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NCT04236986

Study Imaging Microglial Activation in PTSD with PET

Cover page: Protocol, Study Imaging Microglial Activation in PTSD with PET, HIC# 2000020347

HRP-503B – BIOMEDICAL RESEARCH PROTOCOL
(2016-1)

Protocol Title: Imaging Microglial Activation in PTSD with PET

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Version Date: Click or tap here to enter text.

Version Number: 1.33

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

SECTION I: RESEARCH PLAN

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

Post-traumatic stress disorder (PTSD) is associated with abnormalities in the peripheral immune system(1-6), but the role of the *neuro*immune system is not clear. In the healthy immune system, the response of the central nervous system to an insult or damage is mediated by the activation of microglia, which carry out repair functions. However, excessive activation can lead to neuronal dysfunction and damage through the release of inflammatory cytokines and other mediators, and may contribute to the neuro-degeneration found in individuals with PTSD(7-11). When microglia are activated, there is a robust increase in the expression of translocator protein (TSPO)(12). Positron emission tomography (PET) radiotracers such as [^{11}C]PBR28, which bind to TSPO, can therefore be used to measure levels of or increases in activated microglia *in vivo*. We have exciting preliminary PET data demonstrating that individuals with PTSD have higher levels of TSPO compared to controls throughout the brain. This suggests that we can measure neuroinflammation in individuals with PTSD with [^{11}C]PBR28 and PET brain imaging (Aim 1).

In addition, we have developed a paradigm that **allows us to probe the function of the neuroimmune system** – a neuroinflammation "stress test." This is done by systemic administration of *Escherichia coli* lipopolysaccharide (LPS; also known as Clinical Center Reference Endotoxin(CCRE), *Escherichia coli* endotoxin or simply endotoxin), a potent immune activator. By performing a [^{11}C]PBR28 PET scan before and after administration of LPS, we have recently demonstrated robust *increases* in brain microglial activation, as well as increases in peripheral inflammatory cytokines (i.e., blood-based markers of inflammation) and associated mood and anxiety symptoms in healthy human adults(13). We also have new preliminary data demonstrating that a greater LPS-induced neuroinflammatory response is associated with specific and robust declines in neurocognitive function. We propose to use this novel paradigm to probe the function of the neuroimmune system in individuals with PTSD. *We hypothesize that, compared to controls, individuals with PTSD have a dysfunctional neuroimmune system, with higher baseline levels of activated microglia (Aim 1), an exacerbated and prolonged neuroinflammatory response to a pharmacological stressor (LPS), which contributes to increased mood, anxiety, and neurocognitive dysfunction (Aims 2 and 3); and, that neuroinflammation mediates the*

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relationship between peripheral inflammation and PTSD related symptomatology (Aim 4). Results of the proposed study have the potential to identify a novel and treatable(14, 15) neuroimmune mechanism implicated in PTSD.

In Aim 1, we will determine whether individuals with PTSD have higher levels of activated microglia compared to trauma-exposed control subjects as measured with [^{11}C]PBR28 PET brain imaging. Eighty subjects (n=40 with a primary diagnosis of PTSD; n=40 trauma-exposed controls) will participate in a [^{11}C]PBR28 PET scan to assess baseline levels of activated microglia. We will perform clinical assessments and measure cytokine levels, mood, anxiety and neurocognitive function on PET scan day. *Based on our preliminary data, we hypothesize that individuals with PTSD will have higher levels of neuroinflammation compared to trauma-exposed healthy controls, particularly in the amygdala, hippocampus, ventral striatum and frontal cortex, and that higher levels of neuroinflammation will be associated with greater severity of mood and anxiety symptoms and neurocognitive dysfunction.*

In Aim 2, we will use our novel neuroinflammation "stress test" to determine whether individuals with PTSD vs. trauma-exposed controls have a dysfunctional neuroinflammatory response to systemic administration of LPS. The same subjects from Aim 1 will be administered LPS 3 h prior to a second [^{11}C]PBR28 PET scan on the same day, consistent with our established paradigm(13). Cytokine and cortisol levels, mood and anxiety symptoms, and neurocognitive function will be assessed pre- and post-LPS administration. *We hypothesize based on preclinical studies(16) that, compared to controls, individuals with PTSD will have an exacerbated neuroimmune response to LPS evidenced by greater increases in activated microglia. We further expect that greater LPS-induced increases in activated microglia will be associated with greater increases in cortisol levels, mood and anxiety symptoms, and reductions in neurocognitive function.*

In Aim 3, we will determine the role of activated microglia in mediating the relationship between peripheral inflammatory markers (e.g., TNF-α) and PTSD-related symptomatology to discover potential biomarkers of PTSD. It is not known whether peripheral inflammation indicates neuroinflammation. However, peripheral cytokines cross the blood-brain barrier and induce both neuroinflammation and psychological distress(18-20). We will evaluate whether both baseline and LPS-induced levels of activated microglia mediate the relation between peripheral inflammatory markers, and mood and anxiety symptoms and neurocognitive function in individuals presenting with the full dimensional spectrum of PTSD symptoms. Based on prior work(19) and our preliminary data, *we hypothesize that (a) higher baseline levels of activated microglia will mediate the relation between higher levels of peripheral inflammatory markers (particularly TNF-α and IL-8(13)), and more severe mood and anxiety symptoms, and neurocognitive dysfunction; and (b) that greater LPS-induced levels of activated microglia will mediate the relation between greater LPS-induced peripheral inflammatory markers and increased mood and anxiety symptoms, and neurocognitive dysfunction.*

In Aim 4, we will image the relationship between neuronal and arterial inflammation in PTSD. A) In order to define the relationship between neuronal and arterial inflammation we will recruit 40 subjects with PTSD and 40 trauma-exposed controls to participate in one whole body [^{18}F]FDG scan. Performing a "whole body" scan, i.e., brain to torso, allows us to measure brain and arterial inflammation in the same scan. B) These subjects will also have the option of participating in a whole body [^{11}C]PBR28 scan (on the same or different day). While currently FDG is the established method of measuring and quantifying arterial inflammation(21), there are many caveats. PBR28 has been established as the current gold standard for measuring brain inflammation – but has not been explored for arterial inflammation. Thus, as we are conducting our studies using FDG we wish to use PBR28 in the same subjects to determine whether PBR28 may be a more specific marker of both brain and arterial inflammation. *We hypothesize that individuals with PTSD will have greater*

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amygdala activity than trauma-exposed controls and it will be related to levels of arterial inflammation as measured with FDG.

In Aim 5, we will determine whether individuals with PTSD vs. trauma-exposed controls exhibit a dysfunctional neuroimmune recovery from LPS-induced neuroinflammation (i.e. return to basal levels). The same subjects from Aims 1 & 2 will have the opportunity (optional; not required for study participation) to participate in additional [^{11}C]PBR28 scans on days following the systemic LPS challenge in Aim 2. Subjects will have the option to participate in no more than 2 additional [^{11}C]PBR28 scans within one month following LPS administration. Cytokine and cortisol levels, mood and anxiety symptoms, and neurocognitive function will also be assessed on subsequent [^{11}C]PBR28 scan days. *Our preclinical data indicate that healthy non-human primates recovered (i.e. TSPO levels returned to 'basal' pre-LPS levels) within 14 days following LPS administration (17). However, building on Aim 2, we hypothesize that, compared to controls, individuals with PTSD will exhibit a slower neuroimmune recovery (i.e., TSPO levels will return to basal levels more slowly than controls) following LPS, evidenced by persistent activated microglia for up to 21 days. Further, we hypothesize a slower neuroimmune recovery will correspond with higher cortisol levels, mood and anxiety symptoms, and worse neurocognitive performance.*

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities.
   10 years. Aims 1-3 and 5 will take approximately 5-6 years, including data analysis and publication. Aim 4 is currently in the preliminary stages and will require several additional 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.

Background and Significance. Nearly 9 in 10 Americans will be exposed to trauma in their lifetimes and a significant percentage (10.1%)(22) will develop post-traumatic stress disorder (PTSD). PTSD is characterized by elevated threat (e.g., intrusions, avoidance, anxious arousal), loss (e.g., anhedonia, negative affect) and neurocognitive (e.g., verbal learning, processing speed, attention) symptoms(10, 23-31). In addition, individuals with PTSD have elevated rates of physical health morbidities(32, 33), as well as functional impairment(27, 34-36), with loss symptoms in particular contributing to a significantly decreased quality of life(28, 35-37). The immune system is responsible for maintaining health, which includes mounting a response to physical (e.g., virus, injury) and psychological (e.g., stress) insults(38-41), as well as modulating the progression of neurodegenerative conditions such as Alzheimer's disease(42, 43). All of these—physical health conditions, psychological distress, and neurodegenerative disorders—are more prevalent in individuals with than without PTSD(9, 27, 33). While the *peripheral* immune system may play a role(4, 44, 45), the role of the *neuro*immune system in PTSD has not been evaluated. We hypothesize that individuals with PTSD have a dysfunctional neuroimmune system that is chronically activated and thus over-reactive to immune challenges, which may contribute to the chronicity of symptoms. Understanding the role the neuroimmune system plays in PTSD may help identify biomarkers or "proxies" of a dysfunctional neuroimmune response, which can inform etiology and treatment of this disorder. For example, pharmacotherapies that target systems that tune the immune pathway, such as the endocannabinoid(46) or cholinergic system(47), may be evaluated for their efficacy in treating PTSD and related symptoms.

The Neuroimmune System Modulates PTSD-Related Psychopathology. While markers of peripheral inflammation have long been associated with a variety of psychiatric disorders, including PTSD, major depression, and addiction(1-6, 48, 49), the role that *neuro*inflammation plays is unclear. Microglia, the

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resident macrophages of the brain, are involved in a variety of physiologic and pathologic processes, most notably in the initiation and maintenance of neuroinflammation. Resting microglia are tightly regulated by interactions with neurons, and microglia normally protect neurons(50). For example, when provided with signals that indicate the presence of tissue damage or pathogens, microglia become activated and carry out repair functions. However, excessive activation may lead to the release of substances that cause neuronal dysfunction and death, such as inflammatory cytokines, chemokines, reactive oxygen species, nitric oxide, and glutamate(51-54). The degree of activation depends in part on the type of molecular signal: administration of LPS leads to the release of pro-inflammatory cytokines (TNFα, IL-1β)(52), which can enter the brain and induce microglial activation, while exposure to anti-inflammatory cytokines, e.g., by nonsteroidal anti-inflammatory medication, induces a neuroprotective phenotype in microglia(55, 56). Therefore, activation of microglia by pro-inflammatory signals, such as those that are increased peripherally in PTSD (e.g., IL-6, TNF-alpha, IFN-gamma, CRP)(1-6) may lead to chronic levels of neuroinflammation. To the best of our knowledge, in humans, levels of activated microglia in individuals with PTSD have not been measured - with PET, or postmortem.

As described above, neuroinflammation can be measured using PET radiotracers that bind to TSPO, which is found on activated microglia in the brain. In particular, PET has been used to document higher levels of activated microglia—interpreted as neuroinflammation—compared to controls in individuals with Alzheimer's disease(57) and other neurodegenerative disorders(16, 58). A recent study by Setiawan et al.(18) found that, relative to controls, individuals with major depressive disorder (MDD) had significantly higher levels of activated microglia in the insula (33% higher), anterior cingulate cortex (ACC; 32% higher), and prefrontal cortex (26% higher); further, higher levels of activated microglia in the ACC were linked to greater severity of depressive symptoms ($r=0.63$, $p=0.005$). Our group previously reported no difference in neuroinflammation levels in individuals with milder MDD vs. controls(59); however, a reanalysis of these data found higher levels of activated microglia in those individuals with the most severe depression symptoms (see Preliminary Data section C.1.c), which is consistent with Setiawan and colleagues' findings. Interestingly, higher levels of activated microglia have not been observed in individuals with schizophrenia(60) or cocaine dependence(61), suggesting that neuroinflammation may be more specific to individuals with severe mood symptoms and cognitive dysfunction, both of which are common in individuals with PTSD(30, 35, 62). In Aim 1, we will determine whether individuals with PTSD have higher levels of activated microglia than trauma-exposed controls, and ascertain possible links between levels of activated microglia, mood and anxiety symptoms, and neurocognitive function. Thus, this study has the potential to provide compelling evidence for a role of the neuroimmune system in the etiology and manifestation of PTSD-related psychopathology.

Trauma-related Psychopathology is Dimensional in Nature. PTSD-related psychopathology is often considered to be relatively homogeneous in nature and characterized by general psychological distress; however, factor analytic studies have revealed that PTSD-related psychopathology is characterized by heterogeneous clusters of symptoms that span the severity spectrum(62-69) and are differentially linked to neurobiological systems(70-76). While exposure to trauma is associated with increased risk of developing PTSD, MDD, and generalized anxiety disorder (GAD)(66, 77, 78), these disorders are highly comorbid and share common underlying dimensions of threat and loss symptoms(24, 25, 28, 62, 64, 66, 79, 80). Threat symptoms include intrusive/re-experiencing symptoms such as intrusive memories and nightmares about a traumatic event; avoidance of trauma-related reminders; and anxious arousal symptoms, e.g., hypervigilance. Loss symptoms include emotional numbing (e.g., diminished interest in activities, restricted range of affect), as well as negative affect, anhedonia, and dysphoric arousal (e.g., sleep and concentration difficulties)(28, 62, 64, 66, 80-82). The phenotypic expression of threat and loss symptoms is thus best conceptualized as dimensional in nature, spanning the full spectrum of symptom severity ranging from no symptoms to severe symptoms(66, 83-85).

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Given the impetus of the NIMH RDoC project to identify neurobiological substrates that underlie dimensional aspects of psycho-pathology, we propose to determine the role of neuroinflammation in contributing to the full dimensional spectrum of trauma-related threat and loss symptoms, and neurocognitive dysfunction using both objective endophenotypic and clinician-assessed phenotypic measures.

Psychological Stress Sensitizes Microglia to LPS Administration. Preclinical studies using a psychological stressor, namely chronic restraint stress, document a robust increase in microglial activation, both in number(86, 87) and in morphology(87). Another model of psychological stress – inescapable shock (IS) - also led to neuroinflammation(88), and the drug minocycline, which inhibits microglial activation, reduced the shock-induced neuroinflammatory response(89). Most importantly, there is evidence that psychological stress (i.e., inescapable shock) can activate microglia, which then sensitizes the pro-inflammatory reactivity of microglia to subsequent immune challenges(16). There is cross-sensitization between stress and peripheral pro-inflammatory cytokine responses(90), and exposure to a stressor can sensitize LPS-induced cytokine production(91). One study in particular helps form the basis for our hypothesis for Aim 2. Frank and colleagues(16) found that inescapable shock robustly increased activated microglia and potentiated the pro-inflammatory response to LPS in the hippocampus; **Figure 1**). In other words, stress primed the microglia and they became hyper-reactive to subsequent immune challenges. In **Figure 1**, black bars show the increase in IL-1beta gene expression—a protein found on activated microglia—in the hippocampus, and white bars show the media control. HCC are the home cage controls that received no shock and do have a robust neuroimmune response to LPS like healthy humans in our study(13). We hypothesize that individuals with PTSD have a much larger response to LPS, which is analogous to the group that received inescapable shock (IS) 24 hours prior to the LPS challenge. Thus, we hypothesize that individuals with PTSD will have an exacerbated neuroinflammatory response to an LPS challenge compared to controls. Our overall hypothesis is that chronic stress leads to chronically higher levels of neuroinflammation (Aim 1), which primes the system to respond excessively to an immune challenge (Aim 2).

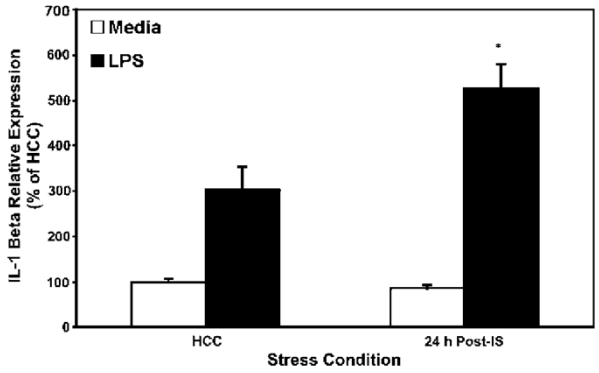

Figure 1. Effect of inescapable shock (IS) on the microglia pro-inflammatory response to LPS ex vivo. Hippocampal microglia were isolated from HCC and 24 h post-IS and exposed to LPS. Gene expression of the pro-inflammatory cytokine IL-1β was then measured. IS significantly potentiated the pro-inflammatory response of microglia to LPS compared to HCC (N=5/group). HCC LPS vs. IS LPS. *p<0.05

Potential Mechanism. The precise mechanism by which neuroinflammation may contribute to the pathophysiology of PTSD is unknown but we hypothesize that activation of the hypothalamic-pituitary-adrenal (HPA) axis plays a critical role. It is well documented that stress (emotional, psychological, physical) can induce an inflammatory response(92, 93). And, it is well known that cytokines – which are released during an inflammatory response - activate the HPA axis, by increasing levels of stress hormones, i.e., cortisol and catecholamines(94, 95). For example, administration of Salmonella abortus equi endotoxin(96) and LPS(97) lead to increases in peripheral cytokines and cortisol, with subsequent increases in anxiety, poor mood, and memory deficits. This suggests that inflammatory induced increases in pro-inflammatory cytokines and cortisol may activate the HPA axis and lead to symptoms that increase threat, loss, and cognitive dysfunction. There is also evidence that repeated vs. single immune stimulation (such as with LPS) may amplify the increase in the HPA axis(98), which is consistent with the finding that stress can sensitize the pro-inflammatory reactivity of microglia to subsequent immune challenges(16) and our hypothesis for Aim 2. Thus, a chronically activated HPA response could lead to persistent anxiety, poor mood, and impaired cognitive function and thus contribute to the pathophysiology of PTSD.

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Microglial Activation is Linked to Mood and Neurocognitive Dysfunction. Studies documenting the role of microglia in psychiatric disorders are scarce(99); however, it is becoming increasingly clear that the brain-cytokine system may have a key role in driving physiological and pathological behavior in psychiatric disorders(19). From our recent study(13), we know that LPS administration in humans leads to robust microglial activation which occurs in concert with particular behavioral changes—an increase in mood symptoms(13) and a decline in neurocognitive function. We also have preliminary data suggesting that LPS-induced changes in activated microglia play a role in mediating the relationship between peripheral inflammation and behavior (see Preliminary Data). Taken together, these studies suggest that systemic inflammation and activated microglia are associated with increased mood dysregulation and neurocognitive dysfunction (see **Figure 2**). In Aim 3, we will determine relationships between the central and peripheral immune pathways, trauma-related threat and loss symptoms, and neurocognitive dysfunction.

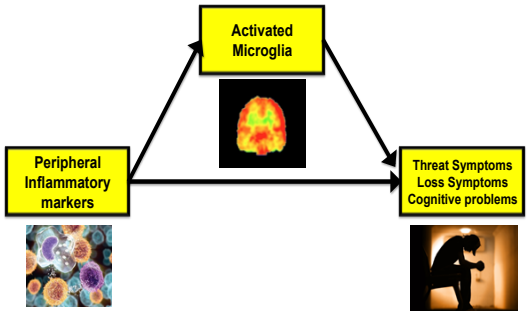

Figure 2. Model of how peripheral inflammation and neuro-inflammation may contribute to PTSD-related symptoms.

Preliminary studies

a) [^{11}C]PBR28 PET imaging. [^{11}C]PBR28 PET is used to measure levels of TSPO, which are expressed mainly on activated microglia. In our previous study(100), we confirmed that LPS-induced TSPO reactivity occurred mainly in microglia, rarely in astrocytes. The specific outcome measure used is volume of distribution (V_T), which is a measure of levels of TSPO binding sites in a given region of interest. One caveat is that [^{11}C]PBR28 binding is influenced by a single point mutation in the TSPO gene(101). This polymorphism results in high, medium and low binding in the brain and low binders do not have measurable TSPO availability with [^{11}C]PBR28. Thus, blood samples are drawn for genotyping during screening and low binders are excluded. Because there is a ~2-fold difference in [^{11}C]PBR28 V_T at baseline between high and medium binders, this genotype is entered as a fixed factor in the analysis(102). In our hands, [^{11}C]PBR28 has excellent test-retest variability (TRV of V_T : 7-9% across gray matter regions(103)).

b) Higher levels of activated microglia in individuals with PTSD compared to trauma-exposed controls. We used [^{11}C]PBR28 and PET to evaluate levels of activated microglia in individuals with PTSD (n=6) compared to trauma-exposed controls (n=8). Five women and one man with PTSD (mean age=33.3, SD=9.3, range=21-48; index trauma: 1 sexual assault, 1 witnessed attempted murder of husband,1 physical abuse, 1 robbed at gunpoint, 1 victim of human trafficking, and 1 who lost husband in accident; mean 24.3 years since trauma) were recruited. After adjustment for the rs6971 polymorphism on the TSPO gene, an ANOVA revealed that, relative to trauma controls (TC), the PTSD group had markedly higher global (21.5% higher) and regional (17.3% to 30.1% higher) [^{11}C]PBR28 V_T values (**Figure 3**). The magnitude of this difference was, by convention, large (Cohen d=1.02). In Aim 1, we will build on these preliminary findings by determining whether PTSD is associated with higher

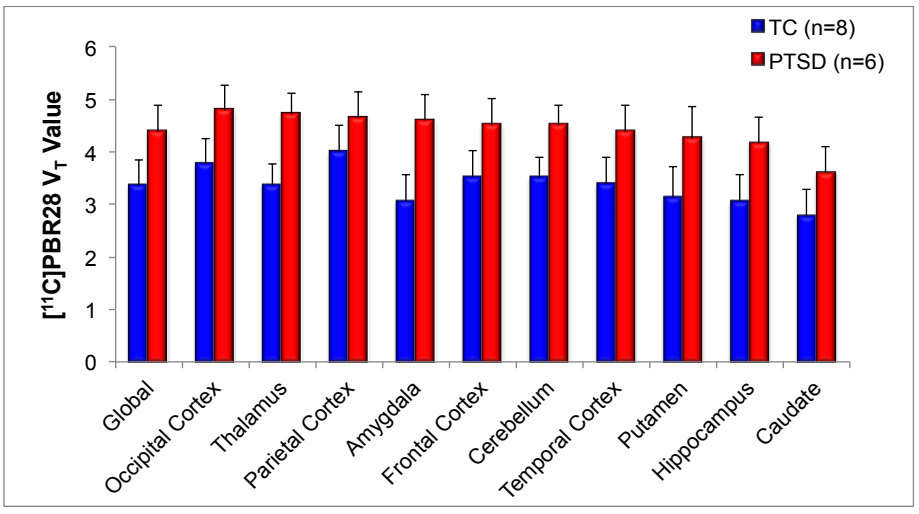

Figure 3. Mean [^{11}C]PBR28 V_T values in individuals with PTSD (n=6) relative to trauma-exposed controls (TC; n=8); Error bars represent SEM.

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levels of activated microglia compared to controls, and by evaluating how levels of activated microglia relate to the expression of threat and loss symptoms, and neurocognitive functioning in trauma-exposed individuals presenting with the full dimensional spectrum of PTSD symptoms.

c) Higher levels of activated microglia are associated with loss (i.e., anhedonic) symptoms. We reanalyzed data from a previous study(59) to compare global activated microglial levels in 5 individuals presenting with moderate or greater severity of MDD symptoms (score >= 20 on the MADRS) to 5 healthy controls matched with respect to age, sex, race, body mass index, and smoking status. Compared to controls, individuals with MDD had substantially higher (27.9%) global and regional (13.6% to 34.5%) [^{11}C]PBR28 V_T values. Composite [^{11}C]PBR28 V_T values correlated strongly with overall severity of depressive symptoms (r= 0.80; **Figure 4**). Dimensional analyses using a 3-factor model of MADRS-assessed MDD symptoms(68, 69) revealed that [^{11}C]PBR28 V_T values were independently associated with severity of anhedonia/loss (i.e., lassitude, inability to feel; r= 0.82), but not psychic anxiety (i.e., pessimistic thoughts; r= 0.36) or vegetative symptoms (i.e., reduced sleep and appetite; r= 0.30). Thus, we hypothesize that higher levels of activated microglia will be strongly related to anhedonic/loss symptoms.

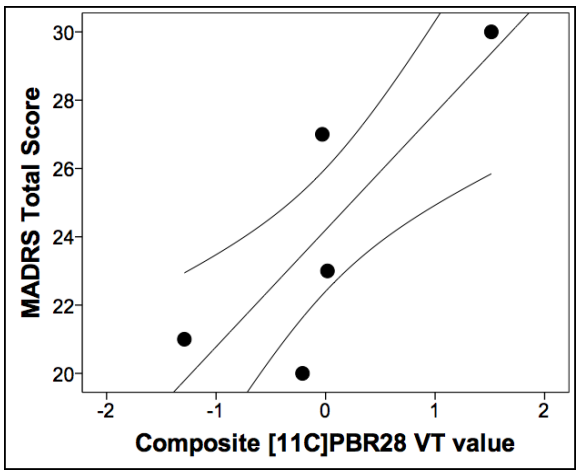

Figure 4. Scatterplot of association between global composite [^{11}C]PBR28 V_T values and severity of depressive symptoms.

d) Higher levels of activated microglia are associated with neurocognitive dysfunction. Preliminary data (n=23) were analyzed to evaluate the relation between global [^{11}C]PBR28 V_T values and performance on a comprehensive neurocognitive test battery developed by Cogstate (www.cogstate.com), which assesses processing speed, visual attention, verbal and visual learning and memory, visual working memory, and spatial problem-solving(104, 105). On average, this sample was 29.7 years old (SD=7.3, range=19-44), predominantly male (65.2%), ethnically diverse (43.5% African American, 30.4% Caucasian, 21.7% Hispanic, 4.3% Hispanic), and comprised of n=13 [56.5%] healthy controls, n=7 [30.4%] individuals with alcohol dependence and 3 [13.1%] with PTSD. Partial correlations adjusted for the rs6971 polymorphism on the TSPO gene and psychiatric disorder revealed that higher global [^{11}C]PBR28 V_T values were associated with significantly worse performance on measures of learning and memory (r= -0.47, p=0.021), and working memory (r= -0.39, p=0.034). These results suggest that higher levels of activated microglia are associated with decrements in learning and memory, as well as working memory. In Aim 1, we will build on these preliminary results to determine how baseline levels of activated microglia contribute to neurocognitive dysfunction in PTSD.

e) LPS administration induces robust microglial activation, anhedonic symptoms, and neurocognitive

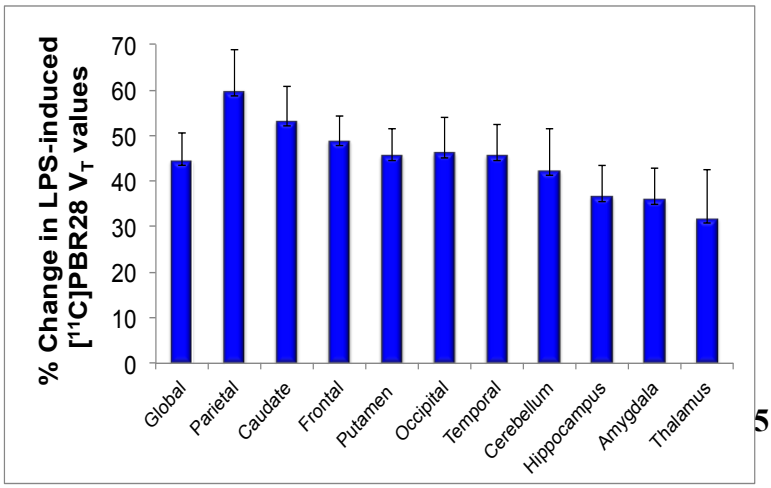

Figure 6. Percent change in LPS-induced [^{11}C]PBR28 V_T values globally and regionally. Error bars represent SEM

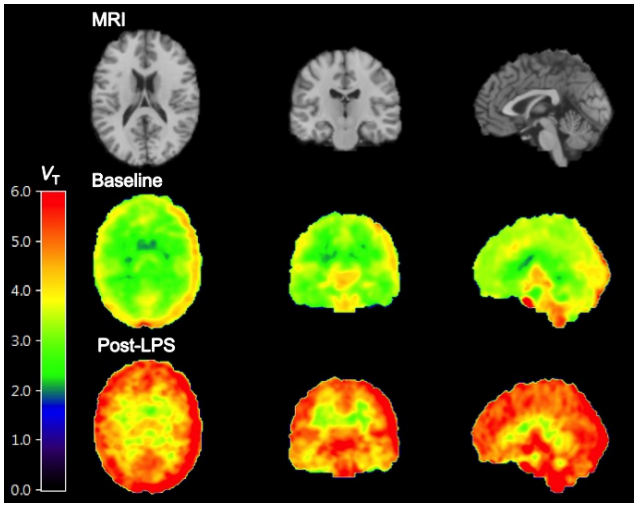

Figure 5. LPS administration significantly increases [^{11}C]PBR28 binding (V_T) from baseline (average parametric images from n=8). Magnetic resonance image (MRI) is shown for anatomical reference (top row).

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dysfunction in healthy adults. After initial studies in baboons(100), we assessed the neuroinflammatory response produced by systemic administration of the *E. coli* lipopolysaccharide (LPS; also called endotoxin) in 8 healthy men (mean age=25, SD=5.5) using [^{11}C]PBR28 and PET(13). In addition, peripheral cytokine levels and depression-related symptoms were assessed before and after LPS administration. Each subject had two [^{11}C]PBR28 PET scans in one day, one before and one 3 h after an LPS (1.0 ng/kg, IV) challenge. **LPS administration significantly increased [^{11}C]PBR28 V_T by 30-60%**, demonstrating robust microglial activation throughout the brain (**Figures 5 and 6**). Notably, the increase occurred in all 8 subjects. This increase was accompanied by an increase in peripheral inflammatory cytokines, vital sign changes, mood symptoms(13) and a significant reduction in learning and memory performance (r= -0.60, p=0.032). These data provide the first demonstration in humans that a systemic LPS challenge induces robust increases in microglial activation in the brain, with concomitant increases in peripheral inflammation, and anhedonic and neurocognitive symptoms. In Aim 2, we will use this established and robust paradigm to evaluate the role of LPS-induced increases in levels of activated microglia in the etiology of PTSD symptomatology.

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.

   Aim 1, we will recruit 40 trauma-exposed individuals and 40 individuals with PTSD to participate in one [^{11}C]PBR28 PET scan and one structural MRI scan, which is necessary for PET data analysis. Measures of PTSD and related symptoms, measures of threat and loss symptoms, and neurocognitive function will be assessed before PET scan.

   Aim 2, the same subjects from Aim 1 will undergo a second [^{11}C]PBR28 PET scan on the same day, 3 hours after LPS administration (1.0 ng/kg, IV). This will allow us to measure LPS-induced changes in microglial activation. The order of the scans is not randomized because it is preferable to obtain both carbon-11 scans (20-min half-life) on the same day to reduce variability; and, because LPS likely has a relatively long-lasting effect on microglial activation/TSPO availability, it cannot be given prior to the first scan without affecting a second scan on the same day. Measures of PTSD and related symptoms, measures of threat and loss symptoms, and neurocognitive function will be assessed before and after LPS administration on scan days (timing of tests is detailed below) to determine whether inducing a systemic immune response dynamically affects these measures differentially between individuals with PTSD vs. trauma-exposed controls.

   Aim 3, blood samples will be obtained on all subjects participating in Aims 1,2, and 5 before and after LPS

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administration on scan day to determine the relationship between peripheral cytokine levels, neuroinflammation, and dependent variables.

Aim 4, A) In order to define the relationship between neuronal and vascular inflammation we will recruit 40 subjects with PTSD and 40 trauma-exposed controls to participate in one whole body [^{18}F]FDG scan. Performing a "whole body" scan, i.e., brain to torso, allows us to measure brain and vascular/arterial inflammation in the same scan. B) These subjects will also have the option of participating in a whole body [^{11}C]PBR28 scan (on the same or different day).

Aim 5, the same subjects from Aims 1 & 2 will have the opportunity to undergo a third and fourth [^{11}C]PBR28 PET scan on subsequent days following LPS administration (these additional scans are optional and are not required for study participation). These additional scans will allow us to measure the approximate time course of neuroimmune recovery following LPS challenge in control and PTSD subjects. Subjects will complete both scans within one month following LPS administration. Measures of PTSD and related symptoms, measures of threat and loss symptoms, and neurocognitive function will be assessed immediately prior to each PET scan.

All subjects participating in PET scans will also have an MRI, which is necessary to rule out any brain abnormalities and for use in the PET image analysis. The MRI scan for an individual subject may be omitted if the required anatomical MRI scan for this subject is on file as part of participation in an approved Yale Protocol and was performed within approximately 6 months prior to the planned scanning time in this study.

Subject Selection, Assessment and Recruitment: All research subjects will be recruited in accordance with the guidelines of the Yale University Institutional Review Board (Human Investigation Committee). Subjects (n=160) will be recruited from the community via IRB-approved advertising (newspaper, radio, internet posting, television, flyers) and by referral from Yale outpatient psychiatric clinics. Over the past 10 years, we have successfully recruited individuals with psychiatric disorders, including PTSD(106), major depression(107), bipolar depression(108), alcohol dependence(109), and schizophrenia(110, 111), as well as healthy control subjects from the community for neuroimaging studies. After completing informed consent, subjects will undergo a physical and neurological examination, and an electrocardiogram (EKG). Laboratory tests will be performed to exclude subjects with medical complications and will include a complete blood count (CBC) and differential, chemistries, kidney function tests, liver function tests, and thyroid stimulating hormone tests. Subjects will be excluded for major medical, neurological or psychiatric illness (except PTSD and MDD, see below for details), abnormal laboratory tests, or contraindication to radiotracer or MRI. A urine drug screen and pregnancy test will be performed at screening and prior to both the PET and MRI imaging session, and subjects who screen positive will be excluded (except for cannabis). Trained, experienced research assistants under the direct supervision of Drs. Cosgrove and Esterlis (both are licensed clinical psychologists) will initially evaluate subjects and administer the Structured Clinical Interview for DSM-5 (SCID-5) to ascertain psychiatric status, and the CAPS-5 to assess severity of PTSD symptoms and determine eligibility for participation. Physical and neurological examinations including a detailed history will be performed or overseen by a study physician who will review all of the clinical information, including laboratory results, and in collaboration with Drs. Cosgrove and Esterlis, will determine eligibility of study subjects.

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PET Scans

PET scans will be performed at the Yale University PET Center. An antecubital venous catheter will be used for IV administration of the radiotracer and for venous blood sampling. A radial artery catheter will be inserted by an experienced health care provider (an experienced physician or a highly skilled APRN trained to complete this procedure)in the morning before the PBR28 PET scan (FDG does not require arterial sampling). 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. The IV catheter will also remain in place for the duration of the scan day. **For Aims 1,2 and 5**, PET scans are acquired as subjects rest using either an HRRT (207 slices, resolution better than 3 mm FWHM in 3D acquisition mode), HR+ PET scanner, 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. A transmission scan is obtained before each emission scan. On the HRRT PET scanner, motion correction will be performed dynamically with measurements from the Vicra (NDI Systems, Waterloo, Ontario) using a dedicated list-mode reconstruction algorithm 74. A dynamic PET scan of up to 2 hours duration will be acquired after IV bolus or bolus plus infusion of up to 18mCi of [^{11}C]PBR28. **For Aim 4**, subjects will be administered up to 10 mCi of FDG and may also be administered up to 20mCi of [^{11}C]PBR28 to be scanned on the Siemens mCT PET/CT scanner (this is the whole body scanner) which includes CT attenuation scans. For the FDG PET scans, subjects are asked to follow a low carbohydrate diet for 24 hours prior to the scan and to fast overnight the night before. For FDG scans, FDG will be administered (up to 10 mCI, IV), and subjects rest for up to 2 hours prior to the 2 hr scanning period. The 2 hr rest period is necessary for the FDG uptake to occur. The PBR28 scans occur immediately following injection.

Vital signs may be taken immediately prior to injection of radiotracer, immediately after injection, at approximately 120 minutes after injection, and at the end of the last emission scan. Any adverse events will be evaluated and recorded continuously through the PET imaging day.

When arterial cannulation has been performed by a qualified health care provider, in the first phase of the scan (5-10 min), the arterial input functions are measured with an automated blood counting system (PBS-101, Veenstra Instruments, Joure, The Netherlands) using a continuous withdrawal system where the radioactivity in whole blood is measured with a calibrated radioactivity monitor. Subsequently, individual blood samples (venous and / or arterial) are taken at various time points and counted in a gamma counter. Samples are centrifuged to obtain plasma, which will be counted, and selected samples will be assayed for the presence of the parent radiotracer compound that has not been metabolized. These measurements will be performed by HPLC. In addition, the fraction of plasma radioactivity unbound to protein will also be determined.

Endotoxin administration

Subjects will receive intravenous administration of open-label endotoxin at a dose 0.8 - 1ng/kg body weight. This will take place at the Yale PET Center. All subjects will fast for at least 8 hours before endotoxin administration to reduce the risk of nausea, but they will be encouraged to hydrate well. They will be asked not to use aspirin, NSAIDs, COX-2 inhibitors, or acetaminophen for at least 3 days prior to each study day. Clinical Center Reference Endotoxin (CCRE) will be produced by List Labs and will be stored and prepared by the YNHH IDS pharmacy. CCRE is an investigational drug, and investigator Kelly Cosgrove has obtained an Investigational New Drug (IND) for its use in humans from the Food and Drug Administration (FDA). CCRE vials will be stored at 2-8°C in a refrigerator in the locked YNHH IDS pharmacy. Preparation of CCRE will be performed by the YNHH IDS research pharmacist. As recommended by NIH, the pharmacist will dissolve the lyophilized CCRE in Sterile Water for Injection USP under a laminar flow hood on the evening before each endotoxin administration. The dissolved CCRE will be stored at 4°C until the following morning.

Dissolved CCRE is stable for at least 24 hours at this temperature.

On the day of endotoxin administration, subjects will rest on a stretcher in the PET Center. A urine drug screen

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and breathalyzer will be done, as will a urine pregnancy test for women. An IV catheter will be placed to allow for hydration with normal saline, endotoxin infusion, and blood draws. In the case of bradycardia or hypotension, this catheter will be used for rapid bolus infusion of normal saline (van Eijk, Pickkers et al. 2004). To reduce the already low risk of adverse cardiovascular events, subjects will be hydrated with 500 mL of saline prior to endotoxin administration, and they will receive approximately 100 mL/hr thereafter for the duration of the endotoxin administration day.

After endotoxin administration, vital signs (blood pressure, heart rate and temperature) will be recorded every 15 minutes for the first two hours. If vital signs change significantly, monitoring will continue at least every 30 minutes, or more frequently as determined by the study MD, until they have returned to within 10% of the baseline values. All subjects will be connected to continuous two-lead EKG monitoring for the duration of the challenge. All subjects will be carefully monitored at all times by a research nurse and by a study physician or APRN (APRNs are trained on the protocol and will have MD telephone back up), who will be present until 3 hours after endotoxin administration. Any subject experiencing prolonged effects of endotoxin or any other adverse event may be transferred to the Yale New Haven Hospital emergency room if appropriate.

There is no evidence that experimental endotoxin administration causes hypoxemia in humans and therefore there is no literature to support monitoring of this parameter (as opposed to parameters such as heart rate and blood pressure, which do change and thus, will be monitored closely). Furthermore, pulse oximetry can be unreliable when there is significant peripheral vasoconstriction, as occurs during e.g. experimental endotoxemia, sympathetic activation, and fever.

If an individual experiences bothersome symptoms after endotoxin administration and wants to stop the study, flu-like symptoms may be treated with acetaminophen (650 mg every 6 hrs), or ibuprofen (400 mg every 6 hrs), and anxiety may be treated with lorazepam (0.5 mg every 2 hrs for a maximum dose of 6 mg every 24 hrs). During screening each subject will be asked about medication allergies, and no medication to which a subject may be allergic will be administered. If a subject requests so, the study will be stopped and these medications may be administered. In addition, if any of the following symptoms occurs, if of a severity such that it prevents normal activity, the study in that individual subject will be stopped: Chills, headache, malaise, myalgias, arthralgias, fatigue, nausea, vomiting. If a subject's body temperature exceeds 39.2°C the study will be halted and the subject will receive ibuprofen or acetaminophen as described above. If these medications are insufficient to lower body temperature, subjects may be transferred to a higher level of care. Subjects will be discharged at the end of the study day, provided that all of the following have occurred: their blood pressure is within 10% of baseline AND their heart rate is within 20% of baseline AND their mental status is normal AND they do not feel physically or mentally unable to return home. The determination will be made by the PI and/or the study physician.

Inflammatory cytokines

Before and at approximately 1, 1.5, 2, 3, and 4 hrs after endotoxin administration all subjects will have an 10 ml blood sample drawn for plasma levels of inflammatory cytokines (including but not limited to: IL-1beta, IL-4, IL-6, TNF alpha, IL-10, IL-12). In addition, plasma levels of inflammatory cytokines will be measured just prior to subsequent PET scans following LPS administration for Aim 5.

Specimen Storage

Specimens will be logged into a specimen tracking log and stored either at the PET Center or YCCI Biorepository. This log will include Study ID, date drawn, label of specimen (i.e. barcode, type of specimen etc), location, and date withdrawn for analysis or destruction.

Behavioral Measures

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We will assess PTSD, depression and anxiety symptoms on all subject participation days with the Civilian M-PTSD, Montgomery-Åsberg Depression Rating Scale (MADRS) (112) and Hamilton Anxiety Rating Scale (HARS) (113) respectively and explore relationships between these measures and radiotracer binding in relevant brain regions.

General Assessments may include:
1). Socio-demographic/General Information: At intake, demographic data and medical history will be assessed with interviews and self-report forms that provide data on age, race, socioeconomic status, marital status, educational and occupational levels, and significant medical history. These are adapted from previous diagnostic and clinical studies at this center.
2) The Structured Clinical Interview for DSM-V (SCID-5) will be used to provide current and past diagnoses of substance use and other psychiatric disorders.
3) *SCID II* is an addendum to the SCID that is used to diagnose axis II disorders including Antisocial Personality Disorder.
4) *Civilian M-PTSD* is an assessment of symptoms of posttraumatic stress disorder.
5.) *Health Status* is a self-report instrument that will be used to assess the lifetime occurrence of illnesses or conditions.
6.) *Concomitant Medication Log* will be used scan day to determine what medications have been taken in the last two weeks.
7.) *Bower VAS and the endotoxin symptoms checklist* will be administered to participants during the endotoxin challenge.

Medical Assessments

Any clinically significant abnormal medical screening laboratories from the initial assessment will be repeated prior to each imaging session.

1) *Physical exam* by a state licensed physician or Advanced Practice Registered Nurse

2) *Laboratory Blood Tests* including a complete blood count with Diff, blood urea nitrogen, creatinine, fasting blood sugar, electrolytes, liver function tests, thyroid function tests (including T3, T4, T3RU, estimated free T4), thyroid stimulating hormone levels and coagulation panel, and DNA genetics testing**.** For all laboratory screening blood and urine tests, one of the study physicians reviews the labs, and in combination with the results from the physical exam, decides if the subject is sufficiently healthy (so that it does not alter the scientific integrity of the study, or risk to the subject for study participation) to participate in the study.

3) *Urine toxicology screens* may be performed at baseline, prior to PET imaging, and more frequently if the clinician or research staff become concerned about possible illicit drug use. Patients will be instructed that random urine drug screens will be performed. Urine samples may be tested for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates.

4) *Pregnancy Tests* female patients will have a blood pregnancy test at baseline and also a urine pregnancy test on the day of each PET scan and MRI scan.

Cognitive Measures

We may obtain these measures at baseline and up to two times on the PET days.

1. Cogstate Battery (30 minutes) – This computerized test battery will assess memory and cognition. The tasks may include:

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a. International Shopping List Task – a computerized task to assess verbal learning and memory.

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/Two Back Tasks – computerized tasks to assess attention and working memory

Probabilistic Reward Task: The PRT has been successfully used to assess reward responsiveness (114-116). 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. This is called the "Face Game" in the consent.

Cold Pressor Task: Subject may be asked to participate in the Cold Pressor Task. The cold pressor task (CPT) is a stress task used to measure pain sensitivity and pain tolerance. This task will be used to determine alterations in pain thresholds as a result of endotoxin administration. Participants will immerse their hand (up to the wrist) for up to 3 minutes in the experimental (ice-cold temperature 0-4°C)) and control (room temperature (20°C)) conditions. Physiological measure (heart rate, blood pressure and subject responses (stress, mood) will be collected 5 minutes before, 1 minute into, and immediately after the CPT.

Dot-Probe Task: The Dot-Probe Task is used to assess attentional biases to threatening and non-threatening information. In the task the subject is instructed to fixate on a cross in the center of the screen while two stimuli, one threatening and one non-threatening are displayed on either side of the screen. The stimuli are presented for a 500 ms and then disappear, and a dot is presented in the location where of one of the stimuli used to be. The subjects are instructed to indicate the location of the dot, left or right, as quickly as possible. The reaction time to the dot when it appears in the location where the threatening stimulus had been, is interpreted as an attentional bias and increased vigilance to threat. This measure can be correlated with clinical features and outcomes of PTSD and other anxiety disorders.

Image Analysis

Outcome Measure and Dependent Variables

For PBR28, the primary outcome measure is total volume of distribution (V_T) which is proportional to *in vivo* TSPO levels. There is no brain region that does not exhibit specific binding of PBR28. Thus, for full quantification of V_T, arterial sampling is required. We will examine the regions-of-interest (ROIs) listed below. For PBR28 we will use two-tissue compartmental analyses with a metabolite-corrected arterial input function to estimate total distribution volumes for each ROI (117).

MRI-Based ROI Definition

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We will use the MR image to define regions of interest (ROIs). The PET scans alone do not have sufficient resolution to identify brain regions. The PET image sets are aligned and resliced to yield images in the same planes and spatial system as the MRI images using AAL template. Primary ROIs will include the hypothalamus, thalamus, amygdala, striatum, and the cingulate and frontal cortices. Other ROIs will be examined *post hoc* to assess radiotracer binding.

SPM Analysis

Statistical parametric mapping software (SPM2, Wellcome Department of Imaging Neuroscience in the Institute of Neurology at University College London) implemented in Matlab 6.5 (Mathworks Inc., Sherborn, MA) will be used for voxel-based statistical analysis. Each scan will be transformed into sterotactic space by normalizing to a template image. The images are then smoothed using a Gaussian kernel (8x8x8 FWHM).

For outcome measures for cardiac FDG, using the CT as guide, the ascending aorta, aortic arch and carotid arteries will be delineated. For aorta, ROIs will be drawn every 3 mm around the artery along the aorta, starting 1 cm distal to aortic valve (to avoid spillover from the heart) and ending after the takeoff of the left subclavian artery. For carotid arteries, serial regions of interest will be drawn around each carotid, starting 2 cm below carotid artery bifurcation and extending 2 cm into internal carotid artery. The mean and maximum standardized uptake value [SUV, decay-corrected tissue concentration of [18]F-FDG (in Bq/mL) divided by the injected dose per body weight (Bq/g)] on co-registered PET images will be determined for each ROI, and will be averaged along each artery. Background (blood) activity will be determined in 5 ROIs separated 3 mm apart drawn around superior vena cava (for aorta) and ipsilateral jugular vein (for carotid artery). Target to background ratio (TBR) will be calculated by dividing the average SUVmax by background activity. For each subject, the artery with the highest TBRmax will be selected as the index vessel for all analyses.

Sample Size Calculation

Power calculations were conducted by a statistician and Co-I, Dr. Pietrzak. Preliminary data from 6 individuals with PTSD and 8 trauma-exposed controls revealed robustly higher [^{11}C]PBR28 V_T values globally (21.5% higher; d=1.02) and regionally (17.3-30.1% higher; d's=0.83-1.15) in PTSD vs. controls.}} Assuming a two-sided analysis of covariance (ANCOVA) with α=0.05, recruitment of 40 trauma-exposed controls and 40 individuals with PTSD (quartiles 3-4) will provide greater than 94.2% statistical power to detect effect sizes as small as d=0.80. This translates into minimum detectable differences between controls and individuals with PTSD of 15.0%. To examine relations between continuous measures of global and regional [^{11}C]PBR28 V_T values, and measures of post-traumatic threat and loss symptoms, and neurocognitive functioning, preliminary data from 5 individuals with moderate-or-greater severity of MDD symptoms revealed a strong association between global [^{11}C]PBR28 V_T values and severity of loss/anhedonic symptoms (r=0.82). Further, preliminary data from 23 individuals revealed that global [^{11}C]PBR28 V_T values correlated significantly with measures of learning and memory (r= -0.47) and working memory (r= -0.39). Thus, assuming a two-sided linear multiple regression model with α=0.01 (0.05/5 dependent variables: dot probe and PRT scores, CAPS-5-assessed threat and loss symptoms, global cognition) and conservative overall model effect size (R^2) of .25, and up to 10 predictors, an n of 80 will provide 99.2% statistical power to detect this magnitude effect. Our previous study in 8 healthy controls revealed robust LPS-induced increases in [^{11}C]PBR28 V_T values ranging in magnitude from 31% to 63%(13). We have preliminary data indicating an overall 8.8% difference (d=0.38)in global [^{11}C]PBR28 V_T values between 2 individuals with PTSD and 2 matched trauma-exposed controls. Thus, assuming a minimum 8.0% differential increase in LPS-induced [^{11}C]PBR28 V_T values in the PTSD group relative to trauma-exposed controls(16), α=0.05, correlation among repeated measures=0.50, and nonsphericity correction ε =1, recruitment of 40 individuals with PTSD and 40 trauma-exposed controls will provide greater than 91.9% statistical power to detect this magnitude differential effect size.

MRI

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MR imaging will be performed on a Prismab MRI with a circularly-polarized head coil located at the Anlyan Center, a member of the research team will accompany the subject to the MRRC and will stay for the MRI exam. MR acquisition will be a Sag 3D magnetization-prepared rapid gradient-echo (MPRAGE) sequence with 3.34 ms echo time, 2,500 ms repetition time, 1,000 ms inversion time, 7degree flip angle, and 180 Hz/pixel bandwidth. The image dimensions will be 256 x 256 x 176 and pixel size is 0.98 x 0.98 x 1.0 mm.

Serum Markers
On each PET scan day, blood samples will be collected prior to radiotracer administration to measure hormone levels in all subjects. Blood samples will also be drawn before and approximately every 30 min after LPS administration to determine LPS concentration, stress hormone levels (cortisol and catecholamine levels), and cytokine levels (e.g., TNFα, IL-1β, IL-6, IL-10 and others).

Genetic Analyses
As an exploratory part of this study we may assess associations between microglial activation, anxiety and depressive symptoms, and carrier status of polymorphism in genes that encode for TSPO and IFN-α receptors, TNF and TNF receptors, IL-6 and IL-6 receptors, and other polymorphisms of interest.

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 PTSD. We will also test for alleles of the Ala147Thr polymorphism of the TSPO gene to avoid non-binding of the PBR28 radiotracer.

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 for polymorphisms of interest.

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.

iv. the methods to uphold confidentiality. The results of genetic testing in locked file cabinets and by separating 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? No sharing is planned

C. Is widespread sharing of materials planned? No

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

F. Describe the provisions for protection of participant privacy Risks associated with genetic testing will be minimized by keeping the results of genetic testing in locked file cabinets 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 in locked file cabinets and by separating the personal identifying information of the subjects from the genetic information

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

   We will recruit men and women aged 18-55 who have been exposed to trauma, with and without PTSD into the study. To ensure that we study a representative cohort of both PTSD and trauma-exposed individuals whose symptoms span the full dimensional spectrum, we will use a sampling strategy that is more inclusive than exclusive, and we will recruit subjects who present with a wide range of PTSD symptoms, ranging from unaffected to severely affected trauma survivors. Notably, we will enrich the sample by requiring that 50% meet criteria for a primary PTSD diagnosis. This will allow us to pursue both categorical and dimensional analyses of our study aims. In doing so, the proposed study aligns with both traditional case-control approaches of evaluating the neurobiology of psychiatric disorders, as well as more contemporary approaches, such as the NIMH RDoC project, which embraces a dimensional approach to classifying the neurobiological underpinnings of psychopathology.

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

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

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects?
Yes ☐ No ☒

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

<u>Inclusion criteria</u>:
- Men and women, aged 18-55 years, any race, with a history of trauma exposure
- Subjects with posttraumatic stress disorder (PTSD) will have a primary, current diagnosis of PTSD according to DSM-V criteria (i.e., CAPS-5 ascertained diagnosis)
- Trauma-exposed controls will have a CAPS-5 score of 0-22 (no/minimal to mild/subthreshold symptoms)
- Able to read and write English and to provide voluntary, written informed consent

<u>Exclusion criteria:</u>
- Current medical condition such as neurological, cardiovascular, endocrine, renal, liver, or thyroid pathology including COPD, anemia, uncontrolled daily asthma or asthma requiring the use of an inhaler more than 1x/week with an ACT score below 20. A history of vasovagal syncope (fainting) in the last 10 years or any history of unexplained vasovagal syncope will be exclusionary for Aim 2.
- Past or current neurological disorder or disorders affecting the brain including but not limited to multiple sclerosis, history of stroke, brain tumors, traumatic brain injury with loss of consciousness, seizure disorder
- Current drug dependence, current psychotic symptoms and current suicidality, self-injurious behavior or aggressive behavior; MDD and Alcohol Dependence are <u>not</u> exclusionary due to the high comorbidity.
- Current use or regular use of prescription, psychoactive or herbal medications with the exception of marijuana

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(e.g., antipsychotics, anxiolytics, ecstasy, cocaine), with no current drug use confirmed by urine toxicology, which may be associated with inflammation(118). We will not exclude for SSRIs and TRIs due to high prevalence of use within the PTSD population and due to evidence suggesting no effect of these drug classes on endotoxin response (128). Benzodiazepines may not be exclusionary per physician discretion due to high prevalence of use within the PTSD population; dose, history of use, and current frequency of use will be taken into consideration. No subject will be asked to stop taking medication to participate in the study.

- Current or regular use of over-the-counter medication that may affect the immune system (e.g., ibuprofen), including corticosteroids or immunosuppressant drugs; no use 3 days prior to the PET scan
- Vaccination in the last month
- 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 [oral, implant, injection, patch, or ring], contraceptive sponge, double barrier [diaphragm or condom plus spermicide], or IUD
- Contraindications to MRI such as claustrophobia or metal in their body
- Individuals who are classified as "low binders" for the rs6971 polymorphism (<10% of the population)
- Subjects whose participation would cause them to exceed yearly radiation limits for research subjects
- Subjects with resting pulse >100 at challenge
- Subjects with >Grade 2 laboratory abnormalities on screening based on Serum Grading scale on page 26

Note Regarding Marijuana: We do not wish to exclude for recreational marijuana use, just like we do not exclude for recreational alcohol use – which is also known to impact microglia. It is likely that a great variety of things will affect microglial activation; which is why we will carefully document subject characteristics such as age, sex, BMI and also drug/alcohol/marijuana/tobacco use so that we may covary for these parameters in the analysis.

**

9. How will **eligibility** be determined, and by whom? Eligibility to participate will be determined by the PI of this study after completion of the medical and psychiatric evaluation of the potential participant.

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

Potential Risks

1. Risks associated with screening and evaluation. During the screening interview, we will ask about psychiatric and medical history. Certain questions may make subjects uncomfortable or anxious. Only trained and experienced research assistants will perform these interviews, which will be done in a sensitive and gentle manner.

2. Risks associated with confidentiality. Although all information collected about each subject in this study will be protected by HIPAA and stored in locked cabinets and in password-protected computers, it is possible that information could be accessed by individuals who are not part of the study team. Such illegitimate access could have deleterious consequences for the subjects with regards to employment, access to health insurance, and stigma. The protection of subject information is not absolute. It does not apply to any State requirement to report certain communicable diseases or to report cases of physical or sexual abuse. Such duty to report will be explained to each subject during the consent process and if reporting becomes necessary, we will inform the subject. We

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may release information that identifies subjects in some circumstances, without their permission. For example, we may disclose medical information in the event of an emergency. We may take steps, including notifying the authorities, to protect the subject or someone else from serious harm (including child abuse and elder abuse).

3. Risks associated with blood drawing and with IV and radial catheter placement. Blood drawing and venous catheter insertion can be associated with mild pain, bruising, infection, or clot formation. These risks are minimized by the use of proper technique. No more than a total of 150 mL of blood will be drawn during the entire study. This amount of blood loss over a period of 10 or more days should have no deleterious consequences in a non-anemic individual. On the morning of the PET scan, a radial artery catheter will be inserted by an experienced physician or skilled aprn. The site will be anesthetized with lidocaine prior to arterial line insertion. The arterial line will remain in place for the duration of the scans, after which it will be removed. Arterial sampling may be associated with mild-to-moderate pain or bruising at the puncture site. In rare instances, blocking of the artery, poor healing, hematoma, inflammation, or infection at the catheter insertion site may occur. Certain individuals may feel light-headed during arterial catheter placement.

4. Risks associated with radiation. The Yale-New Haven Hospital Radiation Safety Committee (Y-NHH RSC) and the Yale University Radiation Safety Committee (YU-RSC) will review the use of radiation in this research study, and no subjects will be scanned until approval is obtained. This research study involves exposure to radiation from [^{18}F]-FDG and [^{11}C]PBR28 PET scanning 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 from participating in Aim 1,2 and 5 of this study is from four injections of up to 18mCi of [^{11}C]PBR28 PET, plus attenuation correction (i.e., transmission). Although each organ will receive a different dose, the maximum amount of radiation exposure subjects will receive if participating in Aims 1, 2 and 5 is an effective dose of **1.75 rem** for a total of up to 72 mCi of [^{11}C]PBR28 in four injections. If participating in just Aim 1, the effective dose would be **0.437** rem from one injection of up to 18mCi of [^{11}C]PBR28 plus attenuation correction. If participating in just Aims 1 and 2, the effective dose would be **.874** rem from two injections of up to 18mCi of [^{11}C]PBR28 plus attenuation correction.

Subjects will also receive radiation exposure from low dose head CT scans or transmission scans, depending upon which scanner is used. If scans are completed on the HRRT, subjects will receive up to 0.0056 rem from up to 4 transmission scans (0.0014 rem each) If scans are conducted on the mCT, each subject may receive an additional .18 rem (0.045 rem per CT x up to 4 Head CT scans), this results in a maximum effective dose from four injections of [^{11}C]PBR28 and 4 head CT scans to an Effective Dose of **1.93 rem**

The ***targeted*** amount of radiation an individual subject will receive from participating in Aim 4 is from up to 10mCi (1.234 rem) from one injection of [^{18}F]-FDG , from up to 20 mci from one injection of [^{11}C]PBR28 PET (0.486 rem), and up to 2 whole body attenuation CT scans (1.25 rem) per whole body PET scan. Whole body CT attenuation scans are necessary for motion correction; previous studies have shown that one before and after PET injection provides ideal data, should we find that one attenuation scan provides sufficient motion correction we will only do the one. Effective Dose for both FDG and PBR28 scans including attenuation will be **4.220 rem.**

In the event that a scan failure occurs, the subject may repeat the scan as long as their dose limit remains below 5 rem for the year. The amount of radiation that subjects will receive in this study is below the dose limit of 5 rem per year occupational guideline followed by the Yale RSC and PET center.

5. Risks of MRI: Magnetic resonance (MR) imaging carries a risk for subjects who are claustrophobic or have pacemakers, metal pieces, aneurysm clips, large colored tattoos, or any other contraindications for MR. MR is a

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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 MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly but we will ask subjects to tell the research staff if they have them. There are some risks with an MR study for certain people. If subjects have a pacemaker or some metal objects inside their body, they may not be in this study because the strong magnets in the MR scanner might harm them. Another risk is the possibility of metal objects being pulled into the magnet and hitting 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 to read and answer very carefully the questions on the MR Safety Questionnaire related to their personal safety. We will be sure that subjects have read the MR Safety Questionnaire and tell us any information they think might be important. This MR study is for research purposes only and is not in any way a clinical examination. The scans performed in this study are not designed to find abnormalities. The PI's, the lab, the MR technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a diagnostic evaluation of the images. If a worrisome finding is seen on a subject's scan, a radiologist or another physician will be asked to review the relevant images. Based on his or her recommendation (if any), the PI 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 their 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 MR exam and for that reason, they will not be made available for diagnostic purposes.

6. Pregnancy and Breast Feeding: Since the acceptable levels of radioactivity are lower for pregnant individuals, women will be excluded from participating in this study if they are currently pregnant or might become pregnant during the study. They will be tested for pregnancy as part of the routine laboratory tests. If the test is positive, they will not be included in the study. Before starting the study, we will ask women to avoid becoming pregnant and what precautions they plan to take. We ask women to tell us immediately if they change their minds about becoming pregnant. If the woman is currently breast-feeding, she will be excluded from the study. Women will be given one pregnancy test during the initial physical evaluation, and in addition on day of the MRI scan and PET scans, prior to radiotracer injection.

7. Lipopolysaccharide Administration: Lipopolysaccharide (LPS, also called endotoxin) is a large molecule found on the outer membrane of gram-negative bacteria that has been widely used to evoke a robust immune response. We propose to use the same dose and protocol of LPS that we have successfully used in our recent study(13): 1.0 ng/kg, IV bolus. Specifically, on the day of the [^{11}C]PBR28 PET scans, LPS (0.8-1.0 ng/kg, IV) will be administered 3 hours prior to the second PET scan. Vital signs (systolic and diastolic blood pressure, heart rate and respiration rate) and sickness and psychiatric symptoms will be monitored before drug administration and every 15 minutes for the first two hours. If vital signs change significantly, monitoring will continue at least every 30 minutes, or more frequently as determined by the study MD, until they have returned to within 10% of the baseline values. Because of the risk of nausea and emesis, subjects are required to fast for the day of the study until after the scan. In our experience to date, no subjects have reported nausea that was significant enough to require stopping the study. If significant nausea or other adverse events occur, the study will be stopped.

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If an individual experiences bothersome symptoms after LPS administration and wants to stop the study, flu-like symptoms may be treated with acetaminophen (650 mg every 6 hrs), or ibuprofen (400 mg every 6 hrs), and anxiety may be treated with lorazepam (0.5 mg every 2 hrs for a maximum dose of 6 mg every 24 hrs). During screening each subject will be asked about medication allergies, and no medication to which a subject may be allergic will be administered. If a subject requests, the study will be stopped and these medications may be administered. If a subject's body temperature exceeds 39.2°C the study will be halted and the subject will receive ibuprofen or acetaminophen as described above. If these medications are insufficient to lower body temperature, subjects may be transferred to a higher level of care.

At the end of the PET scan day, subjects will be assessed (physical and vital signs) by the study physician. Subjects will be discharged when vital signs are within normal limits and when behavioral changes (if any) are found to be not clinically significant by the MD attending to the subject at the PET Center. If subjects experience any adverse events, they will be treated until they become asymptomatic, prior to discharge. In the proposed study and in our ongoing studies, psychiatric patients who are administered a challenge drug may be admitted to an inpatient unit if psychiatric symptoms worsen or become dangerous on the PET scan day. Arrangements for a guaranteed bed will be made in advance of each PET scan day with the Clinical Neuroscience Research Unit (CNRU), located at the Community Mental Health Center (CMHC), which is 1 block from the Yale PET center. To date, no psychiatric patient has required admission. All subjects are followed for 1 week (in person the following day and by phone thereafter) following LPS administration or until potential symptoms (self-report and by assessment) resolve.

Background and potential side effects of LPS administration: LPS administration in human subjects is a generally safe experimental procedure that has been used in at least 2,200 subjects(119). Intravenous LPS administration at doses from 2 to 4 ng/kg causes flu-like symptoms such as fever, chills, rigors, malaise, and increased heart rate(120-122). These effects peak at around 90 minutes, and completely disappear within 6-8 hours or less. Of the 2,200 subjects who have received LPS, only four severe adverse events have been reported, all involving bradycardia or sinus arrest(119). This occurred in subjects who either had a history of vasovagal syncope and/or who participated in a protocol in which they were not adequately hydrated. In large cohorts of subjects who received adequate hydration, no such effects occurred(119). We have administered LPS (0.8-1.0 ng/kg) to >30 subjects, including both healthy controls and individuals with psychiatric disorders, with no unexpected or serious adverse effects. A small study(122) detected gender differences in peripheral cytokine levels after LPS administration, with females having a more pronounced response than males; 50% of our sample will be women so we will be able to explore sex differences. We have not noted particular behavioral differences (e.g., sickness behavior) between men and women receiving LPS to date.

A review of the literature published in 2007(123) describes all the studies wherein LPS was given to human subjects. All articles were reviewed for any potential adverse effects, morbidity or mortality; however, no long-term morbidity or mortality was reported in these more than 1000 healthy volunteers. At a 5 ng/kg dose of EC-5, subjects experienced nausea, vomiting and fever. Consequently, doses of ≤ 4 ng/kg have been subsequently used. We are giving 1/4 of that dose. LPS given at 2 ng/mg is not associated with known long-term sequelae or serious adverse events in healthy populations. Subjects (up to 4 total) who did have side effects at doses as low as 2 ng/kg were found to have underlying medical issues. When side effects have occurred, they are remarkably similar. Typically, within 50-90 min following LPS, subjects describe flu-like symptoms such as fatigue, malaise, arthralgias, myalgias, headache, nausea, and often chills that resolve within 3-4 h. Fever, however, is the hallmark of LPS infusion. After a latent period up to 120 min, there is a monophasic increase in fever which peaks at 1-2°C above baseline. The fever may last up to 3-4 h (depending on dose) and resolves without residual effect.

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This critical review of all the cases of LPS administration in human subjects concludes as follows, "for well over a century, endotoxin has been intentionally administered to humans for therapeutic purposes, for the evaluation of anti-inflammatory reagents as well as to address basic scientific questions of endotoxin biology. Much has been learned from the use of this model, and it has proved to be remarkably safe. Well over 1,000 subjects have been studied, yet we were able to find documentation of only five serious adverse events, and these were in subjects who were later shown to have vagal hypersensitivity,"(123).Thus, all the available data support that LPS administration in healthy human subjects is safe.

Lipopolysaccharide, or Clinical Center Reference Endotoxin (CCRE) will be produced by List Labs and will be stored and prepared by the Yale New Haven Investigational Drug Service (IDS). CCRE is an investigational drug, and the PI, Dr. Kelly Cosgrove has obtained an Investigational New Drug (IND) for its use in humans from the Food and Drug Administration (FDA). CCRE vials will be stored at 2-8°C in a refrigerator in the locked IDS pharmacy. Preparation of CCRE will be performed by the IDS research pharmacist. As recommended by NIH, the pharmacist will dissolve the lyophilized CCRE in Sterile Water for Injection USP under a laminar flow hood on the evening before each endotoxin administration. The dissolved CCRE will be stored at 4°C until the following morning. Dissolved CCRE is stable for at least 24 hours at this temperature.

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

To minimize the risk associated with screening and evaluation we will use trained personnel who will treat each subject with respect and compassion and who will be sensitive to any psychological discomfort produced during the screening or during any of the assessments.

To minimize the risk associated with confidentiality information about current and past psychiatric and medical history and results of screening labs will be stored in hardcopy files in a locked file cabinet or on a password-protected computer in a locked office on the locked unit or the PIs locked office. Any data that is stored on a moveable digital media will be unlinked from the subject's identifying information, and the codes linking the data to the subject will not be stored on such moveable media. Samples sent to laboratories for analysis will be coded by a study identifier that does not include any personal identifiers. All information obtained from participating subjects will be kept in locked confidential files. This information is not available to anyone except the Yale Human Investigations Committee (HIC), FDA, and the investigators and study personnel identified in this protocol. Access is only for study-related purposes. All data collected in this study will be kept for 7 years after study completion. Destruction of paper research files will be done by confetti-cut shredding. Destruction of digital files will be done by zeroing or degaussing. Personally identifiable information will not be used for future research purposes without the subject's written permission.

To minimize risks associated with blood drawing and venous and arterial catheter insertion,
Risks of radial artery cannulation are minimized by having the procedure performed by an experienced health care provider. The health care provider would be either a physician or an advanced practice registered nurse (APRN) with experience in critical care and placement of arterial catheters, as is the practice at Yale-New Haven Hospital. For an APRN to place the arterial line at the Yale PET Center, they must meet the following criteria:
1.) Be currently credentialed at Yale-New Haven Hospital or similar institute and
2.) Perform 3 arterial line procedures supervised by a currently privileged PET Center physician.
The 3 supervised arterial line placements will be documented and signed off by both the APRN and supervising physician. The completed document must be on file at the Yale PET Center prior to an APRN performing any arterial line catheterizations independently.

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Pain is minimized by local anesthesia. Infection is avoided by adequate cleansing of the skin prior to 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 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. All medical staff at the PET center (RNs and MDs) are ACLS-certified and immediately available for urgent or emergency responses. In such a case that a subject would require further evaluation or stabilization, 911 would be called and the subject would 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.

To minimize the risks associated with radiation we will ensure that the radiation exposure in each subject does not exceed Federal guidelines. The dose of radiation will be submitted for approval to the Yale-New Haven Hospital Radiation Safety Committee (RSC) and also reviewed by the Yale University Radiation Safety Committee (YU-RSC). All scans will be done in the presence of medical supervision and trained nursing staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the Yale University PET scan facilities have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiopharmaceuticals and execution of PET scans will be performed 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 radionuclides. 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.

To minimize risks associated with MRI, 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 will be eligible for the study. In addition, subjects will remove all metal (watch, hair pins, jewelry) and change into scrubs immediately prior to the study and pass 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.

To minimize risks associated with pregnancy and breastfeeding we will not enroll women who are pregnant or breast-feeding.

To minimize risks associated with LPS administration we are administering a dose (1 ng/kg) that is 1/4 of the maximum dose (4 ng/kg) that can safely be used in human subjects research. Because we will enroll only physically healthy subjects under the age of 55 who have no cardiovascular problems and no history of vasovagal syncope, we do not expect that any subject will experience a serious adverse event. Subjects will be screened for a history of fainting or vasovagal syncope, and will be adequately hydrated prior to and during LPS administration. These measures are used to reduce the already very low odds of cardiovascular adverse events

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.)

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a. What is the investigator's assessment of the overall risk level for subjects participating in this study? The investigator views this as overall moderate risk for participation.
b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? No children will be enrolled in to this study.
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

DATA SAFETY MONITORING PLAN

Although this study qualifies as clinical research, it does not meet NIH criteria for Phase III clinical trial research. Nonetheless, this study will be monitored every 6 months by a Data Safety Monitoring Board (DSMB). Adequate surveillance and protections will be put in place to discover adverse events promptly and keep their effects to a minimum. There is an established DSMB at the Yale PET Center that meets every 6 months.

Expected and Unexpected Adverse Events will be recorded according to PET Center and NIH guidelines. Should an unexpected or serious AE occur, the PI will be notified immediately and appropriate report forms will be completed. AE's will be captured either in paper form or through digital RedCap forms.

Attribution of Adverse Events.

We will use the Yale University School of Medicine – Human Investigation Committee recommended guidelines for assessing attribution adverse events.

Attribution of Risk Categories:

Definite:	Adverse event(s) will clearly be related to investigational agent(s) or other intervention
Probable:	Adverse event(s) will likely be related to investigational agent(s)
Possible:	Adverse event(s) may be related to investigational agent(s)
Unlikely:	Adverse event(s) will doubtfully be related to investigational agent(s)
Unrelated:	Adverse event(s) will clearly not be related to the investigational agents(s)

Plans for Grading Adverse Event.

For grading the severity of adverse events noted during the study we will adopt a general scheme from FDA publication *Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (FDA, Center for Biologics Evaluation and Research, September 2007):*

Grades of Risk:

0	No adverse event or within normal limits
1	Mild adverse event: Does not interfere with activity
2	Moderate adverse event: Some interference with activity not requiring medical intervention
3	Severe adverse event: Prevents daily activity and requires medical intervention
4	Potentially Life-threatening or disabling adverse event: Results in an ER visit or hospitalization

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5 Fatal adverse event

Mild (Grade 1)	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

1- For most of the anticipated adverse effects we will use this guidance and parameters set:

For arterial cannulation:

	Mild (Grade 1)	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 48hours or interferes with activity	Required use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness *	2.5 - 5 cm	5.1 - 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	2.5 - 5 cm and does not interfere with activity	5.1 - 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

For vital signs:

Vital Signs *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Fever (°C) ** (°F) *	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	Increased by >30% baseline beats per minute and then	Increased by 40% over baseline and then resolving over 15-30	Increase of >40% over baseline for greater than 30	HR >200 for >2 min, ER visit, or hospitalization

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	resolving over 15-30 Minutes	minutes	minutes	
Bradycardia - beats per minute***	Decreased by >30% baseline beats per minute and then resolving over 15-30 minutes	Decreased by 40% over baseline and then resolving over 15-30 minutes	Decreased of >40% over baseline for greater than 30 minutes	ER visit or hospitalization
Hypotension (systolic) – mm Hg	85 – 89 resolving in less than 5 minutes	80 – 84 resolving in less than 5 minutes	< 80 not resolving after 5 minutes	ER visit or hospitalization for hypotensive shock

* Subject should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.
*** When resting heart rate is between 60 - 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

For systemic symptoms:

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Nausea/vomiting	No interference with activity or 1 - 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity	ER visit or hospitalization for hypotensive shock
Diarrhea	2 - 3 loose stools in 24 hours	4 - 5 stools in 24 hours	6 or more watery stools in 24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Prevents daily activity; required prescription of narcotic pain reliever	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Prevents daily activity	ER visit or hospitalization

Serum for Screening *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6

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Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 140 126 – 200	 >140 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23-26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
White blood cell count	1.1 x ULN	1.2-1.3 x ULN	1.4 – 1.9 x ULN	Greater than 2 if accompanied by >60% neutrophils of immature white cells
HgB	10-13.2 g/dl	8-10 g/dl	6.5-8 g/dl	<6.5 g/dl
Platelet count	100000 - 140000	70000-100000	50000 -70000	< 50000

***collected for screening purposes and subjects with grade 3 or higher ranges will not be considered for the study.**
****grade 3 and below will not be considered reportable events.**

STUDY STOPPING CRITERIA

a. >1 SAE at least possibly related to endotoxin administration
b. >2 Grade 3 (severe) adverse events at least possibly related to endotoxin administration, based on appropriate grading scale (e.g., Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials)

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Plans for Reporting Serious Anticipated and Unanticipated Adverse Events.
Serious anticipated adverse events occurring with a greater frequency than expected, and unanticipated adverse events that are possibly, probably, or definitely related to study procedures will be reported.

All SAEs will result in the completion of an SAE Form and a verbal report within one hour to the corresponding Principal Investigator (Dr. Cosgrove). Within 24 hours, the following additional individuals will be informed: 1) the Co-PI and all Co-investigators; 2) the HIC; 3) the DSMB; 4) and NIMH. The Principal Investigator (Kelly Cosgrove, Ph.D.) will assume full responsibility for reporting serious adverse events. All of these individuals will receive a copy of the SAE Form at which point a decision will be made whether to convene a meeting of the DSMB. The DSMB and principal investigator will evaluate the adverse event and determine whether the adverse event affects the Risk/Benefit ratio of the study and whether modifications to the protocol (at Risks to Participants, or in Procedures) or consent form (at Risks and Inconveniences) are required.

The procedures for SAE reporting include written documentation using the clinical notes related to the adverse event and specific forms detailing the event with a sign-off by all appropriate supervisory personnel. Communication of recommendations and decisions from all parties (DSMB, Yale Human Investigations Committee) are made back to the investigator in a timely manner. We will report all protocol amendments or changes in the informed consent form to NIMH as well as any temporary or permanent suspension of patient accrual.

For Data and Safety Monitoring Plan templates, see
http://www.yale.edu/hrpp/forms-templates/biomedical.html

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

Study Hold Criteria: The following is criteria that will be used to halt enrollment or further participation of enrolled subjects pending safety review based on: the number of adverse events considered possibly probably or definitely related to endotoxin administration. ≥ 2 grade 3 adverse events, > 1 grade 4 clinical or laboratory adverse event, or 1 grade 5 adverse event [
.

13. **Statistical Considerations:** Describe the statistical analyses that support the study design.
Frequency distributions and descriptive statistics for all variables will be computed prior to conducting analyses. Should Shapiro-Wilk normality tests indicate non-normality for any continuous variable, we will compute necessary

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data transformations (e.g., logarithmic base 10) prior to conducting analyses. Preliminary data revealed large (i.e., r's>.80) magnitude correlations among [^{11}C]PBR28 V_T values in all of the brain regions assessed. Thus, we will conduct a principal components analysis with oblique (promax) rotation of [^{11}C]PBR28 V_T values in all brain regions assessed to calculate a global composite measure of [^{11}C]PBR28 V_T values.

A univariate ANCOVA will be used to compare global [^{11}C]PBR28 V_T values between trauma-exposed controls and individuals with PTSD. Group (Control vs. PTSD) and rs6971 genotype on the TSPO gene will be entered as fixed factors(102), variables that differ between Control and PTSD groups as additional fixed factors (categorical variables *such as major depressive disorder*) or covariates (continuous variables *such as time since index trauma*), and global [^{11}C]PBR28 V_T values as the dependent variable. A post-hoc multivariate ANCOVA will be conducted with the same independent variables and [^{11}C]PBR28 V_T values in individual brain regions entered as dependent variables; α will be set to 0.005 (0.05/10 brain regions) in this analysis to control for Type I error. To evaluate the relation between continuously distributed global [^{11}C]PBR28 V_T values and measures of post-traumatic threat and loss symptoms, and neurocognitive functioning, we will conduct a linear regression model using Mplus version 7.31(124). In this analysis, global [^{11}C]PBR28 V_T values will be modeled as the independent variable, and continuously-distributed scores on objective endophenotypic measures of threat and loss symptoms, and neurocognitive functioning, and clinician interview-based measures of threat and loss symptoms will be modeled as dependent variables. Should correlation analyses reveal large magnitude associations (i.e., r ≥ 0.70) among variables assessing a common construct (e.g., dot probe and clinician-assessed threat symptoms), observed indicator variables will be modeled and analyzed as latent variables.

Linear mixed-effects models will be used to compare LPS-induced changes in [^{11}C]PBR28 V_T values in the Trauma Control and PTSD groups. Group (Control vs. PTSD) and rs6971 polymorphism in the TSPO gene will be entered as fixed factors, subject as a random effect, pre-LPS global [^{11}C]PBR28 V_T values as a covariate, and post-LPS global [^{11}C]PBR28 V_T values as the dependent variable; variables that differ between Trauma Control and PTSD groups will be entered as additional fixed factors (categorical variables) or covariates (continuous variables). An ANCOVA with the rs6971 polymorphism in the TSPO gene and other variables that differ by PTSD status entered as covariates will be conducted to compare %change in [^{11}C]PBR28 V_T values in the PTSD vs. Trauma Control group. Post-hoc analyses adjusted for multiple comparisons (α=0.005; 0.05/10 brain regions) will be conducted to evaluate LPS-induced changes in [^{11}C]PBR28 V_T values in individual brain regions in these two groups. Interrelationships among changes in outcome measures will be evaluated in the full sample and by PTSD group status using parallel process analyses in Mplus version 7.31.

Partial correlations adjusted for the rs6971 polymorphism will be conducted to examine associations between baseline and LPS-induced measures of global [^{11}C]PBR28 V_T values, peripheral inflammatory markers, and endophenotypic and phenotypic measures of threat, loss, and neurocognitive function. Associated peripheral inflammatory markers will then be entered into a bias-corrected bootstrap test of mediation to evaluate whether global [^{11}C]PBR28 V_T values mediate the relation between peripheral inflammatory markers, and measures of threat and loss symptoms, and neurocognitive function. A parallel set of tests of mediation will be conducted to examine whether LPS-induced increases in activated microglia mediate the relation between LPS-induced increases in peripheral inflammatory markers and the measures of threat and loss symptoms, and neurocognitive function. In this analysis, LPS-induced changes in peripheral inflammatory markers will be modeled as the independent variable, percent change in global [^{11}C]PBR28 V_T values as the mediator variable, and LPS-induced changes in threat and loss symptoms and neurocognitive function as dependent variables. These tests employ a non-parametric bootstrapping procedure (10,000 samples) to generate estimates of direct and indirect effects, and confidence intervals(125-127).

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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 or *N*-acetyl-*N*-(2-[^{11}C]methoxybenzyl)-2-phenoxy-5-pyridinamine**

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)

1a. Name of the radiotracer: **[^{18}F]FDG or [^{18}F]2-Fluro-deoxyglucose (2-deoxy-2-[^{18}F]fluoro-D-glucose**

1b. Is the radiotracer FDA approved? ☒**YES** ☐**NO**

B. DRUGS/BIOLOGICS ☐**N/A**

Endotoxin (lipopolysaccharide) is an experimental drug used under IND # 13598
Lots: 94332B1 and 94332B6

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)*:

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.

   PET tracer: [^{18}F]FDG tracer will be administered i.v. in doses of 10 mCi or less

   PET tracer: [^{11}C]PBR28 tracer will be administered i.v. in doses of 20 mCi or less for aim 4, doses of 18mci or less for aims 1, 2, and 5.

   Endotoxin
   In 1976, endotoxin derived from *E. coli* (group O 113:H10:K negative) was processed to serve as a national standard for experimental studies of inflammation in humans. When this lot was depleted in 1998, the NIH Clinical Center in conjunction with FDA processed, under good-manufacturing-practice guidelines, endotoxin derived from the original bulk material extracted from *E. coli* O:113. This Clinical Center Reference

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Endotoxin (CCRE) has been used in multiple human research studies since then. CCRE is an investigational drug, and the PI has been allowed by FDA to use it in humans under an IND (BB IND#13598). Each vial produced by List Labs, when reconstituted with 0.5 ml water, contains 1 microgram of endotoxin in 1.0% lactose, 0.1% PEG6000.This product has been manufactured according to cGMP using procedures compliant with 21 CFR 211. All specifications and lot disposition requirements have been met.This product is supplied as lyophilized powder, aseptically filled into depyrogenated, sterile, 2 ml glass vials and sealed under vacuum. It is recommended that this material be stored at 2 - 8°C prior to and after reconstitution. Prior to using LPS, vortex for 15 minutes after reconstitution with sterile water for injectionVials should be stored refrigerated and reconstituted in 5 cc of Sterile Water for Injection at the time of use. CCRE is administered intravenously or by lung instillation through bronchoscopy. The most commonly used dose is 2-4 ng/kg body weight by IV bolus. Preparation of the standardized CCRE will be performed by our clinical research pharmacist.

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

[11C]PBR28 Radiotracer will be synthesized and radiolabeled by the Yale University PET Center under the supervision of Drs. Henry Huang and Nabeel Nabulsi.

Endotoxin (CCRE) will be provided by the List Labs and shipped to the Investigational Drug Pharmacy.

[^{18}F]FDG will be produced and shipped to the Yale University PET Center by an approved vendor.

a) Is the drug provided free of charge to subjects? ☒**YES** ☐**NO**
If yes, by whom? *Write here*

1. **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.

Due to the short half-life, PET drugs are prepared 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.

The preparation of sterile PET drug products is validated prior to human use. Sterility is achieved by passing the PET drug product through a 0.22 micron membrane filter during the last step in 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 after administration for further confirmation.

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

[^{11}C]PBR28
[^{11}C]PBR28 will be prepared at the Yale PET Center in accordance with procedures and quality specifications described in Yale IND # 123984.Briefly, [^{11}C]PBR28 is radiolabeled by *O*-methylation of the phenolic group of the *O*-desmethyl-precursor with [^{11}C]methyl iodide or [^{11}C]methyl triflate. The PET drug is purified first by semi-preparative HPLC, and then followed by solid-phase extraction to remove the HPLC buffer mixture. Finally, the

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PET drug is formulated in <10% ethanolic saline solution, and the resulting PET drug product is finally passed through a 0.22 micron membrane filter for terminal sterilization.

FDG

[^{18}F]FDG will be produced and shipped to the Yale University PET Center by an approved vendor and will be administered immediately upon receipt at the Yale PET Center.

Endotoxin

Preparation and storage of CCRE will be done by the YNHH IDS pharmacy, according to the recommendations by the NIH Clinical Center and List Labs. CCRE is inherently pyrogenic and will therefore not be tested for 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.*

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

3. **Continuation of Drug Therapy After Study Closure ☒Not applicable to this project**

B. DEVICES ☒N/A

SECTION III: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. Targeted Enrollment: Give the number of subjects:

a. Targeted for enrollment at Yale for this protocol: 160 total

b. If this is a multi-site study, give the total number of subjects targeted across all sites: *Write here*

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

☒ Flyers	☒ Internet/web postings	☒ Radio
☐ Posters	☐ Mass email solicitation	☐ Telephone
☐ Letter	☒ Departmental/Center website	☒ Television
☐ Medical record review*	☐ Departmental/Center research boards	☒ Newspaper
☒ Departmental/Center newsletters	☐ Web-based clinical trial registries	☐ Clinicaltrails.gov
☐ YCCI Recruitment database	☒ Social Media (Twitter/Facebook):	
☐ Other:		

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

3. Recruitment Procedures:

a. Describe how potential subjects will be identified. *Write here*

b. Describe how potential subjects are contacted. *Write here*

c. Who is recruiting potential subjects? *Write here*

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Subjects will be recruited through flyers and business cards, public advertisement (newspaper, radio, internet posting), by word of mouth, contact with community service groups, and clinics and local treatment facilities (the VA Hospital, West Haven, CMHC, the Yale Psychiatric Hospital, Mood Disorders Research Program, the Yale Depression Research Program, CTNA). The subjects will be asked to call us if they are interested in participating in the research study. Trained RA's, under direct supervision of the PI, are responsible for subject recruitment.

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: We post advertisements in which subjects are asked to call us if they are interested in participating in the studies. When the subjects call, we need to obtain their name, DOB, phone number, etc, in order to contact them regarding eligibility to participate/schedule further appointments, assess study eligibility correctly. Some subjects prefer we email them appointments and directions; thus we need to obtain their email address. We also need to obtain their city of residence to ensure they are within appropriate distance to arrive to all appointments in a timely fashion and to provide accurate directions to our labs.

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.
   Potential subjects will initially be screened over the telephone by one of the research staff. The study will be described to individuals who appear to satisfy the study criteria. During an intake appointment, the nature

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of the project, the procedures, the relative risks and benefits, and the alternatives to participation in the project will be discussed with the subject. If, following this discussion, the subject continues to be interested in the project; written informed consent will be obtained. The decision not to participate will not affect an individual's eligibility to participate in future studies, to receive treatment at the Connecticut Mental Health Center, or to receive treatment on a private basis from a referring clinician. A copy of the signed consent form will be provided to all participating subjects.

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.
   In cases in which capacity is in doubt, the PI will assess the subject's understanding of the study and the subject's capacity to decide to participate.

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 speaking subjects will not be invited to participate in the studies. All of our materials are in English only, and staff members are fluent in English. Furthermore, cognitive testing is validated in English-speaking subjects only.

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

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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.

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

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?

   All staff members that come into contact with the data are fully trained to the current HIPAA regulations and are informed as to the proper use of all data. Identifiable paper information is kept in locked file drawers and password protected computer files. Results are published as group data without the use of characteristics that would identify individual subjects. We quote information only by number in conference discussions, scientific reports, or publications, in order to maintain anonymity. Identifiable research data, including recruitment and screening information and code keys, are stored on a secure database located on the internal PET Center Network. The PET network is protected by a Cisco PIX firewall operated by ITS. All research data are backed up nightly to a Dell PV-136T library wit 4 IBM Ultrium-TD2 tape drives using the backup software Legato Networker 7.3 from EMC. Human subjects enrolled in the study are assigned a subject-specific random identifier. Subject identifiers and the means to link the subject names and codes with the research data are stored in separate locations within the database. The software of the database limits the ability to connect the random identifier to the actual subject identification information to research team members only. Access to the database is password protected and each research team member is required to have a unique ID and password to gain access to the database. Authorized users employ their netid and authentication is performed using Yale's central authentication server. Users always access research data through the random identifier only. Direct identifiers belonging to subjects who withdraw from the study, will be stripped from the key.

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 authorized personnel.

6. If appropriate, has a Certificate of Confidentiality been obtained? *NA*

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.)

There is no direct benefit for participation in this study. All subjects in this study may derive subjective benefit from volunteering to take part in a study for the advancement of scientific knowledge. Subjects with PTSD could benefit from future treatments developed based on the results of this study. Psychiatric and physical examinations are also potential benefits. Because this study may not have direct benefit to individual subjects, they will be remunerated for time spent in PET and MR imaging sessions. The potential benefits for individuals and society at large are great; and the risk/benefit ratio appears favorable.

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 (see attached Consent Form).

2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.
   The subjects will be compensated for their time commitment and inconveniences necessary for completing the study. Subjects will have no financial responsibilities for any portion of the study. Subjects may have the opportunity to receive approximately $1,210 ($1,610 the event a scan cancels): They may receive up to $50 for baseline assessments ($40 cog testing, $10 dot probe), $350 for each PET scan and $50 for each arterial line placement on PET scan day, $50 for the MRI, $150 for endotoxin administration, and a $100 bonus for completing the study. Subjects who participate in the Probabilistic Reward Task may also be compensated for the amount that they “win” during the task up to $50, $10 for cold pressor task. 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. Subjects will be provided with a light meal on PET imaging day and may be provided and/or reimbursed for meals during inpatient stay. Reasonable transportation costs will be reimbursed. Receipts must be submitted. If participation in the PET scan has already begun, then compensation will be based on involvement in the study, and will be up to the discretion of the PI. Cancellations: If a PET scan should get cancelled 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.

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

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.

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** ☒

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

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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.**

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APPROVED BY THE YALE UNIVERSITY IRB 12/20/2022

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