# Title: Imaging Extrastriatal Dopamine Release in Tobacco Smokers and Nonsmokers

# NCT: *NCT02348385*

**Principal Investigator:** ***Kelly Cosgrove***

**Latest Approval Date: 08/22/2017**

## RESEARCH PLAN

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

Our goal is to examine sex differences in amphetamine-induced dopamine (DA) release in tobacco smokers and nonsmokers.

**Specific Aim 1: To determine sex differences in amphetamine-induced dopamine release in healthy tobacco smokers and nonsmokers.**
[$^{11}$C]FLB-457 has been used in several PET centers and has recently been approved for use at the Yale University PET Center (HIC#100900739, PI: Cyril D'Souza). We would like to determine whether there are sex differences in amphetamine induced DA release in healthy tobacco smokers and nonsmokers. Specifically, 40 healthy tobacco smokers and 40 healthy nonsmokers will have an magnetic resonance imaging (MRI) scan followed on another day by two [$^{11}$C]FLB scans (ideally, the two PET scans will be carried out in the same day). Up to 3 hours before the second PET scan, amphetamine (0.4mg/kg, PO) will be administered. Smokers may be asked to return for a second MRI scan 3-5 weeks after their PET scans. We hypothesize women will have greater amphetamine-induced DA release than men in extra-striatal brain regions.

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.

***Tobacco Smoking:*** Tobacco smoking continues to be a major health problem. Despite the overwhelming evidence of the medical risks associated with tobacco smoking, approximately 20-25% of the adult US population continues to smoke and the vast majority of those who do attempt to quit relapse within the first month(1). Nicotine is the main active and addictive chemical in tobacco and acts as an agonist at the $ß_2$subunit containing nicotinic acetylcholine receptor ($ß_2$-nAChR). The few existing approved medications for tobacco smoking cessation (including various forms of NRT and bupropion) are minimally effective at preventing relapse; however, varenicline (Chantix) shows greater promise. **There is an urgent need to understand the fundamental brain mechanisms involved in tobacco smoking to direct future medications development.** The studies herein are a first step. Specifically, we will determine sex-differences in amphetamine-induced dopamine release at "baseline". In a future study, we will then treat tobacco smokers with smoking cessation medication and determine if their amphetamine-induced DA release is reduced. Our long-term goal is to establish whether certain medications are effective by reducing drug-induced DA release, i.e., the drugs make cigarette smoking less rewarding.

***Amphetamine-induced DA release.*** Due to the difficulty and unreliability of obtaining nicotine- or tobacco smoking-induced dopamine release, the majority of PET studies examining changes in synaptic DA levels have relied on amphetamine. Amphetamine, as opposed to nicotine, acts directly at the DA-ergic system and is a potent reuptake inhibitor of dopamine and a dopamine releaser. While nicotine results in 200% increases in dopamine release as measured with microdialysis, amphetamine results in up to 1000% increases in dopamine release(2). Therefore, there is a much larger signal to detect, which has been reliably measured in many PET studies(19-25). In the current study, we will use amphetamine-induced DA release to measure DA neurotransmission.

***Measuring dopamine release in vivo with the occupancy model.*** Typically, dopamine release has been most reliably measured using stimulants such as cocaine and amphetamine with [$^{11}$C]raclopride PET or [$^{123}$I]IBZM SPECT (see (22) for review). Thus, challenges, e.g., injections of drugs that increase synaptic dopamine will interfere with radiotracer binding by decreasing the availability of the dopamine receptors to bind the radiotracer. This allows calculation of the "occupancy" of the receptors by dopamine or a change in binding potential, and is an indirect measure of dopamine release. Specifically, in these studies, because synaptic dopamine will compete with [$^{11}$C]FLB for high affinity $D_{2/3}$ receptor binding, an increase in synaptic dopamine will result in a decrease in [$^{11}$C]FLB binding potential.

The exact mechanism by which the binding potential is reduced is unknown. It is most likely that synaptic dopamine interferes with radiotracer binding, but it is also possible that $D_{2/3}$ receptor internalization also plays a role in the prolonged reduction in binding potential, or displacement of [$^{11}$C]raclopride after amphetamine injection(22, 26). $D_{2/3}$ or $D_2$ receptor internalization after nicotine has not been reported. Currently, the occupancy model together with *in vivo* PET imaging provides a noninvasive technique for measuring drug-induced dopamine release.

***[$^{11}$C]FLB.*** [$^{11}$C]FLB-457 ([$^{11}$C]FLB) has advantages over the more typically used [$^{11}$C]raclopride, because whereas [$^{11}$C]raclopride is useful for measuring DA release in the striatum, [$^{11}$C]FLB can be used to measure extra-striatal DA release. The extra-striatal DA release most critically includes the prefrontal cortex and also other cortical areas and the thalamus. A number of studies have determined that [$^{11}$C]FLB is sensitive to amphetamine-induced DA release(27-29).

***Sex Differences.*** Nicotine replacement therapies (NRTs), e.g., nicotine patch, gum, lozenge, inhaler, as well as the nicotinic partial agonist therapy varenicline, are the most widely used treatments to help people quit smoking. Many studies show that men respond better to NRT than women(30), and women often have a more difficult time quitting smoking than men(31-35). In a series of studies, Perkins and colleagues(36) demonstrated that men are better able to identify nicotine in a nasal spray and to discriminate nicotine from placebo than are women, indicating that men are better able to detect the interoceptive cues of nicotine. Consistently, women experience greater craving relief then men from a denicotinized tobacco inhaler(37), highlighting the role sensory cues play in nicotine dependence for women. These results are consistent with findings showing that women are more reinforced by the non-nicotine conditioned stimuli that are strongly associated with smoking than men, while men are more reinforced by the nicotine *per se* in cigarettes and in NRT than women(38).

Sex differences have been reported in DA neurotransmission and receptor availability. Higher DA $D_2$ receptor availability was found with [$^{11}$C]FLB in women compared to men in the frontal cortex(39). Women also have higher striatal presynaptic dopamine synthesis capacity than men(40). Sex differences have been reported in healthy (nonsmoking) men and women in amphetamine-induced DA release with two other PET radiotracers. Men had greater DA release in the ventral striatum than women when measured with [$^{11}$C]raclopride PET(25). Men had greater DA release in the dorsal striatum than women, and women had greater DA release in the globus pallidus and frontal gyrus than men when measured with [$^{18}$F]fallypride PET(24). Taken together this suggests that amphetamine-induced DA release is greater in men in the striatum, and in women in the frontal cortex. Both of these areas are key players in the addiction to tobacco smoking. This study will help shed light on the neurochemical mechanisms involved in the sex differences in tobacco smoking behaviors.

3. **Research Plan:** Provide an orderly scientific description of the study design and research procedures as they directly affect the subjects.

**3.1 Overall Research Design:**

**Specific Aim 1: To determine sex differences in amphetamine-induced dopamine release in healthy tobacco smokers and nonsmokers.**

[$^{11}$C]FLB-457 has been used in several PET centers and has recently been approved for use at the Yale University PET Center (HIC#100900739, PI: Cyril D'Souza). We would like to determine whether there are sex differences in amphetamine induced dopamine release in healthy tobacco smokers and nonmokers. Specifically, 40 healthy tobacco smokers and 40 healthy nonsmokers will have an MR scan followed on another day by two [$^{11}$C]FLB-457 scans (ideally, the two PET scans will be carried out in the same day). Up to three hours before the second PET scan, amphetamine (0.4mg/kg, PO) will be administered. We hypothesize women will have greater amphetamine-induced DA release than men in extra-striatal brain regions.

**3.2 Subjects:**

**Screening Evaluation**

Potentially eligible subjects will undergo an initial screening evaluation within 6 months of PET scanning, they may be recruited under HIC#0808004163 (PI:McKee). The purpose of this evaluation is to ensure that subjects meet study criteria (as listed above). After informed consent is obtained, a medical history, vital signs, complete physical examination, and ECG will be performed. Several laboratory tests will be performed at this visit, including 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 serum pregnancy test at the time of screening. *Additionally, urine pregnancy tests and urine drug toxicology screening will be done on the day of the PET scans before imaging*.

**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 including but not limited to the below will be measured during the intake evaluation and on the PET scan days.

**Procedures:**

**Screening, Evaluation and Clinical Ratings**

Screening, evaluation and clinical ratings are obtained during the screening process, described above. Subject may be screened under HIC#0808004163 (PI:McKee).

**Magnetic Resonance Imaging** (up to 1 hour)

Structural and functional (resting state) magnetic resonance imaging (MRI) scans (3 T) will be collected in each subject to co-register PET and MRI for image analysis. 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. MR images provide a matching anatomical atlas for creating individualized region-of-interest templates for each subject. Subjects may be asked to participate in up to two MR sessions (one MR prior to each PET scan date).

**PET experiments**
PET experiments will be conducted at the Yale University PET Center.

For Aims smokers and nonsmokers will have up to two [$^{11}$C]FLB457 scans (2/day when feasible). If technical difficulties arise the second PET scan will be rescheduled as soon as possible.

We may ask subjects to refrain from eating 4-6 hours prior to injection and remain fasting until the study is over. Subjects will also be asked to refrain from drinking caffeinated beverages that day. Smokers may also be asked to abstain from smoking cigarettes starting at 10 p.m. the night before their PET scans day. For all women, a urine pregnancy test will be performed at the beginning of the imaging day at the Yale PET Center.

The preparation of the subject may include the placement of up to two venous lines and an arterial line. The purpose of the arterial line is for blood sampling of [$^{11}$C]FLB-457 to measure arterial concentration. The purpose of the two venous lines is for the [$^{11}$C]FLB-457 injection and to measure amphetamine or nicotine levels, respectively.

The arterial line may be placed after infiltration of the skin with 1 to 2% lidocaine by an experienced physician. A study physician or nurse will be present in the PET suite while the arterial line is in place.

Partial immobilization of the head will be done using a felt chin strap. PET scans are acquired as subjects rest using the HR+ or HRRT scanner. A transmission scan is obtained immediately before or after each emission scan.

For the emission scans, 10 mCi or less of [$^{11}$C]FLB457 will be injected intravenously.

Up to two injections of [$^{11}$C]FLB457 are administered to the subject for the emission scans. Following each injection, scan emission data will be acquired for up to 120 minutes. When possible both FLB scans will be performed on the same day, if the second scan is cancelled, e.g., the radiotracer synthesis fails, the second scan will be rescheduled as soon as possible on a different day, and the amphetamine or stress reactivity paradigm will be administered again, using the same protocol. For Aim 1, we will wait at least 4 days in order to allow the amphetamine to clear.

At -1, 5, 20, 40, and 60 minutes post visualization, plasma samples will be obtained. Plasma samples will be obtained to analyze for cortisol, ACTH, NE, EPI, and other stress related hormones. We will assess hypothalamic-pituitary-adrenal (HPA) axis function using samples of adrenocorticotrophin hormone (ACTH) and cortisol and plasma norepinephrine (NE) and epinephrine (EPI) during the stress behavior challenges.

*Amphetamine administration (oral)*
Dextroamphetamine sulfate is the dextro isomer of the compound d,l-amphetamine sulfate, a sympathomimetic amine of the amphetamine group. It is an FDA approved drug available for the treatment of narcolepsy and attention deficit hyperactivity disorder (maximum approved total daily dose of 5-60 mg). After the first scan, dextro-amphetamine (0.4 mg/kg, PO) will be given in increments of 5 mg, as it is available in 5 mg tablets, and will be rounded up to approximate 0.4 mg/kg total dose **without exceeding it**. The dextro-amphetamine will be given at 0.4mg/kg.

The total dextro-amphetamine dose will not exceed 40 mg per scan. A second transmission and emission scan will be acquired 3-hours post amphetamine identical to the methods outlined previously. The timing of the second [$^{11}$C]FLB 457 administration and subsequent PET scanning (i.e., 3 hours) corresponds to the time of maximum plasma concentration of amphetamine as stated in the respective FDA-approved product labeling. EKG and frequent BP monitoring may occur throughout the study and until the vital signs are within normal limits. Supplemental oxygen will be provided via nasal cannula if necessary. If the systolic BP reaches or exceeds 200 mmHg for more than 5 minutes, the study doctor will take the appropriate clinical measures in order to lower the BP, which may include phentolamine administration (5 mg IV over 10 min) or other appropriate measures.

Following the post-amphetamine PET scan, subjects will be assessed (physical, EKG and vital signs) by one of the investigators. Subjects will be discharged when vital signs are within normal limits and when behavioral changes (if any) are found to be not clinically significant by the MD attending to the subject at the PET Center. If subjects experience any adverse events, they will be treated until they become asymptomatic, prior to discharge.

Behavioral response to amphetamine will be measured by self-ratings with a simplified version of the Amphetamine Interview Rating Scale (van Kammen and Murphy, 1975). Four items will be investigated: euphoria ("feel good"), alertness ("feel energetic"), restlessness ("feel like moving") and anxiety ("feel anxious"). Self ratings will be obtained by analog scales at the following times relative to the d-amphetamine administration: -5 minutes, 0, and hourly thereafter until end of the second scan.

*Blood sampling*
During the [$^{11}$C]FLB PET scans, samples may be drawn through the arterial line for determination of [$^{11}$C]FLB arterial concentration. Samples will be drawn through a venous line to measure amphetamine levels. In the first phase of the study (5-10 min), the arterial input functions are measured with an automated blood counting system (PBS-101, Veenstra Instruments, Joure, The Netherlands) using a continuous withdrawal system where the radioactivity in whole blood is measured with a calibrated radioactivity monitor. Subsequently, individual blood samples may be taken at various time points and will be counted in a gamma counter. Additionally, samples are centrifuged to obtain plasma, which will be counted in a gamma counter, 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 analysis. In addition, the fraction of plasma radioactivity unbound to protein will also be determined by ultrafiltration. During the second, post-amphetamine [$^{11}$C]FLB PET scan, up to 15 blood samples will be collected through a venous line for measurement of plasma amphetamine at selected time points following radiotracer administration.
The total blood sampling volume drawn during the PET Day will be up to 295 mL (125 mL per PET scan) for [$^{11}$C]FLB analysis, plus up to 100 mL for amphetamine analysis.

*Discharge*
After the completion of the two [$^{11}$C]FLB PET scans, the catheters will be removed (arterial line will be removed by a nurse). Subjects will be discharged from the PET suite, if they do not have a ride, a taxi cab will be called for them if they have had amphetamine. They will be called by a research staff member the following morning for a check on their health status.
All subjects will be provided the telephone number of a research psychiatrist on call for any problems that arise in the immediate time period after discharge from the unit. They will also be

given a discharge form that has specific instructions for calling one of the physicians if they have any questions or concerns following the study.

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

**Statistical Considerations:** Describe the targeted number of subjects and the statistical analyses that support the study design.

Aim 1 is designed to obtain data in a sample size of 40 smokers and 40 nonsmokers, with equivalent numbers of men and women in each group. This is sufficient to determine whether we can reliably measure amphetamine induced dopamine release in vivo of at least 1000%, which is equivalent to a change in $BP_{ND}$ of at least 20%. Regional [$^{11}$C]FLB-457 uptake for each subject will be quantified as $BP_{ND}$ described above for each ROI and will represent the primary outcome measure for the proposed experiments. Repeated measures ANOVA will be used to evaluate the change between scans (deltaBP) for brain measures for the 80 pairs of scans. Delta BP will be computed as [$(BP_{baseline}-BP_{condition})/BP_{baseline}$ X 100]. Analysis will be conducted using SAS version 9.1 (Cary, NC). N=40 subjects per group is necessary to detect sex differences.

## RESEARCH INVOLVING DRUGS, DEVICES, BIOLOGICS & PLACEBOS

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

**[$^{11}$C]FLB457** ((S)-(-)-N-([1-ethyl-2-pyrrolidinyl]methyl)-5-bromo-[C-11]-2,3-dimethoxybenzamide): 2 injections of ≤10 mCi each of [$^{11}$C]FLB457 will be administered to each subject.

Application to the RDRC for use of the radiotracer [$^{11}$C]FLB457 in this protocol will be submitted through the Yale-New Haven Hospital Radiation Safety Committee (Y-NHH RSC). Approval will be obtained and submitted to the HIC at Yale.

**d-Amphetamine,** dose 0.4 mg/kg, PO to healthy controls so no IND necessary

**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.

<u>The risks of [$^{11}$C]FLB injection are radiation exposure and toxicology.</u>

*Radiation exposure*

Absorbed radiation dose calculation for [$^{11}$C]FLB was provided to us by Raj Narendran, MD and Scott Mason, PhD. at the University of Pittsburgh in collaboration with Dr. Michael Stabin, Department of Radiology, Vanderbilt University (formerly at ORISE).

*Single Study Limit.* Biodistribution data for [C-11] FLB457 was obtained in female and male Sprague-Dawley rats. The dose estimates indicate that the maximum, permissible single study dosage of [C-11]FLB457 in human subjects, to remain below the 21 CFR 361.1 dose limit, is 20 mCi (i.e., calculations based upon the ovaries as the critical organ; 3 rem per single study limit and 7.39 x 10-2 rad per mCi of [C-11]FLB457 to the ovaries of female subjects). The female dose estimate is the worst case, as dose estimates to the critical organ of male subjects (the kidneys; 2.73 x 10-2 rad per mCi [C-11]FLB457) were less than that to the critical organ of female subjects. Thus, the dose of up to 10 mCi per single injection, or up to 20 mCi for the whole study (2 injections) is below this limit.

*Yearly Cumulative Exposure.* The radiation exposure from all radiotracer injections (2 total) indicates that the total exposure resulting from the study (2 * 10 mCi) will remain well below the FDA 21 361.1 dose limits for yearly cumulative exposure to research subjects (dose limits of 5 rads per year for whole body, active blood forming organs, lens of the eye and gonads; 15 rads per year for other organs). See table: limiting organ is ovaries for women, and kidneys for men.

*Toxicology*
The dose of FLB457 used in this study is a trace amount and is negligible ($\leq$ 0.6 µg per administration) and is expected to induce less than 5% occupancy of D2 receptors. The use of [$^{11}$C]FLB-457 at a tracer dose is not known to cause any clinically detectable effect in human beings. In 12 human studies at the University of Pittsburgh (total of 24 human injections), Dr. Raj Narendran did not observe any subjective or objective effects from administration of 0.6 µg. However, the risk of an idiosyncratic reaction is acknowledged in the consent form. Any significant adverse reaction will be reported to the FDA and the IRB.

<u>d-Amphetamine:</u>
Amphetamine is administered to measure changes in [$^{11}$C]FLB-457 binding due to dopamine release. This dose was chosen because it is expected to produce a quantifiable displacement of the radiotracer. The risks are outlined in Section VI. Oral(20, 24, 27, 59) and IV(19, 25, 60, 61) d-amphetamine administration to healthy humans and individuals with psychiatric disorders has been safely used in many PET imaging studies. We will use the oral route due to the greater safety and ease of administration.

**Source:** a) Identify the source of the drug, device or biologic to be used.
**[$^{11}$C]FLB** will be manufactured on site in the radiochemistry lab of the Yale University PET center.
The radiotracer will be administered by PET Imaging technologists in the Yale PET Center.

The radioligand is produced according to the local Standard Manufacturing Procedure and to local quality control procedures in effect at the PET Center, Yale University School of Medicine.

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

[11C]FLB457 is manufactured at the Yale PET center radiochemistry lab. The final product solution is passed through a 0.22 uM membrane filter for terminal sterilization to afford a non-pyrogenic, sterile IV solution ready for injection. The manufacturing procedures quality control methods for [11C]FLB457 have been approved by the RDRC.

**Use of Controlled Substances:**

Will this research project involve the use of controlled substances in human subjects?
☒ Yes ☐ No (*See 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*.

Dextro-amphetamine (0.4 mg/kg) will be given by mouth to each subject starting up to 3 hours prior to the second [$^{11}$C]FLB PET scan.

## HUMAN SUBJECTS

1. **Recruitment Procedures:** How will potential subjects be identified, contacted and recruited? *Upload copies of any recruitment materials that will be used.*

Subjects will be recruited through flyers, public advertisement (newspaper, radio, internet posting), by word of mouth, contact with community service groups, and clinics and local treatment facilities (the VA Hospital, West Haven, CMHC, the Yale Psychiatric Hospital, Mood Disorders Research Program, the Yale Depression Research Program). The subjects will be asked to call us if they are interested in participating in the research study. Interested individuals contacting the clinic by phone in response to advertisements are told that the information they give over the phone is written down and discussed by the research team. They are advised that if they do not enroll in research with the clinic the information is destroyed, and that if they do, it becomes part of their research chart. If an individual appears to meet enrollment criteria and is interested in participating, a face-to-face interview is conducted by one of the project investigators. A release of information is obtained for review of any available historical and clinical data. A written authorization form is also obtained from each subject, permitting the research team to use, create, or disclose the subject's PHI for research purposes. The nature of the project, procedures, relative risks and benefits, and alternatives to participation in the project are discussed with the individual. Following this discussion, the individual is given a copy of the consent form to review, and any questions are answered. The PI of the protocol will seek written consent from all participants.

2. **Subject Population** Provide a detailed description of the targeted population of human

subjects for this research project.

**Tobacco smokers:** n = 40
**Healthy nonsmokers**: n = 40
**Age range:** 18-55
**Gender:** Both groups will include an equal proportion of males and females.

**Racial/ethnic group:** We will recruit from all ethnic groups available.

3. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion? How will eligibility be determined, and by whom?

The following are the inclusion and exclusion criteria used. Eligibility to participate in screening session will be determined by the research staff under the guidance of Dr. Kelly Cosgrove and may be screened under HIC# 0808004163 (PI: McKee). Study participation will be determined after the screening session by the study physician and study PI.

**General inclusion criteria:**

- men and women, aged 18-55 years
- who are able to read and write
- who are able to give voluntary written informed consent
- have no current uncontrolled medical condition such as neurological, cardiovascular, endocrine, renal, liver, or thyroid pathology
- have no history of a neurological or psychiatric disorder, e.g., no DSM-IV Axis 1 diagnosis in 2 preceding years)
- drink less than 21 drinks/week for women and less than 35 drinks per week for men
- do not suffer from claustrophobia or any MRI contradictions
- able to participate in imaging studies including 2 PET scans and 1 MRI scan
- nonsmokers (smoked < 100 cigarettes in lifetime with urinary cotinine levels 0-30 ng/mL both at intake evaluation and on scan day)
- smokers (smoked at least 10 cigarettes/day for at least one year with an FTND$\geq$3, urine cotinine >150 ng/mL and CO $\geq$12 ppm at intake)

**General exclusion criteria:**

- psychosis
- presence of acute or unstable medical or neurological illness. Subjects will be excluded from the study if they present with any history of serious medical or neurological illness or if they show signs of a major medical or neurological illness on examination or lab testing including history of seizures, head injury, brain tumor, heart, liver or kidney disease, eating disorder, diabetes.
- regular use of any psychotropic drugs including anxiolytics and antidepressants and other over-the-counter medications and herbal products within the last six months, that in the PI's assessment would put the subject at increased risk or would be detrimental to the study data.
- pregnancy/Breast feeding (as documented by pregnancy testing at screening or at days of the imaging studies),
- suicidal ideation or behavior
- pacemaker or other ferromagnetic material in body.
- use of medications which affect dopamine transmission within 2 weeks of the PET study

- Participation in other research studies involving ionizing radiation within one year of the PET scans that would cause the subject to exceed the yearly dose limits for normal volunteers.
- Blood donation within 8 weeks of the start of the study.
- history of a bleeding disorder or are taking medication to thin their blood

Subjects will be asked to abstain from alcohol and marijuana for one week prior to the PET scan. If subjects endorse use within one week of the scan, their continued participation will be up to the discretion of the PI.

* For women of child-bearing age: The serum pregnancy test is performed during the screening procedure and a urine pregnancy test is done on the day of the PET scans prior to imaging. Since this test cannot detect the very early stage of pregnancy (period between fertilization and implantation), an effective birth control method or sexual abstinence is required during study participation.

## CONSENT/ ASSENT PROCEDURES

1. **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.

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

We will not recruit subjects with limited decision-making capacity. All of the subjects who sign the consent to participate in the current protocol or in HIC# 0808004163 (PI: McKee) 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. The screening and enrollment processes are supervised by a study doctor.

4. **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.

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 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.

5. **Non-English Speaking Subjects:** Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. 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.

6. **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

## SECTION VI: PROTECTION OF RESEARCH SUBJECTS

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

a. Risks of [$^{11}$C]FLB457

The Yale-New Haven Hospital Radiation Safety Committee (RSC) will review the use of radiation in this research study, and no subjects will be enrolled until RSC approval is obtained. This research study involves exposure to radiation from [$^{11}$C]FLB457 PET scanning. This radiation exposure is not necessary for medical care and is for research purposes only. The total amount of radiation an individual subject will receive in this study is from **up to 4** injection(s) with a total of ≤ **10** mCi (per injection) from [$^{11}$C]FLB457**,** plus transmission scans of the brain.

Although each organ will receive a different dose, the maximum amount of radiation exposure subjects will receive from this study is equal to an effective dose of **1.24 rem** for a total of **up to 40** mCi of [$^{11}$C]FLB457 in **4** injections (10 mCi per injection). That is, a total of **1.24** rem for **4** scan(s) is the amount of radiation exposure that a subject will receive from the study. This calculated value is used to relate the dose received by each organ to a single value.

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

c. Risks of oral d-amphetamine

Risks of amphetamine administration include both medical and psychiatric risks.

The frequent somatic side effects of d-amphetamine administration are cardiovascular (hypertension, palpitations, tachycardia, bradycardia, orthostasis). General effects such as sweating, feeling warm or cold, nausea, diarrhea, muscle and abdominal cramping,have been reported frequently. Behavioral effects in this dose range are increased level of alertness, talkativeness, restlessness, agitation, mood changes (usually euphoria) and anxiety. In our experience, these effects are generally transient and well tolerated. This dose of amphetamine has not been reported to induce psychotic symptoms in non-schizophrenic subjects. Infrequently blurred vision, headaches and chest tightness, and changes in EKG have been reported. There is a rare risk of permanent neurological damage and death as a result of cardiac arrest or stroke.

*Psychiatric or behavioral side effects:* General behavioral effects of the injection of amphetamine in this dose range are increased level of alertness, talkativeness, restlessness, agitation, mood changes (usually euphoria) and anxiety. In our experience, these effects are generally transient and well tolerated. This dose of amphetamine has not been reported to induce psychotic symptoms in non schizophrenic subjects and we confirm this observation.

c. Risks of placing an intra-arterial catheter

On the PET scan day a radial arterial catheter will be inserted. Arterial sampling may be associated with mild-to-moderate pain or bruising at the puncture site. In rare instances blocking of the artery, poor healing, hematoma, inflammation, or infection at the catheter insertion site may occur. Certain individuals may feel light-headed during arterial catheter placement.

d. Risks of placing an intra-venous catheter

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, include screening laboratories, will be approximately **19** tablespoons. This is not expected to have any serious negative effects on a study participant.

e. Risks of an MRI scan

MR carries a risk for subjects who are claustrophobia or have pacemakers, metal pieces, aneurysm clips, large colored tattoos, or any other contraindications for MR. Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug

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

Subjects will be watched closely throughout the MR study. Some people may feel uncomfortable or anxious. If this happens, the subject may ask to stop the study at any time and we will take them out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly but we will ask subjects to tell the research staff if they have them. There are some risks with an MR study for certain people. If subjects have a pacemaker or some metal objects inside their body, they may not be in this study because the strong magnets in the MR scanner might harm them. Another risk is the possibility of metal objects being pulled into the magnet and hitting a subject. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. We also ask all people involved with the study to walk through a detector designed to detect metal objects. It is important to know that no metal can be brought into the magnet room at any time. Also, once subjects are in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet.

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

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

a. For risks associated with [$^{11}$C]FLB457

The dose of radiation will be submitted for approval to the Yale-New Haven Hospital Radiation Safety Committee (Y-NHH RSC). All scans will be done in the presence of medical supervision and trained nursing staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the PET scan facilities have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiopharmaceuticals and performance of PET scans will be by radiochemists, physicians, and technologists of the Department of 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.

b. For risks associated with oral d-amphetamine administration

*Medical side effects:* Subjects will be screened for absence of significant medical history and current medical conditions with a complete medical history, physical examination, routine blood tests, urine toxicology and EKG. The study will be canceled on the day of the scan if several of the subject's blood pressure readings are recorded at >90 for diastolic BP or >140 for systolic BP while at rest. Any automated blood pressure results that are abnormal will be repeated manually.

The manual reading will be the official reading.
Inclusion in the study will be limited to individuals who are between the ages of 18-55.
Patients will be excluded if they have any h/o severe medical or neurological illness, any clinically significant brain abnormality, low Hgb, insulin dependent diabetes, more than one risk factor for coronary artery disease , a history of cardiovascular disease, or hypertension.
Patients will be excluded if they have recently donated blood.
Administration of oral d-amphetamine will take place at the PET center with a physician on site. Constant EKG and frequent BP monitoring will occur until the vital signs are within normal limits. If the systolic BP reaches or exceeds 200 mmHg for more than 5 minutes, an infusion of phentolamine (5 mg IV, over 10 min) will be initiated to control the blood pressure response. The study physician will be notified if those parameters are reached and he/she will supervise the treatment.
A twelve lead EKG, a code cart and defibrillator are available in the room in case of complications. In case of chest pain, chest tightness or other symptoms suggestive of cardiac ischemia, the experiment will be cancelled and a twelve lead EKG will be immediately obtained to rule out angina (ST segment elevation or depression as compared to the baseline EKG). Appropriate treatment will be initiated.
Upon discharge, patients will be given the phone numbers of the study physicians from the Division of Translational Imaging and will follow up at least weekly with the study physician, with in-person sessions in each of the first two weeks after amphetamine administration.

*Psychiatric/behavioral side effects:* Inclusion in the study will be limited to individuals who are between the ages of 18-55. No individuals with psychiatric disorders will be recruited into this protocol, on or off of medications. All subjects will receive a follow up all 30 days after receiving amphetamine to check that they are feeling well and do not still have effects from the amphetamine challenge.

c. For risks associated with placing an intra-arterial catheter
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 using aspirin, NSAIDS or anticoagulants. 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. 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.

d. For risks associated with placing an intravenous catheter
The risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained and experienced personnel under sterile conditions. To avoid injury due to fainting, the antecubital vein catheter will be inserted when the subjects are recumbent. The blood draws during PET scanning sessions will be obtained from the already inserted catheter, to minimize discomfort.

e. For risks associated with the MRI
To minimize risks, each subject will fill out the 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). 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.

3. **Data and Safety Monitoring Plan:**

**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. During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. Either the principal investigator, the HIC, the FDA, or the RDRC, 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, amphetamine administration, and arterial line placement as moderate.

Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as 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:

a. Mild adverse event
b. Moderate adverse event
c. 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:

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

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

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

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

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

**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):

X All Co-Investigators listed on the protocol

X National Institutes of Health

X Yale New-Haven Hospital Radiation Safety Committee (RSC/RDRC)

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.

4. **Confidentiality & Security of Data:**

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.

Yale PET Center staff will collect required research data through study procedures as outlined in this protocol, and record it in confidential research records and protected computer files.

Identifiable information is kept in locked file drawers and password protected computer files. Results are published as group data without the use of characteristics that would identify individual subjects. We quote information only by number in conference discussions, scientific reports, or publications, in order to maintain anonymity.
Identifiable research data, including recruitment and screening information and code keys, are stored on a secure database located on the internal PET Center Network. The PET network is protected by a Cisco PIX firewall operated by ITS. All research data are backed up nightly to a Dell PV-136T library wit 4 IBM Ultrium-TD2 tape drives using the backup software Legato Networker 7.3 from EMC. Human subjects enrolled in the study are assigned a subject-specific random identifier. Subject identifiers and the means to link the subject names and codes with the research data are stored in separate locations within the database. The software of the database limits the ability to connect the random identifier to the actual subject identification information to research team members only. Access to the database is password protected and each research team member is required to have a unique ID and password to gain access to the database. Authorized users employ their netid and authentication is performed using Yale's central authentication server. Users always access research data through the random identifier only.
At the VACHS, we will use coded records and keep signed consent forms in a locked cabinet. Forms that contain the subjects' names and identifying information will be stored in the medical chart in a locked file. Electronic data is stored in a password-protected database at the VACHS. Access to this database is limited to investigators and research staff within the PIs laboratory.

Their MRI report is transferred to the Yale PET Center and kept on their secure servers described above.

Procedures to ensure confidentiality follow the regulations and policies of the Yale University School of Medicine. The security mechanisms specified above in 4d will continue to be in place to protect study data.

Investigators and research staff at Yale University School of Medicine, VACHS, Yale University Human Investigation Committee (HIC), the FDA, and NIH may have access to the protected health information.

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.

5. **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. *(Note: Payment of subjects is not considered a benefit in this context of the risk-benefit assessment.)*

There are no direct benefits from a subject's participation in this study. This research will benefit scientific knowledge by contributing to the understanding of the role of sex differences and dopamine in tobacco smoking addiction. Society may benefit from these insights, as they may have clinical application in the future.

## RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

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

The alternative to participation in this study is to decline participation.

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

The subjects will be compensated for their time commitment and inconveniences necessary for completing the study. Subjects will have no financial responsibilities for any portion of the study. Compensation will be up to $50 for each MRI scan, $350 for each PET scan, and $50 for each arterial line. In addition, smoker subjects from Aim 3 will be given a $100 bonus for completing all portions of the study. Subjects who participate in the Probabilistic Reward Task may also be compensated for the amount that they "win" during the task, up to $50. Subjects may be provided with payment in a form of a pre paid credit card, cash or a check. In addition, subjects will be provided with a light lunch. They will also be reimbursed for parking on study visit days or they will be compensated for reasonable transportation costs. If participation in the PET Scan has already begun, then compensation to will be based on involvement in the study, and will be up to the discretion of the PI. In addition, subjects participating in Aim 3 will be paid $20 for the script development session. 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 pre paid credit card, or cash. Subjects may be compensated for parking for research study appointments. They must show their parking ticket to the Research Staff for verification. Parking tickets will be returned to the subject so they are able to exit the parking lots and they will sign a Parking Voucher for the money received.

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

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

There is no cost to subjects for any of the research procedures.

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?
    Yes
    b. Where and from whom may treatment be obtained?

Treatment may be provided by Yale-New Haven Hospital, or any health care provider chosen by the study subjects.

c. Are there any limits to the treatment being provided?
Only emergency care will be provided. Limits to treatment may be imposed by the insurance carrier of the study subjects.

d. Who will pay for this treatment?
The study participant or his insurance carrier will be expected to pay the costs of this treatment.
No additional financial compensation for injury or lost wages is available. The cost of treatment will be borne by the subject or their insurance carrier.

e. How will the medical treatment be accessed by 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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