

# STUDY PROTOCOL

**Study Official Title:**

The Effects of Transcranial Temporal Interferential Electrical Stimulation on Cognitive Function, Dual-Task Performance and Neuroplasticity in Individuals with Mild Cognitive Impairment

**Unique Protocol ID:** 102772025RT208

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## 1. ABSTRACT

**Background:** Mild cognitive impairment (MCI) is a critical window for Alzheimer's disease (AD) intervention. Patients with MCI often exhibit both cognitive decline and impaired dual-task performance (e.g., walking while talking). Non-invasive deep brain stimulation using temporal interference (tTIS) can target the hippocampus, a key region for memory and motor-cognitive integration.

**Objective:** To investigate the immediate effects of a single session of individualized hippocampal tTIS on cognitive function, dual-task performance, and working memory in MCI, and to elucidate the underlying neuroplasticity mechanisms using multimodal neuroimaging.

**Design:** Randomized, double-blind, sham-controlled, crossover trial.

**Population:** 40 community-dwelling adults aged 60–80 years with MCI (MoCA < 26, CDR = 0.5, ADL < 26, no dementia).

**Intervention:** Single 20-minute session of individualized 5 Hz tTIS targeting the left hippocampus (2 mA, 2000/2005 Hz carriers) vs. sham stimulation, with a 7-day washout.

**Outcomes:** Primary: change in MoCA score, gait and balance dual-task cost, and working memory performance. Secondary: change in brain function (fMRI, EEG) and brain structure (MRI).

## 2. INTRODUCTION

### 2.1 Background and Rationale

MCI affects 15–20% of older adults in China, with an annual conversion rate to AD of 10–15%. Current pharmacological treatments have limited efficacy, highlighting an urgent need for non-pharmacological interventions. The hippocampus is one of the earliest brain regions affected in AD and is crucial for memory and motor-cognitive integration. Non-invasive brain stimulation techniques such as tTIS can modulate deep brain structures without surgery. Preliminary data from our pilot study (n=14) showed that a single session of

hippocampal tTIS significantly increased left hippocampal neural complexity ( $p<0.001$ ) and improved face-name memory accuracy ( $p=0.003$ ), with excellent tolerability.

2.2 Study Objectives

Primary Objective:

- To determine whether a single session of individualized 5 Hz hippocampal tTIS, compared to sham, improves cognitive function (MoCA), dual-task performance (gait and balance), and working memory in MCI patients.

Secondary Objectives:

- To evaluate the safety, tolerability, and blinding effectiveness of tTIS in MCI.
- To elucidate the immediate neuroplasticity mechanisms using multimodal MRI (fMRI, MRS, ASL, QSM, DTI) and EEG, focusing on changes in brain function and brain structure.

3. STUDY DESIGN

3.1 Overall Design

This is a **randomized, double-blind, sham-controlled, crossover trial**.

Participants will receive both active and sham tTIS in random order, with a 7-day washout period between sessions.

Study Flow Diagram:

Visit	Activity
Screening (V0)	Informed consent, medical history, MoCA, CDR, ADL, GDS, MRI safety check.

Visit	Activity
<b>Visit 1 (Baseline)</b>	Randomization, T1 MRI for individualized modeling, baseline assessments (cognitive, dual-task, fMRI, EEG).
<b>Visit 2 (Intervention 1)</b>	First stimulation (active or sham), post-stimulation assessments (MoCA, dual-task, working memory, fMRI, EEG).
<b>Washout (7 days)</b>	No intervention.
<b>Visit 3 (Intervention 2)</b>	Second stimulation (the opposite type), post-stimulation assessments.

### 3.2 Study Population

#### Inclusion Criteria:

- Age 60–80 years, community-dwelling
- Montreal Cognitive Assessment (MoCA) score < 26
- **Clinical Dementia Rating (CDR) score = 0.5**
- Activities of Daily Living (ADL) score < 26 (intact daily function)
- Does not meet criteria for dementia (DSM-5)
- No MRI contraindications (no metallic implants, no claustrophobia)
- Able to provide written informed consent

#### Exclusion Criteria:

- Neurological disorders (e.g., stroke, Parkinson's disease)
- Severe sensory or communication impairments
- Severe depression (Geriatric Depression Scale > 10)
- History of epilepsy or seizures
- Prior brain surgery or deep brain stimulation
- Alcohol or substance abuse

- Concurrent participation in another interventional trial
- Contraindications for tTIS or MRI (as above)

### **Vulnerable Population Protection:**

Given that this study enrolls older adults (aged 60–80 years) with mild cognitive impairment, additional safeguards are implemented. A multidisciplinary team (including a geriatrician, a neuropsychologist, and a clinical pharmacist) will conduct comprehensive assessments of cognitive function, psychological status, and social support systems before enrollment. Potential participants with severe comorbid conditions (e.g., uncontrolled hypertension, heart failure, malignancy) or severe psychiatric disorders (e.g., major depression, schizophrenia) will be excluded. Participants with unstable living situations or inadequate caregiver support will also be excluded to ensure safety throughout the study.

### **3.3 Randomization and Blinding**

Randomization will be performed by an independent statistician using a computer-generated sequence (1:1 allocation to sequence “Active→Sham” or “Sham→Active”). Allocation concealment will be maintained using sequentially numbered opaque envelopes.

Blinding: Participants, outcome assessors, and data analysts will be blinded to treatment assignment. The interventionist (not involved in assessments) will program the stimulator. Sham stimulation will use the same ramp-up/down and sensory experience but no effective current during the stimulation period.

## **4. INTERVENTIONS**

### **4.1 Individualized tTIS (Active)**

- **Target:** Left hippocampus (individualized based on each participant’s T1-weighted MRI using finite element modeling).
- **Parameters:** 5 Hz target frequency, 2000 Hz and 2005 Hz carrier frequencies, 2 mA baseline-to-peak intensity.

- **Duration:** 20 minutes, with 30-second ramp-up and ramp-down.
- **Electrode placement:** Determined by SimNIBS simulation to maximize electric field in the left hippocampus.

## 4.2 Sham Stimulation

- Same electrode placement, same ramp-up/down, and same total duration.
- Active current delivered only during the 30-second ramp-up and ramp-down; no current during the 20-minute stimulation period.

## 5. OUTCOME MEASURES

### 5.1 Primary Outcome Measures

#	Outcome	Description	Time Frame
1	Change in Montreal Cognitive Assessment (MoCA) Score	MoCA assesses multiple cognitive domains (memory, attention, executive function). Score range 0–30; higher scores indicate better cognition.	Baseline, immediately post-intervention
2	Change in Gait-Related and Balance-Related Dual-Task Cost	<b>Gait dual-task cost:</b> (Single-Task Gait Speed – Dual-Task Gait Speed) / Single-Task Gait Speed × 100% during walking + serial subtraction. <b>Balance dual-task cost:</b> Center of pressure sway path length and area during standing + serial subtraction.	Baseline, immediately post-intervention

#	Outcome	Description	Time Frame
3	Change in Working Memory Performance	Working memory assessed using the <b>n-back task</b> (1-back and 2-back conditions) during fMRI, with accuracy and reaction time as primary metrics.	Baseline, immediately post-intervention

## 5.2 Secondary Outcome Measures

#	Outcome	Description	Time Frame
4	Change in Brain Function	Multimodal assessment including: hippocampal neural complexity (resting-state fMRI multi-scale sample entropy), theta-gamma coupling (EEG), functional network efficiency (graph theory metrics), and cerebral blood flow (ASL).	Baseline, immediately post-intervention
5	Change in Brain Structure	Multimodal assessment including: hippocampal subfield volumes (T1 MRI), white matter integrity (DTI: FA, MD, AD, RD), and iron deposition (QSM).	Baseline, immediately post-intervention

## 5.3 Safety Outcomes

- Adverse events (structured questionnaire after each session)

- Tolerability (0–10 scale for each symptom: itching, tingling, burning, headache, etc.)
- Blinding effectiveness (participant guess of treatment assignment)

## 6. SAMPLE SIZE AND POWER

Based on our pilot study (n=14) and a previous meta-analysis of tDCS in MCI, we anticipate a moderate effect size (Cohen's  $d = 0.65$ ) for the primary outcome (MoCA). For a crossover design with a correlation of  $r = 0.5$  between periods,  $\alpha = 0.05$  (two-tailed), and power = 85%, the required sample size is 32. Accounting for 20% dropout, we will enroll **40 participants**.

## 7. STATISTICAL ANALYSIS

### 7.1 Analysis Sets

- **Full Analysis Set (FAS):** All randomized participants who received at least one intervention.
- **Per Protocol Set (PPS):** FAS participants with no major protocol deviations.
- **Safety Set (SS):** All participants who received any intervention.

### 7.2 Primary Analysis

For the crossover design, a linear mixed-effects model will be used with fixed effects for period, treatment, and sequence, and a random effect for participant. Baseline MoCA score will be included as a covariate. The primary contrast is the difference between active and sham conditions.

### 7.3 Secondary Analysis

Continuous secondary outcomes will be analyzed similarly. Categorical variables (e.g., adverse events) will be analyzed using generalized estimating equations.

## 7.4 Missing Data

Missing data will be handled using multiple imputation (chained equations) for sensitivity analyses.

## 7.5 Neuroimaging Analysis

- **fMRI:** Preprocessing with SPM12; group-level analysis using general linear models; network metrics computed with GRETNA.
- **EEG:** Preprocessing with FieldTrip; time-frequency analysis; phase-amplitude coupling.
- **MRI Structural:** VBM and surface-based morphometry with CAT12; DTI with FSL; QSM with STI Suite.

## 7.6 Mediation Analysis

- To test the hypothesized cascade (“neural oscillation → brain network → brain structure → cognitive improvement”), mediation analysis will be conducted using the PROCESS macro for SPSS or the \*lavaan\* package in R. The intervention (active vs. sham) will serve as the independent variable, changes in neuroimaging/EEG metrics as mediators, and changes in cognitive outcomes as dependent variables. The indirect effect will be estimated using bootstrapping (5,000 resamples) with 95% confidence intervals.

# 8. DATA MANAGEMENT AND MONITORING

## 8.1 Data Collection and Storage

- Case report forms (CRFs) will be used for all assessments.
- Data will be entered into a secure electronic database (password-protected, encrypted).
- Backup copies will be stored on a secure institutional server.

## 8.2 Data Safety Monitoring Board (DSMB)

An independent DSMB comprising a neurologist, a biostatistician, and an ethicist will review safety data every 6 months and will have the authority to recommend study termination if serious adverse events occur.

### **8.3 Adverse Event Reporting**

All adverse events will be recorded and reported to the ethics committee and DSMB within 24 hours if serious. Mild events will be reported quarterly.

### **8.4 Safety Monitoring for Older Adults with MCI**

**Pre-intervention preparation:** Before each stimulation session, vital signs (blood pressure, heart rate) will be recorded. Participants will be screened for any acute illness or discomfort that may affect participation.

**Real-time monitoring:** During stimulation, a trained investigator will remain with the participant, monitor for any signs of distress, and inquire about subjective sensations every 5 minutes. If any participant reports intolerable discomfort, stimulation will be stopped immediately.

**Emergency preparedness:** Emergency equipment (e.g., oxygen, suction) and medications (e.g., antihypertensives, glucose) will be readily available. All investigators have completed basic life support training. A direct emergency contact with a nearby tertiary hospital (Yangpu District Central Hospital) will be established for rapid transfer in case of serious adverse events.

**Fatigue prevention:** Each study visit is limited to a maximum of 2.5 hours, with mandatory rest breaks. Participants may rest or postpone tasks if they feel tired.

## **9. ETHICS AND REGULATORY COMPLIANCE**

### **9.1 Ethics Approval**

The study protocol has been approved by the **Shanghai University of Sport Research Ethics Committee** (Approval No. 102772025RT208, date: January 28, 2026). Approval from the partner hospital (Yangpu District Central Hospital, Shanghai) is pending and will be obtained before recruitment begins at that site.

## **9.2 Informed Consent**

All participants will provide written informed consent after a full explanation of the study, including potential risks and benefits. The consent form will be approved by the ethics committee.

## **9.3 Confidentiality**

Participant data will be coded and stored separately from identifiers. Only the principal investigator and authorized study staff will have access to the key.

## **9.4 Registration**

The study is registered with the Chinese Clinical Trial Registry (ChiCTR) and ClinicalTrials.gov (pending final approval).

## **9.5 Additional Safeguards for Cognitively Impaired Older Adults**

**Dual consent:** For participants with MCI who retain decision-making capacity, both the participant and a legally authorized representative (e.g., family member) will co-sign the informed consent form. For participants with impaired capacity, the legal representative will make the primary decision, while the participant is still informed and asked for assent.

**Simplified consent materials:** The informed consent form will be written in plain language (font size  $\geq 12$  pt) with key information highlighted. An illustrated information sheet and an audio-recorded explanation (slow-paced, gentle tone) will be provided to facilitate understanding.

**Extended discussion period:** The consent discussion will be conducted by two trained investigators in a quiet, comfortable setting, lasting at least 30 minutes. All questions will be answered thoroughly before signature.

**Ongoing assent monitoring:** Throughout the study, investigators will monitor participants' willingness to continue. If a participant shows signs of distress or reluctance, participation will be paused and reassessed.

## **10. PUBLICATION AND DATA SHARING**

### **10.1 Publication Plan**

Results will be published in peer-reviewed journals regardless of outcome. Authorship will follow ICMJE guidelines.

### **10.2 Data Sharing**

De-identified individual participant data for primary and secondary outcomes will be made available on Figshare or OpenNeuro within 6 months of publication. The study protocol, SAP, and ICF will be posted on ClinicalTrials.gov.

## **12. DECLARATIONS**

### **12.1 Integrity Statement**

All investigators involved in this study commit to strictly adhering to the study protocol and relevant clinical trial regulations. All study data will be recorded truthfully, completely, and in a traceable manner. No fabrication, falsification, or manipulation of research data will occur. The study procedures, intervention protocols, and assessment standards will be followed rigorously to ensure the scientific validity and reliability of the research outcomes.

## **12.2 Conflict of Interest**

All investigators declare no commercial or academic conflicts of interest related to this study. The study is funded by the National Natural Science Foundation of China (NSFC). All funds are allocated solely for participant recruitment, data collection, participant compensation, and other direct research expenses. No improper financial relationships exist.

## **12.3 Intellectual Property**

The data, results, manuscripts, and any patents arising from this study will be owned by Shanghai University of Sport. The principal investigator and major contributors will have authorship rights in accordance with ICMJE recommendations. Collaborating institutions (e.g., Yangpu District Central Hospital, Shanghai) will be acknowledged based on their contributions.

## **12.4 Publication Plan**

Results will be submitted for publication in peer-reviewed journals (e.g., Brain Stimulation, NeuroImage, Alzheimer's & Dementia) within 6 months of study completion, regardless of outcome. Authorship will follow ICMJE guidelines. Interim findings may be presented at scientific conferences but will be clearly marked as preliminary.

## **12.5 Data Sharing**

De-identified individual participant data for primary and secondary outcomes will be made available upon reasonable request to the corresponding author after publication of the main results. Data requestors must agree to: (1) not attempt to re-identify participants; (2) cite the original study; and (3) not use data for commercial purposes.