correction (framewise-displacement-based), nuisance regression, temporal filtering, spatial normalization to standard MNI space, and spatial smoothing. For tb-fMRI, subject-level activation maps (dorsiflexion vs. rest) will be generated via a General Linear Model, followed by group-level analyses evaluating the intervention (active vs. sham rTMS) by time interaction through whole-brain and predefined region-of-interest (lower-limb motor-related precentral region) analyses. Finally, intervention-related changes in intrinsic brain activity and functional connectivity from the rs-fMRI data will be assessed using the Amplitude of Low-Frequency Fluctuations (ALFF), fractional ALFF (fALFF), and Regional Homogeneity (ReHo).

(5) Safety Evaluation

Any adverse events during the treatment, such as mild headaches, were recorded and monitored, and participants completed an adverse reaction questionnaire following the intervention.

3.5 Statistical Analysis

In the fMRI data analysis, a T-contrast boxcar analysis was conducted for each participant,

retaining only voxels with a significance level of $P < 0.05$ at the voxel-wise threshold. Family-wise error (FWE) correction was applied. Clusters were defined using a cluster-defining threshold of $P = 0.001$, with FWE-corrected cluster significance set at $P < 0.05$, and only clusters exceeding 10 voxels were considered. For group-level analysis, a one-sample t-test was employed. Cohen's d coefficient was used to quantify effect sizes between groups. Mean differences below 0.2 were disregarded, 0.2 – 0.5 were classified as small, 0.5 – 0.8 as moderate, and values > 0.8 as large. Statistical outputs from SPM software included cluster counts, voxel counts, peak voxel t-values, and Montreal Neurological Institute (MNI) coordinates. Contiguous voxels were aggregated into clusters, with peak voxel t-values representing the most statistically significant voxel within each cluster, and MNI coordinates indicating the location of the specific peak voxel in standardized brain space. Finally, activated regions were identified using the Automated Anatomical Labeling (AAL) atlas within the WFU PickAtlas toolbox.

Statistical analyses for efficacy evaluation were performed using SPSS 22.0. Data are presented as mean ± standard deviation. Between-group differences were compared using independent samples t-tests. Overall differences across time points between groups were assessed via two-way repeated-measures ANOVA or mixed-effects models. If a significant overall effect was detected, pairwise comparisons were conducted using the Sidak method. A threshold of $P < 0.05$ was applied to determine statistical significance.

4. Research Timeline

March 2025 – August 2027: Participant recruitment, intervention implementation, and data collection.

August 2027 – October 2027: Data analysis.

October 2027 – December 2027: Continued data analysis and manuscript drafting.