Title: Imaging Synaptic Density in Cocaine and Opiate Addiction In Vivo Using 11UCB-J PET

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HRP-503B – BIOMEDICAL RESEARCH PROTOCOL
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Protocol Title: Imaging Synaptic Density in Cocaine and Opiate Addiction In Vivo using 11C-UCB-J PET

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Version Date: August 2022

(*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.

Exploratory/Developmental Aim: To measure synaptic density in the brains (including in ventral striatum [VS] and medial prefrontal cortex [mPFC]) of abstinent subjects with Cocaine Use Disorder (CUD) or Opiate Use Disorder (OUD) as compared to healthy control (HC) subjects using 11C-UCB-J PET. Thirty CUD, 30 OUD and 30 HC subjects will undergo a single 11C-UCB-J (also known as 11C-APP311) PET scan. CUD and OUD subjects will be studied during states of urine toxicology confirmed abstinence (2 weeks), times at which preclinical (i.e., rodent) studies have demonstrated enduring (albeit opposite) changes in dendritic spine density in both medium spiny (NAc) and pyramidal (mPFC) cell neurons. Thus, we hypothesize that synaptic density will be increased in NAc and mPFC of CUD and decreased in NAc and mPFC of OUD as compared to HC subjects.

This would be the very first research study to image synaptic density in human cocaine and opiate users, thereby testing whether altered synaptic (i.e., dendritic spine) density in the rodent brain is recapitulated in CUD and OUD humans. If confirmed, the current study would provide compelling clinical-translational support for an important pathophysiological mechanism of addiction – aberrant structural synaptic plasticity. As such, the current study has considerable potential for advancing our neurobiological understanding of human cocaine and opiate addiction.

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

   6 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.

APPROVED BY THE YALE UNIVERSITY IRB 9/21/2022

3A. Background: Beyond their transiently intoxicating effects, drugs of abuse produce, in vulnerable individuals, sustained changes in behavior that persist for periods far beyond acute intoxication and withdrawal. With repeated drug use, initially flexible, goal-directed, and adaptive everyday pursuits yield progressively to more restricted, habitual, and maladaptive patterns of compulsive drug seeking and taking. In clinical populations, such behaviors are highly recalcitrant, and for those addicted to cocaine and opiates, highly resistant to available treatments. Even those successfully achieving abstinence face the risk of relapse for years following discontinuation of these drugs.

A major goal of modern addiction research is to understand the neural adaptations that underlie these enduring, drug-induced behaviors – ones so seemingly 'hard-wired' in those suffering from the disorder. Considerable optimism exists in this regard, as powerful preclinical tools and well-established animal models have dramatically advanced our understanding of the molecular and cellular mechanisms of cocaine and opiate-related plasticity (Nestler, 2001). Nonetheless, a considerable gap remains in the clinical-translational testing and validation of such preclinical findings in human cocaine and opiate addiction.

3B. Cocaine and Opiate Effects on Synaptic Structure/Connectivity: Preclinical Evidence: In seminal studies nearly 20 years ago, Robinson & Kolb (Robinson, Gorny, Savage, & Kolb, 2002; Robinson & Kolb, 1997, 1999) hypothesized that addiction-related behaviors (e.g., compulsive drug taking, craving, and the persistent vulnerability to relapse despite sustained abstinence) might result from drug-induced adaptations in synaptic structure/connectivity (Robinson & Kolb, 2004). Using sensitizing regimens of experimenter-administered amphetamine or cocaine in rodents (Robinson & Kolb, 1999) as well as self-administered amphetamines or cocaine (Robinson et al., 2001; Robinson & Kolb, 2004), they demonstrated robust and long-lasting (> 1 month) increases in dendritic spines. In contrast, both experimenter- (Robinson et al., 2002; Robinson & Kolb, 1999) and self-administered morphine (Robinson et al., 2002) produced robust and long-lasting (> 1 month) decreases in dendritic spines (by Golgi-staining), post-synaptic markers of the synapse (Greenough & Bailey, 1988; Greenough, Withers, & Wallace, 1990). Both medium spiny neurons (~95% of striatal neurons) in the nucleus accumbens shell (NAc) and pyramidal cells (the principal output neurons) of the medial prefrontal cortex (mPFC) were affected – cell types and brain regions strongly implicated in cocaine and opiate actions. Their results were compelling, and suggested a preclinical model of addiction as aberrant, drug-specific, experience-dependent, structural synaptic plasticity (Robinson & Kolb, 2004).

Since these reports, findings of altered synaptic/dendritic spine density have been replicated by other groups (Spiga et al., 2014; Spiga et al., 2005), in other species (Spiga et al., 2014), with other drugs such as nicotine (increased dendritic spines) (Robinson & Kolb, 2004), cannabinoids (decreased) and alcohol (decreased) (Spiga et al., 2014), and using more modern methods (Russo et al., 2010; Spiga et al., 2014; Spiga et al., 2005). In addition, molecular mechanisms/mediators (e.g., Cdk5, MEF2, NFκB) (Russo et al., 2010) of such plasticity have been identified, and initial progress towards relating structural alterations to relevant behaviors (e.g., sensitization) has been made (Ferrario et al., 2005).Gene expression studies in rodents support the morphological findings, showing downregulation of structural synaptic transcripts (e.g., synaptotagmin, synaptophysin) in NAc during abstinence from chronic opiates (Russo et al., 2010; Spijker et al., 2004). As such, the preclinical data are strong.

Needless to say, complexities exist. First and foremost, it remains unclear why addictive drugs of different classes (e.g., stimulants and opiates) might produce such opposite changes in synapses in the same brain regions/networks. For example, in addition to those above, orbital frontal cortex (OFC), a region clearly implicated in addiction (Volkow & Fowler, 2000), appears to be oppositely regulated by amphetamines/nicotine and morphine with respect to spine density (i.e., decreased by amphetamines/nicotine and increased by morphine) (Robinson et al., 2002) whereas cocaine showed no changes (Robinson & Kolb, 2004). Similarly, debate remains as to the functional nature of such changes (e.g., causal/Hebbian vs. compensatory/ homeostatic), and their relationship to specific drug- and/or addiction-related behaviors/phases (Kobrin et al., 2016; Russo et al., 2010; Spiga et al., 2014).

For instance, the association between changes in brain synaptic density and consequential clinical phenomena, such as changes in pain sensitivity, has never been studied. The prevalence of ongoing pain and its functional interference are increasingly recognized as a treatment target for addiction therapeutics. Opioid and glutamate receptors are densely expressed in brain regions at the crux of pain and reward systems, which are responsible for salience-based decision-making (ventral striatum [VS]) and affective modulation of pain (medial prefrontal cortex [mPFC]) [5-18]. Over time, the binding of drugs — especially opioids — to these mesolimbic brain strucutres may provoke a shift away from classic nociception, towards brain networks engaged in maintaining cognitive and affective pain processes — thereby sustaining a state of unrelenting pain (Baliki, Petre et al. 2012, Hashmi, Baliki et al. 2013, Kucyi and Davis 2015, Vachon-Presseau, Tétreault et al. 2016). Still, biomarkers of pain sensitivity persons with substance use disorders (SUD) remain elusive, hindering timely pain/SUD treatment selection. While aberrant structural synaptic plasticity constitutes a compelling preclinical hypothesis, it is one largely uninformed by clinical data. Thus, studies that attempt to validate these findings in humans and to understand the clinical correlates of such changes across individuals, addiction phases, and disorders are sorely needed.

3C. Aberrant Structural Synaptic Plasticity in Human Cocaine and Opiate Addiction: An Untested Hypothesis: To date, we are not aware of any clinical studies, post-mortem or in vivo, directly examining synaptic density in the cocaine or opiate-addicted brain. As such, basic scientific findings of altered synaptic density remain essentially untested in clinical populations. This stands in stark contrast to a substantial functional (including PET) neuroimaging literature in which preclinical hypotheses of sensitized subcortical dopamine function [25-27] and/or reduced prefrontal cortical metabolism [28, 29] have been successfully tested and either refuted [30, 31] or replicated [19, 20], respectively. Structural magnetic resonance imaging (MRI) studies of cocaine dependent populations have suggested changes/dissociations in striatal (increased) and/or PFC (decreased) gray matter volume in some [32-39] but not all [40-44] studies. Literature also suggests abnormalities in brain blood flow/connectivity (Daglish et al., 2003), metabolism (Galynker et al., 2007; Galynker et al., 2000), dopamine function (Martinez et al., 2012; Shi et al., 2008; Zijlstra, Booij, van den Brink, & Franken, 2008) and changes in opioid receptors/occupancy in current, former and/or opiate-maintained, opiate dependent (OD) subjects (Greenwald et al., 2007; Kling et al., 2000; Zubieta et al., 2000). A limited structural magnetic resonance imaging (MRI) literature of OD populations has suggested reductions in prefrontal cortical grey matter density / volume (Liu et al., 2009; Lyoo et al., 2006). However, such gross anatomic, gray matter measures are highly insensitive to and non-specific for synaptic number/density.

Compellingly, a post-mortem gene expression study (39,000 transcripts) in cocaine and heroin abusers demonstrated directionally consistent increases and decreases, respectively, in nucleus accumbens (NAc) transcript levels for the for synaptic vesicular glycoprotein 2a (SV2a). SV2a is a presynaptic marker of vesicular number (Albertson, Schmidt, Kapatos, & Bannon, 2006) and as we present evidence below, synaptic density. We believe that efforts to test this important preclinical hypothesis of addiction stem primarily from the lack of a suitable clinical-translational tool for measuring human synaptic density in vivo.

3D. SV2a: A Candidate Target for PET Imaging of Synaptic Density: Synaptic vesicle glycoprotein 2 (SV2) is one of several, essential presynaptic vesicular membrane proteins (e.g., including synaptophysin [SYN] and synaptotagmin) that is present in all vertebrate species. SV2 consists of three known isoforms, including SV2a, SV2b and SV2c, all of which are expressed in the rat and human brain (Bajjalieh, Frantz, Weimann, McConnell, & Scheller, 1994; Bajjalieh, Peterson, Linial, & Scheller, 1993; Janz & Sudhof, 1999). However, SV2 isoforms differ dramatically in their brain distribution, and only SV2a is ubiquitously present in presynaptic nerve terminals throughout the brain (exceptions being the trigeminal and facial nuclei) (Bajjalieh et al., 1994; Bajjalieh et al., 1993; Janz & Sudhof, 1999). While its precise role in synaptic function is still poorly understood (Mendoza-Torreblanca, Vanoye-Carlo, Phillips-Farfan, Carmona-Aparicio, & Gomez-Lira, 2013), SV2a does not appear to play a role in vesicle formation, as vesicular density and synaptic morphology are unaltered in SV2a knockout animals (Crowder et al., 1999; Morgans et al., 2009) (i.e., suggesting that it may be involved in synaptic vesicle cycling, a continuous process within nerve terminals) (Haucke, Neher, & Sigrist, 2011). That being said, SV2a is of critical importance for normal synaptic functioning, as demonstrated by the occurrence of spontaneous seizures/death in SV2a -/- knock-out mice (Crowder et al., 1999; Janz, Goda, Geppert, Missler, & Sudhof, 1999). In fact, considerable clinical therapeutic interest in SV2a has been generated by recognition

of its pathophysiological importance for epilepsy (Kaminski, Gillard, & Klitgaard, 2012) and identification of SV2a as the molecular target of the anticonvulsant medication, levetiracetam (Keppra®) (Mendoza-Torreblanca et al., 2013).

Beyond its specific pathophysiological relevance for epilepsy, we hypothesized that PET imaging of SV2a might provide a novel approach to the study of synaptic density / plasticity more generally, including in other neurobiological disorders such as addiction. For these reasons, we sought to develop an appropriately selective SV2a radiotracer with optimal imaging properties that might be tested for such purposes.

3E. Development of 11C UCB J: A PET Imaging Biomarker of Synaptic Density: Recently, compounds based on levetiracetam (LEV) have been developed with appropriate SV2a affinity and selectivity for PET imaging (Mercier et al., 2014). Syntheses for favorable candidates, including 11C UCB A, 18F UCB-H, and 11C UCB-J (12, 21, and 23, respectively, in (Mercier et al., 2014)) were implemented at Yale and tested in nonhuman primates (NHP). Despite initial positive reports in mini-pigs/rodents (Estrada, Thibblin, Johansen, & al, 2014; Lubberink, Estrada, Thibblin, & al, 2014), 11C UCB A showed unfavorable (slow) brain kinetics (unpublished data). Although 18F UCB-H displayed better kinetics in rodents/NHP (Bretin et al., 2015a, 2015b; Warnock et al., 2014; Zheng, Holden, & Nabulsi, 2014) and reasonable dosimetry in humans (Bretin et al., 2015b), low specific binding and radiosynthetic challenges made it less than optimal. In contrast, our characterizations of 11C UCB-J showed it to possess superior PET imaging properties, including 1) reliable radiochemical synthesis (via the Suzuki–Miyaura cross-coupling method) at high (>98%) purity; 2) high in vitro potency (7 nM) and selectivity for SV2a in humans (> 10x/100x vs. SV2b/c, respectively and negligible affinity for other receptors tested); 3) rapid kinetics and high tracer uptake in rodents/NHP; 4) high levels of specific binding (>90% blockade by 30 mg/kg LEV) in gray matter regions (VT = 22-55 mL/cm3); 5) reliable in vivo quantitation of SV2a volume of distributions (VT) by 1T compartmental modeling; and 6) favorable dosimetry estimates for human studies, based on NHP data (Nabulsi et al., 2016), as well as human whole-body dosimetry data (Bini, et al., 2020).

Given 11C-UCB-J's favorable imaging properties, we have conducted studies (now published) (Finnema et al., 2016) validating its use as a marker of synaptic density in primate brain (see Preliminary Data). More specifically, we directly compared in vivo 11C-UCB-J uptake (VT) to ex vivo measures of both SV2a and an established, 'gold-standard,' in vitro marker of synaptic number (synaptophysin) (Finnema et al., 2016). Furthermore, we have conducted first-in-human demonstrations of the tracer's superior imaging properties, test-retest reliability and sensitivity to synaptic loss (i.e., in patients with temporal lobe epilepsy). Thus, 11C-UCB-J PET constitutes the first valid clinical translational tool for measuring synaptic density in the living human brain. As such, we believe it represents a methodological breakthrough for examining structural plasticity at the synaptic level in clinically addicted populations.

Preliminary Data:
Validation of 11C-UCB-J as a Marker of Synaptic Density in Non-Human Primate Brain: 11C-UCB-J PET scanning, western blot analysis and confocal microscopy were performed in an olive baboon (Papio anubis) to compare brain levels of in vivo and in vitro SV2a with those of another an established (i.e., "gold standard") in vitro marker of synaptic density, synaptophysin (SYN) (Finnema et al., 2016). During PET, 11C-UCB-J uptake was rapid, with highest concentrations in cortex (standardized uptake values, or SUVs = 10-15) and lowest in white matter (e.g., centrum semiovale; SUV ≤ 5) (Figure 1A-B). Regional measures of equilibrium binding (VT) were calculated using a one-tissue (1T) compartment model as previously described (Nabulsi et al., 2016). After 11C-UCB-J PET scanning, the animal was euthanized, brain harvested, and 12 brain regions dissected for in vitro correlations between SV2a and SYN, as well as their comparison with in vivo PET measures of SV2a availability (VT).

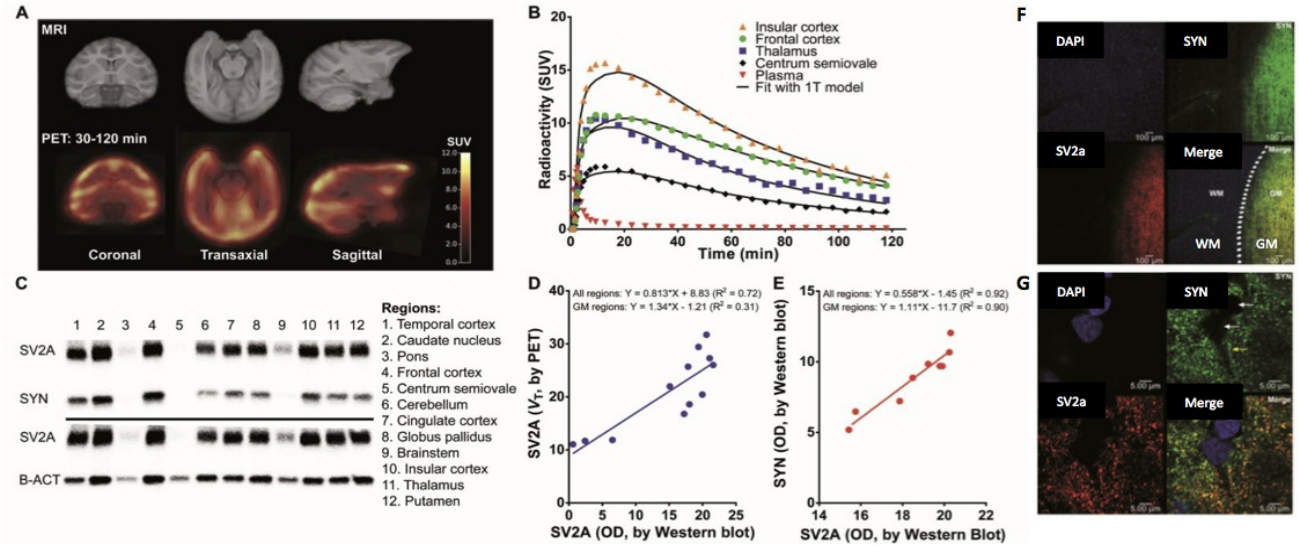

Figure 1. Validation of ^{11}C-UCB-J as a marker of synaptic density in the primate (baboon) brain

Western blotting used selective antibodies for SV2a, SYN, and the housekeeping protein beta-actin (β-ACT). We found clear SV2a and SYN signal in all gray-matter regions, but not in the centrum semiovale (CS) (Figure 1C). The in vitro regional distribution of SV2a (optical density; noted OD in figure) correlated well with PET measures of 11C UCB J VT (R2=0.72) (Figure 1D). Importantly, western blot results also demonstrated a strong linear correlation (R2=0.92) between SV2a and SYN across the brain regions examined (Figure 1E). Confocal microscopic imaging confirmed selective localization of SYN/SV2a to gray matter (GM) (Figure 1F) and neuropil surrounding cell bodies/dendrites (but not DAPI-labeled cell bodies/nuclei) (Figure 1G). Together, these data support the validity of 11C-UCB-J PET imaging for the quantification of synaptic density in vivo (Finnema et al., 2016).

Evaluation of 11C-UCB-J in humans with PET: Five healthy subjects (37±13 yrs, 4M/1F) underwent two 11C-UCB-J PET scans on the same day after bolus tracer injection with full radiometabolite analysis (Finnema et al., 2016). Arterial blood samples were collected for measurement of radioactivity in blood/plasma. Metabolism of 11C-UCB-J was measured at 3, 8, 15, 30, 60 and 90 min post-injection using a modified automatic column-switching HPLC method (Hilton et al., 2000). The free fraction of 11C -UCB-J in plasma (fp) was measured in triplicate with an ultrafiltration method (Millipore Centrifree micropartition device, 4104, Billerica, MA, USA) using arterial blood taken immediately before radioligand injection (Gandelman, Baldwin, Zoghbi, Zea-Ponce, & Innis, 1994). Blood analysis indicated that 11C-UCB-J was metabolized at a moderate pace, with parent fraction of 27±8% at 60 min post-injection and fp of 32±1%.

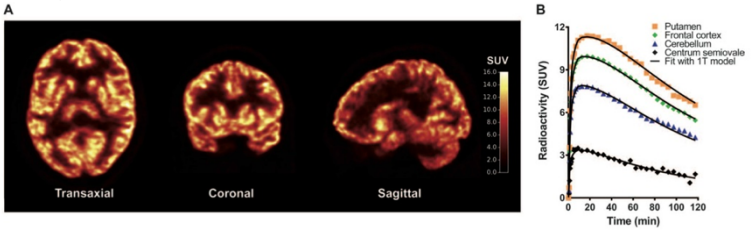

Figure 2. ^{11}C-UCB-J PET Imaging in a Healthy Human Subject

As in the baboon, 11C-UCB-J showed excellent signal-to-noise and favorable kinetics in humans (Figure 2A & B). Radioactivity concentration in brain was high (SUV = 7-11) and homogenous in GM regions. In contrast, as predicted, radioactivity was significantly lower in regions devoid of neuronal synapses (i.e., white matter [WM] / centrum semiovale [CS]; SUV ≤ 3). Regional time-activity curves (TACs) demonstrated rapid entry into the brain and fast clearance kinetics (Figure 2B), suggesting its suitability for kinetic modeling of equilibrium binding.

11C-UCB-J Analysis in Humans: Kinetic Modeling and Test-Retest Reliability: Kinetic modeling showed that regional TACs (Figure 2B) were well described by the 1TC model and that fitting was not improved with the 2TC model. VT values were highest in the striatum (22.5±2.0) and cortex (18.4±2.4 in frontal) and lowest in the WM/CS (5.2±0.5). Percent coefficient of variation was quite low for VT, (9 & 13% for striatum and frontal cortex, respectively) consistent with low inter-subject variability in controls. Furthermore, the test-retest variability of 11C-UCB-J was very low (i.e., exceptional), with mean % differences in VT between test and retest scans ranging from -2% to +2% across regions (and mean absolute differences £4% for all brain regions studied).

PET Imaging of Synaptic Loss in Epilepsy using 11C-UCB-J: To evaluate the sensitivity of 11C-UCB-J to synaptic loss, 3 males with temporal lobe epilepsy/mesial temporal sclerosis (52 ± 6 yrs) were scanned. Binding potential (BPND) maps were generated using the simplified reference tissue model 2 using WM/CS as a reference region. Representative PET/MRI images from a single epilepsy subject are depicted (Figure 3A). Regional 11C-UCB-J uptake was decreased (per elevated asymmetry indices) in a region-specific fashion in hippocampus and amygdala (but not fusiform gyrus) of epilepsy patients (ipsilateral/affected vs. contralateral/unaffected lobe; purple circles; N=3) but not controls (right vs. left; orange circles) (Figure 3B).

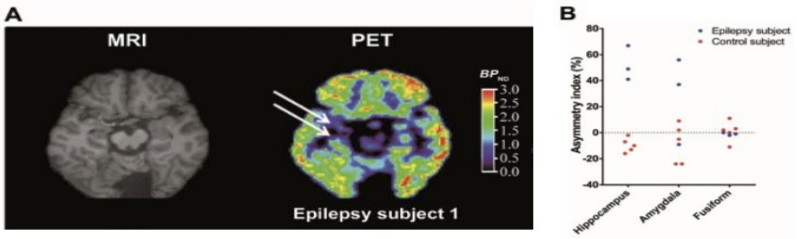

Figure 3. ^{11}C-UCB-J PET Imaging of Synaptic Loss in a Patient with Temporal Lobe Epilepsy

PET Imaging Experience in Substance Dependence at Yale: Our group has >20 years of experience in patient-oriented, clinical-translational study of the neurobiology/genetics of drug dependence. We have a well-established track-record of functional (SPECT & PET) brain imaging of receptors/ neurochemistry (e.g., D2, D3 and 5HT1B receptors; DA, 5HT, and NE transporters, and DA release) (Ding et al., 2010; Gallezot et al., 2014; R. Malison et al., 1998; R. Malison et al., 1995; R. T. Malison, Mechanic, Klummp, & al., 1999; D. Matuskey et al., 2014b; D Matuskey et al., 2014; Matuskey et al., 2011). A majority of this work has involved inpatient study designs requiring extended inpatient stays of 2-8 weeks (Kalayasiri et al., 2007; Lynch et al., 2006; R. Malison et al., 1998; P. Morgan, Pace-Schott, Pittman, Stickgold, & Malison, 2010; P. T. Morgan et al., 2008). Thus, we believe that our expertise in PET imaging of addicted populations not only makes the proposed studies immensely feasible, but also makes us ideally suited to the proposed work.

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.

4A. General Design / Study Overview: A total of 90 subjects will be studied, including 30 with Cocaine Use Disorder (CUD), 30 with Opiate Use Disorder (OUD) and 30 healthy controls (HC) matched for age, race, sex, cannabis, alcohol, and tobacco use (by self-report and the Fagerstrom Test for Nicotine Dependence; FTND) (Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991). All subjects (N=30 CUD, 30 OUD and 30 HC) will undergo a single 11C-UCB-J PET scan with arterial sampling / full radiometabolite analysis to obtain measures of synaptic density (VT and BPND). Structural magnetic resonance imaging (MRI) scans will also be obtained for anatomical registration/partial volume correction. Finally, subjects will participate in a battery of behavioral assessments for exploratory correlations with regional 11C-UCB-J measures of synaptic density. Inpatient subjects who smoke cigarettes will have the option of using nicotine gum and/or nicotine patch while on the unit in order to prevent or minimize nicotine withdrawal.

CUD subjects will be studied as inpatients after 2 weeks of verified (per urine toxicology testing) cocaine abstinence.

OUD subjects will be studied as either inpatients or outpatients. The main difference being that the outpatient route will be offered to participants who are currently undergoing or have recently completed detoxification in another clinical setting, refuse medication assisted options or are not interested in pharmacological detoxification. Additionally, subjects who are treated with buprenorphine/methadone as part of their standard of care will also be included. The PI will work closely with the subject's outpatient prescribers to optimize the doses of buprenorphine/methadone which will be done as the standard of care, to help the subject stay abstinent.

OUD inpatient subjects will be studied after 1-5 weeks of abstinence, verified per urine toxicology testing while on the unit. OUD outpatient subjects will be studied after 1-5 weeks of abstinence verified per outpatient urine toxicology testing and completion of the substance use calendar, 3x week until the scheduled 11C-UCB-J PET scan. The length of the abstinence period will mainly be determined by PET scan availability.

Healthy controls will be studied as outpatient subjects.

4B. Cocaine Use Disorder (CUD) and Opiate Use Disorder (OUD) Subjects: We will recruit 30 medically healthy CUD subjects and 30 medically healthy OUD subjects according to the following inclusion/exclusion criteria:
Inclusion Criteria: 1) Age 21-55 years; 2) Voluntary, written, informed consent; 3) Physically healthy by medical history, physical, neurological, ECG and laboratory examinations; 4) DSM-5 criteria for Cocaine Use Disorder or Opiate Use Disorder; 5) Documented evidence (by urine toxicology) of 2 weeks abstinence from cocaine and 1-5 weeks abstinence from abused opiates; 6) For females, a negative serum pregnancy (bHCG) test at screening and admission, and a negative urine pregnancy test (bHCG) on the PET scan day.

Exclusion Criteria: 1) A DSM-5 diagnosis of other moderate to severe substance use disorders (e.g., alcohol, sedative hypnotics), except for nicotine; 2) A diagnosis of schizophrenia or schizoaffective disorder as determined by the Structured Clinical Interview for DSM-5 (SCID-5) (First, Williams, Karg, & Spitzer, 2015), or an acute psychiatric condition requiring intensive outpatient, inpatient, or emergency room care; 3) A history of significant and uncontrolled medical (e.g., cardiovascular, diabetic/metabolic) or neurological (e.g., cerebrovascular, seizures, traumatic brain injury) illness; 4) A history of seizures; 5) Current use of psychotropic and/or potentially psychoactive prescription medications beyond opiate abstinence initiation agents or management of symptoms not meeting threshold for DSM-5 major conditions (i.e., insomnia, anxiety, etc,.)(see table in section 8 for comprehensive list of medications allowed); 6) Medical contraindications to participation in a magnetic resonance (MR) imaging procedure (e.g., ferromagnetic implants/foreign bodies, claustrophobia, cardiac pacemaker, prosthetic valve, otologic implant, etc.) as recorded on the MR safety sheet; 7) For females, physical or laboratory (bHCG) evidence of pregnancy; 8) PTT and PT/INR lab results not appropriate for arterial line placement; 9) History of a bleeding disorder or are currently taking anticoagulants (such as Coumadin, Heparin, Pradaxa, Xarelto); 10) Participation in other research studies involving ionizing radiation within one year of the PET scans that would cause the subject to exceed the yearly dose limits.

4C. Healthy control (HC) subjects: We will recruit 30 age- (± 5 yrs), race-, sex- cannabis, alcohol, and tobacco use-matched HC subjects (i.e., in an effort to control for factors other than cocaine and opiates that might influence measures of synaptic density). Inclusion/exclusion criteria will be identical, except for those criteria related to cocaine and opiates.

All CUD, OUD, and HC subjects will complete screening for the study, as outpatient, at the Clinical Neuroscience Research Unit (CNRU) of the Connecticut Mental Health Center (CMHC).

4D. 11C-UCB-J PET Imaging Methods: PET procedures will be conducted at the Yale University PET Center. Female subjects will be given a urine pregnancy test prior to the initiation of any imaging procedures. If the test is positive, the scans will be canceled. Each subject will undergo one 11C-UCB-J PET scan. Depending on scheduling issues healthy controls may be asked to serve as a backup for another subject's study day. In this circumstance they will be compensated additionally (see economic considerations below).

Venous catheters will be used for intravenous administration of the radiotracer and possibly for additional venous blood sampling. A radial artery catheter may be inserted by an experienced health care provider (an experienced physician or a highly skilled APRN trained to complete this procedure) before the PET scan to draw arterial blood samples for metabolite analysis and for determination of the fraction of plasma radioactivity unbound to protein. The goal of the arterial line is to be able to measure absolute physiological functions by mathematically relating the signal (from the PET scanner) to the tracer availability (from the blood). This approach is the gold standard for obtaining quantitative PET data. In some cases, for well-established tracers, methods have been developed and validated that provide comparable results to the gold standard. In those cases, the arterial samples may not be necessary. However, validation of such an approach must be performed in each patient group and for each unique experimental design. In this study, if an arterial line cannot be placed, a second intravenous line may be placed for venous blood sampling.

11C UCB J will be prepared as previously described (Nabulsi et al., 2016). PET scans are acquired using an HRRT scanner (207 slices, resolution < 3 mm FWHM), the highest resolution human PET scanner available (de Jong et al., 2007). The scan will be acquired as the subject lies supine on the scanner bed. A transmission scan is obtained before or after the emission scan. List-mode data are reconstructed with all corrections (attenuation, normalization, scatter, randoms, deadtime, and motion) using the MOLAR algorithm (R.E Carson, Barker, Liow, Adler, & Johnson, 2003). Motion correction is based on an optical detector (Vicra, NDI Systems, Waterloo, Canada) and is performed on an event-by-event basis (Jin, Mulnix, Gallezot, & Carson, 2013). This methodology has been used in >2500 human studies on the HRRT. The 11C UCB J scan of up to 120 min is acquired following a single bolus administration of up to 20 mCi of tracer. After completion of the PET scanning session the IV and arterial lines will be removed and the subject will be discharged from the PET Center.

4E. Quantitative Sensory Testing (QST) Methods: Quantitative Sensory Testing (QST) is a nociceptive assessment tool that is widely used in clinical and research settings (Yale HIC #2000029286 and #200027065), which allows forthe standardization of pain assessment procedures (Davis 2019). For instance, QST has been used assess the therapeutic response among patients with diabetic neuropathy (Yarnitsky, Granot et al. 2012), and to predict the outcomes of musculoskeletal pain treatment (Georgopoulos, Akin-Akinyosoye et al. 2019). The technique involves the administration of nociceptive stimuli (e.g. heat or cold) in a controlled environment. Thermal stimuli are highly reliable and repeatable. They also ensure that specific fibers are engaged, allowing inferences about pain sensing and modulation mechanisms — and their relationship with PET imaging biomarkers. In this study, we will use heat stimuli (rate of rise of 0.5°C/ second from a pre-set baseline of 32 C) applied to the palm of the dominant hand, to collect information on participant's pain threshold and tolerance. Pain threshold is the level of nociceptive stimuli at which the participant reports the first instance of pain, by pressing a button *as soon as* the heating sensation changes from "warm" or "hot" to "burning" or "stinging". Pain tolerance is the level of nociceptive stimuli at which the participant *as soon as* finds the heat stimuli no longer tolerable and discontinues the test, also by pressing a button.
We will also conduct two additional QST paradigms to assess participants' nociceptive profile (i.e., overall health of pain sensing and modulation systems): Conditioned Pain Modulation (CPM) and Temporal Summation of Pain (TSP). The first paradigm, CPM, uses both heat and cold stimuli to measure top-down pain inhibition, by leveraging the "pain inhibits pain phenomena". First, participant's pain responses are measured during the administration of isolated heat stimulus (47°C [starting from 37°C at an increase rate of 13° C/s], applied to the palm of dominant hand); second, cold stimulus (5°C) is applied to the ventral surface of the contra-lateral arm; third, the response to the first stimulus is measured again. Higher CPM reflects supra-spinal pain modulation (Kennedy, Kemp et al. 2016). The second paradigm, TSP, involves the repeated administration of heat stimuli (47.5°C, ten times for 1 second each time) to the palm of the dominant hand. Higher TSP indexes bottom-up pain facilitation, indicating the increased firing of ascending pre-synaptic neurons, a mechanism of of central sensitization (Suzan, Midbari et al.

2016). All QST assessments will be conducted at baseline and following the induction onto buprenorphine (the latter, for participants with OUD). The Thermal Sensory Analyzer (TSA-II platform - Medoc, Ramat Yishai, Israel) will be used to assess thermal nociception this study. All pain responses will be collected in real-time, using a computerized visual analog scale (co-VAS).

4F. Sublingual Buprenorphine Detoxification Dosage and Methods: OUD inpatient subjects may undergo detoxification with sublingual buprenorphine as they experience opiate withdrawal. Buprenorphine will be uptitrated during the first week based on the Clinical Opiate Withdrawal Scale (COWS). On the first day of buprenorphine administration, subjects will receive a dose in the morning of 4mg which could be increased up to 8 to 12mg that day, depending on the individual's symptoms/signs. Over the week, the dose will be uptitrated at increments no higher than 4mg per day. Subsequent to stabilization of withdrawal, dosages will begin to be tapered down throughout a period no longer than 21 days. No subject will ever receive a dose higher than 24mg, as NIDA funded studies have found that sublinguals as high as 24mgs are safe and effective for detoxification. The subjects will be monitored through the induction phase and the taper by trained and certified physicians familiarized with opioid addiction and buprenorphine on the CNRU. Participants who are already receiving standard of care medication assisted treatment and are not in need of detoxification, will not receive buprenorphine from research team nor will they complete the detoxification component of the study. This matches with the sub group of the population we are trying to target who are persons who may be/are receiving medication assisted treatment, are still using, don't need detoxification, likely need optimization of their outpatient prescriptions, want to do study as outpatient, and don't need admission in order to optimize their prescribed medications. Among the ones who are already receiving medication assisted treatment but are still using, it will be possible to discuss optimization of their medications with outpatient prescribers. However; the nature of OUD is dynamic, thus participants within the above categories could be better fit for transition to inpatient component if, as part of discussions with outpatient providers, it is considered that they need higher/inpatient level of care (i.e., participant who is receiving buprenorphine in the community, still using, and it is considered that they may benefit from transitioning to a different medication such as vivitrol). This latter transition may require the inpatient component.

Behavioral Assessments:
1) Structured Clinical Interview for DSM-5 (SCID5; Screening): The SCID5 (First et al., 2015) will be used upon screening to confirm the diagnostic eligibility of all CUD, OUD and HC subjects, including the presence/absence of Cocaine Use Disorder or Opiate Use Disorder as well as absence of exclusionary psychiatric and/or substance use disorders (see above). If an acute psychiatric condition (see exclusion criteria) is detected upon screening, we can offer subjects the option to be connected with outpatient treatment instead of participating in the study. If the study physician determines that symptomatology does not pose a risk to subject safety, we can offer the option to be connected with treatment after study procedures. Subjects not requiring acute care who are currently on psychiatric medication will be permitted to continue medication treatment in accordance with table of allowed medications, below.

2) Semi-Structured Assessment of Drug Dependence and Alcoholism (SSADDA), Cocaine or Opiate Subsection (Week 1): The SSADDA is a comprehensive psychiatric interview schedule designed to assess the physical, psychosocial and psychiatric manifestations of substance abuse and dependence (Pierucci-Lagha et al., 2007; Pierucci-Lagha et al., 2005). In addition to diagnostic verification, we will use the Cocaine or Opiate Subsection to obtain a host of quantitative cocaine or opiate use variables in CUD and OUD subjects;

3) Fagerstrom Test for Nicotine Dependence (FTND; week 1): The FTND is an established, 6-item, self-report instrument used to assess the severity of physical dependence upon nicotine (scored on a scale of 0-10). We will use the FTND to match tobacco use/dependence in CUD, OUD and HC subjects (Heatherton et al., 1991);

4) The Substance Use Calendar (Screening, Outpatient visits, and Follow-up). The Substance Use Calendar, developed by Miller and Del Boca (1994) and based on the Time-Line Follow-Back Method of Sobell and Sobell (Sobell, 1992) assesses day-to-day use of alcohol and other substances during the last 90 days;

5) Cocaine Selective Severity Assessment (CSSA; daily, among CUD subjects, during week 1): The CSSA is a clinician administered scale that reliably and validly measures cocaine withdrawal signs and symptoms for the past 24 hours (Kampman, 1998).

6) Clinical Opiate Withdrawal Assessment (COWS; daily, among OUD inpatient subjects, during week 1): The COWS is an 11-items clinician administered scale to assess and rate signs and symptoms of opioid withdrawal (Wesson, 2003).

7) Pain Assessments: 7a) We will assess participant's pain severity and interference using the Brief Pain Iventory – Short Form (BPI-SF). The BPI-SF is a self-report questionnaire that assesses severity of pain, impact of pain on daily function, location of pain, pain medications, and amount of pain relief in the past 24 hours or the preceding week (Cleeland and Ryan 1994). The BPI-SF will be used to assess pain severity and interference at baseline and after the induction onto buprenorphine . 7b) Pain attentional bias (an automatic attentional process that favors pain-related stimuli) will be measured by the difference between response times for pain vs. neutral cues, using a well-validated visual probe task employed in Yale-HIC approved protocols (HIC# 2000029286) (Haggman, Sharpe et al. 2010). The attentional bias visual prob task relies on a tendency for individuals to respond faster to probes (e.g., small dots) when they are presented in an attended region of a visual display. In the standard attentional bias task, a drug or pain-related word is presented next to a neutral word for 500ms. Subsequently, a probe (i.e., a "q" or "p") replaces the drug/pain word or the neutral word at an equal rate. The participant's task is to indicate the location of the probe as quickly as possible by pressing "q" or "p". Attentional bias is calculated from the difference in reaction times (i.e., neutral cue minus drug/pain cue) to indicate the location of the probe, with higher values indicating greater attentional bias. For all tasks, opioid and pain words will be presented in separate blocks in counterbalanced order. Neutral words paired with opioid- or pain-related words will be matched for length and frequency of use in the English language. The choices of opioid (e.g., syringe, needle, high, blues) and pain sensory (e.g., stuff, throbbing, shooting, burning) and affective (e.g., miserable, tiring, unbearable, exhausting) words is based on prior research demonstrating attentional bias in OUD. 7c) The Situational Catastrophizing Questionnaire (SCQ) will assess pain catastrophizing (the propensity to magnify, ruminate over, and feel helpless about the pain experience) in response to QST-evoked pain (Sullivan, Bishop et al. 1995, Campbell, Kronfli et al. 2010). Notably, the self-report components of the pain assessment (questionnaires on pain severity, interference, and catastrophizing) may be conducted using a REDCap survey.
8) Other Behavioral Assessments (week of PET scan for all subjects): In addition to measures of diagnostic severity (SCID) and cocaine/opiate use (SSADDA), we will collect assessments of reward (e.g., Hedonic Response Questionnaire) (O'Brien, Gastfriend, Forman, Schweizer, & Pettinati, 2011) and cognitive function (e.g., Probabilistic Reversal Learning Task (PRLT) (Swainson et al., 2000), Barratt Impulsivity Scale (BIS) (Patton, Stanford, & Barratt, 1995), and Delayed Discounting Questionnaire (Kirby & Marakovic, 1996) to be used in secondary/exploratory correlations with measures of synaptic density.

Below is a timeline of study procedures:

Timeline of Study Procedures for All Subjects

Procedures	CUD Subjects	OUD Subjects	Healthy Controls

SCID5, MR safety sheet, Urine Tox, Pregnancy Test, Blood work, ECG	Outpatient Screening	Outpatient Screening	Outpatient Screening
SSADDA and FTND	Week 1	Week 1	FTND only, at screening
Hedonic Response Questionnaire, PRLT, BIS, Delayed Discounting Questionnaire BPI-SF, QST, SCQ, Visual Probe Task*	Week of PET scan	Week of PET scan	Week of PET scan
Inpatient Detoxification with Buprenorphine BPI-SF, QST, SCQ, Visual Probe Task*	N/A	Up to 21 days	N/A
Weekly Outpatient uTox Screening + Substance Use Calendar	N/A	3x week, up to 5 weeks	N/A
Structural MRI	0-14 days	0-21 days	As available
11C-UCB-J PET scan	After 2 weeks of abstinence	Between 1-5 weeks of opiate abstinence	As available
Follow Up	90 days	90 days	90 days

* The first pain sensitivity assessment will be performed within 24 hours of the PET scan for persons with OUD. The second pain sensivitiy assessment will be performed within 24 hours before discharge, upon induction onto buprenorphine. The second BPI-SF assessment may be performed using a REDCAp survey.

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 *Write here*

ii. the plan for the collection of material or the conditions under which material will be received *Write here*

iii. the types of information about the donor/individual contributors that will be entered into a database *Write here*

iv. the methods to uphold confidentiality *Write here*

B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects? *Write here*

C. Is widespread sharing of materials planned? *Write here*

D. When and under what conditions will materials be stripped of all identifiers? *Write here*

E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials? *Write here*

i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)? *Write here*

F. Describe the provisions for protection of participant privacy *Write here*

G. Describe the methods for the security of storage and sharing of materials *Write here*

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

A total of 90 subjects will be studied; including 30 with Cocaine Use Disorder (CUD), 30 with Opiate Use Disorder (OUD) and 30 healthy controls (HC) matched for age, race, sex, cannabis, alcohol, and tobacco use. Detailed inclusion and exclusion criteria are provided in sections 4B and 4C.

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

☐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?

Cocaine Use Disorder (CUD) and Opiate Use Disorder (OUD) Subjects: We will recruit 30 medically healthy CUD subjects and 30 medically healthy OUD subjects according to the inclusion/exclusion criteria listed in sections 4B and 4C.

Table: Concomitant medications that are prohibited, allowed with restrictions, or permitted.

Use category	Type of medication	Details
Prohibited	MAOIs	
	VNS, ECT, deep brain stimulation	VNS, ECT, or within 6 months at randomization is exclusionary
	Topiramate	
	Opiates	
	Memantine	
	Barbiturates	
Permitted with restrictions	Benzodiazepines (stable dose)	Benzodiazepines are permitted only when used to help with detoxification from opioids for symptoms such as anxiety or insomnia. The daily dosage can't exceed: ï Alprazolam 2.0 mg/day ï Clonazepam 2.0 mg/day ï Diazepam 40 mg/day

Hypnotics	One of the following medications can be used for insomnia at the restricted and maximum allowable dose of: ï Zolpidem tartrate 10 mg ï Ambien CR 12.5 mg ï eszopiclone 3 mg ï zopliclone 7.5 mg

Concomitant use of benzodiazepines and hypnotics is prohibited

Table: Concomitant medications that are prohibited, allowed with restrictions, or permitted.

Use category	Type of medication	Details
Permitted		
	Atypical Antipsychotics	When prescribed mainly for symptoms such as insomnia, anxiety, at doses lower than therapeutic doses for bipolar disorder and schizophrenia (i.e., Seroquel up to 150 mg per day)
	Non-psychoactive medications, including over-the-counter medications	
	Antidepressants	SSRI, SNRI, trazodone, Bupropion.
	Medications required to treat illnesses or complaints that occur during the study	May be used at the discretion of the investigator.
	Medications that are considered necessary for the patient's safety and well-being	May be given at the discretion of the investigator. Includes medication and devices for contraception.

Other permitted drugs	Includes other psychoactive drugs indicated for attention deficit hyperactivity disorder, except stimulants (e.g., atomoxetine, clonidine, guanfacine.) or anxiety (diphenhydramine, etc.).

9. How will **eligibility** be determined, and by whom? Write here

Physical health will be determined via telephone interview, medical history, a physical and neurological examination, ECG and laboratory examinations. DSM-V criteria for CUD or OUD, absence of primary Axis I psychiatric disorder and history of other substance dependence will be determined by the Structured Clinical Interview for DSM-V (SCID) performed by a member of the research staff. In addition, medical or psychiatric information can be gathered as part of the screening by reviewing medical records or by talking with primary care providers. Contraindications to participate in PET imaging procedures will be assessed by looking at the Yale PET Center Database, which has information about prior participation in research studies. Contraindications to participate in the MRI will be assessed with the MR safety questionnaire. All female subjects will undergo serum or urine pregnancy (β-HCG) test to determine evidence of pregnancy. Once screening is completed, the research assistant will meet with the study physician to review the eligibility checklist.

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

The risks from this study include 1) intravenous and arterial line and blood drawing 2) radiation exposure during PET scans, 3) cocaine abstinence / detoxification, 4) opioid withdrawal / sublingual buprenorphine detoxification, and 5) MRI procedures, and 6) Loss of Privacy.

10A. Risks Associated with Phlebotomy, Intravenous Line, and Arterial Line: As part of study assessments of medical eligibility and PET Scan procedures subjects will undergo phlebotomy and intravenous cannulation, respectively. Venous sampling may be associated with mild-to-moderate pain or bruising at the puncture site. Bruising and thrombosis can occur during phlebotomy and the placement of the intravenous line. In rare instances poor healing, or infection at the catheter insertion site may occur. Certain individuals may feel light-headed during venipuncture; to avoid injury due to fainting, procedures will be performed when the subjects are seated/recumbent. The volume of blood collected during this study, may include screening laboratories, and PET scans, will be less than 15 tablespoons.

On the PET scan day, a radial arterial catheter will be inserted. Arterial sampling may be associated with mild-to-moderate pain, hematoma, inflammation, bleeding, or bruising at the puncture site. If this occurs, signs and symptoms will dissipate over time, usually 24 to 72 hours after the event. In rare instances blocking of the artery, tearing of the artery, arterial leakage, poor healing, or infection at the catheter insertion site may occur. Certain individuals may feel light-headed during arterial catheter placement.

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10B. Risks Associated with Radiation Exposure / PET Scan Procedures: The Yale University Radioactive Drug Research Committee (YU RDRC) and The Yale University Radiation Safety Committee (YU RSC) have reviewed and approved the use of radiation in this research study. This research study involves exposure to radiation from [^{11}C] UCB-J (aka [^{11}C]APP311) PET scanning. This radiation exposure is not necessary for medical care and is for research purposes only.

The maximum amount of radiation exposure subjects will receive from this study is equal to an effective dose of 0.607 rem for a total of up to 20 mCi of [^{11}C]UCB-J in one injection, plus transmission scans of the brain. This calculated value is used to relate the dose received by each organ to a single value.

However, in the event of scan failure post-injection (i.e., PET camera malfunction), a second injection may be completed for a total of 1.214 rem from the two [^{11}C] UCB-J injections, plus transmission scans of the brain.

Transmission Scan Dose: Up to 2 transmission scans may be completed per PET scan, for a total of 0.0028 rem to the head per PET scan session (0.0014 rem per transmission scan). The maximum dose to the head received would be 0.0056 rem from up to 4 transmission scans.

Maximum Radiation Exposure: Including transmission dose, and an additional scan (if needed), the maximum amount of radiation from participating in this research study would be 1.219 rem (1.214 rem from [^{11}C] UCB-J,plus 0.0056rem from transmission scans).The amount of radiation subjects will receive in this study is below the dose guidelines established by the federal government and adhered to by the Yale University Radioactive Drug Research Committee (YU RDRC) for research subjects. This guideline is an effective dose of 5 rem (or 5,000 mrem) per year.

The amount of radiation involved in this research is small, but may slightly increase the risk of getting cancer. Scientists are not certain about the actual cancer risk at these low doses, and there may be no risk at all, but to be conservative we assume that any amount of radiation may pose some increased cancer risk.

Another concern some people may have about radiation exposure is the effect on fertility or on the possibility of causing harm to future children (i.e., genetic effects). The doses subjects will receive in the study are well below the levels needed to affect fertility. In addition, genetic effects have not been seen in humans who have been exposed to radiation. The information on genetic effects currently available is based on animal studies using much larger doses of radiation than the amount individuals will receive in this study.

Subjects will tell the clinician of the research team if they have taken part in other research studies or received any medical care at any hospitals or any other place that used radiation. This is done to confirm that subjects will not receive excessive radiation. Examples of the types of radiation exposure considered include x-rays taken in radiology departments, cardiac catheterization, and fluoroscopy as well as nuclear medicine scans in which radioactive materials were injected into their body.

No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing during evaluation and on each scan day before initiation of any scan procedures. If subjects are breastfeeding they will not be able to participate in this research study. Adverse effects of the radiopharmaceuticals in this study have not been reported. However, the possibility exists for a rare reaction to any of the substances or procedures to which a subject is exposed.

10C. *Risks Associated with Quantitative Sensory Testing:* The Thermal Sensory Analyzer (TSA-II platform - Medoc, Ramat Yishai, Israel) will be used to assess thermal nociception this study. This equipment has been widely and safely used for quantitative assessment of nociception, including in ongoing Yale-HIC approved studies (#2000029286 and #200027065). Using a 30mm x 30mm Peltier thermode, the TSA-II is leveraged in conjunction with a computerized visual analog scale (Co-VAS) software to deliver precise thermal stimuli. The

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risks of QST assessments include subtle and temporary increase in pain; and a mild, temporary increase in blood pressure and/or heart rate. Generally, the nociceptive effects induced by the QST battery do not last more than a few seconds, subsiding promptly after the stimuli are removed.

10D. Risks Associated with Cocaine Withdrawal: Cocaine withdrawal symptoms may include depressed mood, lack of motivation, fatigue, sleep changes, increased appetite, and suicidal ideation. Unlike some substances of abuse (e.g. alcohol, benzodiazepines), cocaine withdrawal does not typically cause physiological changes that are life threatening. In our experience with cocaine research, withdrawal symptoms are uncommon, typically mild, and short-lasting.

10E. Risks Associated with Opioid Withdrawal / Sublingual Buprenorphine Induction: All subjects will be actively abusing some combination of illicit street opioids (e.g., heroin) and/or prescription opioids (e.g., oxycodone) at the time of recruitment/screening/study enrollment. Those who choose to participate as inpatients will be admitted to the Clinical Neuroscience Research Unit (CNRU) for buprenorphine induction/detoxification. Withdrawal from opioids, while symptomatically uncomfortable (e.g., lightheadedness, yawning, sneezing, diaphoresis, anxiety, irritability, insomnia, nausea, vomiting, diarrhea, tremor/shivering, muscle aches/cramps/pains, depression, restlessness, drug craving, lacrimation, rhinorrhea, and piloerection [goosebumps]) is medically safe and mild withdrawal is necessary for buprenorphine induction. Medication assisted treatment with sublingual buprenorphine is associated with a variety of side effects/risks, including drowsiness, weakness/fatigue, musculoskeletal pain, sweating, nausea, abdominal pain, constipation, infection, anxiety, high blood pressure, peripheral edema, insomnia, itching, lack of appetite, mood swings, skin rashes, difficulty urinating, insomnia, headaches, oligomenorrhea/amenorrhea, decreased sex drive and dental problems. When taken in larger doses (i.e., 24mg/day sublingual), long-acting partial μ-opioid receptor agonists (i.e., buprenorphine) can cause slow breathing, irregular heartbeat and death (although risks of such complications are rare unless buprenorphine is taken with other agents such as benzodiazepines). Rare but serious side effects include vomiting, decreased blood pressure, dizziness, sore throat, runny eyes, and indigestion.

10F. Risks Associated with Outpatient Abstinence from Opiates: Attaining abstinence as an outpatient, without any pharmacological assistance, is not recommended. It is much more difficult to achieve in this setting (i.e. greater risk of relapse) than doing so as an inpatient with medication assisted treatment. Additionally, the discomfort associated with opiate withdrawal symptoms is expected to be greater than it would be with medication assisted treatment and clinical supervision. Therefore, the primary situation in which we would recommend participating as an outpatient is if a subject is currently undergoing or has recently undergone detoxification in another clinical setting.

10G. Risks Associated with MRI Scans: MR carries a risk for subjects who are claustrophobia or have pacemakers, metal pieces, aneurysm clips, large colored tattoos, or any other contraindications for MR. Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines. 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.

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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 primary investigator, the lab, the MR technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a diagnostic evaluation of the images. If a worrisome finding is seen on a 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 primary investigator or consulting physician will contact the subject, inform them of the finding, and recommend that they seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie solely with the subject and 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.

10H. *Loss of Privacy:* The use of RECap mobile surveys for data collection (follow-up pain assessment) in this study introducres a degree of risk should the participant lose the phone, with the possibility that individuals outside the study could access an uncompleted survey via link on the participant's phone. Such theoretical risk will be drastically reduced due to steps taken to limit the type of data collected using REDCap (pain experience survery), as well as safety features built into Yale's REDCap survery administration.

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

11A. *Minimizing Risks of Phlebotomy, Intravenous Line, and Arterial Line*: These risks can be minimized by having these procedures performed by experienced personnel using good clinical technique. Subjects will have no more than 15 tablespoons of blood drawn. These amounts are well within the Red Cross blood standards. The blood draws during the PET sessions will be obtained from the already inserted cannula, to minimize discomfort. Also, subjects will be asked to abstain from using aspirin or NSAIDS. Subjects taking anticoagulants will be excluded. Bleeding from intravenous site insertion is prevented by local pressure applied for 5 minutes after catheter removal. Infection is avoided by adequate cleansing of the skin prior to intravascular line insertion, and the exclusion of immunocompromised subjects. To avoid injury due to fainting, procedures will be performed when the subjects are seated/recumbent.

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.

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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.

Pain is minimized by local anesthesia. Bleeding from arterial line insertion is prevented by local pressure applied for a minimum of 15 minutes after catheter removal. Subjects will have their hand and finger blood supply examined after arterial cannulation and again following catheter removal. Also, subjects will be asked to abstain from NSAIDs for 3 days prior to arterial line insertion as well as following arterial line removal. Subjects will be provided a 24-hour emergency physician telephone number to call if they encounter pain, discoloration, numbness, tingling, coolness, hematoma, inflammation, or any other unusual symptoms in the wrist or hand, or fever, chills or drainage from the vascular puncture sites, following the procedure. In addition, if an emergency arises at the time of cannulation or scanning, 911 will be called, and the subject will be sent to the Emergency Department for evaluation and treatment. Nurses will provide the subjects an instruction sheet documenting problems to watch for and procedures to follow should such problems occur. Infection is avoided by adequate cleansing of the skin prior to intravascular line insertion.

11B. *Minimizing Risks of Radiation Exposure/ PET Procedures*: This dose of radiation has already been approved by the Yale-University Radioactive Drug Research Committee (YU RDRC) and The Yale University Radiation Safety Committee (YU RSC). All scans will be done in the presence of medical supervision and trained staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the 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, health care providers, nurses, and technologists of the Department of Radiology and Biomedical Imaging, or the Department of Psychiatry, 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.

11C. *Minimizing Risks Associated with Quantitative Sensory Testing:* Upper and lower temperature limits are pre-programmed with cut-off temperatures being 50°C for Thermal Pain Limits, and 5°C and 47.5°C for TSP and CPM, respectively, to avoid thermal damage to the skin. The baseline temperature to which the thermode returns before and after each test is 32°C. While thermal testing can produce transient pain, risks to the individual are minimal because 1) the pain subsides immediately after the procedure; 2) participants are instructed that they may stop any procedure at any time, with no adverse consequences; and 3) the level of pain experienced by subjects is below their tolerance level. Furthermore, the TSA-II has built-in safeguarding mechanisms to ensure the safety and protection of the participant: 1) In the unlikely event that the thermode temperature reaches 56°C (which is **not** a destination temperature in this study) the system disconnects, and the thermode is cooled by the coolant flowing through it; 2) the delivery of prolonged or high-intensity stimuli is prevented through the systems automatic "Safe Mode" wherein the system rapidly cools off, making it impossible to further run any tests until a system Self-Test is performed.

Our comprehensive QST system is currently found in virtually every major pain research laboratory in the U.S. Among the various institutions using this technique are General Hospital and Brigham & Women's Hospital, in Boston, MA (Edwards, Wasan et al. 2011); Beth Israel Medical Center, in New York, NY (Aniskin, Fink et al. 2011); University of California San Francisco, in San Francisco, CA (Abrecht, Cornelius et al. 2019); Medical University of South Carolina, in Charleston, SC (Imperatore, McCalley et al. 2020); Johns Hopkins University, in Baltimore, MD

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(Taylor, Campbell et al. 2018); Vanderbilt University, in Nashville, TN (Sevel, Boissoneault et al. 2019); Dartmouth University in Lebanon, NH (Reddy, Vergo et al. 2016); and the University of Pennsylvania, in Philadelphia, PA (Compton, Wasser et al. 2020). Two protocols approved by the Yale HIC (#2000029286 and #200027065) involve safe QST assessments among persons with opioid use disorder. Further, as with all study procedures, participants will be informed of the nature of this assessment, and will be encouraged to express their concern to the study staff should they feel particularly uncomfortable or fearful; though, as noted above, the equipment is safe and offers only subtle thermal stimulation.

11D. *Minimizing Risks Associated with Cocaine Withdrawal*: The medical and nursing staff on the inpatient unit is trained to assess for withdrawal symptoms, and will be available 24 hours a day should subjects experience any symptoms of withdrawal. In addition; nursing staff will complete assessments such as the Cocaine Selective Severity Assessment (CSSA), on a daily basis for 1 week, in order to identify symptoms of withdrawal. This is a valid and reliable scale to assess signs and symptoms of cocaine abstinence (Kampman, 1998). If at any time there is a concern that a subject is experiencing potentially unsafe symptoms related to cocaine withdrawal (e.g., suicidal ideation), we will have trained psychiatric professionals 24 hours a day who are able to provide treatment or who can order a change in the level of subject's supervision (e.g., from checks every 30 minutes to checks every 15 minutes or to constant visual observation, etc., if necessary). If a subject endorses significant anxiety, insomnia, irritability or depressed mood, investigators will offer standard of care pharmacological treatment for these symptoms, as needed.

11E. *Minimizing Risks of Opioid Detoxification / Buprenorphine Induction:* The recruitment of opioid-abusing subjects will be done locally. Subject reimbursement will be provided in amounts and forms explicitly approved in advance by the local (e.g., Yale) IRB. Subjects actively abusing opioids, who choose to stay inpatient, will undergo monitored induction and taper by trained / certified physicians familiarized with opioid addiction and buprenorphine (e.g. Dr. Angarita) on the CNRU (where all inpatients remain under 24/7/365 supervision of trained psychiatric research medical/nursing staff). Standard induction and taper protocols will be observed (uptitrated, as needed on an individual subject basis based on objective agonist and withdrawal symptoms) most typically over the course of up to 3 days and then tapered down based on withdrawal symptoms. There are different standard of care detoxification protocols with different durations as well as rate of buprenorphine's up titration/taper(Ling, Amass et al. 2005, Lee, Nunes et al. 2018). Our protocol will be conservative allowing a total duration of detoxification up to 21 days, enhancing subject's tolerability/minimizing dropout rates. Once subjects complete study procedures, they will have the option of resuming buprenorphine maintenance or starting other FDA approved agents for OUD (i.e., naltrexone), continuing active psychosocial treatment / rehabilitation, and be referred to further residential treatment or outpatient level of care.

11F. *Minimizing risks associated with outpatient abstinence from opiates:* It is most likely that subjects who choose to do this study as an outpatient will have already undergone detoxification in a different clinical setting or by their own healthcare provider. In this case, subjects will have a reduced risk of withdrawal when they begin the abstinence period. However, if at any point in the study subjects wish to transition to inpatient or receive medication assisted treatment, we can either explore an admission at the CNRU or immediately withdraw them from the study and help refer them to the appropriate clinical resources.

11G. *Minimizing the Risk of MRI*: A member of the research team will accompany subjects to the scanner and stay there for the entire study. To minimize risks, each subject will fill out the Yale Magnetic Resonance Research Center MRI Safety Questionnaire before the study. Only subjects who fulfill the criteria by this questionnaire will be eligible for the study. In addition, subjects will remove all metal (watch, hair pins, jewelry) and change into

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scrubs immediately prior to the study and pass through the metal detector in the MRRC before entering the MRI room.

11F: *Minimizing Risks of Loss of Privacy:* The REDCap service is housed on Yale University research servers and will not contain any PHI or PII other than mobile telephone number. Futther, surveys will not include open text responses, eliminating the potential for a participant to enter identifiable information or PHI. Disclose of mobile telephone number is included in HIPPA authorization. Surverys will be downloaded directly from the Yale REDCap server (i.e., https://poa-redcap.med.yale.edu/) using authorized research staff login, and then kept behind firewall. The master list linking names to code numbers (study ID and REDCap ID) will be kept in a password protected excel file. Only auhorized members of the research team will have access to this information. Study with REDCap training (e.g., Joao De Aquino and Julia Meyerovich) will have access to REDCap data and study measures. Data will be analized in accordance with study procedures by authorized study staff listed on the study protocol. Data will be kept in accordance with Yale University security practices.

12. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.)
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study? Moderate
    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? N/A
    c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are available here http://your.yale.edu/policies-procedures/forms/420-fr-01-data-and-safety-monitoring-plans-templates for
        i. Minimal risk
        ii. Greater than minimal

    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?

Adverse events will be monitored by the research team for each subject participating in the study and attributed to the study procedures / design by Dr. Gustavo Angarita, MD or the study physician according to the following categories:

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

Plan for Grading Adverse Events:

The PI or a study physician will review safety data, after every test day, during weekly research team meetings, and will suspend or modify the study (with IRB approval) if indicated. The IRB will be duly informed if there are

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any reasons to warrant "holding" the study. A review of the study will be submitted to the IRB annually. Adverse events will be graded in severity as follows:

0 No adverse event or within normal limits
1 Mild adverse event
2 Moderate adverse event
3 Severe adverse event resulting in hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.
4 Life-threatening or disabling adverse event
5 Fatal adverse event

Adverse events > level 3 will be reported to the IRB within 24 hours. Other adverse events will be reported to the IRB in a timely manner, using the following predefined causal relationships:

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

Plan for Determining Seriousness of Adverse Events:

Serious Adverse Events: In addition to grading the adverse event, the PI or study physician will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it:

1) is life-threatening OR
2) results in in-patient hospitalization or prolongation of existing hospitalization OR
3) results in persistent or significant disability or incapacity OR
4) results in a congenital anomaly or birth defect OR
5) results in death OR
6) based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition, OR
7) adversely affects the risk/benefit ratio of the study

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

Plan for reporting Reportable Adverse Events and other unanticipated problems involving risks to subjects or others to the IRB

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The principal investigator / research team will report the following types of events to the HIC and the funding agency: a) serious AND unanticipated AND possibly, probably or definitely related events; b) anticipated adverse events occurring with a greater frequency than expected; and c) other unanticipated problems involving risks to subjects or others.

These adverse events or unanticipated problems involving risks to subjects or others will be reported to the HIC within 24-48 hours of it becoming known to the investigator, using the appropriate forms found on the website.

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

13A. Statistical Analysis: Between-group comparisons of our primary outcome measures (VT and BPND) will be conducted by analysis of variance. Linear mixed models will be employed in order to evaluate the independent and joint effects of group (OUD vs. HC or CUD vs. HC), and brain region (VS and mPFC) as well as to account for potential covariates (lifetime years of cocaine or opioid's use, DSM-5 Axis 1 major psychiatric disorders, etc,). Based on the preclinical evidence (see above), we hypothesize that synaptic density will be increased in VS and mPFC in CUD and decreased in OUD subjects. In secondary (exploratory) analyses, potential associations between clinical measures (i.e., QST/pain and behavioral assessments) and 11C-UCB-J PET measures of synaptic density will be evaluated by correlation analysis. In all analyses, nonparametric alternatives will be used as necessary, tests will be two-sided, and considered significant at the alpha=0.05 threshold. Analyses will use SAS, v. 9.4 (Cary, NC).

13B. Power/Sample Size: Assuming a two-tailed alpha=0.05, 30 subjects per group will provide 80% statistical power (Cohen, 1988) to detect a moderate effect (d=0.74) in between-group comparisons of primary outcome measures.

13C. Data Analysis: A summed image (0–10 min postinjection) is created from the motion-corrected PET data and registered to the subject's MPRAGE image. The individual's MRI will then be nonlinearly coregistered to the template MNI MRI to obtain the regions of interest (ROIs) defined in the automated anatomical labeling (AAL) template (Tzourio-Mazoyer et al., 2002). All trans¬formations are performed with BioImagesuite (version 2.5; http://www.bioimagesuite.com). Regional VT estimates and parametric maps will be calculated using conventional compartmental analyses with the 1T model (R. E. Carson, 2003). If the white matter VT values provide an accurate estimate of VND, these values will be used to calculate the binding potentials BPF, BPP, and BPND (Innis et al., 2007). Reference tissue models (e.g. SRTM2) (Lammertsma & Hume, 1996; Wu & Carson, 2002) will be used to produce parametric images of binding potential. In addition, analysis of striatal subregions (i.e., including for ventral striatum [VS]) will be based on guidelines from Mawlawi et al. (Mawlawi et al., 2001) and Martinez (Martinez et al., 2009), as will analyses of cortical subregions (e.g., medial and orbital PFC) as we have performed on PET imaging data in CUD populations (D. Matuskey et al., 2014a). Finally, statistical parametric analyses (using SPM12; Wellcome Trust Centre for NeuroImaging, London, UK) will be employed to confirm/replicate ROI results in candidate regions and, in an exploratory fashion, uncover brain regions with unexpected findings. Corrections for multiple comparisons, either over the whole brain or, using the small volume correction procedure over regions targeted in this proposal in primary analyses (i.e., VS and mPFC). Typically, the threshold for significant clusters is punc < 0.001. All analyses will be conducted blind to subject group.

For kinetic modeling methods, estimates of tracer delivery (K1 from compartment models or R1 from reference models) will be correlated with VT and binding potential values, to assess whether tracer delivery, which is dependent on blood flow, is related to synaptic vesicle density. This consideration is proposed based on the well-

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established coupling of blood flow and metabolism, and the sensitivity of metabolism to neuronal activity (Fox, Raichle, Mintun, & Dence, 1988; Sokoloff et al., 1977). At present, it is not clear to what extent neuronal activity is coupled to synaptic density.

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 [^{11}C]APP311 ☐N/A

1. Name of the radiotracer: [^{11}C]APP311, iv (aka [^{11}C]UCB-J) is a novel PET tracer for measuring SV2A in vivo in the brain. **This will be under the purview of the RDRC.

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# *Write here* or ☒RDRC oversight (RDRC approval will be required prior to use)

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

PET tracer: [^{11}C]APP311 ((aka [^{11}C]UCB-J) tracer will be administered i.v. in doses of 20 mCi or less. To date, more than 500 PET administrations have been conducted with [^{11}C]APP311 at the Yale PET Center in healthy subjects, cocaine subjects (for example see HIC # 1311013082), epilepsy patients (for example see HIC# 1404013781), PTSD, MDD, AD, and Schizophrenia, with a maximum mass dose to date of 8.1 µg. There have been no adverse events associated with the administration of the radiopharmaceutical.

4. **Source:** Identify the source of the radiotracer to be used. [^{11}C]APP311 PET drug (radiotracer): Yale University PET Center.

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

[^{11}C]APP311
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. [^{11}C]APP311 in particular is stable for 150 min after preparation.
The PET drug (radiotracer) will be prepared at the Yale PET Center in accordance with local Chemistry Manufacturing & Control (CMC) procedures and quality specifications described in local Drug Master File (DMF) that are approved by YU-RDRC. Briefly, [^{11}C]APP311 is radiolabeled by C–[11C]methylation of the boronate precursor with [11C]methyl iodide ([11C]MeI) according to the Suzuki cross-coupling method. The resulting PET

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drug is purified first by semi- preparative HPLC, and then followed by solid-phase extraction to remove the HPLC buffer mixture. Finally the PET drug is formulated in <10% ethanolic saline solution (USP) containing <1% sodium bicarbonate (USP), and the resulting PET drug product is then passed through a 0.22 micron sterile membrane filter for terminal sterilization and collected in a sterile pyrogen free collection vial to afford a formulated I.V. solution ready for dispensing and administration.
The preparation of sterile PET drug products is validated prior to human use. Sterility is achieved by passing the PET drug product through a sterile 0.22 micron membrane filter during the last step of the formulation process. Prior to release for administration, a bubble point test is performed on the membrane filter used for terminal sterilization in order to validate and verify its integrity during the filtration process. Due to the short half-life, a sample of the PET drug product is tested for sterility 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).

B. DRUGS/BIOLOGICS Buprenorphine ☐N/A

1. If an **exemption from IND filing requirements is** sought for a clinical investigation of a drug product that is lawfully marketed in the United States, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

Exempt Category 1: The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:	
1. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug.	☒
2. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the product.	☒
3. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product	☒
4. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56).	☒
5. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs.	☒

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.

Buprenorphine is an FDA-approved partial μ-opioid agonist that is used as a method of treatment for opioid dependence. NIDA funded studies have found that sublingual doses as high as 24 mg are safe and effective for detoxification (Ling, Amass et al. 2005)

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3. **Source:** Identify the source of the drug or biologic to be used.
Sublingual Buprenorphine will be obtained from the Connecticut Mental Health Center's Pharmacy.

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

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.
Sublingual Buprenorphine is stored at the Connecticut Mental Health Center's Pharmacy.

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

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

SECTION III: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. Targeted Enrollment: Give the number of subjects:
   a. Targeted for enrollment at Yale for this protocol: We anticipate needing to enroll as many as 360 individuals in order to obtain a total of 90 completers (30 CUD, 30 OUD, 30 HC).
   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 ☒ Clinicaltrials.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. Subject will be recruited through the methods listed above (see written material attached to protocol) as well as through referrals from other participants, research groups/clinicians at CMHC, the VA, and/or the APT Foundation.

b. Describe how potential subjects are contacted. Potential subjects will be encouraged to contact our study recruitment line or email address for inclusion in our study.

c. Who is recruiting potential subjects? Subjects are recruited through our recruitment phone line and/or research email address. Subjects are asked to call or write in to our dedicated recruitment line/email address. A member of our research staff will describe the study to participants who call or write in, answer any questions the potential subjects has, and then complete a phone screening questionnaire to determine the subject's eligibility for a screening visit. If an individual appears to meet enrollment criteria and is interested in participating, a face-to- face interview is conducted by the study staff and study physician. A release of information is obtained for review of any available historical and clinical data. A written authorization form is also obtained from each subject, permitting the research team to use, create, or disclose the subject's PHI for research purposes. The nature of the project, procedures, relative risks and benefits, and alternatives to participation in the project are discussed with the individual. Following this discussion, the individual is given a copy of the consent form to review, and any questions are answered. The process of informed consent will be obtained in accordance with local IRB standards by study personnel who have participated in institutionally approved training in human subject protection. Upon obtaining voluntary, written, informed consent, medical and psychiatric screening procedures will be used to confirm study eligibility. Subjects are free to discontinue their participation in the research at any time by requesting this verbally or in writing.

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.

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

ii: If requesting a waiver of **signed** authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data: The information we are collecting over the phone is required prior to the subject being considered for a screening appointment.

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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.

   All subjects will be consented through a multi-step consent process. Subjects are first described the study in detail over the phone during the phone screen. They are then invited to come the in-person screening appointment. Consenting personnel will meet with the subject, explain both the consent form and the protocol and answer any questions the subject might have. The subject will be given a copy of the consent to read, at their leisure, prior to signing. Once the subject has signed the consent form the screening visit procedures will begin.

   Remote screening option: Subjects who pass telephone screening will be invited to a more in-depth screening to determine study eligibility (as listed in section on inclusion/exclusion criteria). Potential subjects who pass the phone screen portion will have the option to do the consenting screening portion remotely. They will be sent two copies of the consent form and a release of information to review at their leisure through a method that is most convenient for them (i.e., email, fax, or mail). This way subjects have time to review the consent prior to signing and they have their own copy to follow along and ask questions during the consenting phone call. If they agree to participate, subjects can keep one copy of the consent and sign, date, and return the other copy along with a signed released of information back to our research office either by fax, email, or mail in a prepaid envelope. When the consent is also signed by a member of the research team, it will be filed in the subject's research record. Using tele-health platforms (i.e., Zoom) the researcher and subject will thoroughly review and sign the consent form, complete a structured clinical interview (SCID) by a member of the research team, and a medical history and psychiatric interview by a study physician. This will take up to two hours to complete in total. If deemed eligible by initial Zoom call and interviews, potential subjects will be asked to complete the rest of the study procedures as detailed in the protocol.

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.

   Study staff is trained to assess the subject's understanding of pertinent information given to them and whether or not subjects can appreciate the implications of their decision. Study staff will ask the subject to reiterate the study procedures back to them and will ensure that the subject has had substantial time to ask questions about any/all study procedures.

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.

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Non-English speaking subjects will not be enrolled in this study.

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

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

Several translated short form templates are available on the HRPP website (yale.edu/hrpp) and translated HIPAA Research Authorization Forms are available on the HIPAA website (hipaa.yale.edu). If the translation of the short form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via modification prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

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

9. Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study. If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☒Not Requesting any consent waivers

☐Requesting a waiver of signed consent:

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

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

For a waiver of signed consent, address the following:

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

OR

- Does the research pose greater than minimal risk? YES ☐ NO☒ Providing eligibility information by phone is a minimal risk research procedure.
- Does the research include any activities that would require signed consent in a non-research context? YES ☐ NO ☒

☐ Requesting a waiver of consent:

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

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☐ Entire Study

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

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

SECTION IV: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

1. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?

   Subjects who decide to participate in the study will have a medical record at the Connecticut Mental Health Center (CMHC). If subjects already have a medical record at CMHC, some information about their participation in the study will be included here. If they do not have a medical record at CMHC, one will be made for their visit. The information that will be entered into this medical record will include: name, date of birth, date of admission to the CNRU, or scan at the Yale University PET Center / Yale MRRC, date of discharge from the CNRU, phone number, address, medical history, individual and family history of psychiatric problems, and substance abuse history. One of the instruments used to assess substance abuse history will include questions pertinent to legal problems. Subject's name, date of birth, date of admission to the CNRU, date of discharge from the CNRU, and phone number will not be used in the analysis neither in the presentation of the results.

2. How will the research data be collected, recorded and stored?

   Research data will be collected over the phone, by paper and pencil questionnaires, by interviews, by behavioral assessments, by neurocognitive assessments, by PET scanning, MR scanning, and laboratory work. This information will be recorded/transferred in each subject's Case Report Form and on a password protected University computer servers that are kept in locked offices.

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 of the information obtained in this study is stored in locked files or password protected and encrypted computer files. The only ones who have access to this information are the medical personnel and the research staff at the CNRU. Information obtained in this study may be made available to the researchers and Yale's Human Investigation Committee.

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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.

   This information will be kept in a locked office during five years. Afterwards, identifying information will be discarded, but the other research data is kept indefinitely. Paper and electronic data will be destroyed by research assistants and study personnel at Yale University. Paper media will be destroyed by shredding and electronic media will be destroyed by zeroing, degaussing, or physical destroying as applied to the medium. De-identified MRI/PET imaging data will be kept for a minimum of 7 years.

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

   This study is covered by a Certificate of Confidentiality from the National Institutes of Health.

SECTION V: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)

These studies are of no direct benefit to patients. These studies offer no direct individual benefit to participants aside from financial compensation for inconvenience. All participants in this study may derive subjective benefit from volunteering to take part in a study for the advance of scientific knowledge.

SECTION VI: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. Alternatives: What other alternatives are available to the study subjects outside of the research?

   Participants can choose not to participate in this research study.

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.

Inpatient subjects:

Cocaine/opiate dependent subjects who complete inpatient study procedures will be paid up to a total of $800/1500 for their participation, including $25 for screening, $50 for behavioral and neurocognitive testing, $25 for the structural MRI, $150 for the [11C]APP311 PET scan, and $50 for arterial line insertion. Cocaine subjects will receive $25 for the first week of abstinence and $50 for the second week, for a total of $75. They will also

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receive $375 bonus for completion of all study procedures on the unit. Opiate users will be paid $350 for completing the buprenorphine induction/detoxification period, $350 for completing the two weeks of abstinence, and a $350 bonus for completion of all study procedures on the unit. Opiate subjects will receive an additional $50 for each one of two quantitative sensory testing (QST) assessments completed. Notably, compensation for pain sensitivity assessments will be defrayed from IMPOWR-YOU Pilot Funds. All subjects will also receive $50 for completion of the 90-day follow-up appointment. If a PET scan were to be repeated, then an additional $150 would be provided for the 11C-UCB-J PET scan. If the subject needs to return to the research clinic for an additional QST assessment, we will provide $20 to cover transportation costs.

Outpatient Opiate Dependent Subjects:

Opiate dependent subjects who complete outpatient study procedures will be paid up to a total of $1250 for their participation, including $25 for screening, $50 for behavioral and neurocognitive testing, $25 for the structural MRI, $40 per urine toxicology screening visit (3x week, up to 5 weeks, ranging from $120 for 1 week to $600 for 5 weeks), $150 for the [11C]APP311 PET scan, $50 for arterial line insertion, and a $200 bonus if subjects complete all study procedures. All subjects will also receive $50 for completion of the 90-day follow-up appointment, and $50 for each of the QST assessments completed (I.e. total of $100).

Opiate Dependent Subject Referrals:

Participants will be compensated $20 if they refer a potential opiate dependent participant who enrolls in the study (i.e. passes telephone screening and signs consent form). The referral fee will be provided once the referred participant enrolls in the study.

Healthy Controls:
Healthy control subjects will be paid up to a total of $300 for their participation, including $25 for screening, $50 for behavioral and neurocognitive testing, $25 for the structural MRI, $150 for the [11C]APP311 PET scan, and $50 for arterial line insertion. Subjects may also be reimbursed $50 for acting as back up on MRS and PET scan days.

In the event of scan failure post-injection (i.e., PET camera malfunction) and a PET scan were to be repeated, then an additional $150 would be provided for the 11C-UCB-J PET scan. It is also possible that for technical reasons the PET study is canceled. In those cases, subjects will be paid $50 for coming to the appointment.

Payments will typically be made in loadable increments after completing individual study procedures via a Bank of America debit card that will be mailed to subjects after completing the initial screening process. In some instances, reimbursement may be divided between small initial cash payments and subsequent check(s).

Subjects will be given the option to have their payments applied to non-monetary vouchers and/or given to designated relatives / significant others for whom they provide specific approval. In no instance will a single, large (i.e., over $100) cash payment be provided to CUD or OUD participants (i.e., to preclude subjects immediately spending the entire amount on drug of choice).

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.

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Subjects will incur no costs for participation in this research outside of potential costs for parking or transportation that may be reimbursed.

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).

   Subjects will be assisted by research staff in obtaining medical care through information and/or referral if injury occurs during their participation in this study. Subjects and/or their insurance would be responsible for the cost of any medical care required.

IMPORTANT REMINDERS

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

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

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

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

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

a. Does your YNHH privilege delineation currently include the specific procedure that you will perform? Yes ☐ No ☐

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure? Yes ☐ No ☐

c. Will a novel approach using existing equipment be applied? Yes ☐ No ☐

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

IMPORTANT REMINDER ABOUT RESEARCH AT YNHH

Please note that if this protocol includes Yale-New Haven Hospital patients, including patients at the HRU, the Principal Investigator and any co-investigators who are physicians or mid-level practitioners (includes PAs, APRNs, psychologists and speech pathologists) who may have direct patient contact with patients on YNHH premises must have medical staff appointment and appropriate clinical privileges at YNHH. If you are uncertain whether

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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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