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HRP-503B – BIOMEDICAL RESEARCH PROTOCOL
(2018-1)

Protocol Title: Imaging the Neuroimmune Response to Alcohol

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Version Date: .06

(If applicable) **Clinicaltrials.gov Registration #:** Click or tap here to enter text.

INSTRUCTIONS

This template is intended to help investigators prepare a protocol that includes all of the necessary information needed by the IRB to determine whether a study meets approval criteria. **Read the following instructions before proceeding:**

1. Use this protocol template for a PI initiated study that includes direct interactions with research subjects. Additional templates for other types of research protocols are available in the system Library.
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3. Once completed, upload your protocol in the “Basic Information” screen in IRES IRB system.

SECTION I: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested. Alcohol use disorder affects nearly 14% of the population, accruing considerable cost to individual families and society. Much of this cost stems from alcohol's influence on the immune system. Alcohol impairs peripheral immune function, evidenced by increased susceptibility to infection related diseases such as liver cirrhosis and pancreatitis¹. The neuroimmune consequences of alcohol are subtler. Preclinically, alcohol triggers neuroimmune abnormalities that contribute to cognitive dysfunction, neurodegeneration, and alter alcohol drinking behaviors^{2,3}. Yet, limited experimental tools hamper translational efforts to study alcohol's effects on neuroimmune function in people. **We propose to address this deficit by developing an innovative human imaging paradigm that measures neuroimmune response to alcohol.** Our team developed an exciting imaging paradigm using positron emission tomography (PET) to measure 18-kDa translocator protein (TSPO) levels before and after a dose of the classic immune stimulus lipopolysaccharide (LPS)⁴. TSPO is a protein located on the outer mitochondrial membrane⁵ that is overexpressed during microglial activation^{6,7}. Acute LPS increased TSPO levels by 40-60% throughout the brain in a variety of species with this paradigm^{8,9}, establishing an imaging approach measuring the magnitude of acute neuroimmune response. However, LPS and alcohol act on microglia through different mechanisms¹⁰, motivating the important need to validate alcohol as an immune stimulus to study alcohol use disorder. To this end, a recent PET study reported robust increases in TSPO levels after alcohol infusion in monkeys¹¹. **We propose to translate this finding by measuring neuroimmune response to a fixed dose of alcohol in people.** The clinical relevance of this approach will be assessed with a battery designed to evaluate reward dysfunction, anxiety, and cognition at the time of peak 'sickness behaviors'. We will accomplish these goals with the following two specific aims:

Aim 1: To measure TSPO levels with PET imaging before and after a fixed-dose alcohol session.

We will recruit 50 moderate drinkers to participate in this study. A baseline PET scan with [¹¹C]PBR28, a TSPO-specific radioligand, will be conducted^{9,12}. Next, subjects will drink a fixed alcohol dose, followed a post-alcohol [¹¹C]PBR28 PET scan timed to capture acute neuroimmune response¹¹. [¹¹C]PBR28 distribution volumes (V_T), which are proportional to TSPO number, will be measured throughout the brain. We will test the hypothesis that acute alcohol robustly increases [¹¹C]PBR28 V_T , consistent with microglial activation. The percent change in [¹¹C]PBR28 V_T (ΔV_T) from baseline will quantify the magnitude of neuroimmune response.

Aim 2: To compare neuroimmune response in moderate drinkers and heavy drinkers.

In addition to the 50 moderate drinkers recruited for Aim 1, we will recruit 50 heavy drinkers (>21 drinks/week, women; >28 drinks/week, men). Subjects will participate in the study design described in Aim 1 (a baseline [¹¹C]PBR28 PET scan, drink a fixed alcohol dose, followed by a post-alcohol [¹¹C]PBR28 PET scans). The magnitude of neuroimmune response, quantified by ΔV_T , will be compared between moderate drinkers and heavy drinkers to test the hypothesis that the neuroimmune response to alcohol is greater in heavy drinkers compared to moderate drinkers, consistent with the concept of alcohol 'priming microglia'.

Aim 3: To compare the neuroimmune response to alcohol in women and men.

There are well-established differences between men and women in the peripheral immune response to alcohol, however, differences in the brain's response to alcohol are not known. Of the

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moderate drinkers and heavy drinkers, we will recruit equal numbers of women and men (25 in each group). Within each group, we will compare the magnitude of neuroimmune response, quantified by ΔV_T , between women and men in each group. Consistent with the existent preclinical and peripheral literature, we hypothesize that women will exhibit an exaggerated neuroimmune response to alcohol compared to men.

Aim 4: To relate neuroimmune response with 'sickness behaviors' relevant to alcohol use disorder.

Ratings of reward processing, anxiety, and cognitive function will be acquired first before baseline scanning, and a second time the morning after the alcohol session, timed to capture peak 'sickness behaviors'^{13,14}. We will assess the relationships of neuroimmune response (ΔV_T) with subsequent changes in reward processing, anxiety, and cognitive function by testing the following hypotheses:

- SubAim 4A: Greater neuroimmune response in basal ganglia predicts greater reward dysfunction
- SubAim 4B: Greater neuroimmune response in amygdala predicts heightened anxiety
- SubAim 4C: Greater neuroimmune response in hippocampus predicts poorer cognitive performance

Aim 5: To determine peak neuroimmune response with PET imaging after a fixed-dose alcohol session

The same participants from Aim 1 and Aim 2 will have an additional [¹¹C]PBR28 PET scan approximately 20hrs post alcohol administration. The magnitude of neuroimmune response, quantified by ΔV_T , will be compared with the first post challenge scan to determine when peak response occurs.

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

5 Years

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

1. NEUROIMMUNE RESPONSE TO ALCOHOL: THE ROLE OF MICROGLIAL ACTIVATION

Microglia are the primary immunocompetent cells in the brain. These macrophages maintain homeostasis and provide the first line of defense against immune challenges to the brain¹⁵. Under normal conditions, microglia exist in a 'resting' state featuring motile processes that survey the environment for pathogens or brain injury¹⁶. Resting microglia also interact with neurons and synapses, influencing synaptic plasticity^{17,18}. When microglia encounter pathogens or injury signs they become activated. **Microglial activation is a hallmark of neuroimmune response.** Activated microglia transform to a ramified shape and release immune signaling substances such as cytokines and chemokines¹⁷. This activation occurs in stages, and microglia fully activated to an amoeboid shape are phagocytic¹⁹. Microglial activation is a healthy process necessary for cell repair and re-establishing homeostasis¹⁹. However, excessive microglial activation can cause toxicity and cell death²⁰, while insufficient microglial activation can allow the immune stimulus to persist¹⁴. **Dysregulated microglial activation is implicated in many neuropsychiatric conditions²¹, including alcohol use disorder^{2,3}.**

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2. MEASURING ALCOHOL-INDUCED IMMUNE RESPONSE IN PEOPLE

Human studies identify altered immunity in alcohol use disorder. Peripheral pro-inflammatory cytokine levels are higher in people with alcohol use disorder compared to controls²²⁻²⁴, where greater cytokine levels are associated with more alcohol craving²⁵⁻²⁷. Increased gut permeability^{26,28,29} likely contributes to these cytokine elevations by leaking inflammatory molecules such as LPS and peptidoglycans²⁷. Post-mortem brain analyses indicate higher levels of pro-inflammatory cytokines, micro-RNA, genes, and immunohistochemical markers in alcohol dependent individuals compared to controls³⁰⁻³⁵. In contrast, imaging studies suggest lower or no different levels of immune-related biomarkers in alcohol use disorder compared to controls. This includes the magnetic resonance spectroscopy (MRS) measures of NAA³⁶⁻⁴² and Cho^{36,38,39,42-44}, related to neuronal integrity and glial density, respectively; and the 18 kDa translocator protein (TSPO)⁴⁵⁻⁴⁷, a protein sensitive to activated microglia. These human data demonstrate alcohol's intricate effects on the immune system, but provide a snapshot of immune state when measured. Yet, it is clear that a single measurement inadequately captures the dynamic nature of microglial activation. To this end, effects of alcohol on immune response in people are less well characterized. Peripheral monocytes exhibit a blunted pro-inflammatory response to LPS stimulation in alcohol use disorder^{9,27,48}. While peripheral cytokines influence brain immune signaling, this relationship is neither direct nor temporally in sync^{13,49,50}; thus, no studies have directly studied the neuroimmune response to alcohol in people. **We propose to address this knowledge gap by validating an imaging approach directly measuring TSPO levels before and after an alcohol dose.** This measurement is needed to elucidate clinically relevant aspects of microglial activation in alcohol use disorder.

3. TSPO IS AN IMAGING BIOMARKER SENSITIVE TO MICROGLIA ACTIVATED BY ALCOHOL

TSPO is an extensively used biomarker associated with neuroimmune function¹². TSPO is found on the outer mitochondrial membrane of microglia and reactive astrocytes, with low expression in other neuronal cells⁵. PET radioligands with high affinity and specificity to TSPO, such as [¹¹C]PBR28⁵¹⁻⁵³, can be used to estimate the ligand distribution volume (V_T)⁵⁴, which is proportional to the number of *in vivo* sites available for [¹¹C]PBR28 binding B_{avail} where $V_T = B_{avail}/K_D + V_{ND}$. We therefore term [¹¹C]PBR28 V_T as 'TSPO availability'. TSPO expression co-localizes with activated microglia^{6,55}, where binding to TSPO sites correlates strongly with immunohistochemical markers of microglial activation but not those of astrocyte activation⁵⁶. Thus TSPO is a biomarker sensitive to microglial activation¹². Yet, abnormal TSPO levels can also stem from altered TSPO expression in non-microglial cells, change in numbers of cells expressing TSPO, or other processes such as abnormal cell metabolism⁵⁷. This context presents challenges interpreting data from single TSPO PET scans.

A TSPO imaging strategy that maintains unambiguous interpretation of TSPO availability acquires imaging data before and after an acute immune stimulus. For example, LPS triggers 'classic' microglial activation⁵⁸. In non-human primates, we and others reported that LPS robustly increased [¹¹C]PBR28 V_T by 40-60%^{8,9}. Post-mortem analyses indicated similar morphology in cells immunoreactive for TSPO and cells immunoreactive for CD68 (marker of microglia), but not with cells immunoreactive for GFAP (marker of astrocytes; Fig. 1)⁸, demonstrating that increased TSPO expression in microglia mostly accounted for the observed increase in TSPO availability. A team led by Dr. Kelly Cosgrove, a collaborator on this proposal (see letter) translated this paradigm to image people, where 1 ng/kg LPS increased [¹¹C]PBR28 V_T by 46±8% across the brain⁵⁹. **These data demonstrate strong scientific rigor**

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validating TSPO as an imaging biomarker of acute microglial activation. Yet, since LPS indirectly acts on microglia while alcohol's actions are both direct and indirect¹⁰, LPS inadequately models alcohol's complex effects on the neuroimmune system. Thus, **validation of alcohol as an immune stimulus is needed to extend this exciting imaging paradigm to study alcohol use disorder.**

Microglia activated by alcohol overexpress TSPO^{2,60-63}. In a comprehensive preclinical study, a four-day binge alcohol paradigm increased both OX-42 immunoreactivity, a cardinal index of microglia activation⁶⁴, and TSPO radioligand binding⁶² (Fig. 2A-B). **This report validates TSPO as a biomarker of alcohol-induced microglial activation.** Indeed, TSPO radioligand binding increased following ethanol injected directly into rodent striatum^{65,66}, and alcohol administered systemically^{62,67}. **An exciting recent baboon study reported that i.v. alcohol targeting 1 g/L in blood increased TSPO radioligand V_T by 58-138%¹¹ (Fig. 2C).** Critically, the radioligand binding in brain was displaceable, demonstrating signal specificity. These data strongly support translation of TSPO PET imaging as a biomarker of microglia activated by alcohol in people. **Aim 1** will measure [¹¹C]PBR28 V_T before and after a fixed dose alcohol session (Fig. 3). We hypothesize that acute alcohol will increase [¹¹C]PBR28 V_T throughout the brain. **This paradigm will establish a novel assay of alcohol-induced microglial activation in humans**

4. MICROGLIA ACTIVATION IN CHRONIC ALCOHOL EXPOSURE

Alcohol activates microglia by interacting (indirectly via 'alarmins'⁶⁸ or directly) with the toll-like receptor 4^{31,69} (TLR4). TLR4 signaling activates transcription factors such as NF- κ B and AP-1⁷⁰, which regulate production of cytokines, chemokines, and other alarmins. Alcohol increases the alarmin HMGB1⁷¹ and pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 in culture⁶¹ and in vivo^{31,49,72}. Oxidative stress markers, such as the enzymes COX-2 and iNOS, also increase following alcohol doses in rodents⁷³⁻⁷⁶. These pro-inflammatory responses to alcohol can cause gliosis and apoptosis^{60,69,70}. Interestingly, alcohol can also increase anti-inflammatory cytokines⁷⁷ and markers of growth-promoting (M2-like) microglial activation⁷⁸ depending on experimental conditions. Moreover, alcohol has dynamic effects on neuroimmune response. For example, in some reports a single alcohol bout did not elicit microglial activation or cytokine release^{31,79,80}, but intermittent alcohol exposure did^{31,76,79}. These findings could reflect microglia priming⁸¹, where a first alcohol bout partially activates microglia and subsequent alcohol elicits exacerbated immune responses⁸². **Aim 2 of this study will determine if the phenomenon of microglia priming has clinical relevance in alcohol use disorder by comparing neuroimmune response to alcohol in moderate drinkers and heavy drinkers.**

5. SEX DIFFERENCES IN IMMUNE RESPONSES TO ALCOHOL

A growing literature indicates that women exhibit a greater immune response to alcohol than men. This sex difference may have important implications for sex-dependent trajectories and consequences of AUD, including exacerbated health risks⁸³. Intermittent alcohol exposure increased microglia number and neuronal loss in hippocampus and prefrontal cortex for female but not male rats^{84,85}. Similarly, in female mice acute²³ and chronic⁸⁶ ethanol induced higher cortical levels of pro-inflammatory cytokines, chemokines, and enzymes associated with oxidative stress compared to males. These findings translated to human studies, where acute alcohol induced plasma levels of endotoxin⁸⁷ and pro-inflammatory cytokines and chemokines²³ that were higher in women compared to men. In

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populations with problematic drinking behaviors, women exhibited higher plasma levels of the alarmin HMGB1^{22,88}, the pro-inflammatory cytokine TNF- α ⁸⁹, and TIMP-1⁹⁰, a protease inhibitor involved in immune response⁹¹, compared to men. While this work establishes sex differences in peripheral immune response to alcohol, similar differences in the brain's immune response have not been examined. **Aim 3 of this proposal will address this knowledge gap by comparing the neuroimmune response to alcohol in men and women.**

6. CLINICAL IMPLICATIONS OF DYSREGULATED NEUROIMMUNE RESPONSE

Microglia activation elicits a cluster of symptoms called 'sickness behaviors', which include reward dysfunction, anxiety, and mild cognitive impairment^{13,92}. For each symptom, neuroimmune effects converge on a specific brain region. Reward response (via BOLD signal)^{93,94} and dopaminergic signaling⁹⁵⁻⁹⁸ are modulated by neuroimmune stimuli (including alcohol⁹⁷) in the basal ganglia. Stress and anxiety (via BOLD signal)⁹⁹⁻¹⁰¹ are associated with immune effects in amygdala, an important hub for alcohol use disorder and anxiety¹⁰². Indeed, a growing preclinical literature identifies the amygdala as mediating anxiety-like behaviors associated with alcohol¹⁰³⁻¹⁰⁵. Cognitive performance tracks with glucose metabolism¹⁰⁶ and TSPO levels in alcohol dependent patients⁴⁶ in hippocampus. Alcohol exposure that elicited a neuroimmune response in hippocampus also impairs rodent learning and memory performance^{67,76,107,108}. Taken together, this literature indicates the basal ganglia, amygdala, and hippocampus as brain regions susceptible to alcohol-induced immune influences on the respective behaviors of reward processing, anxiety, and cognitive impairment. **Aim 4 of this study will determine if alcohol triggers a neuroimmune response in these specific regions that predicts the extent of specific behaviors clinically relevant to alcohol use disorder.**

4. **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.

4.1 Overall Research Design:

This research plan proposes experiments that are critical to advance our understanding of the brain's immune response to alcohol in people. We will recruit moderate drinkers and Alcohol Use Disorder subjects between the ages of 21 to 50 years to participate in the recruitment, evaluation, and imaging. All subjects will be seen for an initial intake, including a full physical exam and medical coting. The study design consists of up to three [¹¹C]PBR28 PET imaging sessions, a laboratory alcohol session, an overnight stay at the CNRU or HRU, and behavioral and cognitive testing acquired twice, on the morning of each study day. A magnetic resonance imaging session will be acquired on a separate day.

4.2 Subject Selection:

Moderate drinker participating patients will self-report drinking consistent with at least one binge alcohol event in the past three months but not meet DSM-5 criteria for AUD to ensure that accustomed drinking levels are not exceeded in the laboratory. AUD subjects will meet DSM-5 criteria for current Alcohol Use Disorder. Subjects will be recruited mainly from New Haven County, although enrollment is not limited to this region. All research subjects will be recruited under guidelines of the Yale University Institutional Review

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Board (Human Investigation Committee). Subjects will be recruited from the community at large via IRB-approved advertising (television, newspaper, postings in community locations, Craigslist, Facebook).

Screening Evaluation

All screening procedures will be performed by a trained research assistant or study coordinator. Initial screening will be performed over the telephone. Potentially eligible subjects will undergo an initial in-person screening evaluation within 6 months of PET scanning, this will be located either at 2 Church St South Suite 511, the Yale PET Center, or CMHC. Current non treatment seeking inpatient subjects at the CNRU will be able to participate if participation in their current study is not a conflict with this study and vice versa. The purpose of this evaluation is to ensure that subjects meet study criteria. Breath alcohol (BAC) levels will be monitored for subjects before the intake session. After informed consent is obtained, the structured clinical interview for DSM-5 Axis I Disorders (SCID-5) will be used to exclude primary psychiatric and substance use disorders other than alcohol use disorder. A medical history, vital signs, physical examination, and EKG will be performed. The Timeline-Follow Back interview for Alcohol (TLFB) will be used to characterize quantity and frequency of alcohol drinking up to 90 days prior to enrollment. The Lifetime Drinking History, a retrospective interview procedure, will be used to characterize lifetime patterns and quantities of alcohol. Subjects will also indicate the maximum amount of drinks consumed in a single 24-hour period over the last three months. Several laboratory tests will be performed at this visit, which include a complete blood count (CBC), chemistry profile, thyroid function studies, HIV, serum β -HCG (women only), urinalysis, and urine toxicology screen. All female subjects will undergo a pregnancy test at the time of screening. Additionally, urine pregnancy tests will be done on the day of each PET scan before radiotracer injection. Urine drug screens will be done at intake, mri, and on PET days.

4.3 Assessments:

All participants will be screened initially using a telephone screen that will include questions to evaluate medical history, personal and familial psychiatric and smoking history. A wide range of measures, such as psychiatric and substance abuse history, medical assessments and affective symptoms will be measured during the intake evaluation.

4.3.a. General Intake Assessments

1. Structured Clinical Interview for DSM-5 Axis I Disorders The psychotic screening and depression sections of the Structured Clinical Interview for DSM-5 Axis I Disorders (SCID-5) will be used to determine whether subjects meet exclusion criteria for diagnosis of primary psychiatric and substance use disorders other than alcohol use disorder.
2. Illicit Drug/Pregnancy Screen A urine sample may be collected to determine current illicit drug use (for all potential subjects). In addition, serum samples will be collected at the intake visit and urine samples will be collected on each PET scan day to confirm that the subject is not pregnant.
3. Demographic Questionnaire This questionnaire will obtain: (1) basic demographic information including age, gender, marital status, employment status, occupation, (2) alcohol/drug history, (3) family history of alcohol/drug use, depression, anxiety, and smoking history.
4. Medical History This questionnaire will obtain a basic medical history (personal and family) including past or current conditions such as neurological, endocrine, cardiovascular, renal, liver, and thyroid pathology. Current body weight and current medications will also be assessed.
5. Medical Assessments will include a physical exam by a state licensed physician, an EKG, and laboratory tests which may include a complete blood count, blood urea nitrogen, creatinine, fasting blood sugar, electrolytes, liver function tests, thyroid function tests (including T_3 , T_4 , T_3RU , estimated free T_4), thyroid stimulating hormone levels, urine toxicology, and urinalysis. Female subjects will have serum pregnancy tests.

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6. Multidimensional Scale of Perceived Social Support (MSPSS) is a brief research tool designed to measure perceptions of support from 3 sources: Family, Friends, and a Significant Other.
7. Barratt Simplified Measure of Social Status (BSMSS) is a measure of socio-economic status.

4.3.b. Mood and Sleep Measures

We may obtain these measures at intake, up to two times on the PET scan day, and the morning following the alcohol session.

1. Center for Epidemiological Studies Depression Scale (CES-D) The CES-D³⁹ is a 20-item self-report instrument, which has been extensively used in both clinical and nonclinical populations to measure the frequency and severity of depressive symptoms over the past week. The CES-D, which has been used to document the severity of depressive symptoms in adults and has been shown to be a sensitive measure of negative affect in smokers, will be used in the proposed studies to measure levels of mild depressive symptoms.
2. Anxiety: The State-Trait Anxiety Inventory⁴⁰ is a 40-item, self-report measure, comprised of two subscales. The State-Anxiety scale is 20 items and assesses transitory states characterized by feelings of tension, apprehension, and heightened autonomic reactivity. The Trait-Anxiety scale is 20 items and assesses stable individual differences in anxiety proneness.
3. Impulsivity: Barratt Impulsiveness Scale (BIS; ¹⁰⁹) is a 30 item self-report instrument designed to assess the personality/behavioral construct of impulsiveness.
4. Beck Depression Inventory: This widely used 21 item self-report instrument will be used to assess depressive symptomatology in addition to the SCID (43).
5. PSQI

4.3.c. Alcohol and other Drug Measures

We may obtain these measures at intake and also on the PET scan day.

1. Timeline Followback for alcohol, tobacco, and illicit drugs. Questionnaire to measure the amount of alcohol, tobacco, and other drugs that have been used in the past 30 days.
2. Lifetime Drinking History is a structured interview designed to provide quantitative indices of alcohol consumption patterns from the onset of regular drinking.
3. Fagerstrom Test for Nicotine Dependence (FTND). This will be used to measure the severity of nicotine dependence. It is a 6-item scale with an internal consistency of .61 and its total score is closely related to biochemical measures of intensity of smoking. A score of at least 3 is necessary for inclusion in the study.
4. Smoking History. This questionnaire will assess basic smoking status and history such as number of years smoked, number and length of quit attempts, reasons for quitting, and second hand smoke exposure.
5. Nicotine Withdrawal Checklist. This measures the severity of eight withdrawal symptoms on 5-point Likert scales.

4.3.d. Cognitive Measures

We may obtain these measures at intake, up to two times on the PET scan day, and the morning following the alcohol session.

1. Cogstate Battery (30 minutes) – This computerized test battery will assess memory and cognition. The tasks may include:
 - a. International Shopping List Task – a computerized task to assess verbal learning and memory.

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- b. Groton Maze Learning Task – a computerized task to assess executive function and spatial problem solving.
- c. Detection Task – a computerized task to assess psychomotor function and speed of processing.
- d. Identification Task – a computerized task to assess visual attention and vigilance.
- e. One Card Learning Task – a computerized task to assess visual learning and memory.
- f. One Back Task and Two Back Task – computerized tasks to assess attention and working memory.

2. Probabilistic Reward Task (PRT) – The PRT has been successfully used to assess reward responsiveness (51-53). In each trial, subjects choose which of two difficult-to-differentiate stimuli was presented. Stimuli consist of simple cartoon faces (diameter: 25 mm; eyes: 7 mm) presented in the center of the monitor. At the beginning of the trial, the face has no mouth. After a given delay, either a straight mouth of 11.5 mm (“short mouth”) or 13 mm (“long mouth”) is presented for 100 ms. Subjects are instructed to press an appropriate button to decide whether a long or small mouth had been presented. Unbeknownst to subjects, correct identification of one stimulus (“rich stimulus”) is rewarded three times more frequently (“*Correct! You won 20 cents*”) than the other (“lean”) stimulus. In healthy controls, this reinforcement schedule leads to a response bias (i.e., a preference for the more frequently rewarded stimulus). The degree of response bias toward the more frequently reinforced alternative will be used for operationalizing sensitivity to reward.

3. Probabilistic Reversal Learning Task (PRLT) – The PRLT has been shown to effectively detect “belief updating” behavior through the use of the Hierarchical Gaussian Filter model. This task uses a 3-option probabilistic reversal-learning paradigm which requires participants to choose between options with unknown, unequal probabilities of reward. Three decks of cards are displayed on a computer monitor for 160 trials. Participants select a deck on each trial by pressing a predesignated key on a keyboard. We advise participants that each deck contains either winning or losing cards (e.g. +100, -50 points, etc.), but in different amounts. We also state that the best deck may change. Participants are instructed to find the best deck and earn as many points as possible. Probabilities switch between decks when the highest probability deck is selected in 9 out of 10 consecutive trials (performance-dependent reversal). It is predicted that AUD participants may interpret probabilistic errors (e.g., the occasional instance when a good option does not yield reward), as reversal errors (the world has changed) and thus switch their choices prematurely.

4.4 Procedures

Screening, Evaluation and Clinical Ratings

Screening, evaluation and clinical ratings (as described above) are obtained during the screening process.

Magnetic Resonance Imaging (up to 1 hour)

Magnetic resonance imaging (MRI) scans (3T) will be collected in each subject to co-register PET and MRI for image analysis as well as collect resting state data. MRI is without contrast. Within approximately two weeks of the PET study, an MRI will be acquired at the Yale University MRI Center. Subjects will be taken through a ferromagnetic metal detector before entering the scan room.

3T Scan sequence:

Series 1: 3 plane localize

Series 2: Sag 3d tfl; 256fov; 1mm thick slices; 176 slices total; TE 2.77; TR 2530; TI 1100; FA 7; 256X256 1 average.

Series 3 – Resting state: Ep2d bold; 210fov; 2.5mm thick slices; TE 30; TR 3400; FA 85; 84x84 (Run twice).

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MR images provide a matching anatomical atlas for creating individualized region-of-interest templates for each subject. We will also examine functional connectivity at rest.

If an MR image is already on file at the PET Center, and has been collected within a reasonable time period, a new MRI may not need to be collected.

A member of the research staff will accompany the subject and stay for the duration of the MRI.

PET Scans

PET experiments will be conducted at the Yale University PET Center. Subjects will have up to three [^{11}C]PBR28 PET scans. Subject will also be asked to refrain from drinking caffeinated beverages that day, and from drinking alcoholic beverages 48 hours prior to study day.

Upon arrival at the PET center, subjects will complete questionnaires on urges, withdrawal symptoms, and mood. Plasma will be drawn before scans to measure drug levels in the blood. Urine drug tests will be performed before all PET scans. To ensure overnight abstinence for individuals with alcohol use disorder, subjects who are at a higher risk of withdrawal will be required to stay inpatient at the CNRU the night before their PET scans unless there are scheduling conflicts with the subject. Confirmation of alcohol abstinence over the previous 48 hours will be made via self-report and BAC measures, and session will be rescheduled if positive.

For all women, a urine pregnancy test will be performed at the beginning of the imaging day, prior to radiotracer injection, at the Yale University PET Center.

Up to three total [^{11}C]PBR28 PET scans will be acquired per subject. The first scan (baseline) will occur the morning of the alcohol session before alcohol is consumed. The post-alcohol PET scan aims to capture the peak neuroimmune response^{8,59}, however, this exact timing is not known. In human studies, peak pro-inflammatory protein levels occurred 20-30 min after peak BAL^{87,110,111}. Since peak BAL commonly occurs 30 min after drinking cessation¹¹², a second [^{11}C]PBR28 PET scan will begin approximately 60 min after completing the alcohol session. This timing is consistent with the report of increased TSPO levels 30 min after alcohol infusion in baboons¹¹. In contrast, rodent studies identify peak microglia responses 12-18 hours after ethanol gavage^{65,113}. Therefore, a third post-alcohol [^{11}C]PBR28 PET scan may be acquired approximately 20 hours after the alcohol session. Blood samples will be collected throughout each PET scan and during the alcohol administration.

PET Data Acquisition and Analysis

Subject preparation consists of intravenous (IV) catheterizations for IV administration of the radiotracer and blood sampling. A radial artery catheter will be inserted by an experienced physician in the morning before the PET scan. The site will be anesthetized with lidocaine prior to arterial line insertion. The arterial line will remain in place for the whole day of scanning, after which it will be removed by trained nursing staff at the Yale PET Center. PET data are acquired while subjects rest with either an HRRT PET scanner (207 slices, resolution better than 3 mm FWHM) or a Siemens mCT PET scanner (resolution better than 5 mm FWHM). A transmission scan, or low dose CT scan, will be obtained for each emission scan. Up to a total of 4 low-dose CT scans may be completed for the entire study. Motion correction may be performed dynamically with measurements from the Vicra (NDI Systems, Waterloo, Ontario) used by a dedicated list-mode reconstruction algorithm. Acquisition of list-mode emission data will begin shortly before IV bolus administration of up to 20 mCi of high-specific activity [^{11}C]PBR28 and will continue for up to 120 minutes post-injection. Dynamic images of radioactivity concentration will be reconstructed with corrections for measured attenuation, normalization, random events, scatter, and deadtime. Subjects will be asked to void after the scan is completed to reduce radiation exposure to the bladder. Blood samples to measure plasma blood alcohol

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concentration (BAL) and peripheral cytokine levels will also be acquired from i.v. lines used for PET sessions. After the completion of the days final PET scan, the IVs will be removed. Monitoring of vitals will continue for at least 30 minutes before subject returns to the CNRU/HRU or discharged.

List mode data will be reconstructed with MOLAR¹¹⁴, with corrections for attenuation, normalization, and motion to create high resolution images. Early PET image data will be registered to the subjects T1-weighted MR image. The T1-weighted MR image will be non-linearly registered to MNI space for region of interest (ROI) identification. ROIs will be selected using the Anatomical Automatic Labeling template for SPM8. To minimize possible partial volume effects, only gray-matter voxels will be included in ROI definition based on MRI segmentations performed with the Computational Anatomy Toolbox¹¹⁵. Since TSPO responses are observed throughout the brain^{9,11,59}, ROI analyses will focus on the primary brain regions of frontal, parietal, temporal, and occipital cortices; striatum, amygdala, hippocampus, thalamus, and cerebellum. The primary outcome measure will be [¹¹C]PBR28 V_T ⁵⁴, which will be estimated with multilinear analysis (MA1)¹¹⁶ with $t^*=30$ min, which yields a test-retest variability of 7-9%¹¹⁷. [¹¹C]PBR28 V_T will be estimated throughout the brain at both the ROI level and at the voxel level.

Laboratory Alcohol Session

Laboratory sessions will occur at the Yale University PET Center. Subject body weight will be acquired during intake as part of the medical history and used for alcohol dose calculations, this will work out to roughly 3 standard drinks for females and 4 standard drinks for males over 90 minutes depending on weight, height, and age. Subjects will be asked not to diet between intake and study days. Alcohol dose will be recalculated if the subject's weight varies by more than 5 pounds for women and 10 pounds for men. Confirmation of alcohol abstinence will be made via self-report and breathalyzer on study day. Subjects will be given a light standardized breakfast on arrival to the laboratory and the morning prior to discharge.

After the first baseline [¹¹C]PBR28 PET scan, subjects will have a standardized light lunch to increase tolerability of the alcohol dose while minimizing the potential for variations in food intake that could affect alcohol metabolism. Alcohol will be administered in the form of 80 proof vodka and a mixer based on the subject's choice of water or a decarbonated noncaffeinated noncaloric soda. The dose will be prepared by the research team and take into account the participant's total body water (based on gender, age, height, and weight), duration of drinking, and ratio of alcohol to mixer, based on Watson et al's update of the Widmark equation. The total volume will be divided into six equal drinks, with each consumed over a 15-minute period to control the rate of consumption. Pacing the drinking over 90 min aims to reduce risk of nausea while maintaining a rate of consumption consistent with a binge alcohol event. After the session subjects may drink water ad lib until scanning. Subjects will be asked to void prior to the second PET scan. Afterwards, subjects will be given dinner based on food preferences. Water, crackers, and other snacks will be available ad lib for the rest of the evening until 12:00 A.M. Subjects may have an mri the same day prior to alcohol administration, subjects will not have an mri the same day post alcohol administration.

Assessments following alcohol administration:

BAL will be measured at the end of drinking, every 30 minutes for two hours, and then hourly. BAC and vital signs will be measured concurrently. BAL measurements will be discontinued following measurement of negative BAC levels. Other assessments that may be collected during these times include inflammation markers, self-reports of alcohol effects and mood.

1. Blood Alcohol Concentrations: Blood samples will be collected in gray stoppered (oxalate, fluoride) tubes and analyzed with gas chromatography analyses in the Yale-New Haven Hospital Clinical Laboratories. After microfuging, serum will be transferred into microtubes and stored at -20°C until assay.

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2. Breath Alcohol Concentrations will be determined with an Alcohol-Sensor III (Intoximeter Inc., St. Louis, MO).

3. Inflammation Markers: Serum levels of cytokines, such as TNF- α , IL-1 β , IL-4, IL-6, and IL-8, and cortisol will be measured at baseline and when blood is drawn to measure BAL. We may also collect monocytes to assess immune function *in vitro*.

4. Visual Analog Scale (VAS) self-ratings for drug effects. Subjects will answer questions to assess behavioral changes induced by substance use.

5. Subjective Effects of Alcohol Scale is a self-report measure that assesses subjective experience of alcohol effects following alcohol administration¹¹⁸.

6. Biphasic Alcohol Effects Scale is a self-report questionnaire that assesses subjective experiences of alcohol stimulation and sedation¹¹⁹.

The total amount of blood drawn during the study will not exceed 32 tablespoons total.

Discharge

Participants can be discharged after the alcohol session once they reach a BAC of 0.02 and vitals are within normal limits, transportation will be provided for them. Moderate Drinkers and Moderate AUD subjects will also have the option to stay at either the CNRU or HRU. People with high risk for continued risk drinking or withdrawal symptoms, defined as people with any of: history of medicated detoxification, CIWA-R scores greater than 5 during intake or scan day, or people characterized by heavy alcohol use disorder, will stay overnight at the CNRU no matter what their BAC. Participants who stay overnight will be discharged from the CNRU or HRU the morning following the alcohol session after confirming that vital signs are within normal limits. If subjects are participating in Aim 5, they will be escorted once discharged from the CNRU or HRU to the PET center. After the Aim 5 scan, subjects will be allowed to leave when vital signs are confirmed within normal limits. All subjects will be negative for BAC by this time and thus can return home unescorted. A research staff member will contact subjects the following business day for a check on their health status. All subjects will be provided the telephone number of a research psychiatrist or physician on call for any problems that arise in the immediate time period after discharge from the unit. They will also be given a discharge form that has specific instructions for calling one of the physicians if they have any questions or concerns following the study.

5. 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 One 10 mL tube of blood will be collected for DNA for testing of polymorphisms in genes of interest to drug dependence. This sample may be collected during the screening appointment or on one of the PET scan days
- ii. the plan for the collection of material or the conditions under which material will be received as above, a blood sample will be collected at intake to test polymorphisms of interest for drug dependence.
- iii. the types of information about the donor/individual contributors that will be entered into a database the genetic polymorphism results will be entered. The results of this testing will be confidential, will not be entered into the subject's medical record, and will not be made available to the subject.

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- iv. the methods to uphold confidentiality The results of genetic testing will be kept on a University encrypted computer or secure server. We will separate the personal identifying information of the subjects from the genetic information.

- B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects? Some samples and related information may be stored (banked) for future research. The specimens will be stored for an unlimited time and may be used to make a cell line that will live indefinitely. When specimens and information are stored, we are careful to try to protect subject identity. Samples and information will receive a unique code. Other researchers will only receive coded samples and information.
- C. Is widespread sharing of materials planned? Yes
- D. When and under what conditions will materials be stripped of all identifiers? It Will Not
- E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials? Yes
 - i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)? Subjects will be informed that their material has been anonymized and after anonymization, destruction is not possible
- F. Describe the provisions for protection of participant privacy Risks associated with genetic testing will be minimized by keeping the results of genetic testing on encrypted computers or a secure server, and by separating the personal identifying information of the subjects from the genetic information.
- G. Describe the methods for the security of storage and sharing of materials The results of genetic testing will be kept on an encrypted computer or secure server and personal identifying information of the subjects will be separated from the genetic information. Materials will not be shared.

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

Up to 100 subjects (50 men, 50 women) will participate in the study. According to census figures, minority groups comprise approximately 50% of the population of New Haven (36.1% African-American, 13.2% Hispanic, 0.3% Native American, 2.4% Asian, and 4.1% other). Subjects will self-report a binge alcohol even in the previous 3 months to ensure that subject's customary drinking levels are not exceeded in the laboratory.

All subjects will be 21-50 years of age and physically healthy. All research subjects will be recruited under guidelines of the Yale University Institutional Review Board (Human Investigation Committee). Subjects will be recruited from the community at large via IRB-approved advertising (television, newspaper, postings in community locations, Craigslist, Facebook). All participants will be screened initially on the telephone by a research assistant who will schedule the initial intake assessment. The screening and scanning sessions will involve psychological tests in the form of rating scales and questionnaires. These are all noninvasive questionnaires, e.g., to assess mood and craving, and should add no risk.

7. **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.

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- | | | |
|--|--|--|
| <input type="checkbox"/> Children | <input checked="" type="checkbox"/> Healthy | <input type="checkbox"/> Fetal material, placenta, or dead fetus |
| <input type="checkbox"/> Non-English Speaking | <input type="checkbox"/> Prisoners | <input type="checkbox"/> Economically disadvantaged persons |
| <input type="checkbox"/> Decisionally Impaired | <input type="checkbox"/> Employees | <input type="checkbox"/> Pregnant women and/or fetuses |
| <input type="checkbox"/> Yale Students | <input type="checkbox"/> Females of childbearing potential | |

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects?

Yes ☐ No ☒

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

General Inclusion Criteria:

1. Men and women, aged 21-50 years
2. Willing and able to give voluntary written informed consent
3. Able to read and write English and communicate effectively with the investigators, and comply with all study requirements, restrictions, and directions of the clinic staff
4. AUD Subjects will meet DSM-5 criteria for current Alcohol Use Disorder
5. Moderate Drinkers will report consuming alcohol on at least one occasion in the past three months that would result in an estimated blood alcohol level greater than 100 mg/dl but not meet DSM-5 criteria for AUD. This is to ensure that subjects have prior drinking exposure consistent with levels proposed in this study. Prospective subjects will be asked to recall the heaviest two days of drinking in the previous three months. Using this information, approximate BAC will be calculated for those prior episodes.
6. Medically healthy upon physical examination and laboratory testing.

General Exclusion Criteria:

1. Individuals whom the investigators deem may not be able to comply with alcohol abstinence for 48 hours prior to study day.
2. Current significant medical condition such as neurological, cardiovascular, endocrine, renal, liver, or thyroid pathology.
3. History of or current neurological or significant psychiatric disorder such as schizophrenia or bipolar disorder (DSM-5 Axis 1).
4. Other substance use disorder with the exception of nicotine dependence in smokers as assessed with the SCID or positive urine screen for drugs of abuse.
5. Participants with any significant current medical conditions that would contraindicate the consumption of alcohol, such as history of neurological trauma or diseases, seizures, delirium or hallucinations, hepatic, or other unstable medical conditions.
6. Current suicidal or homicidal intent or behavior, or history of suicidal or homicidal behavior.
7. No barbiturates or other known microsomal enzyme inducers or inhibitors in the past month.
8. History of significant head trauma.
9. Women who are pregnant or nursing or fail to use one of the following methods of birth control unless she or partner is surgically sterile or she is postmenopausal (hormone contraceptives

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[oral, implant, injection, patch, or ring], contraceptive sponge, double barrier [diaphragm or condom plus spermicide], or IUD).

10. Regular or current significant use of any prescription, herbal or illegal psychotropic medications (e.g., antidepressants, antipsychotics, anxiolytics, ecstasy) in the past 6 mo, with no current illegal drug use confirmed by urine toxicology (except for cocaine and marijuana when relevant).
11. Have MRI-incompatible implants and other contraindications for MRI, such as a pacemaker, artificial joints, non-removable body piercings, claustrophobia, etc.
12. Subjects with 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.
13. Subjects with current, past or anticipated exposure to radiation in the work place within one year of proposed research PET scans.
14. Subjects with history of IV drug use which would prevent venous access for PET tracer injection.
15. Blood donation within eight weeks of the start of the study
16. History of bleeding disorder or currently taking anticoagulants (such as Coumadin, Heparin, Pradaxa, Xarelto).

9. How will **eligibility** be determined, and by whom? [Write here](#)

Eligibility to participate in screening session will be determined by the research staff under the guidance of Dr. Ansel Hillmer. Study participation will be determined after the screening session by the study physician and Dr. Hillmer.

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

Risks Associated with Administration of Alcohol

Numerous medical conditions, such as liver disease, cardiac abnormality, pancreatitis, diabetes, neurological problems, and gastrointestinal disorders, could potentially be worsened by acute alcohol administration. Therefore, subjects with medical problems identified by physical exam and laboratory findings will be excluded from the study.

Alcohol may induce nausea in high doses; however, significant nausea is not expected at the targeted dose for this study because subjects will have consumed a light lunch and they will have had recent (in the last three months) experience with the targeted alcohol dose. Subjects will be permitted to decline further drinking if they so choose.

Another area of potential risk to subjects under the influence of alcohol is their safety during intoxication. All subjects will be under the supervision of the experimenters to prevent possible accidents such as falls. Subjects will not leave the laboratory during the self-administration procedures. By staying in the CNRU or HRU overnight, the possibility that the subject might leave the session and continue to drink alcohol, which would place them at risk for accidents, is prevented. Ensuring that moderate drinkers have reached a BAC of 0.02 prior to discharge reduces the possibility of falls or impaired judgment once discharged. If moderate drinkers are discharged the same day as their alcohol challenge, a taxi will be provided for them.

Risks Associated with Use of an Arterial Catheter.

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On the PET scanning days a radial arterial catheter will be inserted. Some individuals may feel light-headed during placement of the arterial catheter. This procedure may be associated with mild-to-moderate pain, hematoma, inflammation, bleeding, or bruising at the punctures site. If any of these, or any other symptoms occur, and they do not diminish within 24 to 72 hours after removal of the arterial line, or in the event that they worsen, subjects will be advised to call the on-call doctor listed on the PET discharge instructions. In rare instances blocking or tearing of the artery, arterial leakage, poor healing, or infection at the catheter insertion site may occur.

Risks Associated with Blood Drawing and IV Line Insertion

At the screening visit, a routine venipuncture will be performed for screening laboratory studies. On the PET scan day(s), up to two venous catheters will be inserted for bolus injection and/or constant infusion of the radiotracer, and for blood draws.

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 will be up to 32 tablespoons. Blood samples will be drawn for routine labs and drug screening; measurement of radiopharmaceutical parent and metabolites, for analysis of plasma drug levels, serum estrogen, progesterone, and follicle stimulating hormone levels. This is not expected to have any serious negative effects on a study participant.

Risks Associated with Radiation

The Yale New Haven Radiation Safety Committee (YNHH RSC) and Yale University Radiation Safety Committee (YU-RSC) will review the use of radiation in this research study, and no subjects will be scanned until RSC approval is obtained. This research study involves exposure to radiation from [¹¹C]PBR28 and, if the mCT camera is used, low dose whole body CT scanning. 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 up to three injections of ≤ 20 mCi of [¹¹C]PBR28 (0.814 rem each), plus a small amount of radiation from the transmission scans of the brain. If scans are conducted on the mCT, each subject may receive an additional ~ 2.5 rem (0.625 rem per CT x up to 4 CT scans).

Although each organ will receive a different dose, the targeted amount of radiation exposure subjects will receive from this study is equal to an effective dose equivalent of **3.51 rem** from participating in Aim 1 or Aim 2, plus up to **1.44 rem** for participating in Aim 5. Therefore, the maximum possible radiation exposure for this study would be up to **4.95 rem**.

This includes a total of up to 60 mCi of [¹¹C]PBR28 from three injections plus up to 4 CT scans. This calculated value is used to relate the dose received by each organ to a single value.

The amount of radiation subjects will receive in this study is below the dose guidelines established by the FDA and monitored by The Yale New Haven Radiation Safety Committee (YNHH RSC) for research subjects. This guideline sets an effective dose limit of 5 rem per year.

Adverse effects of the radiopharmaceuticals in this study have not been reported. However, the possibility exists for a rare reaction to any of the substances or procedures to which a subject is exposed.

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Risks Associated with MRI

MR carries a risk for subjects who are claustrophobic or have pacemakers, metal pieces, aneurysm clips, large colored tattoos, or any other contraindications for MR.

Magnetic resonance imaging (MRI) 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.

Subjects 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 MRI 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 any of these symptoms.

There are some risks with an MRI study for certain people. If subjects have a pacemaker or some metal objects inside their body, they may not be in this study because the strong magnets in the MRI scanner might harm them. Another risk is the possibility of metal objects being pulled into the magnet and hitting a 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 all people involved with the study to walk through a detector designed to detect metal objects. It is important to know that no metal can be brought into the magnet room at any time. Also, once subjects are in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet.

We want subjects read and answer very carefully the questions on the MRI Safety Questionnaire related to your personal safety. We will be sure that subjects have read the MRI Safety Questionnaire and tell us any information they think might be important.

This MRI 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 MRI 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 subject's scan, a radiologist will be asked to review the relevant images. Based on his or her recommendation (if any), the primary investigator or consulting physician 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 your physician. **The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that a subject receives based on these findings. The images collected in this study are not a clinical MRI exam and for that reason, they will not be made available for diagnostic purposes.**

Risks Associated with Drug Use and Suicidal/Homicidal Behavior

Participants will be asked about current or past use of illicit "street" drugs and urine drug screens will be performed at the initial intake appointment to rule out substance abuse that is not relevant to the study. Participants currently using drugs or who have a history of using drugs may not be eligible to participate. Suicidal thoughts and behaviors will also be assessed. Any reports of homicidal or suicidal tendencies will be immediately directed to treatment for this condition; and appropriate authorities will be notified.

Risks Associated with Unanticipated Events

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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 unanticipated event, 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 any unanticipated events/complications.

Risks Associated with Allergic Reactions

All drugs have a potential risk of an allergic reaction, which if not treated promptly, could become life threatening.

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

Risks Associated with Administration of Alcohol:

Risks will be minimized by adhering to the recommended guidelines established by the National Advisory Council on Alcohol Abuse and Alcoholism – Recommended Council Guidelines on Ethyl Alcohol Administration in Human Experimentation – Revised May 2005. People for whom alcohol consumption is contraindicated will be excluded based on the screening process including interviews, physical exams, and laboratory findings.

To reduce the risk of nausea or gross impairment from acute alcohol consumption, we will recruit enroll participants with recent (within the last three months) drinking experience consistent with the blood alcohol concentration targeted for this study. We will provide a light lunch prior to the alcohol session. Subjects will have 90 min to consume the drinks and can refuse to consume all of the drinks if they choose to.

Personnel trained in the conduct of alcohol challenges will conduct the sessions. All participants will be under supervision to prevent possible accidents. Following the alcohol sessions, all Heavy Drinker subjects will remain at the CNRU or HRU overnight to prevent the possibility that they might continue drinking after the session, thereby placing themselves at further risk of accidents. Moderate drinkers will have the option to stay overnight at either the HRU or CNRU or be discharged after reaching 0.02 BAC. If a subject should insist on leaving the research setting prematurely, we will provide transportation to their home. While subjects are free to discontinue the experiment at any point, we will strongly encourage them to remain in the research setting until their breath alcohol level is below 0.02. The consent form addresses this contingency under voluntary participation.

Aud Subjects with a higher risk for withdrawal or a history of withdrawal symptoms will stay at the CNRU. Moderate Aud subjects who are not at a high risk for withdrawal will be able to stay the night at the HRU with the below criteria:

Not at risk subjects will be defined as those who meet both of the following requirements:

- 1) No prior history of medicated detoxification
- 2) CIWA-R score less than 5 during intake, scan day and just before admission to the HRU
- 3) Subjects who are characterized by more moderate AUD.

Documentation: Moderate Aud subjects that are considered for the HRU stay will be evaluated again on scan day by the covering physician before and after alcohol consumption which will be documented in the

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admission note. If the covering physician is concerned there is a risk of withdrawal, subjects will not be brought to the HRU and be admitted to the ER.

Risks Associated with Use of an Arterial Catheter:

The risks of radial artery cannulation are minimized by having the procedure performed by an experienced physician. Pain is minimized with the use of local anesthesia. Infection is avoided by adequate cleansing of the skin before intravascular line insertion. After arterial catheter removal, bleeding is prevented by direct pressure applied to the site for a minimum of 15 minutes followed by a pressure dressing (coban) that should be kept clean and dry until evening. Subjects will have their hand and finger blood supply examined after arterial cannulation throughout the study, and again following catheter removal. Also, subjects will be asked to abstain from using aspirin and other NSAIDS 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 contact 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. A nurse will provide discharge instructions outlining general instructions in addition to post-arterial catheter precautions, problems to watch for, and procedures to follow should such problems occur.

Risks Associated with Blood Drawing & IV Line Insertion:

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. Subjects who have donated blood within 8 weeks of the present study will be excluded. Participants will be told that they should not give blood for at least 8 weeks.

Risks Associated with Radiation:

The dose of radiation will be submitted for approval to the Yale New Haven Hospital Radiation Safety Committee (YNHH RSC). All scans will be done in the presence of medical supervision and trained 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 Diagnostic Radiology, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radiopharmaceuticals. Subjects will be asked about their previous radiation exposure and those who have had research exposure within the past year will be excluded if their cumulative annual exposure (including the present study) exceeds FDA limits. The information on the previous radiation exposure of study subjects will be notified to the study doctor.

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.

Risks Associated with MRI Scanning

To minimize risks, each subject will fill out the Yale Magnetic Resonance Research Center MRI Safety Questionnaire before the study. Only subjects who fulfill the criteria by this questionnaire

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will be eligible for the study. In addition, subjects will remove all metal (watch, hair pins, jewelry) and walk through the metal detector in the MRRC before entering the MRI room. If the subject has any metallic prostheses/implants they will be excluded from the study. If a subject becomes anxious during the scan they can request that the MRI scan be stopped.

Risks Associated with Drug Use and Suicidal/Homicidal Behavior

Effective screening will exclude all subjects who would be at greater risk for complications because of medical or psychiatric illnesses. Any subject with a prior suicide attempt or with active suicidal ideation at baseline as determined by psychiatric evaluation and C-SSRS, will be excluded from this study.

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.

Risks Associated with Unanticipated Events

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

Risks Associated with Allergic Reactions

Subjects will be asked to monitor and report the following symptoms: trouble breathing, or swelling of the face, mouth, lips, gums, tongue or neck. Other allergic reactions may include rash, hives, or blisters.

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.

12. **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.)
 - a. What is the investigator's assessment of the overall risk level for subjects participating in this study? Greater than minimal Risk
 - 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://your.yale.edu/policies-procedures/forms/420-fr-01-data-and-safety-monitoring-plans-templates> for
 - i. Minimal risk
 - ii. Greater than minimal

1. Personnel responsible for safety review and its frequency:

The principal investigator, in collaboration with Kelly Cosgrove, Ph.D., and Stephanie O'Malley, Ph.D., 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 (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. The principal investigator, the HIC, the FDA, or the RSC, 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:

We view the risks associated with radiation exposure as greater than minimal.

We do not view the risks associated with alcohol administration as minimal.

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Although we have assessed the proposed study as one of greater than minimal 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 follows:

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 Ansel Hillmer, PhD. according to the following categories:

- a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
- b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
- c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
- d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
- e.) Unrelated: Adverse event is clearly not related to investigational procedures(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:

- 1. Mild adverse event
- 2. Moderate adverse event
- 3. Severe

5. Plan for Determining Seriousness of Adverse Events:

Serious 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 results in any of the following outcomes:

- 1. Death;
- 2. A life-threatening experience in-patient hospitalization or prolongation of existing hospitalization;
- 3. A persistent or significant disability or incapacity;
- 4. A congenital anomaly or birth defect; OR
- 5. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

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 IRB is necessary.

6. Plan for reporting UPIRSOs (including Adverse Events) to the IRB

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The principal investigator will report the following types of events to the IRB:

Any incident, experience or outcome that meets ALL 3 of the following criteria:

Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND

Is related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND

Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include – but are not limited to – *serious, unexpected, and related adverse events* and *unanticipated adverse device effects*. **Please note** that adverse events are reportable to the IRB as UPIRSOs **only** if they meet all 3 criteria listed above.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. All related events involving risk but not meeting the *prompt* reporting requirements described in IRB Policy 710 should be reported to the IRB in summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented. In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

7. Plan for reporting adverse events:

For the current study, the following individuals, funding, and/or regulatory agencies will be notified (choose those that apply):

- ☒ All Co-Investigators listed on the protocol.
- ☒ National Institutes of Health
- ☒ Yale- New Haven Hospital Radiation Safety Committee (Y-NHH)(if applicable)
- ☒ Yale University Radiation Safety Committee (if applicable)

The principal investigator, Ansel Hillmer, PhD, in collaboration with David Matuskey, MD will conduct a review of all adverse events upon completion of every study subject. The principal investigator 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?

d. For multi-site studies for which the Yale PI serves as the lead investigator:

- iv. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed? *Write here*

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- v. What provisions are in place for management of interim results? *Write here*
- vi. What will the multi-site process be for protocol modifications? *Write here*

13. **Statistical Considerations:** Describe the statistical analyses that support the study design.

Frequency distributions and descriptive statistics for all variables will be computed before conducting analyses. Should Shapiro-Wilk normality tests indicate non-normality for any continuous variable, we will compute necessary data transformations (e.g., logarithmic) before conducting analyses.

AIM 1: To test the hypothesis that acute alcohol robustly increases [^{11}C]PBR28 V_T , regional values of [^{11}C]PBR28 V_T will be analyzed with a linear mixed model featuring *rs6971* genotype as a between-subject fixed factor, scan state (baseline, post-alcohol scan 1, post-alcohol scan 2) as a within-subject fixed factor, and region as a within-subjects factor. The main effect of scan state will be examined to test the null hypothesis of no difference in V_T between scan states. Post-hoc linear contrasts will examine regional differences in [^{11}C]PBR28 V_T between baseline and post-alcohol conditions.

From the imaging data we will calculate an index of microglial activation as $\Delta V_T = (V_{T(\text{Alcohol})} - V_{T(\text{Baseline})}) / V_{T(\text{Baseline})}$. The value of ΔV_T will then be used as the primary outcome measurement for subsequent analyses.

AIMS 2 and 3: To test the hypotheses that neuroimmune responses to alcohol are 1) greater in heavy drinkers compared to moderate drinkers and 2) greater in women compared to men, a linear mixed model featuring group (heavy alcohol use vs. moderate use) and sex as between-subject fixed factors and region as a within subjects factor will be constructed. The genotype for *rs6971* will not be included in the model since ΔV_T is unaffected by genotype. The main effect of group will be examined to test the null hypothesis of no difference in ΔV_T between study groups; the main effect of sex will be examined to test the null hypothesis of no difference in ΔV_T between men and women. The interaction term between the two factors will also be examined. Post-hoc linear contrasts will examine regional differences in [^{11}C]PBR28 ΔV_T between groups.

AIM 4: To relate neuroimmune response with 'sickness behaviors'. This outcome measurement will be used to test the hypotheses that 1) ΔV_T in basal ganglia (average of caudate and putamen) is negatively related to change in PRT response bias. 2) ΔV_T in amygdala is negatively related to change in STAI score. 3) ΔV_T in hippocampus is negatively related to changes in the Cogstate domains of executive control and working memory. The percent changes in all outcome measures will be standardized with the Fisher Transformation¹⁶⁹, and Pearson product-moment correlations will be computed between z-scores for each *a priori* ROI and z-scores for the reward/anxiety/cognitive measures.

Exploratory analyses at the voxel level will be conducted for all aims using whole brain data and SPM12.

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

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

A. RADIOTRACERS

☐ N/A

1. Name of the radiotracer: [^{11}C]PBR28 (radiotracer for PET studies), IV

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2. Is the radiotracer FDA approved? ☐ YES ☒ NO

If NO, an FDA issued IND is required for the investigational use unless RDRC assumes oversight.

3. Check one: ☒ IND# 123984 or ☐ RDRC oversight (RDRC approval will be required prior to use)

4. **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 radiotracer is being administered to humans, include relevant data on animal models.

[¹¹C]PBR28 is a commonly used PET radioligand to image the 18-kDa translocator protein (TSPO), a marker related to neuroimmune state. Up to 20 mCi of [¹¹C]PBR28 will be administered by iv per single dose which is below the 21 CFR 361.1 estimated permissible single administration dose limit of 25.6 mCi based on dosimetry calculations.

As of Dec 2018, more than 210 preparations of [¹¹C]PBR28 have been administered in human subjects at the NIMH and had no clinically detectable pharmacological effects or side effects from the radiopharmaceutical. To date, 204 preparations of [¹¹C]PBR28 have administered to human at the Yale PET Center under various FDA (IND # 123984) and RDRC approved clinical studies and had no clinically detectable effects or side effects associated with the radiopharmaceutical.

4. **Source:** Identify the source of the radiotracer to be used. [¹¹C]PBR28

Preparation of radiotracers:

[¹¹C]PBR28 will be synthesized at the Yale University PET Center radiochemistry laboratory under the supervision of Dr. Henry Huang and Dr. Nabeel Nabulsi.

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

Due to the short half-life, PET drugs are produced *ex tempore* and formulated immediately before administration, and therefore there are no issues with storage or stability. PET drug products are stored at room temperature and are stable for at least 60 min after preparation.

[¹¹C]PBR28 will be prepared at the Yale PET Center in accordance with procedures and quality specifications described in local Drug Master File (DMF) approved by the FDA (IND#123984) and the Yale University Radioactive Drug Research Committee (YU RDRC).

The preparation of sterile PET drug products is validated prior to human use. Sterility is achieved by passing the PET drug product through a sterile 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.

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B. DRUGS/BIOLOGICS ☐ N/A

1. If an **exemption from IND filing requirements** is sought for a clinical investigation of a drug product that is lawfully marketed in the United States, 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:	
1. 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.	<input checked="" type="checkbox"/>
2. 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.	<input checked="" type="checkbox"/>
3. 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	<input checked="" type="checkbox"/>
4. 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).	<input checked="" type="checkbox"/>
5. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs.	<input checked="" type="checkbox"/>

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.

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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.

Write here

2. **Source:** Identify the source of the drug or biologic to be used.

a) Is the drug provided free of charge to subjects? ☒ YES ☐ NO

If yes, by whom? *Write here*

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

☐ West Haven VA

☒ PET Center

☐ None

☐ Other:

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.

5. Use of Placebo: ☒ Not applicable to this research project

If use of a placebo is planned, provide a justification which addresses the following:

- Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this.
- State the maximum total length of time a participant may receive placebo while on the study.
- Address the greatest potential harm that may come to a participant as a result of receiving placebo.

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d) Describe the procedures that are in place to safeguard participants receiving placebo.

6. 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. *Write here*

☐ **NO** If no, explain why this is acceptable. *Write here*

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 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**

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. **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.

Write here

3. **Source:**

a) Identify the source of the device to be used. *Write here*

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

4. **Investigational device accountability:** 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:

a) 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): *Write here*

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

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

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- d) Ensures that the device is stored in a secure area with limited access in accordance with applicable regulatory requirements: *Write here*
- e) Distributes the investigational device to subjects enrolled in the IRB-approved protocol: *Write here*

SECTION III: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. Targeted Enrollment: Give the number of subjects:

- a. Targeted for enrollment at Yale for this protocol: 200 subjects will be screened to have 100 subjects participate.
- b. If this is a multi-site study, give the total number of subjects targeted across all sites: N/A

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

- | | | |
|--|---|--|
| <input type="checkbox"/> Flyers | <input type="checkbox"/> Internet/web postings | <input type="checkbox"/> Radio |
| <input type="checkbox"/> Posters | <input type="checkbox"/> Mass email solicitation | <input type="checkbox"/> Telephone |
| <input type="checkbox"/> Letter | <input type="checkbox"/> Departmental/Center website | <input type="checkbox"/> Television |
| <input type="checkbox"/> Medical record review* | <input checked="" type="checkbox"/> Departmental/Center research boards | <input type="checkbox"/> Newspaper |
| <input type="checkbox"/> Departmental/Center newsletters | <input type="checkbox"/> Web-based clinical trial registries | <input checked="" type="checkbox"/> Clinicaltrials.gov |
| <input type="checkbox"/> YCCI Recruitment database | <input type="checkbox"/> Social Media (Twitter/Facebook): | |
| <input checked="" type="checkbox"/> Other: Clinical Referral | | |

* Requests for medical records should be made through JDAT as described at

<http://medicine.yale.edu/ycci/oncology/availableservices/datarequests/datarequests.aspx>

3. Recruitment Procedures:

All subjects will be 21-50 years of age and physically healthy. All research subjects will be recruited under guidelines of the Yale University Institutional Review Board (Human Investigation Committee). Subjects will be recruited from the community at large via IRB-approved advertising.

- a. Describe how potential subjects are contacted.

The subjects will be asked to call us if they are interested in participating in the research study. Interested individuals contacting the clinic by phone in response to advertisements are told that the information they give over the phone is written down and discussed by the research team. They are advised that if they do not enroll in research with the clinic the information is destroyed, and that if they do, it becomes part of their research chart. If an individual appears to meet enrollment criteria and is interested in participating, a face-to-face interview is conducted by one of the project investigators. A release of information is obtained for review of any available historical and clinical data. A written authorization form is also obtained from each subject, permitting the research team to use, create, or disclose the subject's PHI for research purposes. The nature of the project, procedures, relative risks and benefits, and alternatives to participation in the project are discussed with the individual. Following this discussion, the individual is given a copy of the consent form to review, and any questions are answered. The PI of the protocol will seek written consent from all participants.

- b. Who is recruiting potential subjects?
- c. Who is recruiting potential subjects? Members of the research team, as identified in the consenting staff section.

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4. 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. *Write here*

5. 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/screening purposes only

☐ For inclusion of non-English speaking subject if short form is being used and there is no translated HIPAA research authorization form available on the University's HIPAA website at hipaa.yale.edu.

- i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data: *Write here*
- 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: *Write here*

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.

6. 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.

The consent process is a 2-step process, whereby the subject initiates contact via telephone and will undergo a phone screen with a member of the research team. Thereafter, potentially eligible candidates will be scheduled for a face-to-face interview. All subjects who will be asked to volunteer are informed that no immediate personal medical benefits will be derived from participation. The study procedures will be described as a research tool with potential to enhance our knowledge about treating cognitive dysfunction. Subjects are also informed of all potential risks of participation. Informed consent is documented using specific forms for each study, reviewed and approved by the Yale University Human Investigation Committee (HIC).

Informed consent will be obtained by physicians involved in this study or their designated representatives (see list of consenting personnel above). Subjects are required to read the informed consent form, and the entire form is then reviewed with them by study personnel. In addition, the study physician will ask if the

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subject has any additional questions and further describe any risks and discomforts. Subjects will be informed that they can decline to participate in the study without penalty, and given the opportunity to withdraw from the study prior to analysis of their data. Following the resolution of any questions, the subjects will be asked to sign the consent form, if he/she agrees to participate.

- 7. Evaluation 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. All of the subjects who sign the consent to participate in the protocol will have completed and met medical (urine and blood tests, EKG, and physical) and psychological (SCID and clinical interview) criteria. 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 understand 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.

- 8. 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 Speakers will not be enrolled in this study.

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? 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 available on the HRPP website (yale.edu/hrpp) and translated HIPAA Research Authorization Forms are available on the HIPAA website (hipaa.yale.edu). 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 modification 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.

- 9. 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 any consent waivers

☒ Requesting a waiver of signed consent:

☒ Recruitment/Screening only (if for recruitment, the questions in the box below will apply to recruitment activities only)

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☐ Entire Study (Note that an information sheet may be required.)

For a waiver of signed consent, address the following:

- Would the signed consent form be the only record linking the subject and the research? YES ☐ NO ☐
- Does a breach of confidentiality constitute the principal risk to subjects? YES ☐ NO ☐

OR

- Does the research pose greater than minimal risk? YES ☐ NO ☒
- Does the research include any activities that would require signed consent in a non-research context? YES ☐ NO ☒

☐ Requesting a waiver of consent:

☐ Recruitment/Screening only (if for recruitment, the questions in the box below will apply to recruitment activities only)

☐ Entire Study

For a full waiver of consent, please address all of the following:

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

SECTION IV: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

1. 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, and family history, will be collected by research staff and be used for research purposes and charting after consent is obtained. Required private identifiable information about individuals, such as their medical history, current medications, psychiatric problems, and family history, will be collected by research staff and be used for research purposes and charting after consent is obtained.

Subjects will also provide information about their smoking history, their family smoking and psychiatric history, drinking and drug use history, mood, cardiovascular health, and other demographic variables. Biological specimens to be taken from subjects include urine samples to rule out or confirm drugs of abuse prior to imaging, and blood samples (during imaging) to measure plasma concentration of tracer and other times as well as to measure blood drug levels.

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Subjects who stay at the Connecticut Mental Health Center (CMHC) will have a medical record at the Connecticut Mental Health Center (CMHC). If subjects already have a medical record at CMHC, some information about their participation in the study will be included here. If they do not have a medical record at CMHC, one will be made for their visit. The information that will be entered into this medical record will include: name, date of birth, date of admission to the CNRU, date of discharge from the CNRU, phone number, address, medical history, individual and family history of psychiatric problems, and substance abuse history. Subjects will also sign a separate Notice of Privacy Practices specific to CMHC.

2. How will the research data be collected, recorded and stored? T on University encrypted computers. 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.

Identifiable brain research data 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.

3. How will the digital data be stored? ☐CD ☐DVD ☐Flash Drive ☐Portable Hard Drive ☒Secured Server
☒Laptop Computer ☒Desktop Computer ☐Other
4. 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?

Identifiable paper information is kept in locked file drawers and password protected computer files on University encrypted computers. 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.

Identifiable brain research data 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.

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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

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

5. 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 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.
6. If appropriate, has a Certificate of Confidentiality been obtained? This protocol is funded by NIH. As such, according to the NIH policy issues in October 2017, the information collected from subjects is automatically protected by a Certificate of Confidentiality (CoC).

SECTION V: 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.)

These studies are not of direct benefit to subjects. This study will help advance knowledge regarding the brain's neuroimmune response to drinking alcohol.

In the long term, the knowledge mentioned above can guide scientists in their efforts to develop new treatments for drug addiction. There is no direct benefit to these subjects. They will be able to withdraw from the study at any time.

SECTION VI: 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 to not 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 (per the 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.

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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 the following:

- \$50 for completing the in-person screening process
- \$400 for each PET scan (i.e., up to \$1200 if participating in all three scans)
- \$50 per arterial line placement (up to \$100 if scans occur on two separate days)
- \$50 for the MRI scan.
- Up to \$60 for Probabilistic Reward Task
- \$100 per night of inpatient stay
- \$150 bonus upon completion of all study procedures

Subjects participating will receive a total of up to \$1710.

Subjects will be paid either by check, and are advised to allow 4-6 weeks for receipt of payment, or they will be given a credit card or cash. In addition, subjects will be provided with a light lunch, valued at no more than \$10. They will also be reimbursed for parking on their PET scan days, or they may be compensated for reasonable transportation costs, including taxi fees to and from scans.

Cancellations: If a PET scan should be cancelled by the PET Center for a reason outside of the subject's control (i.e. radiotracer synthesis failure) the subject will be paid \$50 minimum, or a higher amount not to exceed the payment for a full scan day. The amount of the payment for cancellation will be based on the subject's length of participation on that scan day prior to the cancellation, and will be up to the discretion of the PI.

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.

Subjects will not be financially responsible for tests, examinations, and medical care provided to them during their participation in this study.

4. **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? *Write here*
- b. Where and from whom may treatment be obtained? *Write here*
- c. Are there any limits to the treatment being provided? *Write here*
- d. Who will pay for this treatment? *Write here*
- e. How will the medical treatment be accessed by subjects? *Write here*

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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Treatment may be provided by Yale-New Haven Hospital or any health care provider chosen by the study subjects. The study team will provide assistance to the study subjects in accessing medical treatment through referrals, or the study subjects may choose to access treatment on their own.

References

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IMPORTANT REMINDERS

Will this study have a billable service? Yes ☐ No ☒

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.

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

Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes ☐ No ☒

If Yes, please answer questions a through c and note instructions below.

- a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform? Yes ☐ No ☐
- b. Will you be using any new equipment or equipment that you have not used in the past for this procedure? Yes ☐ No ☐
- c. Will a novel approach using existing equipment be applied? Yes ☐ No ☐

If you answered "no" to question 4a, or "yes" to question 4b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

IMPORTANT REMINDER ABOUT RESEARCH AT YNHH

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 submitting 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.**