HIC# 2000020186

YALE UNIVERSITY
HUMAN INVESTIGATION COMMITTEE

Application to Involve Human Subjects in Biomedical Research
100 FR1 (2016-1)

SECTION I: ADMINISTRATIVE INFORMATION

Title of Research Project: Imaging mGluR5 and synaptic density in psychiatric disorders			
Principal Investigator: Irina Esterlis, Ph.D.		**Yale Academic Appointment:** Professor	
Department: Psychiatry			
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Yale Cancer Center CTO Protocol Correspondent Name & Address (*if applicable*):			
Campus Phone:	**Fax:**	**E-mail:**	
Business Manager:			
Campus Phone :	**Fax :**	**E-mail**	

Faculty Advisor:(required if PI is a student, resident, fellow or other trainee) ☐ **NA**		**Yale Academic Appointment:**	
Campus Address:			
Campus Phone:	**Fax:**	**Pager:**	**E-mail:**

Investigator Interests:

Does the principal investigator, or do any research personnel who are responsible for the design, conduct or reporting of this project or any of their family members (spouse or dependent child) have an incentive or interest, financial or otherwise, that may affect the protection of the human subjects involved in this project, the scientific objectivity of the research or its integrity? Note: The Principal Investigator (Project Director), upon consideration of the individual's role and degree of independence in carrying out the work, will determine who is responsible for the design, conduct, or reporting of the research.

APPROVED BY THE YALE UNIVERSITY IRB 8/1/2024

See Disclosures and Management of Personal Interests in Human Research
http://www.yale.edu/hrpp/policies/index.html#COI

☐ Yes ●No

Do you or does anyone on the research team who is determined by you to be responsible for the design, conduct or reporting of this research have any patent (sole right to make, use or sell an invention) or copyright (exclusive rights to an original work) interests related to this research protocol?

☐ Yes ● No

If yes to either question above, list names of the investigator or responsible person:

The Yale University Principal Investigator, all Yale University co-investigators, and all Yale University individuals who are responsible for the design, conduct or reporting of research must have a current financial disclosure form on file with the University's Conflict of Interest Office. Yale New Haven Hospital personnel who are listed as co-investigators on a protocol with a Yale University Principal Investigator must also have a current financial disclosure form on file with the University's Conflict of Interest Office. If this has not been done, the individual(s) should follow this link to the COI Office Website to complete the form: http://www.yale.edu/coi/

NOTE: The requirement for maintaining a current disclosure form on file with the University's Conflict of Interest Office extends primarily to Yale University and Yale-New Haven Hospital personnel. **Whether or not they are required to maintain a disclosure form with the University's Conflict of Interest Office, all investigators and individuals deemed otherwise responsible by the PI who are listed on the protocol are required to disclose to the PI any interests that are specific to this protocol.**

SECTION II: GENERAL INFORMATION

1. **Performing Organizations:** Identify the hospital, in-patient or outpatient facility, school or other agency that will serve as the location of the research. Choose all that apply:

a. Internal Location[s] of the Study:

☒ Magnetic Resonance Research Center (MR-TAC)
☐ Yale Cancer Center/Clinical Trials Office (CTO)
☐ Yale Cancer Center/Smilow
☐ Yale-New Haven Hospital
☐ Cancer Data Repository/Tumor Registry
☐ Specify Other Yale Location:
☒ Yale University PET Center
☐ YCCI/Church Street Research Unit (CSRU)
☒ YCCI/Hospital Research Unit (HRU)
☒ YCCI/Keck Laboratories
☐ Yale-New Haven Hospital—Saint Raphael Campus

b. External Location[s]:

☐ APT Foundation, Inc.
☐ Haskins Laboratories

☒ Connecticut Mental Health Center
☐ Clinical Neuroscience Research Unit (CNRU)
☐ Other Locations, Specify:
☐ John B. Pierce Laboratory, Inc.
☐ Veterans Affairs Hospital, West Haven
☐ International Research Site
(Specify location(s)):

c. Additional Required Documents *(check all that apply)*: ☐ N/A

☐ *YCCI-Scientific and Safety Committee (YCCI-SSC)	Approval Date:
☐ *Pediatric Protocol Review Committee (PPRC)	Approval Date:
☐ *YCC Protocol Review Committee (YRC-PRC)	Approval Date:
☐ *Dept. of Veterans Affairs, West Haven VA HSS	Approval Date:
☒ *Radioactive Investigational Drug Committee (RIDC)	Approval Date:
☐ YNHH-Radiation Safety Committee (YNHH-RSC)	Approval Date:
☒ Yale University RSC (YU-RSC)	Approval Date:
☐ Magnetic Resonance Research Center PRC (MRRC-PRC)	Approval Date:
☐ *Nursing Research Committee	Approval Date:
☐ YSM/YNHH Cancer Data Repository (CaDR)	Approval Date:

☐ Dept. of Lab Medicine request for services or specimens form
☐ Imaging on YNHH Diagnostic Radiology equipment request form (YDRCTO request) found at http://radiology.yale.edu/research/ClinTrials.aspx)

Approval from these committees is required before final HIC approval is granted. See instructions for documents required for initial submission and approval of the protocol. Allow sufficient time for these requests. Check with the oversight body for their time requirements.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities. 10 years

3. **Research Type/Phase: (Check all that apply)**
   a. **Study Type**
   ☒ Single Center Study
   ☐ Multi-Center Study
   Does the Yale PI serve as the PI of the multi-site study? Yes ☐ No ☐
   ☐ Coordinating Center/Data Management
   ☐ Other:

   b. **Study Phase** ☒ **N/A**
   ☐ Pilot ☐ Phase I ☐ Phase II ☐ Phase III ☐ Phase IV
   ☐ Other (*Specify)*

4. **Area of Research: (Check all that apply)** Note that these are overlapping definitions and more than one category may apply to your research protocol. Definitions for the following can be found in the instructions section 4c:
☐ Clinical Research: Patient-Oriented
☐ Clinical Research: Epidemiologic and Behavioral
☐ Clinical Research: Outcomes and Health Services

☐ Translational Research #1 ("Bench-to-Bedside") ☒ Interdisciplinary Research
☐ Translational Research #2 ("Bedside-to-Community") ☐ Community-Based Research

5. Is this study a clinical trial? Yes ☒ No ☐

NOTE the current ICMJE (International Committee of Medical Journal Editors) definition of a clinical trial: "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes." Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example, drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events"

If yes, where is it registered?

Clinical Trials.gov registry ☒
Other (*Specify*)

*Registration of clinical trials **at their initiation** is required by the FDA, NIH and by the ICMJE.*

If this study is registered on clinicaltrials.gov, there is new language in the consent form and compound authorization that should be used.

For more information on registering clinical trials, including whether your trial must be registered, see the YCCI webpage, http://ycci.yale.edu/researchers/ors/registerstudy.aspx or contact YCCI at 203.785.3482)

6. Does the Clinical Trials Agreement (CTA) require compliance with ICH GCP (E6)?
Yes ☐ No☐

7. Will this study have a billable service? *A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital or Yale Medical Group to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by professionals within either Yale-New Haven Hospital or Yale Medical Group (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects*.

Yes ☐ No☒

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

8.. Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes ___ No X *If Yes, please answer questions a through c and note instructions below. If No, proceed to Section III.*

a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform?

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure?

c. Will a novel approach using existing equipment be applied?

If you answered "no" to question 8a, or "yes" to question 8b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

Please note that if this protocol includes Yale-New Haven Hospital patients, including patients at the HRU, the Principal Investigator and any co-investigators who are physicians or mid-level practitioners (includes PAs, APRNs, psychologists and speech pathologists) who may have direct patient contact with patients on YNHH premises must have medical staff appointment and appropriate clinical privileges at YNHH. If you are uncertain whether the study personnel meet the criteria, please telephone the Physician Services Department at 203-688-2615. ***By signing this protocol as a PI, you attest that you and any co-investigator who may have patient contact has a medical staff appointment and appropriate clinical privileges at YNHH****.*

SECTION IV: PRINCIPAL INVESTIGATOR/FACULTY ADVISOR/ DEPARTMENT CHAIR AGREEMENT

As the **principal investigator** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- I assume full responsibility for the protection of human subjects and the proper conduct of the research.
- Subject safety will be of paramount concern, and every effort will be made to protect subjects' rights and welfare.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- All members of the research team will be kept apprised of research goals.
- I will obtain approval for this research study and any subsequent revisions prior to my initiating the study or any change and I will obtain continuing approval of this study prior to the expiration date of any approval period.
- I will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set by the University and qualify to serve as the principal investigator of this project or have acquired the appropriate approval from the Dean's Office or Office of the Provost, or the Human Subject Protection Administrator at Yale-New Haven Hospital, or have a faculty advisor.
- I will identify a qualified successor should I cease my role as principal investigator and facilitate a smooth transfer of investigator responsibilities.

_____ _____

PI Name (PRINT)nd Signature Date

SECTION V: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.

About one fifth of the US population suffers from mood disorder in their lifetime. However, currently available treatments take weeks to months to produce beneficial effects and they fail to work for a large proportion of individuals suffering from mood disorders. The past decade of research has strongly implicated the glutamatergic system in the pathophysiology of mood disorders and the mechanism of antidepressant action. Thus, there is great interest in the glutamatergic system for the development of novel diagnostic tests and to provide unique targets for drug development. Results from magnetic resonance spectroscopy studies ([^{1}H]MRS) in individuals with depression suggest an alteration in the brain glutamate levels; however, to date, there are no studies of the functionality of the associated receptors in the brain, nor is there a clear understanding of the functionality of the glutamate dysfunction in depression. With the recent advancements in the positron emission tomography (PET) and radioligand development, **we are now able to image and quantify the metabotropic glutamatergic system (mGluR5) *in vivo* in human subjects**. We propose a novel investigation of mGluR5 (using [^{18}F]FPEB) in depression to obtain critical data to advance our understanding of the etiology of depression and its associated symptoms of cognitive dysfunction. Furthermore, recent evidence suggests differences in mGluR5 availability as a function of nicotine and also as a function of sex. We propose to incorporate this into our work to evaluate the relationship between these measures as emotion and behavior.

The findings with mGluR5 by themselves are limited. Changes in mGluR5 availability could be due to changes in synaptic density. Recently, the Yale PET center synthesized a new radioligand [^{11}C]UCB-J (referred to as [^{11}C]APP311 at the Yale University PET Center) that binds to synaptic vesicle glycoproteins (SV2A), which represent the number of synapses in the brain. Thus, we will also measure synaptic density in the brain and relate to mGluR5 availability. We will achieve this through the following aims:

Aim 1: To determine mGluR5 availability in individuals with mood disorders compared to healthy controls as measured with PET brain imaging using [^{18}F]FPEB.
Hypothesis 1: We hypothesize a decrease in mGluR5 availability in individuals with mood disorders in regions responsible for emotional and cognitive processes, including the amygdala, hippocampus, thalamus, anterior cingulate, and frontal cortices.
Aim 2: To determine if glutamate cycling in individuals with mood disorders is altered as compared to healthy controls as measured with [^{1}H]MRS.
Hypothesis 2: We hypothesize an increase in glutamate number in individuals with mood disorders as compared to controls.
Aim 3: To determine if the PET alterations in the glutamatergic system of depressed individuals are associated with cognitive deficits observed in depression, including concentration, attention, and memory (cognitive testing performance), and distractibility and startle.

Hypothesis 3: We hypothesize a positive relationship between mGluR5 availability and cognitive functioning, such that individuals with higher receptor availability will perform better on tests of concentration, attention, memory, distractibility, and startle than individuals with lower receptor availability.
Aim 4: To examine whether changes in mGluR5 availability are dependent on state, or whether the lower availability is due to trait.
Hypothesis 4: Due to changes in endogenous GLU shown with MRS studies, we hypothesize normalization (or increase) in mGluR5 availability in euthymia as compared to depressed state.
Aim 5: To examine synaptic density changes associated with mood disorders using [^{11}C]APP311.
Hypothesis 5: We hypothesize lower synaptic density in individuals with MDD and BD, and associations between synaptic density changes and mood severity. We also hypothesize there might be a relationship between synaptic density and mGluR5 availability.

2. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.

Mood disorders (e.g., Major Depressive Disorder – MDD and Bipolar Disorder – BD) are the most prevalent type of psychiatric disorders [1]; however, while the current treatments, developed almost exclusively from the monoamine hypothesis of depression, have greatly eased the burden of the disorder, it is now clear that many individuals fail to respond appreciatively and many more fail to achieve remission with these medications. Moreover, the currently available medications typically require weeks to months to exert their beneficial effects, which are commonly lost within the first year of treatment [2]. It is also well established that individuals with mood disorders suffer from specific cognitive deficits, such as poor attention and concentration, and worsened learning and memory, which contribute to the poor long-term outcome of this disorder (i.e., higher rates of suicidality than the general population [3], higher rates of unemployment [4], and higher rates of comorbid substance use [5]). Several lines of emerging evidence now suggest that dysfunction of the glutamate (GLU) neurotransmitter system is associated with the pathophysiology of mood disorders, including studies conducted with Magnetic Resonance Spectroscopy (MRS) at Yale University and other institutions [6,7].
GLU, the major excitatory neurotransmitter, is widespread throughout the brain, and likely modulates some of the constellation of symptoms present in individuals with MDD (e.g., dysfunctional sleep, appetite, motivation, and concentration) [8]. GLU neurotransmission is regulated by ionotropic and the G-protein coupled metabotropic glutamate receptors (mGluR), which are divided into 3 groups: group I (mGluR1 and 5), group II (mGluR2 and 3) and group III (mGluR4, 6, 7, 8). The group I mGluRs couple to phospholipase C, and stimulate cyclic AMP formation and arachidonic acid release [9] and thus impact neuroplasticity, neuronal excitability, synaptic transmission and gene expression. mGluR5 receptors are located postsynaptically and on glia, and have highest density in the hippocampus, intermediate in the caudate/putamen, cerebral cortex, deep cerebellar nuclei, and thalamus, and lowest in the cerebellum [10,11]. The mGluR5 have been considered to be pivotal in the functioning of the glutamatergic system [12] especially as it pertains to cognitive performance [13], and thus are an excellent candidate for study in mood disorders and associated cognitive difficulties [14]. Evidence from preclinical [15,16] and recent clinical [17] studies support mGluR5 involvement in MDD. Specifically, Deschwanden and colleagues used [^{11}C]ABP688 and positron emission tomography

(PET) to image mGluR5 *in vivo* in MDD (n=11) and healthy subjects (n=11) and detected a significantly lower mGluR5 availability associated with diagnosis of MDD (difference of 10% in the thalamus to 15-22% in the cortical regions) [17] and thus provided preliminary clinical *in vivo* evidence of mGluR5 compromise in MDD.

The study proposed herein will use a higher affinity radiotracer [^{18}F]FPEB and a higher resolution PET scanner (HRRT) to replicate and extend these initial findings in a larger cohort of MDD and control individuals. As several studies now suggest significant reductions in GLU measures in the cingulate and frontal cortices of MDD patients (for review [18]) we propose to also obtain brain GLU neurotransmitter levels measured with [^{1}H]MRS in these same subjects in order to observe the relationship between the receptor and its endogenous neurotransmitter in healthy and diseased brain. Although MRS detects all GLU in the tissue, its alterability in psychiatric disorders suggests that its level might be alterable when neurotransmission is changed. Furthermore, in the proposed interdisciplinary study we will investigate how the changes in the glutamatergic system relate to cognitive outcomes in depression. It is critical to evaluate the neurochemistry behind these cognitive deficits since although it is well known that cognitive deficits are present in MDD, it is surprising to learn that the deficits commonly remain after the MDD episode has resolved [19] and may be a trait marker of depression. To date, we do not have a good understanding of the pathophysiology underlying these deficits nor is there a well-established treatment to help individuals with mood disorders to overcome cognitive difficulties. **Using state-of-the-art PET and MRS imaging technologies, we propose to directly relate these clinical characteristics to GLU levels in the frontal and anterior cingulate cortices and mGluR5 availability in the amygdala, hippocampus, thalamus, anterior cingulate and frontal cortices, the brain areas responsible for processing of emotional information, learning and memory, planning, and attentional skills, respectively** [20,21]. **The proposed project is likely to be directly relevant to the development of treatments to improve the quality of lives of those suffering from mood disorders. The results obtained will guide drug development to target the glutamatergic system with the goal of reducing depressive symptoms and improving cognitive functioning. The multimodality approach to the study of glutamatergic involvement in depression is an essential evaluation and critical to the future investigation of the effect of glutamatergic agonists and antagonists on depression and cognitive dysfunction. In light of the fact that several mGluR5 modulating drugs are now available for human use, findings from this study could be rapidly implemented in the clinic.**

SV2A

MDD is characterized by a variety of symptoms including loss of pleasure, fatigue, changes in arousal, including sleep and appetite, and cognitive dysregulation, among others (http://www.nimh.nih.gov/health/topics/depression/index.shtml). MDD can be extremely debilitating, leading to loss of employment and social circles, and can be of a great burden to the patient, family, and society. Unfortunately, currently approved medications to treat MDD take weeks to months to exert their benefits and are only effective in about 50% of individuals, and advances in available treatments have been slow in the past 50 years since the development of serotonin selective reuptake inhibitors. **Thus, there is a great need to develop more effective pharmacotherapies that will provide rapid relief to patients. In order to do this, we need to**

have a better understanding of the pathophysiology involved in MDD in living human patients.

Pathophysiology of MDD includes consistent evidence of decreased volume of cortical regions (mainly the PFC - a region that controls emotion, mood, and cognition) as assessed by magnetic resonance imaging (MRI) and functional MRI (fMRI) studies comparing individuals with MDD to controls[22-25]. FMRI studies also report changes in connectivity between the PFC and subcortical structures, including the hippocampus, suggestive of disruption in brain emotion circuits in MDD. However, MR studies do not provide information on the causes of the changes in volume and connectivity. Evidence from postmortem studies of structural and molecular changes in MDD by our collaborator Dr. Ron Duman suggest decreases in synapses in the dorsolateral PFC (dlPFC)[26]. This evidence is also supported by findings of reduced synaptic signaling proteins in MDD that includes reduction in glutamatergic receptors, pre-synaptic neurotransmitter vesicle-associated proteins, and postsynaptic structural and functional proteins in the dlPFC, as well as other regions important in emotional and cognitive processes in MDD[26-29].

There is also strong preclinical evidence showing that chronic stress and depression lead to structural changes, which include neuronal atrophy, reduced synaptic density and cell loss. For example, chronic unpredictable stress (CUS) – a rodent model of depression and stress employed by Dr. Duman's group to study structural and molecular changes preclinically – leads to behavioral abnormalities which are core symptoms of depression, including anhedonia. Relevant to the current proposal, the CUS animals show reduction in synaptic density in the PFC (see Figure 1, middle panel)[30]. This loss in synapses extends to other models of stress paradigms in rodents, showing reductions in dendritic complexity and synaptic density in the PFC[31-33]. Critically important, this reduction in synaptic density is rapidly reversed by one administration of ketamine (Figure 1, right panel) and lasts about 1 week[30,34]. This corresponds to ketamine's rapid anti-depressant response in individuals with MDD, even those with treatment-resistant depression[35]. In our group (previous Nancy Taylor Foundation award), we replicated published findings[35] and showed a 60% decrease in depressive symptomatology immediately after ketamine administration, and a 70% decrease 24 hrs after ketamine.

Thus, here we seek to quantify synaptic density changes in individuals with mood disorders. Furthermore, given that the mGluR5 receptors are mostly postsynaptic, quantifying synaptic changes and mGluR5 in the same individual will allow for the investigation of the causes of mGluR5 change – whether it is due to glutamate excitotoxicity, synaptic density changes, or a combination of both.

PET Imaging of mGluR5 receptors with [^{18}F]F-PEB

[^{18}F]F-PEB has been synthesized in the Radiochemistry Lab at the Yale University PET Center using a modification of a procedure previously described for the radiolabeling of the nitro precursor with [^{18}F]fluoride [36]. In our human studies, we compared bolus (B) to a bolus plus constant infusions (B/I) administration paradigm for quantification of the mGluR5 radioligand [^{18}F]F-PEB in the human brain.

SV2A as a Novel Synaptic Density PET Imaging Biomarker

We recently developed [^{11}C]UCB-J (referred to as [^{11}C]APP311 at the Yale University PET Center)as a novel PET radioligand for synaptic vesicle glycoprotein 2A (SV2A)[37]. SV2A is an integral membrane protein located in presynaptic vesicle membranes, similar to synaptophysin

(SYN). SV2 has three isoforms, with SV2A being the only isoform which is ubiquitously located in synaptic vesicles across the brain[38]. Thus, PET quantification of SV2A signal may be an excellent *in vivo* biomarker of synaptic density. We hypothesize that PET imaging of SV2A may provide a novel approach to study synaptic pathology in MDD. Validation studies in baboon in our group confirm that *in vivo* regional [^{11}C]APP311 binding is highly correlated with *in vitro* measured SV2A and SYN densities. First in human PET studies demonstrated that [^{11}C]APP311 has excellent imaging properties. [^{11}C]APP311 binding was high in all gray matter regions, could be quantified with a one tissue compartment model, provided reliable volume of distribution (V_T) values, and had excellent test retest reproducibility (<5%). SV2A specific binding of [^{11}C]APP311 was confirmed by displacement with the anticonvulsant levetiracetam in human subjects. In summary, it may be postulated that alterations in [^{11}C]APP311 binding will reflect changes in synaptic vesicles, and can be related to changes in synaptic density.

3. **Research Plan:** Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. **Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths**. Describe the setting in which the research will take place.

   Overview: We will recruit 3 different subject groups for this study over up to 5 years.
   Group 1: 60 psychiatrically-healthy subjects will be enrolled as controls
   (Note: As of 5/20/2021, 30 healthy control subjects have been completed under RDRC purview. 30 more healthy subjects will be enrolled under IND# 155250)
   Group 2: 30 subjects with major depressive disorder (MDD)
   Note: As of 5/20/2021, 3 MDD subjects have been completed under RDRC purview. 27 more will be enrolled under IND# 155250)
   Group 3: 30 subjects with bipolar disorder

   Each subject will undergo a screening appointment to determine study eligibility. Thereafter, they may have an anatomical MRI scan, MRS scans, and [^{18}F]FPEB, and/or [^{11}C]APP311 PET scanning sessions. Subjects will also participate in cognitive testing. Depending on camera time, staff availability and subject schedule, total study participation may last 1-2 months.

   REDCap may be used for eConsent and obtaining study related information (including questionnaires and rating scales). Part 11 Compliant version of REDCap will be used. eConsent may take place in-person or remotely. If occurring remotely, the consent process will take place during a video conference call, subject identify will have already been established through our screening protocol or will be established by subjects' showing identification to the research assistant consenting them.

   Screening: After completing the informed consent process, subjects will have a physical and neurological examination. The following lab tests will be performed at screening to exclude other medical illnesses: complete blood count (CBC) and differential, chemistries, kidney function tests (creatinine, BUN, urinalysis), liver function tests, and TSH. HIV and hepatitis B and C will also be tested for. A urine drug screen will be done at screening and may also

be performed at PET scan day. A pregnancy test (for women) will be done at screening and before radiotracer administration. The psychiatric assessment will include a psychiatric history, a structured clinical interview (SCID), and assessment of depressive symptoms with the Montgomery-Åsberg Depression Rating Scale (MADRS) and Hamilton Depression Rating Scale (HDRS). The screening appointment may last 3-4 hours. If the subject will be determined ineligible to participate in the rest of the studies, their information, which includes drug screen, will be discarded as per HIPAA.

Subjects may be already partially or fully screened at the VA under HSS # IHR0010 (Screening for Depressive, Anxiety, and Trauma-and Stress-Related Disorders Studies). Physical, neurological, and psychiatric assessments will be done and mood and anxiety questionnaires will be administered. Routine laboratory tests and ECG will be done to exclude subjects with significant medical problems. Female subjects will have a pregnancy test, and all subjects will have a drug screen.

Screening may also be performed under screening protocol HIC 2000027842, 'Recruitment, screening, and repository protocol for neuroreceptor imaging studies,' (PI Esterlis). This is a screening protocol to determine if subjects meet physical and psychological health criteria to participate in neuroreceptor imaging studies (including PET and MRI). It is also a research recruitment repository and an (optional) information and sample repository for future research including possible genetic testing. Under 2000027842, subjects will also be asked to participate in an optional repository portion that includes long term storage of their samples and related information for genetic testing and research, which they can decline. Contact information, assessment and screening data from 2000027842 will be shared with this study upon referral and enrollment of the participant. It is this study's responsibility to verify eligibility according to protocol.

In the case of medicated bipolar subjects, treating physician will be contacted to confirm stable medication regimens.

Experimental Methods:
All subjects will participate in one MRI scan. Thereafter, subjects may participate in PET scans ([^{18}F]FPEB and/or [^{11}C]APP311) and/or [1H]MRS.
. Decisions regarding which radiotracer subjects will participate in will be based on the particular grant that will be covered by the current protocol.

Session 1 – *Magnetic Resonance Imaging:* Anatomical MRIs will be performed on a 3T Prisma at Yale. MR acquisition will be a Sag 3D magnetization-prepared rapid gradient-echo (MPRAGE) sequence with 2.81 ms echo time, 2,530 ms repetition time, 1,100 ms inversion time, 7 degree flip angle, and 180 Hz/pixel bandwidth. The image dimensions will be 256 x 256 x 176 and pixel size 0.98 x 0.98 x 1.0 mm. Resting state MRIs will also be obtained as follows: we will be doing 2 six minute scans with subjects in the scanner, eyes open, fixating on a cross. (Voxel size 2mm3), multiband=6, flip=60°, 29.6ms echo time, 60 slices, 220x220mm FOV, GRAPPA acceleration with iPAT factor of 2, number of measurements = 300, and 1976 Hz/Pixel bandwidth.

A member of the research team will accompany the subject to MRRC and will stay with the subject for the duration of the MR study.

Some subjects may be asked to do an fMRI rather than an MRI. This decision will be based upon grant needs and PI discretion. In this scan we will acquire: structural MRI, resting state MRI, diffusion tensor imaging data (DTI), MPRage, and functional and activation fMRI. We will also ask the subjects who participate in the fMRI to complete an emotional capture task as follows

Functional and activation fMRI: Subjects who participate in fMRI may be asked to perform a neuropsychological task. These tasks involve the presentation of visual or auditory stimuli related to cognitive or emotional functions. They will not include material that would be considered offensive, threatening or degrading. Visual stimuli may be words, stars, pictures of objects or human faces standardized by computer and back-projected onto a screen at the end of the patient gurney using an active matrix LCD Projection system. Two such systems are in current use on the Siemens systems. The subjects view the screen using a mirror system. Tasks will alternate to provide information at baseline (e.g. while passively viewing a cross hatch on a screen), during a cognitive activation task of interest (e.g. during a Stroop interference task while naming the color of the letters in words when the words do not match the color such as "blue" written in green letters) and a sensorimotor control (e.g. during a Stroop task naming the color of words presented in the congruent colors such as "blue" in blue letters). Images will be acquired in multiple runs. Conditions will be present in a pseudo-random order. Responses will be made by soft verbalization or by button press using a fiber optic response box. Accuracy and response time will be recorded.

Session 2 – ^{1}H MRS of GABA and glutamate levels: A ^{1}H MRS study will be performed on a 4T or 7T spectroscopy system located in the Yale MRRC. Proton MRS will be obtained with 3D localization in voxels in cortical and/or subcortical regions of interest on a 4T magnet, maintaining specific absorption rates within FDA limits. Placement of regions of interest is guided by the neurocircuitry. The subjects will be lying on their backs with the head resting in a special holder containing the ^{1}H observation coil. The study will last about 2 hours or less.

Session 3 – Positron Emission Tomography:
PET procedures will be conducted at the Yale University PET Center. Female subjects of child bearing potential will be given a urine pregnancy test prior to the initiation of any imaging procedures. If the test is positive, the scan session will be canceled.

Subjects will participate in [^{18}F]FPEB and/or [^{11}C]APP311 scans. High Resolution Research Tomograph (HRRT), the highest resolution human brain scanner available will be used to image subjects. PET scans are acquired as subjects lie supine on the scanner bed. Vital signs (blood pressure, pulse, respiration) will be obtained before and after radiotracer administration. A venous catheter will be used for IV administration of the radiotracer and for venous blood sampling. A radial artery catheter will be inserted by an experienced healthcare provider for [^{11}C]APP311 scans. At the beginning of each scan, the subject's head will be immobilized and a transmission scan is obtained before or after the scan, and used for

attenuation correction. PET scans will be acquired using bolus or bolus plus constant infusion administration of ≤5 mCi of [^{18}F]FPEB and/or ≤20 mCi of [^{11}C]APP311.

Dynamic images of radioactivity concentration are reconstructed with corrections for attenuation, normalization, random events, scatter, and deadtime. Subject motion is corrected automatically on an event-by-event basis with the Vicra motion tracking system if the images are acquired on the HRRT. Blood samples are taken during the scan for analysis of the fraction of plasma radioactivity unbound to protein and for metabolite analyses (~90 mL total).

Subject preparation for PET scanning consists of intravenous and (for [^{11}C]APP311 scans) arterial catheterization. An intravenous line placement will be performed by the research nurse or a certified nuclear medicine technologist. The arterial catheter will be placed by an experienced health care provider (an experienced physician or a highly skilled APRN trained to complete this procedure). Blood pressure will be monitored after arterial catheterization and throughout PET scanning via an indwelling catheter.

The goal of arterial lines is to be able to measure absolute physiological functions by mathematically relating the brain signal (from the PET scanner) to the tracer availability (from the blood). This approach provides the gold-standard data. In some cases, for well-established tracers, methods have been developed and validated that provide comparable results to the gold standard. In those cases, the arterial samples may not be necessary. However, validation of such an approach must be performed in each patient group and for each unique experimental design. In this study, if an arterial line cannot be placed, a second intravenous line may be placed for blood sampling (this is also the case for FPEB scans, as those do not require arterial line).

Subjects receiving arterial lines must refrain from taking over-the-counter aspirin, ibuprofen/naproxen, or another agent that can alter clotting for a period of two weeks prior to arterial line. Subjects taking prescribed aspirin on a daily schedule must receive clearance from their treating doctor to stop the aspirin. Clearance by the treating physician must be documented. The prescribed dose of aspirin, ibuprofen/naproxen, or another agent that can alter clotting cannot be stopped without the sign off of the subjects' primary care provider.

Individual blood samples (venous and/or arterial) are taken at various time points and counted in a gamma counter. Up to 6 tablespoons of blood will be drawn per PET scan. Samples are centrifuged to obtain plasma, which will be counted, and selected samples will be assayed for the presence of the parent radiotracer compound that has not been metabolized. These measurements will be performed by HPLC. In addition, the fraction of plasma radioactivity unbound to protein will also be determined. We will also obtain additional blood samples in all subjects to evaluate changes in plasma GABA/glutamate, glucocorticoid functioning, BDNF and VEGF, thyroid function, inflammatory cytokines (including TNF-alpha, IL-6, IL-10, IL-1beta), and other markers that may differ between groups. Blood will also be collected to measure gonadal hormone levels in all subjects. These levels may include FSH, estradiol, progesterone, LH, and testosterone. The volume of

blood collected during this study, including screening laboratories and PET scans, will be approximately 32 tablespoons.

Samples will be stored at the Yale PET Center until analysis. The GABA/BDNF/VEGF samples will be analyzed by co-investigator Dr. Tore Eid at Yale (CB465) and the Inflammatory markers by the YCCI (lab located at LMP 5042).

Research subjects may be asked to fast overnight (except for water) and report to the PET Center on PET scanning days.

In the event that scans are not able to be completed as scheduled, subjects may be asked to return on another day to complete the scan.

Cognitive Testing
Subjects will participate in cognitive testing at baseline, as well as on MRS and PET scan days, or within a few days of the scan. At baseline, 2-3 sessions will be administered to account for practice effects and novelty of testing. The following tests may be included from the CogState battery: International Shopping List task, Fixed Response Mapping task, Card Detection task, Groton Maze Learning task, One Back task. We may supplement cogstate with other cognitive measures.

Cold Pressor Task
Subjects may be asked to participate in the cold pressor task if their mental status and time allow. The cold pressor task (CPT) is a stress task used to measure pain sensitivity and pain tolerance. Participants will immerse their hand (up to the wrist) for up to 3 minutes in the experimental (ice-cold temperature (0-4°C)) and control (room temperature (20°C)) conditions. Physiological measures (heart rate, blood pressure) and subject responses (stress, mood) will be collected 5 minutes before, 1 minute into, and immediately after the CPT.

Electrophysiological Measures

Electroencephalography (EEG)
These tests use measures of electrophysiological activity recorded from the surface of the skin using conventional clinical electrodes. On the testing day, participants will be brought to the CNRU which has an electrically shielded and sound attenuated EEG testing booth. In the EEG (brainwave) recording, an elastic cloth cap with Ag/AgCl electrodes is worn by the subject. A conductive gel is then placed between the skin and the electrode prior to recording. The subject will listen to sounds or view visual images produced by a computer. By varying the characteristics of the auditory and visual stimulation, different brain systems are activated [39,40]. In some recordings, the subject will be asked to press a button to indicate what sort of stimulation he or she experienced. The EEG recordings will be obtained using a commercial psychophysiological system (Biosemi; see below). The entire EEG procedure will take approximately two hours.

EEG recording will be done with the commercially available ActiveTwo system (Biosemi, the Netherlands) using sintered Ag/AgCl electrodes. The ActiveTwo system is one of the

only commercially available systems with active electrodes (first amplifier stage on the electrode). The use of active electrodes eliminates 60 Hz interference pickup by the electrode wires, even when the electrode impedance is high (in the 100 kOhm range). Due to high electrode impedances being tolerated, the system can be used without the usual skin preparation (scrubbing) to lower the impedance. This makes the application of the electrodes very fast, minimizes discomfort of the subject, and eliminates the risk for infection. Further, subjects are fully protected from shocks by the isolation barrier between the amplifier and the recording computer (i.e. the optical fiber data-link combined with battery power supply provides complete safety; http://www.biosemi.com/faq/limit_current.htm).

EEGs will be recorded at a sampling rate of 1024 Hz with on-line low-pass filter of 256 Hz to prevent aliasing of high frequencies. A 64-channel electrode cap according to the extended 10-20 system will be utilized (http://www.biosemi.com/pics/cap_64_layout_medium.jpg). Seven additional electrodes will be placed: two on the mastoid processes, two horizontal EOG channels lateral to the left and right eyes' lateral canthi, two vertical EOG channels, one below (infraorbital) and one above (supraorbital) the right eye, and a channel on the tip of the nose. All electrodes will be referenced during recording to a common-mode signal (CMS) electrode between POz and PO3 and will be subsequently re-referenced digitally. A conductive gel will be placed between the skin and the electrodes prior to recording. The cap and all electrodes that come into contact with the skin will be cleaned and disinfected after each use in an antibacterial soap, which kills almost all vegetative microbial and viral life forms.

Prepulse Inhibition (PPI)

In order to examine brain inhibitory properties, a standard acoustic PPI paradigm adapted from Swerdlow et al. (1994) will be used on test days [41]. In this paradigm, either brief single white noise stimuli or pairs of white noise stimuli will be presented to subjects through headphones at sound levels above a constant 70 dB white noise background. Trials with single stimuli played at 118 dB SPL (40 ms duration) typically elicit a mild startle response, which will be quantified via eyeblinks using two electromyographic (EMG) electrodes placed under the right orbicularis oculi. On paired trials, a 78 dB SPL "prepulse" stimulus (20 ms in duration) will be administered 100 ms prior to the onset of the 118 dB SPL stimulus, which typically attenuates the startle response. A total of 15 single stimulus trials and 15 paired stimulus trials will be presented in a pseudorandom order with a variable intertrial interval of 15-45 seconds. PPI will be defined as the percentage of reduction in startle-blink amplitude in the presence of the prepulse compared with the amplitude in the absence of the prepulse (100 — [100 X amplitude on prepulse trial/amplitude on P-Alone trial]). Thus, a higher percentage score will indicate a high degree of PPI, while a low percentage score will indicate less or disrupted PPI. The PPI procedure will be performed and analyzed using commercially available hardware and software (http://www.sandiegoinstruments.com/product/sr-hlab-emg/). The entire task will take approximately 20 min.

Electrophysiology

The electrophysiological recordings described above (EEG and EMG) use standard, non-invasive clinical procedures, and are NOT considered dangerous or experimental. For EEG

and EMG collected at the CMHC, an active system (in which high electrode impedances are tolerated) will be used, and the system can be used without the usual skin preparation (scrubbing) to lower the impedance. This makes the application of the electrodes very fast, and minimizes discomfort of the subject.

Return Scans

Subjects who participated in an FPEB or SV2A scan may be asked to return to complete one or two additional FPEB and/or SV2A scans if their mood state changes from that which it was during their first scan. Subjects who were scanned in one mood state may be invited to return when they have met criteria for a different mood state. Subjects will be asked to contact the study team if there are any changes in their mood after study participation. Research staff may also make follow-up calls to subjects to survey their mood. Depression rating scales will be done over the phone to gauge any changes. Thus, individuals with bipolar disorder may be scanned up to 3 times with each tracer (depressed, euthymic, manic) and individuals with MDD may be scanned twice (depressed and euthymic) (see table on page 22/23). This is similar to the HIC7933. Depending on length of time between first scan and return scan, some screening procedures may need to be repeated (labs, physical, EKG) as well as MRI or MRS scan. Determination of repeat EKG and MRI will be made by the PI and/or study MD based on time since last EKG/MRI appointment and what happened since then. For example, if the subjects have had changes in medication or significant life events. Time period of >3 months will warrant repeat of all labs and EKG. MRIs will likely be repeated for all subjects unless a very short time period between the scans (e.g. 1 month). We will ask subjects to contact us when their mood changes, and assess them for repeat scan at that time.

Data Analysis

a. MRS of GABA levels

Using software written in MATLAB (The Mathworks, Inc.), each sub-FID will be line-broadened with a –2 Hz/6 Hz Lorentzian-Gaussian conversion, zero-filled to 16K points, and Fourier- transformed. The sub-spectra will be phased based on the spectrum with the inversion pulse applied, and the area of the creatine resonance will be measured. The two sub-spectra will be subtracted, and the area of the GABA resonance will be measured. If patients moved during any of the GABA editing scans, the much larger and sharper Cr and Cho resonances will cause well-defined subtraction errors that prevent the measurement of GABA, and any pairs of sub- spectra with such patient movement will not be processed further. The concentration of GABA will be determined from the ratio of GABA and total creatine resonances by comparison with solutions of GABA and creatine.

b. MRS of glutamate levels

Using software written in MATLAB (The Mathworks, Inc.), each sub-FID will be line-broadened with a –2 Hz/6 Hz Lorentzian-Gaussian conversion, zero-filled to 16K points, and Fourier- transformed. The spectra will be phased, and the peak heights of glutamate and several other metabolites in the spectrum will be measured and compared to the peak height of creatine for purposes of quantification.

c. Image segmentation analysis
The quantitative imaging of T_1 requires the acquisition of images of radio frequency distribution around the ^1H transceivers in each study, as well as a series of inversion-recovery images. The images will be interpreted together to derive the value of the T_1 relaxation rate in every image pixel in the voxel of CSF. The pixel percentages within the MRS voxel will be added together and divided by the total number of pixels to determine the values of percent grey matter, which is (grey matter)/(grey matter + white matter) as well as percent tissue, which is (grey matter + white matter)/(grey matter + white matter + cerebral spinal fluid).

d. PET data will be reconstructed into images at Yale PET Center with all standard corrections using the appropriate reconstruction protocols and filters.

SV2A

PET Image Analysis: To compute regional values, a summed PET image will be registered to each subject's T1-weighted MR image using a six-parameter mutual information algorithm (FLIRT, FSL 3.2, Analysis Group, FMRIB, Oxford, UK), which in turn will be registered to an MR template image with nonrigid registration (Bioimage Suite, Yale University). Regions of interest (ROIs) will be defined on the MRI template using AAL template.

Outcome Measure Calculation: The V_T images obtained from compartment modeling will be spatially normalized to a standard template (MNI, Montreal Neurological Institute) and analyses will be performed to assess ROI differences between groups in the PFC (dlPFC, vmPFC, and vlPFC) and HIP (right and left for all). To determine if there is a ketamine-induced change in synaptic density, we will calculate % change in V_T between scan 1 and scan 2 [$((V_{Tpost}-V_{Tpre})/V_{Tpre})*100= \Delta V_T$].

FPEB

PET Image Analysis: To compute regional values, a summed PET image will be registered to each subject's T1- weighted MR image using a six-parameter mutual information algorithm (FLIRT, FSL 3.2, Analysis Group, FMRIB, Oxford, UK), which will then be registered to an MR template by a non-rigid registration (BioImage Suite, Yale University, www.bioimagesuite.org). VmPFC and vlPFC ROIs will be defined in BioImage Suite. Additional ROIs will be explored.

Outcome Measure Calculation: As described in the preliminary sections, we use V_T[42] as our outcome measure. For regional analysis, outcome measures will be calculated using the equilibrium approach[43], which we found to be a superior approach. The V_T images will be spatially normalized to a standard template (MNI, Montreal Neurological Institute) and statistics will be performed to assess regional (ROI) differences between groups. These data will then be used for statistical analyses for Aims 1 and 3. Primary ROIs will be the vmPFC and vlPFC. Voxel-wise analyses using SPM will follow to localize the vmPFC and vlPFC for fMRI analyses, as well as to examine whether there are group differences in other regions. Partial volume corrections will be applied. Following the examination of the between-group differences in V_T, we will also evaluate potential effects of the reference region approach on between-group differences. Although we did not observe a significant difference in the CB mGluR5 availability between groups, this could be due to the small sample size in the BD-Dep group. In order to

compare our results with those that may already have been published, and that have used the CB as a reference region, we will explore DVR or BP_{ND} as outcome measures to better compare our results to others.

SPM analysis
Statistical parametric mapping software (Wellcome Department of Imaging Neuroscience in the Institute of Neurology at University College London) implemented in Matlab, version 6.5 (Mathworks Inc., Sherborn, MA) will be used for voxel-based statistical analysis. Each scan is transformed into stereotactic space by normalizing to a template image. The images are then smoothed using a Gaussian kernel (8x8x8 FWHM). Group will be performed using an unpaired or paired t-test, as appropriate. Correlational analyses will be completed to examine relationships between PET data and clinical/behavioral characteristics.

Future analyses
fMRI connectivity data will be used to predict symptoms associated with psychiatric disorders. Connectivity data will also be combined with mGluR5 PET data to assess whether this improves prediction of symptoms. Specifically, we will use connectome-based predictive modeling (CPM), which applies machine-learning algorithm to fMRI data to predict behavior, and offers important advantages over a purely correlational approach, such as generalizability. This analysis will be performed on anonymized fMRI/PET data.

4. **Genetic Testing N/A** ☒
   A. Describe
      i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned
      ii. the plan for the collection of material or the conditions under which material will be received
      iii. the types of information about the donor/individual contributors that will be entered into a database
      iv. the methods to uphold confidentiality

   B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects?
   C. Is widespread sharing of materials planned?
   D. When and under what conditions will materials be stripped of all identifiers?
   E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials?
      i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)?

   F. Describe the provisions for protection of participant privacy
   G. Describe the methods for the security of storage and sharing of materials

5. **Subject Population:** Provide a detailed description of the types of human subjects who will be recruited into this study.

Group 1: 60 psychiatrically healthy subjects will be enrolled as controls
Group 2: 30 subjects with major depressive disorder (MDD)
Group 3: 30 subjects with bipolar disorder

6. **Subject classification:** Check off all classifications of subjects that will be specifically recruited for enrollment in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

☐ Children	☒ Healthy	☐ Fetal material, placenta, or dead fetus
☐ Non-English Speaking	☐ Prisoners	☒ Economically disadvantaged persons
☐ Decisionally Impaired	☐ Employees	☐ Pregnant women and/or fetuses
☐ Yale Students	☒ Females of childbearing potential	

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects? ☐ Yes ☒ No (If yes, see Instructions section VII #4 for further requirements)

7. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion?

General inclusion criteria:
1. 18-80 years old
2. English speaking
3. No other DSM-5 diagnosis present, besides required as below

Inclusion criteria for healthy controls (n=60):
No current, or history of, any DSM-5 diagnosis, except for nicotine use disorder

Inclusion criteria for MDD subjects (n=30):
Clinical diagnosis of major depressive disorder

Inclusion criteria for bipolar subjects (n=30)
Clinical diagnosis of bipolar disorder

Exclusion criteria:
1. Have a current or past significant medical, neurological or metabolic disorder such that would contraindicate study participation based on above criteria and PI/MD review
2. History of head injury that lead to significant long term decline in cognitive abilities as seen by decline in grades or work performance
3. Have active, significant suicidal ideation
4. Have implanted metallic devices or any MR contraindications
5. Are women who are pregnant or breastfeeding
6. Met DSM-5 criteria for mild substance use disorder in the past 6 months or moderate to severe substance use disorder within the past year (except marijuana or nicotine)

7. Have history of prior radiation exposure for research purposes within the past year such that participation in this study would place them over FDA limits for annual radiation exposure. This guideline is an effective dose of 5 rem received per year
8. Subjects with current, past or anticipated exposure to radiation in the work place within one year of proposed research PET scans.
9. Have given a blood donation within eight weeks of the start of the study
10. Have history of a bleeding disorder or are currently taking anticoagulants (such as Coumadin, Heparin, Pradaxa, Xarelto).

8. How will **eligibility** be determined, and by whom?
   Eligibility will be determined based on the above criteria by the study PI in collaboration with co-investigators and study medical providers.

9. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

1) Risks associated with unknowns, 2) Risks associated with venous catheter insertion and with blood drawing, 3) risks associated with arterial catheter, 4) risks associated with radiation exposure, 5) risks associated with MR

1. **Risks Associated with Unknowns**
   The subject's health and safety will always be the primary concern of the doctors and staff performing the study. In the event of an unexpected outcome, all necessary medical action will be taken.

   Medication might be administered as needed, per the Yale PET Center standard operating procedure for medical emergencies, in order to treat complications.

2. **Risks Associated with Blood Drawing and IV Line Insertion**: Drawing blood and inserting an intravenous line (IV) into an arm vein are safe and standard medical procedures. Sometimes a bruise will occur at the puncture site and rarely a blood clot or infection will occur in the vein. Certain individuals may feel light-headed during venipuncture. The volume of blood collected during this study, including screening laboratories, MRS and PET scans, will be approximately 32 tablespoons. This is not expected to have any serious negative effects on a study participant.

3. **Risks Associated with Arterial line:** On the [^{11}C]APP311 PET scanning day, a radial arterial catheter will be inserted. Certain individuals may feel light-headed during arterial catheter placement. Arterial sampling may be associated with mild-to-moderate pain, hematoma, inflammation, bleeding, or bruising at the puncture site. If any of these, or other symptoms occur and do not diminish within 24 to 72 hours after the arterial line removal, or in the event that they worsen, subjects should be advised to call the on-call doctor listed on the PET discharge instructions. In rare instances blocking of the artery, tearing of the artery, arterial leakage, poor healing, nerve damage, or infection at the catheter insertion site may occur.

4. **Risks Associated with Radiation:** The Yale University Radioactive Investigational Drug Committee (YU RIDC) and the Yale University Radiation Safety Committee (YU-RSC) have reviewed and approved the use of radiation in this research study. This research study involves exposure to radiation from [^{18}F]FPEB and/or [^{11}C]APP311, and transmission scans. This radiation exposure is not necessary for medical care and is for research purposes only.

   The targeted amount of radiation an individual subject will receive in this study is from 1 injection of ≤ 5 mCi from [^{18}F]FPEB and/or 1 injection of ≤ 20 mCi from [^{11}C]APP311 plus transmission scans.

   [^{18}F]FPEB: Although each organ will receive a different dose, the amount of radiation exposure subjects will receive from this study is equal to an effective dose of 0.312 rem for a total of up to 5 mCi of [^{18}F]FPEB in 1 injection. This calculated value is used to relate the dose received by each organ to a single value. For subjects who return for repeat FPEB scans due to a change in their mood state, a total of 0.624 rem for up to 2 scans, or a total of up to 0.936 rem for up to 3 scans will be the radiation exposure they will receive from this study.

   [^{11}C]APP311*:* Although each organ will receive a different dose, the amount of radiation exposure subjects will receive from this study is equal to an effective dose of 0.607 rem for a total of up to 20 mCi of [^{11}C]APP311 in 1 injection. This calculated value is used to relate the dose received by each organ to a single value. For subjects who return for a repeat [^{11}C]UCB-J scan, a total of 1.214 rem for up to 2 scans, or a total of up to 1.821 for 3 scans, will be the radiation exposure they will receive from this study.

   <u>Transmission Scan Dose</u>
   Up to 2 transmission scans may be completed per PET scan, for a total of 0.0028 rem to the head per PET scan session (0.0014 rem per transmission scan). The maximum dose to the head received would be 0.0056 rem from up to 4 transmission scans for Psychiatrically Healthy Subjects, up to 0.01112 rem from up to 8 transmission scans for MDD, and up to 0.0168 rem from up to 12 transmission scans for Bipolar.

   Maximum Dose for the Entire Study
   Although each organ will receive a different dose, the maximum amount of radiation exposure each subject population will receive from participating in this study is the following:

Subject Group	Targeted # of [^{11}C]APP311 injections	Targeted # of ^{18}F-FPEB injections	ED (rem)	ED (rem), including transmission scans**	Maximum* # of [^{11}C]APP311 injections	Maximum* # of ^{18}F-FPEB injections	Maximum ED (rem)	Maximum ED (rem), including transmission dose**
Psychiatrically Healthy	1	1	0.919	0.924	1	1	0.919	0.924
MDD	1	1	0.919	0.924	2	2	1.838	1.849

Bipolar	1	1	0.919	0.924	3	3	2.756	2.773

*MDD subjects may be scanned with each radiotracer in up to two mood states (depressed, euthymic). Bipolar subjects may be scanned with each radiotracer in up to three mood states (depressed, euthymic, manic).
** Includes an additional 0.0014 of radiation exposure to the head, per transmission scan.

5. **Risks Associated with Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy:** Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines.
The subject will be watched closely throughout the MR study. Some people may feel uncomfortable or anxious. If this happens, the subject may ask to stop the study at any time and we will take them out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly but we will ask subjects to tell the research staff if they have them.
There are some risks with an MR study for certain people. If a subject has a pacemaker or some metal objects inside their body, they may not be in this study because the strong magnets in the MR scanner might harm them. Another risk is the possibility of metal objects being pulled into the magnet and hitting the subject. To reduce this risk, we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. We also ask that all people involved with the study walk through a detector designed to detect metal objects. No metal may be brought into the magnet room at any time. Once the subject is in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet. Subjects will fill out an MR Safety Questionnaire and be asked to tell research staff of any information that they think may be important.
The MR study is for research purposes only and is not in any way a clinical examination. The scans performed in this study are not designed to find abnormalities. The primary investigator, the lab, the MR technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a diagnostic evaluation of the images. If a worrisome finding is seen on a scan, a radiologist or another physician will be asked to review the relevant images. Based on his or her recommendation (if any), the primary investigator will contact the subject, inform them of the finding, and recommend that they seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie solely with the subject and their physician.
The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that subjects receive based on these findings. The images collected in this study are not a clinical MR exam and for that reason, they will not be made available for diagnostic purposes.

10. **Minimizing Risks:** Describe the manner in which the above-mentioned risks will be minimized.

Radiation: The dose of radiation has been reviewed and approved by the Yale University Radioactive Investigational Drug Committee (YU RIDC) and Yale University Radiation Safety Committee (YU RSC). All scans will be done in the presence of medical supervision and trained nursing staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the PET scan facilities have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiopharmaceuticals and performance of PET scans will be by radiochemists, physicians, and technologists of the Department of Radiology and Biomedical Imaging, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radiopharmaceuticals.

No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing during evaluation and on each scan day before initiation of any scan procedures. If subjects are breastfeeding they will not be able to participate in this research study.

Blood Drawing: The risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained and experienced personnel using aseptic technique. To avoid injury due to fainting, the catheter will be inserted when the subjects are in a recumbent position. The blood draws during PET scanning sessions will be obtained from the already inserted catheter, to minimize discomfort.

Arterial Catheter: Risks of radial artery cannulation are minimized by having the procedure performed by an experienced health care provider. The health care provider would be either a physician or an Advanced Practice Provider (APP): for example, a physician assistant (PA) or an advanced practice registered nurse (APRN) with experience in placement of arterial catheters. For an APP to place the arterial line at the Yale PET Center, they must meet the following criteria:
1.) Be currently credentialed at Yale-New Haven Hospital or similar institute and
2.) Perform 3 arterial line procedures supervised by a currently privileged PET Center physician.
The 3 supervised arterial line placements will be documented and signed off by both the APP and supervising physician. The completed document must be on file at the Yale PET Center prior to an APP performing any arterial line catheterizations independently.

Pain is minimized by local anesthesia. Bleeding is prevented by local pressure applied for a minimum of 15 minutes after catheter removal. Subjects will have their hand and finger blood supply examined after arterial cannulation and again following catheter removal. Also, subjects will be asked to abstain from anticoagulants for 7-10 days prior to arterial line insertion and 7-10 days following arterial line removal. Subjects will be provided a 24-hour emergency physician telephone number to call if they encounter pain, discoloration, numbness, tingling, coolness, hematoma, inflammation, or any other unusual symptoms in the wrist or hand, or fever, chills, or drainage from the vascular puncture sites, following the procedure. In addition, if an emergency arises at the time of cannulation or scanning, 911 will be called, and the subject will be sent to the Emergency Department for evaluation and

treatment. Nurses will provide the subjects an instruction sheet documenting problems to watch for and procedures to follow should such problems occur. Infection is avoided by adequate cleansing of the skin prior to intravascular line insertion.

MRI/MRS: All subjects will be screened for any metallic objects and other MR contraindications that they may be holding or have implanted in their bodies using a questionnaire and all potential subjects with contraindications for MR will be excluded. This questionnaire will be repeated immediately before each measurement to ensure that no metallic materials are brought into close proximity of the magnet, where they might be pulled toward the magnet. For additional security, subjects will be taken through a ferromagnetic metal detector immediately before going to the scan room.

11. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.) For more information, see the Instructions, page 24.
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study? Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as below.
    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? n/a
    c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are available here http://www.yale.edu/hrpp/forms-templates/biomedical.html for
        i. Minimal risk
        ii. Greater than minimal

    1. Personnel responsible for the safety review and its frequency:
       The principal investigator as well as supervising physicians (Drs. Gerard Sanacora and David Matuskey) will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal investigator and Drs. Sanacora/Matuskey will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. Either the principal investigator, the HIC or HSC have the authority to stop or suspend the study or require modifications.
    2. The risks associated with the current study are deemed moderate for the following reasons:
        a. We do not view the risks associated with the radiotracer imaging studies as minimal
        b. Given the now established safety and validity of the current study in our prior work, we do not view the proposed study as high risk

3. Attribution of Adverse Events:
   Adverse events will be monitored for each subject participating in the study and attributed to the study procedures/design by the principal investigator Irina Esterlis, Ph.D. and Drs. Sanacora or Matuskey according to the following categories:
   a.) Definite: Adverse event is clearly related to investigational procedure(s)/agent(s)
   b.) Probable: Adverse event is likely related to investigational procedure(s)/agent(s)
   c.) Possible: Adverse event may be related to investigational procedure(s)/agent(s)
   d.) Unlikely: Adverse event is likely not to be related to the investigational procedure(s)/agent(s)
   e.) Unrelated: Adverse event is clearly not related to investigational procedure(s)/agent(s)
4. Plan for Grading Adverse Events:
   The following scale will be used in grading the severity of adverse events noted during the study:
   a. mild adverse event
   b. moderate adverse event
   c. severe
5. Plan for Determining Seriousness of Adverse Events:
   In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it:
   a. is life-threatening
   b. results in in-patient hospitalization or prolongation of existing hospitalization
   c. results in persistent or significant disability or incapacity
   d. results in a congenital anomaly or birth defect OR
   e. results in death
   f. based upon appropriate medical judgement, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition, or
   g. adversely affects the risk/benefit ratio of the study

   An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate, but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the HIC or HSC is necessary.

6. Plan for reporting serious AND unanticipated AND related adverse events, anticipated adverse events occurring at a greater frequency than expected, and other unanticipated problems involving risks to subjects or others to the HIC or HSC.

The investigator will report the following types of adverse events to the HIC or HSC:

a. serious AND unanticipated AND possibly, probably or definitely related events
b. anticipated adverse events occurring with a greater frequency than expected
c. other unanticipated problems involving risks to subjects or others

These adverse events or unanticipated problems involving risks to subjects or others will be reported to the HIC or HSC within 48 hours of it becoming known to the investigator, using the appropriate forms found on the website.

7. Plan for reporting adverse events to co-investigators on the study, as appropriate the protocol's research monitor(s), e.g., industrial sponsor, Yale Center for Clinical Investigation Research Subject Advocates (RSAs), Cancer Center's Quality Assurance, Compliance and Safety Committee (QUACS) Protocol Review Committee (PRC), DSMBs, study sponsors, funding and regulatory agencies, and regulatory and decision-making bodies.
   For the current study, the following individuals, funding, and/or regulatory agencies will be notified:
   All co-investigators listed on the protocol
   YU RIDC
   National Institutes of Health
   Food and Drug Administration

   The principal investigator Irina Esterlis Ph.D. and Drs. Sanacora and Matuskey will conduct a review of all adverse events upon completion of every study subject. The principal investigator and physicians will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

d. For multi-site studies for which the Yale PI serves as the lead investigator: n/a
   i. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed?
   ii. What provisions are in place for management of interim results?
   iii. What will the multi-site process be for protocol modifications?

12. **Statistical Considerations:** Describe the statistical analyses that support the study design.
We propose a sample size of 30 per psychiatric group to permit a large attrition rate in psychiatric populations. *Hypothesis 1 analysis:* Multivariate analyses of variance (MANOVA), which corrects for multiple comparisons, will be employed to assess for between-group differences in the amygdala, hippocampus, thalamus, anterior cingulate and frontal cortices. *Hypothesis 1 exploratory analysis:* Statistical parametric mapping (SPM) will be used for whole brain between-group comparisons of mGluR5. *Hypothesis 2 analyses:* MANOVA will be used to examine the differences in glutamate and other neurotransmitter cycling in mood disorders as compared to healthy controls. *Hypothesis 3 analyses:* Cognitive outcomes will be correlated with mGluR5 availability in the associated brain regions. Further, principal component analyses will be applied to the cognitive data to reduce the dimensionality of the cognitive outcome variables. Results will

be considered significant at $p<0.05$. *Hypothesis 4 analyses:* Multivariate analyses of variance (MANOVA), which corrects for multiple comparisons, will be employed to assess for between-group differences in the amygdala, hippocampus, thalamus, anterior cingulate and frontal cortices between subject groups. *Hypothesis 5 analyses:* Multivariate analyses of variance (MANOVA), which corrects for multiple comparisons, will be employed to assess for between-group differences in the amygdala, hippocampus, thalamus, anterior cingulate and frontal cortices between subjects with mood disorders and controls. *Hypothesis 6 analyses:* MANOVA will be employed to examine the effect of nicotine on mGluR5/glutamate and SV2A, as well as relate to cognitive and behavioral outcomes (Pearson's test). *Hypothesis 7 analyses:* MANOVA will be employed to examine the effect of sex/gender on mGluR5/glutamate and SV2A, as well as relate to cognitive and behavioral outcomes (Pearson's test).

SECTION VI: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS AND DEVICES

If this section (or one of its parts, A or B) is not applicable, state N/A and delete the rest of the section.

A. DRUGS, BIOLOGICS and RADIOTRACERS

1. **Identification of Drug, Biologic or Radiotracer:** What is (are) the **name(s)** of the drug(s) biologic(s) or radiotracer(s) being used? Identify whether FDA approval has been granted and for what indication(s).

Radiotracers for PET studies:

[^{18}F]FPEB is a radiotracer used for imaging the metabotropic glutamatergic system (mGluR5). For each PET up to 5mCi of [^{18}F]FPEB i.v. will be administered. This product is not FDA approved and is being administered under Yale PET Center IND# 155250.

[^{11}C-APP311], (aka [^{11}C]UCB-J) is a radiotracer specific for imaging synaptic density. For each PET scan, up to 20 mCi of [^{11}C]APP311 i.v. will be administered. This product is not FDA approved and is being administered under Yale PET Center IND# 155250 (The Yale PET Center [^{11}C]APP311 eIND#123271 will be cross-referenced).

All protocols which utilize a drug, biologic or radiotracer **not** approved by, but regulated by, the FDA, or a radiotracer regulated by the RDRC, must provide the following information:

a. What is the Investigational New Drug (IND) **number** assigned by the FDA? **155250**
b. Who holds the IND? **The Yale University PET Center**
c. All protocols which utilize a radiotracer not approved by, but regulated by the FDA must provide the IND number: _____
Alternatively, use of the investigational radiotracer may be under RDRC/RSC oversight: (check if appropriate)_____

For all investigational radiotracers, attach a copy of the RDRC/RSC application (for radioisotopes used in the PET Center, PET Center personnel may complete this step)

Go to http://rsc.med.yale.edu/login.asp?url=myApps.asp. When you have logged in, complete the application and attach a copy to this submission.

Alternatively, an **exemption from IND filing requirements** may be sought for a clinical investigation of a drug product that is lawfully marketed in the United States. If there is no IND and an exemption is being sought, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

Exempt Category 1
The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:

i. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug. ☐ Yes ☐ No
ii. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the product. ☐ Yes ☐ No
iii. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. ☐ Yes ☐ No
iv. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56). ☐ Yes ☐ No
v. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs. ☐ Yes ☐ No

Exempt Category 2 (all items i, ii, and iii must be checked to grant a category 2 exemption)

☐ i. The clinical investigation is for an *in vitro* diagnostic biological product that involves one or more of the following (check all that apply):
- ☐ Blood grouping serum
- ☐ Reagent red blood cells
- ☐ Anti-human globulin

☐ ii. The diagnostic test is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure; and

☐ iii. The diagnostic test is shipped in compliance with 21 CFR §312.160.

Exempt Category 3

☐ The drug is intended solely for tests in vitro or in laboratory research animals if shipped in accordance with 21 CFR 312.60

Exempt Category 4

☐ A clinical investigation involving use of a placebo if the investigation does not otherwise require submission of an IND.

2. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

2.1 [^{18}F]FPEB

To date, the PET Center has completed 393 administrations of ≤ 5 mCi [^{18}F]FPEB i.v. in multiple subject populations, with a maximum mass dose to date of ≤ 0.93 µg. There has been no clinically detectable pharmacological effects or side effects associated with the administration of the radiopharmaceutical

2.2 ^{11}C-APP311 ([^{11}C]UCB-J)

To date, the PET Center has completed over 500 administrations of ≤ 20mCi [^{11}C]APP311, i.v. in multiple subject populations, with a maximum mass dose to date of <4 µg. There have been no clinically detectable side effects associated with the administration of the radiophamaceutical.

3. **Source:** a) Identify the source of the drug or biologic to be used.

Both radioactive drugs, [^{18}F]FPEB and [^{11}C]APP311 will be synthesized at the Yale University PET Center radiochemistry Laboratory under the supervisions of Dr. Henry Huang and Dr. Nabeel Nabulsi.

b) Is the drug provided free of charge to subjects? ☒ Yes ☐ No
If yes, by whom?

4. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.

Check applicable Investigational Drug Service utilized:

☒ **YNHH IDS**
☐ **CMHC Pharmacy**
☒ **PET Center**
☐ **Other:**
☐ **Yale Cancer Center**
☐ **West Haven VA**
☐ **None**

Note: If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation.

Due to the short half-life, PET drugs are prepared *ex tempore* and formulated immediately before administration, and therefore there are no issues with storage or stability. PET drug products are stored in their respective dose vial (final product container) at room temperature and are stable for at least 60 min after their preparation – 1 hour for [^{11}C]APP311, 8 hours for [^{18}F]FPEB.

The preparation of sterile PET drug products is validated prior to human use. Sterility is achieved by passing the PET drug product through a 0.22 micron membrane filter during the last step of the formulation process. Prior to release for administration, a bubble point test is performed on the membrane filter used for terminal sterilization in order to validate and verify its integrity during the filtration process. Due to the short half-life, a sample of the PET drug product is tested for sterility *ex post facto* for further confirmation.

The level of endotoxin in each batch of the final PET drug product is determined quantitatively prior to release for administration using the FDA approved Charles River Laboratory's Portable Testing System (Endosafe®-PTS).

[^{18}F]FPEB: will be prepared in accordance with local Chemistry Manufacturing & Control (CMC) procedures and quality specifications described in our FDA approved Drug Master File (DMF, IND#155250).

[^{11}C]APP311 ([^{11}C]UCB-J): will be prepared at the Yale PET Center in accordance with local Chemistry Manufacturing & Control (CMC) procedures and quality specifications described in our FDA approved local Drug Master File (DMF, IND 123271).

5. **Use of Placebo:** ☒ **Not applicable to this research project**
If use of a placebo is planned, provide a justification which addresses the following:
   1. Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this.
   b. State the maximum total length of time a participant may receive placebo while on the study.
   c. Address the greatest potential harm that may come to a participant as a result of receiving placebo.
   d. Describe the procedures that are in place to safeguard participants receiving placebo.

6. **Use of Controlled Substances:**
Will this research project involve the use of controlled substances in human subjects?
☐ Yes ☒ No *See HIC Application Instructions to view controlled substance listings*.

If yes, is the use of the controlled substance considered:
☐ Therapeutic: The use of the controlled substance, within the context of the research, has the potential to benefit the research participant.
☐ Non-Therapeutic: *Note, the use of a controlled substance in a non-therapeutic research study involving human subjects may require that the investigator obtain a Laboratory Research License. Examples include controlled substances used for basic imaging, observation or biochemical studies or other non-therapeutic purposes. See Instructions for further information.*

7. **Continuation of Drug Therapy After Study Closure** ☒ **Not applicable to this project**
Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended?
☐ Yes If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access.

☐ No If no, explain why this is acceptable.

B. DEVICES N/A

1. Are there any investigational devices used or investigational procedures performed at Yale-New Haven Hospital (YNHH) (e.g., in the YNHH Operating Room or YNHH Heart and Vascular Center)? ☐Yes ☒No *If Yes, please be aware of the following requirements*:

a. A YNHH New Product/Trial Request Form must be completed via EPIC: **Pull down the Tools tab in the EPIC Banner, Click on Lawson, Click on "Add new" under the New Technology Request Summary and fill out the forms requested including the "Initial Request Form," "Clinical Evidence Summary, " and attach any other pertinent documents. Then select "save and submit" to submit your request**; and

b. Your request must be reviewed and approved **in writing** by the appropriate YNHH committee before patients/subjects may be scheduled to receive the investigational device or investigational procedure.

2. What is the name of the device to be studied in this protocol?

Has this device been FDA approved? ☐ Yes ☐ No
If yes, state for what indication.

3. **Background Information:** Provide a description of previous human use, known risks, and any other factors that might influence risks. If this is the first time this device is being used in humans, include relevant data on animal models.

4. **Source:**
   a) Identify the source of the device to be used.

   b) Is the device provided free of charge to subjects? ☐ Yes ☐ No

5. What is the PI's assessment of risk level (significant or non-significant) associated with the use of the device?

☐ **Significant Risk (SR) Device Study:** A study of a device that presents a potential for serious risk to the health, safety, or welfare of a participant and 1) is intended as an implant; 2) is used in supporting or sustaining human life; or otherwise prevents impairment of human health; 3) is of substantial importance in diagnosing, curing, mitigating or treating disease, or otherwise prevents impairment of human health; or 4) otherwise presents a potential for serious risk to the health, safety, or welfare of a participant.

Significant Risk Devices require an Investigational Device Exemption (IDE) issued by the FDA.

What is the **IDE number** assigned by the FDA?

Did the FDA approve this IDE as **Category A** (experimental/investigational) or as **Category B** (non-experimental/investigational)?

Who holds the IDE?

☐ **Non-Significant Risk (NSR) Device Study:** A study of a device that does not meet the definition for a significant risk device and does not present a potential for serious risk to the health, safety, or welfare of participants. Note that if the HIC concurs with this determination, an IDE is not required.

6. **Abbreviated IDE or Exempt IDE:** There are abbreviated requirements for an IDE and there also are exemptions to the requirement for an IDE. *See the criteria in the HIC Application Instructions,* Section VI.B.4 at http://www.yale.edu/hrpp/resources/docs/100FR1aHICProtocol_Application_Instructions5-25-11.pdf *to determine if these pertain to this study.*

☐ **Abbreviated IDE or Exempt IDE** – *If criteria set forth in the HIC Application Instructions are met, copy and paste the completed relevant section from the Instructions into this application.*

7. **Investigational device accountability**:
   a. State how the PI, or named designee, ensures that an investigational device is used only in accordance with the research protocol approved by the HIC, and maintains control of the investigational device as follows:

      Maintains appropriate records, including receipt of shipment, inventory at the site, dispensation or use by each participant, and final disposition and/or the return of the investigational device (or other disposal if applicable):

Documents pertinent information assigned to the investigational device (e.g., date, quantity, batch or serial number, expiration date if applicable, and unique code number):

Stores the investigational device according to the manufacturer's recommendations with respect to temperature, humidity, lighting, and other environmental considerations:

Ensures that the device is stored in a secure area with limited access in accordance with applicable regulatory requirements:

Distributes the investigational device to subjects enrolled in the IRB-approved protocol:

SECTION VII: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. **Targeted Enrollment: Give the number of subjects:**
   a. targeted for enrollment at Yale for this protocol 120
   b. If this is a multi-site study, give the total number of subjects targeted across all sites

2. **Indicate recruitment methods below.** Attach copies of any recruitment materials that will be used.

☒ Flyers	☒ Internet/Web Postings	☒ Radio
☒ Posters	☐ Mass E-mail Solicitation	☒ Telephone

☐ Letter
☐ Medical Record Review*
☐ Departmental/Center Newsletters
☐ YCCI Recruitment database
☐ Other (describe):

☐ Departmental/Center Website
☒ Departmental/Center Research Boards
☐ Web-Based Clinical Trial Registries
☒ Clinicaltrials.gov Registry (do not send materials to HIC)

☒ Television
☒ Newspaper

***Requests for medical records should be made through JDAT as described at**
http://medicine.yale.edu/ycci/oncore/availableservices/datarequests/datarequests.aspx

3. Recruitment Procedures:

a. Describe how potential subjects will be identified.
b. Describe how potential subjects are contacted.
c. Who is recruiting potential subjects?

Subjects will be recruited through flyers, public advertisement (newspaper, radio, internet postings), by word of mouth, contact with community service groups, and clinics and local treatment facilities (the VA Hospital, West Haven, CMHC, the Yale Psychiatric Hospital, Mood Disorders Research Program, the Yale Depression Research Program). The subjects will be asked to call us if they are interested in participating in research study. The PI, in collaboration with study investigators Drs. D'Souza, Sanacora, and Blumberg, is responsible for subject recruitment.

4. Screening Procedures

a. Will email or telephone correspondence be used to screen potential subjects for eligibility prior to the potential subject coming to the research office? ☒ Yes ☐ No
b. If yes, identify below all health information to be collected as part of screening and check off any of the following HIPAA identifiers to be collected and retained by the research team during this screening process.

HEALTH INFORMATION TO BE COLLECTED:

☒ Psychiatric history
☒ Medical history related to study exclusion criteria

HIPAA identifiers:

☒ Names
☒ All geographic subdivisions smaller than a State, including: street address, city, county, precinct, zip codes and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly-available data from the Bureau of the Census: (1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people, and (2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.
☒ Telephone numbers
☐ Fax numbers
☒ E-mail addresses
☐ Social Security numbers
☐ Medical record numbers
☐ Health plan beneficiary numbers
☐ Account numbers

☒ All elements of dates (except year) for dates related to an individual, including: birth date, admission date, discharge date, date of death, all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older
☐ Certificate/license numbers
☐ Vehicle identifiers and serial numbers, including license plate numbers
☐ Device identifiers and serial numbers
☐ Web Universal Resource Locators (URLs)
☐ Internet Protocol (IP) address numbers
☐ Biometric identifiers, including finger and voice prints
☐ Full face photographic images and any comparable images
☐ Any other unique identifying numbers, characteristics, or codes

5. Assessment of Current Health Provider Relationship for HIPAA Consideration:

Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?

☐ Yes, all subjects
☐ Yes, some of the subjects
☒ No

If yes, describe the nature of this relationship.

6. Request for waiver of HIPAA authorization: (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)

Choose one:

☐ For entire study
☒ For recruitment purposes only
☐ For inclusion of non-English speaking subject if short form is being used **and a translated HIPAA research authorization form is not available on the University's HIPAA website**

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data;
We post advertisements which ask subjects to call us if they are interested in participating in the studies. Once subjects call, we need to obtain their name, DOB, phone number, etc, in order to contact them regarding eligibility to participate/schedule further appointments, assess study eligibility correctly. Some subjects prefer we email them appointments and directions, thus we need to obtain their email addresses. We also need to obtain their city of residence to ensure they are within appropriate distance to arrive to all appointments in a timely fashion and to provide accurate directions to our labs.

ii. If requesting a waiver of **signed** authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data;

By signing this protocol application, the investigator assures that the protected health information for which a Waiver of Authorization has been requested will not

be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

7. **Required HIPAA Authorization:** If the research involves the creation, use or disclosure of protected health information (PHI), separate subject authorization is required under the HIPAA Privacy Rule. Indicate which of the following forms are being provided:
   - ☒ Compound Consent and Authorization form
   - ☐ HIPAA Research Authorization Form

8. **Process of Consent/Assent:** Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.

   Subjects who pass phone screen will be invited to 40 Temple Street (or the VA in the case of subjects who are screened under HSS protocol #KW003) for an in-person screening. The study will be described to individuals who appear to satisfy the study criteria. The nature of the project, the procedures, the relative risks and benefits, and the alternatives to participation in the project will be discussed with the subject. If, following this discussion, the subject continues to be interested in the project, written informed consent will be obtained. The decision not to participate will not affect an individual's eligibility to participate in future studies, to receive treatment at Yale-New Haven Hospital, or to receive treatment on a private basis from a referring clinician. A copy of the signed consent form will be provided to all participating subjects. For subjects who are not eligible, all PHI will be destroyed.

9. E**valuation of Subject(s) Capacity to Provide Informed Consent/Assent:** Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.

   We will not recruit subjects with limited decision-making capacity. As part of the consent process, prospective subjects are asked open-ended questions about the research in order to determine whether the subject recalls and understands the process of the study. If an individual shows poor comprehension of the consent form and study, we will not enroll them. A study doctor supervises the screening and enrollment process. In addition, all subjects complete the WTAR, which is a measurement of premorbid intelligence.

   In cases in which capacity is in doubt, the PI will assess the subject's understanding of the study and the subject's capacity to decide to participate. The PI gained extensive experience with determining decision-making through working with psychiatric populations 2004 to present. Prior to participation in study procedures, the subjects are given an explanation regarding each procedure and the time course for each assessment. They are asked open ended questions to ensure comprehension. If a subject is not able to answer questions, they cannot participate in the

study. However, this usually does not happen on procedure days, as we evaluate subjects during screening (HIC 2000027842) and subjects are disqualified at that time.

10. **Documentation of Consent/Assent:** Specify the documents that will be used during the consent/assent process. Copies of all documents should be appended to the protocol, in the same format that they will be given to subjects.
Compound Authorization and Consent form

11. **Non-English Speaking Subjects:** Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. If enrollment of these subjects is anticipated, translated copies of all consent materials must be submitted for approval prior to use.
Non-English speaking subjects will not be invited to participate in the studies. All of our materials are in English only, and staff members are fluent in English. Furthermore, cognitive testing is validated in English-speaking subjects only.

12(a) As a limited alternative to the above requirement, will you use the short form* for consenting process if you unexpectedly encounter a non-English speaking individual interested in study participation and the translation of the long form is not possible prior to intended enrollment? n/a
YES ☐ NO ☐

Note* If more than 2 study participants are enrolled using a short form translated into the same language, then the full consent form should be translated into that language for use the next time a subject speaking that language is to be enrolled.

Several translated short form templates are found on our website at: http://www.yale.edu/hrpp/forms-templates/biomedical.html. If the translation of the short form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via amendment prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

If using a short form without a translated HIPAA Research Authorization Form, please request a HIPAA waiver in the section above.

12. **Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study.** If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.
    - ☐ **Not Requesting a consent waiver**
    - ☒ **Requesting a waiver of signed consent**
    - ☐ **Requesting a full waiver of consent**

A. Waiver of **signed** consent: (Verbal consent from subjects will be obtained. **If PHI is collected, information in this section must match Section VII, Question 6**)
☒ **Requesting a waiver of signed consent for Recruitment/Screening only**
If requesting a waiver of signed consent, please address the following:

a. Would the signed consent form be the only record linking the subject and the research?
☐ Yes ☐ No
b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☐ No

OR

c. Does the research activity pose greater than minimal risk?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity
☒ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☒ No

☐ **Requesting a waiver of signed consent for the <u>Entire Study</u> (**Note that an information sheet may be required.)

If requesting a waiver of signed consent, please address the following:
a. Would the signed consent form be the only record linking the subject and the research?
☐ Yes ☐ No
b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☐ No

OR

c. Does the research pose greater than minimal risk? ☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** ☐ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☐ No

B. **<u>Full waiver</u>** <u>of consent:</u> (No consent from subjects will be obtained for the activity.)

☐ **Requesting a waiver of consent for <u>Recruitment/Screening</u> only**
a. Does the research activity pose greater than minimal risk to subjects?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity
☐ No
b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No
c. Why would the research be impracticable to conduct without the waiver?
d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

☐ **Requesting a full waiver of consent for the <u>Entire Study</u> (Note: If PHI is collected, information here must match Section VII, question 6.)**

If requesting a full waiver of consent, please address the following:

a. Does the research pose greater than minimal risk to subjects?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.***
☐ No
b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No
c. Why would the research be impracticable to conduct without the waiver?
d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

SECTION VIII: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

a. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?
Required private identifiable information about individuals, such as their medical history, current medications, psychiatric problems, family history, and scan data will be collected by research staff and used for research purposes and charting after consent is obtained.

b. How will the research data be collected, recorded and stored?
The data are collected and recorded by trained research personnel. The data will be recorded on Excel spreadsheets that will be saved onto a server or will be in the form of questionnaires that are filled out by the subject or the researcher. These paper research materials containing confidential information are stored in locked filing cabinets. Additional brain data is collected during the brain imaging scans by trained technologists and is stored on password-protected and encrypted computers with identifying information carefully in compliance with HIPAA regulations.

c. How will the digital data be stored? ☐ CD ☐ DVD ☐ Flash Drive ☐ Portable Hard Drive ☒ Secured Server ☐ Laptop Computer ☐ Desktop Computer ☐ Other

d. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?
All staff members that come into contact with the data are fully trained to the current HIPAA regulations and are informed as to the proper use of all data.
Identifiable paper information is kept in locked file drawers and password protected computer files. Results are published as group data without the use of characteristics that would identify individual subjects. We quote information only by number in conference discussions, scientific reports, or publications, in order to maintain anonymity. The key to coded data will be stored in a locked filing cabinet in our locked suite.
Identifiable research data, including recruitment and screening information and code keys, are stored on a secure database located on the internal PET Center Network. The PET network is protected by a Cisco PIX firewall operated by ITS. All research data are backed up nightly to a Dell PV-136T library with 4 IBM Ultrium-TD2 tape drives using the backup software Legato Networker 7.3 from EMC. Human subjects enrolled in the study are assigned a subject-specific random identifier. Subject identifiers and the means to link the subject names and codes with the research data are stored in separate locations within the database. The software of the database limits the ability to connect the random identifier to the actual subject

identification information to research team members only. Access to the database is password protected and each research team member is required to have a unique ID and password to gain access to the database. Authorized users employ their netid and authentication is performed using Yale's central authentication server. Users always access research data through the random identifier only. Direct identifiers belonging to subjects who withdraw from the study, will be stripped from the key.
Electronic data will be collected and stored using REDCap (Research Electronic Data Capture). REDCap is a secure, web application designed to support data capture for research studies. It includes features for HIPAA compliance including real-time data entry validation (e.g. for data types and range checks), a full audit trail, user-based privileges, de-identified data export mechanism to statistical packages (SPSS, SAS, Stata and R), and integration with the institutional Active Directory. Access to study data in REDCap will be restricted to the members of the study team with authentication through University NetID credentials.

All portable devices must contain encryption software, per University Policy 5100. *If there is a technical reason a device cannot be encrypted please submit an exception request to the Information Security, Policy and Compliance Office by clicking on url* http://its.yale.edu/egrc or email it.compliance@yale.edu

e. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.
The data will be stored in locked filing cabinets, on REDCap, and on the password-protected secure database on the internal Yale University PET Center Network for at least 7 years, accessed only by study personnel. De-identified PET data will be kept for a minimum of 7 years.

f. Who will have access to the protected health information (such as the research sponsor, the investigator, the research staff, all research monitors, FDA, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), SSC, etc.)? (please distinguish between PHI and de-identified data) The investigator and research staff (e.g., PET Center nuclear technologists, recruiters) will have access to the PHI only on as needed to know basis. The FDA and HIC may also have access to the PHI.

g. If appropriate, has a Certificate of Confidentiality been obtained? N/A

h. Are any of the study procedures likely to yield information subject to mandatory reporting requirements? (e.g., HIV testing – reporting of communicable diseases; parent interview -incidents of child abuse, elderly abuse, etc.). Please verify to whom such instances will need to be reported. A positive test result for HIV, hepatitis B or C will be reported to the Connecticut Department of Public Health per Connecticut Law.

SECTION IX: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)
There are no direct benefits from a subject's participation in this study. This research will benefit scientific knowledge by contributing to the understanding of the use of PET imaging of mGluR5 to diagnose and treat depression. This may have clinical application in the future.

SECTION X: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?
The alternative to participation in this research protocol is not to participate. Subjects will be informed that they are free to choose not to participate and, if they do agree to become a subject, they will be free to withdraw from the study at any time during its course. They will also be informed that if they choose not to participate or if they withdraw, it will not adversely affect their relationship with their doctors or the hospital (see attached Consent Form).

2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.
The subjects will be compensated for their time commitment and inconveniences necessary for completing the study. Subjects will have no financial responsibilities for any portion of the study. Compensation will be $250 for each PET scan, $50 for arterial line, $50 per hour of [1H]MRS scan, $75 for the fMRI, $25 for the MRI scan, and a $100 completion bonus. Subjects will be compensated $40 for cognitive testing. Subjects may also receive $10 for each cold pressor task session, $50 for each PPI session, and $100 for each EEG session that they participate in. Study payments may be in a form of a pre-paid credit card, check, or cash. In addition, subjects may be provided with a light lunch on their study days. Reasonable transportation and parking costs will be reimbursed at the discretion of the PI. In situations where a subject only completes partial measures or must return to complete additional or partial measures, payment will be based upon participation at the discretion of the PI.
In certain circumstances (loss of sample, unusable sample, etc.) subjects may be asked to return to provide an additional blood sample, for which they will be paid $20.
Subjects who return for one or two additional scans when they are in a different mood state will be paid $250 for each PET scan, $50 for arterial line, and $20 for cognitive testing. If MRI and/or MRS scans need to be repeated they will be paid $25 for the MRI, $75 for fMRI, and $50 for the MRS.

3. **Costs for Participation (Economic Considerations):** Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.
There will be no costs to subjects related to participation in this research study.

5. **In Case of Injury:** This section is required for any research involving more than minimal risk, and for minimal risk research that presents the potential for physical harm (e.g., research involving blood draws).

a. Will medical treatment be available if research-related injury occurs?
b. Where and from whom may treatment be obtained?
c. Are there any limits to the treatment being provided?
d. Who will pay for this treatment?
e. How will the medical treatment be accessed by subjects?

Medical treatment will be offered to the subjects for any physical injuries that they receive as a result of participating in this research. However, the subject or his/her insurance company is responsible for the cost. Federal regulations require that subjects be told that if they are physically injured, no additional financial compensation is available.

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