Investigation of Low-intensity Focused Ultrasound Parameters

NCT: NCT05134233

09/12/2024

Statistical Analysis Plan (SAP) for ClinicalTrials.gov Submission
Study Title: Investigating Neuromodulatory Effects of Low-Intensity Focused Ultrasound (LIFU) in Humans

1. Objective:
This study aims to assess the effects of varying parameters of low-intensity focused ultrasound (LIFU)—such as intensity, duration, duty cycle, and pulsing—on neuromodulation in the human brain. The primary focus is on evaluating changes in motor-evoked potential (MEP) amplitude when LIFU is applied concurrently with transcranial magnetic stimulation (TMS).

2. Study Design:

2.1 Interventions:
The study involves the application of LIFU using the following 20 parameter combinations:

- **Intensity levels:** 6 W/cm² and 24 W/cm²
- **Duty cycles:** 1%, 10%, 30%, 50%, and 70%
- **Durations:** 100 ms and 500 ms
- **Pulsing conditions:** Pulsed versus continuous delivery evaluated in 4 combinations

2.2 Study Type:
This is a fully parameterized, repeated measures design in which LIFU is delivered concurrently with TMS. Each subject experiences all combinations of LIFU parameters.

2.3 Data Collection:

- **MEP amplitude:** Measured as the peak-to-peak response for each LIFU parameter combination, with 20 stimulations per trial per subject.
- **Normalized MEP values:** Calculated by normalizing MEP amplitudes for each parameter combination to baseline (pre-LIFU) values.

3. Outcome Measures:

3.1 Primary Outcome Measure:

- **MEP Amplitude:** Normalized MEP amplitude, analyzed across the 20 parameter combinations of LIFU.

3.2 Secondary Outcome Measure:

- **Pulsed vs. Continuous Ultrasound:** Effects of pulsing on MEP amplitude will be evaluated.

4. Statistical Analysis Plan:

4.1 Power Analysis:
Based on preliminary data from a pilot study (N=5), we observed maximal changes in MEP amplitude of:

- **Excitatory effects:** 46.35% ± 12.33%
- **Inhibitory effects:** -25.54% ± 15.56%

- **Minimal effects:** 5.99% ± 7.87% with less optimal parameter combinations.

To detect a meaningful change in MEP amplitude, we calculated a required sample size of **N=56 participants**. This is sufficient to detect a **15% change** in MEP amplitude (minimum clinically significant change) with a power of **0.8** and a significance level of **p = 0.05**. This sample size may not detect minimal changes (~6%), but is adequate to capture meaningful excitatory and inhibitory effects across parameter combinations.

4.2 Assumptions Testing:

- **Normality of MEP Amplitudes:** Tested using **Bartlett's test for sphericity** ($p < 0.05$). If assumptions of normality or homogeneity of variances are violated, non-parametric tests will be used.

4.3 Primary Analysis:

- **Paired t-tests:**
  We will compare baseline normalized MEP values with post-intervention MEP values for each LIFU parameter combination. To control for multiple comparisons, a **Bonferroni correction** will be applied, adjusting the significance level to **p < 0.0025** (0.05/20).
- **Three-Way Repeated Measures ANOVA:**
  A 3-way repeated measures ANOVA will be performed to assess the main effects and interactions between the following factors:
  - **Intensity:** 6 W/cm² vs. 24 W/cm²
  - **Duty Cycle:** 1%, 10%, 30%, 50%, 70%
  - **Duration:** 100 ms vs. 500 ms
- **Post-hoc testing:**
  If significant main effects or interactions are observed, **Tukey's Honest Significant Difference (HSD) test** will be applied for pairwise comparisons. Tukey's HSD will control the family-wise error rate, ensuring reliable comparisons between parameter combinations.

4.4 Pulsed vs. Continuous Analysis:
A separate **three-way repeated measures ANOVA** will be conducted to evaluate differences between pulsed and continuous LIFU delivery. The main factors assessed will be:

- **Intensity:** 6 W/cm² vs. 24 W/cm²
- **Number of Cycles:** 35,000 vs. 75,000
- **Pulsing Condition:** Pulsed vs. Continuous

Post-hoc analyses using **Tukey's HSD** will follow if significant results are observed.

5. Handling Missing Data:
If missing data arises, participants with missing measurements for key outcomes will be excluded from specific analyses. However, where possible, **multiple imputation** methods will be used to handle missing data. Sensitivity analyses will be conducted to assess the robustness of the results in the presence of missing data.

6. Assumptions and Corrections:

6.1 Assumptions for Parametric Testing:

- **Normality and sphericity:** The normality of MEP data will be confirmed via **Bartlett's test** ($p < 0.05$). If normality or homogeneity of variances is not met, non-parametric alternatives will be used, such as the **Wilcoxon signed-rank test**.

6.2 Correction for Multiple Comparisons:

- **Bonferroni correction** will be applied to paired t-tests, adjusting the significance level to **$p < 0.0025$** for each comparison (0.05/20 combinations).

7. Data Presentation and Reporting:
Results will be presented as **mean ± standard deviation (SD)** for each LIFU parameter combination. Data will be visually represented using bar charts and line graphs for MEP amplitudes, with appropriate error bars. The statistical significance of comparisons will be indicated with asterisks (*$p < 0.05$, **$p < 0.0025$, etc.).

Results from the ANOVAs, including F-values, degrees of freedom, and p-values, will be reported in tables. Post-hoc comparisons using Tukey's HSD will be detailed with adjusted p-values.

8. Conclusion:
The analysis plan outlined above ensures a robust statistical approach to evaluating the effects of various LIFU parameters on MEP amplitude, providing insights into how different ultrasound intensities, duty cycles, durations, and pulsing patterns influence neuromodulatory outcomes. These findings will contribute to optimizing LIFU for potential therapeutic applications in neuromodulation.

This SAP has been designed to ensure comprehensive evaluation of the hypotheses, with appropriate statistical rigor to control for errors and variability across conditions.