STATISTICAL ANALYSIS PLAN (SAP)

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

NCT Number: not yet assigned

Secondary ID: ChiCTR2600119709

Protocol Version: 1.3

SAP Version: 1.1

Date: April 4, 2026

Principal Investigator: Weijie Fu, PhD

Statistician: Yuqi Zheng, PhD

1. INTRODUCTION

This document describes the statistical analysis plan for the study "Individualized Hippocampal Temporal Interference Stimulation for Improving Cognitive-Motor Function in Mild Cognitive Impairment" (tTIS-MCI). The study is a randomized, double-blind, sham-controlled crossover trial designed to evaluate the immediate effects of a single session of active tTIS versus sham on cognitive function, dual-task performance, and working memory in individuals with mild cognitive impairment (MCI). Secondary objectives include assessing safety/tolerability and exploring neuroplasticity mechanisms via multimodal MRI and EEG.

The SAP was developed prior to any data analysis and adheres to the study protocol (Version 1.2). Any deviations from this plan will be documented and justified in the final study report.

2. STUDY DESIGN OVERVIEW

- **Design:** Randomized, double-blind, sham-controlled, crossover trial with two sequences (Active→Sham, Sham→Active) and a 7-day washout period.
- **Sample Size:** 40 participants (20 per sequence) – see Protocol Section 6 for calculation.
- **Primary Outcomes:**
    1. Change in Montreal Cognitive Assessment (MoCA) score.
    2. Change in gait-related and balance-related dual-task cost.
    3. Change in working memory performance (n-back task accuracy/reaction time).
- **Secondary Outcomes:**

    4. Change in brain function (composite of fMRI-derived neural complexity, EEG theta-gamma coupling, functional network efficiency,

ASL cerebral blood flow).

5. Change in brain structure (composite of hippocampal subfield volumes, DTI FA/MD, QSM iron deposition).

- **Safety Outcomes:** Adverse events, tolerability, blinding effectiveness.

3. ANALYSIS SETS

Analysis Set	Definition	Primary Use
Full Analysis Set (FAS)	All randomized participants who received at least one intervention (active or sham) and have at least one post-baseline measurement.	Main efficacy analysis (intention-to-treat principle).
Per Protocol Set (PPS)	FAS participants who completed both periods with no major protocol deviations (e.g., incomplete stimulation, missing outcome data).	Sensitivity analysis.
Safety Set (SS)	All participants who received any intervention (active or sham).	Safety and tolerability analysis.

4. STATISTICAL PRINCIPLES

- **Significance level:** $\alpha = 0.05$ (two-tailed).
- **Confidence intervals:** 95% for all estimates.
- **Multiple testing:** Primary outcomes are three; to control family-wise error rate, a **Bonferroni correction** will be applied: each primary outcome tested at $\alpha = 0.05/3 \approx 0.0167$. Secondary outcomes are exploratory and will be reported with nominal p-values and 95% CIs

without multiplicity adjustment, but findings will be interpreted cautiously.

- **Statistical software:** R (version ≥4.2) with packages *lme4*, *nlme*, *emmeans*, *mediation*, *pROC*; SPSS (version 26) for descriptive statistics.

5. MISSING DATA HANDLING

- **Missing primary outcome data:** For the crossover design, if a participant misses one period (e.g., no post-stimulation MoCA), they will be excluded from the primary analysis for that outcome (listwise deletion) if the missingness is <10% of total participants. If >10% missing, multiple imputation by chained equations (MICE) will be used, with 20 imputed datasets, assuming missing at random (MAR). Sensitivity analysis will compare results with and without imputation.
- **Missing covariates:** Baseline MoCA (covariate) will be mean-imputed if <5% missing; otherwise, imputed with MICE.
- **Reasons for missing data:** Will be documented and summarized.

6. DESCRIPTIVE STATISTICS

Baseline characteristics will be summarized by sequence group (Active→Sham, Sham→Active) and overall:

- **Continuous variables** (age, MoCA, etc.): mean ± SD, median (IQR), range.
- **Categorical variables** (sex, education level, etc.): frequencies and percentages.

Comparability between sequences will be assessed using independent t-tests (or Wilcoxon rank-sum for non-normal) for continuous variables and chi-square tests for categorical variables.

7. PRIMARY OUTCOME ANALYSIS

For each primary outcome (MoCA change, dual-task cost change, working memory change), a **linear mixed-effects model** (LMM) will be used to account for the crossover design with repeated measurements.

7.1 Model Specification

- **Outcome:** Post-stimulation value (or change score; sensitivity analysis using post value with baseline adjustment).
- **Fixed effects:** Sequence (Active→Sham vs Sham→Active), Period (first vs second), Treatment (active vs sham), and baseline value of the outcome (as covariate).
- **Random effect:** Participant (random intercept).
- **Interaction:** Treatment × Period will be tested; if non-significant ($p>0.10$), removed from final model.

7.2 Estimation

- Model fitted using restricted maximum likelihood (REML).
- Estimated marginal means (EMMs) for active and sham conditions will be computed.
- Primary contrast: difference between active and sham (least squares mean difference), with 95% CI and p-value.
- The p-value will be compared to Bonferroni-adjusted $\alpha = 0.0167$.

7.3 Carryover and Period Effects

- Carryover effect will be assessed by testing the interaction between Sequence and Period ($p>0.10$ considered no significant carryover). If carryover is present, analysis will be restricted to first period data only as sensitivity analysis.

8. SECONDARY OUTCOME ANALYSIS

Secondary outcomes (brain function composite, brain structure composite) will be analyzed using the same LMM approach as primary outcomes, without multiplicity adjustment (nominal p-values reported). For exploratory purposes, individual components of the composites (e.g., hippocampal neural

complexity, theta-gamma coupling, hippocampal volume) may also be examined using the same LMM, with results interpreted as hypothesis-generating.

9. SAFETY AND TOLERABILITY ANALYSIS

- **Adverse events (AEs):** Frequency and percentage of participants reporting any AE, and specific AEs (itching, tingling, etc.) by treatment condition. Comparison between active and sham using McNemar's test (paired) due to crossover design.
- **Tolerability scores:** Mean ± SD of intensity scores (0–10) for each symptom, compared between active and sham using paired t-test or Wilcoxon signed-rank.
- **Blinding effectiveness:** Proportion of participants who correctly guessed their treatment assignment (active vs sham) after each period, compared to chance (50%) using binomial test.

10. NEUROIMAGING AND EEG ANALYSIS

Neuroimaging and EEG data will be preprocessed using established pipelines (SPM12, FieldTrip, FSL, CAT12, etc.) as detailed in the protocol.

10.1 Statistical Models for Imaging Data

- **fMRI/EEG outcomes:** For each participant, the difference between active and sham conditions (Δ = value after active – value after sham) will be computed for key metrics (e.g., hippocampal complexity, theta-gamma coupling). Paired t-tests (or Wilcoxon signed-rank) will be used to test if Δ differs from zero. Multiple comparisons across ROIs will be controlled using false discovery rate (FDR) at $q<0.05$.

10.2 Mediation Analysis

To test the mechanistic hypothesis that improvements in cognitive outcomes are mediated by neural changes, a **mediation analysis** will be conducted

using the bootstrapping method (5000 resamples) with the PROCESS macro (or R *mediation* package). The model:

- **Independent variable (X):** Treatment (active vs sham, coded 1/0).
- **Mediators (M):** Change in candidate neural metrics (e.g., hippocampal neural complexity, theta-gamma coupling, functional connectivity). Each mediator will be tested separately.
- **Dependent variable (Y):** Change in primary cognitive outcomes (MoCA, dual-task cost, working memory).

The indirect effect (ab) will be considered significant if the 95% bootstrap CI does not contain zero.

11. SUBGROUP AND SENSITIVITY ANALYSES

- **Subgroup analyses:** Exploratory analyses based on baseline characteristics (age, sex, education, baseline MoCA) will be performed by adding interaction terms (Treatment × Subgroup) in the LMM. Results will be reported with caution and not used for definitive conclusions.
- **Sensitivity analyses:**
    1. Using PPS instead of FAS.
    2. Using change-from-baseline as outcome without covariate adjustment.
    3. Excluding participants with protocol deviations.
    4. If missing data >10%, comparing results with and without multiple imputation.
    5. For the primary outcome, analyzing only first period data if carryover effect is detected.

12. INTERIM ANALYSIS

No interim analysis for efficacy is planned due to the short duration and small sample size. Safety data will be reviewed by the DSMB after the first 10

participants complete the study; no formal stopping rules are predefined, but the DSMB may recommend termination if serious unexpected adverse events occur.

13. REPORTING

All analyses will be reported in accordance with the CONSORT extension for crossover trials. Results will be presented with point estimates, 95% CIs, and p-values (with correction noted). Statistical analysis will be performed after database lock and unblinding of treatment allocation.