Title: Revitalizing Cognition in Older Adults at Risk for Alzheimer's Disease using Near Infrared Photobiomodulation

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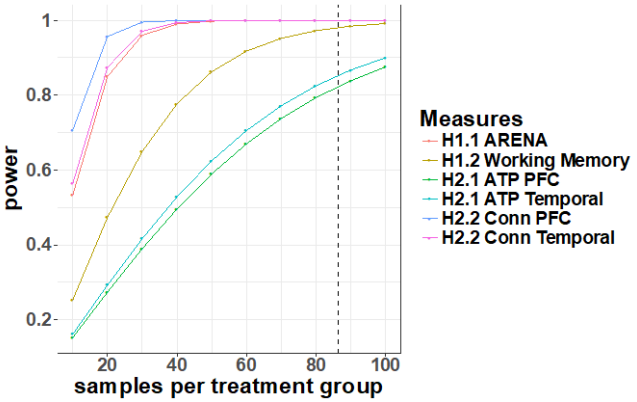
STATISTICAL DESIGN AND POWER

Sample Size Determination/Power Analysis



The sample size calculation was based on our pilot results of all primary and secondary measures in Aim 1 and Aim 2. Here we use ARENA, our primary outcome that is being tested in Aim 1 (see H1.1) to illustrate the power calculation procedure. From our pilot data, the mean difference of ARENA score changes between active stimulation group (n = 8) and sham (n = 5) group was 0.58. Based on a pooled standard deviation of 0.55, the standardized effect size (Cohen's d) is d=1.06. Similarly, we calculated the standardized effect sizes of our secondary outcome variables, including working memory (H1.2, d=0.67); prefrontal cortex ATP (H2.1, d=0.48); temporal lobe ATP (H2.1, d=0.5); prefrontal cortex connectivity (H2.2, d=1.3); and temporal lobe connectivity (H2.2, d=1.1). Based on our experience of 10% attrition in a clinical trial with three months of intervention for the ACT study (MPI: Woods; PI UA Field Site: Alexander) that is currently underway at the UF and UA field sites, we conservatively assumed 15% attrition in the power calculations for the current proposal. At 5% alpha level and assuming the 15% attrition rate, the power trajectories for different measures with respect to number of samples per treatment group are shown in the above figure. In order to guarantee that all these primary and secondary measures have more than 80% power, we plan to enroll a total of 168 participants (84 per group, shown as the dashed line in the above figure). This large sample size will also ensure sufficient statistical power for the exploratory analysis of Aim 3, where we will examine potential mediators and moderators in relation to the intervention response. Note that we are powering the study based on a 2-week session and the actual effect sizes (from a 12-week NIR stimulation session) will likely exceed these minimal estimates.

Statistical Analysis

All data will be checked for out-of-range values, and distributional forms (normality, homogeneity of variance). Decisions regarding parametric vs nonparametric statistics will be based on the results of those analyses. Standard summary statistics will be provided for pre, post- and follow-up time points. The intention-to-treat (ITT) principle will be applied whereby all randomized patients who complete the baseline assessment will be analyzed according to their assigned interventions. For studying the relationship between longitudinal outcomes and NIR stimulation effects, we will fit linear mixed effects models with subject specific random effects to account for the within subject correlation, using R software *lmer* package[158]. Linear mixed model is robust against missing data if they are missing at random (MAR)[159]. Baseline outcomes and covariates including age, sex, education, and other potential confounders (listed in Exploratory Aim 3) will be adjusted to take account for confounding effects. If multiple hypotheses are investigated simultaneously, multiple comparisons will be properly adjusted by using Benjamini-Hochberg correction[160]. Below we describe how these statistical models fit the specific aims in this proposal.

Analyses for Aim 1: To test the hypothesis that the active NIR stimulation will result in greater pre-post intervention improvement in cognition (episodic memory, executive function) relative to the sham group, we will fit the following linear mixed model:

$$y_{ik} = \mu + \alpha_i + \beta \mathbb{I}(z_i = 1) + \sum_{l=0}^{L} \gamma_l x_{il} + \eta \mathbb{I}(k = 2) + \theta \mathbb{I}(z_i = 1, k = 2) + \varepsilon_{ik}, \alpha_i \sim N(0, \sigma_0^2), \varepsilon_{ik} \sim N(0, \sigma^2) \quad (1)$$

where y_{ik} is the outcome variable (e.g., ARENA score for Aim 1) for subject i and visit k ($k = 1$ for post-intervention and $k = 2$ for 3-month follow-up); μ is the intercept term; $\alpha_i \sim N(0, \sigma_0^2)$ is the random effect with variance term σ_0^2 for subject i; z_i is the treatment indicator with $z_i = 0$ for sham group and $z_i = 1$ for active

stimulation group, β is the post intervention treatment effect (active stimulation effect compare to sham group) and $\mathbb{I}(.)$ is the indicator function which equals to 1 if the argument inside $(.)$ is true and equals 0 otherwise; x_{il} is the l^{th} covariates for subject i with $l = 0$ for baseline level (e.g. baseline ARENA for Aim 1) and $l = 1,2,\dots,L$ for L covariates (i.e., age, sex, education…); η is the time effect of 3-month follow-up compared to post-intervention visit; θ is the interaction effect between follow-up and active stimulation; $\varepsilon_{ik} \sim N(0, \sigma^2)$ is the error term with variance σ^2. In equation (1), β is the active stimulation effect for post-intervention, and we will perform the statistical hypothesis testing (H_0: $\beta = 0$) to provide its p-value, point estimate and confidence interval. Similarly, $\beta + \theta$ is the active stimulation effect after 3-month follow-up, where p-value, point estimate and confidence interval will be similarly obtained by testing the null hypothesis H_0: $\beta + \theta = 0$. Note that equation (1) will be applied to all measures in Aim 1 including the primary outcome (ARENA), secondary outcomes (working memory), and other exploratory outcomes (verbal fluency, mnemonic similarity, auditory verbal learning test, and Stroop color word test).

Analyses for Aim 2: To test the hypothesis that NIR stimulation will result in greater pre-post intervention improvement in neuroimaging (31P MRS for ATP and rs-fMRI for connectivity) relative to the sham group, we will fit the following linear mixed model:

$$y_{ik} = \mu + \alpha_i + \beta \mathbb{I}(z_i = 1) + \sum_{l=0}^{L} \gamma_l x_{il} + \varepsilon_{ik}, \alpha_i \sim N(0, \sigma_0^2), \varepsilon_{ik} \sim N(0, \sigma^2) \quad (2)$$

Equation (2) is similar to equation (1), with the adjustment of the same set of covariates, except that the equation (2) doesn't have the 3-month follow-up. β is the active stimulation effect for post-intervention, for which we will perform the statistical hypothesis testing (H_0: $\beta = 0$) to provide its p-value, point estimate and confidence interval. Note that equation (2) will be applied to all measures in Aim 2 including secondary outcomes involving ATP from 31P MRS (H2.1) and connectivity from, rs-fMRI (H2.2).

Analysis for Exploratory Aim 3: We want to further explore whether APOE ε4 carrier status, vascular comorbidities, Fitzpatrick skin type questionnaires, processing speed, and mood are potentially confounders, moderators, or mediators. To examine confounders, we will directly adjust these variables as covariates in the linear mixed model in equation (1) and equation (2). A statistically significant covariate effect will imply the potential confounding roles of these variables. To examine moderators, we will include these variables and their interactions with the intervention effect (one variable at one time) in equation (1) and equation (2). A statistically significant interaction effect will imply the variable can be a moderator. To examine mediators, we will perform mediation analysis[161-162].The mediation effects of these variables and their 95% confidence interval will be determined using the R *mediation* package[163].

For each of the specific aims listed above, we will further conduct exploratory analysis stratifying by sex, to examine its relevant biological effect. In other words, we will disaggregate the data by sex and evaluate the active stimulation effects for male and female, respectively.