INFLAMMATION-RELATED ALTERATIONS IN NEUROCIRCUITRY: REVERSAL WITH LEVODOPA

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Inflammation-related Alterations in Neurocircuitry: Reversal with Levodopa

L-DOPA Depression

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2. Précis/Abstract:

Cytokines released by an activated immune system have been associated with decreased brain dopamine and the development of depression, which afflicts over 20 million adults in the United States. Biomarkers of inflammation, such as inflammatory cytokines and acute-phase proteins like C-reactive protein (CRP), are reliably elevated in a significant proportion of patients with mood and psychiatric disorders. Our recent findings using functional magnetic resonance imaging (fMRI) indicate that depressed patients with high inflammation (defined as plasma CRP >3 mg/L) exhibit reduced connectivity between the striatum and prefrontal cortical brain regions, which associate with symptoms of reduced motivation or anhedonia, a core symptom of depression. The striatum is a key subcortical structure that sends and receives multiple projections to and from the frontal cortex and other brain regions to regulate motivation, and dopamine plays a key modulatory role. Therefore, inflammation-related decreases in striatal dopamine may influence corticostriatal connectivity to mediate symptoms of anhedonia. Our non-human primate work has revealed that inflammatory cytokine effects on striatal dopamine can be reversed by administration of the dopamine precursor, levodopa (L-DOPA). However, whether administration of L-DOPA in humans can reverse inflammation-related disruptions in corticostriatal connectivity and anhedonia is unknown. This study will test the hypothesis that administration of L-DOPA to depressed patients with high inflammation will 1) increase functional connectivity between the striatum and prefrontal cortical brain regions, and 2) improve objective measures of motivation compared to placebo. Medically healthy, medication free depressed patients will be recruited to ensure a range of inflammation from low to high (as determined by plasma CRP) and will undergo resting state fMRI before and after administration of low dose L-DOPA-carbidopa (250/50 mg) and placebo on separate, double blinded visits. This within subject, crossover design has been used previously in a similar study assessing the effects of L-DOPA on corticostriatal functional connectivity in healthy controls.(1) All subjects will undergo MRI scanning as well as neuropsychiatric assessments and blood sampling, when applicable, at baseline and before and after placebo or L-DOPA administration during 2 outpatient visits. This work will establish whether alterations in corticostriatal connectivity may serve as a sensitive and specific brain biomarker for the effects of inflammation on striatal dopamine, while providing supporting data for future studies exploring therapeutic strategies that facilitate availability of dopamine precursors in patients with anhedonia and increased inflammation.

3. Introduction and Background

Mood disorders occur at a high rate (lifetime prevalence >20%) and confer a substantial societal burden. Current pharmacological therapies are effective for many patients; however, more than 30% fail to achieve remission and even responders often exhibit significant residual symptoms, including anhedonia (2-5). Therefore, new conceptual frameworks are needed to reveal pathophysiologic pathways and neurobiological targets for development of novel treatment strategies. Relevant in this regard, recent evidence suggests a cause and effect relationship between inflammation and symptoms of depression (6-8). For example, numerous studies (including meta-analyses) have found increased peripheral and central inflammatory cytokines and acute phase reactants that are induced by cytokines, e.g. C-reactive protein (CRP), which is elevated in up to 40% of patients with depression depending on the sample (9-12). Furthermore, administration of cytokines or cytokine inducers to laboratory animals and humans is associated with development of

depressive symptoms, and particularly those related to anhedonia and psychomotor retardation (13-16). Moreover, inhibition of inflammatory cytokines, such as tumor necrosis factor (TNF), has been shown to reduce anhedonia and psychomotor slowing in patients with inflammatory disorders, and in depressed patients with increased inflammation (12, 17-19). Anhedonia, a deficit in pleasure and motivation is a core symptom of depression and other psychiatric illnesses thought to involve alterations in mesolimbic dopamine (20, 21). Psychomotor retardation is also a prominent feature of many psychiatric disorders, is thought to reflected dysfunction within prefrontal and basal ganglia circuits, and has been found to be related to anhedonia in depressed patients (22-24). Therefore, inflammation may affect dopamine-relevant neurocircuitry that influences motivation and motor activity to lead to symptoms of anhedonia and psychomotor slowing.

Impact of inflammation on striatal dopamine

A growing body of evidence from neuroimaging studies, including previous non-human primate work by the PI, consistently indicates that inflammatory cytokines affect the striatum and dopamine to contribute to symptoms of anhedonia and psychomotor speed (15, 25-28). These studies have utilized administration of cytokine inducers, such as endotoxin or typhoid vaccination, to healthy volunteers (27, 28), or chronic administration of antiviral and antiproliferative, inflammatory cytokines, such as interferon (IFN)-alpha, to patients with hepatitis C or malignant melanoma, or to non-human primates (15, 16). Administration of IFN-alpha induces a number of other inflammatory cytokines that are elevated in depression (29, 30), and produces clinically significant depressive symptoms in up to 50% of treated patients, including anhedonia and psychomotor slowing. For example, functional magnetic resonance imaging (fMRI) has demonstrated decreased ventral striatal activation to hedonic reward (winning in a gambling task) during administration of IFN-alpha that associated with reduced motivation and reduced activity (15). Similarly, typhoid vaccination and endotoxin decreased neural activation of the ventral striatum to hedonic reward and altered substantia nigra activity, which correlated with symptoms of anhedonia and psychomotor slowing (27, 28). To further explore the effects of inflammatory cytokines on synaptic availability and release of striatal dopamine, the PI conducted *in vivo* microdialysis and translational PET neuroimaging with [^{11}C]raclopride displacement following amphetamine (AMPH) challenge (which indirectly measures dopamine release) in IFN-alpha treated monkeys (16). Results indicated that stimulated dopamine release was decreased in the striatum after chronic administration of IFN-alpha, and decreased dopamine release, as measured by *in vivo* microdialysis, was correlated with reduced effort-based sucrose consumption, a measure of motivation and anhedonia (31). Together these findings indicate that inflammatory cytokines target striatal dopamine to contribute to symptoms related to anhedonia and psychomotor retardation.

Inflammation effects on corticostriatal connectivity

Dopamine regulates reward, cognitive and motor activity via projections to ventral and dorsal striatum, as well as prefrontal cortical, limbic and other subcortical brain regions, all of which are highly interconnected. Whereas ventral striatum is thought to mediate motivation and reward, the more dorsal regions are thought to mediate motor and cognitive functions, yet substantial neuroanatomical and functional overlap exists to allowing for integrative processing of information and behavioral output (32, 33). To examine the extent to which inflammation-related decreases in dopamine affect functional connectivity between ventral and dorsal subdivisions of the striatum and other brain regions (15, 32), we conducted resting state fMRI in medically healthy, medication-free depressed patients with varying levels of inflammation as defined by plasma CRP (34). Interestingly, we observed significant negative relationships between inflammation and functional connectivity of the ventral and dorsal striatal with several other cortical, limbic and subcortical brain regions in a whole brain analysis (adjusted $p < 0.01$), either using CRP as a linear variable or when stratifying patients by high versus moderate versus low inflammation (CRP >3 versus 1-3 versus <1 mg/L, respectively) (34). For example, patients with low inflammation exhibited significant positive connectivity between ventral striatum and the ventral medial prefrontal cortex, as well as other cortical, subcortical and limbic structures, consistent with previously published maps of functional connectivity between ventral striatum and other brain regions in healthy controls (1, 32, 35, 36). Conversely, little positive connectivity between ventral striatum and subcortical and limbic brain regions was observed in patients with high inflammation, and connectivity with ventral medial prefrontal cortex was completely absent in these patients, which differed significantly between groups after whole brain correction ($p<0.01$, peak voxel in Brodmann area 32). The ventral medial prefrontal cortex is functionally connected with the striatum and thought to play a role in motivation, reward and anhedonia (21, 37, 38). Interestingly, Z scores representing the degree of correlation between left ventral striatum and ventral medical prefrontal cortex were associated with severity of depressive symptoms as measured by Inventory of Depressive Symptomatology-Self Report, which was significant after adjusting for age, sex and body mass

index. This relationship was mediated by scores for symptoms of anhedonia, indicating that patients with low or negative connectivity scores exhibited higher self-reported symptoms of anhedonia. This ventral medial prefrontal region is consistent with previously identified regions of interest (ROIs) in meta-analyses as activated by a wide variety of rewarding stimuli in healthy controls (39), and decreased connectivity between this region and striatum has been previously observed in anhedonic patients who did not respond to a novel antidepressant treatment (40).

Regarding inflammation-related differences in connectivity with dorsal striatum, patients with high and low inflammation exhibited similar positive connectivity between dorsal striatal subregions and motor cortex. However, loss of functional connectivity between the dorsal caudal putamen and frontal cortical regions were observed in patients with high inflammation. These findings indicate that even within dorsal striatum, inflammation-related changes in corticostriatal connectivity may be specific to frontal cortical, but not motor, brain regions. Decreased connectivity between dorsal caudal putamen and anterior prefrontal cortex (Brodmann area 10) were associated with scores on the Trail Making Test-A, an objective measure of psychomotor speed, demonstrating greater psychomotor retardation in patients with low connectivity ($r=-0.59$, $p<0.05$) that was significant when controlling for age, sex and BMI. Of note, decreased glucose metabolism has been observed in similar prefrontal cortical regions of IFN-alpha-treated and depressed patients that are thought to play a role in psychomotor slowing (26, 41). Together, these findings support the hypothesis that increased inflammation decreases corticostriatal functional connectivity, which may contribute to dopamine-related symptoms of anhedonia and psychomotor slowing. Therefore, corticostriatal connectivity may serve as a brain biomarker of inflammation effects on dopamine that can be used to assess the efficacy of therapeutic targets to reverse inflammation-related changes in neural circuitry and behavior (42).

Inflammation effects on dopamine precursors and reversal with L-DOPA

Inflammatory cytokines may decrease dopamine availability and release by decreasing tetrahydrobiopterin (BH4), an enzyme co-factor required for conversion of phenylalanine to tyrosine by phenylalanine hydroxylase and tyrosine to L-DOPA by tyrosine hydroxylase (43). BH4 is also a co-factor for NO synthases (NOS), and cytokine-induced increases in inducible NOS (iNOS) can usurp available BH4, resulting in NOS uncoupling and the generation of reactive oxygen species rather than NO (44, 45). Inflammation and iNOS-related decreases in BH4 can further increase oxidative stress and contribute to oxidative reduction of BH4 itself (which is highly redox-sensitive), leaving even less BH4 available for dopamine synthesis (44). We and others have examined biomarkers of the dopamine synthetic pathway and observed evidence of reduced BH4 activity in IFN-alpha-treated patients (46-48). For example, IFN-alpha administration was associated with increased peripheral blood phenylanine to tyrosine ratio, which in turn correlated with decreased CSF dopamine and HVA (47). Increased CSF IL-6 was also correlated with decreased BH4 in CSF of IFN-alpha-treated patients (47). Of note, the phenylalanine to tyrosine ratio significantly correlated with IFN-alpha-induced depressive symptoms (47). These findings are consistent with decreased dopamine metabolites in the cerebrospinal fluid (CSF) of both IFN-alpha-treated patients and monkeys that correlated with depressive behaviors (25, 49), and decreased neural activation of the ventral striatum to hedonic reward following dietary depletion of the dopamine precursors phenylalanine and tyrosine (50).

Consistent with the hypothesis that inflammation decreases dopamine precursors, administration of L-DOPA completely reversed IFN-alpha-induced reductions in striatal dopamine release, as measured by *in vivo* microdialysis in rhesus monkeys administered chronic IFN-alpha (51). Of note, no changes were found in the 3,4-dihydroxyphenylacetic acid (DOPAC) to dopamine ratio, which increases when dopamine is not properly packaged in synaptic vesicles and subsequently metabolized via monoamine oxidase (52). Therefore, inflammatory cytokines may reduce the availability of dopamine precursors, without affecting end-product synthesis or vesicular packaging and/or release. A fundamental depletion of dopamine availability by inflammation may manifest as reduced striatal dopamine release and decreased ventral striatal activation to reward, to contribute to symptoms of anhedonia and psychomotor retardation. Of note, these dopamine-related symptoms are often difficult to treat, and a relationship exists between high inflammation and treatment resistance to standard antidepressant therapies (4, 11, 53, 54). Our findings suggest that pharmacologic strategies that boost key components of dopamine synthesis may be effective strategies to treat symptoms of anhedonia and psychomotor slowing in patients with increased inflammation, whereas dopamine reuptake inhibitors may prove less efficacious. Indeed, dopamine reuptake inhibitors have demonstrated limited efficacy

for treating motivation and motor-related symptoms in patients with cancer or other medical illnesses that are associated with increased inflammation (55-57).

Several pharmacological strategies exist to increase BH4 availability, which may subsequently increase dopamine precursors. These include administration of BH4 itself (58), which is currently approved in a synthetic form to treat phenylketonuria (59-61), as well as folic acid, L-methylfolate, or S-adenosyl-methionine (SAMe), all of which have a role in the synthesis and/or regeneration of BH4 (25, 62) and have demonstrated efficacy as adjuvants to antidepressants (63-65). Prior to assessing the efficacy of such strategies to target symptoms of anhedonia and psychomotor slowing in patients with increased inflammation, it is first necessary to establish a sensitive and reliable brain biomarker of the effects of inflammation on striatal dopamine by determining whether this brain biomarker is affected by increasing dopamine release. Resting-state functional connectivity is an increasingly popular technique for understanding alterations in neurocircuitry that may underlie specific neuropsychiatric symptoms or disease states (66-69), and may also serve as a reliable and reproducible method that may be useful in longitudinal studies or clinical trials where repeat testing within the same subject is desired. Corticostriatal connectivity has been shown to be sensitive to drugs that increase dopamine, including the dopamine precursor, L-DOPA (1, 70). Therefore, increasing dopamine with L-DOPA may be used to reverse inflammation effects on corticostriatal connectivity, thereby establishing the specificity of changes in connectivity with changes in dopamine and supporting its use as a brain biomarker. Therefore, the current proposal seeks to establish alterations in corticostriatal connectivity as a sensitive and specific brain biomarker for the effects of inflammation on dopamine in order to lay a foundation for future studies investigating therapeutic strategies that facilitate availability of dopamine precursors in patients with increased inflammation.

4. Objectives

Objective 1: To determine if acute administration of L-DOPA can reverse inflammation-related decreases in corticostriatal connectivity.

Hypothesis 1: Administration of L-DOPA will increase functional connectivity between the striatum and prefrontal cortical regions compared to placebo, in association with increased cerebral blood flow (CBF).

Medically healthy, medication free depressed patients who are recruited to ensure a range of inflammation from low to high (as determined by plasma CRP) will undergo resting state fMRI for connectivity and arterial spin labeling (ASL) for CBF before and after administration of low dose L-DOPA-carbidopa (250/50 mg) and placebo on separate, double blinded visits. Additional MRI measures, including response to hedonic reward (a monetary incentive delay task), and other indices of peripheral inflammation (per Aim 3), will be assessed to aid in interpretation of connectivity and CBF results. Brain activity during the viewing of facial cues (faces task) and heart rate variability will also be collected and used as predictors of response to L-DOPA.

Dependent variables:

Neuroimaging: resting-state fMRI; arterial spin labeling (ASL); task-based fMRI (monetary incentive delay)

Immune Markers: Plasma CRP

Objective 2: **To examine whether L-DOPA-mediated increases in corticostriatal connectivity are associated with improvement in objective measures of motivation and motor performance.**

Hypothesis 2: L-DOPA administration will be associated with improvement in motivation and psychomotor speed compared to placebo, and will correlate with increased corticostriatal connectivity.

Patients described in Objective 1 will undergo performance-based assessments of motivation and motor performance, and self-report assessments of depressed mood, including anhedonia, following administration of L-DOPA and placebo, which will be correlated with changes in functional connectivity.

Dependent variables:

Neuropsychology: Finger Tapping Task; Reaction Time Task (CANTAB); Trail Making Test, Part A; Digit Symbol Test; Effort Expenditure Rewards Task (EEfRT)

Self-report**:** Snaith-Hamilton Pleasure Scale (SHAPS); Inventory of Depressive Symptoms-Self Report (IDS-SR); Beck Depression Inventory (BDI) Anhedonia Subscale; Multidimensional Fatigue Inventory (MFI); Profile of Mood States (POMS); State-Trait Anxiety Inventory (STAI) State Scale

Objective 3: To determine whether peripheral and central cytokines and/or biomarkers of decreased dopamine synthesis predict connectivity and behavior before and after L-DOPA challenge.

Hypothesis 3: Increased biomarkers of central and peripheral inflammation, and decreased cerebrospinal fluid (CSF) dopamine, its precursors and metabolites, will correlate with and predict greater fMRI and behavioral responses to L-DOPA

Patients described in Objectives 1 and 2 will undergo

Dependent Variables:

Neuroimaging and behavior: Functional connectivity and objective measures of behavior

Plasma and CSF Markers: IL-6, sIL-6R, TNF-alpha, sTNFR 2, IL-1 beta, IL-1ra, IL-10 and MCP-1; BH4, dopamine and dopamine metabolites, phenylalanine, tyrosine; mRNA expression of genes

5. Study design and methods

Study Overview: Patients with major depression diagnosed based on DSM-V TR criteria between the ages of 18 and 65 (males, females and minorities) will be recruited to ensure a range of inflammation from low to high (as determined by plasma CRP). Research assessments will occur in the clinical research space of the Principal Investigator in the Emory Clinic Buildings and at the Emory University School of Medicine EUH CRN, a dedicated clinical research unit in the Emory University Hospital. Following a minimum of 2 screening visits to determine study eligibility (see inclusion and exclusion criteria below) and stability of plasma CRP concentrations (or at PI's, study physician or their designee's discretion), patients will undergo psychiatric assessments and ratings and will have a medical history and physical examination along with laboratory testing and research blood draw, and will undergo weight, height, waist circumference, body composition measurements, and a urine drug screen followed by behavioral testing. Patients may also be asked to undergo finger prick at screening or study visits to collect 20 ul of blood that will be used to rapidly measure CRP on a Diazyme hsCRP POC Test Kit (or equivalent as approved by PI or PI's designee) without patients having to undergo venipuncture. Patients may also be asked to have a lumbar puncture (LP) performed by an Emory Anesthesiologist on a separate visit between 7am-12 pm following hydration with 1 L of saline IV using standard sterile technique and local anesthesia with patients in the lateral decubitus position. Approximately 11 cc of CSF will be withdrawn. Upon completion of the LP, subjects will lie flat for approximately one hour and be subsequently discharged after ~4-6 hours from admission. Patients will participate in two additional outpatient study visits (see Table 1 for study protocol). The LP visit may occur approximately 1 week either before the first neuroimaging Study visit or one week after the last neuroimaging Study Visit, unless otherwise approved by the PI. After subjects have completed screening by meeting inclusion/exclusion criteria and moved into the L.P. and/or scanning visit phase of the study, an Abbreviated Clinical Interview and depression self-report (PHQ-9, see below) will be conducted if it has been more than 2 weeks since psychiatric assessments. Additional laboratory and medical assessments will be conducted if more than 4 weeks from screening, unless otherwise approved by the PI, study physician or PI's designee. This information will be used as study data and/or to improve patient safety by enabling monitoring of a subject's physical and psychiatric status during the study. Drastic fluctuations in psychiatric status (i.e. >25% change in depression scores) or medically significant changes in health status from H&P or labs will trigger a review by the Study Team (including the PI and/or study physicians) to evaluate appropriateness for continuation in the study and/or consider referral for further

treatment. Additional CRP testing, psychiatric assessments, or medical assessments may be conducted as indicated by the PI, study physician or PI's designee. Prior to each MRI neuroimaging session, patients will undergo a urine toxicology screening (Testcup for drugs of abuse, or equivalent as approved by PI or PI's designee) for safety and exclusion of substance abuse. On the last screening visit, patients will undergo blood sampling in the morning (collected at the same time as blood draw for safety labs and/or CRP), and complete neurocognitive testing. During Study Visits 1 & 2, subjects will complete a series of self-reports in the morning, have MRI scans (including resting-state fMRI and ASL) pre- and post-L-DOPA or placebo administration from about 9 AM to 12:30 PM, and undergo blood sampling and selected neurocognitive testing after the MRI scans from about 12:30 to 2 PM. L-DOPA or placebo, which will be administered double-blind (randomized by the Emory IDS), will be administered in the waiting area of the Neuroimaging Facilities. Patient's vital signs will be monitored by a Study Physician or designated Nurse Practitioner before, during, and after drug administration. At each study visit, 6 ml of research blood will be collected at 4 degree for extraction of plasma to measure inflammatory markers and/or L-DOPA concentrations. Two 3 ml samples will be collected (at the screening visit #2 only) for whole blood mRNA gene expression. 2 buffy coat samples from the first research blood collected for plasma (screening visit #2) will also be stored for genetic analyses of relevant gene polymorphisms and DNA methylation. In order to further assess immune cell profiles, we will also collect 10 ml of whole blood in EDTA at room temperature for immune cell extraction. Some samples may be sent to other labs for additional analysis. Safety laboratory tests will be conducted at screening, which will include a hematocrit (CBC with automated differential), electrolyte panel (comprehensive metabolic panel), urinalysis with microscopic, and serum quantitative pregnancy test (for women only). A CRP test will be completed at both screening visits to establish stability, and may be repeated during study visits at the PI's or PI's designee's discretion. Subjects who smoke cigarettes must refrain from smoking 30 minutes before all blood draws and 2 hours prior to mRNA sampling. Subjects who smoke half a pack per day, or e-cigarette equivalent, will be offered a nicotine patch during study visits to reduce the effects of withdrawal on fMRI scans.

Enrollment: For this pilot study, we plan to enroll 225 subjects with major depression to analyze data from 80 patients. We will ensure that subjects represent a full range of inflammation from low to high. Therefore, at least one-third of subjects will be studied will have a screening plasma CRP concentration <3 mg/L, one-third of subjects will have CRP 1-3 ml/L, and one-third subjects will have CRP >3 mg/L, measured at least twice to establish stability and rule out acute infection. Patients will be obtained from multiple referral resources including the Emory Clinic, Grady Health System, the Veterans Administration Medical Center (VAMC), and community psychiatrists. Depressed subjects will also be obtained from newspaper and radio advertisements as well as internet solicitation. We anticipate that at least 4 subjects per month will consent to enter our study. This estimate is based on ongoing projects that have been recruiting subjects for related studies over the past 5 years.

Compensation: Participants will be compensated using ClinCard. The ClinCard is a web-based, reloadable debit card that automates reimbursements for clinical research participants. An additional $25 will be provided to cover travel expenses for participants that travel equal to or greater than 50 miles to Atlanta.

Initial visit/consent signing: After signing the consent form, subjects will complete an office intake visit(s) for further evaluation. After providing written informed consent, subjects will complete the Patient Health Questionnaire (PHQ-9) or the Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR). If eligible, subjects may undergo the initial CRP test and possibly other laboratory tests. Subjects will be compensated $25 for this initial intake visit.

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 will only 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 [redacted] 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.

Eligibility for completing further screening and baseline assessments: The PI or PI's designee will review information collected from the initial visit to determine whether subjects are eligible to proceed with further screening and collection of baseline assessments based on the following:

(1) Attainment of informed consent
(2) Psychiatric clinician has determined presence of current depressive symptoms at intake with QIDS score ≥ 14 or PHQ-9 score ≥ 15, 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

Medical and data quality screening criteria for LP and randomization: Subjects determined to be eligible for the study will undergo a minimum of 2 screening visits. Subjects will be compensated $50 at each screening visit. The screening visits will include the following assessments:

(1) Structured Clinical Interview for DSM-V (SCID)
(2) Mini Mental State Exam (MMSE), a standard, 27-item assessment of general cognitive functioning.
(3) Childhood Trauma Questionnaire
(4) Bipolarity Index
(5) Scale for the Assessment of Negative Symptoms (SANS)
(6) Salpêtrière Retardation Rating Scale (SRRS)
(7) Wide Reading Achievement Test (WRAT-3)
(8) Psychiatric History Form
(9) HDRS
(10) SHAPS-C
(11) MGH-ATRQ (Lifetime and Current)
(12) Medical history and physical exam performed by a physician or nurse practitioner.
(13) CRP (This lab is collected at least twice to establish stability – the mean will be used for group assignment. A difference of greater than 50% between values or a CRP ≥10 mg/L will warrant additional CRP sampling.)
(14) Laboratory testing including:
    a) an electrocardiogram
    b) complete blood count with automated differential
    c) comprehensive metabolic panel (with renal and hepatic function tests)
    d) antinuclear antibodies
    e) glycosylated hemoglobin test
    f) thyroid stimulating hormone
    g) tests for HIV Ag/Ab, HBV Ag, and HCV Ab,
    h) urinalysis with microscopic
    i) serum quantitative pregnancy test (if female)
    j) urine toxicology for drugs of abuse
    k) Pt/INR (only done if S will be participating in the LP procedure)
    l) PTT (only done if S will be participating in the LP procedure)

Procedures	Initial Visit	Screening #1†	Screening #2†	Lumbar Puncture Visit	Additional (If needed)	Study Visits #1-3
Consent/re-consent	X	[X]	[X]	[X]	[X]	[X]

Physical exam		X	[X]		[X]	[X]
Health update and vitals			[X]	[X]		[X]
Concomitant Medications		X	X	X	[X]	X
Adverse Events		X	X	X	[X]	X
History of Chronic Conditions		X			[X]	
CRP	[X]	X	[X]	[X]	[X]	[X]
Safety Labs≠	[X]	X	[X]	[X]	[X]	[X]
Additional Safety Labs≠≠	[X]	[X]	[X]	[X]	[X]	[X]
Pt/INR, PTT (for LP only)	[X]	[X]	[X]	[X]	[X]	[X]
Research blood draws‡		[X]	X	[X]	[X]	X
MRI Scan						X
Neurocognitive tests		[X]	X	[X]	[X]	
Neurocognitive tests'				[X]		X
Psychiatric Assessments ´Y	X	[X]	[X]	[X]	[X]	[X]
Psychiatric Assessments ´Y ´Y		X	[X]	[X]	[X]	
Psychiatric Assessments ´Y ´Y ´Y				[X]		X
Self Reports	X	[X]				
Self Reports*			X	[X]	[X]	
Self Reports**						X
MRI Scan Food, Drink and Cigarette Intake Form+						X
Lumbar Puncture				X		
Placement of ECG patches		[X]	[X]		[X]	

[X]: To be completed when indicated.
† Screening 1 & 2 can be completed on the same day if preferred by patient
≠Safety Labs: Complete Metabolic Panel, CBC w diff, Serum Preg Test (females), Urinalysis w micro, Urine drug test
≠≠Additional Safety Labs (screening #1 or screening #2): HIV Ag/Ab, Hep B Ag, Hep C Ab, ANA, TSH, Glycosylated Hemoglobin, EKG, Chem 7: electrolytes, urea, creatine, glucose, and/or hsCRP
Pt/INR, PTT to be completed for subjects who consent to LP within 2 weeks of procedure at any visit
‡ To be completed at one of the two screening visits during collection of safety labs and/or screening CRP
Neurocognitive tests: EEfRT, TMT-A, TMT-B, Digit Symbol Task, FTT, Reaction Time Task, Choice Reaction, Delayed Matching to Sample, Spatial Working Memory, RAVLT, Stroop word and color test to be completed at the end of the last screening visit
Neurocognitive tests' (Visits 1 & 2): EEfRT, TMT-A, Digit Symbol Task, FTT, Reaction Time Task
´Y Psychiatric Assessments (Initial Visit): PHQ-9 or QIDS
´Y ´Y Psychiatric Assessments (Screening #1): MMSE, SANS, SRRS, SCID Modules, Psychiatric History, WRAT-3, Bipolarity Index, Childhood Trauma Questionnaire, HDRS, HAM-A, SHAPS-C, Lifetime MHG-ATRQ, Current MHG-ATRQ, C-SSRS
´Y ´Y ´Y Psychiatric Assessments (Visit 1 & 2): An Abbreviated Clinical Interview will be conducted and PHQ-9 will also be re-administered if more than 2 weeks from last assessment as an update to the SCID to determine MDD status and track suicide risk.
Self report/questionnaires (Initial Visit or Screening #1): Demographics, MRI Screening form
*Self report/questionnaires (Screening #2): MFI, Epworth Sleepiness Scale, Pittsburgh Sleep Quality Index, Habits, STAI State Scale, IDS-SR, Perceived Stress Scale, Visual Analog Rating of Pain, Staging System for Reproductive Aging (female subjects only), Beck Anxiety Inventory, PTSD Checklist for DSM-5
**Self report/questionnaires (Visit 1, 2, & 3): MFI, Snaith-Hamilton Pleasure Scale (SHAPS), IDS-SR, BDI Anhedonia Subscale, Profile of Mood States (POMS), STAI State Scale, Motivation and Pleasure (MAP) Scale
+MRI Scan Food, Drink and Cigarette Intake Form to be completed immediately prior to MRI scan.

Inclusion and Exclusion Criteria:

Inclusion

- informed consent has been discussed with subject, subject has been given opportunity to ask questions, and has signed a current version of the consent and HIPAA documents prior to initiation of study procedures
- age 18-65 years including males, females and minorities and able to comprehend English
- diagnosis of DSM-V major depression and currently off antidepressant medication, unless otherwise approved by the PI or PI's designee
- Depression as the primary axis I disorder
- negative pregnancy test for women of childbearing potential
- not breast feeding
- two (or more) CRP tests conducted to establish reliability

Exclusion:

- evidence of untreated or poorly controlled endocrine, cardiovascular, pulmonary, hematological, renal, or neurological disease
- history of CNS trauma or active seizure disorder requiring medication unless otherwise approved by principal investigator, or PI's designee
- current or history of migraines (>6 debilitating migraines per month), glaucoma, melanoma, or bleeding disorder of any kind
- autoimmune or inflammatory disorder of any kind
- embedded metallic objects, prosthetics made of paramagnetic metals, aneurysmal clips and/or a history of claustrophobia
- chronic infection (e.g. hepatitis B or C or HIV)
- chronic use of agents known to affect the immune system including glucocorticoid therapy within the past 6 months, methotrexate within the past 1 year, chemotherapy of any kind (past or present), immunotherapy of any kind (past or present), aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) (within the past 2 weeks), statins (within the past 1 month), vaccinations (within the past 2 weeks), topical steroids (within the past 2 weeks), and antibiotics (within the past two weeks) unless otherwise approved by principal investigator or PI's designee.
- suicide attempt within six months of screening, or active suicidal intent or plan, or score >2 on HDRS or QIDS/PHQ-9 Suicide Item, unless otherwise approved by the PI or PI's designee
- a positive pregnancy test
- organ transplants
- current or history of cancer within the past five years besides basal cell carcinoma, unless otherwise approved by the PI or PI's designee
- a score of <28 on the Mini Mental Status Exam (MMSE), unless otherwise approved by the PI or PI's designee
- WRAT-3 score indicating less than 8th grade reading level, unless otherwise approved by the PI or PI's designee
- Either QIDS <14 or PHQ-9 <15, or HDRS <18, unless otherwise approved by the principal investigator or PI's designee
- history of the following: schizophrenia, schizoaffective disorder, other (non mood disorder) psychosis, depression secondary to a medical condition, mental retardation, dementia, or delirium
- Substance dependence [or abuse within the past year (except nicotine)], unless otherwise approved by the PI or PI's designee
- BMI >40 to limit the impact of morbid obesity on the results, unless otherwise approved by the principal investigator or PI's designee
- antisocial personality disorder diagnosis as assessed during clinical interview, as well as a history of hospitalization and/or recurrent suicidal behavior judged to be directly due to the personality disorder
- Current eating disorder (aside from binge eating related to depression), unless otherwise approved by the principle investigator or PI's designee
- Current OCD, exclusionary only if impacting daily functioning, as assessed by clinical interview
- any other condition which in the opinion of the investigator would make the patient unsuitable for enrollment, or could interfere with participating in or completing the protocol
- smoking more than one half pack per day, or e-cigarette equivalent, unless otherwise approved by the PI or PI's designee

- initiation of any of the following medications, unless otherwise approved by the PI or PI's designee: Aspirin or Aspirin-like compounds (single dose within 48 hours), Ibuprofen or Naproxen Sodium (single does within 48 hours), Cholesterol medications, Antibiotics, Herbal Medications, Psychiatric or Psychotropic Medications (including sedative hypnotics, anxiolytic medications or pain medications), Omega-3 supplements, Topical Steroids, Vaccinations (within the last 2 weeks)
- Currently on antidepressant medication, unless otherwise approved by the PI or PI's designee

Urine drug screening: A urine drug test (DrugCheck: NxScan Onsite Testcup or equivalent as approved by PI or designee) will be performed for every patient at screening and prior to each MRI scan. The urine test will allow for the qualitative detection of drug or drug metabolites in urine, including benzodiazepines, methamphetamine, cocaine, THC and morphine. Identification of a urine test positive for substance use will exclude the subject from participation, unless substance is a prescribed medication, or otherwise approved by PI or study physician.

Urine Pregnancy Test: A urine pregnancy test may be performed for women of childbearing potential if it has been >30 days since their last screening labwork (which includes a quantitative, serum hCG test). Identification of a positive urine pregnancy test will exclude the subject from participation.

Gender and Minorities: Based on the composition of the patients treated for major depression at the Emory Clinic, Grady Health System, VAMC and community psychiatry clinics, we anticipate that an equal number of men and women will be included in the study. As for the racial composition of the sample, we anticipate 30-40% African Americans, 5-10% Latinos and 2-5% Asians, which is reflective of the community (city of Atlanta) from which our sample will be drawn.

Dropouts: Because participation in this study involves two screening visits followed within approximately 2 weeks by three study visits, we anticipate that there will be few subjects who will drop out between screening and the study visits. In our experience, however, there will be subjects who will have to be removed from the study because of a positive urine toxicology screen. In addition, some subjects may become too severely depressed and/or suicidal between enrollment and completion of the study. Given a conservative estimate of a 20% screen failures and 20% dropout rate, we will need to recruit ~ 225 subjects to reach the target of 80 subjects for analysis. Psychiatric staff is available throughout each subject's participation, and can provide on-site emergency care in the event of crisis.

Study Procedures
Neuropsychiatric Assessments:

Observer-rated assessments
Mini Mental State Exam is an 11 question tool that assesses mental status. The questions cover five areas of cognitive functioning.
Hamilton Depression Rating Scale (HDRS): The HDRS is a 24-item, clinician-administered scale that rates severity of depression.
Scale for the Assessment of Negative Symptoms (SANS): The SANS is a 25-item, clinician-administered scale that rates the severity of negative psychiatric symptoms including affective flattening, poor attention, and anhedonia.
Structured Clinical Interview for DSM-VIV (SCID-Modified):
Mood modules- These modules from the SCID will allow for a categorical diagnosis of the presence or absence of major depression or other DSM-VIV mood disorder. Such categorical diagnoses will complement symptom severity ratings offered by the HDRS.
Alcohol and Drug Abuse/Dependence Module**:** These SCID sections allow for assessment for current or past presence of alcohol and drug abuse/dependence.
Eating Disorder and Anxiety modules: These sections allow for a diagnosis of the presence or absence of eating disorders and anxiety disorders.
Psychotic module: This module from the SCID will allow for the assessment of the presence or absence of psychotic symptoms**.**
Salpetriere Retardation Rating Scale (SRRS): The SRRS is a 15-item, clinician-administered scale to assess clinical psychomotor retardation (71). The 15 items of the scale are either related to motility or mental activity.
Psychiatric History: This form assesses the psychiatric history of the subject.

WRAT-3: The WRAT-3 is a very brief screening measure for reading level.
Bipolarity Index: The bipolarity index assesses five symptom dimensions that are characteristic of patients with bipolar disorder. The degree of severity of each dimension is calculated.
Snaith-Hamilton Pleasure Scale (SHAPS-C): Clinician administered assessment of anhedonia.
Hamilton Anxiety Rating Scale (HAM-A): The HAM-A is a 14-item clinician-administered scale that assesses the severity of symptoms of anxiety.

Self-report questionnaires :
Lifetime and Current Massachusetts General Hospital Antidepressant Treatment Response Questionnaire (MGH-ATRQ): The MGH-ATRQ is a measure of treatment resistance that uses a simple numbering system to quantify both the number of failed antidepressant treatment trials as well as the intensity/optimization of each trial (72). Unlike other resistance classification schema, the MGH-ATRQ does not make assumptions regarding a hierarchy of antidepressant classes (72, 73). The MGH-ATRQ generates a continuous variable reflecting the degree of resistance in depression, with higher scores indicating a greater likelihood of non-response (73).
State-Trait Anxiety Inventory (STAI) State Scale: This 20-item self-report scale is used to measure current anxiety symptoms.
Demographic Questionnaire: This form is used to capture basic demographic information of each subject.
Patient Health Questionnaire (PHQ-9*)* is a nine-item measurement used to assess depressive symptoms and suicidal ideation.
Quick Inventory of Depressive Symptoms (QIDS) is a 16 item clinician-assisted self-report instrument that include assessment of nine diagnostic symptom domains used to characterize a major depressive episode.
Inventory of Depressive Symptoms-Self Report (IDS-SR) is a 30-item self-report instrument with excellent psychometric properties that was designed to measure symptom constructs including psychomotor retardation, fatigue and anhedonia consistent with current DSM nosology (74, 75).
Multidimensional Fatigue Inventory (MFI) To evaluate the presence and severity of fatigue, subjects will complete the self-report, 20-item Multidimensional Fatigue Inventory (MFI-20)(76). Consistent with recent data regarding the structure of fatigue in medically ill patients (77), the MFI assesses 5 dimensions of fatigue, including general fatigue, physical fatigue, mental fatigue, reduced activity and reduced motivation. In addition to scores for each subscale, a total score can be derived by summing the 5-subscale scores (78).
Epworth Sleepiness Scale (ESS) will be used to assess subjective sense of daytime alertness (79). The ESS is a widely used measure of self-reported alertness. The ESS is a 24 point scale, with 8 different items for which the patient rates the likelihood of falling asleep on a 0-3 scale. Higher scores signify a greater likelihood of daytime sleepiness. The ESS has been reported to distinguish conditions characterized by excessive daytime sleepiness (i.e., narcolepsy, sleep apnea) from control populations (79).
Pittsburgh Sleep Quality Index (PSQI) will be used to assess sleep quality over the past month and can be used to distinguish good sleepers from bad sleepers (80). The scale contains 15 multiple-choice items that inquire about frequency of sleep disturbances and subjective sleep quality and 4 write-in items that inquire about typical bedtime, wake-up time, sleep latency, and sleep duration. The PSQI generates seven scores that correspond to relevant domains of sleep, and each score ranges from 0 (no difficulty) to 3 (severe difficulty). The component scores are summed to produce a global score (range of 0–21). A PSQI global score >5 is considered to be suggestive of significant sleep disturbance (80).
Snaith-Hamilton Pleasure Scale (SHAPS) is a 14-item scale used to assess present-state hedonic tone (81). The items 1, 4, 8 and 9 refer to interests, items 3 and 10 to food and drink, items 2, 7, 13 and 14 to social interaction and items 5, 6, 11 and 12 to sensory experiences.
Childhood Trauma Questionnaire (CTQ): The CTQ is a self-report inventory assessing 3 types of childhood abuse: sexual, physical, and emotional. Studies have established the internal consistency, stability over time, and criterion validity of both the original 70-item CTQ and the current brief version (82). The CTQ yields a total score and subscale scores for each of the types of child abuse. CTQ data from Emory have demonstrated good internal consistency reliability (alpha = .99 for physical abuse; alpha = .94 for sexual abuse; alpha = .93 for emotional abuse; and alpha = .98 for the total of these 3 scales). The data from the CTQ will be used to classify subjects into 2 categories for each type of abuse (physical, sexual, and emotional): (1) those with CTQ scale scores in the none to mild range, and (2) those with CTQ scores in the moderate to severe range. We will then create a composite variable across all of the 3 types of abuse. Using this composite, we can divide participants into 2 groups with respect to the numbers of types of abuse that fall into the moderate to severe

range: (1) those with no type of abuse in the moderate to severe range, and (2) those with at least 1 type of abuse in the moderate to severe range (83).
Habits: The Habits form is a brief questionnaire that assesses a subject's daily smoking, daily caffeine consumption, and exercise patterns.
Beck Depression Inventory, **2nd edition (BDI-II), Anhedonia Subscale** is a subscale of the 21-item self-report measure of depressive symptoms, rated on a 4-point Likert scale based on the patient's experience in the last two weeks.
Profile of Mood States (POMS) is a psychological rating scale used to assess transient, distinct mood states. Participants rate the extent to which they feel: unhappy, blue, lonely, gloomy, and worthless on a scale from 0 (not at all) to 4 (extremely).
Post-traumatic Stress Disorder Symptom Scale (PSS): The PSS is a 17-item self-report scale that assesses the presence and severity of symptoms related to a single identified traumatic event in individuals with a known trauma history.
Perceived Stress Scale 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.
Visual Analog Scale of Pain: is a scale between the range of 1 to 10, that measures the perception of pain.
Staging System for Reproductive Aging and Menopause Rating Scale (MRS) is an 11-item self-report scale that assesses the presence and severity of symptoms related to menopause including psychiatric and physical symptoms. This form will be administered to female subjects only.
Columbia-Suicide Severity Scale: The Columbia-Suicide Severity Rating Scale (C-SSRS) is a fully-structured, clinical interview designed to systematically query patients regarding past and current suicidal ideation and behavior (SIB) (100). The psychometric characteristics of the patient- reported C-SSRS have been assessed in multiple contexts. It has well validated to elicit suicidal ideas in depressed subjects and will be consequently used to identify individuals experience acute severe depressive symptoms and refer them for immediate treatment.
Post-Traumatic Stress Disorder Checklist for DSM-5 (PCL-5): 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 based on the patient's experience in the last month.
Beck Anxiety Inventory (BAI): The BAI is a 21-item self-report measure of anxiety symptoms, rated on a 4-point Likert scale based on the patient's experience in the last month.
Motivation and Pleasure Scale (MAP): The motivation and pleasure questionnaire is an 18-item self-report inventory that was created to disentangle state-wise motivational and consummatory components of everyday activities over a 24-hour period, and has been previously validated as a measure of reward-related symptoms in psychiatric populations.[101] This scale will be used to assess self-reported changes in symptoms of anhedonia before and after inflammation blockade.

Neurocognitive Assessments:
A range of neuropsychological assessments will be used to probe basal ganglia function and will be associated with inflammation status as noted above. Assessments will lie along a continuum, progressing from more purely motor tasks (such as finger tapping) which assess circuitry within the basal ganglia to those that involve motor speed with increasing cognitive demand and cortical participation (e.g. the Digit Symbol Task). In addition, we will administer a test of procedural memory, with minimal processing speed or reaction time demands, that has been shown to be sensitive to dysfunction in the basal ganglia, without the executive demands associated with tests of frontal lobe function. Every effort will be made to match groups on the basis of demographic factors affecting test performance, such as age, education level, gender and ethnicity. These factors can significantly influence neuropsychological test results and while relatively small variations between groups can be handled through addition of covariates to statistical analyses, more significant differences in group composition will threaten the integrity and interpretation of the results.
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. Performance norms have been established, and scores have been shown to be stable over time (84). The FTT is designed to assess subtle motor impairment and has been found to be altered in subjects with basal ganglia disorders and lesions (85).
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. Movement times on the CANTAB reaction time task have been shown to be slowed during IFN-α treatment and correlate with IFN-α-induced depression and fatigue (see Preliminary Results). Moreover, reduced DA transmission, as indicated by increased binding of the dopamine $D_{2/3}$ ligand ^{123}IZBM in the striatum, has been correlated with motor slowing in the CANTAB reaction time task in depressed patients (86).

Choice Reaction Task (CANTAB): (87)**: Variables:** Reaction Time, Movement time. Speed and accuracy in responses upon being instructed to choose on among multiple stimuli flashed on a computer screen.

Delayed Matching To Sample (CANTAB): (88, 89) **Variables:** Response time, Discrimination (Hits/False Alarms). A measure of visual memory where subjects are asked to identify objects shown previously at various time points.

Spatial Working Memory (CANTAB):(90) **Variables:** Response time, Correct responses. A test of ability to remember spatial localization of objects presented previously.

Trail Making Tests A and B (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. Part B (TMT-B) requires the patient to connect alternating letter-number sequencing circles in order and reflects basic attention and processing speed.

Digit Symbol Task: The Digit Symbol 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 (1-9). A key is printed above these rows and shows each number paired with a different nonsense symbol. The subject's task is to fill in the blanks with the corresponding symbols as rapidly as possible. This 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 (91). As part of the WAIS, this test is frequently used in neuro-psychology and relevant norms and test-retest reliability have been well established (84). Performance on the Digit Symbol Test has been found to correlate with subcortical atrophy (esp. as measured by the bi-caudate ratio) in disorders involving the basal ganglia including Huntington5psxgwzase and multiple sclerosis (92, 93).

Effort-Expenditure for Rewards Task (EEfRT): In this effort-discounting variant (Treadway et al., 2009), subjects are presented with a choice between two task-difficulty levels in order to obtain monetary rewards. In each trial, subjects must make repeated manual button presses within a certain period of time. To succeed in each trial, subjects must meet or exceed the required number of button presses within the time allotted. Easy-task trials require 30 button presses within 7 seconds, while hard-task trials require 99 button presses within or before 21 seconds have passed. There are also probability cues attached to each trial—88%, 50%, and 12%—that determine whether a trial may result in compensation, upon completion. This information will be made known to the subjects at the start of the task. Upon completion of all trials, two of the trials will be selected and the subject will receive compensation from those trials based on the choices they made. This payment may range from approximately $0.00- $60. This task lasts approximately 15 minutes, and the number of trials subjects complete is based on their choices and button press rate.

Stroop Color and Word Test: Subject is requested to name the color of a word both when the color of the ink matches the name of the color and when the color of the ink does not match the name of the color. Prolongation in reaction time and number of inaccurate responses during the name-ink color mismatch condition are measured.

Rey Auditory Verbal Learning Test (RAVLT): A test of verbal memory and learning using list learning that measures ability to use strategies to improve learning.

Reliability of ratings: All clinicians performing the SCID in this study will be trained to conduct the assessments according to standardized guidelines for the administration of the instrument and will conduct interim training sessions to maintain standardization of ratings. In addition, training on the HDRS and SANS will be provided for relevant study personnel, and interrater reliability will be established. Trained staff will conduct the neurocognitive assessments. New staff joining the study team will go through an apprenticeship for the ratings and training for reliability prior to performing independent ratings.

MRI Neuroimaging: MRI experiments will be conducted on a 3T Siemens Trio MRI scanner at any of the Emory Imagining Facilities. The patients will be informed of the time requirements prior to participation in the

study. All subjects will be required to limit caffeine intake for two hours prior to the scan. Any food or drink intake during this time frame will be recorded using a MRI Scan Food, Drink and Cigarette Intake Form immediately prior to the fMRI scan. MRI scans do not expose patients to radiation or any invasive procedures; however, MRI scanning can affect metallic and other implants. All subjects will be carefully screened for metallic implants and other contra-indications to MRI prior to the procedure, using the standard Emory University Biomedical Information Technology Center MRI screening form. The subject will be pre-screened with the "MRI Screening form" to confirm MRI safety per protocol prior to each scan session. Subjects with contra-indications will not receive a MRI scan. The MRI scanner is an enclosed space and subjects may experience claustrophobia while being scanned. Should a subject develop claustrophobia during the procedure, the scan will be terminated. Patients may also be invited to participate in additional scans on a voluntary basis. The first scan session will occur at study Visit 1, and will include task-based and resting-state fMRI, as well as high resolution anatomical T1 MRI, which will be performed as one continuous procedure. Scans will include resting-state fMRI and ASL scans that will occur before and after administration of L-DOPA or placebo.

Anatomic images: will be obtained using a T1 weighted MPRAGE sequence with the following parameters: TR/TI/TE =2500/1100/3.02 ms; 8° flip angle; FOV of 256×224×192 mm^3; matrix of 256×224×192; 1 mm thickness; GRAPPA 2; total scan time will be 6 minutes. A diffusion tensor imaging (DTI) sequence will also be acquired during the first scan of the initial visit using 128×128 matrix; FOV = 256×256 mm^2; resolution = 2 ×2 ×2 mm^3; parallel imaging with a GRAPPA factor of 2, BW =1954 Hz/pixel, 64 slices, TR/TE = 8700/98 Ms; total scan time will be 12 minutes.

Resting-state fMRI: Images for functional connectivity will be acquired with a 9 minute Z-SAGA sequence to minimize artifacts in the frontal cortex due to sinus cavities. During the resting-state scan, participants will be instructed to lie passively in the scanner and to focus on crosshairs. Image preprocessing will follow standard steps for resting-state fMRI using AFNI software (NIMH, USA). A series of conventional anatomical scans will be obtained for each subject allowing the subsequent superposition of voxels or regions of interest. Regions of interest in ventral and dorsal striatum will be corroborated by regions activated in the task-based fMRI (see MIDT below).

Arterial Spin Labeling (ASL) for Cerebral Blood Flow (CBF): The CBF pre- and post-L-DOPA or placebo administration will be monitored with a 4.5 minute resting scan sequence using a single readout stand-alone SE CBF sequence acquired 5 ascending slices (TR of 4500 ms, 3.43 × 3.43 × 5 mm resolution with 75% partial Fourier acquisition and a TE of 27 ms).

Diffusion Tensor Imaging (DTI)**:** Chronic exposure to increased inflammation may have long-term consequences on brain circuitry, including degeneration of white matter fiber tracts that are necessary to maintain neural connections.(94) Therefore, DTI will be collected at the initial scan to determine whether decreased structural connectivity in key basal ganglia outflow tracts predict or potentially confound responses to L-DOPA. Diffusion weighted images will be obtained using double spin-echo EPI sequence with settings of FOV = 224×224 mm; resolution=1.8×1.8×1.8 mm; flip angle=78°; parallel imaging with a GRAPPA factor 2; bandwidth=1776 Hz/pixel; number of slices=81; slice thickness=1.75mm isotropic; TR/TE=3430/90 ms; GRAPPA accelerated PE=2; multiband accelerating factor=3; EPI factor=128 with a duration of 8.17 minutes.

Task-based fMRI - Faces Task: This is a task that has been developed to measure brain activity in response to viewing of fearful and neutral faces. 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 10^{th} 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.

Task-based fRMI - Modified Monetary Incentive Delay Task (MIDT): This is a task that has been developed to measure behavioral and brain changes in response to reward and non-reward situations. Prior to entering the scanner, the patient will practice the MIDT on a laptop computer for approximately 15 minutes to ensure they understand task instructions. Reaction times for each practice trial will be recorded; the in-scanner MIDT is adapted to each patient's reaction time to ensure that each patient wins or fails to lose on ~66% of trials. The in-scanner session lasts 12 minutes and proceeds as follows: 1) Patient is shown 3 possible phrases that indicate monetary rewards of win (+$), loss (-$) or no change. 2) Patient is instructed to press a button as quickly as possible. 3) If the patient presses the button within a pre-specified time frame (based on their practice trails), they win or do not lose money. This message appears on the screen. Cumulative winnings (average of ~$2) are also shown after each trial. Patients are paid 1/3 of their cumulative winnings (up to $20), and are instructed of this during the consent process. The task has been designed to minimize patient discomfort and enhance cooperation. If anytime the 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.

Baseline Assessments: During screening, a research blood sample (18 ml total) may be collected along with blood draw for safety labs and/or CRP measurement. 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. For female subjects, questions pertaining to menopausal status will also be obtained using a Staging System for Reproductive Aging questionnaire. All patients will also complete a Composite Vascular Risk Profile Scale. A complete series of neurocognitive testing may also be performed during the last screening visit or the LP visit. The baseline research blood and neurocognitive assessments are necessary to complete prior to randomization to study treatment. They may be collected along with screening assessments that are being conducted to determine eligibility for the LP procedure or randomization to study drug, or at the LP visit. Physiological measures including heart rate and activity may also be recorded using adhesive ECG patches. These data may also be used independently of qualification for further study participation. To reduce patient burden, a 15 minute break will be given in between motivational and motor assessments. Participants will receive $25 for this visit, an additional $25 if research blood is collected, and an additional $25 if ECG patches are placed. Patients will also receive $50 for completing motor and motivational assessments, and up to $60 for completing the EEfRT based upon the subject's responses.

ECG patches for collection of biometric data (optional): Patients will be given the option of wearing ECG patches (MC10 BioStamp®) to record physiological measures including heart rate and activity. Trained members of the study team will place the ECG patches on the chest of the subject and they will record heart rate and activity over 24 hours. Another patch may need to be place on the subject's leg; the subject will be trained to do this themselves. This patches will be returned to the study team either at the next study visit or by a pre-paid envelope that will be provided to the subject.

Lumbar Puncture (LP): Patients will have a LP performed by an Emory Anesthesiologist between 7am-12 pm following hydration with approximately ~1 liter of fluid IV as a result of a KVO (keep vein open) flow of normal saline through the indwelling catheter. This administration of IV fluids has been found in our experience to markedly reduce the incidence of post-LP headache. It will also help standardize hydration levels between subjects. An experienced Emory attending anesthesiologist will perform/supervise the LP using standard sterile technique and local anesthesia with patients in the lateral decubitus position. The subject will be given the option to receive a pre-LP sedation (versed) through IV prior to the LP procedure by the anesthesiologist. Approximately 11 cc of CSF will be withdrawn. After discarding the initial 1 ml (to avoid blood contamination), the remaining CSF will be collected into chilled tubes and aliquoted on ice in 1 ml eppendorf tubes, which will be immediately frozen at −80 C for later batched analyses. Upon completion of the LP, subjects will lie flat for approximately one hour and subsequently discharged after 4 hours. Some patients experience some lower back pain after a lumbar puncture. This is usually felt in and around the area where the needle was inserted. In most cases the pain will ease after a few days and it can be treated with analgesics, such as acetaminophen, if necessary. If subjects develop a headache they will be treated with pain medication or the application of a

blood patch. Subjects will be instructed to have a family member or friend be present to transport them home from the ACTSI Unit following the LP procedure. Patients will receive $500 for participating in the LP procedure.

Study Visit 1 & 2 (L-DOPA or placebo administration): To determine whether increasing dopamine release in the basal ganglia with L-DOPA (250 mg with carbidopa 50 mg, also referred to as Sinemet) can reverse the neurobiological changes associated with increased inflammation observed in resting-state fMRI scans. Participants will complete two, 1 day visits (approximately 5-6 hours in duration) based in the EUH CRN or in the Behavioral Immunology Program offices in the Emory Clinic, Building B, and the Emory Neuroimaging Facilities. An anatomical and functional connectivity MRI scan that will last approximately 25-35 minutes will be performed before administration of L-DOPA or placebo (double-blind, randomized by Emory IDS), which will be administered by the study physician or a designated Nurse Practitioner. Vital signs will be monitored before, during, and after administration. A post-treatment scan, including the Monetary Incentive Delay Task (MIDT), will be performed ~45 minutes following administration of L-DOPA and will last approximately 45-55 minutes. Because protein may reduce absorption of L-DOPA, subjects will be asked to eat a light breakfast and not to consume any food within three hours of their scans (water and juices are allowed) and to limit caffeine intake for 2 hours prior to the scan. A low protein snack may be offered by the study team to the participant prior to administration of the L-DOPA or placebo. Participants may also be offered up to 30mL of Maalox (aluminum hydroxide/magnesium hydroxide/simethicone) after scan 2 to prevent nausea. The MRI scans will be conducted as described above as one continuous acquisition (see T1 anatomical, resting-state fMRI, MIDT and ASL protocols). Patients may also undergo a clinical interview if it has been >2 weeks since their last psychiatric assessment, and will be asked to complete self-report questionnaires related to their mood and will be administered objective tests of motivation and psychomotor speed. A blood sample (6 ml total) will be collected into EDTA tubes immediately after the final scan to measure pharmacokinetic parameters of L-DOPA. Participants will be discharged by the CRN staff or the study physician after assessment of vital signs and neurological status including coordination and sedation. Patients can receive up to $280 for completion of each study visit including an extra $20 in winnings from the MIDT and a maximum of $60 for completing the EEfRT.

6. Participant selection

Two hundred twenty-five depressed, medically stable males and females between the ages of 18 and 65 will be recruited for this study. All subjects (50% males and 50% females) will meet criteria for current major depression (MD). An equal number of participants will have CRP values from each of the three risk groups, as defined by CDC/AHA guidelines: <1mg/L (low), 1-3 mg/L (moderate), and >3mg/L (high). Based on retention rates in previous studies by our group utilizing a similar design in depressed subjects, we anticipate that recruiting 225 subjects will provide analyzable data for approximately 80 subjects. There will be no exclusions for race/ethnicity and minority populations and women will be actively recruited to ensure that 50% of the subjects will be female. We will make active attempts to enroll minority patients in a proportion that is reflective of the greater Atlanta metropolitan area 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. Patients will be obtained from multiple referral resources including the Emory Clinic, Grady Health System, the Veterans Administration Medical Center (VAMC), and community psychiatrists. In addition, subjects will be obtained from ongoing clinical trials including the Mental Health Centers for Intervention Development and Applied Research (CIDAR) trial, which is scheduled to enroll 400 subjects over 5 years. Depressed subjects will also be obtained from newspaper and radio advertisements as well as internet solicitation. Subjects in this study may also be referred from our Behavioral Immunology Program "Recruitment Clinic" protocol, Clinical Research in Psychiatry: IRB0000075162, which uses the screening strategy similar to this protocol to recruit and screen patients with depression for our active studies through IRB approved social media campaigns. Subjects who are determined to be eligible for this study will be referred to study staff. Participants who have consented to any Emory Behavioral Immunology Program studies will be given an option to consent to have any screening and study data used in future studies, including this study. If the participant consents to this option, any previously completed screening or study assessments may be used

in the current study to help reduce patient burden. Some labs or evaluations may be repeated at the discretion of the PI or PI's designee, if more current data is needed. We anticipate that at least 2 subjects per month (~24 subjects per year) will consent to enter this study. This estimate is based on ongoing projects that have been recruiting subjects for related studies over the past 5 years. Of subjects who meet entry criteria, our past experience suggests that approximately 20% will discontinue study participation.

Patients with MD enrolled in the study will meet DSM-V-TR criteria for a current major depressive episode without psychosis with a symptom severity score of ≥18 on the Hamilton Depression Rating Scale (HDRS) -17 item scale. To be included, subjects must not demonstrate active suicidal intent or plan and must score ≤2 on the HDRS or QIDS suicide item, and must have no suicide attempt within six months of screening. Enrolled subjects will have no history of the following: schizophrenia, schizoaffective disorder, other (non-mood disorder) psychosis, depression secondary to a medical condition, mental retardation, and substance dependence [or abuse within the past year (except nicotine)], dementia or MMSE <28, unless otherwise approved by the PI or PI's designee, or delirium. Subjects will be at risk for exclusion for a urine toxicology screen positive for ETOH or drugs of abuse. The presence of post-traumatic stress disorder, panic disorder, obsessive compulsive disorder or social phobia will not disqualify subjects from enrollment as long as depression is the primary diagnosis, though post-traumatic stress disorder may be considered as a covariate in statistical analyses. Enrolled subjects may have a comorbid personality disorder; however, subjects who meet criteria in clinical interview for antisocial personality disorder will be disqualified, as will subjects with a history of hospitalization and/or recurrent suicidal behavior judged to be directly due to the personality disorder.

Potential subjects may be excluded for a number of medical conditions that might confound relationships between depression and inflammation, or contraindicate MRI scanning or administration of L-DOPA, including uncontrolled cardiovascular disease, autoimmune condition (i.e. rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, lupus), current or history of migraines or headaches with frequency >6 headaches per month, glaucoma, melanoma, or bleeding disorder of any kind, embedded metallic objects, prosthetics made of paramagnetic metals, aneurysmal clips, a history of claustrophobia, chronic infection (i.e. HIV, hepatitis B or C, herpes), abnormal lab results deemed by study physicians as contraindicated for study participation, or pregnancy. Similarly, patients may be excluded for evidence of medical or neurological abnormality on physical examination. Subjects who develop signs of an infection between screening and commencing the protocol assessments will be rescheduled when symptoms have resolved, if agreeable to the subjects.

7. Statistical Analysis

Power Analysis: To ensure a range of inflammation from low to high, we will analyze an equal number of participants from low to high inflammation levels: approximately 27 patients with CRP <1mg/L, 27 patients with CRP 1-3mg/L, and 27 patients with CRP >3ml/L. We recently completed a study using a similar enrollment strategy and analyzed data from ~80 medication-free subjects over a similar time frame. Based on our preliminary data, we anticipate large effect sizes (Cohen's dz ~1) for L-DOPA-induced increases in connectivity in patients with high inflammation; thus with n=30 per group we will have >80% power at α=0.05.

Interim Monitoring and Early Stopping:

Subjects will receive a physical examination and laboratory testing for safety as well as tests for pregnancy (if female) and substance abuse at screening.

Subjects will be at risk for study discontinuation with the following events:
- Substance abuse
- Protocol violation

The following events are considered sufficient reasons for discontinuing a subject from the study:
- An adverse event (AE) that, in the judgment of the investigator or study physicians, may cause severe or permanent harm
- Subject withdraws consent
- Subject lost to follow-up
- Pregnancy

• Death

Analysis Plan and Statistical Methods:
Descriptive statistics will be used to describe the central tendencies and variances of dependent variables and demographic and clinical characteristics of the patients. Due to the pilot nature of this project, we will use both targeted and exploratory data analysis methods to determine whether L-DOPA increases corticostriatal connectivity in depressed patient with high versus low inflammation. Statistical modeling will be conducted as described below. Log-transformation will be used for non-normally distributed data. Statistics will be two-tailed with alpha <0.05, and conducted in SAS and SPSS.

Analytic Strategy, Objective 1: The primary outcome will consist of a targeted, hypothesis-driven approach including extraction of individual patient level Z scores for connectivity with ventral or dorsal striatum and regions of interest identified in our previous work that 1) exhibited alterations in connectivity between patients with high and low inflammation, and 2) were significantly associated with behaviors of interest. Post-L-DOPA or placebo administration Z scores will be entered into linear models controlling for pre-administration connectivity scores as well as relevant covariates. The difference (delta; post minus pre) in connectivity Z scores before and after placebo and L-DOPA will also be compared by paired T test, and between patients with high and low inflammation (CRP > or < 3mg/L). Generalized linear mixed model (GLMM) will be used to assess whether corticostriatal connectivity is improved by L-DOPA, and whether effects depend on concentrations of CRP, while controlling for clinical and demographic covariates. Linear regression models will be used to determine whether increased CRP is associated with decreased response to hedonic reward, as assed at by fMRI using the MIDT. Response to hedonic reward (as measured by MIDT) will also be used to assist in interpretation of resting-state connectivity data before and after L-DOPA administration in two ways: 1) Striatal regions activated in response to reward will be used to corroborate location of the striatal seed regions for resting-state connectivity analysis, and 2) responses to the MIDT will be entered into GLMM models to determine whether decreased reward sensitivity predicts greater increase in connectivity after L-DOPA. White matter integrity as measured by DTI will also be used to assist in interpretation of responses to L-DOPA, as subjects with less cortical-striatal-thalamic anatomical connections may have less ability to respond to increases in dopamine. To identify other corticostriatal circuits that may be affected by increasing dopamine precursor, striatal seed-to-whole-brain comparisons of L-DOPA versus placebo, will be performed as a secondary analysis strategy. In the event that CRP fails as a classification or correlational variable, exploratory analyses will be conducted using other measures of inflammation described above for Objective 1.

Analytic Strategy, Objective 2: Similar to the analytic strategy for Objective 1, post-L-DOPA or placebo administration neurocognitive performance or self-report scores will be entered into linear models with CRP (or other inflammatory markers) while controlling for pre-administration behavioral scores as well as relevant covariates. The difference (delta; post minus pre) in behavioral scores before and after placebo and L-DOPA will also be compared by paired T test, and between patients with high and low inflammation (CRP > or < 3mg/L). L-DOPA-related changes in functional connectivity in Objective 1 will then be compared to relevant post-LDOPA task-based and self-report behavioral responses using linear modeling.

Analytic Strategy, Objective 3: Relationships between functional connectivity and behavior before and after L-DOPA with central and peripheral inflammatory cytokines and their soluble receptors, and biomarkers of their potential effects on dopamine through decrease BH4 activity will be explored. Gene transcripts associated with inflammatory and metabolic signaling pathways related to inflammation effects on the brain have been used to predict response to pharmacological therapies (54, 95), including our previous work (96, 97). Therefore, genome-wide gene expression will be assayed. Plasma and CSF will be collected on a separate visit to measure IL-6, IL-1β, TNF and their soluble receptors, CSF BH4, dopamine, HVA and DOPAC, and plasma phenylalanine and tyrosine. Linear regression will be used to examine relationships between functional connectivity Z-scores or behavior (dependent variables) and biomarkers of inflammation and dopamine synthesis (independent variables), while controlling for covariates. Cytokines and their receptors will be examined first independently (with and without correction for multiple comparisons), then together in models to identify biomarker(s) that are most significant after backward and forward linear regression. If variables are collinear (as assessed by variance inflammation factor, tolerance and leverage plots), principal component analysis (PCA) will be used.(98) Gene expression that correlates with CRP, or differs between patients with

low versus moderate versus high CRP (<1, 1-3, and >3 mg/L) will be determined (5% FDR, $p<0.05$). Gene lists will be analyzed for over representation of functional pathways and transcription factor binding regions per our previous work, (96, 97, 99) and correlated with connectivity Z-scores.

Relevant Demographic and Illness-Related Variables to be Included as Covariates in the Statistical Analyses:
We will gather information on the number of previous episodes of depression, number of hospitalizations, length of current episode of depression (months), medication/psychotherapy treatment history (number of previous trials in current depressive episode), family history of MDD (yes or no), history of substance abuse (yes or no), use of tobacco (cigarettes/day) and alcohol (drinks/day), marital status, exercise (days/week, intensity, type, reason) and education (highest grade achieved). These variables may be used as covariates in the regression analyses as indicated above.

8. Adverse Event Reporting

Enrolled participants will be monitored closely by study clinicians for any adverse events. If any overt study-related adverse events occur, a decision will be made about study continuation. Additionally, a record of adverse events for study participants will be reported to the DSMB on a regular basis (see below). Subjects will be closely monitored during the course of the study for development of any serious or unexpected adverse reactions. Those events meeting Emory IRB criteria for a reportable event will be reported to the IRB or DSMB according to standard regulations and procedures. The Emory 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.”

9. Data and Safety Monitoring Plan (DSMP)

Although no high risk procedures are being conducted as part of this study, we will be following patients with untreated major depression for a period of up to 4 weeks between the signing of consent and the final study assessment. For this reason, we have elected to utilize the Data Safety Monitoring Board of the Department of Psychiatry and Behavioral Sciences as a third-party oversight committee. The DSMB is described in detail below. In addition, study clinicians will be available by pager 24 hrs/7 days a week during the period between screening and completion of study assessments. Should a subject’s depressive symptoms appreciably worsen or should active suicidal ideation develop, either Dr. Miller or Haroon will be immediately notified. Drs. Haroon and Miller are board certified psychiatrists with extensive experience in the treatment of psychiatric emergencies. If the assessment in question was done by a clinician other than either of them, one of them will immediately contact the subject and will evaluate the need for further psychiatric treatment and will arrange psychiatric follow-up. They will evaluate each case individually to make a determination regarding whether the subject can remain in the study or whether the subject should be terminated in addition to receiving a mental health referral.

Composition of the Data Safety Monitoring Board (DSMB)

Frequency of DSMB review for this protocol will follow recommendations from the IRB based on the assessed risk status of the study. The DSMB for this study will consist of the Clinical Research Oversight Committee with members including Boadie Dunlop, M.D. Chairman, Larry Tune M.D. and Bobbi Woolwine, M.S.W. They have agreed to serve as the external DSMB for investigator initiated clinical trials conducted by Emory researchers in the Department of Psychiatry & Behavioral Sciences. However, Bobbi Woolwine will be recused from the DSMB review of this study. If the DSMB requires additional specialized expertise to evaluate safety issues related to the performance of this study, a relevant specialist will be consulted by the DSMB.

Procedures and Responsibilities of the DSMB

The DSMB will meet quarterly. This protocol will be submitted to the DSMB simultaneously with the initial submission to the Emory IRB. The DSMB will review the research protocol and plans for data and safety monitoring. Once per year (or after 6 months if the protocol is considered 'high risk' by the IRB), the DSMB will

review a report from the study's data manager that includes: 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, an up-to-date consent form, and measures taken to protect confidentiality (e.g., data and tape storage, use of coded ID numbers, etc.). The DSMB will also review the Principal Investigator's summary of any new data or evidence that might alter the risk/benefit ratio for participating in the study (e.g., newly published studies, etc.). After reviewing this information, the DSMB will issue its own report summarizing any 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.

There will be regular, ongoing communication between the PI, Emory's IRB, and the DSMB. The PI will take responsibility for submitting reportable serious and unexpected adverse events or other unanticipated study problems to Emory's IRB according to standard regulations. A copy will be sent to the DSMB. Actions taken by the IRB in response to adverse event reports will be immediately reported to the DSMB.

10. Pharmaceutical Information

Carbidopa-levodopa (Sinemet) is a combination of two drugs, L-DOPA and carbidopa. Carbidopa-levodopa is used in the treatment of Parkinson's disease. 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 improve nerve conduction and assist the movement disorders in Parkinson disease. 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. This combination medicine was approved by the FDA in 1988. Carbidopa-levodopa is taken several times per day for treatment of Parkinson's disease, ~3-8 tablets daily. Patients in this study will receive one tablet of a standard dose of 250 mg L-DOPA with 50 mg carbidopa. Preliminary data using two doses of L-DOPA (100 and 250 mg) indicated that 250 mg was superior in increasing corticostriatal connectivity as measured by fRMI (the primary study outcome) without an increase in adverse events. Before, during and after L-DOPA administration, vital signs will be monitored by the Study Physician or designated Nurse Practitioner.

The following rare but serious side effects have 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 increase eye blinking/twitching, cause fainting, mental/mood changes, and worsening of involuntary movements/spasms.

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