Protocol

Title: Effects of Bupropion versus Escitalopram on Reward Circuitry and Motivational Deficits in Patients with Major Depression and Increased Inflammation and Anhedonia

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1. Title: Effects of Bupropion versus Escitalopram on Reward Circuitry and Motivational Deficits in Patients with Major Depression and Increased Inflammation and Anhedonia

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2. Précis/Abstract:
The overall objective of the proposed research is to determine the mechanism of action of an antidepressant of known efficacy and tie this mechanism of action to a specific biomarker, ultimately supporting precision medicine in the treatment of major depression (MD). More specifically, the proposed research is designed to determine whether bupropion (vs escitalopram) increases functional connectivity (FC) within reward-related neurocircuits and decreases motivational deficits in depressed patients with increased inflammation and anhedonia. Work by our group and others has demonstrated that administration of inflammatory stimuli to humans is associated with significant decreases in neural activity in the ventral striatum in association with symptoms of anhedonia. In studies in laboratory animals and humans, these effects of inflammation appear to be secondary to decreased dopamine (DA) neurotransmission. Data also indicate that MD patients with higher inflammation as indexed by C-reactive protein (CRP) exhibit lower FC between the DA-rich ventral striatum and ventromedial prefrontal cortex in association with motivational deficits and anhedonia that can be reversed by a DAergic drug (L-DOPA). Further relevant to the proposed research, in a recent clinical trial, depressed patients with increased inflammation (CRP≥1mg/L) showed a poor response to the selective serotonin reuptake inhibitor (SSRI) escitalopram alone, while treatment response was markedly increased in escitalopram-treated patients with the addition of bupropion. Bupropion is a DA reuptake inhibitor that exhibits ~25% occupancy of the DA transporter at therapeutic doses and increases DA neurotransmission in animal models. Taken together, these data suggest that inflammation affects DAergic pathways to disrupt reward-related circuits in depressed patients, leading to motivational deficits. Moreover, in the context of inflammation, drugs that target DA may be more efficacious than SSRIs. Thus, the current study proposes to use a mechanistic clinical trial design with drugs of known efficacy to take the first step toward establishing whether antidepressants that target DA (e.g. bupropion) might be a better choice for depressed patients with increased inflammation and anhedonia than an SSRI. Accordingly, 50 depressed patients with a CRP>2mg/L and increased anhedonia will be randomized to 8 weeks of bupropion or escitalopram in order to analyze data from 40 patients (accounting for drop outs). All depressed patients will undergo functional magnetic resonance imaging (fMRI) to examine FC in reward-related circuits at baseline, 4 and 8 weeks along with objective and clinical assessments of RDoC positive (motivational) valence constructs at baseline and 2, 4, 6 and 8 weeks. We hypothesize that patients who receive bupropion versus escitalopram will exhibit increased FC between ventral striatum and ventromedial prefrontal cortex in association with decreased motivational deficits and anhedonia. This pilot study will provide foundational data for design of larger mechanistic clinical trials that will establish the mechanism of action of conventional antidepressants and determine whether biomarkers of inflammation can be used to facilitate antidepressant choice, increase antidepressant efficacy and ultimately personalize antidepressant treatment.

3. Introduction and Background:
<u>Inflammatory Biomarkers May Reveal Differential Responsiveness to Conventional Antidepressants</u>
The goal of the proposed research is to determine the mechanism of action of an antidepressant of known efficacy (bupropion) and to tie this mechanism of action to a biomarker of inflammation in support of precision medicine for the treatment of major depression (MD). MD is a devastating disease affecting ~10% of US adults and being the leading cause of disability worldwide.[1, 2] Despite availability of several classes of antidepressant medications, initial treatment response is low (~30%), and ~1/3 of depressed patients are non-responsive to conventional antidepressant therapies.[3-5] Although extensive reviews of the literature suggest that available antidepressant medications are equally effective,[6, 7] recent studies suggest that there may be differential responsiveness to conventional antidepressants among subgroups of depressed patients.[8, 9]

One subgroup of depressed patients who may exhibit differential antidepressant responsiveness are those with increased markers of inflammation. A meta-analysis on inflammation and treatment response suggests that patients with increased inflammation respond poorly to conventional antidepressants.[10] Nevertheless, as part of this literature, two large community-based trials of over 300 subjects found that patients with increased inflammation as indexed by a C-reactive protein (CRP) ≥1mg/L responded significantly better to the norepinephrine (NE) and dopamine (DA) reuptake inhibitors, nortriptyline and bupropion than the selective serotonin reuptake inhibitor (SSRI) escitalopram, which showed better efficacy in patients with low inflammation.[8, 9] Indeed, in one of these studies, the SSRI escitalopram led to 57% remission in depressed patients with low inflammation (CRP <1mg/L), whereas in patients with higher inflammation (CRP≥1mg/L), the remission rate with escitalopram alone was below 30%.[8] Addition of bupropion to escitalopram in patients with high inflammation increased the remission rate to 51%.[8] These data support the notion that differential responsiveness to conventional antidepressants exists and may be revealed by pretreatment levels of inflammation as indexed by the inflammatory biomarker CRP. However, all studies examining the impact of inflammation on antidepressant treatment response have been post hoc in nature, and no study has prospectively examined whether a priori subgrouping of depressed patients by inflammatory status predicts response to conventional antidepressants. In addition, the mechanism by which bupropion or nortriptyline vs an SSRI may increase antidepressant responsiveness in patients with increased inflammation is unknown. This proposal is designed to address both of these gaps in the literature.

<u>Differential Antidepressant Responsiveness May Relate to the Impact of Inflammation on Monoamine Transporter Activity and Dopamine and Dopamine-Related Reward Circuitry</u>

Inflammatory cytokines including interleukin (IL)-1beta and tumor necrosis factor (TNF) increase expression and function of the serotonin transporter (SERT) through activation of p38 mitogen activated protein kinase (MAPK) pathways.[11, 12] For example, administration of the inflammatory stimulus lipopolysaccharide (LPS) to wild-type mice increased reuptake and clearance of serotonin while inducing depressive-like behavior; effects that were completely reversed by a p38 MAPK inhibitor or the use of IL-1 receptor deficient mice.[12] Interestingly, similar effects of IL-1, TNF and LPS were found on the NE transporter (NET), but not the DA transporter (DAT). These data suggest that inflammation may sabotage SSRIs (and possibly SNRIs) by increasing the activity and function of the very targets of these drugs (the transporters). Thus, the impact of inflammation on SERT and NET suggest that drugs that inhibit DAT may be more effective in states of high inflammation.

In addition to the effects of inflammation on the SERT and NET, data support that inflammation may affect behavior in part through decreasing DA availability and disrupting DA-mediated reward pathways. Chronic administration of IFN-alpha is associated with blunted responses in the DA-rich ventral striatum (VS) to reward anticipation using functional magnetic resonance imaging (fMRI).[13] In addition, IFN-alpha treatment of patients with hepatitis C decreases DA release within the striatum as measured by [18F]Dopa binding using PET.[13] Other inflammatory stimuli including endotoxin and typhoid vaccination administered to healthy volunteers also decrease neural activity in the VS in response to rewarding stimuli using fMRI.[14-16] Consistent with these neuroimaging findings, studies using in vivo microdialysis in VS have demonstrated that chronic administration of IFN-alpha to non-human primates is associated with significant decreases in extracellular DA both at baseline and following stimulation with potassium or amphetamine. It should be noted that subchronic administration of IFN-alpha (2 weeks) and acute administration of LPS can increase DA release, possibly related to the timing of inflammatory exposure.[17-19] Interestingly, chronic effects of IFN-alpha on extracellular DA release were reversed by the DA precursor L-DOPA administered via reverse microdialysis.[20] Decreases in extracellular DA were also found in rodents administered interleukin (IL)-6 and sampling from the nucleus accumbens.[21] Consistent with the effects of inflammation on striatal neural activity and DA and their role in motivated behavior, inflammation also produces concomitant effects on behaviors relevant to motivation including effort expenditure for reward. For example, using a concurrent choice paradigm in rodents, acute administration of IL-1beta or IL-6 was associated with a shift away from preferred foods that required effort in favor of less desirable food that was freely available.[21, 22] Similar inhibitory effects of inflammatory stimuli on effortful behavior occur in rodents,[23] non-human primates,[17] and humans.[24]

Relative to patients with MD, previous studies by our group have shown that higher endogenous inflammation indexed by CRP in medically stable MD patients is associated with lower functional connectivity (FC) between ventral striatum (VS) and ventromedial prefrontal cortex (vmPFC) in

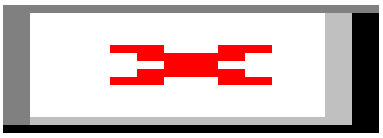
association with anhedonia and motivational deficits including decreased effort expenditure for reward (see preliminary data).[25] These studies indicate that increased chronic endogenous inflammation in patients with MD leads to similar changes in reward circuitry and motivation as chronic exogenously administered inflammatory stimuli.

Bupropion is a Viable Candidate Antidepressant to Test in MD Patients with Increased Inflammation

There are several reasons that bupropion might have increased efficacy in MD patients with increased inflammation.[26] First, as noted above, bupropion in combination with escitalopram was shown to rescue treatment remission in patients with CRP ≥1mg/L, increasing treatment remission from less than 30% to over 50%. Furthermore, in human studies, bupropion (300mg/d) occupies the DAT by as much as 25% using displacement of radiolabeled DAT ligands.[27, 28] One study reported 14% DAT occupancy with bupropion, although several confounds of the study design including a lower dosing regimen make interpretation of these data difficult.[29] Of note, in non-human primates, an acute IV bolus of 5mg/kg of bupropion led to ~85% DAT occupancy in striatum.[30] In addition, several studies in rodents demonstrated that bupropion increases extracellular DA in striatum and nucleus accumbens in a dose and time-dependent manner.[31, 32] Moreover, intraperitoneal administration of bupropion increased effort-based motivation for food rewards in association with pre- and post-synaptic markers of increased DA transmission.[33] Finally, bupropion (but not fluoxetine or desipramine) reversed the inhibitory effects on effort-based motivation by tetrabenazine, a drug that depletes accumbens DA.[34] Taken together, these data support the choice of bupropion as a reasonable candidate to increase FC within DA-related reward circuitry and increase motivation in depressed patients with increased inflammation and anhedonia. It should be noted that while nortriptyline also exhibited efficacy in MD patients with high inflammation, it is unclear whether these effects were related to reported effects of NET inhibition on increasing DA release in frontal cortex.[35] Interestingly, in a systematic review of conventional antidepressants for smoking cessation, only bupropion and nortriptyline exhibited significant efficacy, suggesting some overlap on their effects on reward-related pathways.[36] Nevertheless, given the strong evidence of the impact of inflammation on DA neurotransmission, we chose to proceed with a medication that had more clear-cut effects on DA (bupropion), as opposed to a drug that is a norepinephrine reuptake inhibitor. Of note, there are other drugs that have more selective and potentially more potent effects on DA than bupropion (obviating effects of bupropion on NE reuptake and the related inhibitory impact of NE on microglial activation and proinflammatory cytokine production[37]). Nevertheless, bupropion is an FDA-approved drug of known efficacy in the treatment of depression (a requirement of the funding mechanism to which this proposal is directed – see Introduction).

Working Model and Hypothesis

Bupropion vs escitalopram will increase FC between DA-rich reward regions in the VS and cortical reward regions in the vmPFC and improve associated RDoC positive valence constructs including effort-based motivational deficits in MD patients with increased inflammation and anhedonia.



4. Objectives: (Primary and secondary aims)

The goal of the proposed research is to establish the mechanism of action of an antidepressant medication with known efficacy and to tie this mechanism to a biomarker of inflammation. Specifically, we plan to determine whether bupropion (vs escitalopram) can increase functional connectivity (FC) within reward-related circuitry and improve motivational deficits in patients with major depression (MD) and both increased inflammation and anhedonia. Recent studies suggest that increased inflammation [as indexed by the inflammatory biomarker C-reactive protein (CRP)] is associated with a poor response to serotonin reuptake inhibitors including escitalopram. However, in a recent study, the treatment response to escitalopram in patients with increased CRP (≥1mg/L) was almost doubled with the addition of bupropion. Bupropion is a DA reuptake inhibitor that exhibits ~25% occupancy of the DA transporter at therapeutic doses and increases DA neurotransmission in animal models. These data suggest that the subgroup of

depressed patients with increased inflammation may have unique responsiveness to drugs that target DA. Indeed, preclinical and clinical studies demonstrate that chronic administration of inflammatory stimuli leads to decreased DA availability in ventral striatum (VS) in association with decreased VS activation to rewarding stimuli. In addition, higher inflammation as reflected by CRP is directly linked to lower FC between the DA-rich VS and the ventromedial prefrontal cortex (vmPFC) in MD patients using resting state fMRI. These inflammation-related decreases in VS-vmPFC FC in depressed patients in turn were associated with motivational deficits consistent with an impact of inflammation on RDoC positive valence constructs. Our preliminary data also indicate that administration of the DA-ergic drug L-DOPA increases VS-vmPFC FC (and effort-based motivation) in MD patients with high (CRP>2mg/L) but not low (CRP≤2mg/L) inflammation, using a longitudinal pre- post- neuroimaging design. These data suggest that this circuit and the related behaviors may be especially responsive to DA'ergic medications. Thus, the relationship between CRP and resting state FC within classic reward circuitry in MD patients provides a unique opportunity whereby a functional target in the brain can be used to establish whether there exists differential responsiveness to currently available antidepressants that can be revealed by an inflammatory biomarker (CRP) that is regularly used in clinical practice. Accordingly, this proposal will employ a match/mismatch design, wherein patients with MD and increased inflammation (CRP>2mg/L) and anhedonia will be randomized to bupropion (match) or escitalopram (mismatch). A cut-off of CRP>2mg/L will be used because it predicted increased FC in response to L-DOPA and serves as an excellent translational proxy for a composite of inflammatory mediators in periphery and brain. CRP>2mg/L has also been used in the cardiovascular literature as a threshold for treatment of inflammation. Increased anhedonia will be defined as a score of 2 or greater on item 1 of the PHQ-9. . To examine FC in reward-related circuitry, fMRI will be conducted at baseline and after 4 and 8 weeks of treatment. We hypothesize that bupropion (vs escitalopram) will increase FC within reward circuitry in association with improved objective and clinical measures of motivation in patients with high inflammation and anhedonia at baseline. To test this hypothesis, these aims are proposed:

Specific Aim 1. To determine whether bupropion (vs escitalopram) will increase functional connectivity in reward circuitry in MD patients with high inflammation and anhedonia. Fifty patients (n=50) with a CRP >2mg/L and an anhedonia score ≥ 2 on item #1 of the PHQ-9 will be stratified by sex and randomized to 8 weeks of bupropion XL up to 300mg/d or escitalopram up to 20 mg/d (as tolerated). FC between VS-vmPFC will be examined at baseline and after 4 and 8 weeks using resting state and task-based fMRI. Hypothesis 1: Bupropion vs escitalopram will increase VS-vmPFC FC in MD patients with high CRP and anhedonia.

Specific Aim 2. To determine whether bupropion (vs escitalopram) will improve motivational deficits in MD patients with high inflammation and anhedonia. Objective and clinical measures of RDoC positive valence (motivational) constructs will be assessed in the subjects in Aim 1 at baseline and after 2, 4, 6 and 8 weeks of treatment with bupropion or escitalopram by the Effort-Expenditure for Rewards Task (EEfRT) and the clinician-administered Snaith Hamilton Pleasure Scale and self-report Mood and Pleasure Scale. Hypothesis 2a: Bupropion vs escitalopram will increase the probability of making high effort choices on the EEfRT and improve scores on clinical and self-reported assessments of motivation. Hypothesis 2b: Improvement in VS-vmPFC connectivity will correlate with increased high effort choices on the EEfRT and improved scores on clinical and self-reported assessments of motivation.

Specific Aim 3 (Exploratory). To determine whether bupropion (vs escitalopram) will improve overall depressive symptoms in MD patients with high inflammation and anhedonia. Depressive symptom severity will be assessed by the Hamilton Depression Rating Scale-17 (HAM-D-17) at baseline and after weeks 2, 4, 6 and 8 of treatment in subjects noted in Aim 1. Hypothesis 3: Bupropion vs escitalopram will improve depression severity in MD patients with high CRP and anhedonia at baseline.

In sum, by demonstrating the ability of bupropion vs escitalopram to increase FC in reward circuitry and reduce motivational deficits in MD patients with increased inflammation and anhedonia, this pilot study will not only help establish a mechanism of action for bupropion but will also help provide an important foundation for preferentially using bupropion (and other medications that target DA) in patients with increased inflammatory biomarkers such as CRP and anhedonia, thus supporting precision medicine using available antidepressants.

5. Study design and methods:

Study Population: Fifty male and female subjects ages 25-55 will be randomized to obtain 40 with complete data (20% projected attrition). Subjects will be recruited from a community sample of MD patients through social media.

Participant Selection

Inclusion Criteria: a. willing and able to give written or virtual informed consent; b. men or women, 25-55 years of age or at PI's discretion; c. a primary diagnosis of DSM-V MD or Bipolar II current episode, depressed, as diagnosed by the SCID- *Structured Clinical Interview for DSM-V Axis I Disorders (SCID-V)* V; d. score of >10 on the PHQ-9 and a score of 2 or greater on item #1 of the PHQ-9; e. off all antidepressant or other psychotropic therapy (e.g. mood stabilizers, antipsychotics, and sedative hypnotics) for at least 4 weeks prior to baseline visit (8 weeks for fluoxetine) Concomitant administration of up to 2 mg of clonazepam or its equivalent per day will be allowed, but not within 12 hours of study assessments f. CRP>2mg/L, and g. IDS-SR anhedonia subscale (Items 8,19,21) score ≥5.

Exclusion Criteria: a. current autoimmune disorder; b. history 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 and laboratory testing); e. history of any (non-mood-related) psychotic disorder; active psychotic symptoms of any type; history of Bipolar I Disorder; substance abuse/dependence within 6 months of study entry (as determined by SCID) or any 2 year period of continued substance abuse; f. an active eating disorder or antisocial personality disorder; g. a history of a cognitive disorder unless otherwise approved by the PI; h. pregnancy or lactation; i. chronic use of non-steroidal anti-inflammatory agents (NSAIDS) (excluding 81mg of aspirin), glucocorticoid containing medications; j. use of NSAIDS or oral glucocorticoids at any time during the study; k. any contraindication for MRI scanning; l. failure of more than 2 antidepressant trials in the current episode; m. Intolerance of bupropion or escitalopram and n. BMI >40 (to exclude severe obesity). Due to the high co-morbidity between anxiety disorders and depression, we plan to include patients with anxiety-related disorders excluding OCD if depression is the primary diagnosis. Patients with stable medical conditions and on medications for those conditions will not be excluded. Concomitant administration of up to 2 mg of clonazepam or its equivalent per day will be allowed, but not within 12 hours of study assessments. m. sexually active participants will be asked not to become pregnant during study participation. Pregnancy tests will be conducted at screening, baseline,and treatment weeks 4 and 8.

Description of Study Procedures

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.

Screening: Screening will include: (1) Past psychiatric history and current symptom severity by clinical interview and using a modified Structured Clinical Interview for DSM-V (SCID-V),[38] and the PHQ-9 [39] (2) Psychiatric treatment history in current episode using the MGH Antidepressant Treatment Response Questionnaire (ATRQ),[40] (3) Medical history from patient interview and review of medical records, (4) Complete medical and neurological examination (5) Screening laboratory evaluation, (6) Vital signs, height and weight and (7) hsCRP (obtained twice over a 2-week period at the P.I's discretion as per AHA/CDC guidelines to establish stability, and along with physical examination and laboratory testing to rule out acute inflammation or infection (which may influence DA neurotransmission). The PI may request to perform a rapid CRP test at screening using the Diazyme hsCRP POC Test Kit. This kit produces 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 may be repeated at PI's or PI designee's discretion. Additional assessments will be conducted if more than 4 weeks from screening, unless otherwise approved by the PI, study physician or PI's designee.

Baseline and Study Procedures: After screening, subjects will undergo fMRI and behavioral assessments at baseline followed by stratification by sex and double-blind randomization to either bupropion XL 150mg/d or escitalopram 10mg/d as indicated in Table 1. The randomization scheme will be implemented by research pharmacy, and all patients and study staff will be blinded to treatment assignment. Patients will be randomized in blocks of 4. After 2 weeks, subjects with a HDRS-17_>7_will be increased to bupropion XL 300mg/d or escitalopram 20mg/d as tolerated. fMRI scanning and behavioral assessments will be repeated at 4 and 8 weeks. Vital signs, safety labs, urine toxicology and adverse events will be collected as indicated in Table 1. The primary outcome (Aim 1) will be targeted VS-vmPFC FC as detailed below. Secondary outcomes (Aim 2) will include RDoC positive valence constructs including objective assessments of effort-based decision making and clinician-administered and self-report assessments of motivation/anhedonia (see below). Additional (exploratory) outcomes will include treatment response over time and adverse events.

Table 1. Schedule of Study Procedures and Assessments

	Virtual	Visit 1	Visit 2		Visit 3	Visit 4	Visit 5	Virtual	Visit 6
Assessments	**Intake**	**Screen A**	**Screen B**	**Base-line A**	**Base-line B**	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**
Consent	X								
PHQ-9, Medical / Psychiatric Screening* **	X	[X]	[X]						
FERN MRI Screening Form* **	[X]	X					X		X
ATRQ, Medical History, SCID V-modified (Mood, Anxiety, PTSD modules), Bipolarity index	X								
Laboratory Eval*, Urinalysis, height weight* **		X							
Rapid CRP (Finger Stick)* **		X	X						
Physical Exam			X						
Vital Signs		X	X		X	X	X		X
Adverse events, Concomitant Meds*** **	X	X	X		X	X	X	X	X
Urine toxicology screen, pregnancy test* **		X			X		X		X
Research bloods†				X			X		X
Neurocognitive Battery				X		X	X		X
EEfRT (computer-based)				X	X	X	X		X
Demographics, habits, CTQ				X					
MAP, IDS-SR, STAI, BAI, PCL-5, PSS				X		X	X	X	X
fMRI					X		X		X
SHAPS-C, CSSRS, CGI					X	X	X	X	X
HAM-A [Baseline only]**, HAM-D-17**					X	X	X		X
Randomization to bupropion / escitalopram					X				

Medication dispensing /compliance check§					**X**	**X**	**X**	**X**	**X**
Adjustment of medication#						**X**	**X**	**X**	
Discharge/discharge planning							**X**	**X**	**X**

***** Laboratory evaluation will include the comprehensive metabolic panel, thyroid-stimulating hormone (TSH), CBC with diff, and plasma CRP.
****** Adverse events will be recorded at each visit after study enrollment (Baseline A until Week 8).

§ Compliance check will be conducted starting at Week 2 until Week 8.
# Medication dosage will be adjusted upward to 2 pills at Week 2 or Week 4 as needed based on tolerability.
[]To be completed if needed
***These assessments may be conducted at the Emory Behavioral Immunology program research screening clinic
****HAM-A instrument removed from Baseline visit on 7/5/22.

Criteria for Study Discontinuation ("Stopping Rules"):
Individual:
To reduce the risks associated with active treatment, all subjects will be monitored carefully for the development of adverse events as well as worsening of their condition. Between signing consent and completing the screening assessment, a period of no more than 2 weeks will pass until baseline. Subjects will be closely evaluated throughout the study both virtually and during study visits for worsening of psychiatric symptoms. Moreover, all patients are provided 24/7 access to study personnel in the event of an emergency. 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 CSSRS,[41] 2) the development of psychotic or catatonic symptoms, 3) a 25% increase from baseline of HAM-D-17 score, 4) missing more than 2 consecutive doses of medication more than once 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.
Study:
1) If more than two individuals experience a reportable serious adverse event related to study procedures within a six-month period, the study will be stopped and reviewed by the DSMB.
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.[42] All patients will be evaluated by a modified SCID as part of the screening process. 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.
The MGH Antidepressant Treatment Response Questionnaire (ATRQ):[43] 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.[40] Degree of resistance will be used as a covariate in relevant statistical analyses and to exclude severely treatment resistant subjects.[44] Severity of clinical condition and improvement in overall clinical state over the study period will be evaluated at each assessment with the Clinical Global Impressions Scale (CGI)[45]. *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. *MRI neuroimaging:* Scans will be carried out in Emory's Facility for Education and Research in Neuroscience (FERN) on a Siemens Trim Trio 3T scanner and 32-channel head coil using the following protocols. Anatomic images: High-resolution T1 weighted images [46] will be obtained for co-registration with fMRI data. A localizer scan will be used to orient the 3D volume scan.

Resting fMRI BOLD: Resting bold will be acquired before and after treatment using phase-encoding directions of opposite polarity (anterior-posterior) for distortion correction. Sequences will be optimized for signal-to-noise ratio and sensitivity to head motion, and collected over ~15 minutes.[47] Data will be analyzed with standard preprocessing protocols in AFNI (http://afni.nimh.nih.gov/) and SPM12 (www.fil.ion.ucl.ac.uk/spm12/), including slice-timing correction, realignment/motion-correction, anat-to-epi co-registration, and 5mm spatial smoothing.[25, 48, 49] Resting BOLD analysis will also include nuisance signal regression (head motion, cerebral spinal fluid, and white matter) and band pass filtering (0.009Hz<f<0.08Hz). Individual's fMRI data will be normalized into a standard stereotaxic space, MNI template with 1mm3 resolution. ***MIDT*****:** MIDT will be used to assess FC[50-52] during reward anticipation. This widely-used task for assessing reward function in psychiatric patients[53-55] is one of the few imaging reward tasks with established test-retest reliability.[56] 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.[53, 57, 58] Participants will complete 2 functional runs of 70 trials each (140 trials total) over ~20 minutes.[50, 52] FC during each anticipation condition will be assessed using beta-series correlation[59] shown to be both powerful and sensitive for assessing task-modulated connectivity[60, 61] and has been used with MIDT.[50, 52] *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 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. *DTI*: At the first visit, a cmrr_mbep2d_bold protocol over ~12 minutes will be used calculated fractional anisotropy (FA) maps for each voxel using 3DSlicer.

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)[25, 62] and the vmPFC cluster identified as being reward-sensitive in neuroimaging meta-analyses[63] 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).[25, 62, 64] 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 [13, 25] 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,[65] 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: *Effort-Expenditure for Rewards Task (EEfRT):* The EEfRT task is a multi-trial game in which participants are given an opportunity to choose different task difficulty levels to obtain monetary rewards.[66] The task is 20 min, and first 50 trials are analyzed. The proportion of hard-task choices across each level of probability is calculated. Lower proportions of hard task choices indicate decreased motivation.:

Clinician-Rated: *Snaith-Hamilton Pleasure Scale (SHAPS-C):* The SHAPS-C is a 14-item clinician-administered scale that assesses hedonic tone.[67, 68] In a previous study examining hedonic capacity, confirmatory factor analysis revealed a Hedonic Capacity factor that was largely defined by the SHAPS.[68, 69] *Clinical Global Impression (CGI)*: The CGI provides an overall clinician-determined summary measure of improvement that incorporates all available information, including the patient's history, psychosocial circumstances, symptoms, behavior, and the impact of the symptoms on the patient's ability to function[45].
Self-Report: *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.[70]
Assessments of Depression: Clinician-Rated: Hamilton Depression Rating Scale (HAM-D-17): The HAM-D-17 is a 17-item, clinician-administered scale, that rates severity of depression.[71]
Self-Report: Inventory of Depressive Symptoms- Self Reported: The 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
Assessments of Anxiety:
Hamilton Anxiety Rating Scale (HAM-A): The HAM-A is a 14-item clinician-administered scale that assesses the severity of symptoms of anxiety[72].
Beck Anxiety Inventory (BAI): The BAI is a 21-item self-report measure of anxiety symptoms, rated on a 4-point Likert scale[73] 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[74] 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. 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. Participant selection:

Recruitment and Informed Consent Process
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: IRB0000075162, 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. An overview of the study will initially be provided in person or over the phone by study clinicians or trained study staff. A telephone 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 a study clinician or designee, by virtual consent utilizing the 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. Informed consent will be documented on the Institutional Review Board-approved form. A copy of the signed consent form will be given to the participant or accessible to the participant following virtual consent provided in a REDCap ICF document link and the original document 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.
Telephone screen Interview

A telephone screen interview or RedCap Survey may be conducted with study referrals following verbal consent. Candidates eligible to proceed with the study process will be scheduled for an appointment to see a study clinician in a virtual appointment or in an on campus office visit.

Virtual intake and onsite screening

Trained personnel will obtain either virtual or written informed consent from candidates prior to initiating study procedures. Virtual consents will be obtained using the Emory IRB approved REDCap Platform. Virtual interviews will be conducted by use of ZOOM, a video conferencing service endorsed by Emory University for HIPPA compliant patient encounters. Screening information will be collected by interview and evaluation, as well as obtaining collateral history and data from other relevant sources (e. g. medical records, referring physician). Screening will include the following assessments: (1) History of medical and psychiatric conditions (2) screening laboratory evaluations, (3) subject height and weight measurements and waist circumference obtained by staff 4) Concomitant Medications 5) Adverse Events 6) MRI Safety Form 7) Demographics and 8) assessments below:

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.

Laboratory Variables

Research bloods will be collected between 9-11AM by venipuncture into EDTA-containing vacutainer tubes using standard sterile technique. Plasma for the evaluation of concentrations of CRP will be obtained along with additional plasma, buffy coat and a tempus tube for future studies. Plasma and buffy coat will be removed separately, aliquoted into siliconized polypropylene tubes, and stored at -80°C until batched assay of CRP. Bloods will also be collected by venipuncture into Tempus mRNA tubes and directly placed in freezer storage at -20°C. In order to collect immune cells for future analyses, we will additionally collect 10 ml of whole blood in EDTA at room temperature for immune cell extraction. Some de-identified samples may be sent to other labs for additional analysis for future studies.

C-reactive protein (CRP): Plasma CRP will be assessed with a high sensitivity turbidimetric assay. Sensitivity is 0.18 mg/L, range of measure is 0.2 to 80 mg/L, and functional sensitivity (at 20% CV) is 0.2 mg/ L.

7. Management of MRI Incidental Findings

The purpose of these scans is not to make a clinical determination of patients' brain health. However, if any incidental finding is noticed during the scan, the information will be provided to patients or their medical provider-of-record for further management. Incidental findings are those abnormalities seen in patients' brain images during the scanning process, which may or may not be of clinical importance. Follow-up of the scan findings will be the responsibility of the patients' Healthcare provider-of-record.

8. Sources of Materials: Research material will be derived from clinician-administered and self-report questionnaires, blood, urine, 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, urine 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 virtual or in person interviews and documented by study clinicians. Standardized clinician-administered and self-report questionnaires will be administered and collected by study physicians or study personnel. Blood and urine samples at screening will be obtained by the study team/or trained nurses. Blood and urine for the baseline assessment and subsequent assessment at week -4 and 8 will also be obtained by the study personnel or trained 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 except in the REDCap database accessed by approved study personnel. Subject name and identifying number will be kept in a locked office or in the secure REDCap database. Only approved study team members and study physicians will have access to subject identities. Research personnel analyzing blood

data 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.

9. Data Collection and Management: The data management team will use REDCap.[75] REDCap allows database construction, an interface for collecting data, data validation, and automated export procedures for data downloads to statistical packages (SPSS, SAS, Stata, R). Dr. Miller is currently using REDCap for 3 of his currently funded projects, and he and his staff have extensive experience with its usage.

10. Statistical Analyses

Aim 1: To determine whether bupropion (vs escitalopram) will increase FC in reward circuitry.

Hypothesis 1: Bupropion vs escitalopram will increase VS-vmPFC FC in depressed patients with high CRP and anhedonia at baseline. *Primary Endpoint:* Targeted VS-vmPFC FC at 8 weeks. *Predictors:* group (bupropion vs escitalopram), time (baseline, 8 weeks), and group x time interactions. *Covariates:* Primary: Age, sex, BMI, race. Additional covariates: Number of depressive episodes, length of current depressive episode (in months), treatment trials (ATRQ), age of onset, and family history of depression (yes/no), weeks on targeted dose of medication. Analysis Plan: Subjects will be randomized to two different treatment groups and scanned across two visits. Consequently, a factorial mixed-effects models for repeated measures (MMRM) will be employed with group, time and group x time interaction on targeted VS-vmPFC FC as fixed effects and each subject's baseline (identity) as random effects. Linear and multi-level mixed effects will be evaluated using both unstructured and autoregressive covariance structures and restricted maximum likelihood estimation and will be used to predict random effects (slope and intercept) in addition to fixed effects, optimized using Bayesian Information Criteria (BIC). Primary and additional covariates will be entered in all models as random variables after testing for significant associations. Given the inherently noisy nature of the covariates, model tuning to optimize the balance between explanatory (r^2) and noise variables (root mean square error) will be undertaken to select the most parsimonious models using BIC. The MMRM will also be augmented using Elastic net generalized regression models using group||time nesting approaches. Models will be selected using model comparison metrics. All analyses will be conducted in accordance with published guidelines.[76] Exploratory analysis: A similar analysis as indicated above will be conducted on fMRI scans conducted at 4 weeks. The 4-week scan will be conducted based on the rapid effects seen with dopamine agonists in our preliminary studies with L-dopa (data not shown).

Aim 2. To determine whether bupropion (vs escitalopram) will improve motivational deficits.

Hypothesis 2a: Bupropion vs escitalopram will improve objective and clinical assessments of motivation in depressed patients with high CRP and anhedonia at baseline. *Secondary Endpoints:* EEfRT, SHAPS-C and MAP. *Predictors and Covariates:* Same as above. *Analysis plan:* MMRM with random intercepts will be used to examine effects of group, time and group x time interaction controlling for variations in baseline scores. Relevant covariates as indicated in Aim 1 will be included in these analyses as described above.

Hypothesis 2b: Improvement in VS-vmPFC FC will correlate with improved objective and clinical assessments of motivation. *Analysis plan:* Multivariate regression models will be used to examine linear associations between primary (VS-vmPFC FC) and secondary endpoints (EEfRT, SHAPS-C and MAP). Models will be adjusted for relevant covariates as indicated above. Separate analyses will be run for each behavioral outcome of motivation. Type I errors as a result of multiple testing will be controlled by Family-Wise Error correction.[77]

Aim 3. To determine whether bupropion (vs escitalopram) will improve depression severity.

Hypothesis 3 (Exploratory): Bupropion vs escitalopram will improve depressive symptoms in MD patients with baseline high CRP and anhedonia. *Analysis plan:* MMRM will be used to examine effects of group, time and their interaction on depression severity using the HAM-D-17. Relevant covariates as in Aim1 will be included.

Interim Analysis: After 16 subjects have completed the study, the blind will be broken by Dr. Miller, and an effect size estimation will be made of between group differences in VS-vmPFC FC.* If the effect size is less than Cohen's d =0.3 at both fMRI time points (weeks 4 and 8), the study will be terminated. If the effect size is between a Cohen's d of 0.3 and 0.6 at either 4 or 8 weeks, the study will be continued

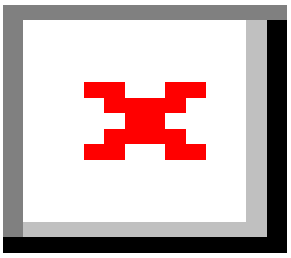
focusing on the fMRI time point with the largest effect size. If the between group difference in VS-vmPFC FC is >0.6 (Cohen's d), the possibility of using the data as proof-of-concept for a larger trial will be discussed with the study sponsor (NIMH).

* Due to slow recruitment secondary to COVID 19, we have decided to conduct the interim analysis at 16 subjects as opposed to 20.

11. Power Analysis

Hypothesis 1: Our preliminary data from depressed (major depression – MD) patients with high inflammation (CRP >2 mg/L, n=7) administered a dopaminergic agent, L-DOPA, and placebo control was used to estimate the anticipated change in VS-vmPFC FC in response to treatment (bupropion) and control (escitalopram) conditions in the proposed study. The expected change in FC in response to bupropion and escitalopram (the within-subject effects of these conditions) was then used to calculate the effect size (ES) and sample size per group that will be required to detect a significant difference between the response to bupropion versus escitalopram (the between-subjects effect) with alpha= 0.05 and power >0.80. Accordingly, based on the mean and standard deviation for the change in FC after L-DOPA (0.2±0.16), we can expect a large ES for the expected within-subject effects of bupropion (Cohen's d_z = 1.25, which would require only 8 subjects to detect). Based on the mean and standard deviation for the change in FC after placebo (0.07±0.10), we expected a medium effect size for the effects of escitalopram (Cohen's d_z = 0.7), representing ~30-40% increase in FC and consistent with a ~30% placebo effect commonly observed in trials in MD patients.[78] Based on these expected within-subject effects for our treatment and control groups and the corresponding mean and standard deviations from our preliminary data, we estimate the between-group effect of the response to bupropion and response to escitalopram to have an ES Cohen's d = 0.97, which would require a total sample size of 36 (18 per group) for power >0.8 at alpha=0.05. Using 5000-fold resampling, with alpha = 0.05, sample power= 0.8, sample sizes of 18 per group (total sample size= 36) was large enough to estimate group differences. **Figure 1** presents a graphical representation of the total sample sizes required for between-subject group comparisons of the FC response to bupropion versus escitalopram across the full range of power, demonstrating >80% power at the proposed total sample size of n=40.

Figure 1: Estimated power for between-subject comparisons of the FC response to bupropion versus escitalopram. Sample size (x-axis) and power (y-axis) for ES (d) = 0.97. The proposed total sample size of 40 (dashed line, n=20 per group) will yeild power >0.8.



Hypothesis 2: For hypothesis **2a**, the expect difference in motivation after bupropion versus escitalopram is based on the improvement in the EEfRT that has been previously established to be clinically and pharmacologically meaningful (≥10% change,[79, 80] representing a 0.10 increase in probability of hard choice choices) and standard deviations from our preliminary data for the EEfRT in MD patients. Thus, we expect a 0.10 ± 0.10 difference in mean probability of hard choices after bupropion compared to placebo and a medium to large ES (Cohen's d = 1.0), which will require a total sample size of 34 subjects (17 per group) for power >0.8 at alpha=0.05. For hypothesis **2b**, our preliminary data on the relationship between VS-vmPFC FC and motivation, as measured by the EEfRT, demonstrated a correlation coefficient (r) of 0.43 (r^2 = 0.185). We expect a similar effect size for the relationship between change of FC and change in motivation and will need a sample size of 37 subjects to detect an overall relationship between change in FC and motivation with power >0.8 at alpha=0.05. Therefore, based on our sample size of n=40 (20 per group) we will have at least 80% power to detect significant differences for the proposed hypotheses to be tested under both Aims 1 and 2 at alpha=0.05.

12. Potential Study Risks: 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. Use of bupropion has been associated with several side effects including anorexia, dry mouth, rash, sweating, tinnitus, tremors, hypertension and seizures. Use of escitalopram has been associated with appetite changes, headache, sweating, indigestion, nausea, tremors and sexual dysfunction. Both drugs can induce mania and suicidal ideation. 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.

Alternatives to study participation include not participating in the proposed study. Although both medications to be used in this study have demonstrated efficacy in major depression, there are an array of other demonstrated effective treatments for depression including but not limited to: other selective serotonergic antidepressants (i.e. fluoxetine, paroxetine, etc.), serotonin-norepinephrine reuptake inhibitors (i.e. venlafaxine, duloxetine), atypical antidepressants (i.e. 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 for more severe depressions 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.

13. Adequacy of Protection Against Risks:

Recruitment and Informed Consent:

Subjects will be recruited from social media campaigns including Facebook and Instagram. An overview of the study will initially be provided over the phone by study clinicians. 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 designee who provides this information. 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 and a copy will be placed in a case-book containing relevant demographic data for the subject. Of note, because this case-book will contain personal identifiers (i.e. name), it will be kept will be kept in a locked office or in a password protected REDCap electronic casebook

Protection Against Risk:

Every effort will be taken to prevent injury or distress that may result from this study.

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. If a subject becomes unduly distressed, Drs. Miller or Haroon will be immediately contacted, and an appropriate clinical intervention plan will be developed. Drs. Miller and Haroon 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.

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 200 ml over a maximum 8-week period (from screening to study completion).

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.

Bupropion or Escitalopram Treatment:

Risks and side effects of these medications vary in type and severity depending on the drug, but include gastrointestinal distress, headaches, sexual dysfunction, anxiety, high blood pressure, induction of mania, suicidal ideation, seizures and potential lethality in overdose. To reduce the risks associated with active treatment, all subjects will be monitored carefully for the development of adverse events as well as worsening of their condition. Specific indications for discontinuation between screening and baseline will include any of the following at the PI's discretion: 1) the development of significant suicidal ideation, plan or intent as determined by spontaneous subject report or as determined by CSSRS,[41] 2) the development of psychotic, manic/hypomanic or catatonic symptoms, 3) a 25% increase from baseline of HAM-D-17-17 score, 4) missing more than 2 consecutive doses of medication more than once during the study, and 5) a positive pregnancy test at any point during the study. 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. Upon discontinuation, subjects will be immediately evaluated by a study psychiatrist and determination of the appropriate level of care, including emergency hospitalization, will be determined. Subjects with non-urgent symptoms but who disqualify for further participation and who are in active treatment will be referred to their primary psychiatric physician. Participants will be required to identify a primary care or psychiatric physician at study entry to ensure seamless psychiatric care following study completion. All depressed subjects without a current psychiatrist will be offered an appropriate referral. The unblinded study physician may break the treatment blind following study completion or early termination from study participation. Participants with adequate treatment response at study completion may receive a 4 week supply of identified study medication. At the end of the study, or in the event of Early Termination, patients not achieving a 50% or greater treatment response as measured by the Ham-D-17 total score, they will be given a prescription to continue medication for 1 tablet daily for 7 days before stopping the medication (if on 2 pills of study medication at the end of the study). 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. During the treatment study itself, 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 the Columbia Suicide Severity Rating Scale, 2) the development of psychotic or catatonic symptoms, 3) a 25% increase from baseline of HAM-D-17 score, 4) missing more than 2 consecutive doses of medication more than once during the study and 5) positive pregnancy test at any point during the study. Subjects requiring discontinuation will be referred for further evaluation and treatment by one of the study psychiatrists as described above.

<u>Confidentiality and Data Management:</u>

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 regular recertification. Each subject will be assigned a unique ID number. 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 data will include unique patient identifiers to maintain patient confidentiality Any protected personal health information will be stored in a restricted access area or a password protected electronic casebook in REDCap System. The link between identifying information (e.g., name, contact information) and project data stored on a password-protected computer or in source documentation 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.

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 at the PI's discretion: 1) the development of significant suicidal ideation, plan or intent as determined by spontaneous subject report or as determined by CSSRS,[41] 2) the development of psychotic, manic/hypomanic or catatonic symptoms, 3) a 25% increase from baseline of HAM-D-17 score, 4) missing more than 2 doses of medication more than once during the study, and 5 a positive pregnancy test at any time during the study. Subjects requiring discontinuation will be referred for further evaluation and treatment by one of the study psychiatrists or clinicians.

Compensation to Participants:

Participants will receive up to $400-$1500 for participation of the study. 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. Candidates deemed ineligible by virtual evaluation(s)prior to the first in person office visit will not be compensated.

Visit	Total Amount	Amount put on Clincard immediately after visit	Amount put on Clincard at the end of study participation
Intake (Virtual when possible)	No Payment	No Payment	No Payment
(Visit Total)			
Visit 1 (Screen A)			
Screening lab work, UDS, Preg Test, AEs, Concomitant Meds	$50*	$50*	
(Visit Total)	$50		
Visit 2 (Screen B & Baseline A)			
Physical Exam, Rapid CRP, Vitals, research bloods, urine toxicology and pregnancy test, self-report surveys	$50	$50	
Computer Task (depending on choice made on the computer)	$5-$10	$5-$10	
(Visit Total)	$55-$60		
Visit 3 (Baseline B)			
Medical and psychiatric assessments, vitals, self-report surveys and medication check and randomization and medication administration	$50	$50	
Computer Task (depending on choice made on the computer)	$5-$30	$5-$30	
Complete Scan	$200		$200
Incomplete Scan	$25**	$25**	
(Visit Total)	$80-$280		
Visit 4 (Week 2)			
Medical and psychiatric assessments, self-report surveys and medication check	$50	$50	

Computer Task (depending on choice made on the computer)	$5-$10	$5-$10	
(Visit Total)	$55-$60		
Visit 5 (Week 4)			
Medical and psychiatric assessments, urine toxicology, self-report surveys and medication check	$50	$50	
Computer Task (depending on choice made on the computer)	$5-$30	$5-$30	
Complete Scan	$150		$150
Incomplete Scan	$25**	$25**	
(Visit Total)	$80-$230		
Virtual (Week 6)	$25	$25	
(Visit Total)	$25		
Visit 6 (Week 8)			
Medical and psychiatric assessments, research bloods, urine toxicology, self-report surveys and medication check	$50	$50	
Computer Task (depending on choice made on the computer)	$5-$30	$5-$30	
Complete Scan	$150		$150
Incomplete Scan	$25**	$25**	
(Visit Total)	$80-$230		
Total Study Compensation	$425-$935		

*Screening visit may be divided across 2 visits and paid $25 per visit
**Unable to complete scan leading to termination from the study

Potential benefits of the proposed research to the subjects and others:
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. In addition, subjects will all receive an active treatment with known efficacy. 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. 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 coupled with the likelihood that many patients will obtain at least some symptomatic benefit, we feel that the risks of the study are reasonable in relationship to the benefits of study participation.

Importance of the knowledge to be gained:
Successful treatment modalities both arise from, and contribute to, our understanding of disease pathophysiology. Mounting data indicate that depression is associated with evidence of immune/inflammatory activation, and that patients with major depression may be especially likely to demonstrate increased proinflammatory cytokine production/release. Furthermore, alterations in dopamine neurotransmission have been demonstrated extensively in individuals exposed to inflammatory stimuli, and patients with major depression have been shown to exhibit inflammation-associated alterations in functional connectivity within dopamine-related reward circuitry. Nevertheless, studies conducted to date have not explored whether currently available antidepressant treatments have

differential effectiveness in reversing inflammation-related brain changes and whether any observed differential effects on the brain are manifested in behavior. Understanding the mechanisms by which conventional antidepressant treatments interact with immune-related pathologies may not only lead to new treatments for depression but also may help guide antidepressant choice by measuring inflammatory state prior to treatment. For these reasons, we feel that risks to subjects are reasonable in relationship to the value of the knowledge (both theoretical and practical) to be gained.

Investigational Drug Status

All drugs to be used in this study (bupropion and escitalopram) are FDA-approved for the treatment of depression and will be used at doses and duration recommended in the package inserts of these medications.

14. Data and Safety Monitoring Plan (DSMP)

The DSMB for this study will consist of Larry Tune, M.D. Chairman, Boadie Dunlop, M.D., Tanja Mletzko, M.S. and Marian Evatt, 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. 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. The frequency of the Department of Psychiatry and Behavioral Sciences DSMB review for this protocol will be once every year based on IRB recommendations consistent with the assessed risk status of the study.

Procedures and Responsibilities of the Emory DSMB

Prior to each DSMB meeting, the data manager/research coordinator will prepare a 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 may 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 Department of Psychiatry and Behavioral Sciences DSMB, once every year the DSMB will issue a letter to the PI to be included in the annual Emory IRB Continuing Review submission. The letter will inform the Emory IRB that the study is approved to proceed or state any safety related concerns expressed by the committee. Reports will not specifically disclose the treatment arm of the study for relevant subjects unless this disclosure is required for safety reasons.

PI and designated trained study personnel will review all pertinent aspects of study conduct including patient safety, compliance with protocol, data collection and efficacy. Periodic chart monitoring will be conducted by study personnel to validate integrity of the data.

Adverse Event Reporting

Note that any adverse event or serious adverse event (SAE) meeting Emory IRB criteria for on Unanticipated Problem will be reported to the Emory IRB and the study DSMB 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.” According to the Emory IRB, an AE meets criteria for an Unanticipated Problem (UP) if all of the following criteria are met: 1) The AE is unexpected 1) the AE is related or possibly related to participation in the research 3) the AE suggests that the research places subjects or others at a greater risk of harm that was previously know.

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