Dynamics of Inflammation and its Blockade on Motivational Circuitry in Depression

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Research Protocol

Title of Protocol: Dynamics of Inflammation and its Blockade on Motivational Circuitry in Depression

Short Title: Infliximab

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Abstract
Motivational anhedonia–a subset of anhedonic symptoms involving dopamine-linked impairments in effort- based decision-making, reward anticipation and reinforcement learning–are common in psychiatric disorders such as major depression, and are notoriously difficult to treat. In recent years, these symptoms have been associated with alterations in dopaminergic corticostriatal circuitry, yet the underlying causes of this circuit dysfunction remain unknown. One candidate mechanism is inflammation; increased inflammatory cytokines have been reliably found in depressed patients, and administration of inflammatory cytokines or cytokine inducers has been shown to foment depressive symptoms of apathy, anhedonia and fatigue. In addition, inflammatory cytokines have been found to disrupt dopamine synthesis, alter basal-ganglia metabolism, and blunt striatal responsivity during reward anticipation. To date, however, the majority of data supporting the relationship between cytokines and symptoms in patients with major depression and other disorders is correlational in nature, and thus alternative experimental strategies are required to elucidate causal relationships. One strategy is to block inflammatory cytokines in a sample of depressed patients with high inflammation so as to determine which symptom domains are most affected and through which molecular pathways. The current study will assess neuroimaging measures of corticostriatal circuitry before and after a placebo-controlled pharmacologic blockade of inflammation in 80 depressed patients (n = 40 per group) recruited to ensure high levels of peripheral inflammation [C-reaction protein (CRP) > 3mg/L]. We will also recruit two comparison samples consisting of 20 depressed patients with low levels of peripheral inflammation [CRP <2mg/L] and 40 healthy controls. Primary aims are to evaluate whether 1) corticostriatal function during reward motivation and anticipation are associated with change in peripheral inflammation following pharmacologic blockade relative to placebo 2) the temporal dynamics of change in inflammation, gene-expression, reward motivation and reinforcement learning behavior and motivational

symptoms assessed at baseline, and 24 hours, 3 days, 1 week and two weeks post infliximab infusion, and 3) test an integrative multi- level path model to determine whether change in corticostriatal circuitry following inflammation blockade mediates the relationship between change in inflammation and change in motivational anhedonia symptoms. These data will provide further validation of inflammatory cytokines as therapeutic targets for motivational symptoms in depression and will define symptom targets and biomarkers of response for future studies.

Introduction and Background

Development of new treatments for major depression has been substantially hindered by the marked heterogeneity of the disorder, leaving remission rates as low as 33% with conventional antidepressant therapies.[24] To address this problem, the National Institute of Mental Health (NIMH) has emphasized transdiagnostic approaches focused on identifying pathophysiological mechanisms for specific symptoms, rather than disorders as a whole. Anhedonia and other reward-related symptoms represent an excellent opportunity for this type of translational neuroscience approach, given the vast basic science literature from which to draw upon.[25] Additionally, anhedonic symptoms reflect an area of critical need as they frequently fail to respond to widely available psychosocial and pharmacological treatments.[26,27]

To date, however, application of this important preclinical work to humans has been hampered by the enormous heterogeneity in psychiatric disorders, and the reliance on clinical symptom definitions that are underspecified for the purposes of identifying their neurobiological mechanisms. For example, the *DSM-V* (p. 163) states that individuals meeting criteria for anhedonia "may report feeling less interested in hobbies, 'not caring anymore,' or not feeling any enjoyment in activities that were previously considered pleasurable." In other words, clinical assessment of anhedonia does not discriminate between a decrease in motivation and a reduction in experienced pleasure. However, a wealth of preclinical data suggest that this distinction is crucial for purposes of specifying the neural processes involved in motivation and pleasure, with dopamine-dependent corticostriatal function implicated in the former[28-30] but not the latter.[31,32] In addition to distinct substrates, these different sub-domains of anhedonia likely have distinct etiopathophysiologies. Indeed, as summarized below, there is excellent evidence to suggest that one path towards the development of an anhedonic presentation is the effect of inflammation on dopaminergic circuits and subsequent corticostriatal dysfunction resulting in impaired motivation, anticipation and reinforcement learning (referred to herein as "motivational anhedonia"[6]).

The goal of the proposed research is to establish the validity of an inflammation-dependent path towards the development of motivational anhedonia. Importantly, we do not propose this model as a means of accounting for *all* forms of anhedonic symptoms; rather, we suggest this as a possible mechanism that may occur in the context of high inflammation and altered dopamine-dependent corticostriatal network functions

Objectives. There are three primary aims of this study, each of which generates one or more hypotheses to consider and explore in the protocol design. These are elaborated on

below.

Aim 1: Determine the relationship between inflammation and corticostriatal circuit function in patients with major depression *and high inflammation* before and *after a placebo-controlled anti-inflammatory challenge.*
80 medication-free depressed patients pre-selected for high inflammation (CRP >3mg/L), 20 medication-free depressed patients with low inflammation (CRP<2mg/L), and 40 healthy controls will be enrolled. fMRI assessments of corticostriatal circuit function during effort-based decision-making, reward anticipation and reward attainment will be assessed using the EEfRT[21], MID[22], and a Reinforcement Learning (RL)[23] task, respectively, at baseline and 2 weeks after double-blind, random assignment to receive administration of the TNF antagonist infliximab or placebo (n = 40 per group). Peripheral protein and gene expression markers of inflammation will also be assessed. Hypothesis 1a: Infliximab-induced decreases in inflammation will predict pre-post increases in neural activity in ventral striatal and vmPFC during the EEfRT and, MID tasks, as well as increased prediction-error signals in ventral striatum during the reward attainment. Hypothesis 1b: Decreases in inflammation will additionally be associated with pre-post increases in vmPFC–striatal connectivity during the EEfRT task.

Aim 2: Determine the temporal dynamics of change in *CRP and interleukin-6 (IL-6)* and change in behavioral and clinical measures of motivational anhedonia in depressed patients before and after an anti-inflammatory challenge.
Behavioral measures of effort-based decision-making and reinforcement learning as well as symptoms of anhedonia will be collected in the subjects in Aim 1 at 4 time-points before and after anti-inflammatory challenge with infliximab *or placebo*: baseline (pre), 24 hours post-infusion, 3 days, 1 week, and 2 weeks. Peripheral protein and gene expression markers of inflammation will also be assessed at each time point. *Hypothesis 2:* Individual differences in the magnitude of infliximab-induced decreases in plasma *CRP and (IL-6) as well gene expression changes in TNF signaling pathways* will be associated with reduced motivational anhedonia as assessed by behavioral and clinical measures.

Aim 3: Explore the inter-relationship among inflammation, corticostriatal circuit function and reward motivation using path analysis. *Hypothesis 3:* Across all subjects, the association between change in peripheral inflammation and symptoms of motivational anhedonia following inflammation blockade *or placebo* will be mediated by changes in corticostriatal circuitry (ventral striatal BOLD signal & striatal-vmPFC connectivity). Mediation will be assessed using a well-validated bootstrapping procedure for indirect effects analysis. We will also test a model of moderated-mediation to examine whether indirect mediational effects of changes in corticostriatal circuitry are moderated by *treatment with infliximab or placebo*.

STUDY PROTOCOL AND METHODS.

Virtual Study Visits: Due to COVID-19, the informed consent process, clinical interview, neuropsychiatric assessments, task administration, and self-report surveys may be completed virtually with trained research staff using Emory's HIPPA-Compliant Zoom Account and REDCap. Participants will be paid $15/hour for time spent in study visits on

Zoom. Please see the updated TReAD Lab Suicide Policy for information specific to virtual study visits.

The overall procedure for this study is summarized in the table, below.

Schema/Study Summary

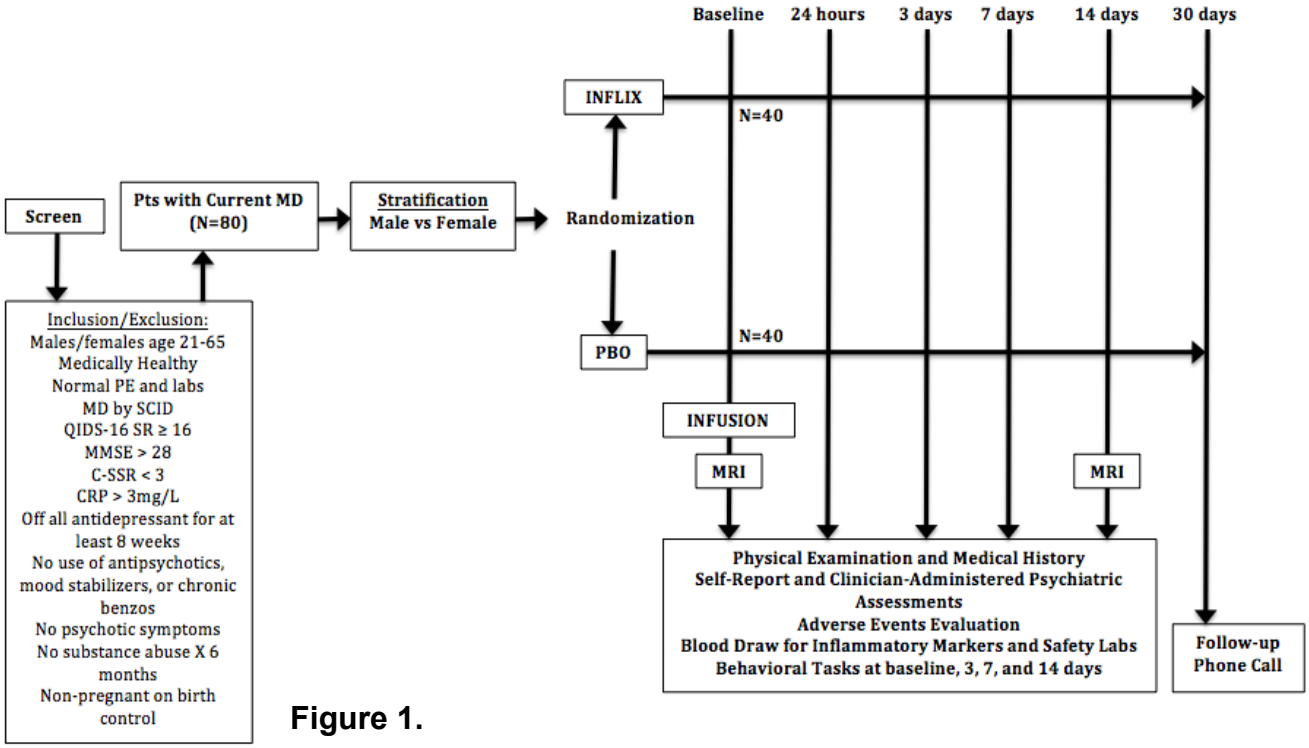

Figure 1.

Legend: **PE** = physical examination**; MD** = major depression; **SCID** = structured clinical interview for DSM-V; **QIDS-SR** = 16-item Quick Inventory of Depressive Symptoms; **MMSE** = Mini Mental State Examination; **C-SSR** = Columbia Suicide Severity Rating Scale; **CRP** = C-reactive protein; **INFLIX** = infliximab; **PBO** = placebo; **MRI** = magnetic resonance imaging; **Psych Ass.** = Interviewer based assessments using the SCID Mood Disorders module, MGH Antidepressant Treatment Response Questionnaire (ATRQ), Mini Mental State Examination (MMSE), SCID substance abuse modules, and Columbia Suicide Severity Rating scale (C-SSR). Self-report questionnaires will include the Multidimensional Fatigue Inventory (MFI), Inventory of Depressive Symptoms Self-Report (IDS-SR), 16-item Quick Inventory of Depressive Symptoms (QIDS-16 SR), Snaith-Hamilton Pleasure Scale (SHAPS), Fatigue Severity Scale (FSS), Mood and Pleasure scale (MAP-SR), and Positive Affect/Negative Affect Scale - Now (PANAS-X) **Immune Eval** = blood draw for assessment of plasma concentrations of TNF-alpha, sTNF RI and R2, IL-1, IL-1ra, IL-6, sIL-6R and CRP, intracellular TNF-alpha expression and NF-κB activity; the kynurenine to tryptophan ratio **Adv. Events** = clinician-based assessment of medication side effects/adverse events.

Description of Study Procedures

Research Evaluation Clinic: Some subjects in this study will be recruited after completion of a separate "evaluation clinic protocol", in which screening will be conducted to assess a subject's eligibility to participate in this study and/or others. Screening may include a structured clinical interview, administration of self-reports and computer tasks, physical examination and EKG, urine drug and/or pregnancy screen, and blood work (CBC with differential platelet count, comprehensive metabolic panel, etc.). This pre-screening process will take place at the Behavioral Immunology screening

clinic, where subjects who are determined to be eligible for this study will be referred to study staff. An overview of the study may initially be provided in person or over the phone by study clinicians or trained study staff after a subject has completed the evaluation protocol and consented to be contacted about other studies. Before enrollment of a subject, study staff will review the screening test results and medical record of a referred subject to ensure he or she is medically healthy and eligible to participate. Any screening tests and procedures necessary to verify eligibility not performed through the Research Evaluation Clinic will be administered for a subject as a part of this protocol. Subjects who are recruited through this route may not undergo some of the procedures described under the "Pre-Screening" and "Screening" visits if these were completed through the Research Evaluation Clinic and the results were still valid through completion of the study protocol. Screening tests and procedures may be repeated during the study as needed if the validity of a test has expired before completion of the study visits. For example, an EKG performed through the Research Evaluation Clinic could be deemed sufficient to assess for safety criteria for this study, if performed within a reasonable amount of time before study enrollment (under advisement of PI and co-investigators). However, a serum pregnancy test would be completed during screening for this study regardless of whether a test was previously performed as a part of the other protocol. Serum Pregnancy will be repeated on infusion date, regardless of the date of the last negative result.

Pre-Screening: To obviate the full screening assessment, an initial CRP and QIDS-16 SR will be obtained before embarking on the full screening process.

Following pre-screening, participants will be scheduled to complete one or more screening appointments. The procedures described under "Screening" may be divided between multiple visits depending on subject scheduling and preference, staff availability, and other factors. Screening visits will occur at the Emory Department of Psychology and/or the Emory Clinics and Emory University Hospital. If screening is performed in a single visit, it will occur at the locations listed above.

Screening: Subjects potentially qualified for enrollment will undergo a screening evaluation after written informed consent. Screening will involve a subject interview and evaluation, as well as obtained collateral history and data from other sources (i.e., medical records, referring physician). Screening will include the following assessments: (1) Assessment of past psychiatric history and current symptom severity using a) the Structured Clinical Interview for ***DSM-V*** (SCID-V) and QIDS-16 SR, (2) Assessment of past and current psychiatric treatment history, (3) Evaluation of medical history obtained from interview of the patient and review of pertinent medical records, (4) Complete medical and neurological examination, (5) Screening laboratory evaluations as described in "medical exclusionary criteria" above, (6) Height and weight (provided to the pharmacy to calculate appropriate infliximab dose), and (7) CRP. *We anticipate that 50 full-screens will be required annually to enroll 16-20 subjects per year.*

Randomization and Blinding:

A list containing the randomly generated sequence of assignments to infliximab or placebo will be maintained in the Emory University Investigational Drug Service pharmacy. Independent pharmacists will dispense either infliximab or placebo in a 250ml saline bag according to the randomization list. Subjects will be stratified on the basis of sex to obviate any confounding effect of gender on the outcome variables. Study personnel responsible for administering the infusion, conducting psychiatric evaluations, conducting medical assessments, drawing blood and performing lab analyses will be blinded to subject group assignment. In the event of a significant adverse reaction to the study medication, the study blind will be broken if medically necessary, and the subject will be referred for appropriate medical care. Unblinded subjects will be discontinued from the protocol. Based on our previous experience, infliximab was well-tolerated with no serious adverse events, no differences in side effects compared to placebo, and no ability to be distinguished from placebo by patients or staff.[20]

Important Note: In some cases, an FDA-approved infliximab biosimilar, Inflectra (infliximab-dyyb) may be used in place of infliximab. Please note at each place where infliximab is referenced in this protocol that Inflectra may be used as its substitute.

Phone Calls:
If a participant misses a study visit, a study staff member will contact that participant to determine the reason behind the missed visit and will actively follow up with him or her as appropriate until the conclusion of the study (at the 30 day follow-up call).

Healthy Controls and Low CRP Depressed Patients:
Healthy controls and low CRP depressed patients will go through a pre-screen and screening process that will consist of psychiatric evaluations, research and safety labs, baseline assessments (clinician-rated assessments, self-reports, behavioral tasks) and will take part in one scan visit. In total, these participants will take part in up to 3 study visits (prescreening, screening, scan visit); however, some participants may request to consolidate their visits into just 2 study visits (prescreening, screen/scan visit).

Healthy controls and low CRP depressed patients will undergo all research-related screening procedures that high CRP patients would undergo but **will not** receive a Quantiferon Gold TB Test, EKG or Chest X-Ray, as these are only required in order to ensure safety and suitability for the study drug. Healthy controls and low CRP depressed patients will not receive an infusion of either drug or placebo.

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.[96] All patients will be evaluated by the SCID as part of the screening process. In addition to providing information for inclusion/exclusion purposes, the SCID will provide data about relevant psychiatric covariates including number of past major depressive episodes, length of the current episode and presence of co-morbid anxiety disorders and/or dysthymia. Substance

abstinence will be additionally confirmed by urine drug screen at multiple points over the course of the study.

The MGH Antidepressant Treatment Response Questionnaire (ATRQ)[97]: 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[98] Degree of resistance will be used as a covariate in relevant statistical analyses.

Mini-Mental State Exam (MMSE) is a 27-item interviewer-administered questionnaire widely used for the evaluation of general cognitive functioning and identification of altered mental status. At screening, subjects will be excluded for score ≤24, indicating evidence of more than mild cognitive impairment.[95]

Bipolarity Index (BI): Bipolarity will be measured as a continuous variable using the NIMH funded Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD) study.[99] The STEP-BD computed bipolarity index scores by rating the patient's profile relative to five traits characteristic of Bipolar I disorder, including episode characteristics, age of onset, course of illness/associated features, response to treatment and family history. The Bipolarity Index (BI) assigns common clinical features along each of five dimensions using a 0–20 score, where 20 points represents the presence of traits considered most characteristics of Bipolar I Disorder.

Hamilton Rating Scale for Depression (HAM-D): The HAM-D is a 21-item interviewer-administered questionnaire widely used for the evaluation of severity of symptoms of depression.[144] This clinician-rated assessment may be administered at the PI's discretion.

Wide Range Achievement Test (W-RAT3): The WRAT III is a very brief screening measure for achievement. It covers reading recognition, spelling, and arithmetic. It contains very easy beginning items (letter reading, basic counting, and dictation of letters) followed by spelling, pronouncing words, and written math problems.

Note: Neuroimaging Measures and Computer tasks will be drawn from the tasks listed below. Participants will be required to complete a selection of these tasks.

<u>Neuroimaging Measures</u>

All neuroimaging will be acquired on a 3T Siemens Prisma magnetom scanner with a 32-channel headcoil. Collection of structural and functional data includes: a 13-s localizer scan; an "auto-align scout" scan that uses a reference database to ensure consistent slice positioning across subjects; a rapidly acquired (~2 min), T1-weighted, multi-echo MPRAGE volume for structural analysis and localization of fMRI data (0.8 mm isotropic voxels; TR = 2.3 s; flip angle = 8 deg; TE1 = 3.15 ms; 224 slices); and multiband echo

planar imaging for task-related fMRI data (2x2x2.5 mm voxels; TR = 1.0 s; TE = 30 ms; flip angle = 46 deg; 56 transverse slices, no skip). This last sequence is re-used to acquire task-evoked fMRI data. The scanning session may also include diffusion tensor imaging (DTI) and MR spectroscopy (MRS) scans. All neuroimaging data will be analyzed in SPM using standard preprocessing routines involving realignment/motion-correction, co-registration, normalization and smoothing with a 6mm Gaussian kernel.

fMRI-adapted EEfRT (approx. 15-20 min) Assessment of reward motivation will be accomplished using an fMRI-adapted version of the EEfRT task. During each trial, subjects are presented with a choice between two levels of task difficulty, a High Effort option and a Low Effort option, which require different amounts of speeded manual button pressing for differing levels of monetary reward. The reward magnitude for a No Effort option remains constant ($1.00), while the reward magnitude for the High Effort option varies from $1.00 to $5.00. Additionally, the amount of effort required for the High Effort option will vary between 20%, 50%, 80% and 100% of the subject's max effort (set for each individual prior to scan). The task will use a rapid event-related design with an exponential jitter between trials drawn from a Poisson distribution in order to optimize HRF estimation. Responses will be made using MRI-compatible button-boxes and images will be presented on a rear projection screen visible with a mirror mounted on the head coil.

After the scan, subjects will be asked to complete a "post-scan" effort task. This task will present the same trial options the subject viewed and decided on in the scanner (e.g., $3.70 for 80% max effort or $1 for no effort). The subject may then choose to re-select the choice previously made or change it, and then complete the effortful button pressing or not (depending on the choice made).

Main Effects Analysis: Based on subject choices, indifference curves for each level of effort will first be established using a logistic model, and then fit to a parabolic discounting effort function where the subjective value (SV) of each choice is defined as $SV = Reward / (1-k*effort^2)$. A parabolic discounting function is used based on prior work suggesting superior fits for effort-discounting as compared to hyperbolic or exponential functions [89]. Per-trial subjective values will then be used as a parametric regressor in a first-level model, such differences in subjective value between the high and low effort options will be modeled against BOLD signal for each subject. Single-subject parametric contrast maps will then be entered into an RFX GLM, which is expected to isolate areas of vmPFC and striatum.

Note: For this task, participants will be instructed that they may complete the EEfRT "post-scan" task during a mandatory 30-minute observation period, to follow the MRI scan session. In reality, an observation period is not needed after an MRI scan. This instruction will help ensure that participants do not believe that visit length is dependent choices made in the EEfRT post-scan task. Once all study procedures are complete at the 14-day follow up visit, this will be explained to participants and a debriefing form will be provided.

Monetary Incentive Delay Task (approx. 15 min): Assessment of reward anticipation will be achieved using the Monetary Incentive Delay Task.[22] This is one of the most widely-used tasks for assessing reward function in psychiatric populations,[8,10,100] and is one of the few imaging reward tasks with established test-retest reliability.[91] Briefly, during this task participants have the opportunity to win or lose money by making a rapid button press in response to a target visual stimulus. The primary epoch of interest is the "anticipatory delay" – a period of ~2000ms that occurs after participants have been informed how much money they can win or lose on a given trial, but prior to the presentation of the target. This epoch has repeatedly been associated with robust ventral striatal activity[10,22,101-103] and shows excellent test-retest reliability. Participants will complete 2 functional runs of 90 trials each, resulting in a total of 180 trials (72 win; 72 loss, 36 no-change), with evenly distributed reward magnitudes of $0.20, $1.00 and $5.00. An optional variant of this task may involve an additional cue at the beginning of each trial signifying different ranges of possible reward values with the precise amount for each trial revealed only at the outcome phase.

Main Effects Analysis: Primary contrasts of interest will focus on reward anticipation, and include Win > Neutral trials, Loss > Neutral trials, and Win > Loss trials. We will additionally examine these contrasts after restricting Win and Loss trials to only high reward amounts ($5.00).

Adaptive MID (one run AdaptMID; approx. 9-10 min. Total run time approx. 18-20 min.): The adaptive MID task was designed to show how brain value signals adapt to the specific context that a person is experiencing. The AdaptMID includes both training and a test phase. During training subjects will see stimuli identical to what they see in the test portion. Subjects are first shown a cue signal informing them of the context they are in. The two contexts are low context rewards, where the trial could be worth $0.25 or $1.00, and high context rewards, where the trial could be worth $1.00 or $4.00. The contexts are indicated by either one "+" symbol (low) or by three "+" symbols (high). After the cue there is a jittered delay (mean 2.84 sec.) and the reward amount is displayed to them ($0.25, $1.00, or $4.00). The lower value option and higher value option are displayed an equal number of times within each context. After all of the cue information has been presented there is an anticipation phase delay (mean 2.36 sec.) before a target appears. A target red box flashes briefly on the screen and the subject's role is to quickly press a button before the box disappears. An accurate response results in a "hit" and the subject is rewarded the amount shown previously. An inaccurate response results in a "miss" and the subject is not rewarded. The subject is shown feedback on whether they hit or missed, and how much money they earned if any. The primary dependent variables are how subjects respond to low and high contextual situations and whether the reward amount revealed is the low value or high value within that context. The AdaptMID will be administered as two runs with a novel order of trials for each of the runs.

Gamble Task (approx. 30 min): To examine the relationship between chosen certain rewards the expected values of chosen gambles, the difference between experienced and predicted rewards and happiness; we have added a Gamble Task. During this task,

participants will have the option of selecting between a certain choice and a gamble choice, with equal probabilities of the two outcomes. There will between 50 and 150 trials with an equal proportion of three trial types: mixed (a certain amount of $0 with a gain and loss amount), gain (with a certain gain or a gamble with $0 and a larger loss), and loss (with a certain loss or a gain of $0 and a greater loss). Further, participants will be asked to rate their current level of happiness after every three trials. Also, before and after the task is complete, participants will be asked to measure their life happiness. Previous studies by Rutledge et. al. have demonstrated that this technique can be used to establish a relationship between task earnings and happiness. Further, fMRI measures have demonstrated a relationship between ventral striatum activities with happiness ratings from this task.

RL Task (approx. 15-20 min): To examine effects of infliximab on neural responses to reward attainment we have added an instrumental conditioning task (90 trials). Trials for this task involve a 3s cue presentation during which subjects choose between two abstract stimuli, followed by an exponentially jittered delay, and then a 3s feedback presentation with positive (monetary win), negative (monetary loss) or neutral outcomes. Behavioral responses will be analyzed by fitting a standard reinforcement learning (Q-learning) model[104] to each subject's sequence of choices. Based on individual choices and outcomes for each trial, the Q-learning algorithm will compute the expected values (Q) of choosing a given stimulus: $Q_t = Q_{t-1} + alpha*RPE_t$, where $RPE_t = Reward_t - Q_{t-1}$ and alpha represents the learning rate.[104] Model-based expected values will be related to subject choices using a softmax selection rule[105] to determine learning-rate parameters and generate behavioral RPE values for each trial, consistent with past studies from our group (Preliminary Study 5) and others.[23,106] We note that while some past work has suggested minor test-retest reliability issues with fMRI RL tasks[107], these have only been observed using task designs with a very low number of trials (~25 as compared to 90 in the current proposal), which likely produce less reliable measurements for purposes of repeated assessments.

Main Effects Analysis: Primary contrasts of interest will focus on parametric modulation of RPE during reward attainment reward, which is expected to robustly recruit ventral striatal activity.

Delay-discounting Task (approx. 5-15 min): Participants are presented with choices between receiving one reward amount now, and a different reward amount that will be delivered some number of days later. Reward values will range between approximately $5.00 to $50.00, and number of days will range between today and 52 weeks. To ensure the ecological validity of this task, participants will be told that two of the choices they made will be added to their final compensation, with this added payment received either at the end of the study or some days later (depending on the choice made by the participant).

Main Effects Analysis: Primary contrasts of interest will focus on the parametric modulation of reward amount and delay of reward.

Ramping Task (approx. 10 min/run, 20 min total)*:* In this task, subjects will be trained on different computerized mazes with varying difficulties/lengths. There will be a group of

easy mazes and a group of hard mazes. After a subject has sufficiently learned the mazes (defined as reaching the end of a maze by a specified goal time), he or she will begin the task itself. In this task, subjects may be exposed to different trial types, like "navigate" and "passive viewing". In "navigate" trials, subjects will have to reach the end of the maze for a varying known or unknown reward amount (ranging from $0-10). Trials may be set to "time out" after a predetermined period of time has elapsed to ensure subject compliance and performance. In "passive viewing" trials, subjects will watch as they are navigated through the maze without needing to actively respond or move.

Main Effects Analysis: Primary contrasts of interest will focus on the parametric modulation of reward amount and effort differences between 'passive viewing' and 'navigate' trial types.

Behavioral Assessments of Reward Motivation and Reinforcement Learning

Behavioral Effort-Expenditure for Rewards Task (behEEfRT; approx. 20-25 min): The behEEfRT task is a multi-trial game in which participants are given an opportunity on each trial to choose between two different task difficulty levels in order to obtain monetary rewards.[21] For all trials, participants make repeated manual button presses within a short period of time. Each button press raises the level of a virtual ''bar'' viewed onscreen by the participant. Participants are eligible to win the money allotted for each trial if they raise the bar to the ''top'' within the prescribed time period. Each trial presents subjects with a choice between two levels of task difficulty, a 'high-effort' and 'low-effort' task that require different amounts of speeded button pressing. Reward magnitudes for the high effort task vary between $1.24 and $4.33, while reward magnitudes for the low effort task remain constant ($1.00). Trials also vary in terms of 3 levels of probability of winning the amount associated with the choice selected. Subjects participate in the task for about 20 minutes and the first 50 trials are used for analysis. For statistical analyses the proportion of hard-task choices across each level of probability is calculated. Lower proportions of hard task choices indicate decreased motivation for monetary rewards. The EEfRT will be administered 5 times. The task has excellent test-retest reliability (test-retest $r > 0.85$), and has been successfully used in prior multi-session studies with similar time intervals between administrations. [43,108]

Probabilistic Stimulus Selection Task (PST; approx. 15-20 min): The PST was designed to differentiate between "go" and "no-go" reinforcement learning and has been validated tested using a bio-realistic model of basal ganglia function, and empirical data in humans.[37,109,110] The PST includes a training phase and a test phase. During training, participants are presented with three different stimuli pairs (AB, CD, EF) in random order, and are instructed to choose one. After each choice, participants are informed if their selection was "correct" or "incorrect." Correct/incorrect feedback is probabilistic such that for AB trials, A is correct 80% of the time and B 20%; for CD trials C is correct 70% and D 30%, and for EF trials, E is correct 60% and F 40%. After subject behavior demonstrates that they have accurately learned the "correct" option for each stimulus pair, they enter the testing phase. In this phase, subjects are presented with novel pairs involving either the A stimulus (AC, AD, etc.) or B stimulus (BC, BD, etc.). Since A always has the highest probability of being correct across all stimuli (80%), the extent

that subjects choose A in this novel pairs represents a learned "go" association. Conversely, since the B stimulus has the lowest probability of being correct (20%), the extent to which subjects do NOT choose B in the novel pairs indicates a "no-go" association. The primary dependent variables are the proportions of correct go and no-go trials during the testing phase. The PST will be administered 5 times with a novel stimuli set used each time, consistent with past repeated measures designs using the PST.[111]

Go No-Go Variant Task (approx. 32 min): On each trial of this task, one of four possible fractal images is briefly presented to the subject. Each represents the combination between action (either "go" or "no-go", carried out by button pressing or withholding button pressing), and valance at outcome (either win or lose). Action will be required in response to a circle that follows the fractal image, after a brief variable delay. The circle will appear on the screen for approximately 500-2500ms. In go trials, subjects will be asked to indicate which side of the screen the circle appeared on, by pressing a button. Subjects must make a response within a brief amount of time (e.g. 700 ms). In no-go trials, subjects will simply be asked to withhold any response. After a short delay, the outcome will be presented to a subject (win, loss, or neutral). A green upward symbol indicates a win of $1, a red downward symbol indicates a loss of $1, and a horizontal bar indicates an absence of win or loss. In "go to win" trials, a correct button press is rewarded. In "go to avoid losing" trials, a correctly avoided button press avoids punishment. In "no-go to" win trials, withholding the button press leads to reward. In "no-go to avoid losing" trials, withholding a button press avoids punishment. The outcome will be designed in a probabilistic manner, so that 70% of correct responses are rewarded in win trials and 70% of correct responses are not punished in lose trials. On 50% of trials, target detection and outcome will be omitted. Participants will be asked to complete 4 blocks of this task, each approximately 8 minutes in length.

Behavioral Assessment of Emotional Memory Pre/Post-Infusion

Emotional Memory Task: To examine the effects of infliximab on emotional memory, we have added a two-part (i.e., encoding & retrieval) emotional memory task that may be performed before and after the infusion. Participants will complete the two-part emotional memory task before the infusion and again after the infusion. We will use 360 happy, angry, and neutral faces selected from the Carnegie Mellon University Multi-PIE Face Database (Gross et al., 2010) as stimuli. All images will be cropped to ovoid shape in order to remove distinctive hairstyles, jewelry, or clothing. During encoding, we will present each participant with 90 images of faces: 30 happy faces, 30 angry faces, and 30 neutral faces. On each trial, the participant will view the face for 2 seconds before indicating by button press whether the expression is happy, angry or neutral—the participant will have an additional 2 seconds to make this judgment. Trials will be separated by a jittered intertrial interval (ITI) during which time a fixation cross will be presented (mean duration: 2 seconds; range: 1-5 seconds). Thus, the total trial time will by 6 seconds, on average, for a total encoding time of about 10 minutes (including time for setup and brief instructions). On the following day (or as close to the following day as possible we will administer a recognition memory test for the faces. Briefly, participants will view the 90 "old" faces from the encoding session mixed with 90 "new" faces—

lures—and will be asked to indicate whether each face is old or new and also how confident they are in their judgment (4 seconds). A fixation ITI will again separate the trials (mean: 2 seconds). Given 180 faces and an average trial length of 6 seconds, the recognition test is expected to last about 25 minutes. Identical procedures will be used before and after treatment with infliximab, with different faces presented at each session.

We will first analyze the recognition memory data by computing *d'*, which is a signal detection measure of memory accuracy unconfounded by response bias; *d*' is calculated as *Z*(hit rate) – *Z*(false alarm rate). Next, we will submit the *d*' data to a 2 *Treatment* (pre, post) x 3 *Emotion* (happy, angry, neutral) repeated measures ANOVA. We expect to obtain a significant *Treatment* x *Emotion* interaction. We further predict that follow-up *t*-tests will reveal significantly better memory for angry vs. happy faces before treatment, but no difference between angry and happy faces after treatment (i.e., memory for happy faces will be selectively improved by treatment with infliximab). Furthermore, we expect a series of "post minus pre" within-subject *t*-tests to show no effect of treatment on memory for neutral or angry faces, but a positive effect of treatment on memory for happy faces. If possible, we will seek to correlate any observed change in memory for happy faces with measures of improved dopamine function. We predict that depressed adults will show poor memory for positive relative to negative stimuli before treatment, but that memory for positive stimuli will improve significantly after treatment.

Behavioral Assessments of Psychomotor Slowing

Finger Tapping Task (FTT): This task uses a specially adapted tapper that the subject taps as fast as possible using the index finger. The subject is given 5 consecutive 10-second trials for the preferred and non-preferred hands. The finger tapping score is the mean of 5 trials and is computed for each hand. Performance norms have been established, and scores have been shown to be stable over time.[112] The FTT is designed to assess subtle motor impairment and is altered in subjects with basal ganglia disorders and lesions.[113]

Reaction Time Task (RTT)(CANTAB): The RTT provides measures of simple and choice movement and 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-alpha treatment and correlate with IFN-alpha-induced depression and fatigue.[114]

Digit Symbol Task (DST): 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. The test involves graphimotor speed, visual scanning and memory, with about half of the variance being accounted for by graphimotor speed, a third by visual scanning and 4-5% by memory.[115] This test is one of the most frequently used in neuropsychology and relevant norms and test-retest reliability have been well established.[112] Performance on the Digit Symbol Test has been found to correlate with

subcortical atrophy in disorders involving the basal ganglia including Huntington's disease and multiple sclerosis.[116,117]

Self-Report Assessments

Snaith-Hamilton Pleasure Scale (SHAPS): The SHAPS is a 14-item self-report scale that assesses hedonic tone.[121] 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.[122]

Multidimensional Fatigue Inventory (MFI): is a 20-item self-report instrument designed to measure fatigue, covering the dimensions General Fatigue (GF), Physical Fatigue (PF), Mental Fatigue (MF), Reduced Motivation (RM) and Reduced Activity (RA).[123] Separate scores for each of these five dimensions are generated from the MFI. We will only use the score from the RM dimension in this domain. The Reduced Motivation score from the MFI has been shown to highly correlate with reduced neural activation in the ventral striatum to a reward task during IFN-alpha administration as determined by fMRI.[124]

Motivation and Pleasure Scale: The mood and pleasure questionnaire is a recently-developed 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.[125] This scale will be used to assess self-reported changes in motivational anhedonic symptoms before and after inflammation blockade.

PANAS-X: The Positive Affect/Negative Affect Scale (PANAS) is a widely used measure of in-the-moment aspects of positive and negative affectivity[126], and will be used as an additional measure of self-reported changes in anhedonic symptoms following inflammation blockade.

Fatigue Severity Scale (FSS): is a 9-item self-report scale that is one of the best known and most used fatigue scales. It has high internal consistency, has good test–retest reliability and is sensitive to change with time and treatment. It has been used in multiple medically ill populations as well as in those with primary depression.[127,128]

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 consistent with current DSM nosology and that has been widely used as a self-report outcome measure of depression in treatment trials.[94,118]

Apathy Motivation Index (AMI): is an 18-item self-report index of apathy and motivation. It provides a useful means of probing different mechanisms underlying sub-clinical lack of motivation in otherwise healthy individuals. It covers motivation within three dissociable domains: cognitive, emotional/affective and behavioral. This index will take about 5 minutes to complete.

Apathy Evaluation Scale (AES): The AES addresses characteristics of goal directed behavior that reflects apathy including behavioral, cognitive and emotional indicators. It consists of 18 items; Items are scored on 4-point Likert scale with descriptors for the "self" version (not at all true, slightly true, somewhat true, very true). It takes approximately 10 minutes to complete.

Childhood Trauma Questionnaire (CTQ): The self-report questionnaire includes a 28-item test that measures 5 types of maltreatment – emotional, physical, and sexual abuse, and emotional and physical neglect and assesses individual's understanding of their childhood trauma. This questionnaire should take about 5 minutes to complete.

Dysfunctional Attitudes Scale – Short Form (DAS-SF): is a self-report questionnaire containing nine items taken from the original DAS, using item-response analysis to provide an efficient and accurate assessment of dysfunctional attitudes. It is designed to negative self-belief construct. This questionnaire should take about 5 minutes to complete.

HEXACO: HEXACO Personality Inventory-Revised, an instrument that assesses the six major dimensions of personality, Honesty-Humility, Emotionality, eXtraversion, Agreeableness (versus Anger), Conscientiousness, and Openness to Experience.

Perceived Stress Scale (PSS): The Perceived Stress Scale (PSS) is the most widely used psychological instrument for measuring the perception of stress. It is a measure of the degree to which situations in one's life are appraised as stressful. Items were designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The scale also includes a number of direct queries about current levels of experienced stress.

UCLA Loneliness Scale: A 20-item scale designed to measure one's subjective feelings of loneliness as well as feelings of social isolation. Participants rate each item on a scale from 1 (Never) to 4 (Often). This measure is a revised version of the original UCLA Loneliness Scale.

State-Trait Anxiety Inventory (STAI): The State-Trait Anxiety Inventory (STAI) is a commonly used measure of trait and state anxiety (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). It can be used in clinical settings to diagnose anxiety and to distinguish it from depressive syndromes. It also is often used in research as an indicator of caregiver distress
Form Y, its most popular version, has 20 items for assessing trait anxiety and 20 for state anxiety. State anxiety items include: "I am tense; I am worried" and "I feel calm; I feel secure." Trait anxiety items include: "I worry too much over something that really doesn't matter" and "I am content; I am a steady person." All items are rated on a 4-point scale (e.g., from "Almost Never" to "Almost Always"). Higher scores indicate greater anxiety.

Stress and Adversity Inventory (STRAIN): The STRAIN, or Stress
and Adversity Inventory, is a NIMH/RDoC-recommended instrument that efficiently and

reliably assesses a person's cumulative exposure to stress over the life course. The measure is entirely online and systematically inquires about a diverse array of acute life events (e.g., deaths of relatives, job losses, negative health events) and chronic difficulties (e.g., ongoing health problems, work problems, relationship problems, financial problems, etc.) that have implications for human health and well-being. Stressors occurring in early life (e.g., childhood maltreatment or neglect, parental loss/separation, etc.) are also queried in detail. Respondents are asked to rate the severity, frequency, timing, and duration of each stressor they endorse. The average time needed to complete the STRAIN is 25 minutes, with a range of approximately 18-30 minutes based on the population being interviewed.

Index of Race-Related Stress (IRRS): The IRRS is a 46-item instrument developed according to the theoretical framework of daily hassles (R. S. Lazarus & S. Folkman, 1984) and integrated with P. Essed's (1990) concept of everyday racism. The self-report survey takes approximately 10 minutes to complete.

Menstrual Cycle Questionnaire: Female participants may be asked to complete a short form with the following information: first day of last period, usual length of menstrual cycle, approximate onset of next period, and use of hormone contraceptives or hormone therapy. This self-report may be administered at the PI's discretion.

Demographics Questionnaire: Participants will be asked to complete a short form with basic demographic information like race, income level, and years of education.

Mobile Assessments

At the end of a participant's screening appointment, we may ask him or her to install a mobile application on their cell phone to complete assessments from home. The application is free of charge and can be deleted at any time if the subject decides not to continue. If the subject does decide not to continue, we will stop collecting data from him or her upon notice of withdrawal. Each day, following the screening visit (up to 2 weeks following the last study visit [30-day Follow-Up Call]) we may send a notification to the subject (via phone or email) to complete a variety of computer tasks and/or short surveys through the application. Subjects may be asked to complete experiments at various points during the day or will be free to complete experiments as frequently as desired, depending on the study design. These will include a subset of the same measures used for the behavioral components of the study (described in detail elsewhere in the protocol).

Insomnia Severity Index: Participants may be asked to complete a short form with information about their sleeping habits.

Laboratory and Biological Variables:

Blood collection: At the designated assessments, blood will be collected by venipuncture into EDTA-containing vacutainer tubes using standard sterile technique. Some research

blood may be collected by point of care testing. Plasma for the evaluation of plasma concentrations of cytokines and their receptors as well as CRP will be obtained by centrifugation of whole blood at 1000 x g for 10 minutes at 4°C. Plasma will be removed and aliquoted into siliconized polypropylene tubes and stored at -80°C until batch assay. Blood for mRNA analyses will be collected in Tempus tubes and stored at -80°C for later RNA extraction. In order to further assess immune cell profiles, we will also collect approximately 10 ml of whole blood in EDTA at room temperature for immune cell extraction at Infusion Visit, 24-Hour Visit, and 14-Day Visit.

Plasma cytokines and soluble cytokine receptors: Customized Fluorokine MAP Multiplex Human Biomarker Panels (R&D Systems, Minneapolis, MN) will be used to measure plasma IL-1ra, IL-6, sIL-6R, IL-10, TNF-alpha, sTNFR2 and MCP-1. These inflammatory markers were chosen based on their reliable changes in depression and/or their relationship to symptoms of anhedonia and corticostriatal function in subjects administered inflammatory cytokines and cytokine inducers.[14,16,129] Each determination requires 50-100 µl, and all samples will be assayed in duplicate according to manufacturer's instructions. Quality control plasma of both low and high cytokine concentrations will be included with every assay. The mean inter- and intra-assay coefficients of variation for control samples are reliably 10% or less.

C-reactive protein (CRP): Plasma CRP will be assessed with a high sensitivity turbidimetric assay. Sensitivity of the assay is rated at 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.

Gene Expression: RNA will be isolated from Tempus tubes using 5 Prime PerfectPure RNA Blood kit (Gaithersburg, MD). RNA quality will be verified using the Agilent Bioanalyzer, and only samples with a RIN factor >7.0 will be included. Samples will be analyzed in the Emory Integrated Genomic Core for gene expression analysis using an Illumina platform (Illumina, San Diego, CA). Raw microarray scan files from the Illumina HT-12 v4.0 arrays will be exported using the Illumina Beadstudio program and loaded into R for downstream analysis (http://www.R-project.org). The data will be transformed and normalized using the variance stabilizing normalization method.[130] An Illumina probe detection p-value of <0.01 in 5% of the individuals will be used to filter non-expressed transcripts which will be excluded from all subsequent analyses. To correct for confounding due to batch effects, the expression profiles will be normalized using ComBat, an empirical Bayes method for batch correction.[131] The results will be corrected for multiple testing using the permutation of regressor residuals test as implemented in the R package glmperm (http://cran.r-project.org/web/packages/glmperm/ index.html).[132]

Urine Samples: At one of the study visits where a urine sample is collected, up to 500mL of urine may be collected and sent to a collaborating laboratory led by study Co-Investigator Zhexing Wen. The collaborator will use cells from this urine sample to create induced pluripotent stem cells (iPSC). Over the past decade, researchers—including Dr. Wen (Wen, et al., 2014; Tang, et al., 2016; Wen et al., 2016)—have harnessed the power of cellular-reprogramming technologies to turn differentiated cells in the adult, like those found in urine or skin, into induced pluripotent stem cells (iPSCs).

Importantly, patient-derived iPSCs feature the same mutations as those found in the donor individual (Wen et al., 2016). Thus, researchers can use these cells to model a condition or disease in the context of an individual person. A description of how the cells will be used is laid out in detail in the consent form. If the amount of sample collected is not sufficient or a sample cannot be collected at a visit, additional samples may be collected at following visits while the participant is enrolled in the study. If the amount of sample collected is not sufficient or a sample cannot be collected at a visit, additional samples may be collected at following visits (if applicable) while the participant is enrolled in the study. If the sample collected is not sufficient or if a sample is not collected, participants may be asked to come back to the laboratory to provide a sample. Participants will have an opportunity to indicate consent for re-contact for this purpose in the study consent form.

Participants

140 medically stable male and female subjects between the ages of 21 and 65 will be included in this study. Subjects will meet criteria for current major depression (MD) or be recruited as healthy controls. MD subjects will be further screened based on levels of peripheral inflammation as determined by plasma CRP concentrations. The high CRP patient group (n=80) will have a CRP greater than 3mg/L at screening whereas the low CRP patient group (n=20) will have a CRP less than 2mg/L. Healthy controls (n=40) will also be recruited. Based on a screening failure rate of 30-50% and a dropout rate of 10%, we anticipate that including up to 350 subjects (65 full screens per year) will provide 140 subjects for final data analysis. Because this study is not a treatment trial, only subjects who complete all study procedures will be included in the analysis. This will be determined at the PI's discretion. Subjects will continue to be enrolled until 140 completers are included. There will be no exclusions for race/ethnicity and minority populations, and women will be actively recruited. Indeed, based on our previous work ~65% of the sample will be female. We will make active attempts to enroll minority patients in a proportion that is reflective of the areas surrounding Atlanta from which patients will be recruited. No patients will be enrolled from vulnerable populations, including neonates, children, prisoners, or institutionalized individuals.

Subjects may be recruited after completion of an "evaluation clinic protocol" within the Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine and from advertising in radio, television, Internet (Facebook) and print media. Alternatively, subjects may also express interest in the study directly to our lab personnel without first going through the Behavioral Immunology Research Evaluation Clinic. These participants may contact the lab through our master prescreen survey (see TReADLab_MasterPrescreen.pdf), phone, or email to request additional information. This information is provided on our website, clinicaltrials.gov and through our social media (Facebook, Instagram, Twitter) platforms. The evaluation clinic protocol evaluates potential subjects for appropriateness for participation in several on-going trials of various modalities for major depression. Most frequently, subjects come to these clinics as a result of referral from their primary physicians or as a result of seeing/hearing advertisements. An

overview of the study will initially be provided in person or 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 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 casebook containing relevant demographic data for the subject. Of note, because this casebook will contain personal identifiers (i.e. name), it will be kept separate from any data gathered as part of the study, and will be kept in a locked office. Some participants may elect to complete all screening and infusion-related visits under IRB Protocol #90667, Infliximab-Glutamate Study, PI Dr. Andrew Miller, and participate in the task-based MRI visits only as part of this study. These participants will sign a consent form (MRI-Only Version) under the current protocol (IRB87941) only for completion of the fMRI study visits (Baseline, 14-Day) and be paid under this protocol for those visits.

To compensate for time required for participation, inconvenience and travel expenses, high CRP MDD patients will receive $100 after completion of an outpatient 24-Hour visit (OPTIONAL), $60 for a 3-Day visit, and $200 for an optional 7-Day (OPTIONAL) visit post infusion. Subjects will receive $100 for the pre-screening visit, and $100 for screening procedures ($50 for each screening visit). Subjects will receive $410 for the infusion visit, and $310 for the study visit at 14 days (due to the MRI scan). Subjects may have the option of completing the MRI scan portion of the infusion visit one day before infusion. If they do so, the compensation for the MRI scan will be paid as a separate visit. During the visits where subjects undergo an MRI scan (on the day of infusion and at 14 days after infusion), subjects will have the opportunity to earn an additional $150 per visit based on choices and performance on the computer tasks. At visits 3 and 7 days after infusion, subjects will have the opportunity to earn an additional $50 based on task choices and performance, making the total amount possible from task performance at study visits $400.

MRI-Only Participants will be paid up to $800 for the MRI related visits and task compensation, detailed below.

Lab Visits	**Total Amount Earned**
Baseline Task-Based fMRI Visit	$250
14-Day Task-Based fMRI Visit	$250
Task Performance	**Total Amount Possible**
Baseline Task-Based fMRI Visit	Up to $150
14-Day Task-Based fMRI Visit	Up to $150
Minimum for Task-Based fMRI Study Procedures **Maximum for Task-Based fMRI Study Procedures**	**$500** **$800**

Healthy controls and low CRP depressed patients will receive $100 for the screening visit and $100 for the scan visit. Subjects will also have the opportunity to earn an addition $50 on the scan visit based on choices and performances in the computer tasks.

Individuals who choose to participate in the daily assessment portion of this study on their mobile phones, will receive an additional $2-10 dollars for each day of mobile assessments completed. The exact compensation received will be based on their choices and performance in the task(s) on the mobile app. If a participant completes 3 days of assessments, they could receive between $6 and $30. If they complete 7 days of assessments, they could receive between $14 and $70, and so on. Participants will be prompted to complete two days prior to the infusion visit and 8 days following infusion visit, making the max amount possible $100 based on task performance. Subjects who complete at least 8 out of 10 days will receive an additional $50 as a completion bonus. Total possible compensation from the mobile assessments is $150.

If requested by the PI or designee to return for follow-up assessments (i.e. repeat measures or confirm eligibility/safety), compensation will be provided at a rate of $25/hour.

Subjects who travel over 30 miles to participate in study-related activities may receive an additional payment as compensation for time and travel, at the discretion of the PI or designee.

Participants who agree to be re-contacted for an additional urine or blood sample and come to the laboratory to provide the sample will be compensated an additional $30 for their time.

Note: Participants may be compensated by cash, check or Emory ClinCard (see below) for their time and efforts in the study. If participants are compensated by check or Emory ClinCard, they will be asked to fill out a tax form, including mailing address and Social Security or Taxpayer Identification Number, in order to be reimbursed. Subjects will be informed that they are able to decline payment if there are concerns around confidentiality, or that they can talk to the study team to see if there are other payment options (i.e., cash). If subjects elect to receive payment via the Emory ClinCard, compensation earned from task performance will be paid at the end of study participation. If participants elect to be paid via check, all funds will be paid at the conclusion of the study.

- The payment card (Emory ClinCard) is a prepaid debit card issued to participants for free. It can be used exactly like a Mastercard. Study staff load money onto the individual's card electronically every time they need to be paid. The card scheme is run by Greenphire, an independent company specializing in payments for research studies and clinical trials. To issue the card, we need to provide Greenphire with some of the participant's personal information including SSN, Name, DOB, and Address. Banks and other financial institutions can access this information if they need to verify the

participant's identity when using the card. If a participant wants to receive e-mail or text alerts when payments are made, we will ask them to provide an e-mail or phone number. All of this information will be stored on computers owned by Greenphire. Greenphire will not have access to any other information collected during this study. Full instructions about using the card will be provided to the participant when it is issued.

- It will be explained to the participant that payment by check would involve study-related mail coming to his or her house, which may be seen by others in the household.

Visit	**Total Amount Earned**
PRE-SCREENING	$100
Visit Total	$100
SCREENING VISIT(S)	
Visit A	$50
Visit B	$50
Visit Total	$100
INFUSION VISIT(S)	
fMRI	$250
Infusion	$100
Scheduled Assessment	$60
Visit Total	$410
24-HOUR VISIT (OPTIONAL)	
Scheduled Assessments	$100
Visit Total	$100
3-DAY VISIT	
Scheduled Assessments	$60
Visit Total	$60
7-DAY VISIT (OPTIONAL)	
Scheduled Assessments	$200
Visit Total	$200
14-DAY VISIT	
fMRI	$250
Scheduled Assessments	$60
Visit Total	$310
TASK PERFORMANCE	
Infusion Visit	Up to $150
3-Day Visit	Up to $50
7-Day Visit (OPTIONAL)	Up to $50
14-Day Visit	Up to $150
Assessment Total	Up to $400
MOBILE APP (Optional)	
Pre-Infusion Assessments (2 Days)	$4-20
Post-Infusion Assessments (8 Days)	$16-80
80% Completion Bonus (8 of 10 Days)	$50
Assessment Total	Up to $150
Minimum Over Course of Study	**$880**
Maximum Over Course of Study	**$1,730**

The inclusion and exclusion criteria for this study were formed on the basis of two general concerns: participant safety and data quality. Criteria related to participant safety (as indicated in the table below) will be assessed for each enrolled participant using study-specific eligibility checklists. These can be accessed through the eIRB smartform for this study. For data quality criteria, assessments may rely on participant self-report or observations by the PI or designee. In certain cases, not all data quality criteria are necessary, and exclusion criteria that solely affect data quality may be waived at the discretion of the PI or his designee. Inclusion and exclusion criteria that ONLY affect data quality (as indicated below) will be enforced at the discretion of the PI or designee. Inclusion/Exclusion criteria related to participant safety will never be waived.

Inclusion Criteria for High CRP Depressed Subject Safety

1. All subjects will be fully ambulatory and in good medical health.
    a. Note: By DSM-V definition of depression, subjects will report impairment in ability to carry out daily activities as a result of their major depression.
2. Subjects will be able to read and understand English.
3. Women must be postmenopausal (no menstrual period for a minimum of 2 years) or surgically sterilized and/or have a negative serum pregnancy test on entry in the study and negative urine pregnancy tests throughout the study (performed at each visit after Screening). Additional serum pregnancy tests will be completed at the Infusion and 14-Day Visits.
4. Men and women of childbearing potential must use adequate birth control measures (e.g., abstinence, oral contraceptives, intrauterine device, barrier method with spermicide, implantable or injectable contraceptives or surgical sterilization) for the duration of the study and should continue such precautions for 6 months after receiving the last infusion. Participants will also be required to abstain from sexual intercourse for two weeks prior to the schedule infusion visit. Appropriate methods will be documented and on a Birth Control Method Form and signed by both the participant and the PI, NP, or designee (see Birth Control Method Form).
5. The following are considered eligible according to the following tuberculosis (TB) screening criteria:

    a. Have no history of latent or active TB prior to screening.

    b. Have no signs or symptoms suggestive of active TB upon medical history and/or physical examination.

    c. Have had no recent close contact with a person with active TB or, if there has been such contact, will be referred to a physician specializing in TB to undergo additional evaluation to rule out infection. The candidate will be excluded from study participation if the specialist diagnoses active TB and or determines TB treatment is warranted.

d. Have a chest radiograph (both posterior-anterior and lateral views), taken within 3 months prior to the first administration of study agent and read by a qualified radiologist, with no evidence of current active TB or old inactive TB.

e. History of negative PPD test; or documentation of a negative blood test (Quantiferon-TB-Gold). Any candidate testing positive for tuberculosis in the medical screening evaluation, will be excluded from study participation.

Inclusion Criteria for High CRP Depressed Data Quality

1. Patients will have a primary diagnosis of DSM-V MD current, or Bipolar, depressed type as diagnosed by the SCID-V
    a. Individuals meeting criteria for both PTSD and MDD will only be included if depression is the primary diagnosis.
2. Subjects will have a CRP of > 3 mg/L.
3. Subjects will be required to be off all antidepressant therapy for a period of at least 8 weeks prior to the baseline visit.
    a. Note: Subjects must agree to maintain this treatment status until the final assessment is complete.
4. Subjects will be between the ages of 21-65.

6. Able to comfortably fit in MRI scanner.

Exclusion Criteria for High CRP Depressed Subject Safety

1. Subjects will be excluded for any prior use of a TNF-alpha antagonist (i.e. etanercept, infliximab, adalimimub) and/or use of any other immunosuppressant agent (i.e. systemic corticosteroids or anti-proliferative agents such as methotrexate) within one year of study entry.
2. Subjects taking more than 2 mg of lorazepam (or equivalent benzodiazepine) for one month daily at the time of study participation will be excluded.
3. Subjects will be required not to use anti-inflammatory agents, non-steroidal anti-inflammatory agents (NSAIDs) (excluding 81mg of aspirin), glucocorticoid containing medicines or statins, or COX-2 inhibitors during the study as these agents may interfere with assessment of the relationship between inflammatory markers and treatment response.
    a. Note: Acetaminophen will be allowed.
4. Potential subjects will be excluded for a <u>history</u> of any of the following conditions:
    a. Abnormal electrocardiogram
    b. Auto-immune condition as confirmed by laboratory testing (i.e. rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, lupus)
    c. History of significant infectious sequelae, including but not limited to, abscess or sepsis

d. Infection within one month prior to screening that required antibiotic or antiviral therapy
e. History of a cognitive disorder or ≤ 24 on the Mini-Mental State Exam (MMSE), unless otherwise approved by PI or his designee
f. Unstable cardiovascular or endocrinologic disease (as determined by physical examination and/or laboratory testing)
g. Any other current or past medical condition that might increase the risk of infliximab-related adverse events

5. Potential subjects will be excluded for any of the following conditions:
    a. Active suicidal ideation defined as a score of >3 on Columbia Suicide Severity Rating Scale (C-SSR).
        i. Note: PI will be consulted if score is > 1
    b. Suicide attempt within six months of study entry
    c. Schizophrenia or Schizoaffective Disorder
    d. Active eating disorder unless limited to binge eating in the context of a mood disorder and in absence of purging
    e. Comorbid PTSD and MDD where MDD is the secondary diagnosis
    f. History of any (non-mood related) psychotic disorder or active psychotic symptoms of any type
6. Subjects will have had no infectious illnesses for one month prior to infusion. Should a subject develop an infection (i.e. flu, upper respiratory viral infection) between screening and infusion, the infusion will be delayed until 4 weeks after resolution of symptoms. As noted above, patients with a chronic infectious condition or with a past history of serious infectious complications will be excluded.
7. Subjects will be excluded for any evidence on laboratory testing (or by history) of hematologic, renal or hepatic abnormality. Subjects will be excluded if the anti-nuclear antibody (ANA) test indicates the presence of a possible autoimmune disease.

Inclusion Criteria for Low CRP Depressed Patients & Health Controls for Safety

1. All subjects will be fully ambulatory and in good medical health.
    a. Note: By DSM-V definition of depression, subjects will report impairment in ability to carry out daily activities as a result of their major depression.
2. Subjects will be able to read and understand English.
3. Women must be postmenopausal (no menstrual period for a minimum of 1 year) or surgically sterilized and/or have a negative serum pregnancy test on entry in the study and negative urine pregnancy tests throughout the study (performed at each visit after the serum pregnancy test is completed).

Inclusion Criteria for Low CRP Depressed Patients & Healthy Controls for Data Quality

1. Patients will have a primary diagnosis of DSM-V MD current, or Bipolar, depressed type as diagnosed by the SCID-V

a. If subject is a Healthy Control, then no diagnosis for current disorder will be present
b. Low CRP patients meeting criteria for both PTSD and MDD will only be included if depression is the primary diagnosis.

2. Patients will have a CRP of < 2 mg/L.
    a. Healthy Controls will not be ruled out for CRP, but this information will be collected
3. Patients will be required to be off all antidepressant therapy for a period of at least 8 weeks prior to the baseline visit.
    a. Note: Subjects must agree to maintain this treatment status until the final assessment is complete.
4. Subjects will be between the ages of 21-65.

6. Able to comfortably fit in MRI scanner.

Exclusion Criteria for Low CRP Depressed Patients & Healthy Controls

1. Subjects will be excluded for any prior use of a TNF-alpha antagonist (i.e. etanercept, infliximab, adalimimub) and/or use of any other immunosuppressant agent (i.e. systemic corticosteroids or anti-proliferative agents such as methotrexate) within one year of study entry.

2. Subjects taking more than 2 mg of lorazepam (or equivalent benzodiazepine) for one month daily at the time of study participation will be excluded.

3. Subjects will be required not to use anti-inflammatory agents, non-steroidal anti-inflammatory agents (NSAIDs) (excluding 81mg of aspirin), glucocorticoid containing medicines or statins, or COX-2 inhibitors during the study as these agents may interfere with assessment of the relationship between inflammatory markers and treatment response.
    a. Note: Acetaminophen will be allowed.
4. Potential subjects will be excluded for a <u>history</u> of any of the following conditions:
    a. Auto-immune condition as confirmed by laboratory testing (i.e. rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, lupus)
    b. History of significant infectious sequelae, including but not limited to, abscess or sepsis
    c. Infection within one month prior to screening that required antibiotic or antiviral therapy
    d. History of a cognitive disorder or ≤ 24 on the Mini-Mental State Exam (MMSE), unless otherwise approved by PI or his designee
    e. Unstable cardiovascular or endocrinologic disease (as determined by physical examination and/or laboratory testing)
    f. Any other current or past medical condition that might increase the risk of infliximab-related adverse events
5. Potential patients will be excluded for any of the following conditions:
    a. Active suicidal ideation defined as a score of >3 on Columbia Suicide Severity Rating Scale (C-SSR).
        i. Note: PI will be consulted if score is > 1
    b. Suicide attempt within six months of study entry

c. Schizophrenia or Schizoaffective Disorder
d. Comorbid PTSD and MDD where MDD is the secondary diagnosis
e. Active eating disorder unless limited to binge eating in the context of a mood disorder and in absence of purging
f. History of any (non-mood related) psychotic disorder or active psychotic symptoms of any type

6. Subjects will have had no infectious illnesses for one month prior to study enrollment. Should a subject develop an infection (i.e. flu, upper respiratory viral infection) between screening and infusion, the infusion will be delayed until 4 weeks after resolution of symptoms. As noted above, patients with a chronic infectious condition or with a past history of serious infectious complications will be excluded.
7. Subjects will be excluded for any evidence on laboratory testing (or by history) of hematologic, renal or hepatic abnormality. Subjects will be excluded if the anti-nuclear antibody (ANA) test indicates the presence of a possible autoimmune disease.

All Subjects (High CRP MDD, Low CRP MDD, Healthy Controls): Study-Specific Exclusion Criteria

a. Have had any previous treatment with monoclonal antibodies or antibody fragments.

b. History of receiving human/murine recombinant products or a known allergy to murine products. A known allergy to murine product is an exclusion criterion.

c. Documentation of seropositive for human immunodeficiency virus (HIV). Any candidate testing positive for HIV, in the medical screening evaluation, will be excluded from study participation.

d. Documentation of a positive test for hepatitis B surface antigen or hepatitis C. Any candidate testing positive for hepatitis B or hepatitis C, in the medical screening evaluation, will be excluded from study participation

e. Are unable or unwilling to undergo multiple venipunctures because of poor tolerability or lack of easy access.

f. Use of any investigational drug within 30 days prior to screening or within 5 half-lives of the investigational agent, whichever is longer.

g. Presence of a transplanted solid organ (with the exception of a corneal transplant > 3 months prior to screening).

h. Have a concomitant diagnosis or history of congestive heart failure.

i. Have a history of alcohol or substance abuse within the preceding 6 months that, in the opinion of the investigator, may increase the risks

associated with study participation or study agent administration, or may interfere with interpretation of results. (As determined by SCID)

j. Have a known history of serious infections (e.g., hepatitis, pneumonia, or pyelonephritis) in the previous 3 months.

k. Have or have had an opportunistic infection (e.g., herpes zoster [shingles], cytomegalovirus, *Pneumocystis carinii*, aspergillosis, histoplasmosis, or mycobacteria other than TB) within 6 months prior to screening.

l. Have a history of lymphoproliferative disease, including lymphoma or signs suggestive of possible lymphoproliferative disease such as lymphadenopathy of unusual size or location (e.g., nodes in the posterior triangle of the neck, infraclavicular, epitrochlear, or periaortic area), or splenomegaly.

m. Currently have any known malignancy other than the condition being treated or have a history of malignancy within the previous 5 years, with the exception of basal cell or squamous cell carcinoma of the skin that has been fully excised with no evidence of recurrence.

8. Any contraindication(s) to fMRI scanning (as assessed with standard MRI screening form from the Facility for Research and Education in Neuroscience (FERN)), including but not limited to:

    a. Cardiac pacemaker or pacemaker wires,

    b. Metallic particles in body,

    c. Vascular clips in the head,

    d. Previous neurosurgery,

    e. Prosthetic heart valves

    f. Claustrophobia.

    g. Notes: All MR-Safe Intra-Uterine Devices (IUDs) will be allowed

Assessments to Evaluate Eligibility

Laboratory values and other evaluations: The following medical evaluations will be obtained after consent is signed and prior to randomization:

- Comprehensive metabolic panel,
- HIV, Hepatitis B Surface Antigen, and Hepatitis C Antibody
- Complete blood count with differential
- C-Reactive Protein

- Serum pregnancy test
- TSH
- Urinalysis with microscopic
- Anti-nuclear antibody (ANA).
- Urine drug screen
- Quantiferon-TB-Gold blood test prior to randomization to rule out tuberculosis - High CRP Patients Only
- A complete physical examination will be performed and a medical history will be obtained prior to randomization.
- An electrocardiogram (EKG) – High CRP Patients Only
- A chest X-ray - High CRP Patients Only

The following psychiatric instruments will be administered after consent is signed and prior to randomization to evaluate eligibility:

- SCID
- MMSE
- ATRQ
- C-SSR
- Self-Reports: QIDS-16 SR, MFI, IDS-SR, PANAS-X, SHAPS, FSS, MAP-SR

Early Detection of Active Tuberculosis (High CRP Patients Only):

To aid in the early detection of TB reactivation or new TB infection during trial participation, subjects must be evaluated for signs and symptoms of active TB at scheduled visits or by telephone contact approximately every 8 to 12 weeks. The following series of questions is suggested for use during the evaluation:
"Have you had a new cough of > 14 days' duration or a change in a chronic cough?"

- "Have you had any of the following symptoms:

  – Persistent fever?
  – Unintentional weight loss?
  – Night sweats?"

- "Have you had close contact with an individual with active TB?" (If there is uncertainty as to whether a contact should be considered "close," a physician specializing in TB should be consulted.)

If the evaluation raises suspicion that a subject may have TB reactivation or new TB infection, an immediate and thorough investigation should be undertaken, including, where possible, consultation with a physician specializing in TB.

Investigators should be aware that TB reactivation in immunocompromised subjects may present as disseminated disease or with extrapulmonary features. Subjects with evidence of active TB must immediately discontinue study agent and should be referred for appropriate treatment.

Subjects who experience close contact with an individual with active TB during the conduct of the trial must have a repeat chest radiograph, a repeat tuberculin skin test, and, if possible, referral to a physician specializing in TB to determine the subject's risk of developing active TB and whether treatment for latent TB is warranted.

Note: The presence of comorbid dysthymia and/or an anxiety disorders (excluding Obsessive Compulsive Disorder and Post Traumatic Stress Disorder) will not disqualify subjects from enrollment as long as the mood disorder is the predominant diagnosis. Bipolar depressed patients will be included, given that these patients also commonly experience anhedonia and increased inflammation. Enrolled subjects may also have a comorbid personality disorder. Patients with stable medical conditions and on medications for those conditions will not be excluded.

History and physical:

At enrollment, a general medical history and comprehensive physical and neurological examinations will be obtained. Medical history evaluation will focus specifically on conditions that might represent an increased risk for receiving infliximab or that might confound results. Such conditions include, but are not limited to, tuberculosis, chronic infection, autoimmune condition, malignancy or neurological condition. Findings will inform eligibility.

Mandatory laboratory assessments:

The following labs will be evaluated to assess study eligibility prior to randomization: comprehensive metabolic panel, complete blood count with differential, antinuclear antibody (ANA), TSH, HIV, Hepatitis B Surface Antibody and Hepatitis C Antibody, urinalysis with microscopic, and a serum pregnancy test (if applicable). A tuberculosis blood test (Quantiferon-TB-Gold) will be drawn to rule out tuberculosis. A chest x-ray will be obtained, and an electrocardiogram will be obtained. Plasma concentrations of high-sensitivity CRP will be measured. A urine drug screen will also be performed.

Healthy Controls and Low CRP Patients will not obtain a TB-Gold test, chest x-ray, or EKG as they will not be infused with the study drug.

Other assessments:

The pretreatment evaluation will also include interviewer-administered questionnaires: the mood and substance abuse modules from the Structured Clinical Interview for DSM-V (SCID), the Bipolarity Index (BI), the Mini Mental State Examination (MMSE), the Columbia Suicide Severity Rating Scale (C-SSR), and the Massachusetts General Hospital Staging (MGH-S) questionnaire (to evaluate degree of treatment resistance). To qualify for entry, subjects will meet criteria for current major depression by SCID, will have a score of > 24 on the MMSE, will a score of <4 on the C-SSR, indicating no active suicidal ideation and will have a MGH-S score of 2 or greater.

Note: If a score >1 is recorded on the C-SSR scale, the PI will be notified to determine if further evaluation is needed.

Healthy Controls should have no current diagnoses as confirmed by the above measures.

Registration/Randomization.

Where and when to call:

Subjects may be recruited through the Behavioral Immunology Program in the Department of Psychiatry and Behavioral Sciences. In addition, the program has an ongoing recruitment program utilizing IRB-approved advertisements. Registration will occur in person either in the offices of the Behavioral Immunology Program or the laboratory of the PI. If questions arise subjects will be able to call the P.I., Michael T. Treadway, at [redacted]. Some participants may also express interest in the study directly to our lab personnel without first going through the Behavioral Immunology Research Evaluation Clinic. These participants may contact the lab through our master prescreen survey, phone, or email to request additional information. This information is provided on our website, clinicaltrials.gov and through our social media (Facebook, Instagram, Twitter) platforms.

Information to provide at entry:

Prior to entry, at the time of obtaining consent, it will be explained to high CRP depressed patients that if they meet entry criteria they will be randomized to receive one infusion of either infliximab or placebo (salt water) in a random manner (like flipping a coin) and that neither they nor personnel who interact with them will know which they received. It will be explained that they will be followed at 24 hours, 3 days, 7 days, 14 days, and one month after infusion for changes in depressive symptoms and/or for the development of physical side effects to the medication. Study visits may deviate from these time points or be split into multiple visits at the approval of the PI to accommodate scheduling needs. Subjects will be given the Medication Guide for REMICADE® (infliximab) from Janssen Biotech, Inc. as well as the Drug Summary for REMICADE® (infliximab) from the Physicians Desk Reference (PDR). A schedule of each subject's participation will be given at study entry. Names and numbers to contact with complaints or in case of an adverse event will be provided at this time. There are some medications that subjects will be asked to avoid during the study. Subjects will be given a take-home instruction sheet, which will explain in detail which medications to avoid. This sheet will also instruct subjects to contact study staff if they start antibiotics or any new medications while in the study. The sheet includes contact information for the study coordinators, and will also tell subjects what to do in the event of an after-hours concern or emergency.

Infusion (High CRP Depressed Patients Only)

Infusion protocol: Blinded infusions of infliximab or placebo will be conducted at the ACTSI or an infusion center at the Emory Clinic. Infusions will be performed following a standard protocol. Specifically, intravenous access will be obtained in a sterile manner and infliximab (5 mg/kg body weight) or placebo will be administered over an approximate 2-hour period. Subjects will be monitored during the infusion and for approximately 10-20 minutes (or longer if deemed clinically necessary) after completion for the development of anaphylaxis (allergic reaction). Significant anaphylactic reactions requiring treatment occur in less than 1% of patients receiving an initial dose of infliximab. All aspects of the infusion will be overseen by a trained nurse, and a physician will be available should subjects develop anaphylaxis during the infusion. A standard protocol is in place in the infusion center for the treatment of anaphylaxis.

Under advisement of the study physicians, NP, and nurses at the infusion site, subjects may be administered a premedication prior to the infusion to prevent a possible hypersensitivity reaction. Subjects may be administered one of the premedications listed below at least 30 minutes before the infusion.

1. Acetaminophen (Tylenol) 650mg PO
2. Diphenhydramine (Benadryl) 25mg or 50mg PO or IV
3. Famotidine (Pepcid) 20mg IV push

In the event of an infusion-related drug hypersensitivity reaction a standard protocol will be followed at the infusion site.

Blinding/Randomization/Placebo Preparation: A list containing the randomly generated sequence of assignments to infliximab or placebo will be maintained in the Emory University Investigational Drug Service pharmacy. Treatment allocation will occur when a patient is eligible for the study and the consent for randomization has been obtained. If a subject drops prior to receiving the initial infusion, he/she will not be included in data analyses and his/her randomization assignment will "roll over" to the next recruited subject. The research pharmacist with the Emory Investigational Drug Service will be responsible for preparing the infusion bag with either infliximab or normal saline based on the pre-determined order of the randomization list. Infusion bags with infliximab will be indistinguishable from bags with normal saline (placebo). Study personnel responsible for administering the infusion, conducting psychiatric evaluations, conducting medical assessments, drawing blood and performing lab analyses will be blinded to subject group assignment. In the event of a significant adverse reaction to the study medication, the study blind will be immediately broken by the unblinded study physician or research pharmacist and the subject will be referred for appropriate medical care. Such adverse reactions include, but are not limited to, anaphylaxis during infusion, serum sickness or development of symptoms of infection.

Graphical Display of Study Procedures

Assessment	Pre-Screening [4]	Screening: Part A or Part B[1,4]	Optional Scan Visit[3]	Infusion (Infliximab or Placebo)	Optional 24 hours	3 days	Optional 7 days	14 days	30 day F/U Call
Clinician-Rated Assessments									
SCID		**X**							
Bipolarity Index		**X**							
MMSE		**X**							
ATRQ		**X**							
Substance Use Assessment			**X**	X	X	X	X	X	
Adverse Events		**X**	**X**	X	X	X	X	X	X
C-SSR		**X**	**X**	X	X	X	X	X	
Self-Report Questionnaires									
IDS-SR, PANAS-X		**X**		X	X	X	X	X	
SHAPS, FSS, MAP-SR, MFI		**X**				X	X	X	
QIDS-16 SR	**X**								
Behavioral Assessments									
behEEfRT, Go No-Go, FTT, RTT, DST[2]		**X**				X	X	X	
Medical Assessments Related to Safety									
Medical F/U Assessment					X	X	X	X	
Physical Examination		**X**		X				X	
Medical History		**X**		X					
Vital signs		**X**		X	X	X	X	X	
Height, weight (BMI) waist circumference		**X**		X					
EKG, CXR		X							
Serum Pregnancy Test (if applicable)		**X**		X				X	
Urine Pregnancy Test (if applicable)			**X**	X	X	X	X	X	
Comprehensive Metabolic Panel		**X**		X (Optional)				X (Optional)	
HIV, Hepatitis B Surface Antigen, and Hepatitis C Antibody		**X**							
TSH		**X**							

Anti-nuclear Antibody (ANA)		**X**							
Quantiferon TB Gold		X							
CBC with differential		**X**		X	X	X	X	X	
Urinalysis with microscopic		**X**		X				X	
	Medical Assessments Related to Research								
Laboratory Testing		**X**	X	X	X	X	X	X	
Plasma CRP (assessed by finger stick and/or blood draw)	**X**	**X**	**X**	X	X	X	X	X	
Fluorokine MAP Multiplex Human Biomarker Panel, mRNA			X	X	X	X	X	X	
Immune Cell Analysis				X	X			X	
Urine drug screen		**X**	**X**	X	X	X	X	X	
	Other Procedures								
MRI scan (including fMRI tasks: EEfRT, Ad. MID, Gambles			**X**	X				X	
Emotional Memory Assessment (Encoding & Recall)		X				X	X		

[1]Following pre-screening, participants will be scheduled to complete one or more screening appointments. The procedures described under "Screening" may be divided among these visits depending on subject scheduling and preference, staff availability, and other factors. Screening visits will occur at the Emory Department of Psychology and/or the Emory Clinics and Emory University Hospital. If screening is performed in a single visit, it will occur at the locations listed above. Follow up appointments may deviate from the specified "3-Day", "7-Day", and "14-Day" dates or be split into multiple dates within a range approved by the PI in order to accommodate scheduling of the participant and study staff.

[2]Behavioral assessments may be skipped in the interest of time on visit days where visit length exceeds what was scheduled

[3]Subjects may be asked to or may prefer to complete the MRI scan portion of the infusion visit one day prior to the infusion.

[4]Bolded "X's" signify prescreening and screening procedures healthy controls and low CRP depressed controls will undergo.

Issues Related to Questionnaires, Rating Scales and Interviews:

1. A copy of each questionnaire that will be used in this study has been included with this IRB submission.

2. Time required for questionnaire completion: On average the SCID will require 90 minutes for completion. This may vary depending on the particular participant. Questionnaires and interviews for the screening visits will require approximately 3½ hours. Completion of questionnaires and interviews for the infusion visit through the visit at 14 days will take approximately 1½ hours. The QIDS-16 SR

will be completed at pre-screening. The SCID, Bipolarity Index, ATRQ, and MMSE will be completed once during screening. The C-SSR and an adverse events assessment will be completed all study visits other than pre-screening, unless a subject completes a screening or study visit over multiple days or the assessment is deemed redundant due to administering of a SCID in which case the C-SSR may not be administered at the PI's discretion. The SHAPS, FSS, MFI and MAP-SR will be completed at screening and at the 3, 7 and 14 day follow up visits. The IDS-SR and PANAS-X will be completed at screening, on the infusion day and each follow up visit. A brief substance use assessment will be completed on the day of infusion and at each study visit following this visit.

3. Volunteers (subjects) in this study who are in active psychiatric treatment during their involvement with this study will be asked to sign a release of information for the study PI to communicate important clinical changes, such as the development of suicidal ideation, to the primary clinician. Psychiatric follow-up following study completion will be with pre-existing primary physician for these subjects.

4. All information gathered for this study and the medical records generated will be kept locked with appropriate protection to maintain confidentiality. Research records will only be released with the subject's written permission. The subject's name will not appear elsewhere in any form and if publications evolve from the study, names will in no way be associated with the reports.

Reliability of Clinical Ratings:

Training on the SCID and other clinician rated assessments will be provided for relevant study personnel and interrater reliability will be established and maintained. New staff joining the study team will go through an apprenticeship for the ratings and training for reliability prior to performing independent ratings.

Safety Monitoring

As noted in Figure 1, all infusions will be conducted at the ACTSI at Emory University Hospital or an infusion center at the Emory Clinic Building A. Subjects will be closely monitored during and after infusions for the development of any adverse reactions. Standard protocols are in place for treatment of any adverse reactions. In addition, all subjects will have access to the study 24 hour pager number through the Emory Clinic operator to report adverse events to a study physician by phone following each infusion and will be evaluated weekly (or bi-weekly**)** in person thereafter. Finally, a designated physician member of the research team will be available 24 hours a day seven days a week by phone to study participants to address questions and evaluate potential adverse events.

Discontinuation of Study Agent

Study agent must be permanently discontinued if any of the following occur:

- Subject is deemed ineligible according to the TB screening criteria:
- A diagnosis of active TB is made.
- A subject receiving treatment for latent TB discontinues this treatment prematurely or is noncompliant with the therapy.

- A subject has symptoms suggestive of active TB based on follow-up assessment questions and/or physical examination, or has had recent close contact with a person with active TB, and cannot or will not continue to undergo additional evaluation.
- A subject undergoing continued screening has a chest radiograph with evidence of current active TB and/or a positive tuberculin skin test, unless active TB can be ruled out and appropriate treatment for latent TB can be initiated either prior to or simultaneously with the next administration of study agent and continued to completion.

Adverse Event Reporting and Follow-Up

An adverse event is any unfavorable and unintended sign, symptom, or disease (including an abnormal laboratory finding), temporally associated with the use of a study agent(s), whether or not related to the study agent(s), occurring at any time during the study (i.e., any time after informed consent is obtained). Should there be an adverse event, PI Dr. Treadway or Co-I Dr. Miller shall inform the IRB within the stipulated time frame. Recording should be done in a concise manner using standard, acceptable medical terms. The adverse event recorded should not be a procedure or a clinical measurement (i.e., a laboratory value or vital sign) but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement. Preexisting conditions identified during screening that worsen in severity or frequency during the study will be reported to the IRB within the stipulated time frame (a preexisting condition that does not worsen is not an adverse event). Further, a procedure or surgery is not an adverse event; rather, the event leading to the procedure or surgery is considered an adverse event and will be reported to the IRB within the stipulated time frame.

If, in the investigator's judgment, a clinically significant worsening from baseline is observed in any laboratory or other test parameter (e.g., electrocardiogram, angiogram), physical exam finding, or vital sign, a corresponding clinical adverse event will be reported to the IRB within the stipulated time frame. If a specific medical diagnosis has been made, that diagnosis will be recorded. For example, if clinically significant elevations of liver function tests are known to be secondary to hepatitis, "hepatitis" and not "elevated liver function tests" will be recorded. If the cause is not known, the abnormal test or finding should be recorded as an adverse event, using appropriate medical terminology (e.g., thrombocytopenia, peripheral edema, QT prolongation). The actual numeric value of a test result will not be recorded.

A serious adverse event is any adverse event occurring at any dose that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (i.e., a substantial disruption in a person's ability to conduct normal activities of daily living)
- Is a congenital anomaly/birth defect
- In addition, an important medical event that may not result in death, be life-threatening, or require/prolong hospitalization may be considered a serious adverse event when, based on appropriate medical judgment, it may jeopardize the subject

and/or may require medical or surgical intervention to prevent one of the outcomes listed above.

An unexpected adverse event is any adverse event, the specificity or severity of which is not consistent with the current Investigator Brochure.

A reasonably related adverse event is one that is, in the opinion of the investigator, possibly, probably or definitely related to study agent.

Any serious adverse event, regardless of relationship to the study agent, must be reported immediately. A serious adverse event must be reported if it occurs during a subject's participation in the study (whether receiving study agent or not) or within [30 days or 5-half lives of study agent – choose whichever is longer; if 5-half lives is chosen, put in the number of days that it represents, followed parenthetically by "5-half lives"] of receiving the last dose of study agent, whichever is longer.

Investigators are also advised that active TB is considered a reportable disease in most countries.

Any serious adverse event that is ongoing when a subject completes his/her participation in the trial must be followed until any of the following occurs:

- The event resolves or stabilizes;
- The event returns to baseline condition or value (if a baseline value is available);
- The event can be attributed to agent(s) other than the study agent, or to factors unrelated to study conduct.

Warnings and Precautions
REMICADE® (infliximab)

Serious infections, including sepsis and pneumonia, have been reported in subjects receiving TNF-blocking agents. Some of these infections have been fatal. Many of the serious infections in subjects treated with REMICADE have occurred in subjects on concomitant immunosuppressive therapy that, in addition to their underlying disease, could predispose them to infections. REMICADE should not be given to subjects with a clinically important, active infection. Caution should be exercised when considering the use of REMICADE in subjects with a chronic infection or a history of recurrent infection. Subjects should be monitored for signs and symptoms of infection while on or after treatment with REMICADE. New infections should be closely monitored. If a subject develops a serious infection, REMICADE therapy should be discontinued. Cases of tuberculosis, histoplasmosis, coccidioidomycosis, listeriosis, pneumocystosis, other bacterial, mycobacterial and fungal infections have been observed in subjects receiving REMICADE. For subjects who have resided in regions where histoplasmosis or coccidioidomycosis is endemic, the benefits and risks of REMICADE treatment should be carefully considered before initiation of REMICADE therapy.

Malignancies

In the controlled portions of clinical trials of some TNF-blocking agents including REMICADE, more malignancies have been observed in patients receiving those TNF-blockers compared with control patients. Caution should be exercised in considering REMICADE treatment in patients with a history of malignancy or in continuing treatment in patients who develop malignancy while receiving REMICADE.

Rare postmarketing cases of hepatosplenic t-cell lymphomas have been reported in adolescent and young adult patients with Crohn's disease treated with infliximab. All of these reports have occurred in patients on concomitant treatment with azathioprine or 6-mercaptopurine. The clinical course of this disease is very aggressive with a fatal outcome in most patients within 2 years of diagnosis. The causal relationship of hepatosplenic t-cell lymphoma to infliximab therapy remains unclear.

In a clinical trial exploring the use of REMICADE in patients with moderate to severe chronic obstructive pulmonary disease (COPD), more malignancies, the majority of lung or head and neck origin, were reported in REMICADE-treated patients compared with control patients. All patients had a history of heavy smoking (see ADVERSE REACTIONS, Malignancies). Prescribers should exercise caution when considering the use of REMICADE in patients with moderate to severe COPD.

Malignancies, including non-Hodgkin's lymphoma and Hodgkin's disease, have also been reported in patients receiving REMICADE during post-approval use.

Hepatotoxicity

Severe hepatic reactions, including acute liver failure, jaundice, hepatitis and cholestasis have been reported rarely in postmarketing data in patients receiving REMICADE. Autoimmune hepatitis has been diagnosed in some of these cases. Severe hepatic reactions occurred between two weeks to more than a year after initiation of REMICADE; elevations in hepatic aminotransferase levels were not noted prior to discovery of the liver injury in many of these cases. Some of these cases were fatal or necessitated liver transplantation. Patients with symptoms or signs of liver dysfunction should be evaluated for evidence of liver injury. If jaundice and/or marked liver enzyme elevations (e.g., ≥5 times the upper limit of normal) develops, REMICADE should be discontinued, and a thorough investigation of the abnormality should be undertaken. As with other immunosuppressive drugs, use of REMICADE has been associated with reactivation of hepatitis B in patients who are chronic carriers of this virus (i.e., surface antigen positive). Chronic carriers of hepatitis B should be appropriately evaluated and monitored prior to the initiation of and during treatment with REMICADE. In clinical trials, mild or moderate elevations of ALT and AST have been observed in patients receiving REMICADE without progression to severe hepatic injury.

Patients with Heart Failure

REMICADE has been associated with adverse outcomes in patients with heart failure, and should be used in patients with heart failure only after consideration of other treatment options. The results of a randomized study evaluating the use of REMICADE in patients with heart failure (NYHA Functional Class III/IV) suggested higher mortality in patients who received 10 mg/kg REMICADE, and higher rates of cardiovascular adverse events at doses of 5 mg/kg and 10 mg/kg. There have been post-marketing reports of

worsening heart failure, with and without identifiable precipitating factors, in patients taking REMICADE. There have also been rare post-marketing reports of new onset heart failure, including heart failure in patients without known pre-existing cardiovascular disease. Some of these patients have been under 50 years of age. If a decision is made to administer REMICADE to patients with heart failure, they should be closely monitored during therapy, and REMICADE should be discontinued if new or worsening symptoms of heart failure appear.

Hematologic Events

Cases of leukopenia, neutropenia, thrombocytopenia, and pancytopenia, some with a fatal outcome, have been reported in patients receiving REMICADE. The causal relationship to REMICADE therapy remains unclear. Although no high-risk group(s) has been identified, caution should be exercised in patients being treated with REMICADE who have ongoing or a history of significant hematologic abnormalities. All patients should be advised to seek immediate medical attention if they develop signs and symptoms suggestive of blood dyscrasias or infection (e.g., persistent fever) while on REMICADE. Discontinuation of REMICADE therapy should be considered in patients who develop significant hematologic abnormalities.

Hypersensitivity

REMICADE has been associated with hypersensitivity reactions that vary in their time of onset and required hospitalization in some cases. Most hypersensitivity reactions, which include urticaria, dyspnea, and/or hypotension, have occurred during or within 2 hours of REMICADE infusion. However, in some cases, serum sickness-like reactions have been observed in Crohn's disease patients 3 to 12 days after REMICADE therapy was reinstituted following an extended period without REMICADE treatment. Symptoms associated with these reactions include fever, rash, headache, sore throat, myalgias, polyarthralgias, hand and facial edema and/or dysphagia. These reactions were associated with marked increase in antibodies to infliximab, loss of detectable serum concentrations of infliximab, and possible loss of drug efficacy. REMICADE should be discontinued for severe reactions. Medications for the treatment of hypersensitivity reactions (e.g., acetaminophen, antihistamines, corticosteroids and/or epinephrine) should be available for immediate use in the event of a reaction.

Neurologic Events

REMICADE and other agents that inhibit TNF have been associated in rare cases with optic neuritis, seizure and new onset or exacerbation of clinical symptoms and/or radiographic evidence of central nervous system demyelinating disorders, including multiple sclerosis, and CNS manifestation of systemic vasculitis. Prescribers should exercise caution in considering the use of REMICADE in patients with pre-existing or recent onset of central nervous system demyelinating or seizure disorders. Discontinuation of REMICADE should be considered in patients who develop significant central nervous system adverse reactions.

PRECAUTIONS

Autoimmunity
Treatment with REMICADE may result in the formation of autoantibodies and, rarely, in the development of a lupus-like syndrome. If a patient develops symptoms suggestive of a lupus-like syndrome following treatment with REMICADE, treatment should be discontinued.

Vaccinations
No data are available on the response to vaccination with live vaccines or on the secondary transmission of infection by live vaccines in patients receiving anti-TNF therapy. It is recommended that live vaccines not be given concurrently.

Pregnancy Category B
Since infliximab does not cross-react with TNFα in species other than humans and chimpanzees, animal reproduction studies have not been conducted with REMICADE. No evidence of maternal toxicity, embryotoxicity or teratogenicity was observed in a developmental toxicity study conducted in mice using an analogous antibody that selectively inhibits the functional activity of mouse TNFα. Doses of 10 to 15 mg/kg in pharmacodynamic animal models with the anti-TNF analogous antibody produced maximal pharmacologic effectiveness. Doses up to 40 mg/kg were shown to produce no adverse effects in animal reproduction studies. It is not known whether REMICADE can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. REMICADE should be given to a pregnant woman only if clearly needed.

Nursing Mothers
It is not known whether REMICADE is excreted in human milk or absorbed systemically after ingestion. Because many drugs and immunoglobulins are excreted in human milk, and because of the potential for adverse reactions in nursing infants from REMICADE, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.
Please see the latest version of the REMICADE package insert and investigator brochure for further information on adverse reactions, warnings, and precautions.

Data Analysis Plan

For all three aims, analyses will focus on a priori inflammatory markers CRP and IL-6. These two inflammatory markers were chosen based on their role in sample selection, response to infliximab and association with reward pathways in our preliminary data as well as published papers by our group[133] and meta-analyses of elevated inflammatory makers in depression.[134] We did not include plasma measures of TNF in this analysis because measurement of plasma TNF is confounded by infliximab, which binds to TNF and leads to arbitrarily high concentrations of TNF on ELISA. Instead, the role of TNF will be investigated using gene expression data examining TNF signaling pathways (See Aim 2 below).

<u>Aim 1: Determine the relationship between inflammation and corticostriatal circuit function in patients with major depression and high inflammation before and after an</u>

anti-inflammatory challenge. Hypothesis 1a: Mixed-effects models for repeated measures (MMRM) will first be employed to examine effects of group (infliximab vs. placebo), time and their interaction on neuroimaging data using an a Region-of- Interest (ROI) approach. For ROI analysis, as described above, first-level GLMs will be created in normalized space for each time-point for each task, and a priori functional ROI in the striatum and vmPFC will be defined for both main effects using the baseline scan. Anatomically-defined ROIs will also be examined. Parameter estimates will be extracted from these ROI for each time-point. Relevant covariates, including age, sex, race (white/non-white) and body mass index (BMI) as well as number of depressive episodes, length of current depressive episode (in months), treatment responsiveness (ATRQ), age of onset, bipolarity index score and family history of depression (yes/no) will be sequentially tested in the models for goodness of fit (Akaike Information Criterion) and included as appropriate. To examine possible neural markers of inflammation change outside of our a priori ROIs, an exploratory voxel-wise approach will also be used. For this analysis, pre-post change in two primary inflammatory markers (CRP and IL-6) will be regressed against whole-brain corrected maps (pFWE < 0.05) for primary contrasts as described above. Hypothesis 1b: A Psychophysiological Interaction Analysis[135] implemented through the gPPI toolbox (http://brainmap.wisc.edu/PPI) will be used to investigate connectivity between vmPFC and ventral striatum during effort-based decisions. Seed-regions will be based on two striatal ROIs corresponding to ventral and dorsomedial striatum that have been previously shown to exhibit robust resting state connectivity with aspects of vmPFC. These ROIs are drawn from a 17-network parcellation of corticostrial connectivity performed in a large (n = 1,000) sample[5]. After single-subject connectivity maps have been generated for each time point, difference maps will be generated reflecting the change connectivity strength before and after infliximab or placebo treatment. A paired t-test as implemented through SPM12 will be used to compare pre-post change in corticostriatal connectivity (whole-brain corrected, pFWE < 0.05).

Aim 2: Determine the temporal dynamics of change in inflammation and change in behavioral and clinical measures of motivational anhedonia in depressed patients before and after an anti- inflammatory challenge. Hypothesis 2: As above, initial analyses will focus on plasma CRP and IL-6. Random intercept linear mixed models will be used to assess whether change in these markers predicts change in self-reported anhedonic symptoms, effort expenditure (BehEEfRT) or reinforcement learning (PST) when controlling for relevant covariates as appropriate (see above). For each of these two inflammatory markers, four separate models will be employed to assess the association between each inflammatory marker at each time point and 1) composite self-report data, 2) behEEfRT performance (proportion of high effort choices) 3) Go learning performance (PST) and 4) No-Go learning performance. To control for multiple comparisons across these 8 models, alpha significance will be set to a Bonferroni-corrected p<0.00625. Given the rapid changes that will likely occur in inflammatory markers prior to changes in behavioral measures, the models described above will be repeated using a "lagged regressor" that will test the associations between gene-expression at time t against inflammation, symptom and behavioral measures at time t+1. Exploratory analyses will be used to test for any incremental predictive power of

additional collected cytokines and their receptors. For gene-expression analysis, an identical set of models will be run using gene-expression within the previously identified transcripts in TNF signaling pathways as described in Preliminary Study 9. In addition to these a priori transcripts, an exploratory gene expression analysis will be performed. Based on change in reported symptoms at week 2, participants will be divided into "responders" and "non-responders". Individuals who cannot easily be assigned to one group or the other will be excluded from the analysis. A list of differentially expressed genes (based on a 1.2 fold difference and a false discovery rate of $p<0.01$) will be generated between infliximab "responders" versus "non responders" in self-report measures. To compare differences in gene expression at baseline between responders versus non-responders within groups, we will use logistic regression, correcting for relevant covariates as described above and including differential expression of white blood cells as determined by the white blood cell count. In addition, we will use a transcript origin analysis (see below). For differentially expressed genes in responders versus non-responders as a function of group (infliximab vs. placebo), pathway analysis will be done using a number of tools including the WebGestalt WikiPathways tool and BibliosphereTM data mining software from Genomatix. Overrepresentation of specific transcription binding factors such as NF-kB in the promoters of the associated transcripts will be tested using the cREMaG interface (http://149.156.177.116/cremag/), TRAP (http://trap.molgen.mpg.de) and the TELIS database. (http://www.telis.ucla.edu).[136] Transcript origin analysis will also be used.[137] This analysis examines specific patterns of gene expression that are associated with immune cell subtypes and generates a cell origin diagnosticity score, which provides information on which cell types are contributing to changes in gene expression observed between responders and non-responders within groups.[137, 138] Expression levels within transcripts identified by the exploratory analysis will be additionally employed as predictors of change in EEfRT and PST performance, as described above. Finally, to test whether changes in gene-expression may serve as an "early indicator" of infliximab response, lagged regressor models will also be explored to determine whether early changes in these inflammatory variables predict subsequent changes in task performance.

Aim 3: Explore the inter-relationship among inflammation, corticostriatal circuit function and reward motivation using path analysis. Hypothesis 3: A final aim of the study is to explore multi-level path modeling between measures of inflammation, corticostriatal circuitry, and motivational anhedonia. Using a bootstrapped estimation procedure, we will test whether change in VS signal and vmPFC-VS connectivity mediate the relationship between change in CRP and/or IL-6 and change in motivational anhedonic symptoms. Bootstrapping will be used to provide an empirical estimate of the sampling distribution for indirect effects and will be used to generate confidence intervals for the purpose of inferential testing.[139] We will additionally test a model of moderated-mediation to assess whether mediation pathways are influenced by the presence of high vs. low baseline inflammation. Path analysis will be implemented using macros developed by Preacher and Hayes (http://quantpsy.org/medn.htm), which enable inclusion of covariates as well as possible moderators.

Power Analysis: For neuroimaging measures in **Aim 1**, the MID, fMRI-EEfRT and RL tasks have excellent power for generating main effect contrasts[140] and our sample is well above the minimum n=50 recommended for individual differences analysis in fMRI studies.[141] For the within (pre-post treatment) by between (infliximab vs. placebo) interaction, we have excellent power (99%) to detect a medium effect size of f = 0.25, and good power (84%) for smaller effect sizes (f = 0.15). For **Aim 2**, because data at multiple time-points are clustered within subject, effect sizes that ignore clustering may be inflated (i.e., design effects).[142] Consequently, power analysis is based on a conservative effect size estimate of slope = 0.3, for which we have adequate power (80%). Finally, **for Aim 3,** the use of bootstrapping to adjust for low sample sizes has received wide-acceptance in the literature[139], and will provide adequate power for partial mediation in the current sample.

Safety Issues and Safety Analyses: We recognize that a single-dose of infliximab is a potent anti-inflammatory challenge with the potential to result in immunosuppression. Nevertheless, in our previous study, we found no differences in adverse events in infliximab versus placebo- treated patients for up to 6 months after 3 infusions (published and unpublished data). Moreover, in more recent studies (derived from large pre- and post-marketing databases) infliximab and other TNF antagonists have not been clearly associated with increased risk of cancer, serious adverse cardiovascular events or adverse de novo neurologic adverse events. Given that all adverse event data is largely derived from patients who have received months to years of treatment with these drugs, we believe a single infusion of an anti-inflammatory challenge poses limited risk under the conditions of careful patient selection and monitoring. Adverse events (AEs) will be recorded and reported using standard Medra categories, and suicidality will be assessed at each visit using the C-SSR. Dropout rates by category (e.g., adverse events, SAE's, non-adherence, etc.) will be reported and compared using Fisher Exact Tests.

GENERAL RISKS AND DISCOMFORTS

There are 7 major areas of potential risk in the study, as outlined below.

Neuropsychiatric assessments: Neuropsychiatric assessments may uncover strong and potentially disturbing feelings about the subject's past or present emotional state.

Blood draws: The risks of blood drawing include discomfort, bruising, infection, bleeding, and fainting.

Infliximab infusion: Use of infliximab has been associated with a number of short and long term risks (in 10%-50% of subjects), including mild allergic reaction to the medication, stomach pain, nausea, diarrhea, heartburn, upper respiratory tract infections, sore throat, sinusitis, coughing, runny nose, rash, fatigue, fever, headache, joint pain, back pain, urinary tract infection and hypertension. It should be noted however that in our previous study of infliximab, there were no differences in the number or severity of adverse events in the infliximab group compared to placebo over the 12 weeks of the study and up to 6 months post study completion.[1](unpublished observations) Very rarely (less than 1%), more serious adverse events have been reported, including severe anaphylactic reaction to

the infusion, development of serum sickness following infusion, reactivation of tuberculosis, development of serious and occasionally life threatening infections, induction of autoimmunity, worsening of congestive heart failure, bone marrow suppression, optic neuritis, seizures, cerebral demyelination and development of lymphoma or other cancers including skin cancer. Of note, early data from pooled randomized clinical trials revealed increased malignancy including lymphoma with TNF inhibitors in rheumatoid arthritis (RA) leading to the current FDA black box warning.[2] Nevertheless, more recent data in RA patients (including analysis of more than 40,000 patients and 150,000 patient-years exposure) as well as patients with inflammatory bowel disease indicate that TNF inhibitors are not clearly associated with increased malignancy.[3,4] In addition, data from pooled databases of over 100,000 patients and post-marketing data reported to the FDA indicate that TNF-inhibitors are also not clearly associated with major adverse cardiovascular events or de novo neurologic events.[5,6] Finally, although there is a possibility of drug-drug interactions with infliximab, according to MicroMedex, an evidence-based drug information resource (used by Emory Healthcare), there are few drug-drug interactions with infliximab excluding other immunosuppressants, other monoclonal antibodies, and "moderate" interactions with the following psychotropic agents: phenytoin, thioridizine and pimozide (all of which are exclusionary – patients will be free of psychotropic medications).

Placebo treatment: Subjects in this study also have a 50% chance of receiving a placebo infusion. Risks of placebo include lack of efficacy relative to active antidepressant treatments that might lead to a worsening of depressive symptoms, including the development of suicidal ideation.

MRI scan: Undergoing fMRI scans 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 fMRI procedure.

Suicidality: Given the fact that patients are not on any standard antidepressant medications, it is possible that they may experience worsening of their symptoms over the course of the study including the experiencing of suicidal ideation. Appropriate measures will be taken if patients exhibit significant suicidal risk.

Confidentiality: Confidentiality of all subjects will be protected per institutional and NIH and other federal requirements, and as described in greater detail below. At screening, study clinicians' will discuss benefits and risks of participation as well as alternative options.

A number of procedures will be in place to prevent a breach of confidentiality from taking place. All potential subjects will be fully informed of their rights pertaining to disclosure of PHI in accordance with HIPAA regulations. Confidentiality will be maintained by assigning participants a study number and numerically coding all data. One hard copy file linking the code number with identifying information will be kept in separate locked file with direct access available to the PI only. All records and research

data will be kept in locked filing cabinets or computers. Only summaries of group data will be reported in any publications or presentations, with no identification of individuals. These precautions should serve to minimize legal risks to participants.

At the completion of the study, all MDD patients will be provided with a comprehensive list of treatment options for depression and related mental health issues. A copy of this information has been included as a separate attachment in our resubmission (please see "MDD_PostStudy_Treatment_Resource_List.doc").

The key investigators will meet quarterly to discuss any potential adverse event and side effects. We will involve the IRB for consultation if any additional potential risks arise. Adverse events and unanticipated problems involving risks to subjects or others will be reported to the IRB in accordance with reporting guidelines.
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 404-727-7541 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.

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."

Data and Safety Monitoring Plan (DSMP)

This study utilizes an anti-inflammatory pharmacological challenge paradigm to explore the relationship among inflammation, corticostriatal circuit function and motivational anhedonia in patients with MD. Because study subjects will be receiving an active pharmacologic medication that has rare potentially serious side effects, we have elected to utilize a Data and Safety Monitoring Board as part of our data and safety-monitoring plan. 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. Treadway, Dr. Miller or their designee will be immediately notified. Dr. Treadway is a licensed clinical psychologist and Dr. Miller a board-certified psychiatrist. Both have 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)

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 and 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 Emory DSMB review for this protocol will be once every six months based on IRB recommendations consistent with the assessed risk status of the study. Of note, based on a random internal audit (not for cause) of the previous infliximab trial, accolades were given for regulatory compliance and documentation of all adverse events as well as timely reporting and processing with the Emory DSMB and IRB.

Procedures and Responsibilities of the DSMB

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

Based on the information provided to the Emory DSMB, once every six months the DSMB will issue a report to the Emory IRB that summarizes the following: All serious and unexpected adverse events or other unanticipated problems that involve risk to study participants, and whether these appear related to the study- based interventions or research assessment protocols. Note that any serious adverse event (SAE) will be reported to the Emory IRB within 24 hours according to standard regulations.

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

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

Literature Cited

1. Schultz W. Multiple dopamine functions at different time courses. *Annu Rev Neurosci.* 2007;30:259-288.

2. Schultz W, Dickinson A. Neuronal coding of prediction errors. *Annu Rev Neurosci.* 2000;23:473-500.

3. Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. *Science.* 1997;275(5306):1593-1599.

4. Schultz W. Neuronal reward and decision signals: from theories to data. *Physiol Rev.* 2015;95(3):853-951.

5. Choi EY, Yeo BT, Buckner RL. The organization of the human striatum estimated by intrinsic functional connectivity. *J Neurophysiol.* 2012;108(8):2242-2263.

6. Treadway MT, Zald DH. Reconsidering anhedonia in depression: lessons from translational neuroscience. *Neurosci Biobehav Rev.* 2011;35(3):537-555.

7. Treadway MT, Zald DH. Parsing Anhedonia Translational Models of Reward-Processing Deficits in Psychopathology. *Current Directions in Psychological Science.* 2013;22(3):244-249.

8. Pizzagalli DA, Holmes AJ, Dillon DG, Goetz EL, Birk JL, Bogdan R, Dougherty DD, Iosifescu DV, Rauch SL, Fava M. Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. *Am J Psychiatry.* 2009;166(6):702-710.

9. Kumar P, Waiter G, Ahearn T, Milders M, Reid I, Steele JD. Abnormal temporal difference reward-learning signals in major depression. *Brain.* 2008;131(Pt 8):2084-2093.

10. Juckel G, Schlagenhauf F, Koslowski M, Wustenberg T, Villringer A, Knutson B, Wrase J, Heinz A. Dysfunction of ventral striatal reward prediction in schizophrenia. *Neuroimage.* 2006;29(2):409-416.

11. Gradin VB, Kumar P, Waiter G, Ahearn T, Stickle C, Milders M, Reid I, Hall J, Steele JD. Expected value and prediction error abnormalities in depression and schizophrenia. *Brain.* 2011;134(Pt 6):1751-1764.

12. Morris RW, Vercammen A, Lenroot R, Moore L, Langton JM, Short B, Kulkarni J, Curtis J, O'Donnell M, Weickert CS. Disambiguating ventral striatum fMRI-related bold signal during reward prediction in schizophrenia. *Mol Psychiatry.* 2011;17(3):280-289.

13. Felger JC, Mun J, Kimmel HL, Nye JA, Drake DF, Hernandez CR, Freeman AA, Rye DB, Goodman MM, Howell LL. Chronic Interferon-Œ± Decreases Dopamine 2 Receptor Binding and Striatal Dopamine Release in Association with Anhedonia-Like Behavior in Nonhuman Primates. *Neuropsychopharmacology.* 2013.

14. Capuron L, Pagnoni G, Drake DF, Woolwine BJ, Spivey JR, Crowe RJ, Votaw JR, Goodman MM, Miller AH. Dopaminergic mechanisms of reduced Basal Ganglia

responses to hedonic reward during interferon alfa administration. *Arch Gen Psychiatry.* 2012;69(10):1044-1053.

15. Eisenberger NI, Berkman ET, Inagaki TK, Rameson LT, Mashal NM, Irwin MR. Inflammation-induced anhedonia: endotoxin reduces ventral striatum responses to reward. *Biol Psychiatry.* 2010;68(8):748-754.

16. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry.* 2009;65(9):732-741.

17. Dantzer R, Meagher MW, Cleeland CS. Translational approaches to treatment-induced symptoms in cancer patients. *Nat Rev Clin Oncol.* 2012;9(7):414-426.

18. Zorrilla EP, Luborsky L, McKay JR, Rosenthal R, Houldin A, Tax A, McCorkle R, Seligman DA, Schmidt K. The relationship of depression and stressors to immunological assays: a meta-analytic review. *Brain Behav Immun.* 2001;15(3):199-226.

19. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctot KL. A meta-analysis of cytokines in major depression. *Biol Psychiatry.* 2010;67(5):446-457.

20. Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF, Haroon E, Miller AH. A Randomized Controlled Trial of the Tumor Necrosis Factor Antagonist Infliximab for Treatment-Resistant DepressionThe Role of Baseline Inflammatory BiomarkersInfliximab for Treatment-Resistant Depression. *JAMA Psychiatry.* 2013;70(1):31-41.

21. Treadway MT, Buckholtz JW, Schwartzman AN, Lambert WE, Zald DH. Worth the 'EEfRT'? The effort expenditure for rewards task as an objective measure of motivation and anhedonia. *PLoS ONE.* 2009;4(8):e6598.

22. Knutson B, Fong GW, Adams CM, Varner JL, Hommer D. Dissociation of reward anticipation and outcome with event-related fMRI. *Neuroreport.* 2001;12(17):3683-3687.

23. Pessiglione M, Seymour B, Flandin G, Dolan RJ, Frith CD. Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature.* 2006;442(7106):1042-1045.

24. Trivedi MH, Fava M, Wisniewski SR, Thase ME, Quitkin F, Warden D, Ritz L, Nierenberg AA, Lebowitz BD, Biggs MM, Luther JF, Shores-Wilson K, Rush AJ. Medication augmentation after the failure of SSRIs for depression. *N Engl J Med.* 2006;354(12):1243-1252.

25. Gold JM WJ, Prentice KJ, Morris SE, Heerey EA. Reward processing in schizophrenia: a deficit in the representation of value. *Schizophr Bull.* 2008;34(5):835-847.

26. Nutt D, Demyttenaere K, Janka Z, Aarre T, Bourin M, Canonico PL, Carrasco JL, Stahl S. The other face of depression, reduced positive affect: the role of catecholamines in causation and cure. *J Psychopharmacol.* 2007;21(5):461-471.

27. Shelton RC, Tomarken AJ. Can recovery from depression be achieved? *Psychiatr Serv.* 2001;52(11):1469-1478.

28. Salamone JD, Correa M, Farrar A, Mingote SM. Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology (Berl).* 2007;191(3):461-482.

29. Walton ME, Kennerley SW, Bannerman DM, Phillips PE, Rushworth MF. Weighing up the benefits of work: behavioral and neural analyses of effort-related decision making. *Neural Netw.* 2006;19(8):1302-1314.
30. Floresco SB, Ghods-Sharifi S. Amygdala-prefrontal cortical circuitry regulates effort-based decision making. *Cereb Cortex.* 2007;17(2):251-260.
31. Berridge KC, Robinson TE. Parsing reward. *Trends Neurosci.* 2003;26(9):507-513.
32. Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl).* 2007;191(3):391-431.
33. Schultz W DP, Montague PR. A neural substrate of prediction and reward. *Science.* 1997;275(5306):1593-1599.
34. Schott BH, Minuzzi L, Krebs RM, Elmenhorst D, Lang M, Winz OH, Seidenbecher CI, Coenen HH, Heinze HJ, Zilles K, Duzel E, Bauer A. Mesolimbic functional magnetic resonance imaging activations during reward anticipation correlate with reward-related ventral striatal dopamine release. *J Neurosci.* 2008;28(52):14311-14319.
35. D'Ardenne K MS, Nystrom LE, Cohen JD. BOLD responses reflecting dopaminergic signals in the human ventral tegmental area. *Science.* 2008;5867(319):1264-1267.
36. Frank MJ, O'Reilly RC. A mechanistic account of striatal dopamine function in human cognition: psychopharmacological studies with cabergoline and haloperidol. *Behav Neurosci.* 2006;120(3):497.
37. Frank MJ, Seeberger LC, O'Reilly RC. By carrot or by stick: cognitive reinforcement learning in parkinsonism. *Science.* 2004;306(5703):1940-1943.
38. Kravitz AV, Tye LD, Kreitzer AC. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nat Neurosci.* 2012;15(6):816-818.
39. Salamone JD, Correa M. The mysterious motivational functions of mesolimbic dopamine. *Neuron.* 2012;76(3):470-485.
40. Bardgett ME, Depenbrock M, Downs N, Points M, Green L. Dopamine modulates effort-based decision making in rats. *Behav Neurosci.* 2009;123(2):242-251.
41. Floresco SB, Tse MT, Ghods-Sharifi S. Dopaminergic and glutamatergic regulation of effort- and delay-based decision making. *Neuropsychopharmacology.* 2008;33(8):1966-1979.
42. Venugopalan VV, Casey KF, O'Hara C, O'Loughlin J, Benkelfat C, Fellows LK, Leyton M. Acute Phenylalanine/Tyrosine Depletion Reduces Motivation to Smoke Cigarettes Across Stages of Addiction. *Neuropsychopharmacology.* 2011.
43. Wardle MC, Treadway MT, Mayo LM, Zald DH, de Wit H. Amping Up Effort: Effects of d-Amphetamine on Human Effort-Based Decision-Making. *J Neurosci.* 2011;31(46):16597-16602.
44. Rushworth MF, Noonan MP, Boorman ED, Walton ME, Behrens TE. Frontal cortex and reward-guided learning and decision-making. *Neuron.* 2011;70(6):1054-1069.
45. Hunt LT, Kolling N, Soltani A, Woolrich MW, Rushworth MFS, Behrens TEJ. Mechanisms underlying cortical activity during value-guided choice. *Nat Neurosci.* 2012;15(3):470-476.

46. Hare TA, O'Doherty J, Camerer CF, Schultz W, Rangel A. Dissociating the role of the orbitofrontal cortex and the striatum in the computation of goal values and prediction errors. *J Neurosci.* 2008;28(22):5623-5630.
47. Rangel A, Camerer C, Montague PR. A framework for studying the neurobiology of value-based decision making. *Nat Rev Neurosci.* 2008;9(7):545-556.
48. Hauber W, Sommer S. Prefrontostriatal Circuitry Regulates Effort-Related Decision Making. *Cereb Cortex.* 2009.
49. Pizzagalli DA, Iosifescu D, Hallett LA, Ratner KG, Fava M. Reduced hedonic capacity in major depressive disorder: evidence from a probabilistic reward task. *J Psychiatr Res.* 2008;43(1):76-87.
50. Treadway MT, Bossaller NA, Shelton RC, Zald DH. Effort-based decision-making in major depressive disorder: a translational model of motivational anhedonia. *J Abnorm Psychol.* 2012;121(3):553.
51. Yang X-h, Huang J, Zhu C-y, Wang Y-f, Cheung EFC, Chan RCK, Xie G-r. Motivational deficits in effort-based decision making in individuals with subsyndromal depression, first-episode and remitted depression patients. *Psychiatry Res.* 2014;220(3):874-882.
52. Barch DM, Treadway MT, Schoen N. Effort, anhedonia, and function in schizophrenia: Reduced effort allocation predicts amotivation and functional impairment. *J Abnorm Psychol.* 2014;123(2):387.
53. Treadway MT, Peterman JS, Zald DH, Park S. Impaired effort allocation in patients with schizophrenia. *Schizophr Res.* 2014.
54. Fervaha G, Graff-Guerrero A, Zakzanis KK, Foussias G, Agid O, Remington G. Incentive motivation deficits in schizophrenia reflect effort computation impairments during cost-benefit decision-making. *J Psychiatr Res.* 2013;47(11):1590-1596.
55. Felger JC, Mun J, Kimmel HL, Nye JA, Drake DF, Hernandez CR, Freeman AA, Rye DB, Goodman MM, Howell LL. Chronic interferon-α decreases dopamine 2 receptor binding and striatal dopamine release in association with anhedonia-like behavior in nonhuman primates. *Neuropsychopharmacology.* 2013;38(11):2179-2187.
56. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, Knapp DJ, Crews FT. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia.* 2007;55(5):453-462.
57. Felger JC, Alagbe O, Hu F, Mook D, Freeman AA, Sanchez MM, Kalin NH, Ratti E, Nemeroff CB, Miller AH. Effects of interferon-alpha on rhesus monkeys: a nonhuman primate model of cytokine-induced depression. *Biol Psychiatry.* 2007;62(11):1324-1333.
58. Gentile A, Fresegna D, Federici M, Musella A, Rizzo FR, Sepman H, Bullitta S, De Vito F, Haji N, Rossi S. Dopaminergic dysfunction is associated with IL-1β-dependent mood alterations in experimental autoimmune encephalomyelitis. *Neurobiol Dis.* 2015;74:347-358.
59. Sarkar C, Basu B, Chakroborty D, Dasgupta PS, Basu S. The immunoregulatory role of dopamine: an update. *Brain, behavior, and immunity.* 2010;24(4):525-528.

60. Yan Y, Jiang W, Liu L, Wang X, Ding C, Tian Z, Zhou R. Dopamine Controls Systemic Inflammation through Inhibition of NLRP3 Inflammasome. *Cell.* 2015;160(1):62-73.
61. Harrison NA, Brydon L, Walker C, Gray MA, Steptoe A, Critchley HD. Inflammation causes mood changes through alterations in subgenual cingulate activity and mesolimbic connectivity. *Biol Psychiatry.* 2009;66(5):407-414.
62. Vichaya EG, Hunt SC, Dantzer R. Lipopolysaccharide Reduces Incentive Motivation While Boosting Preference for High Reward in Mice. *Neuropsychopharmacology.* 2014.
63. Nunes EJ, Randall PA, Estrada A, Epling B, Hart EE, Lee CA, Baqi Y, Mueller CE, Correa M, Salamone JD. Effort-related motivational effects of the pro-inflammatory cytokine interleukin 1-beta: studies with the concurrent fixed ratio 5/chow feeding choice task. *Psychopharmacology (Berl).* 2013:1-10.
64. Felger JC, Miller AH. Cytokine effects on the basal ganglia and dopamine function: the subcortical source of inflammatory malaise. *Front Neuroendocrinol.* 2014.
65. Couch Y, Anthony DC, Dolgov O, Revischin A, Festoff B, Santos AI, Steinbusch HW, Strekalova T. Microglial activation, increased TNF and SERT expression in the prefrontal cortex define stress-altered behaviour in mice susceptible to anhedonia. *Brain, behavior, and immunity.*29:136-146.
66. Thorne R, Hanson L, Ross T, Tung D, Frey Ii W. Delivery of interferon-Œ≤ to the monkey nervous system following intranasal administration. *Neuroscience.* 2008;152(3):785-797.
67. Capuron L, Ravaud A, Miller AH, Dantzer R. Baseline mood and psychosocial characteristics of patients developing depressive symptoms during interleukin-2 and/or interferon-alpha cancer therapy. *Brain Behav Immun.* 2004;18(3):205-213.
68. Capuron L, Miller AH. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacol Ther.* 2011;130(2):226-238.
69. Berridge KC. 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. *Physiol Behav.* 2009;97(5):537-550.
70. Pecina S, Smith KS, Berridge KC. Hedonic hot spots in the brain. *Neuroscientist.* 2006;12(6):500-511.
71. Cannon CM, Palmiter RD. Reward without dopamine. *J Neurosci.* 2003;23(34):10827-10831.
72. Hnasko TS, Sotak BN, Palmiter RD. Morphine reward in dopamine-deficient mice. *Nature.* 2005;438(7069):854-857.
73. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry.* 2009;65(9):732-741.
74. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med.* 2009;71(2):171-186.
75. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctot KL. A meta-analysis of cytokines in major depression. *Biol Psychiatry.* 2010;67(5):446-457.
76. Pincus T, Griffith J, Pearce S, Isenberg D. Prevalence of self-reported depression in patients with rheumatoid arthritis. *Br J Rheumatol.* 1996;35(9):879-883.

77. Dickens C, Creed F. The burden of depression in patients with rheumatoid arthritis. *Rheumatology.* 2001;40(12):1327-1330.
78. Blume J, Douglas SD, Evans DL. Immune suppression and immune activation in depression. *Brain Behav Immun.* 2011;25(2):221-229.
79. Raison CL, Miller AH. Is depression an inflammatory disorder? *Curr Psychiatry Rep.* 2011;13(6):467-475.
80. Mehta D, Raison CL, Woolwine BJ, Haroon E, Binder EB, Miller AH, Felger JC. Transcriptional signatures related to glucose and lipid metabolism predict treatment response to the tumor necrosis factor antagonist infliximab in patients with treatment-resistant depression. *Brain, behavior, and immunity.* 2013;31:205-215.
81. Hyman SE. Neuroscience, genetics, and the future of psychiatric diagnosis. *Psychopathology.* 2002;35(2-3):139-144.
82. Hyman SE. The diagnosis of mental disorders: the problem of reification. *Annu Rev Clin Psychol.* 2010;6:155-179.
83. Treadway MT, Buckholtz JW, Cowan RL, Woodward ND, Li R, Ansari MS, Baldwin RM, Schwartzman AN, Kessler RM, Zald DH. Dopaminergic mechanisms of individual differences in human effort-based decision-making. *J Neurosci.* 2012;32(18):6170-6176.
84. Treadway MT, Buckholtz JW, Zald DH. Perceived stress predicts altered reward and loss feedback processing in medial prefrontal cortex. *Front Hum Neurosci.* 2013;7:180.
85. Treadway MT, Grant MM, Ding Z, Hollon SD, Gore JC, Shelton RC. Early adverse events, HPA activity and rostral anterior cingulate volume in MDD. *PLoS ONE.* 2009;4(3):e4887.
86. Treadway MT, Waskom ML, Dillon DG, Holmes AJ, Park MTM, Chakravarty MM, Dutra SJ, Polli FE, Iosifescu DV, Fava M. Illness Progression, Recent Stress, and Morphometry of Hippocampal Subfields and Medial Prefrontal Cortex in Major Depression. *Biol Psychiatry.* 2014.
87. Raison CL, Borisov AS, Majer M, Drake DF, Pagnoni G, Woolwine BJ, Vogt GJ, Massung B, Miller AH. Activation of central nervous system inflammatory pathways by interferon-alpha: relationship to monoamines and depression. *Biol Psychiatry.* 2009;65(4):296-303.
88. Rushworth MFS, Kolling N, Sallet J, Mars RB. Valuation and decision-making in frontal cortex: one or many serial or parallel systems? *Curr Opin Neurobiol.* 2012;22(6):946-955.
89. Hartmann MN, Hager OM, Tobler PN, Kaiser S. Parabolic discounting of monetary rewards by physical effort. *Behav Processes.* 2013;100:192-196.
90. Buckholtz JW, Treadway MT, Cowan RL, Woodward ND, Benning SD, Li R, Ansari MS, Baldwin RM, Schwartzman AN, Shelby ES, Smith CE, Cole D, Kessler RM, Zald DH. Mesolimbic dopamine reward system hypersensitivity in individuals with psychopathic traits. *Nat Neurosci.* 2010;13(4):419-421.
91. Wu CC, Samanez-Larkin GR, Katovich K, Knutson B. Affective traits link to reliable neural markers of incentive anticipation. *Neuroimage.* 2014;84:279-289.
92. Reader BF, Jarrett BL, McKim DB, Wohleb ES, Godbout JP, Sheridan JF. Peripheral and central effects of repeated social defeat stress: Monocyte trafficking, microglial activation, and anxiety. *Neuroscience.* 2015.

93. Rutgeerts P, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Hanauer SB. Comparison of scheduled and episodic treatment strategies of infliximab in Crohn's disease. *Gastroenterology.* 2004;126(2):402-413.

94. Trivedi MH, Rush AJ, Ibrahim HM, Carmody TJ, Biggs MM, Suppes T, Crismon ML, Shores-Wilson K, Toprac MG, Dennehy EB, Witte B, Kashner TM. The Inventory of Depressive Symptomatology, Clinician Rating (IDS-C) and Self-Report (IDS-SR), and the Quick Inventory of Depressive Symptomatology, Clinician Rating (QIDS-C) and Self-Report (QIDS-SR) in public sector patients with mood disorders: a psychometric evaluation. *Psychol Med.* 2004;34(1):73-82.

95. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975;12(3):189-198.

96. First MB, Spitzer RL, Gibbon M, Williams JB. *Structured Clinical Interview for DSM-IV*. Washington DC: American Psychiatric Press; 1997.

97. Chandler GM, Iosifescu DV, Pollack MH, Targum SD, Fava M. RESEARCH: Validation of the Massachusetts General Hospital Antidepressant Treatment History Questionnaire (ATRQ). *CNS Neurosci Ther.* 2010;16(5):322-325.

98. Fava M. Diagnosis and definition of treatment-resistant depression. *Biol Psychiatry.* 2003;53(8):649-659.

99. Sachs GS. Strategies for improving treatment of bipolar disorder: integration of measurement and management. *Acta Psychiatr Scand.* 2004;110(s422):7-17.

100. Knutson B, Bhanji JP, Cooney RE, Atlas LY, Gotlib IH. Neural responses to monetary incentives in major depression. *Biol Psychiatry.* 2008;63(7):686-692.

101. Knutson B, Cooper JC. Functional magnetic resonance imaging of reward prediction. *Curr Opin Neurol.* 2005;18(4):411-417.

102. Knutson B, Fong GW, Bennett SM, Adams CM, Hommer D. A region of mesial prefrontal cortex tracks monetarily rewarding outcomes: characterization with rapid event-related fMRI. *Neuroimage.* 2003;18(2):263-272.

103. Samanez-Larkin GR, Gibbs SE, Khanna K, Nielsen L, Carstensen LL, Knutson B. Anticipation of monetary gain but not loss in healthy older adults. *Nat Neurosci.* 2007;10(6):787-791.

104. Sutton RS, Barto AG. *Reinforcement Learning: An Introduction*. Cambridge, MA: MIT Press; 1998.

105. Luce RD. *Individual choice behavior: a theoretical analysis*. New York, NY: Wiley; 1959.

106. Palminteri S, Justo D, Jauffret C, Pavlicek B, Dauta A, Delmaire C, Czernecki V, Karachi C, Capelle L, Durr A, Pessiglione M. Critical roles for anterior insula and dorsal striatum in punishment-based avoidance learning. *Neuron.* 2012;76(5):998-1009.

107. Chase HW, Fournier JC, Greenberg T, Almeida JR, Stiffler R, Zevallos CR, Aslam H, Cooper C, Deckersbach T, Weyandt S. Accounting for Dynamic Fluctuations across Time when Examining fMRI Test-Retest Reliability: Analysis of a Reward Paradigm in the EMBARC Study. 2015.

108. Wardle MC, Treadway MT, de Wit H. Caffeine increases psychomotor performance on the effort expenditure for rewards task. *Pharmacol Biochem Behav.* 2012;102(4):526-531.
109. Frank MJ, Moustafa AA, Haughey HM, Curran T, Hutchison KE. Genetic triple dissociation reveals multiple roles for dopamine in reinforcement learning. *Proceedings of the National Academy of Sciences.* 2007;104(41):16311-16316.
110. Frank MJ, Samanta J, Moustafa AA, Sherman SJ. Hold your horses: impulsivity, deep brain stimulation, and medication in parkinsonism. *Science.* 2007;318(5854):1309-1312.
111. Turi Z, Mittner M, Opitz A, Popkes M, Paulus W, Antal A. Transcranial direct current stimulation over the left prefrontal cortex increases randomness of choice in instrumental learning. *Cortex.* 2015;63:145-154.
112. Spreen O, Strauss, E. *A Compendium of Neuropsychological Tests*. New York: Oxford University Press; 1991.
113. Aparicio P, Diedrichsen J, Ivry RB. Effects of focal basal ganglia lesions on timing and force control. *Brain Cogn.* 2005;58(1):62-74.
114. Majer M, Welberg LA, Capuron L, Pagnoni G, Raison CL, Miller AH. IFN-alpha-induced motor slowing is associated with increased depression and fatigue in patients with chronic hepatitis C. *Brain Behav Immun.* 2008;22(6):870-880.
115. Joy S, Fein D, Kaplan E. Decoding digit symbol: speed, memory, and visual scanning. *Assessment.* 2003;10(1):56-65.
116. Bermel RA, Bakshi R, Tjoa C, Puli SR, Jacobs L. Bicaudate ratio as a magnetic resonance imaging marker of brain atrophy in multiple sclerosis. *Arch Neurol.* 2002;59(2):275-280.
117. Starkstein SE, Brandt J, Folstein S, Strauss M, Berthier ML, Pearlson GD, Wong D, McDonnell A, Folstein M. Neuropsychological and neuroradiological correlates in Huntington's disease. *J Neurol Neurosurg Psychiatry.* 1988;51(10):1259-1263.
118. Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The Inventory of Depressive Symptomatology (IDS): psychometric properties. *Psychol Med.* 1996;26(3):477-486.
119. Wildocher D, Ghozlan, A. The measurement of retardation in depression. In: Hindmarch I, Stonier, P.D., ed. *Human Psychopharmacology.* Vol 2. New York: John Wiley and Sons; 1989:1-22.
120. Bonin-Guillaume S, Sautel L, Demattei C, Jouve E, Blin O. Validation of the Retardation Rating Scale for detecting depression in geriatric inpatients. *Int J Geriatr Psychiatry.* 2007;22(1):68-76.
121. Smith RS, Maes M. The macrophage-T-lymphocyte theory of schizophrenia: additional evidence. *Medical Hypotheses.* 1995;45(2):135-141.
122. Leventhal AM, Chasson GS, Tapia E, Miller EK, Pettit JW. Measuring hedonic capacity in depression: a psychometric analysis of three anhedonia scales. *J Clin Psychol.* 2006;62(12):1545-1558.
123. Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J Psychosom Res.* 1995;39(3):315-325.
124. Capuron L, Pagnoni G, Drake DF, Woolwine BJ, Spivey JR, Crowe RJ, Votaw JR, Goodman MM, Miller AH. Dopaminergic mechanisms of reduced basal ganglia

responses to hedonic reward during interferon alfa administration. *Arch Gen Psychiatry.* 2012;69(10):1044-1053.

125. Llerena K, Park SG, McCarthy JM, Couture SM, Bennett ME, Blanchard JJ. The Motivation and Pleasure Scale–Self-Report (MAP-SR): Reliability and validity of a self-report measure of negative symptoms. *Compr Psychiatry.* 2013;54(5):568-574.

126. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol.* 1988;54(6):1063-1070.

127. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol.* 1989;46(10):1121-1123.

128. Dittner AJ, Wessely SC, Brown RG. The assessment of fatigue: a practical guide for clinicians and researchers. *J Psychosom Res.* 2004;56(2):157-170.

129. Brydon L, Harrison NA, Walker C, Steptoe A, Critchley HD. Peripheral inflammation is associated with altered substantia nigra activity and psychomotor slowing in humans. *Biol Psychiatry.* 2008;63(11):1022-1029.

130. Huber W, von Heydebreck A, Sultmann H, Poustka A, Vingron M. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. *Bioinformatics.* 2002;18 Suppl 1:S96-104.

131. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics.* 2007;8(1):118-127.

132. Potter DM. A permutation test for inference in logistic regression with small- and moderate-sized data sets. *Stat Med.* 2005;24(5):693-708.

133. Felger JC, Li Z, Haroon E, Woolwine B, Jung MY, Hu X, Miller AH. Inflammation is associated with decreased functional connectivity within corticostriatal reward circuitry. *Mol Psychiatry.* in press.

134. Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimäki M. Cumulative meta-analysis of interleukins 6 and 1 b, tumour necrosis factor a and C-reactive protein in patients with major depressive disorder. *Brain Behav Immun.* 2015;49:20.

135. Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ. Psychophysiological and modulatory interactions in neuroimaging. *Neuroimage.* 1997;6(3):218-229.

136. Cole SW, Yan W, Galic Z, Arevalo J, Zack JA. Expression-based monitoring of transcription factor activity: the TELiS database. *Bioinformatics.* 2005;21(6):803-810.

137. Cole SW, Hawkley LC, Arevalo JM, Cacioppo JT. Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes. *Proc Natl Acad Sci U S A.* 2011;108(7):3080-3085.

138. Felger JC, Cole SW, Pace TW, Hu F, Woolwine BJ, Doho GH, Raison CL, Miller AH. Molecular signatures of peripheral blood mononuclear cells during chronic interferon-alpha treatment: relationship with depression and fatigue. *Psychol Med.* 2011:1-13.

139. Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav Res Methods.* 2008;40(3):879-891.

140. Gitelman DR PW, Ashburner J, Friston KJ. Modeling regional and psychophysiologic interactions in fMRI: the importance of hemodynamic deconvolution. *Neuroimage.* 2003;19(1):200-207.
141. Braver TS, Cole MW, Yarkoni T. Vive les differences! Individual variation in neural mechanisms of executive control. *Curr Opin Neurobiol.* 2010;20(2):242-250.
142. Liu G, Liang K-Y. Sample size calculations for studies with correlated observations. *Biometrics.* 1997:937-947.
143. Gross, R., Matthews, I., Cohn, J., Kanade, T., & Baker, S. (2010). Multi-PIE. *Image and Vision Computing*, *28*, 807-813.
144. Hamilton, M. A rating scale for depression. *J. Neurol. Neurosurg. Psychiatry* **23**, 56-62 (1960).
145. Bastien CH, Vallières A, Morin CM. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. Sleep Med. 2001;2:297–307