Title: Dopaminergic Therapy for Inflammation-Related Anhedonia in Depression

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PROTOCOL TITLE: Dopaminergic Therapy for Inflammation-Related Anhedonia in Depression.

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FUNDING SOURCE: NIMH R61 Award.

REVISION HISTORY

Revision #	Version Date	Summary of Changes
2	04/10/20	Revisions from initial IRB review
3	12/30/20	Revision of recruitment procedures (now virtual and in-person) before start of recruitment; provide additional study details; add personnel
4	12/30/20	Revisions requested for Modification #3
5	01/28/21	Fix errors in reimbursement table
6	06/16/21	Revisions of travel compensation (reduce mileage coverage to 30 miles from 50 miles)
7	11/02/21	Revisions to the visit order of screening data collection
8	7/11/22	HAM-A instrument removed from Baseline Visit.
9	8/23/23	Clarified language regarding repeat CRP testing, clarified method of screening and monitoring of suicide, and clarified/fixed typos for HAM-D and IDS-SR in Table 2.

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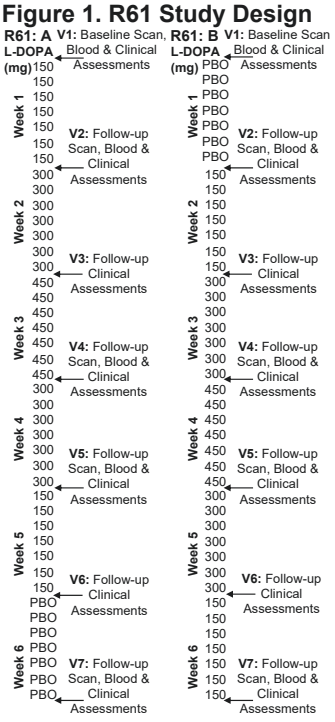
1.0 Study Summary

Study Title	Dopaminergic Therapy for Inflammation-Related Anhedonia in Depression
Study Design	6-week randomized, double-blind, placebo-controlled crossover
Primary Objective	To determine a daily dosing strategy for the dopamine precursor, levodopa (L-DOPA), that will increase functional connectivity (FC) in ventral striatum to ventromedial prefrontal cortex (VS-vmPFC) reward circuitry in major depressive disorder (MDD) patients with high inflammation and anhedonia.
Secondary Objective(s)	Data regarding safety and tolerability, and preliminary data on the potential for L-DOPA to improve anhedonia and motivation will also be collected. Additional behavioral, biologic and MRI measures (at baseline) will be collected for use as predictors of response and to enhance interpretation of results.
Research Intervention(s)/Interactions	L-DOPA; placebo
Study Population	Medically stable and medication-free adults (ages 25-55) with Major Depressive Disorder, plasma CRP >2 mg/L, and high anhedonia (score >/=2 on the anhedonia question of Patient Health Questionnaire [PHQ]-9)
Sample Size	35
Study Duration for individual participants	≥8 Visits; ~8 week duration
Study Specific Abbreviations/ Definitions	Levodopa (L-DOPA) Major depressive disorder (MDD) Functional connectivity (FC) C-reactive protein (CRP) Ventral striatum to ventromedial prefrontal cortex (VS-vmPFC)
Funding Source (if any)	NIMH R61 Grant (R61MH121625)

2.0 Objectives

The primary objective of the proposed research is to determine a daily dosing strategy for the dopamine precursor, levodopa (L-DOPA), that will increase functional connectivity (FC) in ventral striatum to ventromedial prefrontal cortex (VS-vmPFC) reward circuitry in major depressive disorder (MDD) patients with high inflammation and anhedonia. More than 30% of patients with MDD fail to achieve remission with conventional antidepressants and even responders exhibit significant residual symptoms including anhedonia. Inflammation is elevated in a significant portion of patients with MDD, particularly in those patients who are resistant to conventional antidepressant therapies. Preclinical and clinical findings indicate that inflammation can affect striatal dopamine availability and release, to contribute to symptoms of anhedonia and reduced motivation. We have previously reported a relationship between inflammation and FC in dopaminergic reward circuitry in patients with MDD whereby patients with increased inflammation, as defined by plasma C-reactive protein (CRP), exhibited lower VS-vmPFC FC. This VS-vmPFC FC was in turn correlated with symptoms of anhedonia. Clinical and translational evidence from our group and others suggests that inflammation impacts corticostriatal circuits by decreasing the synthesis and availability of dopamine. Our preliminary data demonstrate that VS-vmPFC FC connectivity was increased after acute administration of L-DOPA compared to placebo in patients with high CRP (indexed as CRP >2 mg/L), indicating that increasing dopamine with L-DOPA can reverse the impact of inflammation on this circuit. The dopamine precursor, L-DOPA, is taken up in the brain and rapidly converted to dopamine by DOPA decarboxylase. Despite evidence of reduced dopamine availability in MDD, little work has explored the potential for L-DOPA to improve anhedonia and motivation in MDD. By building on our previous work demonstrating a functional link between decreased dopamine availability and impaired FC in reward circuits of MDD patients with high inflammation, this study will test the hypothesis that treatment with L-DOPA will improve depressive symptoms in MDD patients with high inflammation and anhedonia by increasing FC in reward circuitry and improving motivation.

Figure 1. R61 Study Design



In order to obtain 30 study completers (~10-15% expected attrition rate), 35 medication-free, medically-stable and otherwise psychiatrically healthy adults (25-55 yrs) with 1) current MDD per DSM-V criteria, 2) plasma CRP >2 mg/L, and 3) score ≥5 on an anhedonia subscale of the Inventory of Depressive Symptomatology-Self-Report (IDS-SR), will be randomized to two study tracks (A and B) to receive both placebo and L-DOPA (starting dose of 150 mg/day with dose escalation 150 mg/day per week to a final dose of 450 mg/day) in a 6-week, double-blind, placebo-controlled, crossover study (see **Figure 1**). Patients will undergo resting-state functional magnetic resonance imaging (fMRI) at baseline, after 1-week of placebo, and after 1-week at each dose of L-DOPA to establish target engagement in the brain for L-DOPA versus placebo to improve FC in reward circuity. Symptoms of anhedonia and an objective measure of motivation will be assessed as preliminary data regarding L-DOPA effects on behavior in relation to change in FC. Additional behavioral, biologic and MRI measures will be collected at baseline for use as predictors of response and to enhance interpretation of results. Safety and tolerability and data on the effect of L-DOPA on anhedonia and motivation in relation to target engagement in the brain (FC) will also be assessed.

Aim 1: To determine whether L-DOPA increases VS-vmPFC functional connectivity in MDD patients with high CRP (>2 mg/L) and anhedonia.

Hypothesis 1: L-DOPA versus placebo will increase VS-vmPFC FC along with plasma L-DOPA levels, and with maximal effects at higher doses.

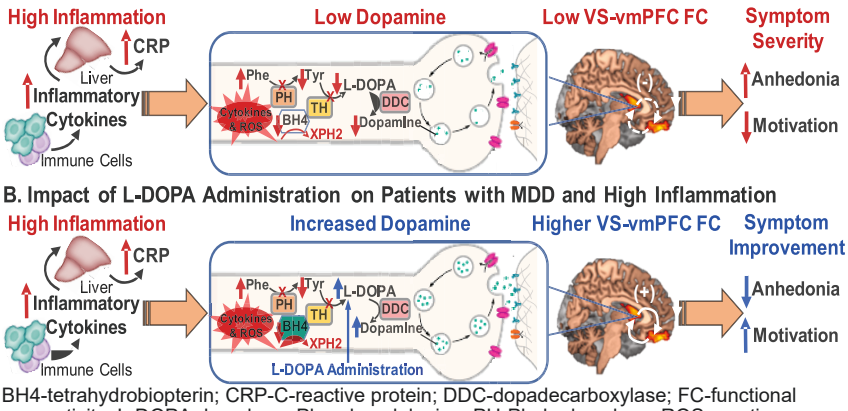
Exploratory aims: To obtain information on the dosing and tolerability of L-DOPA in our patient population, while collecting preliminary data on whether L-DOPA-sensitive fMRI FC brain biomarkers are affected in association with improvement in anhedonia and motivation.

Exploratory hypotheses: L-DOPA will be well-tolerated in otherwise medically-stable MDD adults with CRP >2mg/L and anhedonia at all doses tested, and improvement in FC in reward circuitry will occur in association with improved motivation and motor slowing.

3.0 Background

Depression is common, affecting ~10 million in the US alone, and confers a personal and societal burden. More than 30% of patients with major depressive disorder (MDD) fail to achieve remission with conventional antidepressants, and even responders exhibit significant residual symptoms including anhedonia.[1-4] Therefore, new conceptual frameworks are needed to reveal pathophysiologic pathways and neurobiological targets for development of novel treatments for subgroups of MDD patients who are not sufficiently responsive to existing therapies. Relevant in this regard, one pathophysiologic pathway thought to contribute to symptoms of depression and particularly anhedonia is inflammation.[5-7] A significant proportion of patients with MDD are reliably found to exhibit increased inflammatory markers in both the periphery and central nervous system, as measured by inflammatory cytokines and acute phase reactants such as C-reactive protein (CRP).[8-11] Elevated CRP and other peripheral blood markers of inflammation have also been found to predict future development of depressive symptoms,[12-14] and patients with high levels of inflammatory markers are more resistant to standard antidepressants such as selective serotonin reuptake inhibitors (SSRIs).[5,15-19]

Figure 2. Working Model



BH4-tetrahydrobiopterin; CRP-C-reactive protein; DDC-dopadecarboxylase; FC-functional connectivity; L-DOPA- levodopa; Phe-phenylalanine; PH-Phehydroxylase; ROS-reactive oxygen species; Tyr-tyrosine; TH-Tyrhydroxylase; vmPFC- ventromedial prefrontal cortex; VS-

Working Model and Study Overview. Administration of inflammatory stimuli or cytokines to laboratory animals and humans affects reward pathways in the brain to lead to anhedonia and reduced motivation, which has been shown to be mediated by reduced synthesis and availability of striatal dopamine.[20-25] We recently reported a relationship between increased inflammation (as defined by plasma CRP) and low functional connectivity between the ventral striatum (VS) and ventromedial prefrontal cortex (vmPFC), key structures that receive mesolimbic dopamine input,[26] in patients with MDD, in association with increased anhedonia.[27] Furthermore, our preliminary results also indicate that VS-vmPFC FC in MDD patients can be increased by acute administration of the dopamine precursor levodopa (L-DOPA) in association with improved motivation, but only in patients with high inflammation. Together these findings indicate that MDD patients with high inflammation have low striatal dopamine availability that drives decreased FC in reward circuitry and increased anhedonia/reduced motivation (**Figure 2A**).[7] These data also support our hypothesis that VS-vmPFC FC in these patients can be strengthened by L-DOPA administration to lead to reduced anhedonia and improved motivation (**Figure 2B**).

This study will determine a daily dosing strategy for L-DOPA that will increase FC in VS-vmPFC reward circuitry in a 6-week randomized, double-blind, placebo-controlled, crossover study with 3 doses of L-DOPA in the R61. Data regarding the potential for L-DOPA to improve anhedonia

and motivation will also be collected. Milestones for R61: To establish target engagement in the brain, we expect that at least one of 3 doses of L-DOPA will produce a mean increase in VS-vmPFC FC that is ≥20% higher than that of placebo.

This study aims to address an unmet need to investigate novel therapeutic strategies that may be targeted to sub-populations of patients with MDD based on a known pathophysiology that can be identified by a combination of peripheral blood and behavioral biomarkers. Despite evidence of low dopamine function in MDD,[3,28,29] the ability of existing dopaminergic therapies with well-characterized pharmacology, like L-DOPA, to affect brain circuits in MDD has yet to be explored. Further novelty and innovation exist in targeting such therapies to MDD patients with high inflammation and anhedonia, a population frequently reported-on but understudied in experimental medicine or clinical trials. The choice of L-DOPA for this study is particularly well-matched to this target population as inflammation is known to impede dopamine synthesis (see **Figure 2**), which is rapidly increased by the dopamine precursor, L-DOPA. Therefore, this work involving a dopamine agonist with known pharmacology will not only test a potential new therapy[30] but will also provide the foundation for the development of novel dopaminergic therapies for the ~30-50% of MDD patients with high inflammation[19,31-35] who are less responsive to conventional antidepressants like SSRIs.[5,15-19]

Inflammation Impacts Reward Circuitry through Decreased Dopamine Synthesis and Availability. Numerous neuroimaging studies in subjects peripherally administered cytokines or other inflammatory stimuli (either as clinical therapy or in laboratory experiments) have found that inflammation preferentially affects corticostriatal circuitry that is relevant to anhedonia and motivation, as well as psychomotor speed.[21,22,36-39] For example, functional magnetic resonance imaging (fMRI) has demonstrated decreased ventral striatal neural activation to hedonic reward in patients administered the inflammatory cytokine interferon (IFN)-α to treat hepatitis C, which correlated with reduced motivation and activity.[21] Typhoid vaccination and endotoxin administered in the laboratory to healthy subjects produced similar effects on the basal ganglia, including decreased neural activation of the ventral striatum to hedonic reward and decreased corticostriatal FC, which also correlated with symptoms of anhedonia and psychomotor slowing.[22,38-40] Positron emission tomography (PET) neuroimaging investigating cytokine effects on dopamine metabolism revealed increased uptake and decreased turnover (indirect measures of dopamine capacity and release) of radiolabeled L-DOPA, [18F]fluorodopa, in the caudate, putamen, and ventral striatum of IFN-α-treated patients.[21] Our translational work in non-human primates and studies in rodents have directly linked the effects of inflammation on corticostriatal reward circuits to decreased availability and release of striatal dopamine.[20,24,41,42] Indeed, we conducted in vivo microdialysis and PET neuroimaging with [^{11}C]raclopride displacement following amphetamine challenge in IFN-α-treated monkeys.[20] Results indicated that stimulated dopamine release was decreased in the striatum (including nucleus accumbens) after chronic administration of IFN-α, and decreased dopamine release, as measured by in vivo microdialysis, was correlated with reduced effort-based sucrose consumption.[20] A primary pathway by which inflammation impacts dopamine is by decreasing the availability of tetrahydrobiopterin (BH4), an enzyme co-factor required for synthesis of dopamine via the conversion of phenylalanine to tyrosine and tyrosine to L-DOPA (see **Figure 1**).[43] We and others have observed evidence of reduced BH4 activity in patients administered IFN-α, which correlated with decreased cerebrospinal concentrations of dopamine and increased depressive symptoms.[23,44] To examine whether inflammation reduces availability of dopamine by depleting dopamine precursors, we administered L-DOPA via reverse in vivo microdialysis to monkeys treated with chronic IFN-α, and the inflammation-induced reductions in striatal dopamine release were restored by L-DOPA.[24] Therefore, pharmacological agents that improve dopamine synthesis may effectively treat anhedonia and reduced motivation in MDD patients with high inflammation.

Pharmacology of L-DOPA and Potential to Improve Reward Circuit Function, Motivation and Anhedonia in Depression with High Inflammation. Unlike dopamine itself, L-DOPA crosses the blood-brain-barrier (BBB) and is rapidly converted to dopamine by DOPA

decarboxylase (DDC) then packaged and either stored or released from presynaptic dopaminergic terminals. L-DOPA is currently used in combination with carbidopa (which does not cross the BBB) to increase dopamine transmission in dopamine-deficient patients with Parkinson's disease (PD) or dopamine-mediated sleep movement disorders like restless legs syndrome (RLS).[45,46] Carbidopa blocks the conversion of L-DOPA to dopamine in the periphery, allowing more L-DOPA to be taken up by the brain and to reduce peripheral side effects from dopamine receptor activation. In addition to having known pharmacology, the pharmacokinetics of L-DOPA and its effects on brain dopamine are well established.[24,47] Despite evidence of reduced dopamine availability in depression,[24] little work has explored the potential for dopaminergic therapies like L-DOPA to improve anhedonia and motivation in MDD. Case series beginning in the 1960s report improved depressive symptoms with L-DOPA monotherapy or augmentation, particularly in patients considered to have a slowed or "retarded" phenotype of depression.[48-51] In new onset PD, L-DOPA was shown to improve symptoms in 28 out of 31 patients with a mean decrease in the Hamilton Depression Rating Scale (HAM-D) of 11.7,[52] whereas others have reported beneficial effects on depressive symptoms, quality of life and/or cognitive performance.[53-55] With regard to hedonic tone and motivation, L-DOPA increased the frequency by which healthy subjects chose high-probability gains in an instrumental learning task.[56] Similarly, L-DOPA selectively improved motivation for (wanting), versus sensitivity for (liking) or learning about, reward in mice with a genetic depletion of dopamine.[57] These findings support the hypothesis that L-DOPA is able to improve motivation, particularly in subjects with low dopamine synthesis and availability. Agents that increase dopamine signaling, such as the dopamine receptor agonist pramipexole, have demonstrated efficacy in treatment-resistant depression,[58,59] and MDD patients with moderately increased CRP (≥1 mg/L) were found to exhibit a robust response to the combination of a SSRI plus bupropion (an antidepressant with stimulant properties) compared to a poor response to the SSRI alone.[19] These findings provide support for our hypothesis that MDD patients with high inflammation, who are often resistant to SSRIs, may be especially responsive to dopaminergic therapies. However, dopamine receptor agonists bind to receptors for other systems such as serotonin,[60] and bupropion is not specific for the dopamine transporter.[61] Furthermore, inflammation is thought to decrease dopamine neurotransmission by interfering with rate-limiting aspects of dopamine synthesis that occur upstream of the conversion of L-DOPA to dopamine by DDC. Thus, L-DOPA is the most direct and specific pharmacological approach to increase dopamine transmission and improve corticostriatal reward circuits in association with behavior in MDD patients with high inflammation who may exhibit a fundamental depletion of dopamine availability. Accordingly, L-DOPA is the most appropriate compound for the R61/R33 mechanism as it is 1) well-matched to the underlying mechanism and 2) will isolate a specific dopaminergic effect in the target population, which will have implications for development of novel therapeutics, or the targeting of existing compounds like bupropion,[19,62] pramipexole[58] or even L-DOPAf[30] (as will be explored in the R33) to patients with MDD, high inflammation and anhedonia. The R33 can then inform future studies that examine L-DOPA as a monotherapy or adjunct and compare the response to other dopaminergic therapies (or SSRIs) and in low CRP patients.

4.0 Study Endpoints *

Milestone for R61: Target Engagement of Brain. Targeted VS-vmPFC connectivity will serve as the primary outcome and will be assessed for determining the Go/No-Go Criteria. Targeted FC for each subject and treatment condition will be calculated as the degree of correlation in activity between a 3mm^3 radius sphere in VS[27,65] and the vmPFC ROI identified as being reward-sensitive in neuroimaging meta-analyses[105] (MNI coordinates x=0, y=44, z=-8, cluster size=1408 mm^3 and encompassing parts of BA11 and ventral BA32 of ACC) or other similar ROIs used to define vmPFC in our previous work.[27,64,65,106] This method will ensure rigor and reproducibility and application of results to potential future trials because the seeds and ROI are predefined, as opposed to those derived from whole-brain and data-driven analyses which are determined based on the specific patient sample. Z-scores will be extracted and the change in mean FC values after each dose of L-DOPA or placebo compared to baseline will be calculated.

FC between bilateral VS seeds and the vmPFC ROI will be examined separately and change in FC in either the right or left VS can contribute to meeting the Go/No-Go criteria. However, based on our previous work in MDD and IFN-a-induced depression[21,27] and our preliminary studies, we anticipate that this change will be more pronounced on the left side. Response may also be strongest in subjects with greatest bioavailability of L-DOPA. If more than 1 dose of L-DOPA meets criteria, % of patients and mean number of reported side effects will be compared across doses to determine the effective dose that was most tolerated by patients. If doses are similarly tolerated, % change in behavior (EEfRT > anhedonia) will be used. Statistical significance, ES or sensitivity analyses may be used to assist with selection of the optimal dose.

Additional Analyses for Publication and Dissemination: Brain Target- Targeted FC Z scores (as described above) will be entered into linear regression models including clinical covariates (see below) and both conservative (Bonferroni) and powerful (Fisher LSD) post-hoc analyses to asses change in FC across the 3 doses of L-DOPA compared to placebo and to each other. Linear models will also be used to determine whether FC increases as a function of increasing plasma L-DOPA, and whether patients with low bioavailability exhibit low ΔFC. In addition to the targeted FC that will be used to assess Go/No-Go criteria, hypothesis-driven and data-driven whole-brain analysis will also be explored and compared before and after L-DOPA and placebo. Consistent with our previous data, these analyses will include VS seed-to-voxel-wise comparisons of L-DOPA versus placebo, as well as network analyses using GBC and PBNA, as a secondary outcome measure and to identify other circuits that may be affected by L-DOPA. For all analyses, concentration of CRP and final dose will be explored as a linear predictor of response to L-DOPA. Clinical covariates such as age, sex, race, BMI and ATQR and/or bipolarity scores will be explored as potential confounders or moderators of L-DOPA effects on FC. Integration of Brain and Behavior: As described for FC, linear regression models and post-hoc analyses will be used to asses change in behavioral outcomes across the 3 doses of L-DOPA compared to baseline, to placebo and to each other, and whether response was modified by plasma bioavailability of L-DOPA, CRP, cytokine or sex hormone concentrations, or clinical covariates. Random intercept linear mixed models will be used to assess whether change in FC predicts change in anhedonia and motivation and in relation to clinical covariates. Overall depression scores and SHAPS-C and MAP-SR will be explored as secondary outcomes. Summary scores for dose-response (e.g. AUC) may be used to assess relationships among variables.

5.0 Study Intervention/Investigational Agent

Description: Carbidopa-levodopa (Sinemet) is a combination of two drugs, L-DOPA and carbidopa. Carbidopa-levodopa is used primarily in the treatment of Parkinson's disease (although it is also FDA approved for other movement disorders such as Restless Legs Syndrome). Parkinson's disease is believed to be caused by low levels of dopamine in certain parts of the brain. When L-DOPA is taken orally, it crosses into the brain through the "blood- brain barrier." Once it crosses, it is converted to dopamine. The resulting increase in brain dopamine concentrations is believed to modulate nerve conduction and improve movement impairments. Carbidopa does not cross the blood-brain barrier. Carbidopa is added to L-DOPA to prevent its breakdown before crossing into the brain. The addition of carbidopa allows lower doses of L-DOPA to be used. This reduces the risk of side effects from L-DOPA such as nausea and vomiting. Nausea and vomiting are frequent side-effects of taking levodopa, and often improve with increasing time on medication. This combination medicine was approved by the FDA in 1988. Carbidopa-levodopa is taken several times per day for treatment of Parkinson’s disease, up to 800 mg daily. Patients in this study will receive L-DOPA at doses ranging from 150 to 450 mg administered with at a ratio of 1 mg carbidopa for every 4 mg L-DOPA. Our preliminary data using

acute administration of 250 mg L-DOPA to MDD patients with CRP >2mg/L increased corticostriatal FC in reward circuitry as measured by fMRI (the primary study outcome) without only mild adverse events that are anticipated during initial exposure to the medication, primarily nausea (with vomiting in some patients). Other common symptoms that were experienced included headache and drowsiness or lightheadedness. Furthermore, in a similar recent study in aged adults with depression (>/=60 years),[30] L-DOPA administered at the same doses proposed herein (also 1 week at each dose) decreased depressive symptoms and was well-tolerated. Indeed, the percent of patients reporting nausea (the most common symptom) decreased from 19.4% in Week 1 to 9.1% in Week 3, despite dose increases by 150 mg/week. Symptoms of headache and drowsiness occurred in less than 10% of patients throughout the duration of the study, and insomnia occurred in 8% in the first week which decreased to 3% in week 3. Moreover, patients were monitored for dyskinesias (a common symptom in Parkinson's Disease that may be specific to neurodegenerative disease pathology) and scores were 0.0 at Week 3 for all subjects.

Table 1. Expected Frequency and Duration of Common Anticipate Mild Adverse Events Associated with L-DOPA

Anticipated Mild Adverse Events Associated with L-DOPA	**Approximate Expected Frequency**	**Anticipated Expected Duration**
Nausea (may include vomiting in some patients)	20-30% 10-20% ~10% or less	First few days First few weeks After week 3
Insomnia	~10% ~5% or less	First week After the first week
Headache	~5-10%	Anytime during treatment
Lightheadedness, dizziness or drowsiness	~5-10%	Anytime during treatment

The following rare but serious side effects have also been reported following the use of chronic high doses of L-DOPA for treatment of Parkinson's disease: easy bleeding/bruising, signs of infection (e.g., fever, persistent sore throat), tingling of the hands/feet, vision changes (e.g., blurred/double vision), chest pain, seizures, vomit that looks like coffee grounds, black/tarry stools, unusual muscle stiffness, severe confusion, sweating, fast/irregular heartbeat, rapid breath or trouble breathing, painful or prolonged erection in males, rash, itching/swelling (especially of the face/tongue/throat), and severe dizziness. Transient and mild side effects that may occur following acute administration of the doses used in this study include: dizziness, nausea, vomiting, increased sleepiness or trouble sleeping, and headache. L-DOPA may also increased eye blinking/twitching, cause fainting, mental/mood changes, and worsening of involuntary movements/spasms.

5.1 Drug/Device Handling: L-DOPA and placebo will be dispensed to by Emory Investigational Drug Services (IDS) under an IDS-SOP and delivered to the principal investigator or study team member. The principal investigator or

health care providers on his/her research team will provide the medicine to the subjects. To maintain blinding during dose escalation and across tracks, study medication will be dispensed to each participant at their weekly follow-up visits. In order to assist participants and the monitoring of adherence, medication will be dispensed in blister packs and all unused medication will be returned to the pharmacy. Compliance will be monitored and recorded. Falling below 85% compliance on more than one week may be grounds for dismissal.

5.2 N/A. Investigator Justification for IND Exemption is attached.

6.0 Procedures Involved*

6.1 Full participation in this study will require a minimum of 9 visits, including, 1) at least two screening visits (Screen A and B, which may be conducted on the same day), 2) baseline blood draws and behavioral assessments, and fMRI scan, 3) 4 follow-up visits during dose-escalation of L-DOPA or washout to placebo that include blood, fMRI scan and behavioral assessments, and 4) two follow-up visits without scans to collect self-report and safety and tolerability information. An initial intake visit (to be conducted on the phone or virtually) will also be conducted either within the study (after study consent) or prior to study consent within our Behavioral Immunology Program Screening Clinic. At screening (in-person visit to occur after study consent), patients will receive a physical examination and laboratory testing (blood sampling) and will be requested to provide detailed information on past medical and psychiatric history and treatment, as well as current symptom status. Height, weight and vital signs will be recorded. Some aspects of laboratory testing (blood work and urinalysis) and medical and psychiatric history may be collected in the Screening Clinic to help match participants to this or other ongoing studies. During screening, a fasting research blood sample (18 ml total) may be collected (along with blood draw for additional safety labs and/or CRP measurement if needed) as a Pre-Baseline assessment. Research blood will be used for measurement of plasma inflammatory markers, mRNA gene expression for inflammatory signaling pathways, circulating and mRNA gene expression markers related to metabolism and endocrine hormones, and buffy coat to examine polymorphisms in relevant inflammatory proteins. Pre-Baseline assessments will be collected during the Screen B visit, and will also include practice neurocognitive and motivation assessments.

Eligibility for completing further screening and Pre-Baseline assessments: The PI or PI's designee will review information collected from the initial intake/consent visit and any subsequent screening to determine whether subjects are eligible to proceed with further screening and collection of Pre-Baseline assessments based on the following:

(1) Attainment of informed consent

(2) Psychiatric clinician has determined presence of current depressive symptoms at intake with PHQ9 score >10, unless otherwise approved by the principal investigator or PI's designee

(3) The patient has not informed study staff or the psychiatric clinician of any condition that, in the opinion of the PI or PI's designee, would make the patient unsuitable for or unable to complete further screening

Following the in-person screening visits and Pre-Baseline data collection, the patient will undergo the Baseline visit including MRI scan and randomization. At the Baseline visit, urine will be obtained for toxicology and pregnancy testing (where appropriate) and a fMRI scan will be performed. Subjects will receive standardized clinician-administered interviews and ratings for psychiatric symptoms, will be asked to fill out self-report questionnaires and will undergo objective tests of motivation and motor activity. Blood will also be drawn for the measurement of inflammatory marker/hormone levels after the scan procedures, and patients will receive study medication. At the end of each indicated week of treatment (7 days after each dose change in weeks 1, 3, 4 and 6), subjects will undergo fMRI scans, receive standardized clinician-administered interviews and ratings for psychiatric symptoms, will be asked to fill out self-report questionnaires, have blood drawn for L-DOPA concentrations and inflammatory markers, urine will be obtained for toxicology, and pregnancy testing, and will perform objective tests of motivation/motor activity. Safety and tolerability will also be assessed. For follow up visits at weeks 2 and 5, patients will either be seen in person (where possible) or virtually to fill out self-report questionnaires, height, weight and vital signs will be recorded (in-person only), update psychiatric symptoms, and safety and tolerability will be assessed. If the visit occurs virtually, medication will be mailed to the participant and unused medication will be returned at the next in-person visit.

Table 2. Schedule of Study Procedures and Assessments.

Assessments	Intake/ Consent	Screen A	Screen B- (Incl. Pre-Baseline Assess.)	Baseline	Weeks 1, 3, 4, 6	Weeks 2 & 5
Consent, Intake Registration Form, PHQ-9 [Intake or Screen A, optional at Screen B], Med-Psych Prescreen Form, History of Chronic Conditions, Medication Avoidance List*	X	[X]	[X]			
SCID V-modified (Mood, Anxiety, PTSD modules), Bipolarity index, ATRQ, Clinical Review for Screening**	X	[X]	[X]			
C-reactive protein (CRP), Rapid CRP Finger Prick [if necessary to verify CRP]*		X	[X]	[X]		
MRI Safety Screening, Anti-nuclear AB, TSH, Serum Pregnancy (hCG), Hepatitis B surface antigen, Hepatitis C antibody, HIV antigen/antibody*		X	[X]			
EKG		X	[X]			
Urine pregnancy test*			[X]	X	X	[X]
Physical Exam			X			

Adverse Events, Concomitant Medications*	X	X	X	X	X	X
Vital Signs, weight, height [screen only], waist-hip ratio [screen only]		X	X	X	X	[X]
CBC with differential, urinalysis w/ micro, comprehensive metabolic panel*		X	[X]			
Urine toxicology		X	[X]	X	X	[X]
Demographic Data, Habits, CTQ, IDS-SR			X			
Randomization (Track A or Track B)			X			
Study Medication Dispensing, Dose Change and/or Compliance Check				X	X	X
Research Blood (fasting)			X			
Research Blood (Non-Fasting)				X	X	
HAM-D item #3, Columbia Suicide Severity Rating Scale (CSSRS), Clinical Review for Follow Up, Dyskinesia Symptom Review				X	X	X
Psychiatric Assessments (HAM-D, SHAPS-C), HAM-A [Baseline only, optional for all other visits]***				X	X	X
Self-Report Forms (IDS-SR/IDS-Anhedonia, MAPS-SR, BAI, STAI, PSS, PCL-5, Habits)				X	X	X
EEfRT (Motivation), Neurocognitive Testing			X	X	X	
MRI Scan				X	X	

*These assessments may be conducted in the Emory behavioral Immunology Program research screening clinic

** A Clinical Review will be conducted if more than 2 weeks from last assessment or as an update to SCID modules conducted in the Behavioral Immunology Program within the past 6 months

***HAM-A removed from Baseline visit on 7/11/22.

[] to be completed if needed

6.2 **Neuropsychiatric Assessments**: Structured Clinical Interview for DSM-V Axis I Disorders (SCID-V): is a semi-structured clinical interview that provides a wide range of DSM-V diagnoses. All patients will be evaluated by a modified version of the SCID as part of the screening process. These interviews will primarily be conducted virtually. In addition to providing information for inclusion/exclusion purposes, the SCID will provide data about psychiatric predictors of treatment response, including number of past major depressive episodes, length of the current episode and presence of co-morbid anxiety disorders and/or dysthymia. At screening and at each visit, a standardized clinician interview will also be used to assess or monitor symptoms of mania/hypomania, substance abuse (also confirmed by urine toxicology screen), alcohol use, dyskinesia, and gambling (a potential symptom of dopaminergic medications like L-DOPA).

The MGH Antidepressant Treatment Response Questionnaire (ATRQ): The ATRQ provides specific criteria for adequate dose and adequate length of a trial for it to be considered a failure, thus allowing clinicians to systematically collect data aimed at assessing degree of treatment-resistance of the MD episode. The data obtained can then be used to calculate a score using the MGH Staging Method (MGH-S) to classify degree of treatment resistance. Degree of resistance will be used as a

covariate in relevant statistical analyses and to exclude severely treatment resistant subjects (>3 adequate failed trials).

MRI neuroimaging: Scans will be carried out in Emory University's Facility for Education & Research in Neuroscience (FERN) on a Trim Trio scanner and 32-channel head coil. T1 weighted anatomic images will be collected with MPRAGE[148] or a similar high-resolution sequence for co-registration with fMRI data after a localizer. fMRI BOLD for resting and task-based functional connectivity: A multi-echo (ME) ep2 BOLD sequence will be optimized to provide high signal-to-noise ratio in regions of interest that is maintained during minor incidences of head motion. Resting ME BOLD will be acquired over ~10 min, and task fMRI will involve two scans of ~10 minutes each. For resting bold, a single acquisition of phase-encoding in the opposite direction of polarity (anterior-posterior) for distortion correction will be collected over ~ 30 seconds.[149] Data will be analyzed with standard preprocessing protocols in AFNI and SPM1. Individual's fMRI data will be normalized into a standard stereotaxic space, Montreal Neurological Institute (MNI) template with 1mm^3 resolution. MIDT**:** MIDT will be used to assess FC[95,152,153] during reward anticipation. This widely-used task for assessing reward function in psychiatric patients[154-156] is one of the few imaging reward tasks with established test-retest reliability.[157] Monetary outcome depended on patient performance in a simple reaction time task by pressing a button in response to a visual target stimulus. The "anticipatory delay," ~4000 m, occurs after presentation of a pseudo-randomly distributed cue to inform participants whether a given trial will allow them to win or lose money (reward: +$; loss: -$; no incentive: 0$; averaging ~$2) but prior to the target stimulus, has reliably been shown to robustly activate ventral striatum.[154,158,159] Participants will complete 2 functional runs of 70 trials each (140 trials total) over ~20 minutes.[95,152] FC during each anticipation condition will be assessed for comparison. Exploratory Measures (baseline only) - DTI: Inflammation may have consequences on white matter fiber tracts necessary to maintain neural connections.[163] Though not a primary outcome, DTI will be collected at the 1st scan to determine whether decreased structural connectivity in key basal ganglia outflow tracts predict or confound L-DOPA response.[164,165] Briefly, diffusion weighted images will be acquired over ~5 min, with an image acquired in the opposite polar direction for ~30 seconds. Faces task: This task that has been developed to measure brain activity in response to viewing of fearful and neutral faces and will be collected at the 1st scan. For the duration of the task, patients will be instructed to passively view faces as they appear on the screen. The task lasts 5 minutes and proceeds as follows: 15 blocks of 16 trial face stimuli are presented in pseudorandom order. Each block contains 8 fearful faces and 8 neutral faces presented in random order. Each face stimulus is presented for 500ms, followed by a 500ms presentation of a fixation cross. After every 10th block, a 10,000ms rest period is presented. The task has been designed to minimize patient discomfort and enhance cooperation. If at any time a patient experiences negative or unpleasant reaction, they will be removed from the scanner. The addition of these tests does not add to the overall risk to patient safety

Targeted FC will serve as the primary outcome and will be calculated as the degree of correlation in activity between a 3mm3 radius sphere in VS (see

Fig. 1A) and the vmPFC cluster identified as being reward-sensitive in neuroimaging meta-analyses and as used to define vmPFC in our previous work (MNI coordinates x=0, y=44, z=-8, cluster size=1408 mm3 and encompassing parts of BA11 and ventral BA32 of ACC) *or other similar ROIs used to define vmPFC in our previous work.*[27,64,65,106] This method will ensure rigor and reproducibility and application of results to potential future trials because the seeds and ROI cluster are predefined as opposed to those derived from whole-brain and data-driven analyses which are determined based on the specific patient sample. Z-scores will be extracted and the change in mean FC values compared to baseline will be calculated. FC between bilateral VS seeds and the vmPFC ROI will be examined separately and treatment-driven change in FC in either the right or left VS will be considered in the analysis. However, based on our previous work in MD and IFN-alpha-induced depression and preliminary studies above, we anticipate that changes in FC will be more pronounced on the left side.
Additional Analyses for Publication and Dissemination: In addition to the targeted FC that will serve as the primary outcome, hypothesis-driven and data-driven whole-brain analysis will also be explored and compared before and after L-DOPA and placebo. Consistent with our previously published data, these analyses will include VS seed-to-voxel-wise comparisons of bupropion versus escitalopram, as well as network analyses using GBC and PBNA, as a secondary outcome measure and to identify other circuits that may be differentially affected by the two treatments. For all analyses, concentration of CRP and final dose will be explored as linear predictors of response.

Assessments of Positive Valence (Motivational) Constructs

Objective: The EEfRT[28,167,168]: was developed by Co-I, Dr. Treadway. EEfRT is a suggested measure under the RDoC Domain: Positive Valence Systems > Construct: Reward Valuation, and can detect dose-dependent increases in effort expenditure for reward in response to dopaminergic drugs.[169] EEfRT is well-matched to detecting effects of inflammation on motivation considering previous studies in rodents and non-human primates showing that inflammation may target effort expenditure for reward as opposed to simply affecting the ability to experience pleasure.[20,138,139] As described, EEfRT performance correlated with both the IDS anhedonia subscale and with VS-vmPFC FC in our preliminary data. In this multi-trial game, participants are given an opportunity to choose between two different task difficulty levels in order to obtain monetary rewards by repeated manual button presses within a short time.[170] Button presses raises a virtual ‘‘bar’’ viewed onscreen. If they raise the bar to the ‘‘top’’ within the prescribed time, they are eligible to win the allotted money. Each trial presents the subject with a choice between two levels of task difficulty, a ‘hard task’ and an ‘easy task’ and 3 probabilities of winning. Subjects participate in the task for 20 minutes and the first 50 trials are analyzed by calculating proportion of hard-task choices across each level of probability. Lower proportions of hard task choices indicate decreased motivation.

Clinician-Rated Scales for Depression and Anhedonia:

Snaith-Hamilton Pleasure Scale (SHAPS-C): The SHAPS-C is a 14-item clinician-administered scale that assesses hedonic tone.[104,176] In a previous

study examining hedonic capacity, confirmatory factor analysis revealed a Hedonic Capacity factor that was largely defined by the SHAPS. Hamilton Depression Rating Scale (HAM-D-17): The HAM-D-17 is a 17-item, clinician-administered scale, that rates severity of depression.[70] Patient Health Questionnaire (PHQ)-9: The PHQ9 is a clinician administered assessment covering the 2 core (depressed mood, anhedonia) symptoms of depression plus 7 additional symptoms that will be used for screening purposes.

Self-Report Assessments of Depression and Anhedonia:

Anhedonia subscale of IDS-SR: correlates with the SHAPS,[27,104] but probes not only capacity for pleasure and response to desired events, but also interest in work and activities,[27,65,73,104] which may more closely approximate motivational aspects of anhedonia.[177] We have established relationships between this scale and both FC and performance on the EEfRT.

Mood and Pleasure Scale (MAP): The mood and pleasure scale is an 18-item self-report inventory that has been validated in psychiatric populations and is designed to disentangle motivational and consummatory components of everyday activities over a 24-hr period.[178]

Additional Measures of Lifestyle, Stress, Anxiety and PTSD Symptom Severity:

Hamilton Anxiety Rating Scale (HAM-A): The HAM-A is a 14-item clinician-administered scale that assesses the severity of symptoms of anxiety[71].

Beck Anxiety Inventory (BAI): The BAI is a 21-item self-report measure of anxiety symptoms, rated on a 4-point Likert scale[72] modified to be based on the patient's experience in the past week.

State-Trait Anxiety Inventory (STAI) State Scale: This 20-item self-report scale is used to measure current anxiety symptoms.

PTSD Checklist for DSM-5 (PCL-5): The PCL-5 is a The PCL-5 is a 20-item self-report measure that assesses the 20 DSM-5 symptoms of PTSD, rated on a 5-point Likert scale[73] modified to be based on the patient's experience in the past week.

Perceived Stress Scale (PSS): The PSS is a self-report scale measuring the perception of stress. The items are designed to predict how uncontrollable, unpredictable, and overloaded the subjects find their lives.

Childhood Trauma Questionnaire (CTQ): The CTQ is a standardized, retrospective 28-item self-report inventory that measures the severity of different types of childhood trauma. Participants will not be excluded for history of childhood trauma.

Habits**:** The Habits form is a brief questionnaire that assesses a subject's daily smoking, caffeine and alcohol consumption, and exercise patterns.

Neurocognitive Tasks: Additional Measures of Psychomotor Speed and Goal-Directed Behavior

Finger Tapping Task (FTT): This task uses a specially adapted tapper that the subject is asked to tap as fast as possible using the index finger. The subject is given 5 consecutive 10-second trials for both the preferred and

non-preferred hands. The finger tapping score is the mean of the 5 trials and is computed for each hand.

Reaction Time Task (CANTAB): The reaction time test includes simple and choice reaction time tasks and is divided into 5 stages requiring increasingly complex chains of responses and providing distinction between reaction (or decision) time and movement latencies (3).

Trail Making Tests A (TMT): is a commonly used neuropsychological measure with updated norms. Part A (TMT-A) requires the patient to connect numbered circles in order and reflects a measure of basic attention and processing speed.

Digit Symbol Task (DSST): The Digit Symbol Substitution Task is a subtest of the Wechsler Adult Intelligence Scale (WAIS) and consists of rows of blank squares, each printed with a randomly assigned number. The test involves graphimotor speed, visual scanning and memory, with about half of the variance being accounted for by graphimotor speed, a third by visual scanning and 4-5% by memory.

Safety and Tolerability

Adverse Event Recording: All adverse events will be coded in standard MeDRA terms (Version 14.1), and whether events are expected and study-related will be determined by the study PI or designee. In addition, severity and start and end dates will be recorded as well as any evidence of unanticipated problems. This information will be provided to the Department of Psychiatry and Behavioral Sciences DSMB and IRB annually as described in the Human Subjects Section.

6.3 Protection Against Risk:
Every effort will be taken to prevent injury or distress that may result from this study.

Recruitment and Informed Consent:

- Male and female subjects with depression will be recruited from local (inter-Departmental) referrals, medical record queries and/or social media recruitment campaign(s). Subjects in this study may also be referred from our Behavioral Immunology Program “Recruitment Clinic” protocol, Psychiatric Research Screening Clinic: IRB00075162, which uses the screening strategy for this protocol to recruit and screen patients with depression for our active studies through IRB approved social media campaigns including Facebook and Instagram. An overview of the study will initially be provided virtually or over the phone by study clinicians. A telephone or virtual prescreening interview may be conducted on candidates providing verbal consent. If a subject shows interest in the study, research staff members will describe the general procedures involved and will answer relevant questions. If a subject remains interested in participation, the detailed nature, purpose, procedures, benefits risks of, and alternatives to this research study will be explained to each subject, and written informed consent will be obtained by the study clinician or their appropriately trained designee who will provide this information by virtual consent utilizing the Emory IRB approved REDCap platform or in a private

office space. To ensure comprehension of the informed consent information, the clinician will allow time for adequate consent review and questioning. Virtual interviews will be conducted by use of ZOOM, a video conferencing service endorsed by Emory University for HIPPA compliant patient encounters. Informed consent will be documented on an Emory Institutional Review Board-approved form. A copy of the signed form will be given to the subject or accessible to the participant following virtual consent provided in a REDCap ICF document link. The original document will be filed in a central study consent binder. Subject casebooks will be in electronic format in the REDCap database. The consent binder(s) and select regulatory information gathered as part of the study will be kept in a locked office and/or cabinet.

Neuropsychiatric Assessments:
Care will be taken to avoid bringing about undue psychological distress during the neuropsychiatric interviews. This will be accomplished by using trained raters (clinicians) for all neuropsychiatric assessments. In the event that a subject becomes unduly distressed, Dr. Miller will be immediately contacted, and an appropriate clinical intervention plan will be developed. Drs. Miller and is a Board-Certified psychiatrist, and has many years of clinical experience in a wide variety of treatment settings, including emergency, consultation-liaison, inpatient and outpatient psychiatry. In cases where patients need extra time to collect their thoughts and emotions, mental health clinicians will be available for emotional support after all interviews.

Blood Draws:
Standard sterile procedure for blood withdrawal will be used. Blood draws will be conducted by clinicians with significant experience in the technique. In addition, the volume of blood withdrawn for this study will not exceed 400 ml over a ~8-week period (from screening to study completion).

Rapid CRP test:
May be conducted at screening using the Diazyme hsCRP POC (or comparable) Test Kit. The kits produce rapid results using a finger prick method to collect 20µl of blood and is intended for the in vitro quantitative determination of C-reactive protein. Screening laboratory evaluations, including rapid CRP, may be repeated at PI's or PI designee's discretion.

fMRI scans:
To minimize discomfort during the fMRI scanning, patients will be provided with a head cushion and ear plugs. Patients will also be informed about the progress of the procedure through a remote microphone. In the case of patients developing acute anxiety or panic the scanning session will be terminated and patient provided enough support to cope with the feelings induced by the scanner.

L-DOPA Treatment:
To reduce the risks associated with treatment, all subjects will be monitored carefully for the development of adverse events as well as worsening of their condition. Although it remains unclear what risk chronic therapy with L-

DOPA will have in depression, and risks are assumed to be small, procedures will be put in place to ensure that any risks associated with receiving L-DOPA are minimized. At screening, subjects will be carefully evaluated for the presence of any medical conditions that might increase the risk of an adverse events (for example cardiovascular issues uncovered by EKG). Subjects will be evaluated by medical personnel weekly during treatment and will be available 24/7 if problems arise. All procedures will be conducted in the Emory University Hospital facilities where emergency assistance will be available if needed. Between signing consent and completing the screening assessment, a period of no more than 2 weeks will pass until baseline, and during this period either the patient will be taken off-study, or a study clinician or their designee will contact and valuate the subject every 7 days to ensure that symptoms haven't worsened appreciably or that suicidal ideation has not developed. In the case of either of these eventualities, subjects will be discontinued from the study immediately and appropriate psychiatric referral will be made. Specific indications for discontinuation between screening and baseline will include any of the following: 1) the development of suicidal ideation or attempt assessed by spontaneous subject report or by scoring >3 on the HAM-D suicide item [item #3]) at any assessment or 2) the development of psychotic or catatonic symptoms at any assessment. Other criteria for discontinuation between screening and the baseline visit include: hospitalization for any reason or a clinical judgment that a subject is an imminent threat to self or others. Subjects will be evaluated for the appropriate level of care, including emergency hospitalization if indicated. Subjects with non-emergent symptoms but who disqualify for further participation will be referred back to their primary psychiatric physician. Appropriate follow-up care will be arranged for subjects not in active psychiatric treatment. An individualized plan will be crafted for each subject to ensure psychiatric back-up should emergency psychiatric care be required after hours or on weekends. Upon completion of the study protocol, all depressed subjects without a current psychiatrist will be offered an appropriate referral.

Confidentiality and Data Management:
Loss of confidentiality is a risk of research participation. Every effort will be made to maintain subject confidentiality throughout the study. All project personnel, including those involved in data entry, have completed an on-line course in human subjects' protection for patient-related research with annual recertification. Each subject will be assigned a unique ID number that will be used in all data files and on all measures in place of personal identifiers. Data entry forms will be developed using the REDcap database system developed at the Vanderbilt Institute for Clinical and Translational Research (CTSA). REDCap (Research Electronic Data Capture) is an Oracle-based, secure, password-protected, HIPAA-compliant, web-based application designed to support data capture for research studies. REDCap provides: 1) an intuitive interface for data entry (with intrinsic data validation); 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, Stata, R); 4) procedures for importing data from external sources; 5) Real-time data cleaning and

validation; 6) Automatic field computation; 7) Data dropdowns for choice lists (including condition selections based on earlier responses); 8) Data entry warnings for out of range or missing values; and 9) Electronic scheduling. Data entry can be performed anywhere using the internet and provides 128-bit SSL security. All electronic and hard entry forms will include unique patient identifiers to maintain patient confidentiality and will not relate to the patient in any way. Any protected personal health information will be encrypted and stored separately. The only link between identifying information (e.g., name, contact information) and project data will be in a key stored on a password-protected computer accessible only to the PIs and to the project coordinators, who will be making appointments and assigning research personnel to meet with the participant. Paper forms will be stored in locked file cabinets accessible only to study personnel.

Appointment Scheduling & Reminders: All participants will be given the opportunity to receive appointment reminders and scheduling information via text message on their mobile phones. If the subject consents to this form of communication, study staff may use OhMD Texting Service to communicate with participants. This platform provides a Desktop and Mobile Version of OhMD for the research team to securely communicate with participants and maintain subject confidentiality. This service will only be used for communicating relevant appointment information and never used for PHI. Participants will receive the following text message from the study team upon first contact: “Please do NOT send any personal information via text. You may call the study team at 404-727-8229 to discuss any personal or health details.” Any PHI received via text message will be reported to the IRB as a potential breach of confidentiality

Stopping Rules:
Subjects will be closely evaluated throughout the study for worsening of psychiatric symptoms. Subjects will be discontinued at any point for any of the following: 1) the development of significant suicidal ideation, plan or intent as determined by spontaneous subject report or as determined by scoring >3 on the HAM-D suicide item [item #3], 2) the development of psychotic or catatonic symptoms, or 3) a 25% increase from baseline of HAM-D score, 4) below 85% compliance on more than one week during the study, and 5) positive pregnancy test. Subjects requiring discontinuation will be referred for further evaluation and treatment by one of the study psychiatrists. All unanticipated and/or serious adverse events or problems will be reported to the DSMB, IRB and NIH and we will follow their recommendations for stopping the overall study.

6.4 Research material will be derived from clinician-administered and self-report questionnaires, blood, urine, objective behavioral tests and fMRI scans. Material for evaluating baseline medical status and subject safety prior to randomization will be obtained from blood and urine. Data to be recorded from subjects will include standardized ratings of emotional, cognitive and physical symptoms, as well as clinical information derived from medical records when available, interviews with the subject, referring physicians and family members when relevant. Data will be obtained from peripheral blood, including complete metabolic panel, CBC with differential,

serum pregnancy test, plasma concentrations of hs-CRP. Data obtained from urine will include urinalysis, toxicology screen and pregnancy testing. Clinical data that will be used to assess eligibility and safety will be collected via interview and documented by study clinicians. Standardized clinician-administered and self-report questionnaires will be administered and collected by study physicians or study coordinator. Blood and urine samples at screening will be obtained by the study coordinator and nursing staff. Blood and urine for the baseline assessment and follow up visits will also be obtained by nursing staff.

All data, including questionnaires and blood and urine samples will be coded by unique identifying numbers. Subjects' names will not appear elsewhere in any form that would link them specifically to data. Subject name and identifying number will be kept on separate forms in a locked office separate from data files. Only the study coordinator and study physicians will have access to subject identities. Research personnel analyzing blood data or entering data into the database will not be provided information from which they could identify subjects by name. Only the identifying number will be used during fMRI scanning and post processing.

All information will be obtained solely to determine eligibility for study participation or for research purposes or to monitor subject safety. Nevertheless, any abnormalities uncovered will be reported to the subject and his/her treating physician for follow-up or will be used by study clinicians to determine appropriate medical care in the case of adverse events.

6.5 Because we do not anticipate any serious adverse events attributed to L-DOPA in this study, and because both tracks of study participation involve dose de-escalation at the end of study, we do not anticipate a need to conduct long-term follow up.

7.0 Data and Specimen Banking* N/A ☐

7.1 De-identified plasma samples and buffy coat (collected from 2, 6cc tubes EDTA whole blood at 4 degree) for inflammatory markers and L-DOPA concentrations, and Tempus tubes for mRNA (from ~3-4 cc blood) will be collected in this study at baseline and Weeks 1, 3, 4 and 6. 10mL samples (in EDTA at RT) will also be collected for immune cell extraction. Samples will be stored in the -80-degree freezers (or liquid nitrogen for immune cells) in the Behavioral Immunology Program Laboratory on the 5th Floor of Clinical B at Emory. Samples will be analyzed by the PI and the Laboratory Research Associate or other laboratory personnel that are approved on this protocol, or sent to core laboratories within Emory University for analysis (i.e. the Genomics Core lab, Biomarker Core Laboratory) or elsewhere (see section 17.4).

7.2 Samples will be de-identified and only the study personnel approved on this protocol will have access to the samples or to the data that is associated with the identifier for each sample.

7.3 External investigators may obtain specimens and the associated de-identified clinical data for research purposes only through a Materials Transfer Agreement to be prepared and approved by the Emory Technology Transfer Office and the Emory Office of Sponsored Programs.

8.0 Sharing of Results with Participants*

8.1 The results of screening laboratory tests conducted by EML will become part of each participant's Emory Healthcare medical record and will be shared with them. Other results such as computer tests, medical and psychiatric history, health status updates, questionnaires, urine drug screen results, research lab work for looking at inflammation, and MRI scans are not intended for clinical use and will be kept only in a research record. These results will not be placed in their Emory Healthcare medical record and will not be reported to participants or to other healthcare providers who may be providing them treatment.

The Principal Investigator and research staff may also share PHI or results with the following parties to help conduct the study or to ensure safety or regulatory compliance:

1. Emory offices that are part of the Human Research Participant Protection Program and those that are involved in study administration and billing. These include the Emory IRB, the Emory Research and Healthcare Compliance Offices, and the Emory Office for Clinical Research.
2. Government agencies that regulate the research including: Office for Human Research Protections; Food and Drug Administration.
3. Public health agencies including the sponsor of the study, the National Institute of Mental Health (NIMH).
4. Research monitors and reviewer.
5. Accreditation agencies.

8.2 The fMRI scans that will be completed as data for this study are not designed to detect or diagnose problems of the brain. A radiologist will not be reading the scans. However, it is still possible that we will see something on a structural scan that is potentially abnormal. If this happens, we will discuss it with the participants.

8.3 During the consent process, participants will be informed that they will receive the results of their screening laboratory tests performed by EML. Participants will also be informed that their other results (i.e. computerized testing, psychiatric evaluations, as described above) are not intended to be clinical or diagnostic and will not be reported. They will also be informed that although MRI scans are also not diagnostic, will not be reviewed by a radiologist, and that fMRI results will not be reported, they will be informed that if an abnormality is observed during collection of MRI data, this finding will be discussed with them by the PI, Study Physician or their designee. Participants will have the option to opt out of receiving their lab results or being alerted to any incidental findings.

8.4 This study is not deigned to provide treatment and does not include a budget for the diagnosis or treatment of incidental findings uncovered during participation in this research. The study team will discuss the screening laboratory results with the participant, including going over values that are outside of the normal range. Patients with abnormal results will be advised to schedule a visit with their primary care or treating physician. If potential abnormalities are observed during MRI scans, the participants will be informed that a potential structural abnormality was observed and that it

Table 3: Timeline	Year 1 (R61)				Year 2 (R61)				Year 3 (R33)				Year 4 (R33)				Year 5 (R33)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Start-up & training	X								X											
Accrual (completers)		5	10	15	20	25	30		5	10	20	25	30	35	40	45	50	55	60	
Cytokine/image analysis				X		X	X				X					X		X	X	X
Gene expression (R61)							X													
DSMB report				X				X				X				X				X
Annual reports NIH/IRB				X				X				X				X				X
Final data cleaning						X	X	X				X	X	X	X			X	X	X
Final analysis							X	X						X	X	X			X	X
Decision on Go/No-Go criteria								X												X

is recommended that they schedule an appointment with their primary care doctor or a specialist, if applicable.

9.0 Study Timelines*

Because our programs are currently running longitudinal experimental medicine studies and clinical trials, and because we are already administering L-DOPA to a similar MDD population, we expect that start up and training can be completed in the 1st quarter of the project, and that we will be able to study 5 patients by the end of the 2nd quarter. Start up and training will include: a. Study team orientation, b. setting up of data bases, c. IRB approval and stamped

10.0 Inclusion and Exclusion Criteria*

10.1 Seventy medically-stable male and female subjects between the ages of 25 and 55 will be enrolled in this study in order to obtain 35 qualified subjects for the R61 study. All subjects will meet criteria for current major depression. All subjects will have a plasma hs-CRP concentration >2 mg/L and a score ≥2 on the anhedonia item from the PHQ-9 at screening. During screening, CRP will be measured at least twice over a 1 to 2-week interval per AHA/CDC guidelines, and additional testing will occur at 1 to 2-week intervals in patients with CRP >10 mg/L as needed to determine stability and/or, along with exam and lab tests, rule out acute infection. Based on a screening failure rate of up ~50%, we anticipate that enrolling and screening 70 subjects (35 per year) will provide 35 qualified subjects for inclusion in the study. AT screening, participants will undergo laboratory testing (for safety labs including coagulation parameters and determination of the absence of unstable medical illness including infection, thyroid disease and autoimmune disorder). Electrocardiogram (EKG to rule out cardiac contraindications to L-DOPA) will also be conducted followed by a complete psychiatric examination (conducted by a trained clinician) including detailed information on past psychiatric history and treatment, as well as current symptom status. There will be no exclusions for race/ethnicity and minority populations, and women will be actively recruited. Indeed, based on our previous work ~65% of the sample will be female. We will make active attempts to enroll minority patients in a

proportion that is reflective of the areas surrounding Atlanta from which patients will be recruited. No patients will be enrolled from vulnerable populations, including neonates, children, prisoners, or institutionalized individuals. No use will be made of fetal tissue.

10.2 Inclusion Criteria: a. willing and able to give written informed consent; b. men or women, 25-55 years of age; c. a primary diagnosis of DSM-V MD, current, as diagnosed by the SCID-V; d. score of >10 on the Patient Hospital Questionnaire (PHQ-9); e. off all antidepressant or other psychotropic therapy (e.g. mood stabilizers, antipsychotics, anxiolytics, and sedative hypnotics) for at least 4 weeks prior to baseline visit (8 weeks for fluoxetine) f. CRP ≥2 mg/L, g. PHQ-9 anhedonia score ≥2.

Exclusion Criteria: a. history or evidence (clinical or laboratory) of an autoimmune disorder ; b. history or evidence (clinical or laboratory) of hepatitis B or C infection or human immunodeficiency virus infection; c. history of any type of cancer requiring treatment with more than minor surgery; d. unstable cardiovascular, endocrinologic, hematologic, hepatic, renal, or neurologic disease (as determined by physical examination, EKG and laboratory testing); e. history of any (non-mood-related) psychotic disorder; active psychotic symptoms of any type; history or current bipolar disorder; history or current gambling disorder; substance abuse/dependence within 6 months of study entry (as determined by standardized clinician interview); f. active suicidal plan as determined by self-report (question #9 on the PHQ-9) and clinician interview (SCID); g. an active eating disorder (except for patients with binge eating disorder in whom binging is clearly associated with worsening of mood symptoms) ; h. a history of a cognitive disorder or traumatic head injury involving loss of consciousness; i. pregnancy or lactation; j. chronic use of non-steroidal anti-inflammatory agents (NSAIDS) (excluding 81mg of aspirin), glucocorticoid containing medications or statins; k. use of NSAIDS, glucocorticoids, or statins at any time during the study; l. urine toxicology screen is positive for drugs of abuse, m. any contraindication for MRI scanning; n. intolerance, sensitivity or contraindication to carbidopa-levodopa (including history of narrow-angle glaucoma, melanoma, gastric and/or duodenal ulcers, bleeding disorders, or frequent migraines). Due to the high co-morbidity between anxiety disorders and depression, we plan to include patients with comorbid dysthymia and/or anxiety-related disorders excluding OCD if depression is the primary diagnosis. Enrolled subjects may also have a comorbid personality disorder (except for subjects who meet criteria for antisocial personality disorder or with a history recurrent hospitalizations and/or recurrent suicidal behavior due to the personality disorder). Patients with stable medical conditions and on medications/treatments for those conditions will not be excluded.

10.3 INCLUSION OF WOMEN, MINORITIES, AND CHILDREN

a. Recruitment of minorities: Eligible subjects will be offered the opportunity to participate regardless of gender or minority status. We appreciate that minority groups tend to be under-represented in research samples and under-treated for mood and anxiety disorders. Therefore, we will recruit minority patients to ensure that the subject sample represents the community. Census estimates from 2017 for Atlanta, GA, were 40.1% white, 52.4% African American, 0.3% American Indian and Alaskan Native, 4% Asian, and <1% Native Hawaiian and other Pacific Islander; Hispanic or Latino were 4.8%. There will be two factors that will determine recruitment of minorities into the current study. First, although Emory University is located in Atlanta, we tend to draw participants *regionally*; therefore, recruitment is more likely to reflect the minority

distribution of Georgia, which is similar to that of the larger Metro-Atlanta population. Specifically, these distributions are as follows: 60.8% white, 32.2% African American, 0.5% American Indian and Alaskan Native, 4.2% Asian, and <1% Native Hawaiian and other Pacific Islander; Hispanic or Latino were 9.6%. Second, the prevalence rate of depression among non-Hispanic whites (17.9%) is higher than Hispanic whites (13.5%) and African Americans (10.8%) (Breslau J, et al. Psychological Med 2006; 36:57-68). Based on these factors, we anticipate recruitment of minorities with the following distribution: 60-65% white, 30-35% Black or African American, 1-2% American Indian and Alaskan Native, 3-5% Asian, <1% Native Hawaiian and other Pacific Islander, 2.1% Two or More Races, and 6-8% Hispanic or Latino.
Efforts to boost minority enrollment: The following efforts will be undertaken to help maintain minority enrollment: 1) Advertisements in African American or Hispanic television or radio programs, 2) Advertisements in African American or Hispanic-Latino newspapers, 3) Advertising in Emory-affiliated clinics serving communities with high representation of African Americans and Hispanic-Latino groups (e.g. Grady Hospital), and 4) targeting of our online Advertisement campaign to minority social media users. Recruitment of minorities will be assessed quarterly and approaches adjusted accordingly.

b. Inclusion of women: Consistent with NIH policy, women will be included in this study. Women typically comprise approximately 65% of subjects at Emory, distributed over racial and ethnic categories similar to men. Furthermore, epidemiological studies have found that depression is much more prevalent in women than men, with a risk ratio of roughly 2:1 (e.g. Kessler, J Affect Disord, 2003, 74:5-13). Higher enrollment of women than men will more closely approximate the prevalence of depression, making this study sample more representative of and generalizable to the population of depressed patients from which it samples. In addition, because of the potential interactive effects of sex with inflammation and depression, recruitment of women will be assessed quarterly to ensure adequate representation.

c. Inclusion of Children:
In order to limit the variability of the primary outcome variables (neuroimaging and behavioral), we have chosen to limit the age range from 25-55, reflecting +/- ~1-2 standard deviations from the mean age (40 years) in our previously published patient samples. This design consideration precludes the inclusion of children (and older adults), limiting variability contributed by potential neurodevelopmental and neurodegenerative factors on brain connections (a primary outcome of this study). Furthermore, the relationship between inflammatory markers, dopamine and reward responsivity has not been established in children. The study will also exclude younger adults (<25 years) due to the unknown risk of treating subjects in an age range where the brain is still be developing.

11.0 Vulnerable Populations* N/A ☐

N/A

12.0 Local Number of Participants

12.1 Seventy medically-stable male and female subjects between the ages of 25 and 55 will be enrolled in this study in order to obtain 35 qualified subjects to be randomized for the R61 study.

12.2 Thus, n=70 will be enrolled for screening, and n=35 will be randomized in order to obtain and analyze data from n=30 study completers when considering a ~10-15% attrition rate.

13.0 Recruitment Methods

13.1 Recruitment will be made through the use of contact lists, databases or other pre-screening resources, advertisements, outreach, media/social media, and referral networks or groups.

13.2 Community recruitment will encompass the Metro Atlanta are as well as regionally through north and central Georgia.

13.3 We use primarily online advertising to identify interested participants. Approximately 500 potential participants will be screened by phone per year to yield 200 face-to- face prescreens (to be conducted through our program recruitment center) and 35 subjects per year (n=70 total) for enrollment and full screening for this study. As part of daily recruitment monitoring, the study team records all advertisement responses and contacts and schedules potentially eligible participants.

13.4 We use primarily Facebook, Google and Instagram campaigns with the advertising materials attached.

13.5 Participant reimbursement*:*

Table 4. Schedule of Reimbursement

Visit	Total Amount	Amount put on Clincard immediately after visit	Amount put on Clincard at the end of study participation
Intake (phone or virtual)	**No Payment**	**No Payment**	
(Visit Total)	**n/a**		
Screening A*			
Rapid CRP, Vitals and BMI, MRI Safety Questionnaire, Urine Toxicology Screen, Serum Pregnancy Test, Screening Labs, EKG, SCID and Clinician Assessments (may be done virtually or as part of intake)	$50	$50	
(Visit Total)	**$50**	**$50**	
Screening B and Pre-Baseline			
Physical Exam, CRP, Fasting Blood, Demographics and Report Forms, Practice Neurocognitive	$50	$50	
Practice Computer Motivation Tasks In and Out of Scanner (depending on choices made in task)	$5-$10	$5-$10	
(Visit Total)	**$55-60**	**$55-60**	
Visit 1 (Baseline)			
Medical and Psychiatric Update, Urine Toxicology, Urine Pregnancy, Self-report Surveys, Neurocognitive Tasks,	$50	$50	

Research Bloods, Medication Dispense			
Complete Scan (~1 hr)	$100		$100
Incomplete Scan	$25**	$25**	
Computer Motivation Task in Scanner (depending on choices made in task)	$0-$20	$0-$20	
Computer Motivation Task Out of Scanner (depending on choices made in task)	$5-$10	$5-$10	
(Visit Total)	**$25-$180**	**$25-$80**	**$0-$100**
Visit 2 (Week 1)			
Medical and Psychiatric Update, Urine Toxicology, Urine Pregnancy, Self-report Surveys, Neurocognitive Tasks, Research Bloods, Medication Check and Dispense (as tolerated)	$50	$50	
Complete Scan	$100		$100
Incomplete Scan	$25**	$25**	
Computer Motivation Task in Scanner (depending on choices made in task)	$0-$20	$0-$20	
Computer Motivation Task Out of Scanner (depending on choices made in task)	$5-$10	$5-$10	
(Visit Total)	**$25-$180**	**$25-$80**	**$0-$100**
Visit 3 (Week 2)			
Medical and Psychiatric Update, Urine Toxicology and Pregnancy (in-person only), Self-report Surveys, Medication Check and Dispense	$50	$50	
(Visit Total)	**$50**	**$50**	
Week 4 (Week 3)			
Medical and Psychiatric Update, Urine Toxicology, Urine Pregnancy, Self-report Surveys, Neurocognitive Tasks, Research Bloods, Medication Check and Dispense	$50	$50	
Complete Scan (~45 min)	$100		$100
Incomplete Scan	$25**	$25**	
Computer Motivation Task in Scanner (depending on choices made in task)	$0-$20	$0-$20	
Computer Motivation Task Out of Scanner (depending on choices made in task)	$5-$10	$5-$10	
(Visit Total)	**$25-$180**	**$25-$80**	**$0-$100**

Visit 5 (Week 4)			
Medical and Psychiatric Update, Urine Toxicology, Urine Pregnancy, Self-report Surveys, Neurocognitive Tasks, Research Bloods, Medication Check and Dispense	$50	$50	
Complete Scan (~45 min)	$100		$100
Incomplete Scan	$25**	$25**	
Computer Motivation Task in Scanner (depending on choices made in task)	$0-$20	$0-$20	
Computer Motivation Task Out of Scanner (depending on choices made in task)	$5-$10	$5-$10	
(Visit Total)	**$25-$180**	**$25-$80**	**$0-$100**
Visit 6 (Week 5)			
Medical and Psychiatric Update, Urine Toxicology and Pregnancy (in-person only), Self-report Surveys, Medication Check and Dispense	$50	$50	
(Visit Total)	**$50**	**$50**	
Visit 7 (Week 6)			
Medical and Psychiatric Update, Urine Toxicology, Urine Pregnancy, Self-report Surveys, Neurocognitive Tasks, Research Bloods, Medication Check	$50	$50	
Complete Scan (~45 min)	$100		$100
Incomplete Scan	$25**	$25**	
Computer Motivation Task in Scanner (depending on choices made in task)	$0-$20	$0-$20	
Computer Motivation Task Out of Scanner (depending on choices made in task)	$5-$10	$5-$10	
(Visit Total)	**$25-$180**	**$25-$80**	**$0-$100**
Completed Study Compensation	**$980-$1110**		

*=These visits may be combined into a single screening visit with combined compensation
**= Unable to complete scan leading to termination from the study

Participants will also receive tokens for parking expenses incurred at EUH, and an additional $25 will be provided to cover travel expenses for participants that travel equal to or greater than 30 miles one way to Atlanta.

14.0 Withdrawal of Participants*

14.1 Patients may be drawn from the research without their consent if they experience a severe adverse event thought to be attributed to the study procedures, due to repeat non-compliance with study procedures (2 or more of the same violation), worsening of depressive symptoms including suicidal ideation, or for any other reason that the PI or Study Physicians determine to be putting the participant at increased risk or jeopardizing integrity of the study data.

14.2 To withdraw a participant, the study team will contact the subject to inform them that they should discontinue use of all study medication and to schedule a follow-up visit for the participant to be assessed by study clinicians for medical and psychiatric safety. The participant will be asked to return all unused medication at this time, and a member of the study team will take them off study. If the patient is determined to be a potential risk to themselves or others on the call or in the final interview, Drs. Miller or Dunlop will be immediately contacted, and an appropriate clinical intervention plan will be developed.

14.3 When participants withdraw from this study, they will be appropriately off-studied and their participation will be terminated. Because this study is a mechanistic study to demonstrate target engagement using a within-in subject design, no partial withdrawal with continued data collection will be allowed for participants who miss their schedule MRI, blood and neurocognitive follow-up assessments.

15.0 Risks to Participants*

There are 5 major areas of potential risk in the proposed study stemming from 1) neuropsychiatric assessments, 2) blood drawing, 3) fMRI scanning, 4) medication administration, and 5) loss of confidentiality. Neuropsychiatric assessments may uncover strong and potentially disturbing feelings about the subject's past or present emotional state. The risks of blood drawing include discomfort, bruising, infection, bleeding, and fainting. Undergoing fMRI scanning poses no more risk than undergoing a routine MRI scan. Physical discomfort due to lying in the scanner, occasional headaches due to scanner sounds and previously unrecognized claustrophobic attacks are the prominent adverse effects of the procedure. L-DOPA may cause nausea or vomiting, and carbidopa is co-administered with the L-DOPA as a combination pill to reduce side effects. Common symptoms are most pronounced after the first few doses of L-DOPA and may also include dizziness or headache. L-DOPA may also cause increased eye blinking/twitching, fainting, or worsening of involuntary movements and spasms. People who take high-doses of L-DOPA daily for Parkinson's disease have reported more serious but rare symptoms such as dyskinesias, and these are not anticipated following the doses used in this study or in patients with depression who do not have neurodegeneration. Participants' vital signs and adverse events will be monitored at weekly follow visits. If patients are unable to tolerate dose escalations, the study physician will provide the patient with a dose pack or bottle of one pill of study medication (containing 150 mg L-DOPA) a day for 3 days followed by study medication discontinuation. Finally, there is a risk of loss of confidentiality. Confidentiality of all subjects will be protected per institutional and NIH and other federal requirements, and as described in greater detail below.

Alternative options include not participating in the proposed study. In addition, an array of demonstrated effective treatments for depression exists and include, but are not limited to:

selective serotonergic antidepressants (i.e. fluoxetine, paroxetine, etc.), serotonin-norepinephrine reuptake inhibitors (i.e. venlafaxine, duloxetine), atypical antidepressants (i.e. bupropion, mirtazapine, nefazodone), tricyclic antidepressants, monoamine oxidase inhibitors, electroconvulsive therapy (ECT) and psychotherapy (i.e. interpersonal and cognitive behavioral therapies). Risks and side effects of these agents vary in type and severity depending on class, but include sexual dysfunction, gastrointestinal distress, anxiety, anticholinergic symptoms, induction of mania, suicidal ideation and potential lethality in overdose. Common risks of ECT include short term memory loss, head and body aches and risks related to anesthesia. Benefits of antidepressants and ECT include documented efficacy in the treatment of depression. In addition to approved modalities, trials of novel treatments are ongoing and include the use of vagal nerve stimulation and deep brain stimulation. Benefits of these modalities may include efficacy for patients who have failed traditional agents. Risks include the possibility that these modalities are not effective. There are also surgery-related risks of infection and anesthesia involved with these modalities. At screening, study clinicians will discuss benefits and side effects of these alternative treatments with subjects compared to the risks and potential benefits of study participation.

16.0 Potential Benefits to Participants*

The direct benefits of study participation will include receiving a psychiatric and medical evaluation, including standard blood and urine-based laboratory tests and MRI scan. Subjects will also have the chance to contribute to a scientific investigation, which may be of benefit to future patients. Benefits to others may include gaining significant knowledge regarding the role of inflammatory activation in the pathophysiology and treatment of depression and potential for use of novel dopaminergic therapies. As a result of this knowledge, this study may contribute to personalization of therapeutic strategies for patients with major depression.
Given the low risk involved with blood drawing, psychiatric assessments and fMRI scanning and the use of an intervention with only a mild side-effect profile and that has the potential to have some clinical benefit, we feel that the risks of the study are reasonable in relationship to the benefits of study participation.

17.0 Data Management* and Confidentiality

17.1 Statistical Analysis and Power

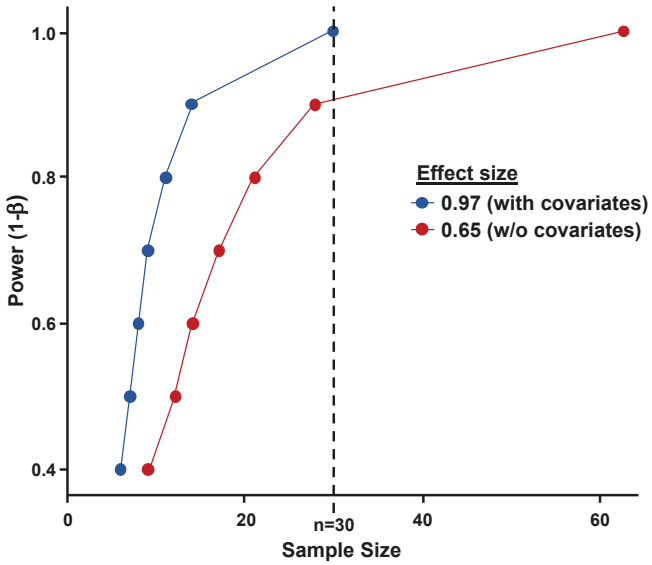
POWER ANALYSIS

Effect Size Estimations for Power and Go/No-Go Criteria: The relationship between VS-vmPFC FC and anhedonia from our previous dataset (n=48)[27] was used to estimate a clinically meaningful effect size (ES) for Go/No-Go criteria and power calculations. Mean FC scores for patients with high (≥5) versus low (<5) anhedonia scores indicated a 50% difference in FC scores. Of note, a 50% difference for FC in patients with low compared to high anhedonia was stable across the low and high ends of the 95% confidence interval of the means, as determined by 1000-repetition bootstrapping of the data. Therefore, we expect that L-DOPA will produce ≧50% increase in FC, corresponding to a mean difference of ~3 points on the anhedonia scale. We also anticipate a ~30% placebo response, consistent with the placebo response rate in MDD[145] and with the percent change in FC after acute placebo administration in our preliminary data in patients with high CRP (32%). Therefore, we expect to observe a ≧20% separation between the L-DOPA and placebo responses, which will serve as Go/No-Go criteria for the R61. Based on a 20% difference in FC response between placebo and L-DOPA and the estimated standard deviation of this change based on

our preliminary data involving acute L-DOPA and placebo administration to high CRP patients (~1.5 x the mean difference between L-DOPA and placebo), an ES (Cohen's d_z for standardized differences) of 0.65 is anticipated.

Power Analysis for R61 Go/No-Go Criteria: Based on our estimated ES of d_z=0.65, 21 patients would be required for 80% and 28 patients would be required for 90% power. To account for uncertainty and bias, clinical covariates (age, sex, race and BMI) were also considered. Although none of the covariates exerted significant effects in the model, per se (all p-values >0.05), they did improve the ES for the mean difference in placebo versus L-DOPA in our preliminary data. Based on this improvement, an ES d_z=0.97 is estimated for the proposed separation of the means by ≥20% when including covariates in the model. A graphical analysis of the sample size across a full range of power at the proposed ESs (with and without covariates) is provided in **Figure 1** as shown below.

Figure 1. Estimated power for within subject comparisons of FC response to L-DOPA versus placebo in the R61 phase. Sample size (x-axis) and power values (y-axis) for ES (d_z) of 0.97 (with covariates) and 0.65 (without covariates) are provided. Relevant to the power analysis of the study based on Go/No-Go criteria for difference in response to L-DOPA versus placebo, both the treatment effect alone and the treatment effect plus covariates will exhibit power >0.8 with the proposed sample size of 30 (dashed line).



A sample size of n=30 will also provide sufficient power for collecting preliminary information about the relationship between change in behavior and target engagement in the brain. For example, based on the correlation between EEfRT and change in VS-vmPFC FC after acute L-DOPA in our prelim data of r=0.6, we will have >95% power to detect significant correlations with 30 patients.

Analytic strategy

Milestones (Go/No-Go Criteria)

Target Engagement in the Brain: VS-vmPFC FC using an objective, a priori analytic strategy, will serve as the target for physiologic response and functional improvement in the brain after administration of L-DOPA versus placebo. The relationship between VS-vmPFC FC and anhedonia from our previous dataset (n=48)[27] and anticipate placebo response was used to estimate a clinically meaningful effect size (ES) for Go/No-Go criteria and power calculations (see **Milestones**).

Milestone for Brain Target: **At least 1 dose of L-DOPA will cause a mean increase in FC that is ≥20% higher than placebo.**

Power Analysis and Sample Size Estimation

Sample size was estimated to have adequate power to observe statistical significance based on our preliminary data and the proposed Go/No-Go criteria described above (see **Power Analysis** section for detail). For the R61 Phase, the proposed threshold criteria for the primary outcome of target engagement in the brain (VS-vmPFC FC) is a mean change with L-DOPA from baseline of ≥20% greater than that of placebo. Based on the means and standard deviations from our previous work, a ≥20% difference in mean change in FC corresponds to a effect size (ES) of 0.65, indicating that with a 2-sided alpha=5%, a total of 30 patients (out of 35 that will be studied to account for potential drop-outs) will be required to have >90% power for statistical analysis (see **Figure 1** in the **Power Analysis** section of **Human Subjects** for detail). Based on our preliminary data on the relationship between change in EEfRT performance and change in VS-vmPFC FC after acute L-DOPA of r=0.6, we will have also have >95% power to detect correlations between change in FC and improvements in motivation, a secondary outcome to be explored.

Statistical Analysis

Target Engagement in the Brain for R61 Go/No-Go Criteria: Targeted VS-vmPFC connectivity will serve as the primary outcome and will be assessed for determining the Go/No-Go Criteria. Targeted fMRI FC will be calculated as the degree of correlation in activity using an a priori defined seed in VS and region of interest (ROI) in vmPFC (per the literature and our previous studies). This method will ensure rigor and reproducibility and application of results to potential future trials because the seeds and ROI cluster are predefined as opposed to those derived from whole-brain and data-driven analyses which are determined based on the specific patient sample. FC Z-scores will be extracted and the change in mean FC values after each dose of L-DOPA or placebo compared to baseline will be assessed. We expect that at least one dose of L-DOPA will increase VS-vmPFC FC by ≥20% greater than placebo. If more than 1 dose of L-DOPA is able to engage the FC outcome at the proposed thresholds, the percent of patients and mean number of reported side effects will be compared across doses to determine the effective dose that was most tolerated by patients. If doses are similarly tolerated, the one with greatest impact on behavior (% change in behavior, EEfRT > anhedonia), or lowest dose meeting all of the above criteria, will be chosen. Statistical significance, ES or sensitivity analyses may be used to assist with selection of the optimal target dose of L-DOPA foe the R33 phase.

Additional Analyses for Publication and Scientific Dissemination: Brain Target- Targeted FC Z scores (as described above) will be entered into linear regression models including clinical covariates and both conservative (Bonferroni) and powerful (Fisher LSD) post-hoc analyses to asses change in FC across the 4 doses of L-DOPA compared to baseline, placebo and to each other. Linear models will also be used to determine whether FC increases as a function of increasing plasma L-DOPA, and whether patients with low bioavailability exhibit low FC. Treatment resistance (as measured by ≥2 on the MGH-ATRQ) will also be assessed in linear models to determine whether it is associated with greater response to L-DOPA. In addition to the targeted FC that will be used to assess Go/No-Go criteria, hypothesis-driven and data-driven whole-brain analysis will also be explored and compared before and after L-DOPA. Consistent with our previous data, these analyses will include VS seed-to-voxel-wise comparisons of L-DOPA versus placebo, as well as network analyses using GBC and PBNA, as a secondary outcome measures and to identify other circuits that may be affected by L-DOPA. For all analysis, concentration of CRP and final dose will be explored as a linear predictor of response to L-DOPA. Clinical covariates such as age, sex, race and BMI will also be explored as potential confounders or mediators of L-DOPA effects on FC. As described for FC, linear regression models and post-hoc analyses will be used to asses change in behavioral outcomes across the 3 doses of L-DOPA compared to baseline, placebo and to each other, and whether response was modified by plasma bioavailability of L-DOPA, CRP or cytokines concentrations or clinical covariates. Overall depression scores, SHAPS-C and MAP-SR will also be explored as an outcome. Integration of Brain and Behavior: Random intercept linear mixed models will be used to assess whether change in FC predicts change in anhedonia and motivation and in relation to clinical covariates. Overall depression scores and motor behaviors will also be explored as outcomes. Summary scores for dose-response (e.g. area under the curve) may be used to assess relationships among variables.

Integration of Brain, Behavior and Inflammatory Markers: Whether plasma levels of inflammatory cytokines and their receptors at baseline, together in models with selection or when combined in a composite or principle component factor, predict FC and behavioral response with be assessed. There is some evidence that dopamine is anti-inflammatory, and change in inflammatory markers and their relationship with response to L-DOPA over time will also be examined. Gene transcripts will be entered into linear regression models in R with change in FC and behavioral measures, and identified genes will be assessed in bioinformatic platforms as described in the methods to determine additional biological and inflammatory pathways that are associated with L-DOPA response. Other behaviors (e.g. locomotor behavior) and imaging markers (e.g. white matter fractional anisotropy) will also be explored as factors that moderate L-DOPA-induced changes in FC, anhedonia and motivation, and depression scores.

17.2 Data entry forms will be developed using the REDcap database system developed at the Vanderbilt Institute for Clinical and Translational Research (CTSA). REDCap (Research Electronic Data Capture) is an Oracle-based,

secure, password-protected, HIPAA-compliant, web-based application designed to support data capture for research studies. REDCap provides: 1) an intuitive interface for data entry (with intrinsic data validation); 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, Stata, R); 4) procedures for importing data from external sources; 5) Real-time data cleaning and validation; 6) Automatic field computation; 7) Data dropdowns for choice lists (including condition selections based on earlier responses); 8) Data entry warnings for out of range or missing values; and 9) Electronic scheduling. Data entry can be performed anywhere using the internet and provides 128-bit SSL security. All electronic and hard entry forms will include unique patient identifiers to maintain patient confidentiality and will not relate to the patient in any way. Any protected personal health information will be encrypted and stored separately. The only link between identifying information (e.g., name, contact information) and project data will be in a key stored on a password-protected computer accessible only to the PIs and to the project coordinators, who will be making appointments and assigning research personnel to meet with the participant. Paper forms will be stored in locked file cabinets accessible only to study personnel.

17.3 Data will be securely entered directly into REDCap. Data will be value checked with double entry. The PI and Co-I's and their staff are currently using REDCap for their funded projects, and have extensive experience with its usage. No names or locating information will be entered in databases. Forms will be maintained in locked offices. Neuroimaging and neurocognitive data will be reviewed weekly and backed-up to local servers. Imaging data will be preprocessed using standard techniques to remove or control for artifact. Descriptive statistics will summarize central tendencies and variances of dependent variables, demographics and clinical characteristics. Log-transformation will be used for non-normally distributed data (i.e. inflammatory markers) and non-parametric tests will be used were appropriate.

17.4 De-identified plasma samples, immune cells and Tempus tubes for mRNA collected in this study will be stored in the -80-degree freezers in the Behavioral Immunology Program Laboratory on the 5th Floor of Clinical B at Emory. Samples will be analyzed by the PI and the Laboratory Research Associate or other laboratory personnel that are approved on this protocol, or sent to core laboratories within Emory University for analysis (i.e. the Genomics Core lab, Biomarker Core Laboratory). Samples will remain stored indefinitely to allow completion of all planned or future analyses. By storing samples for future analyses, we can do research for a long time without needing to ask for fresh samples from new patients. We anticipate transporting one sample per participant for each assay, but understand that there may be the need to repeat some analyses and additional tubes may need to be transported. Samples will be transported by laboratory personal who have completed the sample shipping certification class offered at Emory. In addition to analysis at Emory-affiliated core labs, we also expect to ship de-identified plasma samples to Brainsonline/Charles River (South San Francisco, CA) labs for measurement of concentrations of L-DOPA.

18.0 Provisions to Monitor the Data to Ensure the Safety of Participants*

Because study subjects will be receiving a pharmacological intervention as part of this study protocol, we have elected to utilize a Data and Safety Monitoring Board as part of our data and safety monitoring plan. This board is described below:

Composition of the Emory DSMB

The Emory DSMB consists of Marian Evatt, M.D. from the Department of Neurology, Larry Tune, M.D., Chairman of the DSMB, Dr. Dunlop, Ms. Woolwine and David Goldsmith, M.D. Each of these clinical researchers has agreed to serve as the external DSMB for investigator-initiated clinical trials conducted by Emory researchers in the Department of Psychiatry & Behavioral Sciences. No persons involved in the operation of this study will review DSMB reports for the study. The frequency of Emory DSMB review for this protocol will be once every 12 months based on IRB recommendations consistent with the assessed risk status of the study.

Procedures and Responsibilities of the Emory DSMB

In advance of the DSMB meeting, the data manager/research coordinators will prepare a an annual report to be reviewed during that meeting. The report will include the number of participants who signed consent for the study and were randomized, the number of post-randomization dropouts, reasons for these dropouts, and any safety concerns, adverse events, etc. An up-to-date consent form will be provided, as well as a summary of measures taken to protect confidentiality (e.g. data storage, use of coded ID numbers, etc). The PIs will also prepare a report summarizing any new data/evidence that might alter the risk/benefit ratio for participating in the study (e.g., newly published studies, etc.). Data will be presented to the DSMB in such a way as to maintain patient confidentiality.

Based on the information provided to the Emory DSMB, once every 12 months the DSMB will issue a report to the Emory IRB that summarizes the following: All serious and unexpected adverse events or other unanticipated problems that involve risk to study participants, and whether these appear related to the study-based interventions or research assessment protocols. Reports will not specifically disclose the treatment arm of the study for relevant subjects unless this disclosure is required for safety reasons. Note that any serious adverse event (SAE) will be reported to the Emory IRB within 24 hours according to standard regulations.

The IRB defines a serious adverse event as: “any adverse experiences occurring that result in any of the following outcomes: death, a life-threatening adverse experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. For the purposes of this policy, death is never expected.”

The PIs will take responsibility for reporting any serious and unexpected adverse events in a timely fashion directly to the Emory DSMB. The PIs will also report serious and unexpected adverse events or other unanticipated study problems or variances to the Emory IRB. Actions taken by the IRB in response to adverse event reports will be immediately reported to the Emory DSMB.

19.0 Provisions to Protect the Privacy Interests of Participants

19.1 Loss of confidentiality is a risk of research participation. Every effort will be made to maintain subject confidentiality throughout the study. All project personnel, including those involved in data entry, have completed an on-line course in human subjects’ protection for patient-related research with annual recertification. Each subject will be assigned a unique ID number that will be used in all data files and on all measures in place of personal identifiers. Data entry forms will be developed using the REDcap database

system developed at the Vanderbilt Institute for Clinical and Translational Research (CTSA). REDCap (Research Electronic Data Capture) is an Oracle-based, secure, password-protected, HIPAA-compliant, web-based application designed to support data capture for research studies. All electronic and hard entry forms will include unique patient identifiers to maintain patient confidentiality and will not relate to the patient in any way. Any protected personal health information will be encrypted and stored separately. The only link between identifying information (e.g., name, contact information) and project data will be in a key stored on a password-protected computer accessible only to the PIs and to the project coordinators, who will be making appointments and assigning research personnel to meet with the participant. Paper forms will be stored in locked file cabinets accessible only to study personnel.

19.2 Care will be taken to avoid bringing about undue psychological distress to participants, particularly during the neuropsychiatric interviews. This will be accomplished by using trained raters (clinicians) for all neuropsychiatric assessments. In the event that a subject becomes unduly distressed, Drs. Miller or Dunlop will be immediately contacted, and an appropriate clinical intervention plan will be developed.

19.3 Only research team members approved on this protocol are allowed to access any sources of information about a participant. Approved team members may do so by accessing the casebooks, which are stored in locked file cabinets. The CRC, Director of Projects and Study physicians are also able to view a participant's Emory Medical Record through Power Chart.

20.0 Economic Burden to Participants

No cost is incurred by the participant other than transportation expenses for study visits. an additional $25 will be provided to cover travel expenses for participants that travel equal to or greater than 50 miles one way to Atlanta.

21.0 Consent Process

If a subject shows interest in participation, the detailed nature, purpose, procedures, benefits risks of, and alternatives to this research study will be explained to each subject, and written informed consent will be obtained by the study clinician or trained designee who provides this information in a private setting.

22.0 Process to Document Consent in Writing

Written informed consent will be obtained by the study clinician or their appropriately trained designee who will provide this information by virtual consent utilizing the Emory IRB approved REDCap platform or in a private office space. To ensure comprehension of the informed consent information, the clinician will allow time for adequate consent review and questioning. Virtual interviews will be conducted by use of ZOOM, a video conferencing service endorsed by Emory University for HIPPA compliant patient encounters. Informed consent will be documented on an Emory Institutional Review Board-approved form. A copy of the signed form will be given to the subject or accessible to the participant following virtual consent provided in a REDCap ICF document link. The original document will be filed in a central study consent binder. Subject casebooks will be in electronic format in the REDCap database. The consent binder(s) and select regulatory information gathered as part of the study will be kept in a

locked office and/or cabinet. Active participants will be asked to reconsent with a revised consent if an IRB approved modification is relevant to subjects' participation.

23.0 Setting

This work will be conducted in the Emory Behavioral Immunology Program on the 5th floor of Clinic B, and using the following clinical, laboratory and imaging facilities as described below.

Office Space: The Department of Psychiatry and Behavioral Sciences provides Dr. Felger with an office on the 4th floor of the Woodruff Memorial Research Building, which is down the hall from her laboratory space, shared equipment, and to the offices of Dr. Miller (Co-I and Study Physician) and Ms. Woolwine (Project Manager). Office and desk space for the dedicated laboratory manager, as well as clinical and laboratory staff and students, are provided in the laboratory spaces and adjacent clinical research areas. In addition, 3 offices are available for patient interviews, and one office area is available for storage of patient files and equipment. The Department of Psychiatry and Behavioral Sciences provides Dr. Dunlop with an office on the 3th floor of the Executive Park 12 (EP12) building. Dr. Treadway is Faculty of the Department of Psychology at Emory where he has laboratory and office space on the 4th floor of the Psychology and Interdisciplinary Sciences Building.

Laboratory Resources:
The Emory University Hospital maintains a CLIA-Certified lab for the necessary clinical laboratory testing. Dr. Felger's over 600 square feet of laboratory space is located on the 4th Floor of the Woodruff Memorial Research Building, and is part of the Emory Behavioral Immunology Program spaces. See Equipment for details.

Clinical Resources:
Emory University Hospital Clinical Research Site:
The proposed study will use the Emory University Hospital Clinical Research Network (CRN), which is part of the Georgia Clinical and Translational Science Alliance (GCTSA, formerly Atlanta CTSI) located in the Emory University Hospital. The GCTSA-CRN is staffed around the clock with trained research nurses, who are available to aid in the implementation of all protocol procedures. It should be noted that GCTSA staff have extensive experience in conducting blood sampling procedures as proposed in this protocol and preparing the patients for LP. More detailed information about the CRN includes:
Outpatient Care Area: The outpatient area consists of two beds and four infusion recliners, an outpatient nursing station and a restroom. There is a small waiting area in the hallway adjacent to the Outpatient Room. In addition, the GCTSA outpatient area offers attached lab services, where blood and CSF samples can be processed. The GCTSA has implemented NCATS cost recovery mandated for all CTSAs through fees for use of space, nursing and laboratory support.
Statistical Core: As an active GCTSA investigator, the Dr. Felger has access to the GCTSA statistics faculty and statistical software. FCTSA computers are connected to a local area network that allows sharing software, custom programs, data, and automatic backups of administrative files. Investigators can access their data from any personal computer at the CRN, as well as by dialing into the CRN system.
The Mood and Anxiety Disorders Program (MAP):
The Emory Mood and Anxiety Disorders Program directed by Co-Investigator, Dr. Boadie Dunlop, is the largest clinical research program in the Emory Department of Psychiatry and Behavioral Sciences, and will be the primary clinical site for this project. MAP has been conducting NIH- and industry-funded outpatient research trials of major depression, PTSD, social phobia, generalized anxiety disorder, obsessive-compulsive disorder and panic disorder for 15 years. Trials conducted by MAP include industry- and federally sponsored studies,

including the recently completed PReDICT study with a recruitment target of 400 treatment-naïve MDD patients willing to be randomized to medication or psychotherapy. MAP personnel conduct telephone screening of approximately 1200 people per year for depressive or anxiety disorders, resulting in in-person evaluations of approximately 150-200 people per year who are interested in participating in clinical research. Potential research subjects contact MAP either through our telephone number or complete application forms located on the website for "emoryclinicaltrials". The program is located on the third floor of the Executive Park 12 (EP12) building. MAP operates out of a suite of 12 offices, plus additional rooms for physical exams, phlebotomy, EKG, centrifugation, storage (including dry-ice storage equipment, and -20ºC and -80ºC freezers), and a conference room. The program is fully equipped with modern desktop computers, printers, fax machines, photocopier, supplies, and video-taping equipment. The personnel have extensive clinical experience and all have been trained to conduct high-quality clinical research. There is plentiful free parking directly in front of the EP12 building, and a public transit bus stop directly in front of the EP12 building.

Additional Patient Referral Resources and Support*:* In addition to the MAP, patients will be obtained from multiple internal and external referral resources including the Emory Clinic, Grady Health System, and the Veterans Administration Medical Center (VAMC) as well as community psychiatrists. Advertisement will consist primarily of a social media advertising campaign, but may also include radio, television, and print advertising. We anticipate that up to 3 subjects per month will consent and qualify to undergo full screening for our study (~35 per year), and we will enroll approximately 1-2 patients per month. This estimate is based on current studies on depression in mood disorders research programs within the department. Relevant to this project, we consistently observe that at least 50% of patients exhibit increased C-reactive protein (i.e. CRP >2mg/L).

Core Resources:

Biomedical Imaging Technology Center (BITC): Center for Systems Imaging (CSI)/Biomedical Imaging Technology Center (BITC): The BITC is part of the overall Emory CSI and the Wallace H. Coulter Department of Biomedical Engineering run jointly by Emory University and Georgia Institute of Technology. The BITC facility at Emory University Hospital is housed in approximately 3000 square feet of space. BITC is a core facility of the School of Medicine at Emory University. It was established to focus on the development and application of imaging technology, particularly magnetic resonance imaging (MRI). BITC is equipped with a 3 Tesla Siemens Prisma whole-body MR system, and a 9.4T/21-cm Bruker animal MR imaging/spectroscopy system dedicated to research. In addition, a research agreement was reached with Siemens Medical System such that BITC will have full access to sequence programming software, technical details for data manipulation, and necessary technical support. This MRI system will be used for fMRI studies proposed in this project. The post processing will be conducted in a state-of-art computing facility consisting of a Linux-based 29 nodes computational cluster, disk array with a capacity of 131 TB, and an automated backup system. The core of the processing is accomplished by a Sun Grid Engine powered for maximum data processing efficiency. This state-of-the-art hardware setup is augmented with a number of software packages including Matlab, FSL, AFNI, Hammer, SPM5/8, IDL, LCMODEL, and Stimulation. In addition to the MRI system and computing facility, peripheral equipment including coils, monitoring systems, computers and software are also available in the center described as follows:

<u>RF coils:</u>
CP_Head coil [Siemens]
64, 32 and 20- channel head array coils [Siemens]
Spinal Matrix coil [Siemens]

2x Flexible Body Matrix coil [Siemens]
CP Extremity Coil (Knee coil) [Siemens]
CP Wrist Coil [Siemens]

Managed Peripheral Equipment:
In vivo Vital Sign Monitor (MRI compatible) -- ECG, SpO2, EtCO2, and HR (heart bit rate); also (upgraded to) provide external trigger signal and synchronized vital signal digital recording.
Homemade respiration belt monitoring/recording setup.
Homemade visual stimulation setup -- support computer video display mode 640x480 up to 1280x1024.
Homemade LED goggle -- visual stimulation.
Avotec Silent ScanTM Audio System -- PI-patient communication and acoustic stimulation.
Applied Science Laboratories eye-tracker -- pupil tracking.
Current Designs, Inc. optical button box -- non-audio patient response.
Home-made single large button -- simple response.
Home-made buzzer -- tactile stimulation.

Third Party Peripheral Equipment:
Biopac MP100 -- GSR recorder.
Including: ECG100C-MRI (for ECG), RSP100C (for respiration), EDA100C-MRI (for skin conductivity),
TSD160A (for animal respiration), STM100C (for electrical stimulation), and DTU200/300 (for cardiac and
respiration triggering).

BITC Cluster software available for MRI data processing
fMRI data processing - AFNI
fMRI/DTI data processing - FMRIB Software Library
fMRI data processing for animal image - Stimulate
MRI image converter/viewer - MRIcro
Post-processing software - LC Model
Echoplanar Spectroscopic Imaging - Developed at the University of Miami used in BITC with permission
Generic Scientific Calculation
Matlab

The Emory Integrated Genomics Core (EIGC)*:* Dr. Zwick is the Scientific Director for the EIGC, which provides a host of genomic and related computational services for Emory Investigators. The EIGC is a CLIA-certified (CLIA # 11D1086150) laboratory located on the 7th floor of the Woodruff Memorial Research Building, with 2400 sq ft of dedicated wet-lab space and 500 sq ft of dedicated office space for bioinformatics. The EIGC's laboratory areas include dedicated pre- and post-PCR spaces. The EIGC is composed of three divisions: CLIA, Research, and Computational. Each division provides specialized genomics and computational research services to Emory investigators.

Biostatistics and Bioinformatics Shared Resource: The Biostatistics and Bioinformatics Shared Resource at Winship Cancer Institute is a shared resource that serves as a focal point from which Winship investigators may draw statistical expertise for planning, management and analysis of their studies. The Biostatistics core also develops, tests and implements various techniques of statistical analysis, including mathematical modeling, applicable to cancer research. Types of research supported include clinical trials, translational research,

epidemiology, behavioral, and basic science. This shared resource has Ph.D. and M.S. biostatisticians including whose primary appointments are in the Department of Biostatistics at Rollins School of Public Health of Emory University.

The EIGC offers comprehensive bioinformatics pipelines and services for the analysis of genomics data contained within the Computational Division. Presently, standard analyses are carried out using the Emory Human Genetics Computer Cluster (HGCC) which is composed of 1 head node and 20 compute nodes and serves multiple functions related to genomic projects, including running NGS analysis pipelines, high-performance and parallel computing, web application serving, and data storage. The cluster includes a 143 TB Penguin Computing RAID 6 storage array for user files and data, a 32TB Advanced Clustering RAID 5 storage array for storing and processing NGS data, and a 22TB Apple Xserve RAID 51 storage array for serving web applications. A mix of 10 Gbps ethernet, 1 Gbps ethernet, and 4 Gbps fibre-channel switches are configured to provide a high-speed Storage Area Network (SAN). All storage arrays and compute nodes utilize the SAN for data transfer, and are configured to connect either via 10 Gbps high-speed network, or with dual-bonded connections to the 1 Gbps ethernet or 4 Gbps fibre-channel ports for increased bandwidth. The cluster runs Centos 5.7 64-bit operating system on all nodes. Configuration: One head node: 2 x 2.4 GHz 8-core CPUs, 64 GB RAM. One development and job-submission node: 2 x 2.4 GHz dual-core CPUs, 8GB RAM. Four compute nodes: 4 x 2.4 GHz 8-core CPUs, 256 GB RAM, 10 Gbps ethernet. Two compute nodes: 4 x 2.4 GHz quad-core CPUs, 24GB RAM, 2 x 1 Gbps ethernet. Fourteen compute nodes: 1 x 3 GHz dual-core CPU, 12 GB RAM, 1 Gbps ethernet. Sun Grid Engine is implemented for job submission. Four queues are implemented: shq - for short jobs requiring high memory, lhq - for long jobs requiring high memory, slq - short jobs requiring low memory, and llq - for long jobs requiring low memory. Short jobs are defined as those requiring 12 hours or less to complete. High memory jobs are defined as those requiring greater than 12GB RAM.

The EIGC operates a custom ***EIGC Galaxy server*** for standard analyses. Galaxy is an open, web-based platform for accessible, reproducible, and transparent computational biomedical research. The Galaxy server will use flexible Emory University Isilon storage (up to 1 petabyte total capacity) to store large datafiles and the EHPCC to process large computational jobs in parallel. Galaxy provides a wide variety of bioinformatic tools that allow users to analyze, manipulate, and visualize large genome-wide datasets from a wide variety of platforms, including microarrays and next-generation sequencing instruments.

Emory's Library & Information Technology Services (LITS) supports **High Performance Computing (HPC)** on campus through a relationship with **Amazon Web Services (AWS)** as a preferred vendor. The HPC team will meet directly with clients to help them set up an account, create a virtual private cloud (VPC) as well as Amazon Machine Images (AMI). The team also provides necessary training. The AWS Cloud provides a broad set of infrastructure services, such as computing power, storage options, networking and databases, delivered as a utility: on-demand, available in minutes, with pay-as-you-go pricing.

Computer Resources:
See above. Available to the research staff in this application are 6 IBM desktop computers and 2 laptop computers as well as 2 laser printers (one color), a FAX copier and a dedicated scanner. The computers are all connected to the University server for internet access and are all available for data storage and management, statistical analyses, word processing and presentation graphics. The project staff also has access to the Emory University Computing Center. The Center is available to the investigators via fiber-optic linked campus wide network and supports a wide array of software including a number of statistical packages such as SAS and SPSS.

24.0 Resources Available

24.1 This project will be conducted using the resources described above. Recruitment and referral sources for subject recruitment will include paid online advertisement conducted through the Behavioral Immunology Program, where up to 500 subjects per year will be contacted to yield 200 participants to be seen by our program for pre-screen followed by 35 consents and full screens to be conducted for this study each year to yield 35 patients randomized over 2 years. This recruitment method, which has been successful in our previous studies (including Dr. Felger's ongoing R01 study) will be supplemented by referrals from the clinical of Co-Investigator, Dr. Dunlop (see MAP clinic above).

- Anticipated effort of the faculty and staff involve on the project (may change at any time during the study in accordance with NIH requirements):
  Principal Investigator Jennifer C. Felger, Ph.D., M.S. (3.6 calendar months, both years) Dr. Felger is an Associate Professor of Psychiatry and Behavioral Sciences at Emory University School of Medicine, and has been conducting clinical and translational research in behavioral immunology for over 10 years. As the team lead, she will be responsible for the overall administration and management of the study, and will maintain regular contact with all team members. She will work closely with the Project Manager/Director of Projects to hire, supervise, and train staff in clinical/study procedures, design all data collection instruments, and coordinate patient recruitment. She will consult with Dr. Miller, Dr. Dunlop and the Nurse Practitioner to review recruitment criteria and safety measures for all study participants. Dr. Felger will have weekly meetings with the Postdoctoral Fellow, and quarterly meetings with Dr. Treadway and Dr. Li, to ensure quality control of neuroimaging data acquisition and analysis. She will work with Dr. Treadway and the Postdoctoral Fellow on the implementation and analysis of performance-based reward tasks inside and outside of the scanner. Dr. Felger is the Laboratory Director for the Emory Behavioral Immunology program, and will oversee all specimen collection and bioassays performed by the research Specialist. Dr. Felger will work with Dr. Schettler, a statistician with extensive experience in psychiatry research, and the Postdoctoral Fellow to compile, analyze and upload/share (see Resource Sharing Plan) the collected data, and will prepare the results for presentation and publication.

  Co-Investigator Andrew H. Miller, M.D. (1.8 calendar months, both years) Dr. Miller is Professor of Psychiatry and Behavioral Sciences at Emory University School of Medicine, and will serve as the study physician during the R61 phase. He has over 20 experience in experimental medicine, psychoneuroimmunology research and clinical trials. He will determine if subjects are medically healthy, as demonstrated by a normal history and physical examination and laboratory testing. He will provide physician coverage for any medical or psychiatric problems that arise during subject assessments. He will review entry criteria for all patients (including screen failures) and will oversee the medical management of all study participants. He will

provide on-call availability to study participants 24/7. He also will be involved in data interpretation and manuscript preparation.

Co-Investigator Boadie W. Dunlop, M.D., M.S. (1.2 calendar months, both years) Dr. Dunlop is an Associate Professor of Psychiatry and Behavioral Sciences at Emory University and is the Director of the Mood and Anxiety Disorders Program (MAP) for the Department. During the R61 phase, Dr. Dunlop will assist with recruitment by referring participants from his clinic and research program into the study, and will serve the second (back-up) psychiatrist on the project. He will participate in writing up study findings for publication.

Co-Investigator Michael T Treadway, Ph.D. (1.8 calendar months, both years) Dr. Treadway is an Associate Professor in the Department of Psychology at Emory University. Dr. Treadway has a background in cognitive neuroscience and clinical psychology, with specific training in methods of functional and structural magnetic resonance imaging and application of computational models to neuroimaging data. He is an expert in the evaluation of reward responsivity and has developed innovative strategies for measuring various aspects of reward processing, notably effort-based decision-making. As part of his research, he developed the Effort Expenditure for Rewards Task (EEfRT) task, which will be one of the tasks used in this proposal to assess reward valuation. Dr. Treadway will oversee the implementation of the performance-based reward tasks and will assist with determination of the quality of the data. He also has over 10 years of neuroimaging experience, and will assist in the collection, analysis and interpretation of fMRI data, as well as the integration of imaging findings with the results of the EEfRT task outside of the scanner for presentation and publication.

Other Personnel

Director of Projects Bobbi J. Woolwine, M.S.W. (2.4 calendar months, both years) Ms. Woolwine is a well-trained research clinician with 25 years of experience who will supervise the clinical interviews/assessments of study participants, and will assist Dr. Felger with regulatory oversight and study monitoring during the R61 phase. She has worked closely with the Departmental and University Offices of Grants and Contracts and the Institutional Review Board to assure that all research activities are in compliance with appropriate university and other funding agency guidelines. She will help Dr. Felger to develop budgets and track expenditures. She will also assist with the writing of protocols, operational decision manuals, and provide documentation of these annual reports. The Project Manager will also assist Dr. Felger with the hiring and training of staff in clinical/study procedures, as well as advertisement and patient recruitment, and will oversee the flow from intake through the participation process.

TBD, Clinical Research Coordinator I (12 calendar months, both years) A Clinical Research Coordinator I (CRC) will be needed for

recruiting patients, scheduling patients for study procedures, and handling the day-to-day management of the study including maintaining the case report forms, entering data into the database, and screening of patients. The CRC will report directly to Dr. Felger. He/she will also assist in conducting standardized patient interviews and conducting the performance-based assessments under the supervision of Dr. Treadway. The CRC will conduct intake interviews, administer research rating scales with the validated instruments, and conduct vital signs measurements. He/she will assist the Research Interviewer in the phone and clinic screening of potential research participants. The CRC will also be responsible for recording adverse events, study variances and maintaining up-to-date charting and entry of clinical data into Red Cap. The CRC will help prepare reports for the DSMB and IRB, and help prepare and submit study modifications and renewals to the IRB.

TBD Research Interviewer (6 calendar, both years)The primary role of the Research Interviewer will be to work with the PI, CRC, NP and Director of Projects to recruit study participants. He/she will conduct phone screens and participate in the initial intake interviews. The Research Interviewer will also assist the CRC and PIs in facilitating the flow of participants through the study protocols, maintaining patient records and preparing reports for the DSMB and IRB.

TBD, Research Specialist (1.8 calendar months, both years)
The Research Specialist will be responsible for the overall management of the laboratory aspects of the project including the collection, processing and storing of all biological samples, and conducting assays of inflammatory markers. The Research Specialist will also be responsible for laboratory data entry, data proofing and cleaning, and developing reports and queries for manuscripts and progress reports. She/he will assist with data acquisition, chart cleaning, and data retrieval as needed.
This research will be conducted using the Facilities and Resources described above.

Availability of medical or psychological resources:
Psychological: Care will be taken to avoid bringing about undue psychological distress during the neuropsychiatric interviews. This will be accomplished by using trained raters (clinicians) for all neuropsychiatric assessments. In the event that a subject becomes unduly distressed, Study Physicians Drs. Miller or Dunlop will be immediately contacted, and an appropriate clinical intervention plan will be developed. Drs. Miller and Dunlop are Board Certified psychiatrists, and have many years of clinical experience in a wide variety of treatment settings, including emergency, consultation-liaison, inpatient and outpatient psychiatry. In cases where patients need extra time to collect their thoughts and emotions, mental health clinicians will be available for emotional support after all interviews.
Medical: At screening, subjects will be carefully evaluated for the presence of any medical conditions that might increase the risk of an

adverse events (for example cardiovascular issues uncovered by EKG). Subjects will be evaluated by medical personnel weekly during treatment and will be available 24/7 if problems arise. All procedures will be conducted in the Emory University Hospital facilities where emergency assistance will be available if needed.

- All personnel working on this study will complete appropriate CITI training modules including Good Clinical Practice. The CRCs working on the study will also complete a coordinator training class. Staff will be trained by the PI and designees (Study Physicians or Director of Projects) on all procedures, and adequate training for specific tasks will be documented in the delegation of authority log. After any approved modification to this protocol, the new protocol and IC will be circulated and discussed by study team. Each member will be asked to acknowledge key changes for written documentation that all staff and Investigators were appropriately educated.

25.0 Multi-Site Research when Emory is the Lead Site*N/A ☐

N/A

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