Study Protocol

Title: Inflammation-Induced CNS Glutamate Changes in Depression

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1. Title: Inflammation-Induced CNS Glutamate Changes in Depression (Infliximab Glutamate Study)

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2. Précis/Abstract:

The proposed research is designed to determine the cause and effect relationship between inflammation and CNS glutamate as well as the relationship between CNS glutamate and specific symptoms. To accomplish these aims, we will administer a single infusion of either the TNF antagonist infliximab or placebo (n=30 per group) to patients with high inflammation (CRP≥3mg/L). A CRP≥3mg/L was chosen because it is considered high inflammation according to guidelines by the American Heart Association.[1] Moreover, a CRP≥3mg/L was associated with significantly increased basal ganglia glutamate and was associated with a clinical response to infliximab.[2, 3] Inflammatory biomarkers, basal ganglia glutamate as measured by MRS, and motivation and psychomotor activity will be assessed at baseline and days 1 and 3 and weeks 1 and 2 following infliximab or placebo administration. Inflammation is rapidly reduced (within 6 hours) following infusion, and there is evidence of improved motivation within 2 weeks of the first infusion (see Preliminary Data).[3, 4] Finally, as previously demonstrated, infliximab-treated depressed patients show a 4mg/L drop in plasma CRP within 1 week of infliximab infusion.

3. Introduction and Background:

Two emerging theories regarding the development of mood disorders involve excessive activation of inflammatory pathways and alterations in glutamate metabolism.[5-8] For example, increased inflammatory markers have been reliably found in the peripheral blood and cerebrospinal fluid (CSF) of patients with unipolar and bipolar depression.[5, 9, 10] Moreover, administration of inflammatory stimuli including interferon (IFN)-alpha, typhoid vaccination or endotoxin have all been shown to lead to behavioral changes that characterize depression including depressed mood, anhedonia, and psychomotor slowing.[11-16] In addition, inflammatory cytokines interact with virtually every pathophysiologic domain known to be involved in the regulation of mood including neurotransmitter metabolism, synaptic plasticity, neuroendocrine function and relevant neurocircuitry.[5, 17, 18] Finally, inhibition of inflammatory cytokines has been shown to lead to improvements in depressed mood, anhedonia, and psychomotor retardation in patients with increased inflammation associated with depression or medical illnesses such as autoimmune and inflammatory disorders as well as cancer.[3, 19, 20] Alterations in glutamate metabolism have also been implicated in mood disorders.[6] Indeed, a number of studies using magnetic resonance spectroscopy (MRS) have found alterations in glutamate and glutamate metabolite levels in multiple brain regions of depressed patients, with bipolar depressives showing relatively consistent elevations in CNS glutamate and unipolar depressed patients exhibiting more mixed results.[21-27] In addition, loss of glial elements including astrocytes and oligodendrocytes as well as loss of the transporters for excitatory amino acids, which are responsible for the reuptake and recycling of glutamate, are some of the most reliable changes found in post mortem brain tissue from patients with mood disorders.[6, 7, 28-30] Significant differences in methylation patterns specific for astrocytic dysfunction have also been associated with depressive psychopathology in postmortem brain samples.[31] Probably the most dramatic evidence of the role of glutamate in psychopathology of mood disorders is derived from the profound and rapid response of treatment resistant depressed patients to ketamine, an antagonist of the glutamate N-methyl-d-aspartate (NMDA) receptor.[32-34] Of note, data indicate that anhedonia may be an especially responsive symptom to ketamine administration.[35]

Inflammation and Glutamate Pathology may be Linked Cytokines Can Alter Glutamate Metabolism

Inflammatory cytokines have been shown to interact with glutamate pathways in several important ways that may contribute to altered glutamate metabolism. Inflammatory cytokines have been shown to decrease the expression of glutamate transporters (excitatory amino acid transporters – EAAT) on relevant glial elements (astrocytes and oligodendrocytes) and increase the release of glutamate from astrocytes via reverse efflux from the EAAT and increased secretion through the cysteine/glutamate

antiporter.[36-39] Of note, glutamate released by astrocytes has preferential access to extrasynaptic NMDA receptors which have been shown to decrease brain derived neurotrophic factor (BDNF) and increase excitotoxicity.[5, 40] Moreover, blockade of glutamate reuptake has been shown to lead to anhedonic-like behavior in rats.[41] Glutamine synthetase which converts glutamate to glutamine, potentially leading to a build-up of intracellular and extracellular glutamate, along with cytokine induction of nitrogen and oxygen free radicals, can lead to astrocyte dysfunction and death.[37, 42-44] Cytokines such as TNF can also reduce glutamate transporter expression on oligodendrocytes and in excess is directly toxic to these cells, further compromising glutamate reuptake.[37, 42, 44] Finally, inflammatory cytokines can activate the kynurenine pathway leading to production of quinolinic acid by activated microglia. Quinolinic acid has been shown to act as an agonist at the glycine-binding site of the NMDA receptor, but also can stimulate synaptosomal glutamate release and decrease astrocytic glutamate reuptake both in vitro and in inflammatory pathways and alterations in glutamate metabolism.[5-8] For example, increased inflammatory markers have been reliably found in the peripheral blood and cerebrospinal fluid (CSF) of patients with unipolar and bipolar depression.[5, 9, 10] Moreover, administration of inflammatory stimuli including interferon (IFN)-alpha, typhoid vaccination or endotoxin have all been shown to lead to behavioral changes that characterize depression including depressed mood, anhedonia, and psychomotor slowing.[11-16] In addition, inflammatory cytokines interact with virtually every pathophysiologic domain known to be involved in the regulation of mood including neurotransmitter metabolism, synaptic plasticity, neuroendocrine function and relevant neurocircuitry.[5, 17, 18] Finally, inhibition of inflammatory cytokines has been shown to lead to improvements in depressed mood, anhedonia, and psychomotor retardation in patients with increased inflammation associated with depression or medical illnesses such as autoimmune and inflammatory disorders as well as cancer.[3, 19, 20] Alterations in glutamate metabolism have also been implicated in mood disorders.[6] Indeed, a number of studies using magnetic resonance spectroscopy (MRS) have found alterations in glutamate and glutamate metabolite levels in multiple brain regions of depressed patients, with bipolar depressives showing relatively consistent elevations in CNS glutamate and unipolar depressed patients exhibiting more mixed results.[21-27] In addition, loss of glial elements including astrocytes and oligodendrocytes as well as loss of the transporters for excitatory amino acids, which are responsible for the reuptake and recycling of glutamate, are some of the most reliable changes found in post mortem brain tissue from patients with mood disorders.[6, 7, 28-30] Significant differences in methylation patterns specific for astrocytic dysfunction have also been associated with depressive psychopathology in postmortem brain samples.[31] Probably the most dramatic evidence of the role of glutamate in psychopathology of mood disorders is derived from the profound and rapid response of treatment resistant depressed patients to ketamine, an antagonist of the glutamate N-methyl-d-aspartate (NMDA) receptor.[32-34] Of note, data indicate that anhedonia may be an especially responsive symptom to ketamine administration.[35]

Inflammation and Glutamate Pathology may be Linked

Cytokines Can Alter Glutamate Metabolism

Inflammatory cytokines have been shown to interact with glutamate pathways in several important ways that may contribute to altered glutamate metabolism. Inflammatory cytokines have been shown to decrease the expression of glutamate transporters (excitatory amino acid transporters – EAAT) on relevant glial elements (astrocytes and oligodendrocytes) and increase the release of glutamate from astrocytes via reverse efflux from the EAAT and increased secretion through the cysteine/glutamate antiporter.[36-39] Of note, glutamate released by astrocytes has preferential access to extrasynaptic NMDA receptors which have been shown to decrease brain derived neurotrophic factor (BDNF) and increase excitotoxicity.[5, 40] Moreover, blockade of glutamate reuptake has been shown to lead to anhedonic-like behavior in rats.[41] Glutamine synthetase which converts glutamate to glutamine, potentially leading to a build-up of intracellular and extracellular glutamate, along with cytokine induction of nitrogen and oxygen free radicals, can lead to astrocyte dysfunction and death.[37, 42-44] Cytokines such as TNF can also reduce glutamate transporter expression on oligodendrocytes and in excess is directly toxic to these cells, further compromising glutamate reuptake.[37, 42, 44] Finally, inflammatory cytokines can activate the kynurenine pathway leading to production of quinolinic acid by activated microglia. Quinolinic acid has been shown to act as an agonist at the glycine-binding site of the NMDA receptor, but also can stimulate synaptosomal glutamate release and decrease astrocytic glutamate reuptake both in vitro and in vivo.[45-47] Taken together, these effects of inflammatory cytokines on glutamate metabolism, reuptake and release by astrocytes and oligodendrocytes as well as the fundamental integrity (and survival) of these glial elements

provide an intriguing intersection of the inflammation and glutamate hypotheses of mood disorders including depression. Clinical data supporting the notion that inflammatory cytokines can alter CNS glutamate metabolism have recently been generated by our group in both patients treated with the inflammatory cytokine interferon (IFN)-alpha and in depressed patients with increased inflammation as reflected by elevated concentrations of plasma C-reactive protein (CRP). Indeed, administration of IFN-alpha was found to increase glutamate as measured by the glutamate to creatine (Glu/Cr) ratio using MRS in the left basal ganglia and dorsal anterior cingulate cortex (dACC). The basal ganglia and dACC are brain regions that have been repeatedly implicated as targets of peripherally administered inflammatory stimuli including inflammatory cytokines as well as typhoid vaccination and endotoxin.[11, 12, 14-17] Of note, IFN-alpha-induced increases in glutamate in the basal ganglia in turn were correlated with IFN-alpha-induced depressive symptoms including anhedonia and psychomotor slowing.[48, 49] In addition, IFN-alpha-induced increases in TNF were correlated with increased basal ganglia glutamate,[48] suggesting a direct relationship between increased inflammatory cytokines and increased glutamate (as proposed in this application) and supporting the use of a TNF antagonist to reduce CNS glutamate as planned in Aims 1-3. Our recently published data (MDD) also indicate that increased inflammation as reflected by plasma CRP is associated with increased basal ganglia glutamate in both a linear and stepwise fashion, with MDD patients with a CRP>3mg/L showing significantly higher basal ganglia glutamate than patients with a CRP<1mg/L(see Appendix http://www.nature.com/mp/journal/vaop/ncurrent/full/mp2015206a.html).[2] Interestingly, increased basal ganglia glutamate was also associated with anhedonia and psychomotor slowing in MDD patients. Finally, pretreatment with ketamine has been shown to block the development of depressive-like behavior in mice as a result of the administration of the inflammatory stimulus lipopolysaccharide.[50] Surprisingly, ketamine had no effect on LPS-induced inflammatory activation in the brain. Taken together, these data provide convincing evidence across distinct experimental paradigms that increased inflammation may be linked to altered glutamate metabolism that is manifested as increased CNS glutamate as measured by MRS. Nevertheless, the data to date in humans is correlational in nature, and the opportunity exists to block inflammation in depressed patients with high inflammation and determine whether glutamate is decreased in a cause and effect manner. Relevant in this regard, is a recent study conducted by our group demonstrating that inhibition of TNF in depressed patients with high inflammation using a monoclonal antibody to TNF (infliximab) led to decreased anhedonia and psychomotor retardation, which are the two major symptoms that have been associated with increased basal ganglia glutamate in our previous studies.[2, 48, 49] Whether this effect of the TNF antagonist was a result of effects on CNS glutamate will be tested in Aims 1 and 2 of this proposal.

4. Objectives: (Primary and secondary aims)

Specific Aims: To establish a cause and effect relationship between increased inflammation and basal ganglia glutamate, we propose to administer an anti-inflammatory challenge to depressed patients with high inflammation and determine whether basal ganglia glutamate is decreased in association with increased motivation and psychomotor speed. Recent data suggest that inflammation leads to increased CNS glutamate which may contribute to behavioral alterations in patients with neuropsychiatric disorders including depression. Consistent with this notion, data from our group has shown that administration of the inflammatory cytokine interferon (IFN)-alpha to otherwise non-depressed subjects leads to increased glutamate in the basal ganglia as measured by magnetic resonance spectroscopy (MRS). Increased basal ganglia glutamate was in turn associated with alterations in research domain criteria (RDoC) involving Positive and Negative Valence systems including anhedonia and psychomotor retardation. Our data in patients with major depression also suggest that increased inflammation as reflected by peripheral blood C-reactive protein (CRP) is correlated with increased basal ganglia glutamate in association with anhedonia and decreased psychomotor speed. Taken together, these data suggest that inflammation may increase basal ganglia glutamate, leading to the development of depressive symptoms relevant to motivation and motor activity. Nevertheless, the data to date has been correlational in nature. Our prior data demonstrate that administration of the anti-inflammatory agent infliximab, a highly specific monoclonal antibody to TNF, powerfully reduces inflammation (e.g. CRP) and decreases symptoms of anhedonia and psychomotor retardation in depressed patients with high inflammation. Therefore, through the use of state-of-the-art MRS techniques and a biologic agent that can block inflammation (TNF antagonist) with limited off-target effects, the proposed research will test the hypothesis that increased inflammation leads to increased basal ganglia glutamate, which in turn is associated with changes in

behavior related to motivation and psychomotor performance. Accordingly, the following specific aims are proposed:

Aim 1) To determine whether inhibition of inflammation reduces basal ganglia glutamate following acute administration of the anti-inflammatory agent infliximab versus placebo in depressed patients with high inflammation. 60 medication-free depressed patients ages 21-65 with high inflammation (CRP ≥3mg/L) will be enrolled. Single-voxel MRS assessments of absolute glutamate in the basal ganglia and other regions of relevance including the dorsal anterior cingulate cortex (dACC) as well as the left medial parietal cortex (as a control region) will be assessed at baseline and on day 3 and week 2 after a single infusion of the
TNF antagonist infliximab or placebo. For exploratory analyses, additional metabolites will also be examined, and multivoxel chemical shift imaging (CSI) will be performed. **Hypothesis 1:** Compared to placebo, infliximab treated patients will exhibit decreased absolute glutamate in the basal ganglia on day 3 and week 2.

Aim 2) To determine the relationship between basal ganglia glutamate and anhedonia and psychomotor retardation following acute administration of the anti-inflammatory agent infliximab versus placebo in depressed patients with high inflammation. Objective and clinical measures of anhedonia and psychomotor retardation will be obtained in the subjects in Aim 1 at baseline and day 3 and week 2 after administration of infliximab or placebo. **Hypothesis 2:** Decreased basal ganglia glutamate concentrations will precede and ultimately be associated with improved objective and clinical measures of anhedonia and psychomotor retardation.

Aim 3) To determine the relationship between inflammatory markers and basal ganglia glutamate following acute administration of the anti-inflammatory agent infliximab versus placebo in depressed patients with high inflammation. Peripheral blood protein and gene expression markers of inflammation will be assessed at baseline and on post-infusion days 1 and 3 and weeks 1 and 2 along with central (cerebrospinal fluid) measures of protein inflammatory markers at week 2 in subjects recruited in Aim 1.

Hypothesis 3: Decreased peripheral and central markers of inflammation will precede and predict decreases in basal ganglia glutamate. Determination of dynamic, temporal relationships among inflammation, CNS glutamate and behavior will provide the foundation for biomarker-driven, intelligently-designed studies of novel therapeutic agents that target inflammation and/or glutamate for the treatment of alterations in motivation and motor activity in patients with depression and other neuropsychiatric disorders with increased inflammation.

5. Study design and methods: Two hundred male and female participants ages 21-65 will be enrolled in the study to obtain 60 qualified subjects. Written informed consent will be obtained from all patients before protocol-specified procedures are conducted. Subjects will be drawn from an ambulatory sample of patients with MDD.

Participant Selection

Inclusion Criteria:

a. willing and able to give written informed consent;
b. men or women, 21-65 years of age;
c. a primary diagnosis of DSM-V MDD, current, or Bipolar, depressed type as diagnosed by the SCID-V;
d. score of ≥14 on the Quick Inventory of Depressive Symptomatology (QIDS)-SR-16*[51] or score ≥ 15 on the Patient Health Questionnaire 9 item (PHQ-9)
e. absence of significant suicidal ideation defined using the Columbia Suicide Severity Rating Scale – Screen Version (CSSRS) as follows:

Yes on:	Q6 (<30d)	Q6 (>30d/lifetime)	Q6 (no)
Q3 (- Intent/- Plan)	Exclude**	Include*	Include
Q4 (+ Intent/- plan)	Exclude**	Exclude**	Include
Q5 (+ Intent/+ Plan)	Exclude**	Exclude**	Exclude**
Q6 (s/p SIB)	Exclude**	N/A	N/A
Q6 (>30d/lifetime) (Lifetime SIB)	N/A	Include	Include

*PI discretion or d/w BW, AHM or EH, ** = refer for Rx.

f. off all antidepressant or other psychotropic therapy (e.g. mood stabilizers, antipsychotics, anxiolytics, and sedative hypnotics) for at least 4 weeks prior to the baseline visit (8 weeks for fluoxetine)*. No patients will be removed from their psychotropic medications for the sole purpose of participating in the study.

g. sexually active participants are required to agree to abstain from sexual intercourse 14 days prior to study infusion and are required to use medically approved birth control methods as defined in the Birth Control Method Form below for the duration of the study and advised to delay conception for 6 months post infusion.

BIRTH CONTROL METHOD

Participant name _____

Sex Male___Female___

DOB (___/___/___)

Sexually active participants of childbearing potential must agree to use contraception or abstain from sexual activity as defined below.
Please initial all that apply below:

□ Female participants practice abstinence from sexual activity 14 days prior to date of study infusion.

□ Male and female participants agree to use a medically approved birth control method as defined below for at least 60 days post study infusion. Participants are advised to delay conception for 6 months post-infusion.

□ Female (patient or partner) not of reproductive potential

□ Removal of ovaries and/or uterus (when: _____)

□ Post-menopausal for at least 2 years (when: _____)

□ Medically approved contraception methods (participant or partner)
(Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception)

□ Abstinence from sexual activity that could result on pregnancy

□ Sterilization surgery
Males: vasectomy (when: _____)
Females: Bilateral tubal ligation (when: _____)

□ Condoms (male or female) with spermicide

□ Diaphragm or cervical cap with spermicide

□ Intrauterine device (IUD) (when: _____)

□ Hormone-based therapy, e.g. birth control pills, Norplant, or Dep-Provera

□ I have been informed of and fully understand the potential risks of the study drug (infliximab/inflectra) with pregnancy.

Participant signature _____ Date _____

PI/NP/designee signature _____ Date _____

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Exclusion Criteria: a. any active or history of autoimmune disorder (as confirmed by laboratory testing); b. history of tuberculosis (by history or as discovered by chest X-ray, skin testing or blood testing) or high risk of tuberculosis exposure; c. hepatitis B or C infection or human immunodeficiency virus infection (as established by laboratory testing); d. history of fungal infection; e. history of recurrent viral or bacterial infections, including dental, gum, and ear infections; f. history of any type of cancer; g. unstable cardiovascular, endocrinologic, hematologic, hepatic, renal, or neurologic disease (as determined by physical examination and laboratory testing); h. history of any (non-mood-related) psychotic disorder; active psychotic symptoms of any type; antisocial personality disorder as determined by a clinician; substance abuse/dependence within 6 months of study entry (as determined by SCID)*; i. active suicidal

plan as determined by a score >3 on item #3 on the HAM-D; j. an active eating disorder, unless limited to binge eating in the context of a mood disorder and in absence of purging; k. a history of a cognitive disorder or < 28 on the Mini-Mental State Exam*;[52] l. pregnancy or lactation; m. women of child bearing potential who are not using a medically accepted means of contraception; n. heterosexual males and their partners who do not agree to practice appropriate birth control; o. known allergy to murine products or other biologic therapies; p. chronic use of non-steroidal anti-inflammatory agents (NSAIDS), glucocorticoid containing medications or statins*; q. use of NSAIDS, glucocorticoids, or statins at any time during the study*; r. contraindication to MRI (see Human Subjects), s. previous organ transplant, and t. history of CNS trauma or active seizure disorder.* Due to the high co-morbidity between anxiety disorders and depression, we plan to include patients with anxiety disorders as long as depression is the primary diagnosis. Patients with stable medical conditions and on medications for those conditions will not be excluded. Finally, highly treatment resistant depressed patients who score >5 on the MGH Antidepressant Treatment Response Questionnaire (ATRQ) for current episode will be excluded, because these individuals may represent an extreme subgroup with a qualitatively distinct biology.[53] *unless otherwise approved by the PI.

Description of Study Procedures

Screening: Subjects potentially qualified for enrollment will undergo a screening evaluation after written informed consent. Screening will involve subject interview and evaluation, as well as obtaining collateral history and data from other relevant sources (i.e. medical records, referring physician). Screening will include the following assessments: (1) Assessment of past psychiatric history and current symptom severity using the Structured Clinical Interview for DSM-V (SCID-V), 24-item HDRS, QIDS-16 SR or PHQ-9, and a DSM V-based interview to rule out antisocial personality disorder (2) Assessment of past and current psychiatric treatment history using the ATRQ supplemented by information from medical records and referring physicians when available, (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 infliximab dose for patients randomized to the infliximab arm of the study), and (7) plasma CRP. The PI may request to perform a rapid CRP test at screening using the Diazyme hsCRP POC Test Kit. This kit produces rapid results using a finger prick method to collect 20µl of blood and is intended for the in vitro quantitative determination of C-reactive protein. Screening laboratory evaluations may be repeated at PI's or PI designee's discretion. Additional assessments will be conducted if more than 4 weeks from screening, unless otherwise approved by the PI, study physician or PI's designee.

Baseline: Baseline will occur over 2 days, including MRS, blood sampling and behavioral and neurocognitive assessments on Day 1 (Visit 1), and randomization to infliximab versus placebo on Day 2 (Visit 2). Vital signs, safety labs, urine toxicology and adverse events will be collected as indicated in Table 1.

Table 1A: Schedule of Study Procedures and Assessments

Assessments	Intake	Screening	Baseline*		Day 1	Day 3	Week 1	Week 2		30 Day F/U Phone Call
			Visit 1	Visit 2				Visit 1	Visit 2 (Optional)	
Intake forms and assessments: Consent, HIPPA tracking, Release of Information**, Consent of Means of Communication**, Participant Payment Form**, MRI Screening Form**, Clinician-Assisted QIDS-16 SR** or Patient Health Questionnaire PHQ-9**, Medication Avoidance List**, Lumbar Puncture Information Guide, Schedule Quick Guide	X									
Psychiatric screening: WRAT-3**, MMSE**, HAM-D**, ATRQ, SCID V**, CSSRS, Clinical Interview Review [to be done in place of imported psych assessments from prior study]		X								

Laboratory Screening: Anti-nuclear AB**, C-reactive protein, TSH, Hepatitis B surface antigen**, Hepatitis C antibody**, HIV antigen/antibody**, QuantiFERON-TB Gold or TB Gold Plus, Hemoglobin A1c, Diazyme hsCRP POC Test Kit [if necessary to verify CRP], EKG, CXR		X								
Serum pregnancy test		X		X				X		
Medical History and Physical Examination or medical update		X	X	X	[X]	X	[X]	X	[X]	X
Adverse events, Concomitant medications [Con. Med. not completed at 30 day F/U phone call only]		X	X	X	[X]	X	[X]	X	[X]	X
Vital Signs, weight, height [screen only], waist-hip ratio [screen only]		X	X	X	[X]	X	[X]	X	[X]	
Routine Labs: CBC with differential, urinalysis w/ micro, comprehensive metabolic panel^{0}		X		X			[X]	X	[X]	
Urine toxicology		X	X	X	[X]	X	[X]	X	[X]	
Psychiatric baseline: Demographic data**, Childhood Trauma**, Bipolarity Index**			X							
Infusion (Infliximab or placebo)				X						
Estrogen/progesterone				X						
Urine pregnancy test kit			X			X	X	X		
Research blood: Cytokine Multiplex, mRNA, Plasma CRP			X	X	[X]	X	[X]	X		
Clinician-Administered: SHAPS-C, CSSRS^{0}, SRRS^{0}, HAM-A^{0}			X			X		X		
Self-Report: MFI, MAP, IDS-SR, BAI, PCL-5***, STAI-State			X			X		X		
Neurocognitive Tests: FTT, RTT, DSST, Trails A			X			X		X		
Computer Assessments: Gamble, Go No-Go, RLT, SLT			X					X		
MRS			X			X		X		
PT, PTT								[X]		
Lumbar Puncture (Optional)									[X]	

*Baseline will occur over 2 visits: Visit 1: MRS, blood and Symptom Domain Assessments; Visit 2: Infusion; see below for abbreviations, **Unless prior documentation available from previous study, ^{0}Screening (or Baseline 1) and Week 2 only, [ ] indicates option to complete assessment, *** PCL-5 (with LEC) at Baseline 1 only, and PCL-5 (without LEC) at Day 3 and Week 2

Table 1B: Participants in the combination study will complete the above schedule in addition to the adapted version below:

OPTIONAL: Combination Study

Assessments	Intake	Screening	Baseline*		Day 1	Day 3	Week 1	Week 2		30 Day F/U Phone Call
Self-Report: PANAS-X, IDS-SR			X		[X]	X	[X]	[X]		
Behavioral Computer Task: behEEfRT (in place of Gamble task)			X			X	[X]	[X]		

Infliximab Administration: The dosing protocol and scheduling of the infliximab infusions for this proposal was derived from the standard intravenous regimen for the treatment of inflammatory bowel disease.[54] Independent pharmacists will dispense either infliximab or placebo in a 250ml saline bag according to a computer-generated randomization list provided by the study statistician. The placebo will be matched to

infliximab on the basis of color and consistency when dissolved in saline. Several factors contributed to the decision to use infliximab in this study. First, we have data using infliximab that demonstrates that patients with high inflammation administered this drug exhibit a decrease in both plasma and PBMC protein and gene expression of inflammatory markers as well as certain depressive symptoms.[3, 4] Second, unlike etanercept and anakinra, which require daily or several times a week dosing done at home, infliximab provides significant and long-lasting anti-inflammatory activity after a single infusion. Third, the administration of infusions at our site under our supervision guarantees that all subjects will receive identical medication exposure, thus eliminating adherence issues. Finally, 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.[3]

Infliximab Infusion Protocol: Double-blinded infusions of infliximab or saline will be administered in the research outpatient unit of the Atlanta Clinical and Translational Science Institute- Clinical Research Network (ACTSI-CRN). Infusions will be performed following guidelines in place for patients who receive infliximab at the ACTSI-CRN. Specifically, intravenous access will be obtained in a sterile manner and infliximab (5 mg/kg body weight) or saline will be administered over a 2 to 2½ hour period while subjects rest quietly in an infusion chair solely used for infusions. Subjects will be monitored during the infusion and for 30 minutes after completion for the possible development of anaphylaxis, which occur in less than 1% of patients receiving an initial dose of infliximab. All aspects of the infusion will be overseen by infliximab-trained nurses and the standard protocol for acute adverse reactions will be initiated if subjects develop anaphylaxis during infusion.

Randomization: A randomly generated sequence of assignments to infliximab or saline will be created and maintained by the study pharmacist. Subjects will be stratified on the basis of sex to obviate any confounding effect of gender on the outcome variables. Subjects will be randomized in blocks of 4. Allocation to infliximab or saline will occur when a patient is eligible for the study and the consent for randomization has been obtained. If a subject drops out prior to receiving the infusion, he/she will not be included in data analyses and his/her randomization assignment will "roll over" to the next recruited subject until 60 subjects have received an infusion and at least one post infusion assessment.

Blinding/Saline Preparation: An unblinded pharmacist will be responsible for preparing the infusion bag with either infliximab or normal saline based on the pre-determined order of the randomization list supplied by the study statistician. 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. Such adverse reactions are indicated in section on Human Subjects Protection. Unblinded subjects will be discontinued from the protocol. An unblinded physician not associated with the study will contact the study pharmacist in cases where medical intervention requiring knowledge of group assignment is clinically warranted.

Lumbar Puncture (LP): On Week 2-Visit 2 (within 2 weeks, or at the discretion of the PI) of the Week 2-Visit 1 procedures (blood sampling, neuropsychiatric assessments and MRS), all subjects will receive ~1 liter of normal saline through an indwelling catheter to reduce incidence of post-LP headache and standardize hydration levels. Before the procedure, subjects will be offered pre-LP sedation (e.g. Versed) through IV. An anesthesiologist will review the results of the PT and PTT coagulation tests collected 1-14 days prior. The anesthesiologist will perform the LP using standard sterile technique and local anesthesia with patients in the lateral decubitus position. ~10 cc of CSF will be withdrawn. After discarding the initial 1 ml (to avoid blood contamination), remaining CSF will be collected into chilled tubes and aliquoted on ice in 1ml Eppendorf tubes, which will be immediately frozen at –80 C for later batched analyses. Upon LP completion, subjects will lay flat for ~1 hour and will be observed and treated for any side effects (e.g. headache or local irritation at LP site). The nurse practitioner or designee will also make a follow-up call 1, 3, 7, and 14 days following the LP to check if patients are experiencing any other study emergent side effects including chronic pain. If any patient expresses persisting pain or any other study emergent adverse event, then the information will be forwarded to the study MD for further management.

30 Day (Post-Infusion) Follow-Up Call: The research team will call subjects 30 days post-infusion to assess for any adverse events that may have occurred after study completion.

Optional Combination Study: Department of Psychology Task-Based fMRI Scan Visits:
Study participants may choose to complete 2 additional scan visits as an optional extension of this study. These visits are a part of a Task-Based Functional MRI (fMRI) study under the Department of Psychology, Dynamics of Inflammation and its Blockade on Motivational Circuitry in Depression, IRB#00087941 (see Table 1B). The purpose of the study is to see how the brain processes different types of information, and the way this affects behavior. Both study visits, the Baseline: fMRI Scan Visit and 14 Day: fMRI Scan Visit will take place at the Emory Department of Psychology on campus. The combined protocol scan visits will be consistently scheduled in the following order: Baseline Visits (fMRI scan → MRS Scan) and 14 Day Visits (MRS Scan→ fMRI scan). All scans should be within 3 days of either the infusion visit or week 2. Participants will complete behavioral tasks, self-report questionnaires, fMRI scans, urine drug tests and urine pregnancy tests (if applicable) at each visit. Subjects will sign a separate consent form to participate in the combination study. Participants completing the additional 2 day study option will be paid up to $800 in addition to the compensation received for the main study.

Table 1C:

Baseline: Task-Based fMRI scan **	Baseline (MRS Scan)	Infusion	1 Day	3 Day (MRS Scan)	7 Day	14 Day (MRS Scan)	14 Day: Task- Based fMRI scan **	LP
Optional	IGLU Study	IGLU Study	IGLU Study	IGLU Study	IGLU Study	IGLU Study	Optional	IGLU Study

Subjects participating in the combination I-Glu/Infliximab studies will complete the Additional Measures below as part of participation in the IGLU study schedule:

Self-Report Measures

The below self-report measures will be collected at the Baseline, 24-Hr, 3-Day, and 7-Day Visits.

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

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.

Optional Combination Study (con't):
Behavioral Computer Tasks

The below task will replace the Gambling task administered at Baseline, 3-Day, and 7-Day visits.

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. 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.

Criteria for Study Discontinuation ("Stopping Rules"):
Individual:
Subjects will be closely evaluated for worsening of psychiatric symptoms during the study. Subjects will be evaluated at baseline and at post-infusion Day 3 and weeks 1 and 2. Subjects will be discontinued at any point after screening for any of the following: 1) significant suicidal ideation as determined by spontaneous subject report or as clinically warranted based on the Columbia Suicide Severity Rating Scale – Screen Version (CSSRS),[55] 2) psychotic or catatonic symptoms and 3) serious complications of TNF antagonism (e.g. serious infection). Meeting discontinuation criteria prior to baseline will result in a subject not undergoing infusion. Subjects who meet discontinuation criteria following infusion will be discontinued. Subjects requiring discontinuation will be evaluated for danger to self or others and will receive emergency referral if required. In non-emergent cases, the referring clinician will be contacted immediately and a plan for appropriate psychiatric or medical care will be established and implemented.
Study:
1) If more than two individuals experience a reportable serious adverse event related to study procedures within a six month period, the study will be stopped and reviewed by the DSMB.

Neuropsychiatric Assessments
Structured Clinical Interview for DSM-V Axis I Disorders (SCID-V) is a semi-structured clinical interview that provides a wide range of DSM-V diagnoses.[56] 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 psychiatric predictors of treatment response, including number of past major depressive episodes, length of the current episode and presence of co-morbid anxiety disorders and/or dysthymia. The Mood Disorders and Alcohol/Substance Abuse Modules will be administered at all study assessments to evaluate whether subjects continue to meet criteria for major depression and to evaluate whether subjects have developed substance abuse symptoms (also confirmed by urine toxicology screen at each assessment).
The MGH Antidepressant Treatment Response Questionnaire (ATRQ):[57] 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.[58] Degree of resistance will be used as a covariate in relevant statistical analyses and to exclude severely treatment resistant subjects.[53]
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 < 28, which is a standard cutoff for clinically significant cognitive impairment.[52]
Dependent Variables
MRS acquisition:
The scanner hardware and the scanning sequences described in this section are similar to those described previously,[2] but modified for the PRISMA platform. All images will be acquired using a Siemens 3 Prisma 3T scanner (Siemens Medical Solutions, Malvern, PA, USA) equipped with a maximum gradient strength of 80 mT/m and a rise time of 200 μs, which will provide nearly double the signal-to-noise ratio enabling high-resolution structural imaging data. The new digital RF system equipped with 64-channel RF head-and-neck coil and the two-channel RF excitation coils will provide better spectral resolution with improved signal-to-noise ratio while at the same time providing more uniform excitation pulses. For image guidance and MRS volume of interest prescription, anatomic images will be obtained using a T1-weighted MPRAGE sequence with the following parameters: TR/TI/TE =2300/900/3.02 msec; flip angle=8°; FOV read=256 mm; matrix=256; slice thickness=1mm; GRAPPA factor of 0; total scan time=6 minutes.
Single Voxel MRS Acquisition:
Single Voxel Spectroscopy (SVS) will be used to quantitate of neurometabolites of interest in specific brain regions with a focus on the basal ganglia (see Figure 5 for sample basal ganglia spectrum). SVS

will be acquired using ChemicalShift Selective (CHESS)-based MRS sequence using settings of TR/TE = 3000/30 ms, sampling size=128 averages, bandwidth=1024Hz. Two voxels with sizes of 17 x 30 x 17 mm3 located in the left and right basal ganglia (encompassing parts of the caudate, putamen and pallidum and the associated intervening white matter),one voxel in dACC (BA 24) with a size of 20 x 30 x 10mm3, and one "control" voxel located in the left medial parietal regions of 20x20x20 mm3 (to explore regional specificity of the findings) will be used for SVS (see Figure 6). Spatial localization will be implemented using PRESS technique.[59] Numerically optimized Shinnar-Le Roux (SLR) radio frequency pulses will be used for PRESS (90°, 180°, 180°) and CHESS (90°, 90°, 90°).[60] Four unsuppressed water FIDs (free induction decay) will also be acquired for eddy current suppression and phase correction66 and to compute metabolite/water ratios if needed (LC-Model). The FWHM of the unsuppressed water peak will be optimized to <14 Hz by shimming. Of note, to facilitate spectral acquisition in deeper regions, previously phantom optimized 20-channel coil will be used. The spectral data will not be apodized

Chemical Shift Imaging (CSI): The application of a single voxel to a specific region makes it difficult to evaluate diffuse or global cortical and subcortical glutamate abnormalities that may occur in association with inflammation and have been proposed to occur in unipolar depression.[61] The setting will be similar to those reported in our recent publication. CSI will be acquired for exploratory analyses using 2D Point Resolved Spectroscopy (PRESS)-based MRSI sequence using settings of TR=1590 ms, TE=30 ms, averages= 7, matrix=16×16, voxel size=11. 3×11.3×15 mm3, slice thickness=15mm. The raw files will be processed using LC-Model.[62]

Post Processing:

Metabolite Spectral Fitting: The LC-Model[62] is an operator-independent commercial software package that fits in vivo metabolite spectra using model resonances acquired from multiple compounds in standard phantom solutions. The water suppressed time-domain data will be analyzed between 0.4 ppm and 4.0 ppm without T1 and T2 correction. The basis set provided by the vendor of the LC Model[62] will be used and then scaled to account for receiver gain differences. While the entire 18 metabolite basis set would be entered during metabolite fitting, the analysis will only include metabolite variances (Cramer Rao Lower Bounds or CRLB) <20. Use of a high field strength (Prisma 3T) scanner and short TE (=30 msec) sequence has enabled us to calculate metabolites at very low metabolite variance (CRLB <10) in our previously published MRS studies.

Water scaling and relaxometry: The post processing pipeline will be developed to yield absolute metabolite quantitation as well as metabolite/water and metabolite/creatine values. LC model provides automated water scaling settings which will be used (DOWS). All data (water signal and water suppressed data) will be phased followed by quantification of water relaxometry data. The relaxometry data will then be fitted to obtain water reference data and in the case of gray matter following segregation of the CSF and tissue water constituent. Finally, water signal will be scaled to that of resulting value obtained in previous steps.

Tissue correction and segmentation: T1-weighted images will be segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) compartments using FreeSurfer (https://surfer.nmr.mgh.harvard.edu/fswiki) on the whole brain T1 images. A volume of interest (VOI) will be generated on the T1 images, matching the location and size of MRS voxels indicated above. Volumes of GM, WM and CSF segments in this volume-of-interest will then be generated using FreeSurfer. The absolute metabolite concentrations generated by the LC-Model will then be corrected for CSF using the formula metabolite C = Co x 1/(1-fCSF), where C is corrected metabolite concentrations, Co is metabolite concentrations generated by LC-Model output and fCSF is fraction of CSF volume.[63, 64] The final output of the absolute metabolite quantitation
will be provided in units of molality. CSI data will be assessed using metabolite values normalized to creatine to minimize methodological constraints published elsewhere[65] after ensuring that raw uncorrected concentrations of creatine (Cr) and phosphocreatine (PCr) are not significantly different among the different study groups in the prescribed regions of interest.

Macromolecular separation: To address over-interpretation of MR spectra with respect to quantification of glutamate in the presence of overlapping glutamine and macromolecules (MM), we will conduct a substudy involving 10 healthy control subjects using the approach proposed by Cudalbu and Gottschalk et al. in all three regions.[66, 67] This experimentally acquired prior-knowledge will represent both MM and lipids. This manual metabolite quantitation may have better accuracy than the LC-Model vendor-provided synthetic prior-knowledge, and if so, can be used to statistically correct metabolite

quantitation using LC-Model and assist in interpretation of the data. We will also conduct the analysis using the individual values of Glu, Glx (glutamate+glutamine) and Gln (glutamine); while setting the Crammer-Rao lower bound (CRLB) value for Gln at high (<30%) with LC-Model processing. An exploratory analysis of the impact of inflammation on other metabolites of interest including choline (Cho), myoinositol (mI) and n-acetyl aspartate (NAA) will also be conducted.

Neurocognitive Assessments

In accordance with the recent RDoC proposals by NIMH,[68] for our primary dependent behavioral variables, we have chosen to examine specific symptom domains that are relevant to the function of the basal ganglia and their role in moderating positive and negative valence systems (e.g. anhedonia and psychomotor retardation). The entire assessment will take up to approximately 120 minutes (with breaks as needed). The examiner will code the reliability of each test score, any alteration of standard procedure, and the reason (e.g., frequently repeating instructions due to difficulty with attention). Only trained research personnel will perform neurocognitive ratings.

Positive Valence Domain (Motivation)

Objective

Neurocognitive Assessments

Gamble Task*:* 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 have demonstrated that this technique can be used to establish a relationship between task earnings and happiness. This task takes between 10-20 minutes per session.

Go No-Go Variant Task*:* 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.

Reinforcement Learning Task*:* Trials for this task involve a 1-3s cue presentation during which subjects choose between two abstract stimuli, 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 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: $Q_t = Q_{t-1} + \text{alpha}*RPE_t$, where $RPE_t = \text{Reward}_t - Q_{t-1}$ and alpha represents the learning rate. This task will include between 80-120 trials and will take approximately 10-15 minutes in length.

Subjective Value Task*:* In this task, subjects will be asked to repeatedly choose between two options to obtain a monetary reward. Each option will consist of a horizontal bar (representing reward magnitude) with a percentage written underneath it (representing reward probability). A subset of trials in this task will

feature one option where both the magnitude and probability are higher than the alternative option ('catch' trials). Options will be presented, during which time subjects must wait to indicate their choice. Next, a question mark will appear, to prompt participants to press a button to indicate their choice. Choices will be confirmed by a frame around the chosen option, which will remain on the screen until the outcome is revealed. The outcome (reward or no reward) will be indicated by the bars turning green or red, respectively. Outcomes are then followed by an intertrial interval (ITI). A "prize bar" will be displayed between the options and will grow by the width of the reward bar when a subject options a reward. The subject's goal will be to move the prize bar across a target line to win a certain amount of money. Once this goal is achieved, the prize bar will be reset and the subject will begin again. This task will last a maximum of 20 minutes.

Negative Valence Domain (Psychomotor Retardation)
Objective
Neurocognitive Assessments
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 nonpreferred 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.[73] The FTT is designed to assess subtle motor impairment and is altered in subjects with basal ganglia disorders and lesions.[74] **Variables:** Mean and total number of taps in 90 sec. for both dominant and non-dominant hands
Reaction Time Task (RTT)(CANTAB): The RTT measures simple and choice movement and reaction time 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.[75] **Variables:** Simple and Choice Reaction Time and Movement time
Digit Symbol Substitution Task (DSST): The Digit Symbol Substitution Task is a subtest of the Wechsler Adult Intelligence Scale (WAIS) and consists of rows of blank squares, each printed with a randomly assigned number. The test involves graphimotor speed, visual scanning and memory, with about half of the variance being accounted for by graphimotor speed, a third by visual scanning and 4-5% by memory.[76] Performance on the DSST has been found to correlate with subcortical atrophy in disorders involving basal ganglia including Huntington's disease and multiple sclerosis.[77, 78] **Variables:** # Correct responses within 2 minutes
Trails Making Test A:[79, 80] The scale measures cognitive processing speed using a series of non-sequentially arranged numbers where the participant is asked to sequentially track the numbers occurring to numerical order as quickly as possible. **Variables:** Completion time

Clinician-Administered and Self-Report Instruments
Snaith-Hamilton Pleasure Scale (SHAPS-C): The SHAPS-C is a 14-item clinician-administered scale that assesses hedonic tone.[69, 70] 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. In a previous study examining hedonic capacity, confirmatory factor analysis revealed a Hedonic Capacity factor that was largely defined by the SHAPS.[69, 71]
Motivation and Pleasure Scale (MAP): The motivation and pleasure questionnaire is an 18-item self-report inventory that was created to disentangle state-wise motivational and consummatory components of everyday activities over a 24-hour period, and has been previously validated as a measure of reward-related symptoms in psychiatric populations.[72] This scale will be used to assess self-reported changes in symptoms of anhedonia before and after inflammation blockade.
Retardation Rating Scale: The RRS is a 14-item, clinician-administered scale used to assess psychomotor retardation.[81] The items of the scale are either related to motility or mental activity. In factor analyses, items 1-8 have been specifically associated with motor retardation in elderly depressed patients.[82]
Multidimensional Fatigue Inventory (MFI) is a 20-item self-report instrument designed to measure motivation and fatigue, covering the dimensions General Fatigue (GF), Physical Fatigue (PF), Mental

Fatigue (MF), Reduced Motivation (RM) and Reduced Activity (RA).[83] Separate scores for each of these five dimensions are generated from the MFI. This instrument has reasonable internal consistency (Cronbach's alpha between 0.53 and 0.93) and construct validity. The Reduced Motivation score from the MFI has been shown to highly correlate with reduced neural activation in the ventral striatum to a hedonic reward task during IFN-alpha administration as determined by fMRI.[12]

Assessments of Depression Severity, Suicidality and Bipolarity

Inventory of Depressive Symptoms-Clinician Rating (IDS-SR) (Item 20) 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.[51, 84]

Columbia-Suicide Severity Scale: The Columbia-Suicide Severity Rating Scale (C-SSRS) is a fully-structured, clinical interview designed to systematically query patients regarding past and current suicidal ideation and behavior (SIB)[55]. The psychometric characteristics of the patient- reported C-SSRS have been assessed in multiple contexts. It has well validated to elicit suicidal ideas in depressed subjects and will be consequently used to identify individuals experience acute severe depressive symptoms and refer them for immediate treatment.

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.[85] To quantify the bipolarity risk, the STEP-BD computed bipolarity index scores by rating the patient's profile relative to five traits believed to be most characteristic of Bipolar I disorder. 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. The dimensions include: episode characteristics, age of onset, course of illness/associated features, response to treatment and family history. A theoretical case with the traits most convincing for bipolar disorder on every dimension would score 100 points and most Bipolar I patients score above 60. Most unipolar depressed patients score much lower.

Assessments of Anxiety and PTSD Severity

Hamilton Anxiety Rating Scale (HAM-A):[86] The HAM-A is a 14-item clinician-administered scale that assesses the severity of symptoms of anxiety.

Beck Anxiety Inventory (BAI):[87] The BAI is a 21-item self-report measure of anxiety symptoms, rated on a 4-point Likert scale modified to be based on the patient's experience in the past week.

State-Trait Anxiety Inventory (STAI) State Scale:[88] This 20-item self-report scale is used to measure current anxiety symptoms.

PTSD Checklist for DSM-5 (PCL-5):[89] The PCL-5 is a 20-item self-report measure that assesses the 20 DSM-5 symptoms of PTSD, rated on a 5-point Likert scale modified to be based on the patient's experience in the past week. The accompanying Life Events Checklist (LEC) and Criterion A assessment measures trauma exposure.

Safety and Tolerability

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

Laboratory and Biological Variables:

Blood collection: At Baseline Visits 1 and 2, and post-treatment Days 1 and 3 and Weeks 1 and 2, study bloods will be collected under fasting conditions between 8am-10am by venipuncture into two 6mL and one 10mL EDTA-containing vacutainer tubes using standard sterile technique. 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.

Plasma and CSF cytokines and soluble cytokine receptors: Fluorokine MAP Multiplex Human Biomarker Panels (R&D Systems, Minneapolis, MN) will be used to measure plasma and CSF TNF-alpha, sTNFR2, IL-1ra, IL-6, sIL-6R, IL-10, and monocyte chemoattractant protein (MCP)-1. These

cytokines and their receptors have all been found to be altered in depression,[5] and TNF and sTNFR2 have been found to predict response to infliximab and to correlate with basal ganglia glutamate.[3, 48, 49] 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 and CSF CRP will be assessed with a high sensitivity turbidimetric assay based within the Emory University Hospital. 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.

Estrogen and Progesterone: Serum estrogen and progesterone will be collected at the ACTSI CRN on the day of the subject's infusion and sent to the EUH laboratory for analysis. Processed in the Emory Medical Laboratory.

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.[90] 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 analyses. To correct for confounding due to batch effects, expression profiles will be normalized using ComBat, an empirical Bayes method for batch correction.[91] 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).[92]

6. Participant selection:

Recruitment and Informed Consent Process

Male and female subjects with depression will be recruited from local (inter-Departmental) referrals, medical record queries and/or social media recruitment campaign(s). Subjects in this study may also be referred from our Behavioral Immunology Program "Recruitment Clinic" protocol, Psychiatric Research Screening Clinic: IRB0000075162, which uses the screening strategy for this protocol to recruit and screen patients with depression for our active studies through IRB approved social media campaigns. An overview of the study will initially be provided in person or over the phone by study clinicians or trained study staff. A telephone prescreening interview will be conducted on candidates providing verbal consent. If a subject shows interest in the study, research staff members will describe the general procedures involved and will answer relevant questions. If a subject remains interested in participation, the detailed nature, purpose, procedures, benefits, risks of, and alternatives to this research study will be explained to each subject, and written informed consent will be obtained by a study clinician, in a private office space, who provides this information. To ensure comprehension of the informed consent information, the clinician will allow time for questioning and administer a WRAT-3 brief screening level of reading to determine if participant is at least evaluated at an 8th grade reading level in order to understand the consent language. Informed consent will be documented on the Institutional Review Board-approved form. A copy of the signed consent form will be given to the participant and the original document filed in a central study consent binder. The consent binder(s) and subject casebooks containing information gathered as part of the study will be kept in a locked office and/or cabinet.

Telephone Prescreen Interview

A telephone prescreen interview will be conducted with study referrals following verbal consent. Candidates eligible to proceed with the study process will be scheduled for an appointment to see a study clinician

Onsite visit (s): Trained personnel will obtain written informed consent from candidates prior to initiating study procedures. Screening information will be collected by interview and evaluation, as well as obtaining collateral history and data from other relevant sources (e. g. medical records, referring physician). Screening will include the following assessments: (1) History of medical and psychiatric conditions (2) screening laboratory evaluations, (3) subject height and weight measurements and waist circumference obtained by staff 4) Concomitant Medications 5) Adverse Events 6) MRI Safety Form 7) Demographics and 8) assessments below:

Clinician-Assisted Quick Inventory of Depressive Symptomology (QIDS-16 SR)- A 16-item self-report instrument that includes assessment of nine diagnostic symptom domains used to characterize a major depressive episode.
Patient Health Questionnaire (PHQ-9) is a nine-item measurement used to assess depressive symptoms and suicidal ideation.
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 <28 (unless otherwise approved by the PI), which is evidence of mild cognitive impairment.[83]
WRAT-3 is a very brief screening measure for reading level. Participants evaluated at less than an 8th grade reading level may not participate unless otherwise approved by the PI.
Child Trauma Questionnaire (CTQ) is a standardized, retrospective 28-item self-report inventory that measures the severity of different types of childhood trauma. Participants will not be excluded for history of childhood trauma.

Laboratory Variables
Research bloods will be collected by venipuncture into EDTA-containing vacutainer tubes using standard sterile technique. Plasma and buffy coat for the evaluation of 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 and buffy coat will be removed separately, aliquoted into siliconized polypropylene tubes, and stored at -80°C until batch assay. Bloods will also be collected by venipuncture into Tempus mRNA tubes and directly placed in freezer storage at -20°C. In order to further assess immune cell profiles, we will additionally collect 10 ml of whole blood in EDTA at room temperature for immune cell extraction. Tempus tubes and buffy coats will be sent to the Emory Integrated Genomics Core for RNA extraction, gene expression, mRNA profiling, epigenetic evaluation and measurement of gene polymorphisms. Some samples may be sent to other labs for additional analysis.
Plasma cytokines and soluble cytokine receptors: Customized high sensitivity Fluorokine MAP Multiplex Human Biomarker Panels (R&D Systems, Minneapolis, MN) will be used to measure plasma TNF-alpha, sTNFR1 and 2, IL-1ra, IL-6 and sIL-6R. 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 is 0.18 mg/L, range of measure is 0.2 to 80 mg/L, and functional sensitivity (at 20% CV) is 0.2 mg/ L.
Tryptophan and Kynurenine: Free tryptophan and kynurenine plasma concentrations will be determined by high-performance liquid chromatography in the laboratory of Dr. Dietmar Fuchs or other laboratories with equivalent expertise at the PI's discretion.
Gene expression and epigenetics: As indicated above, blood will be obtained for analysis of inflammatory mediators and signaling pathways.
7. Management of MRS Incidental Findings
The purpose of these scans is not to make a clinical determination of patients' brain health. However, if any incidental finding is noticed during the scan, the information will be provided to patients or their medical provider-of-record for further management. Incidental findings are those abnormalities seen in patients' brain images during the scanning process, which may or may not be of clinical importance. Follow-up of the scan findings will be the responsibility of the patients' Emory Healthcare provider-of-record.
8. Sources of Materials: Research material will be derived from clinician-administered and self-report questionnaires, blood, urine, cerebrospinal fluid and MRS scans. Material for evaluating baseline medical status and subject safety prior to infusion will be obtained from blood and urine. Data to be recorded from subjects will include standardized ratings of emotional, cognitive and physical symptoms, as well as clinical information derived from medical records when available, interviews with the subject, referring physicians and family members when relevant. Data on cardiac functioning will be obtained from EKG. Data will be obtained from peripheral blood, including complete metabolic panel, CBC with differential, serum pregnancy test, anti-nuclear antibodies, rheumatoid factor, serum concentrations of estrogen and

progesterone, blood tests for fungal infection (aspergillus antibody, blastomyces antibody, coccidioides antibody, histoplasma antibody) plasma concentrations of TNF-alpha, sTNFR 2, IL-1 beta, IL-1ra,IL-6, sIL-6R, IL-10, MCP-1, and CRP. CSF will be used to measure concentrations of TNF-alpha, sTNFR 2, IL-1 beta, IL-1ra, IL-6, sIL-6R, IL-10, MCP-1, and CRP. Blood for mRNA expression analyses will also be collected. Data obtained from urine will include urinalysis and toxicology screen. Clinical data that will be used to assess eligibility and safety will be collected via interview and documented by study clinicians. Standardized clinician administered and self-report questionnaires will be administered and collected by study physicians or study coordinator. Blood and urine samples at screening will be obtained by the study coordinator and nursing staff at the Emory Clinical Research Network (CRN). Blood and urine for the baseline assessment (Visits 1 and 2) and subsequent assessments on days 1 and 3 and weeks 1 and 2 for study bloods and weeks 1 and 2 for safety labs will also be obtained by CRN nursing staff. All data, including questionnaires and blood and urine samples will be coded by unique identifying numbers. Subjects' names will not appear elsewhere in any form that would link them specifically to data. Subject name and identifying number will be kept on separate forms in a locked office separate from data files. Only the study coordinator and study physicians will have access to subject identities. Research personnel analyzing blood data or entering data into the database will not be provided information from which they could identify subjects by name. Only the identifying number will be used during MRS scanning and post processing. All information will be obtained solely to determine eligibility for study participation or for research purposes or to monitor subject safety following the infusion. Nevertheless, any abnormalities uncovered will be reported to the subject and his/her treating physician for follow-up or will be used by study clinicians to determine appropriate medical care in the case of adverse events related to the infusion.

9. Data Collection and Management: The data management team will use REDCap.[93] REDCap allows database construction, an interface for collecting data, data validation, and automated export procedures for data downloads to statistical packages (SPSS, SAS, Stata, R). Dr. Miller is currently using REDCap for 3 of his currently funded projects, and he and his staff have extensive experience with its usage.

Caveats and Considerations

It should be noted that in this study we are using peripheral blood CRP as a proxy measure for CNS inflammation. Our previous data in two published reports have demonstrated that plasma CRP is associated not only with basal ganglia glutamate, but also connectivity between ventral striatum and ventral medial prefrontal cortex, both of which were correlated with anhedonia.[2, 94] In addition, plasma CRP was a robust predictor of the clinical response to infliximab.[3] Finally, peripheral CRP highly correlates with CSF CRP (see Figure 7), and CSF CRP not only correlated with left basal ganglia glutamate using CSI in our recently published work,[2] but also significantly correlates with CSF IL-6, CSF sTNFR2 and CSF MCP-1 (all $p<0.05$). Therefore, for the purposes of identifying patients that are likely to exhibit increased inflammation and its consequences in the CNS, we believe plasma CRP is an excellent proxy measure for CNS inflammation. Another caveat is that infliximab does not cross an intact blood brain barrier (BBB). Nevertheless, previous studies have shown that blockade of peripheral inflammation through the use of monoclonal antibodies like infliximab can abrogate stress-induced depressive-like behavior in rodents.[95] These data suggest that peripheral inflammation may drive central inflammatory responses and their effects on behavior, and blocking peripheral inflammation may reduce central inflammation and its effects on the brain.[8] This possibility will be examined for the first time in Aim 3 by sampling CSF inflammatory markers after inhibition of peripheral inflammation by infliximab. Finally, we should note that the MRS glutamate signal is a proxy measure for glutamate concentrations in the brain including both intracellular and extracellular compartments. Moreover, the modeling which determines glutamate concentrations in the brain provides an approximation of several confluent spectral peaks including glutamate, glutamine, GABA and glutathione, with glutamate representing the primary component. As indicated above, however, various strategies are proposed to address this issue**.**

10. Statistical Analyses

Aim 1: To determine whether inhibition of inflammation reduces basal ganglia glutamate.

Hypothesis 1. Compared to placebo, infliximab-treated patients will exhibit decreased absolute glutamate in the basal ganglia on day 3 and week 2. Mixed-effects models for repeated measures (MMRM) will be employed to examine effects of group, time and their interaction on left and right basal ganglia glutamate controlling for baseline glutamate concentrations. Relevant covariates including age, sex, race (white/non-white), menstrual status (estrogen/progesterone) 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 teste tested in the conducted for the dACC and medial parietal voxels.

Aim 2. To determine the relationship between basal ganglia glutamate and anhedonia and psychomotor retardation.

Hypothesis 2. Decreased basal ganglia glutamate concentrations will be associated with improved objective and clinical measures of anhedonia and psychomotor retardation. Random intercept linear mixed models will be used to assess whether change in left or right basal ganglia glutamate predict change in motivation and psychomotor activity. Models will be adjusted for relevant clinical covariates as indicated above. Separate analyses will be run for each behavioral outcome of motivation (Gamble Task, Go No-Go, RLT, PRT SHAPS-C, MAP) and psychomotor speed (FTT, RTT, DSST, Trails, RRS, MFI) as well as total depression severity scores using the IDS-SR. Type I errors as a result of multiple testing will be controlled by using a step down Bonferroni procedure.[96] Given that changes in basal ganglia glutamate may occur prior to changes in behavior, lagged regressor models will also be explored to determine whether early changes in basal ganglia glutamate predict subsequent changes in motivation and motor activity in separate analyses. Finally, exploratory analyses will be conducted to examine the impact of basal ganglia glutamate on suicidality (STS), given that the relationship between inflammation and suicidality may be mediated by glutamate. This possibility is supported by the impact of the glutamate antagonist ketamine on suicidality as well as the relationship between gene expression of glutamate signaling pathways and suicide in postmortem brain tissue.[97, 98]

Aim 3: To determine the relationship between inflammatory markers and basal ganglia glutamate.

Hypothesis 3. Decreased peripheral and central markers of inflammation will precede and predict decreases in basal ganglia glutamate. Random intercept linear mixed models will be used to assess whether change in inflammatory variables predict change in left or right basal ganglia glutamate controlling for relevant covariates as appropriate (see above). In the first model, only the effect of CRP will be tested. Subsequent models will include the cytokines and their receptors in both blood and CSF. In the case of collinearity among inflammatory measures, principal component analyses will be used for the purposes of data reduction. Given the rapid changes that will likely occur in inflammatory markers prior to changes in basal ganglia glutamate, lagged regressor models will also be explored to determine whether early changes in inflammatory variables predict subsequent changes in basal ganglia glutamate. For analysis of mRNA expression, separate delta values between baseline and day 3 and baseline and week 2 will be calculated for both mRNA expression and basal ganglia glutamate. Regression analyses will then be performed on delta values to identify mRNA transcripts that are significantly correlated with delta glutamate (FDR<0.05). RNA transcripts found to significantly predict changes in basal ganglia glutamate will be entered into pathway analyses using a number of tools including the WebGestalt WikiPathways tool and Bibliosphere™ 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).[99] Transcript origin analysis will also be used.[100] 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 groups.[100, 101] Exploratory analyses will be conducted using the strategies indicated above for CSI measurements of glutamate and other metabolites normalized to creatine.

11. Power Analysis: A power analysis was conducted using our preliminary data examining a cohort of 50 unmedicated patients with major depression. The sample size was powered on the basis of Aim 1, which represents the primary hypothesis of the study that decreasing inflammation will decrease basal ganglia glutamate. The effect size (d) of the differences in glutamate concentrations between high (>3mg/L) and low (<1mg/L) CRP was 1.15 using absolute tissue-corrected glutamate concentrations. Using a beta/alpha error ratio of 4, the proposed sample size of 60 patients (30 infliximab and 30 placebo) will yield a power of 0.99 with an alpha error probability of 0.001 and beta error probability of 0.005 (computed using G*power). Linear analysis-based power calculations using r=0.43 and d=0.95 (from our preliminary data) with the proposed sample size of 60 and 5 predictors (age, sex, race, BMI, CRP) will have a power of 0.93, with alpha error of 0.01 and beta error of 0.07. Given our anticipated attrition rate of 10%, we will still have ample power for the proposed analyses.

12. Potential Study Risks: There are 7 major areas of potential risk in the proposed study stemming from 1) neuropsychiatric assessments, 2) blood drawing, 3) lumbar puncture, 4) MRS scanning, 5) infusion of infliximab, 6) placebo treatment, and 7) loss of confidentiality. Neuropsychiatric assessments may uncover strong and potentially disturbing feelings about the subject's past or present emotional state. The risks of blood drawing include discomfort, bruising, infection, bleeding, and fainting. Undergoing MRS scan poses no more risk than undergoing a routine MRI scan. Physical discomfort due to lying in the scanner, occasional headaches due to scanner sounds and previously unrecognized claustrophobic attacks are the prominent adverse effects of the procedure. Some patients experience some lower back pain after a lumbar puncture. This is usually felt in and around the area where the needle was inserted. In most cases, the pain will ease after a few days and it can be treated with analgesics, such as acetaminophen, if necessary. Headache and lower back pain are the most common side effects of a lumbar puncture, usually developing within 24 to 48 hours of the procedure. There is a small risk of a lumbar puncture leading to intracerebral bleeding, although this is very rare. The likelihood of a lumbar puncture causing an infection in the spine is also very small. 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. 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.[102] Nevertheless, more recent data in RA patients (including analysis of more than 40,000 patients and 150,000 patient-years exposure) indicate that TNF inhibitors are not clearly associated with increased malignancy in RA excluding skin cancer.[103] 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). 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. Finally, there is a risk of loss of confidentiality. Confidentiality of all subjects will be protected per institutional and NIH and other federal requirements, and as described in greater detail below.
Alternatives include not participating in the proposed study. An array of demonstrated effective treatments for depression exists and include, but are not limited to: selective serotonergic antidepressants (i.e. fluoxetine, paroxetine, etc.), serotonin-norepinephrine reuptake inhibitors (i.e. venlafaxine, duloxetine), atypical antidepressants (i.e. bupropion, mirtazapine, nefazodone), tricyclic antidepressants, monoamine oxidase inhibitors, electroconvulsive therapy (ECT) and psychotherapy (i.e. interpersonal and cognitive behavioral therapies). Risks and side effects of these agents vary in type and severity depending on class, but include sexual dysfunction, gastrointestinal distress, anxiety, anticholinergic symptoms, induction of mania, suicidal ideation and potential lethality in overdose. Common risks of ECT include short term memory loss, head and body aches and risks related to anesthesia. Benefits of antidepressants and ECT include documented efficacy in the treatment of depression. In addition to approved modalities, trials of novel treatments for more severe depressions are ongoing and include the use of vagal nerve stimulation and deep brain stimulation. Benefits of these modalities may include efficacy for patients who have failed traditional agents. Risks include the possibility that these modalities are not effective. There are also surgery-related risks of infection and anesthesia involved with these modalities. At screening, study clinicians' will discuss benefits and side effects of these alternative treatments with subjects compared to the risks and potential benefits of study participation.

13. Adequacy of Protection Against Risks:

Recruitment and Informed Consent:

Subjects with major depression will be recruited from outpatient evaluation clinics within the Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine and from advertising in

radio, television, internet and print media. The Emory outpatient clinics evaluate potential subjects for appropriateness for participation in several on-going trials of various modalities for MD. 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 a 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 case-book containing relevant demographic data for the subject. Of note, because this case-book will contain personal identifiers (i.e. name), it will be kept separate from any data
gathered as part of the study, and will be kept in a locked office. Protection Against Risk: Every effort will be taken to prevent injury or distress that may result from this study.

Neuropsychiatric Assessments*:*

Care will be taken to avoid bringing about undue psychological distress during the neuropsychiatric interviews. This will be accomplished by using trained raters (clinicians) for all neuropsychiatric assessments. In the event that a subject becomes unduly distressed, Drs. Miller or Haroon will be immediately contacted, and an appropriate clinical intervention plan will be developed. Drs. Miller and Haroon are Board Certified psychiatrists, consultation-liaison, inpatient and outpatient psychiatry. In cases where patients need extra time to collect their thoughts and emotions, mental health clinicians will be available for emotional support after all interviews.

Blood Draws: Standard sterile procedure for blood withdrawal will be used. Blood draws will be conducted by clinicians with significant experience in the technique. In addition, the volume of blood withdrawn for this study will not exceed 200 ml over a maximum 4-week period (from screening to study completion).

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

Lumbar Puncture: On day of the Emory outpatient CRN admission, subjects will receive approximately ~1 liter of fluid iv as a result of a KVO (keep vein open) flow of normal saline through the indwelling catheter. This administration of iv fluids has been found in our experience to markedly reduce the incidence of post-LP headache. It will also help standardize hydration levels between subjects. An anesthesiologist will review the results of the PT and PTT coagulation tests collected 1-7 days prior. The anesthesiologist will perform the LP, using standard sterile technique and local anesthesia with patients in the lateral decubitus position. Approximately 10 cc of CSF will be withdrawn. After discarding the initial 1 ml (to avoid blood contamination), the remaining CSF will be collected into chilled tubes and aliquoted on ice in 1 ml Eppendorf tubes, which will be immediately frozen at –80 C for later batched analyses. Upon completion of the LP, subjects will lie flat for approximately one hour and subsequently discharged. Some patients experience some lower back pain after a lumbar puncture. This is usually felt in and around the area where the needle was inserted. In most cases the pain will ease after a few days and it can be treated with analgesics, such as acetaminophen, if necessary. A headache is a common side effect of a lumbar puncture, usually developing within 24 to 48 hours of the procedure. Most people describe a dull or throbbing pain at the front of their head and this can sometimes spread to the neck and shoulders. The pain is usually worse while standing or sitting up and is usually relieved by lying down. The headache can usually be treated with simple painkillers such as acetaminophen. Some people have also reported that drinks containing caffeine, such as coffee, tea and or cola, have helped reduce the discomfort. If post-lumbar puncture headaches persist, are particularly severe, or are accompanied by sickness and vomiting, the patient will be advised to call the study physician to be triaged appropriately. For intractable post LP headaches, the current standard-of-care is to apply an epidural blood patch. At Emory, anesthesiologists conduct this procedure, which can be performed 24 hours a day for emergencies. There is a small risk of a lumbar puncture leading to intracerebral bleeding, although this is very rare. The likelihood of a lumbar puncture causing an infection in the spine is also very small. However, patients will

be asked to call the study physician, if the patient develops a temperature or sensitivity to bright lights, or if the lumbar puncture site becomes painful and swollen.

Infliximab: Several procedures will be put in place to reduce the risks associated with receiving infliximab. At screening, subjects will be carefully evaluated for the presence of any medical conditions that might increase the risk of an adverse reaction to the medication. For example, because anaphylactic reactions are more common after subsequent dosages of infliximab, all subjects will be naïve to the medication so that they will be receiving their first dose of infliximab in this protocol. It should be noted that the medical exclusion criteria for the protocol are very conservative and address all areas of potential concern, including outcomes such as cancer (including skin cancer), which has been linked to the use of infliximab in some studies. Moreover, to reduce the risk of inducing autoimmunity, subjects will be excluded for a history of autoimmune conditions such as RA and Inflammatory Bowel Disease (IBD), for which infliximab has FDA approval and has been established as safe enough to warrant widespread use. Subjects will be excluded for presence of autoantibodies to nuclear antigens and the presence of rheumatoid factor. A full list of all exclusionary criteria is presented in the text of the proposal (see Research Strategy). To further reduce risks associated with infliximab, all infusions will be conducted in the research outpatient unit of the Atlanta Clinical and Translational Science Institute- Clinical Research Network. All infusions will be conducted by specially-trained infliximab infusion nurses who will follow the standard protocol for acute adverse reactions if necessary. Subjects will be evaluated by medical personnel 1 and 2 weeks post-infusion and will be available 24/7 if problems arise. Safety labs will be drawn prior to and up to 2 weeks after the infusion to screen for adverse responses to the medication. Prior to the infusion, a study clinician will review potential adverse events and will explain symptoms to look for (i.e. signs of infection or sickness, rash, fatigue, etc.) and report. The ACTSI-CRN has standard protocols in place for the treatment of acute allergic reactions to the infliximab infusion as follows:

FOR MILD REACTIONS: Headache, chills, itching, hives, rash, chest tightness, low grade fever:

A. Stop infliximab infusion
B. Monitor patient closely for further side effects (monitor vital signs q 5 minutes x 15-30 minutes)
C. May give acetaminophen 325-1000 mg po
D. May give diphenhydramine 25-50 mg IV or po
E. Restart infliximab infusion at reduced rate (10 cc/hour x 15 minutes and increase by 5 cc/hour @ 15 minute intervals once symptoms subside and patient is reassured by physician

FOR SEVERE REACTIONS: Hypotension, bronchospasm, wheezing, shortness of breath, throat tightening, swelling, body temperature >101 F.

A. Stop infusion of infliximab immediately
B. Call M.D. Administer 02
C. Call 911 if patient acutely unstable
D. 0.9 normal saline IV fluid per physician order
E. Monitor patient closely for airway, breathing, circulation. Monitor vital signs q 5 minutes
F. Give diphenhydramine 25-50 mg IV push (may repeat in 2-3 hours)
G. Give solucortef 250 mg IV push per M.D.
H. Give epinephrine 1 mg in 1:1000 concentrate IV push (may repeat q 2-5 minutes as needed for blood pressure/heart rate support) per M.D.
I. Place oral airway, lay flat, start CPR if needed. STAY WITH PATIENT. Call 911.

Placebo:

To reduce the risks associated with not receiving an active treatment, only subjects judged to have no active suicidal plan or intent will be recruited. Between signing consent and completing the screening assessment, a period of no more than 2 weeks will pass until baseline, and during this period a study clinician will evaluate each depressed subject every 7 days to ensure that symptoms haven't worsened appreciably or that suicidal ideation has not developed. In the case of either of these eventualities, subjects will be discontinued from the study immediately and appropriate psychiatric referral will be made. Specific indications for discontinuation (Stopping Rules) between screening and study endpoint for any subject will include any of the following: 1) the development of significant suicidal ideation, plan or intent as determined by spontaneous subject report or as determined by the Columbia Suicide Severity Rating Scale – Screen Version (CSSRS), 2) the development of psychotic or catatonic symptoms, 3) the development of serious complications of cytokine antagonism (e.g. serious infection), and 4) a 25% increase from baseline of IDS-SR score. Other criteria for discontinuation between screening and the

baseline visit include: hospitalization for any reason or a clinical judgment that a subject is an imminent threat to self or others. Subjects will be evaluated for the appropriate level of care, including emergency hospitalization if indicated. Subjects with non-emergent symptoms but who disqualify for further participation and who are in active treatment will be referred back to their primary psychiatric physician. Appropriate follow-up care will be arranged for subjects not in active psychiatric treatment. An individualized plan will be crafted for each subject to ensure psychiatric back-up should emergency psychiatric care be required after hours or on weekends. Upon completion of the study protocol, all depressed subjects without a current psychiatrist will be offered an appropriate referral.

Confidentiality and Data Management:
Loss of confidentiality is a risk of research participation. Every effort will be made to maintain subject confidentiality throughout the study. All project personnel, including those involved in data entry, have completed an on-line course in human subjects' protection for patient-related research with annual recertification. Each subject will be assigned a unique ID number that will be used in all data files and on all measures in place of personal identifiers. Data entry forms will be developed using the REDcap database system developed at the Vanderbilt Institute for Clinical and Translational Research (CTSA). REDCap (Research Electronic Data Capture) is an Oracle-based, secure, password-protected, HIPAA-compliant, web-based application designed to support data capture for research studies. REDCap provides: 1) an intuitive interface for data entry (with intrinsic data validation); 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, Stata, R); 4) procedures for importing data from external sources; 5) Real-time data cleaning and validation; 6) Automatic field computation; 7) Data dropdowns for choice lists (including condition selections based on earlier responses); 8) Data entry warnings for out of range or missing values; and 9) Electronic scheduling. Data entry can be performed anywhere using the internet and provides 128-bit SSL security. All electronic and hard entry forms will include unique patient identifiers to maintain patient confidentiality and will not relate to the patient in any way. Any protected personal health information will be encrypted and stored separately. The only link between identifying information (e.g.name, contact information) and project data will be in a key stored on a password-protected computer accessible only to the PIs and to the project coordinators, who will be making appointments and assigning research personnel to meet with the participant. Paper forms will be stored in locked file cabinets accessible only to study personnel.

Appointment Scheduling & Reminders:
All participants will be given the opportunity to receive appointment reminders and scheduling information via text message on their mobile phones. If the subject consents to this form of communication, study staff 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 404-727-9828 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.

Compensation to Participants:

Participants will receive up to $560-$2225 for completion of the study. Participants will be compensated using ClinCard. The ClinCard is a web based, reloadable, debit card that automates reimbursements for clinical research participants. An additional $25 will be provided to cover travel expenses for participants that travel equal to or greater than 50 miles to Atlanta.

Visit	Total Amount	Amount put on Clincard immediately after visit	Amount put on Clincard at the end of study participation
Intake	$25	$25	

(Visit Total)	**$25**		
Screening			
Psychiatric evaluation, history and physical, EKG, chest x-ray and lab work	$50*	$50*	
(Visit Total)	**$50**		
Baseline Visit 1			
Medical and psychiatric assessments, lab work research blood, neurocognitive tests, and self-report surveys	$50	$50	
Computer Motivation Tasks (depending on choice made on the computer)	$5-$100		$5-$100
Complete Scan	$300		$300
Incomplete Scan	$25**	$25**	
Extended break	$50***	$50***	
(Visit Total)	**$80-$500**		
Baseline Visit 2			
Infusion (Infliximab or placebo) and all scheduled assessments	$150	$50	$100
(Visit Total)	**$150**		
Post-Treatment Day 1 (Optional)			
Medical assessment, lab work, research blood	$50	$50	
(Visit Total)	**$50**		
Post-Treatment Day 3			
Medical assessment, lab work, research blood, neurocognitive tests, and self-report surveys	$50	$50	
Complete Scan	$300		$300
Incomplete Scan	$25**	$25**	
Extended break	$50***	$50***	
(Visit Total)	**$75-$400**		
Post-Treatment Week 1 (Optional)			
Medical assessment, lab work, research blood	$50	$50	
(Visit Total)	**$50**		
Post-Treatment Week 2			

Medical assessment, lab work research blood, neurocognitive tests, and self-report surveys	$50	$50	
Computer Motivation Tasks	$5-$100		$5-$100
Complete Scan	$300		$300
Incomplete Scan	$25**	$25**	
Extended break	$50***	$50***	
(Visit Total)	**$80-$500**		
Post-Treatment Week 2-Visit 2 (Optional)			
Spinal Tap (Lumbar Puncture)	$500		$500
(Visit Total)	**$500**		
Total Study Compensation	**$560-$2225**		

*=Screening visit may be divided across 2 visits and paid $25 per visit
**= Unable to complete scan leading to termination from the study
***=Subject can leave and return to complete visit if break is a minimum of 2 hours

POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

The direct benefits of study participation will include receiving a psychiatric and medical evaluation, including standard blood and urine-based laboratory tests, EKG and MRI scan – done prior to MRS scan to enable voxel placement. In addition, subjects will have a 50% chance of receiving a medication that may have efficacy for the treatment of their symptoms (infliximab). In addition, placebo interventions are effective in reducing or remitting depressive symptoms in some patients and hence may be of benefit to some study subjects. Subjects will also have the chance to contribute to a scientific investigation, which may be of benefit to future patients. Benefits to others may include gaining significant knowledge regarding the role of inflammatory activation in the pathophysiology and treatment of severe depressions. As a result of this knowledge, this study may contribute to novel therapeutic strategies for patients with major depression. The side effect burden of infliximab is mild and most frequently manifests as minor infusion reaction symptoms such as headache and low grade fever. In our pilot study, no differences in side effects between infliximab and placebo were found at any point during the study, and neither subjects nor staff were able to discern group assignment. These findings suggest that some of the reported reactions to infliximab in patients with autoimmune or inflammatory disorders may represent an interaction between the drug and their disease. Rates of serious adverse events, such as severe anaphylactic reaction or development of serious infection, are rare (less than 1%). In comparison, subjects who will be administered infliximab in this study will meet criteria for major depression, a condition characterized by severe symptoms, a chronic course and a profound decrement in quality of life and social and occupational functioning. Given data that TNF antagonists improve emotional functioning in patients with RA and IBD, it is reasonable to expect that subjects who receive infliximab may derive a benefit that outweighs the risks involved, especially given evidence that effects from a single infusion of infliximab can last for several months. Study subjects who receive a saline infusion will be exposed to the risk of venipuncture and of exposure to a pharmacologically inactive treatment. However, subjects receiving placebo frequently demonstrate symptomatic improvement. Given the low risk involved with the saline infusion and the likelihood that many patients will obtain at least some symptomatic benefit, we feel that the risks of the study are reasonable in relationship to the benefits for subjects receiving placebo.

IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

Successful treatment modalities both arise from, and contribute to, our understanding of disease pathophysiology. Mounting data indicate that depression is associated with evidence of

immune/inflammatory activation, and that patients with major depression may be especially likely to demonstrate increased proinflammatory cytokine production/release. Furthermore, alterations in the functioning and/or metabolic recycling of glutamate and neurotransmission have been demonstrated extensively in bipolar depressions and some unipolar depressions as well as anxiety disorders. Nevertheless, studies conducted to date have not explored the connection between inflammation and glutamate, leaving unanswered questions about the direct relevance of inflammation to the phenomenology and/or treatment of major depression as it relates to alterations in CNS glutamate. In addition, just as evidence for a conclusive role for monoamines in depression first arose from studies of medications that affected the bio-availability of these neurotransmitters, agents that block proinflammatory cytokine activity may represent a novel and important strategy for evaluating whether inflammation contributes to alterations of glutamate systems that are believed to be directly involved in the genesis of depression and responsiveness to treatment. Understanding the mechanisms by which inflammatory changes alter glutamate will provide key insights into the association between glutamate changes and treatment of depression. Given the current evidence that medications that block NMDA glutamate receptor have antidepressant efficacy, this study will reveal mechanisms underlying glutamate alterations in depression. Because a wide range of inflammatory indices are being examined, data from the proposed study may also shed light on novel biomarkers of inflammation that may help identify patients with increased CNS glutamate and may be most likely to respond to therapies targeting the immune system and/or glutamate metabolism. For these reasons, we feel that risks to subjects are reasonable in relationship to the value of the knowledge (both theoretical and practical) to be gained.

Investigational Drug Status

Infliximab has FDA approval for the treatment of RA and IBD. The current proposal represents the use of infliximab as an experimental tool to dissect the role of inflammatory processes leading to changes in glutamate turnover and changes in specific symptom domains. The proposed study meets the requirements for an IND waiver for the following reasons:

1) Results from the proposed study are not intended to be reported to the FDA in support of a new indication for use or to support any other significant change in the labeling for infliximab.

2) Results from this study are not intended to support a significant change in the advertising for the product.

3) The design of the study does not call for any new route of administration or dosage level (we will use a standard dose). There is no evidence that the subject population of the study (medically healthy individuals with treatment resistant depression) are at any greater risk than FDA-approved populations with RA or IBD. Indeed, our population should be at less risk, given that many patients with RA or IBD are also on other immunosuppressive agents. Co-administration of infliximab with other immunosuppressive agents (i.e. methotrexate) appears to increase the risk of serious infection with infliximab. There are no other factors in our design that significantly increase the risk (or decreases the acceptability of the risks) associated with infliximab.Data suggest that depression is associated with greater disability than RA, and we are selecting a patient population with a tremendous clinical need.

4) The proposed study will be conducted in compliance with the requirements for IRB review and informed consent [21 *CFR* parts 56 and 50, respectively].

5) The study will be conducted in compliance with the requirements concerning the promotion and sale of drugs [21 *CFR* 312.7].

6) The study will not involve a waiver of informed consent in an emergency room setting.

14. Data and Safety Monitoring Plan (DSMP)

The DSMB for this study will consist of Larry Tune, M.D. Chairman, Boadie Dunlop, M.D., Tanja Mletzko, M.S. and Marian Evatt, M.D. Each of these clinical researchers has agreed to serve as the external DSMB for investigator-initiated clinical trials conducted by Emory researchers in the Department of Psychiatry & Behavioral Sciences. If the DSMB requires additional specialized expertise to evaluate safety issues related to the performance of this study, a relevant specialist will be consulted by the DSMB. The frequency of the Department of Psychiatry and Behavioral Sciences DSMB review for this protocol will be once every year based on IRB recommendations consistent with the assessed risk status of the study. 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 Emory DSMB

At least four weeks prior to each DSMB meeting, the data manager/research coordinator will prepare a report to be reviewed during that meeting. The report will include the number of participants who signed consent for the study and were randomized, the number of post-randomization dropouts, reasons for these dropouts, and any safety concerns, adverse events, etc. An up-to-date consent form may be provided, as well as a summary of measures taken to protect confidentiality (e.g., data storage, use of coded ID numbers, etc.) The PIs will also prepare a report summarizing any new data/evidence that might alter the risk/benefit ratio for participating in the study (e.g., newly published studies, etc.). Data will be presented to the DSMB in such a way as to maintain patient confidentiality.
Based on the information provided to the Department of Psychiatry and Behavioral Sciences DSMB, once every year the DSMB will issue a letter to the PI to be included in the annual Emory IRB Continuing Review submission. The letter will inform the Emory IRB that the study is approved to proceed or state any safety related concerns expressed by the committee. Reports will not specifically disclose the treatment arm of the study for relevant subjects unless this disclosure is required for safety reasons.

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

Adverse Event Reporting
Note that any adverse event or serious adverse event (SAE) meeting Emory IRB criteria for on Unanticipated Problem will be reported to the Emory IRB and the study DSMB according to standard regulations. The IRB defines a serious adverse event as: “any adverse experiences occurring that result in any of the following outcomes: death, a life-threatening adverse experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. For the purposes of this policy, death is never expected.” According to the Emory IRB, an AE meets criteria for an Unanticipated Problem (UP) if all of the following criteria are met: 1) The AE is unexpected 1) the AE is related or possibly related to participation in the research 3) the AE suggests that the research places subjects or others at a greater risk of harm that was previously know.

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