Statistical Analysis Plan

Combination Treatment with L-DOPA and Exercise for Mood and Mobility Problems in Later Life

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Statistical Design and Power

Overview: The primary aims of the study are to (1) measure individual and combined effects of L-DOPA and Exercise on cognitive, psychiatric, and physical performance measures in depressed older adults with psychomotor slowing and (2) investigate responsivity of reward-related dopamine circuits to L-DOPA and exercise. Exploratory aims are to evaluate correlations between real-world physical functioning metrics (i.e., gait speed, fatigability, physical activity) and behavioral/neural responses on the effort-based decision making (EBDM) and virtual reality maze navigation (VRMN) tasks in the scanner as well as compare differences in L-DOPA and exercise acceptability, side effects, and attrition. As outlined in **Human Subjects 4.3.**, the primary psychiatric/behavioral measure is the Hamilton Rating Scale for Depression (HRSD), the primary cognitive measure is the processing speed latent factor extracted from the Digit Symbol and Pattern/Letter Comparison Tests, and the primary physical functioning measure is gait speed measured on the Gaitrite walkway. The primary neuroimaging measures on the EBDM task are each participant's subjective value discounting curve, the parameter k=the discount rate, and p=the inflection point at which participants begin to discount, and neural activations in ventromedial prefrontal cortex (vmPFC) and dorsal anterior cingulate cortex (dACC). For the VRMN paradigm, the primary measures are dorsomedial ventral striatum (dmVS) activation associated with effort (High-Effort>Low-Effort) and action (Low-Effort>Passive-Motion) during the Cue and Navigation Start phases as well as anterior VS (aVS) activation associated with the effect of reward (Reward>No-Reward, across all conditions) and magnitude of reward (in parametric models) during the Reward phase.

Before the specific techniques are applied, we will examine all variables at all time points for illegitimate values, outliers, and inconsistencies. The distribution of demographic variables and baseline clinical characteristics will be examined and described across the depressed subjects (N=80 total, 20 randomized to each group) in terms of means, standard deviations, proportions, and 95% confidence intervals. As a precaution, indications of inequality between groups in specific features (despite randomization) will trigger examination of whether differences in primary outcome measures can be attributed to an imbalance in group assignment. Intent-to-treat analysis will be implemented for all estimation and testing. All tests for main effects will be performed at two-tailed significance 5%.

Analyses for Specific Aim 1: *Hyp 1:* Analysis of covariance (ANCOVA) models will be used to determine whether there are significant main effects of L-DOPA and Exercise (as compared to Placebo and Stretching/Toning Control, respectively) on change in processing speed, gait speed, and depressive symptoms from baseline to 12-week follow-up. The change from baseline in (1) each of the three processing speed measures (Digit Symbol, Pattern and Letter Comparison tests) and also for the factor score obtained from a one-factor confirmatory factor analysis model, (2) the continuous gait speed (m/s) measure (single and dual task), and (3) 24-item HRSD each will be analyzed as a function of treatment group and baseline values of the outcome. Contrasts of treatment difference in each variable will be formed and tested. *Hyp 2:* The L-DOPA x Exercise interaction will be evaluated by extending the ANCOVA model to include a 4-category treatment group variable and testing the contrast that the L-DOPA + Ex group shows the greatest improvement compared to Placebo + Control.

Analysis for Specific Aim 2: *Hyp 3:* Outputs from the first-level analyses of functional MRI data described in **Research Strategy C.7.** will be entered into ANCOVA models similar to Aim 1. These will test for main effects of L-DOPA and Exercise (as compared to Placebo and Stretching/Toning Control, respectively) on behavioral and neural activation measures derived from the EBDM task (Hyp 3) and the VRMN task (Hyp 4).

Exploratory aims: Correlations between each physical performance measure (e.g., single and dual task gait speed, fatigability scores on the Pittsburgh Fatigability Scale [PFS], Short Physical Performance Battery [SPPB] scores) with behavioral and neural activation measures derived from the EBDM and VRMN tasks will be examined at baseline and 12 weeks across all treatment groups. Correlations between change scores will also be examined. Linear and logistic regression will be used to examine differences in acceptability, side effects, and attrition across the 4 randomized conditions.

Approach to Dropouts and Missing Data: The aims will be analyzed using intent-to-treat (ITT) sample on all randomized subjects. We will account for unobserved data by using inverse probability weighting by building a model with baseline predictors of drop-out (156). This weighting method is appropriate under the missing 'at random' assumption. We also plan to perform sensitivity analyses using various models of missingness and assess the effect of the assumption of missing 'at random' on the inference (157-160).

Power Analysis: For Aim 1, the analysis will include N=80 (N=20 per group) depressed subjects in a randomized, blinded, placebo-controlled mechanistic trial of L-DOPA and Exercise for between-group comparisons on cognitive, motor, and behavioral assessments. For Aim 2, we will evaluate the N=40 subject sub-sample (N=10 per group) for between-group comparisons on behavioral and neural activation data from the effort-based reward tasks. Based on our past pilot data (1), we anticipate a minimal dropout rate of approximately 8%.

Power for Aim 1: *Hyp 1* examines differences between treatment groups (L-DOPA + Exercise, L-DOPA + Control, Placebo + Exercise, Placebo + Control) on change in cognitive processing speed, gait speed, and depressive symptoms. With N=74 LLD subjects providing follow-up data (approximately 37 receiving L-DOPA and 37 receiving Exercise), we will have at least 80% power to detect effect size differences of Cohen's d = 0.63 for the independent main effects of L-DOPA and Exercise, at a fixed significance level of 5%. Such a medium to large effect size would be clinically meaningful in LLD patients and is in a range of what has been observed in prior L-DOPA (1) and exercise studies (55,92). *Hyp 2* evaluates whether an L-DOPA x Exercise interaction is present on these outcomes, such that the greatest improvements occur among participants randomized to L-DOPA + EX. Based on simulations, with N=20 depressed subjects in each of the four groups, we will have >80% power to detect an interaction corresponding to an effect size of Cohen's d=1.28.

Power for Aim 2: Aim 2 will be evaluated using a mechanistic probe of dopaminergic function with a trial of L-DOPA and/or Exercise. We anticipate a low drop-out rate of 8% based on our pilot study of L-DOPA in N=36 depressed adults very similar to the population to be enrolled here and assume roughly equivalent drop-out between groups (1). *Hyp 3-4* examine between-group differences on our neuroimaging outcomes of interest after the acute 12 week across the two tasks (effort-based decision making [EBDM] task and virtual reality maze-navigation [VRMN] task). We will have at least 80% power to detect effect size differences of Cohen's d=0.96 for the main effects of L-DOPA and Exercise on task responses (each participant's subjective value discounting curve, the parameter k=the discount rate, p=the inflection point at which participants begin to discount, and neural activations in vmPFC, dACC, dmVS, and aVS ROIs). K24 Mentor Dr. Treadway's previous studies examining between-group differences in willingness to expend effort using similar fMRI tasks have reported between-group effect sizes in this range (24).