YALE UNIVERSITY
HUMAN INVESTIGATION COMMITTEE

Application to Involve Human Subjects in Biomedical Research
100 FR1 (2015-2)

SECTION I: ADMINISTRATIVE INFORMATION

Title of Research Project: Imaging the neurochemistry of drug addiction with PET			
Principal Investigator: Kelly Cosgrove, Ph.D.		**Yale Academic Appointment:** Associate Professor	
Department: Psychiatry, Radiology, and Biomedical Imaging			
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Protocol Correspondent Name & Address (*if different than PI*): Marc Grasso			
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Yale Cancer Center CTO Protocol Correspondent Name & Address (*if applicable*):			
Campus Phone:	**Fax:**	**E-mail:**	
Business Manager:			
Campus Phone :	**Fax :**	**E-mail**	

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

Investigator Interests:

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

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

Yes X No

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

Yes X No

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

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

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

SECTION II: GENERAL INFORMATION

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

a. Internal Location[s] of the Study:

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

b. External Location[s]:

- ☐ APT Foundation, Inc.
- ☐ Haskins Laboratories

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☒ Connecticut Mental Health Center
☒ Clinical Neuroscience Research Unit (CNRU)
☐ Other Locations, Specify:

☐ John B. Pierce Laboratory, Inc.
☐ Veterans Affairs Hospital, West Haven
☐ International Research Site
(Specify location(s)):

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

☐ *YCCI-Scientific and Safety Committee (YCCI-SSC) Approval Date:
☐ *Pediatric Protocol Review Committee (PPRC) Approval Date:
☐ *YCC Protocol Review Committee (YRC-PRC) Approval Date:
☐ *Dept. of Veterans Affairs, West Haven VA HSS Approval Date:
☐ *Radioactive Drug Research Committee (RDRC) Approval Date:
☒ YNHH-Radiation Safety Committee (YNHH-RSC) Approval Date:
☐ Yale University RSC (YU-RSC) Approval Date:
☒ Magnetic Resonance Research Center PRC (MRRC-PRC) Approval Date:
☐ *Nursing Research Committee Approval Date:
☐ YSM/YNHH Cancer Data Repository (CaDR) Approval Date:
☐ Dept. of Lab Medicine request for services or specimens form
☐ Imaging on YNHH Diagnostic Radiology equipment request form (YDRCTO request) found at http://radiology.yale.edu/research/ClinTrials.aspx)

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

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities. 7 years

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

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

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

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

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

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

If yes, where is it registered?

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

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

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

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

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

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

Yes ☐ No☒

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

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8.. Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes ___ No __X_ *If Yes, please answer questions a through c and note instructions below. If No, proceed to Section III.*
a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform?
b. Will you be using any new equipment or equipment that you have not used in the past for this procedure?

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

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

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

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

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

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

_____ _____

PI Name (PRINT) and Signature Date

As the **faculty advisor** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- This project has scientific value and merit and that the student or trainee investigator has the necessary resources to complete the project and achieve the aims.
- I will train the student investigator in matters of appropriate research compliance, protection of human subjects and proper conduct of research.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- The student investigator will obtain approval for this research study and any subsequent revisions prior to initiating the study or revision and will obtain continuing approval prior to the expiration of any approval period.
- The student investigator will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set forth by the University and qualify to serve as the faculty advisor of this project.
- I assume all of the roles and responsibilities of a Principal Investigator even though the student may be called a PI.

_____ _____

Advisor Name (PRINT) and Signature Date

Department Chair's Assurance Statement

Do you know of any real or apparent institutional conflict of interest (e.g., Yale ownership of a sponsoring company, patents, licensure) associated with this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC.)
☐ No

As Chair, do you have any real or apparent protocol-specific conflict of interest between yourself and the sponsor of the research project, or its competitor or any interest in any intervention and/or method tested in the project that might compromise this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC)
☐ No

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I assure the HIC that the principal investigator and all members of the research team are qualified by education, training, licensure and/or experience to assume participation in the conduct of this research trial. I also assure that the principal investigator has departmental support and sufficient resources to conduct this trial appropriately.

_____ _____

Chair Name (PRINT) and Signature Date

_____

Department

YNHH Human Subjects Protection Administrator Assurance Statement

Required when the study is conducted solely at YNHH by YNHH health care providers.

As Human Subject Protection Administrator (HSPA) for YNHH, I certify that:

- I have read a copy of the protocol and approve it being conducted at YNHH.
- I agree to notify the IRB if I am aware of any real or apparent institutional conflict of interest.
- The principal investigator of this study is qualified to serve as P.I. and has the support of the hospital for this research project.

_____ _____

YNHH HSPA Name (PRINT) and Signature Date

SECTION IV: RESEARCH PLAN

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

Our project has two overarching goals. 1) We will use newly developed positron emission tomography (PET) technology to investigate the dopaminergic neurochemistry of drugs of abuse including marijuana, traditional cigarettes, and 2) We will extend our PET technology to an additional neurotransmitter system – namely, the opioid-ergic system, using the same drugs of abuse.

The majority of the research investigating the neurochemical mechanisms involved in drug addiction has been conducted in animals. From these studies it is well known that the mesolimbic dopamine (DA) system drives the reinforcing effects of drugs of abuse. In particular, a seminal study demonstrated that DA is released (in animals) by all drugs that are abused by

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humans[1]. In addition, it has been hypothesized that DA influences reward "wanting", while the opioid system governs reward "liking"[2], both of which are critical for understanding neurochemical processes underlying compulsive drug use. These findings in animals need to be confirmed in humans to validate the animal models, to improve our understanding of the neurochemistry of drug abuse, and so that we can test new medications in humans. With PET we can noninvasively, and now dynamically, measure neurotransmitter changes in response to drugs of abuse in humans. This investigation will utilize a system previously developed by our group for imaging and modeling DA release in humans from cigarette smoking in the PET scanner. Our experimental and analytical techniques are sensitive enough to detect moderate increases in striatal DA (in magnitude and spatial extent) that are expected with marijuana. Our model is also uniquely able to capture the *temporal profile* of *brief* DA responses. The goals of this study are to extend our paradigm to additional drugs of abuse (marijuana) and to extend our technology to an additional neurotransmitter system (opioid-ergic) with the same drugs of abuse (tobacco, marijuana). This will allow us to determine the spatio-temporal patterns of combined DA and beta-endorphin fluctuations associated with drugs of abuse in the living human brain.

Aim 1: **To examine the magnitude, location and timing of drug-induced dopamine release.**

- **a.** Tobacco smokers (n=20) will be imaged with [^{11}C]raclopride PET, after overnight abstinence from tobacco. Subjects will smoke their preferred brand of cigarette during the PET scan.
- **b.** Marijuana smokers (n=20) will be imaged with [^{11}C]raclopride PET, after overnight abstinence from marijuana. Subjects will smoke a marijuana cigarette during the PET scan.

**Aim 2: To examine the magnitude, location and timing of drug-induced beta-endorphin release. ***We will attempt to use the same subjects from Aim 1 for Aim 2 (Marijuana Exposed Healthy Controls are the exception)

- **a.** Tobacco smokers (n=20) will be imaged with [^{11}C]carfentanil PET, after overnight abstinence from tobacco smoking. Subjects will smoke their preferred brand of cigarette before or during the drug condition PET scan.
- **b.** Marijuana smokers and Marijuana Exposed Healthy Controls (n=20) will be imaged with [^{11}C]carfentanil PET, after overnight abstinence from marijuana smoking. Subjects will smoke a marijuana cigarette before or during the drug condition PET scan.

Aim 3: To examine the magnitude, location and timing of marijuana-induced dynorphin release. Cannabis has diverse effects on the endogenous opioid system. We will image Marijuana Smokers and Marijuana Exposed Healthy Controls (n=20) with [^{11}C]EKAP PET, after overnight abstinence from marijuana smoking. Subjects will smoke a marijuana cigarette before or during the drug condition PET scan.

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

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The neurochemistry of drug addiction. Drug addiction is a widespread public health issue, affecting at least one quarter of the adult U.S. population. A key process of chronic drug use is the transition from voluntary to compulsive use in spite of adverse consequences. The rewarding aspects of drug abuse are known to be critical to both the initiation, i.e., voluntary, and maintenance of drug use[4]. Reward processes are primarily influenced by the DA and opioid neurotransmitter systems. It has been hypothesized that DA influences reward "wanting", while the opioid system governs reward "liking"[2], both of which are critical for understanding neurochemical processes underlying compulsive drug use. For example, initial drug seeking for the pleasurable aspects of the drug ("liking") often gives way to habitual drug use ("wanting"), particularly in response to drug cues, despite individuals reporting little pleasure from the drug itself[5]. While much preclinical work has studied dopamine-opioid interactions in reward response[6,7], extending this work to study the neurochemistry of reward response in ***clinical*** substance abuse disorders has been limited.

Using PET to study to neurochemistry of drug addiction. PET neuroreceptor imaging has revealed a wealth of *in vivo* research examining *basal* levels of dopaminergic and opioid receptor availability in substance abuse disorders. In other words, these studies ask the question, "*In the absence of drug administration, how many receptors are available for radioligand binding?"* Using these methods, lower dopamine D_2/D_3 receptor availability in drug users compared to healthy controls has been identified as a key hallmark of substance abuse disorders, and this currently influences therapeutics development[8,9]. In contrast, different substances have diverse effects on the opioid system. Reduced μ-opioid receptor availability in smoking[10] and nicotine dependence severity[11,12], while increased μ-opioid receptor availability was associated with chronic cocaine use[13-16], heroin dependence[17,18], alcohol dependence[19-21], and alcohol craving[21-23]. Different drugs of abuse may thus affect the opioid receptors in a variety of ways, underscoring the importance of studying the opioid system in substance abuse disorders.

To build on these findings, many groups have attempted to use PET neuroreceptor imaging to examine the *acute drug response* of the dopamine and opioid systems *in vivo*. These studies are designed to ask the question, *"How does the number of receptors available for radioligand binding change after drug administration?"* The common methodology for these experiments is to use two PET scans to measure the neurotransmitter response, inferred by changes in radioligand binding, following drug administration. First, a baseline PET scan is acquired to measure the available receptors indexed by binding potential (BP). Next, the drug is administered, followed by a second PET scan acquired to measure the post-drug BP. If the drug leads to neurotransmitter release, the additional neurotransmitter will bind to the receptor site, decreasing the number of available receptors for radioligand binding (and BP). Measured changes in BP (baseline BP minus post-drug BP) thus enable an indirect measure of neurotransmitter increase. This two-scan paradigm has been used to repeatedly demonstrate a blunted dopamine response to amphetamine administration in individuals chronically using drugs of abuse[24-27]. However, BP measures assume steady-state radioligand binding, and thus steady-state neurotransmitter levels, for the scan duration.

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This assumption works well with amphetamine because this drug induces a robust and long-lived dopamine elevation that remains steady during the entire post-drug scan, allowing for reliable estimates of changes in dopamine levels with the two-scan paradigm. Other drug stimuli, such as cigarette smoking, induce short-lived neurotransmitter release that dynamically changes radioligand binding throughout the post-drug scanning period. The classic BP paradigm can underestimate or even fail to detect such transient neurotransmitter responses[28]. Thus, ***alternative analysis methods are needed to identify short-lived neurotransmitter activation with PET imaging.***

We have developed a new imaging paradigm, known as linear parametric neurotransmitter-PET (lpntPET), to explicitly accommodate time-varying changes in radioligand binding while scanning, enabling accurate detection of short-lived neurotransmitter response using PET imaging[29-33]. lpntPET identifies the spatial extent of neurotransmitter activation at the voxel level with improved sensitivity and specificity relative to two-scan paradigms[28,34], while also characterizing the temporal component of endogenous neurotransmitter activation[33,34]. This approach revealed remarkable differences between men and women both in the brain areas and temporal dynamics of dopamine release in response to cigarette smoking[35]. Importantly, these findings were consistent with men tending to smoke for the reinforcing effects of nicotine than women[36] (i.e., sex differences in cigarette "wanting"). However, to date lpntPET has only been used to image changes in the dopamine[31,35] and acetylcholine[37] systems. Given the close interaction of the dopamine and opioid systems in the dynamics of reward processing, validating ntPET with radioligands sensitive to endorphin activation will provide powerful tools enhancing *in vivo* study of reward processes in the brain with PET imaging.

PET imaging of the opioid system has primarily been conducted with the μ-selective [^{11}C]carfentanil[38] and non-selective [^{11}C]diprenorphine[39] radioligands. Both radioligands are reported to be sensitive to endorphin levels, the opioid peptides that preferentially act at μ- and δ-opioid receptors[40]. Reduced [^{11}C]carfentanil binding, presumably due to increased β-endorphin levels, was reported in response to acute somatic pain[41-43], affective responses[18], placebo administration[44], oral amphetamine administration[45,46], and cigarette smoking[47]. In particular, one study reported variation in average [^{11}C]carfentanil BP over time following acute somatic pain[42] suggesting time-varying β-endorphin response to pain, albeit with biased analysis methods. These initial reports suggest that [^{11}C]carfentanil is a suitable radioligand to detect short-lived changes in β-endorphin levels. Moreover, increasing interest in marijuana's diverse effects on the endogenous opioid system have identified an important role for dynorphin in potentially mediating marijuana's putative analgesic effects. The Yale PET center has developed the novel agonist radioligand [^{11}C]EKAP that exhibits kinetic properties consistent with radiotracer sensitivity to endogenous dynorphin levels. Given repeated observations of short-lived opioid peptide responses to drugs of abuse with microdialysis techniques[48,49] (see [50] for review), ***extension of the lpntPET paradigm to the opioid system could provide groundbreaking* in vivo *characterization of endorphin release following drug stimulus*.**

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In the current proposal we propose to image the dopaminergic and the opioidergic response to drugs of abuse in the same subjects. Thus, we will be able to potentially visualize multiple neurotransmitter systems within the same person and we will be able to compare the neurotransmitter system map between drugs of abuse.

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

3.1 Overall Research Design:

This study will recruit drug users (tobacco or marijuana) to participate in one magnetic resonance imaging scan (MRI),one [^{11}C]raclopride PET scan, two [^{11}C]carfentanil PET scans, and two[^{11}C]EKAP PET scans (for marijuana users/marijuana exposed) . If subjects have never been in a PET scanner, they may participate in a brief "mock" PET scan session prior to their actual scan to familiarize them with the environment (reduce the novelty) and if they are a smoker, to familiarize them with the mechanics of smoking in the scanner.

Aim 1. **To examine the magnitude, location and timing of drug-induced dopamine release.**

- **a.** Tobacco smokers (n=20) will be imaged with [^{11}C]raclopride PET, after overnight abstinence from tobacco smoking. Subjects will smoke their preferred brand of cigarette during the PET scan.
- **b.** Marijuana smokers (n=20) will be imaged with [^{11}C]raclopride PET, after overnight abstinence from marijuana smoking. Subjects will smoke a marijuana cigarette during the PET scan.

Aim 2. **To examine the magnitude, location and timing of drug-induced beta-endorphin release. ***We will attempt to use the same subjects from Aim 1 for Aim 2. (Marijuana Exposed Healthy Controls are the exception)

- **a.** Tobacco smokers (n=20) will be imaged with [^{11}C]carfentanil PET, after overnight abstinence from tobacco smoking. Subjects will smoke their preferred brand of cigarette before or during the drug condition PET scan
- **b.** Marijuana smokers and Marijuana Exposed Healthy Controls (n=20) will be imaged with [^{11}C]carfentanil PET, after overnight abstinence from marijuana smoking. Subjects will smoke a marijuana cigarette before or during the drug condition PET scan.

Aim 3: To examine the magnitude, location and timing of marijuana-induced dynorphin release. Cannabis has diverse effects on the endogenous opioid system. We will image Marijuana Smokers and Marijuana Exposed Healthy Controls (n=20) with [^{11}C]EKAP PET, after overnight abstinence from marijuana smoking. Subjects will smoke a marijuana cigarette before or during the drug condition PET scan.

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For Aim 1 using a single scan paradigm, drugs will be administered beginning approximately 35 minutes after the radiotracer injection, as previously validated[35]. For Aim 2 and 3, since this approach has not yet been done in people with [^{11}C]carfentanil or [^{11}C]EKAP, we will use a two scan paradigm (baseline and challenge). Drugs will be administered either before (approximately 15 min prior to radiotracer injection) or during (30-60 minutes after radiotracer injection) drug condition PET scan, which may occur after a baseline condition PET scan. The scans will be analyzed to produce the magnitude *and* temporal characteristics of the DA and beta-endorphin signature of drug use under each condition.

3.2 Subject Selection:

All research subjects will be recruited under guidelines of the Yale University Institutional Review Board (Human Investigation Committee). Subjects will be recruited from the community at large via IRB-approved advertising (television, newspaper, postings in community locations, Craigslist, Facebook).

Screening Evaluation

Potentially eligible subjects will undergo an initial screening evaluation within 6 months of PET scanning. The purpose of this evaluation is to ensure that subjects meet study criteria. After informed consent is obtained, a medical history, vital signs, physical examination, and EKG will be performed. Several laboratory tests will be performed at this visit, which may include a complete blood count (CBC), chemistry profile, thyroid function studies, serum ß-HCG (women only), urinalysis, and urine toxicology screen. All female subjects will undergo a pregnancy test at the time of screening. Additionally, urine pregnancy tests will be done on the day of each PET scan before radiotracer injection. Urine drug screens will be done at intake and on PET days.

3.3 Assessments:

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

3.3a. General Intake Assessments

1. Demographic Questionnaire This questionnaire will obtain: (1) basic demographic information including age, gender, marital status, employment status, occupation, (2) alcohol/drug history, (3) family history of alcohol/drug use, depression, anxiety, and smoking history.
2. Medical History This questionnaire will obtain a basic medical history (personal and family) including past or current conditions such as neurological, endocrine, cardiovascular, renal, liver, and thyroid pathology. Current body weight and current medications will also be assessed.
3. Medical Assessments will include a physical exam by a state licensed physician, an EKG, and laboratory tests which may include a complete blood count, blood urea nitrogen, creatinine, fasting blood sugar, electrolytes, liver function tests, thyroid function tests (including T_3, T_4, T_3RU, estimated free T4), thyroid stimulating hormone levels, urine toxicology, and urinalysis**.** Female subjects will have serum pregnancy tests.
4. Illicit Drug/Pregnancy Screen A urine sample may be collected to determine current illicit drug use (for all potential subjects). In addition, serum samples will be collected at the intake

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visit and urine samples will be collected on each PET scan day to confirm that the subject is not pregnant.

5. Structured Clinical Interview for DSM-IV Axis I Disorders The psychotic screening and depression sections of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) will be used to determine whether subjects meet exclusion criteria for diagnosis of significant psychopathology such as schizophrenia and bipolar disorder [38].

6. Columbia Suicide Severity Rating Scale (C-SSRS). The C-SSRS is designed to measure suicidal ideation (50).

7. Multidimensional Scale of Perceived Social Support (MSPSS) is a brief research tool designed to measure perceptions of support from 3 sources: Family, Friends, and a Significant Other.

8. Barratt Simplified Measure of Social Status (BSMSS) is a measure of socio-economic status.

3.3.b. Mood Measures

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

1. Center for Epidemiological Studies Depression Scale (CES-D) The CES-D[39] is a 20-item self-report instrument, which has been extensively used in both clinical and nonclinical populations to measure the frequency and severity of depressive symptoms over the past week. The CES-D, which has been used to document the severity of depressive symptoms in adults and has been shown to be a sensitive measure of negative affect in smokers, will be used in the proposed studies to measure levels of mild depressive symptoms.

2. Anxiety: The State-Trait Anxiety Inventory[40] is a 40-item, self-report measure, comprised of two subscales. The State-Anxiety scale is 20 items and assesses transitory states characterized by feelings of tension, apprehension, and heightened autonomic reactivity. The Trait-Anxiety scale is 20 items and assesses stable individual differences in anxiety proneness.

3. Impulsivity: Barratt Impulsiveness Scale (BIS; [51]) is a 30 item self-report instrument designed to assess the personality/behavioral construct of impulsiveness.

4. Beck Depression Inventory: This widely used 21 item self-report instrument will be used to assess depressive symptomatology in addition to the SCID (43).

3.3.c. Smoking and other Drug Measures

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

1.Fagerstrom Test for Nicotine Dependence (FTND). This will be used to measure the severity of nicotine dependence. It is a 6-item scale with an internal consistency of .61 and its total score is closely related to biochemical measures of intensity of smoking. A score of at least 3 is necessary for inclusion in the study.

2. Smoking History. This questionnaire will assess basic smoking status and history such as number of years smoked, number and length of quit attempts, reasons for quitting, and second hand smoke exposure.

3. Nicotine Withdrawal Checklist. This measures the severity of eight withdrawal symptoms on 5-point Likert scales.

4. Tiffany Questionnaire of Smoking Urges (QSU). The QSU-brief is a 10-item questionnaire that evaluates the structure and function of smoking urges. Subjects indicate on a likert-type scale how strongly they agree or disagree with each statement with a score of 1 (strongly agree)

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to 7 (strongly disagree). This characterizes 'urges to smoke' into a negative affect related to relief of withdrawal symptoms and positive affect related to expectancy of reinforcement.
5. Wisconsin Predicting Patients' Relapse Questionnaire (WI-PREPARE). A brief, 7-item questionnaire that predicts relapse rate (49).
6. Timeline Followback for alcohol, tobacco, and illicit drugs. Questionnaire to measure the amount of alcohol, tobacco, and other drugs that have been used in the past 30 days.
7. Visual Analog Scale (VAS) self ratings for drug effects. Subjects will answer questions to assess behavioral changes induced by substance use.

8. Cannabis Problems Questionnaire is a 22 item self-report instrument to document cannabis-related problems.
9. Marijuana Motives Questionnaire is a 25 item self-report instrument to characterize individual's motivations for using marijuana.
10. Marijuana Smoking History Questionnaire is a 23 item self-report instrument to characterize the dose and frequency of marijuana use and quit attempts, both over lifetime and the past 30 days.
11. Marijuana Craving Questionnaire is a 12 item self-report instrument to document marijuana craving.
12. Cannabis Withdrawal Scale is a 19 item self-report questionnaire to document symptoms of cannabis withdrawal.
13. Clinician Administered Dissociative Symptoms Scale is a 27 item instrument administered by trained research staff to characterize present-state dissociative symptoms.

3.3.d. Cognitive Measures

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

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

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

Probabilistic Reward Task (PRT) – The PRT has been successfully used to assess reward responsiveness (51-53). In each trial, subjects choose which of two difficult-to-differentiate stimuli was presented. Stimuli consist of simple cartoon faces (diameter: 25 mm; eyes: 7 mm) presented in the center of the monitor. At the beginning of the trial, the face has no mouth. After a given delay, either a straight mouth of 11.5 mm ("short mouth") or 13 mm ("long mouth") is presented for 100 ms. Subjects are instructed to press an appropriate button to decide whether a long or small mouth had been presented. Unbeknownst to subjects, correct identification of one stimulus

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("rich stimulus") is rewarded three times more frequently (*"Correct! You won 20 cents"*) than the other ("lean") stimulus. In healthy controls, this reinforcement schedule leads to a response bias (i.e., a preference for the more frequently rewarded stimulus). The degree of response bias toward the more frequently reinforced alternative will be used for operationalizing sensitivity to reward.

Cold Pressor Task

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

3.4 Procedures

Screening, Evaluation and Clinical Ratings

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

Magnetic Resonance Imaging (up to 1 hour)

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

3T Scan sequence:

Series 1: 3 plane localize
Series 2: Sag 3d tfl; 256fov; 1mm thick slices; 176 slices total; TE 2.77; TR 2530; TI 1100; FA 7; 256X256 1 average.
Series 3 – Resting state: Ep2d bold; 210fov; 2.5mm thick slices; TE 30; TR 3400; FA 85; 84x84 (Run twice).

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

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

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

PET Scans

PET experiments will be conducted at the Yale University PET Center. Subjects will have one [^{11}C]raclopride PET scan, two [^{11}C]carfentanil PET scans, and/or two [^{11}C]EKAP PET scans with different radiotracer studies on separate days. If technical difficulties arise, additional PET scans

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may be scheduled at a later date, for a total of up to 8 PET scans. We may ask subjects to refrain from eating 4-6 hours prior to injection and remain fasting until the study is over. Subjects may also be asked to refrain from drinking caffeinated beverages that day, and from drinking alcoholic beverages the week prior to the scan.

All participating subjects will be asked to abstain from smoking or drug use for approximately 12 hrs before each scan (e.g., overnight abstinence) to maximize craving). Upon arrival at the PET center, subjects will complete questionnaires on drug urges, withdrawal symptoms, and mood. Plasma will be drawn before scans to measure drug levels in the blood. CO levels will also be measured (MicroDirect CO Monitor) upon arrival for scan. CO < 11ppm indicates that subjects were abstinent as requested prior to tobacco and marijuana sessions. If the CO level is positive, subject participation will be up to the discretion of the PI. .Urine drug tests may be performed before all PET scansSubjects' vitals will be monitored throughout the scans up until at least an hour before discharge.
For all women, a urine pregnancy test will be performed at the beginning of the imaging day, prior to radiotracer injection, at the Yale University PET Center.

PET Data Acquisition and Analysis
Subject preparation consists of intravenous (IV) catheterizations and immobilization of the head. Venous catheters will be used for IV administration of the radiotracer and blood sampling. For [^{11}C]EKAP scans, a radial artery catheter will be inserted by an experienced physician in the morning before the PET scan. The site will be anesthetized with lidocaine prior to arterial line insertion. The arterial line will remain in place for the whole day of scanning, after which it will be removed. PET scans are acquired as subjects rest using an HRRT, PET-CT, or NX-CT scanner . For HRRT scans, a transmission scan using an orbiting ^{137}Cs point-source is obtained for each emission scan. Motion correction will be performed dynamically with measurements from the Vicra (NDI Systems, Waterloo, Ontario) used by a dedicated list-mode reconstruction algorithm [52].

The PET scans will be acquired using IV bolus or bolus plus constant infusion administration of up to 20 mCi or less of high-specific activity [^{11}C]raclopride, and/or[^{11}C]carfentanil, and /or [^{11}C]EKAP using a dynamic PET scan of up to 120 min.

For NX scans, a stereovision motion tracking system will be used. This markerless (no head attachment) monitoring system uses 3D cameras mounted to the PET camera that will track head motion using optical measurement of facial features (face detection). No recognizable images of the subject's face will be recorded. Data about the subject's facial features will be captured in order to detect movement of the head while on the scanner bed. A computer-generated grid that detects movement will generate the motion data. Data may be shared with the manufacturer, United Imaging Healthcare America (UIHA). Data will be coded, and though images of the head will be captured, no other identifying information will be attached to the data acquired and shared with UIHA. A low-dose head CT scan will be acquired before or after each NX-CT and PET-CT scan to be used for attenuation correction.

For HRRT scans, a transmission scan of approximately 6 minutes will be acquired for each emission scan to be used for attenuation correction. Acquisition of HRRT list-mode data will begin shortly before radiotracer injection and will continue for up to 120 minutes post-injection. Dynamic images of radioactivity concentration are reconstructed with corrections for measured attenuation, normalization, random events, scatter, and deadtime. Subject motion is also corrected automatically on an event-by-event basis with the Vicra. The resolution (full width at

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half maximum) of the HRRT is 2-3 mm. The extended scanning time will reduce the noise in data acquisition, and thus, improve the binding potential (BP) estimates. This will be more important if we want to quantitate in small regions with the HRRT, specifically the ventromedial/dorsomedial PFC, NAc, amygdala, ventral/dorsal striatum, GP, and SN. Individual IV blood samples may be taken IV prior to radiotracer injection and during the scan for analysis of the fraction of plasma radioactivity unbound to protein and for metabolite analyses. For [^{11}C]EKAP scans, after arterial cannulation has been performed by a qualified physician, individual blood samples are manually taken at various time points and counted in a gamma counter. Samples are centrifuged to obtain plasma, which will be counted, and selected samples will be assayed for the presence of the parent radiotracer compound that has not been metabolized. These measurements will be performed by HPLC. In addition, the fraction of plasma radioactivity unbound to protein will also be determined. Subjects will be asked to void after the scan is completed to reduce radiation exposure to the bladder.

Tobacco Smoking in the Scanner

All subjects may initially participate in mock PET session during which they will lie in a replica of the PET scanner and be told when the scanner is being “turned on”. In addition, the subjects will be instructed to smoke with minimal to no head movement and to avoid bringing their smoking hand into the field of view of the scanner. They will practice being guided to flick their ashes into a large ashtray held at their side during the smoking epochs. These procedures will minimize the potential for motion during scanning. They will also help reduce order effects in the current design that might otherwise be caused by anxiety or expectation since it has been shown that even a scan of the rest condition can be affected by expectation.

On the scan day(s)*,* subjects will smoke 1 cigarette at a pre-determined time in the scanner (approximately 35 minutes following radiotracer injection for [^{11}C]raclopride; from approximately 15 min before radiotracer injection to 60 min afterwards for [^{11}C]carfentanil). Subjects will understand from the preceding training session how to bring the lit cigarette to their mouths, roughly how many puffs to take (~10), at what speed, and where to deposit their ashes. Subjects positioned normally in the HRRT will still have enough clearance to smoke comfortably (based on dry runs).

Prior to scanning, subjects will be asked to rate their craving immediately before their drug, and their satisfaction from craving, feeling of reward, and drug “liking” periodically thereafter. They will also be asked about unpleasantness, and perceived high pre- and post-scan.

To assess the specific reinforcement from smoking the cigarette in the scanner, the Cigarette Evaluation Scale will be administered immediately after the PET scan. The questions (see below) are answered with reference to the immediate past cigarette (on a scale from 1 = “not at all”, to 7 = “extremely”)

1. Was smoking satisfying?
2. Did cigarettes taste good?
3. Did you enjoy the sensations in your throat and chest?
4. Did smoking calm you down?
5. Did smoking make you feel more awake?
6. Did smoking make you feel less irritable?
7. Did smoking help you concentrate?
8. Did smoking reduce your hunger for food?

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9. Did smoking make you dizzy?
10. Did smoking make you nauseous?
11. Did smoking immediately relieve your craving for a cigarette?
12. Did you enjoy smoking?

Marijuana Smoking in the Scanner

All subjects may initially participate in mock PET session during which they will lie in a replica of the PET scanner and be told when the scanner is being "turned on". They will practice taking deep breaths. These procedures will minimize the potential for motion during scanning. They will also help reduce order effects in the current design that might otherwise be caused by anxiety or expectation since it has been shown that even a scan of the rest condition can be affected by expectation.

Subjects will have abstained from cannabis use overnight and at least for 12 hours (verified by carbon monoxide levels ≤ 11 ppm). At the pre-determined time (approximately 35 minutes following radiotracer injection for [^{11}C]raclopride; from approximately 15 min before radiotracer injection to 60 min afterwards for [^{11}C]carfentanil), subjects will smoke up to the entirety of a standardized cannabis cigarette (Between 3.5% and 10% THC) obtained from NIDA. We will use a protocol similar to other studies in which subjects inhale for 3 sec, hold the smoke in their lungs for 5 sec and exhale. They will smoke up to the entirety of the cigarette at approximately 30 sec intervals per puff, subjects will not be asked to smoke more than they are accustomed to. Subjects will rate their craving immediately before smoking, and their satisfaction from craving, feeling of reward, and drug "liking" periodically thereafter. Subjects will be asked about unpleasantness and perceived "high" pre- and post-scan.

Data Processing

All PET data will be reconstructed with an iterative reconstruction algorithm, MOLAR, designed specifically for the HRRT. MOLAR incorporates attenuation, scatter and deadtime, and subject-motion (detected by the Vicra system), into the forward projection model for the scanner. Emission images are binned into equal frame times (typically 3 minutes). Following image reconstruction, an early summed image (0-12 min) will be registered to the subject's MR image, which will be registered to an MR template so PET can be transformed to a standard (MNI) space. HYPR filtering will be applied to all emission data before analysis. lp-ntPET and voxelwise analysis will be conducted.

4. Genetic Testing N/A ☐

A. Describe

i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned

- One 10 mL tube of blood will be collected for DNA for testing of polymorphisms in genes of interest to drug addiction. This sample may be collected during the screening appointment or on one of the PET scan days.

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

-as above, a blood sample will be collected at intake to test polymorphisms of interest for drug dependence.

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

- the genetic polymorphism results will be entered. The results of this testing will be confidential, will not be entered into the subject's medical record, and will not be made available to the subject.

iv. the methods to uphold confidentiality

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

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

Some samples and related information may be stored (banked) for future research. The specimens will be stored for an unlimited time and may be used to make a cell line that will live indefinitely. When specimens and information are stored, we are careful to try to protect subject identity. Samples and information will receive a unique code. Other researchers will only receive coded samples and information.

C. Is widespread sharing of materials planned?

Yes

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

It will not.

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

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

Subjects will be informed that their material has been destroyed.

F. Describe the provisions for protection of participant privacy

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Risks associated with genetic testing will be minimized by keeping the results of genetic testing on encrypted computers or a secure server, and by separating the personal identifying information of the subjects from the genetic information.

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

The results of genetic testing will be kept on an encrypted computer or secure server and personal identifying information of the subjects will be separated from the genetic information. Materials will not be shared.

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

Up to 110 subjects (55 men, 55 women) will participate in the study. According to census figures, minority groups comprise approximately 50% of the population of New Haven (36.1% African-American, 13.2% Hispanic, 0.3% Native American, 2.4% Asian, and 4.1% other). While we prefer to have 60 subjects complete both Aim 1 and Aim 2, we anticipate up to a 50% drop out rate between scans so we are allowing for the possibility having 110 subjects participate.

All subjects will be 18-55 years of age and physically healthy. All research subjects will be recruited under guidelines of the Yale University Institutional Review Board (Human Investigation Committee). Subjects will be recruited from the community at large via IRB-approved advertising (television, newspaper, postings in community locations, Craigslist, Facebook). All participants will be screened initially on the telephone by a research assistant who will schedule the initial intake assessment. The screening and scanning sessions will involve psychological tests in the form of rating scales and questionnaires. These are all noninvasive questionnaires, e.g., to assess mood and craving, and should add no risk.

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

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

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

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

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General Inclusion Criteria:

1. Men and women, aged 18-55 years
2. Able to read and write English and give voluntary written informed consent
3. Not treatment seeking or using treatment medications

Tobacco Smokers

1. Have a Fagerström Test for Nicotine Dependence (FTND) rating of at least 3.
2. Have been using at least 7 cigarettes per day for at least 1 year
3. Carbon monoxide levels > 10 ppm during intake evaluation
4. Urine cotinine levels of > 150 ng/mL during intake evaluation
5. Are not current users of marijuana or other illicit drugs

Marijuana Smokers

1. Meet DSM-V criteria for cannabis use disorder based on the structured clinical interview diagnostic (SCID) or regular cannabis use of ≥5 times/week
2. Test positive for THC
3. Have been smoking cannabis on a regular basis for ≥ 1 year

Marijuana Exposed Health Controls

1. Do not meet DSM-V criteria for cannabis use disorder based on the structural clinical interview diagnostic (SCID)
2. Have used cannabis in the past recreationally

General Exclusion Criteria:

1. Current significant medical condition such as neurological, cardiovascular, endocrine, renal, liver, or thyroid pathology.
2. History of or current neurological or significant psychiatric disorder such as schizophrenia or bipolar disorder (DSM-IV Axis 1).
3. History of significant head trauma.
4. Women who are pregnant or nursing, or fail to use one of the following methods of birth control unless she or partner is surgically sterile or she is postmenopausal (hormone contraceptives [oral, implant, injection, patch, or ring], contraceptive sponge, double barrier [diaphragm or condom plus spermicide], or IUD).
5. Regular or current significant use of any prescription, herbal or illegal psychotropic medications (e.g., antidepressants, antipsychotics, anxiolytics, ecstasy) in the past 6 mo, with no current illegal drug use confirmed by urine toxicology (marijuana when relevant).
6. Significant substance misuse (including alcohol, and excluding cannabis and marijuana when relevant) that in the PI's determination interferes with the study results or safety of the subject.
7. Have MRI-incompatible implants and other contraindications for MRI, such as a pacemaker, artificial joints, non-removable body piercings, claustrophobia, etc.
8. Subjects with history of prior radiation exposure for research purposes within the past year such that participation in this study would place them over FDA limits for annual radiation exposure. This guideline is an effective dose of 5 rem received per year.

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9. Subjects with current, past or anticipated exposure to radiation in the work place within one year of proposed research PET scans.
10. Subjects with history of IV drug use which would prevent venous access for PET tracer injection.

8. How will **eligibility** be determined, and by whom?

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

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

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

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

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

Drawing blood and inserting an intravenous line (IV) into an arm vein are safe and standard medical procedures. Sometimes a bruise will occur at the puncture site and rarely a blood clot or infection will occur in the vein. Certain individuals may feel light-headed during venipuncture. The volume of blood collected during this study will be up to 32 tablespoons. Blood samples may be drawn for routine labs and drug screening; measurement of radiopharmaceutical parent and metabolites, for analysis of plasma drug levels, serum estrogen, progesterone, and follicle stimulating hormone levels. This is not expected to have any serious negative effects on a study participant.

Risks Associated with Arterial Line
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.

Risks Associated with Radiation

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The Yale University Radioactive Investigational Drug Committee (Yale RIDC) will review the use of radiation in this research study, and no subjects will be scanned until RSC approval is obtained. This research study involves exposure to radiation from [11C]raclopride, [11C]carfentanil PET, and [^{11}C]EKAP scanning, all of which will be under IND **#132971** . This radiation exposure is not necessary for medical care and is for research purposes only.

The maximum amount of radiation an individual subject will receive in this study is from one injection of ≤ 20 mCi of [11C]raclopride (0.46rem), two injections of ≤ 20 mCi of [11C]carfentanil (0.68rem total), two injections of ≤ 20 mCi of [11C]EKAP (0.64 rem total) (plus a small amount of radiation from the transmission scans of the brain). However, in situations where a PET scan is not successful following a radiotracer injection (e.g., problems with the PET camera), the subject may receive an additional [11C]raclopride, [11C]carfentanil, and [11C]EKAP injection, for up to **8** total radiotracer injections during the study, if deemed appropriate.

Although each organ will receive a different dose, the targeted amount of radiation exposure subjects will receive from this study is equal to an effective dose of **2.01 rem** for a total of up to 20 mCi of [11C]raclopride in one injection, up to 40 mCi of [11C]carfentanil in two injections, and up to 40mCi of [11C]EKAP. This calculated value is used to relate the dose received by each organ to a single value.

In the event that a scan fails after radiotracer injection and the subject is scheduled for a second injection of [11C]raclopride, a third injection of [11C]carfentanil, each subject would also receive an additional effective dose of 1.26rem, for a total of up to 40 mCi of [11C]raclopride in two injections, up to 60 mCi of [11C]carfentanil in three injections, and up to 60 mCi of [11C]EKAP in three injections. **Therefore, the maximum possible radiation exposure for this study, taking additional injections due to cancellation into account, would be an effective dose of up to approximately 3.26rem.**

This amount of radiation exposure is below the annual limit of 5 rem, followed by the Yale University PET Center, which is also the occupational exposure limit.

Studies of occupational workers who are chronically exposed to low levels of radiation above normal background have shown no adverse biological effects. However, to be conservative, we assume that any amount of radiation may pose some risk for causing cancer and hereditary effect, and that the risk is higher for higher radiation exposures. This means that any increase in radiation dose, no matter how small, results in an increase in risk. The U.S. Nuclear Regulatory Commission (NRC) accepts this model, referred to as the “linear no-threshold (LNT) dose-response relationship”, for estimating radiation risk. Monitoring of radiation exposure is done by **Yale University Radioactive Investigational Drug Committee** for research subjects.

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.

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

Magnetic resonance imaging (MRI) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States

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Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines.

Subjects will be watched closely throughout the MR study. Some people may feel uncomfortable or anxious. If this happens, the subject may ask to stop the study at any time and we will take them out of the MRI scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly but we will ask subjects to tell the research staff if they have any of these symptoms.

There are some risks with an MRI study for certain people. If subjects have a pacemaker or some metal objects inside their body, they may not be in this study because the strong magnets in the MRI scanner might harm them. Another risk is the possibility of metal objects being pulled into the magnet and hitting a subject. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. We also ask all people involved with the study to walk through a detector designed to detect metal objects. It is important to know that no metal can be brought into the magnet room at any time. Also, once subjects are in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet.
We want subjects read and answer very carefully the questions on the MRI Safety Questionnaire related to your personal safety. We will be sure that subjects have read the MRI Safety Questionnaire and tell us any information they think might be important.

This MRI study is for research purposes only and is not in any way a clinical examination. The scans performed in this study are not designed to find abnormalities. **The primary investigator, the lab, the MRI technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a diagnostic evaluation of the images.** If a worrisome finding is seen on a subject's scan, a radiologist will be asked to review the relevant images. Based on his or her recommendation (if any), the primary investigator or consulting physician will contact the subject, inform them of the finding, and recommend that they seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie solely with the subject and your physician. **The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that a subject receives based on these findings. The images collected in this study are not a clinical MRI exam and for that reason, they will not be made available for diagnostic purposes.**

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

Risks Associated with Marijuana Smoking
THC Effects: Δ^9-THC is the primary active component in cannabis/marijuana and has been administered in very large oral doses (50-712 mg)[53] and modest intravenous doses (1-10 mg) to cannabis users and abusers without any serious adverse events[53]. Cannabis cigarettes have

been used in research studies in cannabis users and abusers without any serious adverse effects[52,66]

The unpleasant behavioral effects of Δ^9-THC include anxiety, panic, paranoia and rarely psychosis; all of these may be extremely distressing. However, the extent to which Δ^9-THC effects are perceived as unpleasant is context dependent. These effects are rare in our experience. Precautions will be taken to minimize the risk of discomfort to subjects.

The acute physical effects of Δ^9-THC include motor incoordination, tremulousness, muscle weakness, hypo or hypertension, tachycardia, conjunctival injection, dry mouth, and increased appetite, which generally resolve spontaneously. The estimated lethal dose of Δ^9-THC (30 mg/kg) is nearly 1000-fold higher than the dose proposed in this study (up to one ~3.5%-10% THC cannabis cigarette). Regular cannabis users recruited from this study are unlikely to report any adverse effects in response to the cannabis cigarette.

An air purifier approved by Yale Environmental Health and Safety will be used to filter cigarette and cannabis smoke from the air during all smoking challenges to minimize exposure to study personnel.

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

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

Risks Associated with Arterial line:
Risks of radial artery cannulation are minimized by having the procedure performed by an experienced physician. Pain is minimized by local anesthesia. Bleeding is prevented by local pressure applied for a minimum of 15 minutes after catheter removal. Subjects will have their hand and finger blood supply examined after arterial cannulation and again following catheter removal. Also, subjects will be asked to abstain from anticoagulants for 7-10 days prior to arterial line insertion and 7-10 days following arterial line removal. Subjects will be provided a 24 hour emergency physician telephone number to call if they encounter pain, discoloration, numbness, tingling, coolness, hematoma, inflammation, or any other unusual symptoms in the wrist or hand, or fever, chills or drainage from the vascular puncture sites, following the procedure. In addition, if an emergency arises at the time of cannulation or scanning, 911 will be called, and the subject will be sent to the Emergency Department for evaluation and treatment. Nurses will provide the subjects an instruction sheet documenting problems to watch for and procedures to follow should such problems occur. Infection is avoided by adequate cleansing of the skin prior to intravascular line insertion.

Risks Associated with Radiation:

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The dose of radiation will be submitted for approval to **the Yale University Radioactive Investigational Drug Committee** All scans will be done in the presence of medical supervision and trained staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the PET scan facilities have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital**.** Preparation of radiopharmaceuticals and performance of PET scans will be by radiochemists, physicians, and technologists of the Department of Diagnostic Radiology, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radiopharmaceuticals. Subjects will be asked about their previous radiation exposure and those who have had research exposure within the past year will be excluded if their cumulative annual exposure (including the present study) exceeds FDA limits. The information on the previous radiation exposure of study subjects will be notified to the study doctor.

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

Risks Associated with MRI Scanning.
To minimize risks, each subject will fill out the Yale Magnetic Resonance Research Center MRI Safety Questionnaire before the study. Only subjects who fulfill the criteria by this questionnaire will be eligible for the study. In addition, subjects will remove all metal (watch, hair pins, jewelry) and walk through the metal detector in the MRRC before entering the MRI room. If the subject has any metallic prostheses/implants they will be excluded from the study. If a subject becomes anxious during the scan they can request that the MRI scan be stopped.

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

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

Risks associated with marijuana smoking

3.5%-10% THC is considered a moderate dose that should not lead to any adverse effects in regular cannabis users. Minimization of the risk entailed by cannabis smoking will be accomplished by (a) criteria for a history of recent, regular use of cannabis $\geq$5 times/week (b) test positive for THC at screening (c) have been smoking cannabis on a regular basis for $\geq$ 1 year and (d) stringent criteria for the absence of clinically significant medical or primary psychiatric disorders.

Measures of heart rate, systolic blood pressure, and diastolic blood pressure will be obtained at baseline and every 15 minutes after cannabis administration. In the event of psychiatric complications such as psychosis or anxiety, and appropriately trained psychiatric support staff involved in the study will be available and medications to alleviate such conditions (e.g., benzodiazepines) will be on hand.

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Risks Associated with Carfentanil

Although Carfentanil can be considered a potent analgesic, side effects from the amount of carfentanil in the tracer dose should be minimal to non-existent. Monitoring of oxygen saturation, blood pressure, pulse, and other vitals are done before, during and for minimum of 30 minutes post scan (2.5hrs post carfentanil). Should the need arise oxygen and naloxone are on hand to reverse any negative side effects that may occur. MD will decide if subject is ready for discharge should there be any clinically significant changes from baseline vitals. Should vitals not return to baseline after additional monitoring and treatment, MD may decide to have subject sent to the ER. In this case a SAE will be filed.

11. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.) For more information, see the Instructions, page 24.
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study?

       This study represents a greater than minimal risk to the participants.

    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? N/A

    c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are available here http://www.yale.edu/hrpp/forms-templates/biomedical.html for
        i. Minimal risk
        ii. Greater than minimal

1. Personnel responsible for safety review and its frequency:

The principal investigator, in collaboration with David Matuskey, MD, will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency, which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. The principal investigator, the HIC, the FDA, or the RSC, have the authority to stop or suspend the study or require modifications.

2. The risks associated with the current study are deemed moderate for the following reasons:

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

Although we have assessed the proposed study as one of greater than minimal risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or

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in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator Kelly Cosgrove, PhD. according to the following categories:

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

4. Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

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

5. Plan for Determining Seriousness of Adverse Events:

Serious Adverse Events:

In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it results in any of the following outcomes:

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

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

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

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

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

1. Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND
2. Is related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

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

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

7. Plan for reporting adverse events:

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

☒ All Co-Investigators listed on the protocol.
☒ National Institutes of Health
☒ Yale University Radioactive Drug Research Committee (if applicable)
☒ Yale University Radiation Safety Committee (if applicable)

The principal investigator, Kelly Cosgrove, PhD, in collaboration with David Matuskey, MD, will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

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

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

a. The presence of neurotransmitter activation is determined, statistically, by comparing the best lp-ntPET fit to MRTM. The F-statistic is used to compare formally the residual sum of squares for each model taking into account differences in degrees of freedom. MRTM has 3 parameters. lpntPET has 7 (4 explicit, 3 implicit). Only those voxels for which the F-test indicates an improved fit ($p<0.01$) are retained for the neurotransmitter movie. The map of all retained voxels is a "Significance Mask".

b. To correct for multiple comparisons (~1000 voxels in our striatal mask) we apply a cluster-size threshold to the Significance Mask. For a scan on the HRRT, 15-20mCi dose of radiotracer, and approximately 3 minute time-frames, we have determined that a cluster-size threshold of 17 contiguous voxels is the minimum size activation cluster to retain so that we achieve a global incidence of less than 1/10 subjects with any false positive clusters.

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

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

A. DRUGS, BIOLOGICS and RADIOTRACERS

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

1. [^{11}C]Raclopride (radiotracer for PET studies), IV

[^{11}C]Raclopride is the most commonly used D2/3 radioligand for PET studies in humans. It has been used in many PET centers through out the world and its dosimetry has been well characterized and published. Studies contained in this protocol will not commence until the Yale New Haven Hospital RSC grants approval for this protocol.

An application will be submitted to the Yale New Haven Hospital RSC for use of [^{11}C]raclopride in this protocol as it is covered under IND# 132971. No subjects will be scanned until approval is obtained fro YNHH RSC.

2. [^{11}C]Carfentanil (radiotracer for PET studies), IV

[^{11}C]Carfentanil is a mu opioid receptor agonist. Its primary actions of therapeutic value are analgesia and sedation. It has been used in many PET centers throughout the world and its dosimetry has been well characterized and published. An application will be submitted to the Yale New Haven Hospital RSC for use of [^{11}C]Carfentanil in this protocol as it is covered under IND# 13297. No subjects will be scanned until approval is obtained from YNHH RSC.

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3. Cannabis cigarettes: standardized cannabis cigarette (approximately 3.5%-10% THC) obtained from NIDA.

4. [^{11}C]EKAP(radiotracer for PET studies), IV**:** is a kappa opioid receptor agonist. The tracer has been used at the Yale PET Center under an eIND and will be submitted to IND #13297 for use in this study.

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

a. What is the Investigational New Drug (IND) **number** assigned by the FDA?

Cannabis cigarettes – (IND #**132971;** Kelly Cosgrove PhD

b. Who holds the IND?

Cannabis cigarettes – Kelly Cosgrove PhD

c. All protocols which utilize a radiotracer not approved by, but regulated by the FDA must provide the IND number: __**132971**_____
Alternatively, use of the investigational radiotracer may be under RDRC/RSC oversight: (check if appropriate)_____

For all investigational radiotracers, attach a copy of the RDRC/RSC application (for radioisotopes used in the PET Center, PET Center personnel may complete this step)
Go to http://rsc.med.yale.edu/login.asp?url=myApps.asp. When you have logged in, complete the application and attach a copy to this submission.

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

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

i. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug. ☐ Yes ☐ No
ii. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the

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product. ☐ Yes ☐ No

iii. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. ☐ Yes ☐ No

iv. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56). ☐ Yes ☐ No

v. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs. ☐ Yes ☐ No

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

☐ i. The clinical investigation is for an *in vitro* diagnostic biological product that involves one or more of the following (check all that apply):

- ☐ Blood grouping serum
- ☐ Reagent red blood cells
- ☐ Anti-human globulin

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

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

Exempt Category 3

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

Exempt Category 4

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

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

Preclinical Characterization of [^{11}C] raclopride: in vitro assays

Using the Cheng-Prusof Method [60], the affinity, KD, of [^{11}C]RAC for the D2 receptor ranges from 1.6 nM [61] to 3.4 nM.[62]. The raclopride affinities for D_2 and D_3 are approximately the same, ~1.5 nM and 1.2-2.1 nM, respectively [63] [64] [65].

Clinical Application of [^{11}C]raclopride

[^{11}C]raclopride is the most commonly used D2 radioligand for PET studies in humans. It has been used in many PET centers throughout the world and its dosimetry has been well characterized and

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published. This tracer has been synthesized at the Yale PET Center radiochemistry lab using an automated module, GE FXc, according to a known procedure and three validation studies have been conducted. This tracer is already used in human studies at the Yale PET center so will be ready for human studies upon HIC/YNHH RSC approval of this protocol.

[^{11}C]carfentanil will be administered at tracer doses of ≤0.03µg/kg, at which significant side-effects of the radiotracer are not expected. In over 20 PET imaging studies of [^{11}C]Carfentanil involving over 270 human volunteers with a range of diagnoses, no clinically-significant side-effects have been observed; mild drowsiness has been reported in some studies [66].

Cannabis cigarettes with a potency of ~3.5%-10% THC will be administered to research subjects. 3.5%-10% THC is considered a medium dose and should be well tolerated in regular cannabis users and cannabis exposed users.

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

PET drugs (radiotracers), [^{11}C]raclopride and [^{11}C]carfentanil are supplied by the Yale University PET Center.

Cannabis: Will be obtained as cannabis cigarettes from the National Institute on Drug Abuse

b) Is the drug provided free of charge to subjects? ☒ Yes ☐ No
If yes, by whom?
NIDA
The Yale University PET Center. The Yale PET Center has an in-house chemistry department capable of producing the radiotracer (PET drug) necessary for the research study. All fees charged by the PET Center for services rendered are covered by the study funding.

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

☒ **YNHH IDS**	☐ **Yale Cancer Center**
☐ **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.

Preparation of radiotracers:

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[^{11}C]Raclopride, [^{11}C]Carfentanil, and [^{11}C]EKAP will be manufactured by experienced radiochemists at the Radiochemistry Laboratory of the Yale University PET Center in accordance with our local Chemistry Manufacturing & Control (CMC) procedures and quality specifications described in local Drug Master File (DMF) that have been approved by the Yale University RDRC (YURDRC).

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

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

[^{11}C]raclopride ([^{11}C]RAC): Briefly, [^{11}C]RAC is synthesized by ^{11}C-methylation of the desmethyl phenolate precursor with [^{11}C]methyl triflate according to known literature methods. The resulting PET drug is purified first by semi-preparative HPLC, followed by solid-phase extraction to remove the HPLC buffer mixture. Finally [^{11}C]RAC is formulated in <10% ethanolic saline solution (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.

[11C]carfentanil ([^{11}C]CFN): Briefly, [^{11}C]CFN is synthesized by ^{11}C-methylation of the carboxylate oxygen of the desmethyl precursor with [^{11}C]methyl iodide according to known literature methods. The resulting PET drug is purified first by semi-preparative HPLC, followed by solid-phase extraction to remove the HPLC buffer mixture. Finally [^{11}C]CFN is formulated in <10% ethanolic saline solution (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.

Preparation of [11C]EKAP: Cyclotron produced [11C]CO2 is transferred into the GE FxC/FxC-pro, Fx-MeI module and trapped in a mixture of nickel and molecular sieve. Hydrogen is then passed through the catalyst and reacted with [11C]CO2 to afford [11C]methane, which is converted to [11C]methyl iodide by reaction with iodine at high temperature (720 °C). Conversion to [11C]methyl triflate is effected by passing [11C]methyl iodide in a stream of inert gas through a silver triflate/graphite column heated at 190 °C. Radiolabeling is performed by trapping the reagent [11C]methyl triflate in a solution of descarboxymethyl-EKAP and DBU-CO2 (1.2 equivalents), in the presence of tetrabutylammonium triflate (TBATf) and cesium carbonate (Cs2CO3), in the solvent N,N-dimethylformamide (DMF, 0.3 mL), at 0 °C, and then

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reacting at 25 °C for 5 min. Purification of the labeled product [11C]EKAP is achieved by semi-preparative HPLC. The desired product fraction from the HPLC is collected, diluted with 0.1% ascorbic acid in water (50 mL), and passed through a C-18 SepPak cartridge. The SepPak is then rinsed with 10 mL of 0.1% aqueous ascorbic acid solution. The radioactive product, trapped on the SepPak, is recovered by eluting the SepPak with 1 mL of USP absolute ethanol, followed by 3 mL of 0.1% ascorbic acid in USP saline, into a product vial containing 7 mL of 0.1% ascorbic acid in USP saline and 200 μL of 4.2% sodium bicarbonate solution, USP. This mixture is then passed through a sterile membrane filter (0.22 μm) for terminal sterilization and collected in a sterile empty vial to afford a formulated solution ready for dispensing and intravenous injection. The radioactive product is stored at room temperature and is stable for at least 60 min after preparation. Pyrogen test is performed for each batch of product. Sterility is achieved by passing product through a membrane filter for terminal sterilization as the last step in the formulation process, and confirmed by sterility test performed after administration.

Cannabis: Will be obtained from NIDA as prepared cannabis cigarettes. Cannabis will be kept in the PET Center located at 801 Howard Ave, New Haven, CT which is a key card only access floor, in room 203A closet which is kept locked at all times. It will be locked in an approved Gardall Light Duty Commercial Utility/Under Counter Safe.
Dimensions: 14"H X 14"W X 14"D
Overall Product Weight: 61 lbs
It is bolted permanently to the concrete floor. Only individuals with Power of Attorney as listed above will have the combination.

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

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

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

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

☐ No If no, explain why this is acceptable.

SECTION VI: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. Targeted Enrollment: Give the number of subjects:

a. targeted for enrollment at Yale for this protocol__250 subjects will be screened to have 110 subjects participate._

b. If this is a multi-site study, give the total number of subjects targeted across all sites__N/A_

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

☒ Flyers	☒ Internet/Web Postings	☒ Radio
☐ Posters	☐ Mass E-mail Solicitation	☐ Telephone
☐ Letter	☒ Departmental/Center Website	☒ Television
☐ Medical Record Review	☒ Departmental/Center Research Boards	☒ Newspaper
☒ Departmental/Center Newsletters	☐ Web-Based Clinical Trial Registries	
☒ YCCI Recruitment database	☒ Clinicaltrials.gov Registry (do not send materials to HIC)	
☐ Other (describe):		

3. Recruitment Procedures:

a. Describe how potential subjects will be identified.

All subjects will be 18-55 years of age and physically healthy. All research subjects will be recruited under guidelines of the Yale University Institutional Review Board (Human Investigation Committee). Subjects will be recruited from the community at large via IRB-approved advertising (newspaper, postings in community locations, Craigslist, Facebook).

b. Describe how potential subjects are contacted.

The subjects will be asked to call us if they are interested in participating in the research study. Interested individuals contacting the clinic by phone in response to advertisements are told that the information they give over the phone is written down and discussed by the research team. They are advised that if they do not enroll in research with the clinic the information is destroyed, and that if they do, it becomes part of their research chart. If an individual appears to meet enrollment criteria and is interested in participating, a face-to-face interview is conducted by one of the project investigators. A release of information is obtained for review of any available historical and clinical data. A written authorization form is also obtained from each subject, permitting the research team to

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use, create, or disclose the subject's PHI for research purposes. The nature of the project, procedures, relative risks and benefits, and alternatives to participation in the project are discussed with the individual. Following this discussion, the individual is given a copy of the consent form to review, and any questions are answered. The PI of the protocol will seek written consent from all participants.

c. Who is recruiting potential subjects?

Members of the research team, as identified under consent personnel below.

4. Screening Procedures

a. Will email or telephone correspondence be used to screen potential subjects for eligibility prior to the potential subject coming to the research office? ☒ Yes ☐ No

b. If yes, identify below all health information to be collected as part of screening and check off any of the following HIPAA identifiers to be collected and retained by the research team during this screening process.

HEALTH INFORMATION TO BE COLLECTED:
personal and family psychiatric, medical, and substance use history.

HIPAA identifiers:
☒ Names
☒ All geographic subdivisions smaller than a State, including: street address, city, county, precinct, zip codes and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly-available data from the Bureau of the Census: (1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people, and (2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.
☒ Telephone numbers
☐ Fax numbers
☒ E-mail addresses
☐ Social Security numbers
☒ Medical record numbers
☐ Health plan beneficiary numbers
☐ Account numbers
☒ All elements of dates (except year) for dates related to an individual, including: birth date, admission date, discharge date, date of death, all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older
☐ Certificate/license numbers
☐ Vehicle identifiers and serial numbers, including license plate numbers
☐ Device identifiers and serial numbers
☐ Web Universal Resource Locators (URLs)
☐ Internet Protocol (IP) address numbers
☐ Biometric identifiers, including finger and voice prints
☐ Full face photographic images and any comparable images
☐ Any other unique identifying numbers, characteristics, or codes

In addition, if a subject agrees to participate in this study, and will be visiting the Connecticut Mental Health Center (CMHC) as part of study procedures, some information about participation in this research study will become part of CMHC medical record that identifies the subject. If they do not already have a medical record at CMHC, one will be made for their visit. The information that will be entered into their medical record may include the HIPAA identifiers mentioned above.

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

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

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

If yes, describe the nature of this relationship.

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

Choose one:

☐ For entire study
☒ For recruitment purposes only
☐ For inclusion of non-English speaking subject if short form is being used

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

As the initial subject screening will be done by telephone, it will not be possible to obtain the subject's signature.

By signing this protocol application, the investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

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

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

8. **Consent Personnel:** List the names of all members of the research team who will be obtaining consent/assent.

   This list is maintained in the IRES database application

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

   Subjects will be given verbal and written information describing the study under conditions in which they have adequate time to consider the risks of participation. Each study subject will be given a copy of the Consent Form/Compound Authorization Form enclosed with this protocol outlining the risks and benefits of participation in this study.

   Prior to signing the Compound Authorization Form, the principal investigator or her designee (Sub-Investigator/Coordinator, Key Personnel) will discuss the Compound Authorization Form with each subject. The prospective subject will be given verbal and written information (the Compound Authorization Form) describing Protected Health Information (PHI) and why and to whom it will be distributed. The subject will have adequate time to consider this information before signing. If the subject decides not to sign the Compound Authorization Form, he/she will not be able to participate in the study. A copy of the signed and dated Compound Authorization form will be given to each subject and informant.

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

    We will not recruit subjects with limited decision-making capacity. All of the subjects who sign the consent to participate in the protocol will have completed and met medical (urine and blood tests, EKG, and physical) and psychological (SCID and clinical interview) criteria. As part of the consent process, prospective subjects are asked open-ended questions about the research in order to determine whether the subject recalls and understand the process of the study. If an individual shows poor comprehension of the consent form and study, we will not enroll them. A study doctor supervises the screening and enrollment process.

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

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The following consent forms will be used:

1. Aim 1 and 2 Compound Authorization Form
2. Aim 3 Compound Authorization Form

12. Non-English Speaking Subjects: Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. If enrollment of these subjects is anticipated, translated copies of all consent materials must be submitted for approval prior to use.

Non-English speaking subjects will not be recruited for this study.

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

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

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

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

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

☐ **Not Requesting a consent waiver**
☒ **Requesting a waiver of signed consent**
☐ **Requesting a full waiver of consent**

A. Waiver of **signed** consent: (Verbal consent from subjects will be obtained. **If PHI is collected, information in this section must match Section VII, Question 6**)

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☒ **Requesting a waiver of signed consent for <u>Recruitment/Screening</u> only**

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

a. Would the signed consent form be the only record linking the subject and the research?

☐ Yes ☐ No

b. Does a breach of confidentiality constitute the principal risk to subjects?

☐ Yes ☐ No

OR

c. Does the research activity pose greater than minimal risk?

☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity

☒ No

AND

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

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

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

a. Would the signed consent form be the only record linking the subject and the research?

☐ Yes ☐ No

b. Does a breach of confidentiality constitute the principal risk to subjects?

☐ Yes ☐ No

OR

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

AND

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

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

☐ **Requesting a waiver of consent for <u>Recruitment/Screening</u> only**

a. Does the research activity pose greater than minimal risk to subjects?

☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity

☐ No

b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No

c. Why would the research be impracticable to conduct without the waiver?

d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

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

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

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

SECTION VII: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

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

Required private identifiable information about individuals, such as their medical history, current medications, psychiatric problems, and family history, will be collected by research staff and be used for research purposes and charting after consent is obtained.
Subjects will also provide information about their smoking history, their family smoking and psychiatric history, drinking and drug use history, mood, cardiovascular health, and other demographic variables. Biological specimens to be taken from subjects include urine samples to rule out or confirm drugs of abuse prior to imaging, and blood samples (during imaging) to measure plasma concentration of tracer and other times as well as to measure blood drug levels.

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, date of discharge from the CNRU, phone number, address, medical history, individual and family history of psychiatric problems, and substance abuse history. Subjects will also sign a separate Notice of Privacy Practices specific to CMHC.

b. How will the research data be collected, recorded and stored?
c. How will the digital data be stored? ☐ CD ☐ DVD ☐ Flash Drive ☐ Portable Hard Drive ☒ Secured Server ☒ Laptop Computer ☒ Desktop Computer ☐ Other
d. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?
Do all portable devices contain encryption software? ☒ Yes ☐ No
If no, see http://hipaa.yale.edu/guidance/policy.html

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Identifiable paper information is kept in locked file drawers and password protected computer files on University encrypted computers. Results are published as group data without the use of characteristics that would identify individual subjects. We quote information only by number in conference discussions, scientific reports, or publications, in order to maintain anonymity.

Identifiable brain research data are stored on a secure database located on the internal PET Center Network. The PET network is protected by a Cisco PIX firewall operated by ITS. All research data are backed up nightly to a Dell PV-136T library wit 4 IBM Ultrium-TD2 tape drives using the backup software Legato Networker 7.3 from EMC. Human subjects enrolled in the study are assigned a subject-specific random identifier. Subject identifiers and the means to link the subject names and codes with the research data are stored in separate locations within the database. The software of the database limits the ability to connect the random identifier to the actual subject identification information to research team members only. Access to the database is password protected and each research team member is required to have a unique ID and password to gain access to the database. Authorized users employ their netid and authentication is performed using Yale's central authentication server. Users always access research data through the random identifier only.

e. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.

The data will be stored in locked filing cabinets and on the password-protected secure database on the internal Yale University PET Center Network for at least 7 years, accessed only by study personnel.

f. Who will have access to the protected health information (such as the research sponsor, the investigator, the research staff, all research monitors, FDA, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), SSC, etc.)? (please distinguish between PHI and de-identified data)

Only very select, authorized individuals in the PET center (appointment scheduler, nurse, nuclear medicine technician, administrator in charge of billing and patient reimbursement) and the PI will have access to the key that identifies patient codes. Relevant research staff at CMHC will also have access to the information entered into the participant's CMHC medical record.

No external agency (other than the FDA) will have access to the data.

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

A Certificate of Confidentiality from NIH will be obtained prior to study enrollment. With this Certificate, the researchers cannot be forced to disclose information that may identify subjects, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The Certificate does not imply approval or disapproval of the project by the Secretary of DHHS. It adds special protection for the research information about subjects. The researchers will use the Certificate to resist any demands for information that would identify subjects, except as explained below. The Certificate cannot be used to resist a demand for information from personnel of the United

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States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA). A Certificate of Confidentiality does not prevent subjects or subject family members from voluntarily releasing information about the subject or their involvement in this research. If an insurer, employer, or other person obtains written consent from the subject to receive research information, then the researchers may not use the Certificate to withhold that information.

h. Are any of the study procedures likely to yield information subject to mandatory reporting requirements? (e.g. HIV testing – reporting of communicable diseases; parent interview -incidents of child abuse, elderly abuse, etc.). Please verify to whom such instances will need to be reported.

The Certificate of Confidentiality does not prevent the researchers from disclosing voluntarily, without subject consent, information that would identify the subject as a participant in the research project under the following circumstances: In the event of relevant discovery related to events of abuse, identified health risks, or threats to self or others, as related to the health and safety of the public and/or individual study participants, a report will be made to the relevant agencies and authorities to be conducted as outlined and mandated by HIC guidelines, and Federal, State, and Local Statutes.

SECTION VIII: POTENTIAL BENEFITS

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

These studies are not of direct benefit to subjects. This study will help advance knowledge regarding the brain's dopamine and beta-endorphin response to drug addiction.

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

SECTION IX: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

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

The alternative to participation in this research protocol is to not participate. Subjects will be informed that they are free to choose not to participate and, if they do agree to become a subject, they will be free to withdraw from the study at any time during its course. They will also be informed that if they choose not to participate or if they withdraw, it will not adversely affect their relationship with their doctors or the hospital (per the consent form).

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2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.

The subjects will be compensated for their time commitment and inconveniences necessary for completing the study. Subjects will have no financial responsibilities for any portion of the study.

For all aims, compensation will be the following:

- $400 for each PET scan (i.e., up to $800 if participating in both scans)
- $50 for the MRI scan.
- $50 for an arterial line (aim 3)
- $100 bonus upon completion of all study procedures

Subjects participating will receive a total of up to $950 for Aim 1, and up to $1000 for Aim 3 . This includes a $100 completion bonus after finishing their participation in each aim (1 MRI and up to 3 PET scans). Subjects who participate in the Probabilistic Reward Task may also be compensated for the amount that they "win" during the task up to 50$. Subjects who complete cogstate will received 40$. may also receive $10 each time they participate in the Cold Pressor Task

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

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

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

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

4. **In Case of Injury:** This section is required for any research involving more than minimal risk.
   a. Will medical treatment be available if research-related injury occurs?
   b. Where and from whom may treatment be obtained?
   c. Are there any limits to the treatment being provided?
   d. Who will pay for this treatment?

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e. How will the medical treatment be accessed by subjects?

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

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

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