Brain Imaging in Tobacco Smokers During a Quit Attempt

NCT04055467

12/4/2021

Date: 12/04/2021
Principal Investigator: Elise M. Weerts
Application Number: IRB00173536

JHM IRB - eForm A – Protocol

- **Use the section headings to write the JHM IRB eForm A, inserting the appropriate material in each. If a section is not applicable, leave heading in and insert N/A.**
- **When submitting JHM IRB eForm A (new or revised), enter the date submitted to the field at the top of JHM IRB eForm A.**

****

1. Abstract

a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

The primary goals of this project are to examine the role of the α7-nicotinic acetylcholine receptors (nAChRs) in tobacco use disorder. Tobacco smoking represents the leading cause of preventable illness and death in the United States (US)[1]with ~22% of adult men and 16% of adult women in the US being current smokers. Only 6% of smokers who attempt to quit are successful long-term[1], and relapse occurs most often during the first 8 days of quitting[2]. One of the greatest barriers to smoking cessation is the nicotine withdrawal syndrome that emerges within 12 hours of abstinence [3, 4], and include intense craving/urges to smoke that are strongly associated with relapse [5]. Nicotine is the main psychoactive component of tobacco, and produces its effects are via nicotinic acetylcholine receptors (nAChRs) which are comprised of arrangements of alpha (α2-α10) and/or beta subunits (β2-β4). The most common types of nAChRs are those containing β2* subunits (*denotes assembly with other subunits) and homomeric versions which contain only α7 subunits. Preclinical and clinical studies have demonstrated that β2* nAChR are important for the reinforcing effects of nicotine, but appear to play a lesser role in expression of nicotine withdrawal. However, preclinical evidence suggests α7 nAChR may modulate the rewarding aspects of smoking and nicotine craving,[6]and thus α7 nAChR may provide an important novel target for pharmacotherapeutic interventions for smoking cessation. Surprisingly, the extent to which chronic tobacco use affects α7 nAChR in humans has yet to be explored.

The proposed outpatient study will quantify α7 nAChR availability in 18 tobacco cigarette smokers with tobacco use disorder (TUD) using human Positron Emission Tomography (PET) with a α7 nAChR selective radiotracer (^{18}F-ASEM) and explore the potential relationship of α7 nAChR availability to clinically relevant measures of tobacco abstinence (e.g., withdrawal and craving, cognitive impairment). Participants' α7 nAChR availability will be compared to α7 nAChR availability of historical controls (healthy volunteers who were nonsmokers). Study participants and historical controls will be matched by race, sex, and age. Smokers will also complete an 8-day quit attempt during which they can receive an incentive (money), contingent upon target exhaled carbon monoxide (CO) in early abstinence, and target urinary cotinine levels at later abstinence stages. These data are critical towards our understanding of the effects of cigarette smoking on α7 nAChR, a potential pharmacotherapeutic target to promote smoking cessation.

2. Objectives (include all primary and secondary objectives)

The proposed studies will help fill gaps in existing research by determining if in α7 nAChR availability in nicotine-dependent cigarette smokers is higher when compared to nonsmoker historical controls using PET imaging and the α7 nAChR selective radiotracer ^{18}F-ASEM. The study will also explore whether distribution volume (V_T), a measure of α7 nAChR availability in tobacco users influences clinically relevant measures of tobacco abstinence (e.g., withdrawal and craving, cognitive impairment), tobacco/nicotine use, and number of days abstinent during an 8-day quit attempt during which smokers can receive escalating payments contingent upon providing objective evidence (breath CO and urinary cotinine) of smoking abstinence.

Primary Objective 1: To examine α7-nAChR availability in tobacco smokers versus healthy nonsmoker controls.

Hypothesis 1: α7-nAChR availability (V_T) will be higher in heavy smokers with TUD than nonsmoker historical controls.

Primary Objective 2: To examine the relationship between α7-nAChR availability and clinically relevant measures of tobacco abstinence.

Hypothesis 2: α7-nAChR availability (V_T) in smokers will be positively associated with severity of nicotine dependence.

Hypothesis 3: Greater α7-nAChR availability (V_T) in smokers will be associated with higher craving/urges to smoke and greater cognitive/memory impairment during abstinence when compared to their baseline levels (smoking as usual).

Hypothesis 4: Higher α7-nAChR availability (V_T) will be correlated with fewer abstinence days during the 8-day practice quit attempt.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Public Health Significance. The World Health Organization (WHO) has estimated smoking-related costs across the globe to be about $500 billion USD a year. There is extensive evidence that nicotine, the primary component in tobacco, produces it effects via nAChR[(7)]. Tobacco smoking (or administration of nicotine) produces mild euphoria, and improvements in mood, attention and cognitive function, as well as physiological effects such as increased heart rate, sweating, dizziness, and nausea. Smoking abstinence is associated with a nicotine withdrawal syndrome, which includes negative affective symptoms such as increased anxiety, depression, irritability, as well as intense craving/urges to smoke, and impairments in attention and working memory. Symptoms of nicotine withdrawal emerge within 12 hours of smoking abstinence, lasting 2-4 weeks, and are influenced by nicotine dependence severity[(4)]. During quit attempts, smokers are most vulnerable to relapse during early abstinence. Although FDA-approved treatments (nicotine replacement therapy, bupropion, and varenicline) improve quit success rates, especially short-term, the fact that 80-85% of smokers still ultimately relapse highlights the need for better understanding of the neurobiological mechanisms involved in smoking maintenance, nicotine withdrawal, and relapse[(8)].

nAChR. α4β2 nAChRs have a high affinity for nicotine and are distributed throughout the CNS, including the mesocorticolimbic dopamine system. While the predominant receptor nAChR subtype consisting of β-subunits are more ubiquitous, the pentameric homomeric α7 nAChR has been implicated as a potential target for therapeutic intervention due to its high calcium permeability and second messenger system involvement [(9)]. α7 nAChRs have lower nicotine affinity than α4β2 nAChRs, and are predominantly located in the hippocampus, cortex, and subcortical limbic regions.

Importantly, α7 nAChRs are localized within the ventral tegmental area (VTA) and nucleus accumbens (NA) terminating to dopamine cell bodies, which suggests a likely influence on the dopamine reward system and contribution to aspects of smoking and relapse[6]. Studies in rodents and humans have shown exposure to chronic nicotine produces an upregulation of nAChRs containing β2* subunits and such upregulation is associated with nicotine dependence. Activation of β2∗ nAChRs facilitates nicotine self-administration. In contrast, α7 nAChRs negatively modulate both nicotine reinforcement and β2∗ nAChR function in the mesolimbic dopamine system. Selective stimulation of α7 nAChRs reduces nicotine reward and motivation to self-administer nicotine, and augments the aversive effects of nicotine [10]. This is consistent with growing evidence that there are compensatory interactions affecting the balance between β2* vs. α7 nAChRs. This suggests a working hypothesis that brain α7 nAChR may play a role in negative craving and withdrawal severity. With high nicotine exposure, α7 nAChRs desensitize more rapidly, and at higher nicotine concentrations than α4β2 nAChRs [11-14]. Such desensitization processes are thought to be a critical step in the development of nicotine tolerance and dependence. Indeed, the most effective FDA-approved medication for smoking cessation (varenicline) acts as a full α7nAChR agonist and a α4β2 nAChR partial agonist consistent with current theories of a role α7 subtype in long-term continuance of smoking cessation[15].

PET Imaging of nAChR. Human *in vivo* imaging study of *β2 nAChRs is well established in the literature with multiple studies demonstrating partial occupancy of *β2 nAChRs after exposure to second hand tobacco smoke (19%)[16], or just a few puffs of a tobacco cigarette (50%)[17] . Other studies have demonstrated an upregulation of *β2 nAChRs in tobacco smokers in first few days of quitting smoking, when compared to non–smokers, and levels normalize by 6 to 12 weeks [18-21]. In contrast, the lack of a suitable radioligand for α7 nAChR hampered this area of research in human subjects. Previous published attempts yielded over 20 radioligands insufficient for clinical use [22-32]. In 2013 our team developed ^{18}F-ASEM, a high α7 affinity (K_i = 0.3 nM) and selective radioligand with excellent specific binding in rodents[32] and baboons[33], and completed the first successful in-human PET experiments[34]. To date, there has been no demonstration of the effects of tobacco use on α7 nAchR *in vivo* in the human brain. α7 nAChRs regulate a variety of behaviors including cognitive process of attention, learning and memory, as well as emotion, and reward; the same processes are involved in the maintenance of smoking behaviors, craving and withdrawal. It is unclear why tobacco users often smoke so many cigarettes each day, given that relatively low amounts of tobacco use saturates the α4β2 nAChRs. Moreover, upregulation of *β2 nAChRs was not correlated with clinical measures of abstinence (severity of nicotine dependence, withdrawal or craving/urges to smoke) in prior studies[20, 35]. Thus, the α7 nAChR receptor is a likely mechanism for modulating the degree to which tobacco users smoke each day. Our study will utilize ^{18}F-ASEM to examine if α7-nAChR availability is higher in heavy tobacco smokers when compared to nonsmoking historical controls. We will also explore the possible role of the α7-nAChR in craving/urges to smoke, severity of nicotine dependence, severity of withdrawal and cognitive impairment during nicotine/tobacco abstinence, and propensity to relapse.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

Protocol Overview

The proposed proof-of-concept pilot research study will be conducted at the Johns Hopkins Behavioral Pharmacology Research Unit (BPRU) and the Johns Hopkins Hospital (JHH) PET Imaging Center. All participants will be healthy volunteers and all procedures will be for research purposes. Tobacco smokers (n=18) will be invited to complete a smoking as usual visit

with an MRI, followed by an 8-day quit attempt with 2 study visits during the 8-day period. Participants who successfully maintain 1 day of abstinence will complete a PET scan with the α-7 nAChR radiotracer ^{18}F-ASEM. At all visits, exhaled CO and urinary cotinine levels will be determined. For Visits 2 and 3, participants will receive incentive payments contingent on biomarkers of smoking abstinence (CO and cotinine) to promote smoking abstinence. Measures that are clinically relevant for smoking cessation (e.g., symptoms of nicotine withdrawal and craving; impaired cognitive performance; changes in resting heart rate), number of cigarettes smoked per day, and number of days abstinent until return to smoking (lapse) will be examined in relation to α-7 nAChR availability. In addition, we will access PET imaging data from healthy non-smoking controls who completed ^{18}F-ASEM PET studies under IRB approved protocols NA_00091295 and IRB00185590 (PI: Dean F. Wong) who match participants from the present study on characteristics such as age, sex, and BMI.

Human Subjects Involvement, Characteristics, and Design

Subjects aged 21-50 will be recruited via include newspaper, radio and other advertisements in the greater Baltimore-Washington D.C. area. Subjects will be recruited to yield a broadly representative sample of the community. Based on census data, the greater Baltimore area population is mostly Caucasian (62%) or African American (29%); the remaining 9% is mostly Asian (6%) and persons reporting more than two or more races (2%). About 8% of the population report Hispanic or Latino origin. We anticipate a similar distribution for the current study sample.

All potential subjects will be screened by telephone using a standardized initial questionnaire, which includes demography, alcohol and drug use patterns and associated problems, and a brief personal medical and mental health status. This telephone screening addresses the major inclusion/exclusion criteria for the study and quickly rules out respondents who disqualify. We anticipate a highly adequate recruitment flow of subjects. In earlier studies, we completed a total of approximately 600 phone call inquiries and telephone screens annually. Based on our recent experience, we estimate we will consent and assess about 4 persons for each subject who completes study all procedures (i.e., approximately 72 participants will be consented in order to obtain 18 study completers). This is necessary to have a sufficient pool of subjects who meet inclusion/exclusion criteria and who can be matched to historical controls by sex, race/ethnicity, and age.

Subjects who appear eligible based on the telephone interview are informed of the general nature of the study, including study duration and commitment, introductory information about the medication, the nature of the measurement procedures, and compensation amount and arrangements. Interested volunteers are then invited for an in-person interview at the BPRU facilities on the Bayview Campus.

Screening.

Subjects who appear eligible and provide written informed consent will complete an in-person screening visit that includes a standard battery of instruments, a medical history, physical examination, vital signs, and standard blood chemistry and hematology laboratory tests. Prior to any research participation, subjects provide written informed consent using the Institutional Review Board (IRB) approved form; consent is obtained by IRB-approved members of the research team. Upon enrollment, subjects are assigned a unique study identifier (ID). Only subject IDs will be used to code all data forms.

Multiple assessment instruments will be done to determine who should be included and who excluded, and to provide measures that may be included as covariates in analyses. The Mini-International Neuropsychiatric Interview (MINI, v.7)[36] for DSM-5 will be used to assess TUD,

other SUD, and psychological disorders. Tobacco, alcohol, and illicit drug use will be characterized using the 90-day Time Line Follow Back (TLFB)[37]. The presence of tobacco use disorder and severity of nicotine dependence will also be assessed using standard questionnaires such as the Fagerstrom test for nicotine dependence. The Shipley Institute of Living Scale [38] will be used to ensure adequate reading comprehension for completion of study assessment instruments; a score of 18 or higher is required for study enrollment. Exhaled breath carbon monoxide (CO) and urinary cotinine will also be assessed at screening to determine smoking status. Volunteers who appear eligible for study participation at the end of the screening visit will complete a screening ECG (see below).

ECG Screening.

The study team physicians with JHBMC privileges will review all health information to determine initial study eligibility. Any participants with a history of heart disease, or blood pressure that exceeds a systolic blood pressure of 150 mmHg or a diastolic blood pressure of 90 mmHg will be excluded. Only healthy volunteers are eligible. A 12-lead ECG will be conducted in those that appear to meet all other study inclusion/exclusion criteria as an additional screening procedure. If the ECG shows any abnormalities, a credentialed cardiologist will determine if the abnormality may be clinically significant (incidental findings). Participants that have clinically relevant ECG abnormalities will be excluded from further study participation.

MRI Procedures.

Eligible participants will have an MRI scan of the head to identify the anatomy of the brain for co-registration with the PET images. The MRI exam will take approximately 60 min. During this MRI, participants will also undergo echo-planar imaging scans during rest, and a diffusion-weighted imaging scan. Resting-state and diffusion-weighted imaging scans will be used to determine resting-state functional brain connectivity. Prior to the MRI, each participant will be asked to complete a standard questionnaire to determine if the participant has any metal in the eyes or head. The purpose of this questionnaire is to ensure that the participants are safely able to enter the MRI area. After answering this questionnaire, a small number of participants may be required to undergo two anterior-posterior (AP) skull x-rays to further determine if it is safe for them to enter the MRI area. If the skull x-rays are necessary, the MRI staff will explain the x-ray procedure to the participant. The results of the x-rays will then determine if it is safe for the participant to enter the MRI area. An extremely small number of participants are determined to be unsafe to enter the MRI area based on the questionnaire and/or x-rays. If this should happen, or if the participant chooses not to have the x-rays, the participant will be removed from the study.

Practice Quit Attempt

Following the screening procedures, eligible smokers will be asked to refrain from tobacco smoking for at least 1 day and try to remain abstinent for 8 days. We selected an a 8-day quit attempt in order to capture data during the period in which relapse occurs most often (i.e., the first 8 days of quitting)[2]. During this 8-day period, smokers will complete 2 study visits (e.g., day 1, and 8 of the quit attempt) during which breath CO and urine samples to determine levels of cotinine and clinically relevant measures for smoking cessation (e.g., withdrawal, craving, cognitive/memory performance, vital signs) will be assessed. Cigarettes smoked per day will also be assessed retrospectively.

To promote smoking abstinence, a validated incentive intervention (contingency management)[39-41] will be used. Specifically, at each visit, smokers will receive $40 payment for attendance, and additional incentive payments for providing breath and urine sample(s) at each visit that meet defined abstinence criteria for breath CO and urinary cotinine consistent with tobacco/nicotine abstinence [42, 43]. CO provides a specific biomarker for recent smoking. A threshold of <6ppm will be used as this level reduces misclassification of individuals who engage in some smoking as "abstinent"[44]. Urinary cotinine has a longer half-life (16 h) than breath CO. Cotinine progressively declines over consecutive days of abstinence, and a lack of such a decrease is a biomarker of nicotine use. Using criteria established previously[39-41], abstinence on day 8 will be cotinine levels below the abstinence threshold (<100 ng/mL). To maximize the number of smokers available for imaging after 1 day of abstinence, a second reinforced quit attempt will be allowed on the following week, if they are unable to reach the abstinence criteria on their first attempt. Once the initial quit is verified, a reset scheme typically used in incentive interventions will be employed. Provision of a sample that exceeds the abstinence criterion will result in no incentive earnings for the day and a reset to the beginning of the schedule. When an incentive is not earned, the $40 payment for making the visit should be sufficient compensation to support continued study attendance, and re-try of the quit attempt. This strategy is based prior BPRU studies that employed the same incentive intervention and reset scheme. The combined use of both CO and cotinine as abstinence biomarkers provides the strong method to verify and reinforce early phase abstinence when residual cotinine is being eliminated, and longer abstinence when cotinine has high sensitivity[42, 44]. CM (using similar parameters and incentive earning schedules) is a well validated intervention. CM is used to promote smoking cessation in treatment-reticent populations including opioid-maintained smokers[48-50] and smokers with schizophrenia as a stand-alone treatment or combined with smoking cessation medication[45, 51].

PET Scan Procedures:

Tobacco smokers (n=18) will complete a single ^{18}F-ASEM PET scan on day 1 of the 8-day quit attempt, assuming they are able to remain abstinent from cigarettes prior to the scan (verified by exhaled breath CO).

Prior to the PET scan participants will complete procedures i-iv.

(i)Facemask Procedures:

Each participant will have a facemask custom-fitted to facilitate maintaining the same position of the head for the PET scan. The facemask will be constructed on the day of the PET scan. To make the mask, a soft, warm piece of plastic will be laid across the participant's face. The plastic touches the participant's face from his/her forehead to the middle of his/her nose. The plastic molds to the shape of the participant's face and hardens as it cools. The facemask will take approximately 10 minutes to construct.

(ii)Pregnancy Testing for Females prior to all PET scans:

All female participants will be required to provide a urine sample for pregnancy testing. The pregnancy test must be negative for the participant to continue with the study.

(iii)Catheters and blood sampling:

All participants will have two catheters, a venous and arterial, placed prior to the start of the PET scan. The first venous catheter will be inserted into a vein of one arm for the injection of the radiotracer. The arterial catheter will be inserted into a radial artery of the other arm by an anesthesiologist for rapid withdrawal of blood specimens for analysis of radiotracer. The wrist of each participant will be infiltrated intradermally via a 30 gauge needle with 2 mL 1% (20 mg (0.3 mg/kg for most adults) lidocaine hydrochloride before the insertion of the arterial line to provide local anesthesia. If participants experience discomfort at the site of insertion of the arterial line, then they will be administered subcutaneous injections of 2 mL 1% (20 mg (0.3 mg/kg for most adults) lidocaine hydrochloride. This dose corresponds to 10% of the maximal recommended dose and 5% of the dose associated with effects on the central nervous system. During the PET scan, up to forty blood samples (a total blood volume of approximately 150 mL) will be collected for metabolite and radioactivity analysis of the radiotracer.

(iv)Transmission and emission scan:

After the catheters are placed the participant will be placed in the PET scanner and complete a 6 to 10 minute transmission (attenuation) scan performed before or after the PET scan. The participant will then have the PET scan performed during which an intravenous injection of 15 mCi of ^{18}F-ASEM will be administered. Dynamic PET data will be acquired during scanning for approximately 90 minutes following tracer injection.

Retrospective Healthy Control Data:

We will use historical PET imaging data from prior studies that used the same ^{18}F-ASEM radiotracer studies under IRB approved protocols NA_00091295 and IRB00185590 (PI: Dean F. Wong), to compare α-7 nAChR availability in the non-smokers in those studies to smokers in the current study. All participants for whom we access their historical data consented to the use of their data in future investigations.

b. Study duration and number of study visits required of research participants.

Participants will have up to 1 month to complete all study procedures, from the time the informed consent is signed up to the completion of all study procedures. The number and type of visits (4 total) for study participants is as follows:

Baseline Visit 1: Baseline (smoking as usual) cognitive tests, self-report instruments, Vital signs, breath and urine sample collection, and MRI.
Set quit date (should be the day before PET on Visit 2).

Visit 2: Vital signs, breath and urine sample collection.
PET scan (pending CO-verified tobacco/nicotine abstinence)
Cognitive tests and self-report instruments

Visit 3: Day 8 of the 8-day practice quit attempt
Vital signs, breath and urine sample collection.
Cognitive tests and self-report instruments

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

This is not applicable for this study, as experimental procedures will be the same for all study participants.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

Participants in this study will be healthy volunteers who use tobacco products and are not seeking smoking cessation treatment. Routine care for any medical illness that may arise during participation will not be affected. Individuals currently attempting to quit smoking using a form of therapy such as nicotine replacement therapy (NRT) or behavioral counseling or who have used a tobacco cessation treatment in the past 3 months will not be eligible.

e. Justification for inclusion of a placebo or non-treatment group.

There is no placebo or non-treatment group in the present study.

f. Definition of treatment failure or participant removal criteria.

Participant participation is voluntary. Participants may withdraw at any time for any reason. Participants will also be withdrawn from the study if the participant experiences an event making it unsafe for the participant to continue to in the study. Participants may also be withdrawn the study if the principal investigator feels it is in the best interest of the participant, or participant is unreliable in attendance of study visits.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Upon completion or premature exit from the study, all participants who are smokers will undergo our standard smoking cessation counseling session, guided by the NCI booklet "Clearing the Air: Quit Smoking Today" and will receive a referral card to a tobacco quit line. Individuals who have used a smoking cessation product in the past 3 months or are currently attempting to quit smoking using a form of treatment (e.g., behavioral counseling) will not be eligible. Thus, the practice quit attempt smokers will engage in for this study will not serve as a substitution for other treatments. For participants who do not meet full study inclusion/exclusion criteria participation ends after screening procedures. Participants who meet all inclusion/exclusion criteria may also be discontinued if they are unable to complete all inpatient procedures (e.g., are unable to schedule or complete procedures in a timely manner) or where continuation may increase risk as determined by study physicians. The study investigators may also discontinue study participants for other reasons not known at this time such as for failing to follow a particular protocol requirement. Participants with a positive toxicology or pregnancy test are excluded from participation. Participants may be discontinued if they are unable to meet the 1-day smoking abstinence criteria prior to the PET scan per the study protocol. Participants will be compensated for each of the procedures completed regardless of whether they continue in the study or not; earnings for uncompleted procedures or completion bonuses will be forfeited. Discontinuation of a study participant can result in the need to recruit additional research volunteers.

5. Inclusion/Exclusion Criteria

Inclusion criteria:

- Healthy volunteers who are regular tobacco smokers
- Aged 21 - 50 years old
- Willing to make themselves available for the duration of the study and are willing to follow study procedures and research unit policies.
- Women of child bearing potential must agree to use a reliable method of birth control during the study and for 1 month after all study procedures have concluded
- Self-report currently smoking 10 or more cigarettes per day
- Self-report regular smoking for a period of 2 or more years
- Provide an exhaled breath carbon monoxide (CO) exceeding 8 ppm
- Provide a positive cotinine urine test
- Provide a negative cannabis urine toxicology screen
- Meet DSM-V criteria for moderate to severe tobacco use disorder (TUD).

Controls (non-smokers): We will access a historical database of research participants that completed intake assessments and ^{18}F-ASEM PET scans under our other comparable research studies (e.g., NA_00091295; IRB00185590; PI: Wong). Specifically, we will select males and females from our database of healthy nonsmokers who meet the following inclusion criteria: a) males and females aged 21-50 years, b) no current or past history of tobacco smoking, b) confirmed negative breath CO at intake and pre-PET ($\leq$4ppm). Exclusion criteria were the same as described below for smokers in the current protocol. We will select controls who are best matched for age, sex and race distributions to smokers in this study.

Exclusion Criteria:

- Meets DSM-V for AUD or SUD Disorder (excluding TUD for smokers)
- Meets DSM-V Psychiatric Disorder; in or in need of treatment
- Use of illicit drugs within last 90 days; positive urine toxicology screen
- Clinically-significant abnormality on ECG
- Unstable hypertension (BP > 150/90)
- Serum creatinine and hepatic enzymes (AST, ALT) outside of normal limits
- History of seizures, seizure disorder or closed head trauma
- HIV positive
- Weight > 350 lbs
- < 5th grade reading level as determined via a Shipley score of less than 18
- Use of smoking cessation products in last 3 months or undergoing current quit attempt using another treatment
- If female: has a positive pregnancy test, is pregnant, lactating, or planning pregnancy
- Significant anatomical abnormalities on MRI of the brain (e.g., enlarged ventricles, brain lesions)
- Use of prescribed medications in last 3 months; over the counter drugs or herbal supplements which may be counter indicated as determined by study physician may also be excluded
- Presence or history of drug allergy, or allergic disease diagnosed and treated by a physician.
- Any serious medical condition in whom participation is contraindicated.
- Any condition which would preclude MRI (e.g., implanted metal, severe claustrophobia).
- Radiation exposure in the last year that when combined with the study protocol would exceed the annual limits.

To determine eligibility, the medical records will be reviewed by study physicians, and psychological assessments and full inclusion/exclusion criteria will be reviewed by Dr. Weerts.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.
b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.
c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

PET radiotracer: ^{18}F-ASEM

^{18}F-ASEM is a selective, high-affinity (K_i = 0.3 nM) antagonist for α7-nAChR that was developed for PET imaging. ^{18}F-ASEM was developed, synthesized and first tested in humans at the JHU PET center[52-54], and will be used in the proposed studies under IND 118,496. Dr. Dean F. Wong (Co-I on this protocol) submitted documents to the FDA which transfers ownership of this IND to Dr. Martin Pomper (Packet date 2/27/2020). ^{18}F-ASEM will be synthesized by our dedicated team of PET radiochemists and will meet all USP Chapter <823> acceptance testing criteria. Radiochemical purity at end of synthesis will be > 99% with high specific activity. A single dose of 15 mCi of ^{18}F-ASEM will be administered to each participant during the PET scanning procedure by intravenous bolus administration. This dose represents an exposure that conforms to the "ALARA" (as low as reasonably achievable) principle for radiation exposure. Based on highly analogous studies in nonhuman primates, this dose should provide the minimum image quality necessary to quantify α7-nicotinic cholinergic receptor densities in the brain while exposing the human volunteers to an ED of radiation that should be less than 5 rem per year (the State of Maryland's approved occupational limit, 21 CFR 361.1). Indeed, a single injection of 15mCi of ^{18}F-ASEM will only expose participants to 0.95 rem, over 5 times less than the recommendation annual radiation exposure limit. ^{18}F-ASEM is the appropriate radiopharmaceutical for our studies. It has excellent test-retest reproducibility[55], and has been used successfully and safely in PET studies in healthy normal volunteers and schizophrenic patients[54].

7. Study Statistics

a. Primary outcome variable.
b. Secondary outcome variables.
c. Statistical plan including sample size justification and interim data analysis.
d. Early stopping rules.

Derivation of outcome variables. There is no appropriate, reliable reference region for ^{18}F-ASEM due to the widespread distribution of α7 nAChR in the human brain. BP_{ND} (non-displaceable binding potential which requires a measure of absolute non-specific binding) is not appropriate for quantification of this radioligand. Volumes of Interest (VOI) will be generated by spatially normalizing[56] a standard VOI template[57] to individual subjects on Spoiled Grass (SPGR) sequenced MRI volumes. VOIs are: amygdala, ventral striatum, cingulate, hippocampus, and frontal cortex. α7 nAChR availability (V_T) will be obtained for each VOI by plasma reference graphical analysis (PRGA); we have previously demonstrated that PRGA showed more robust estimates of V_T than the two-compartment model, as measured with time dependency of estimates and the magnitude of inter-subject variability[54]. V_T is a standard outcome for many receptor binding radiopharmaceuticals.

Sample size and Power Analysis: We propose a total sample size of 36, which includes 18 smokers accrued in the proposed study, plus an equal number (n=18) of healthy nonsmokers selected from a

pooled sample of our historical database, matching for basic demographic variables (age, sex, and race) of our smokers.

The primary outcome variable is V_T determined via ^{18}F-ASEM PET scans in 5 VOI (anterior cingulate, hippocampus, frontal cortex, amygdala, and ventral striatum). We completed a power analyses using R, and the package 'pwr' version 1.2-1, using pooled baseline data (total n=21) and our test-retest data (n=8) for ^{18}F-ASEM V_T. Across all regions, TRV averaged 6% (+/- 0.9%, range 5.1% to 8.8% across regions), which is well below the standard accepted reproducibility level of 10%. Assuming between group comparisons (18 Smokers vs. 18 controls), with an α of 0.05 (one tailed test), we examined our power to detect a 20% difference between groups (smokers vs. nonsmokers). Cohen's d for the VOIs, calculated as the average difference in mean V_T for a 20% increase in smokers/pooled standard deviation (SD), ranged from 1.09 (amygdala) to 1.3 (frontal cortex). Our analysis indicated we will have 0.94 to >0.98 power to detect 20% change in our VOIs. If the change is more modest (e.g.,15%), calculated Cohen's d ranged from 0.82 (amygdala) to 0.98 (frontal cortex), and we would still have >0.80 power to detect group differences of 15% in anterior cingulate, hippocampus, frontal cortex, and ventral striatum, but may be slightly underpowered for amygdala (0.77 power) for a 15% group difference.

Statistical Analysis Plan: First, the baseline characteristics of the participants (age, race, sex, socioeconomic status etc.) will be summarized and compared by group (smokers vs. controls). The primary and secondary outcomes variables as defined in 4.3 Outcome variable, and summarized above, will be our dependent variables. Descriptive statistics will be generated; continuous variables will be reported as means (SD) and categorical variables as frequencies and percentages. The primary outcome variable (V_T), and all secondary outcome variables will undergo data cleansing and CAPA (Corrective/Preventive Measures-based confirmation of accuracy and missing data). We will perform exploratory data analyses (stem-leaf displays, box plots, histograms, and Q-Q plots), and if necessary, appropriate data transformations will be performed. Our intention is to frequency match groups as closely as possible for age, race, and sex, and to match male and female smokers for nicotine dependence severity as appropriate, to reduce these potential confounds. To determine if smokers differ from controls in α7 nAChR availability (hypothesis 1a), we will use a general linear model. V_T (or a transformation, if necessary) will be the dependent variable with group (smokers and controls) as the independent variable. All VOI-based statistical analyses will be done with SPSS (IBM Corp., 2013) or R (Foundation for Statistical Computing, 2011). After the initial VOI-based analyses, we will also use Statistical Parametric Mapping (SPM [56, 58] to identify and confirm differences in V_T between groups without restrictions of VOIs. The same models used in VOI-based analyses will be applied to the voxel-based analyses. We plan to use a threshold level of $p = 0.05$, FDR-corrected, and the minimal cluster size at 50 voxels (~0.4 ml). To determine if V_T will be associated with clinically relevant measures of tobacco withdrawal during abstinence in smokers, we will construct separate models with V_T of hypothesized VOIs as the dependent variable, and change from baseline to abstinence (day 1) for: craving, withdrawal discomfort, negative affect, and cognitive/memory performance as independent variables. The Holm-Bonferroni method will be applied to correct for multiple comparisons.

Resting-state functional brain connectivity analysis: Resting-state data will undergo standard preprocessing step, including slice timing correction, realignment/motion correction, co-registration across runs and to the SPM EPI template [59], with smoothing, motion sensoring or "scrubbing" [60], physiological artifact correction [61], and 36-parameter confound regression and spike regression [62]. DTI and structural MRI data will undergo realignment, registration to an average template, and deterministic tractography will be conducted in order to determine laplacian harmonics for connectome harmonic analysis. Seed-based, region-of-interest-based [63], independent component-based [59], graph-theory/network-analysis-based[64], and connectome-harmonic-based metrics [65] of

connectivity within and among commonly identified resting-state networks [66, 67]), including the default mode, salience, executive control, and fronto-parietal attention networks will be determined using standard software. These metrics will be compared between groups (smokers and matched controls) using a two-sample T-test.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

This study involves several separate procedures, each of which entails some risk of discomfort or side effects. These risks are discussed by procedure. Participants will receive a thorough description of all potential risks in the consent document.

Screening procedures: This study involves questions about dangerous or illegal behavior, psychiatric history, a medical history, and a physical exam. There is risk of a possible breach of confidentiality. There is also a small risk that participants will become upset during the screening interview. Participants' names will be recorded only on the screening, informed consent, and necessary medical and payment forms. Anonymous participant identification numbers will be used on all other forms and labeling of biological fluids and test results.

ECG Screening. A 12-lead ECG will be conducted in those that appear to meet all other study inclusion/exclusion criteria as an additional screening procedure. These screening ECG results may identify a potentially clinically significant cardiac abnormality (i.e., incidental finding). The screening ECG only identifies an "incidental finding" and is not a full diagnostic ECG. One of the study physicians will contact the participant and discuss the nature of the ECG findings, and if results are clinically significant, the physician will recommend that the participant see his/her physician for diagnostic evaluations, or if needed, refer the participant to an appropriate physician. We will provide a copy of the ECG report. Participants that have clinically relevant ECG abnormalities will be excluded from further study participation. Disclosure of these incidental findings to the participant may be distressing to the participant.

MRI. The MRI does not involve any radiation exposure, but does involve exposure to magnetic fields. Although scientists do not know a great deal about the possible long-term effects of exposure to magnetic fields, the risks of the MRI scanning procedure are presumed to be minimal. Because the MRI instrument contains a powerful magnet, it could be painful if subjects have any metal objects in or on their body; Subjects who report metal in their body are excluded from participation. At-risk subjects (e.g., metalworkers) who may have metal in their eye will also complete a skull x-ray to determine presence of metal prior to the MRI exam (see below). Some people find the MRI unpleasant or claustrophobic.

MRIs will be reviewed by a faculty neuroradiologist, who may identify brain abnormalities (incidental findings), some of which may be clinically significant. These MRI results only identify "incidental findings" as the research MRI is not a full diagnostic MRI. One of the study Physicians will contact the participant and discuss the nature of the findings (e.g. benign, congenital abnormalities etc.) if it is not exclusionary, and if results are clinically significant, will recommend that the participant see his/her physician and if needed refer the participant to a physician. Disclosure of these incidental findings to the participant may be distressing to the participant.

Skull x-ray. As indicated in the MRI procedures, participants may be asked to undergo two skull x-rays to determine the participant has no metal in their skull. This is a standard safety procedure employed by the Johns Hopkins MRI staff to verify it is safe for the participant to

enter the MRI area. If participants undergo the skull x-ray there will be an additional 0.01 rem of radiation exposure to the participant.

PET and Radiologic Procedures. Studies that employ PET are approved by the Johns Hopkins University IRB and the Federal Drug Administration (FDA). Subjects will undergo one PET imaging session using the α7-nAChR radiotracer ^{18}F-ASEM. Use of ^{18}F-ASEM in the current protocol will be under IND# 118,496 (approved 1/2/2014), recently transferred from Co-I Dr. Wong to Co-I Dr. Martin Pomper. Potential risks are detailed below.

1. *Catheters:* All participants will have two catheters, one venous and one arterial, placed prior to the start of each PET scan. The venous catheter will be inserted into a vein of one arm for the injection of the radiotracer. The arterial catheter will be inserted into a radial artery of the other arm by an anesthesiologist for rapid withdrawal of blood specimens; up to 40 blood samples (a total blood volume of approximately 150 mL) will be collected for metabolite and radioactivity analysis of the radiotracer.
2. *Lidocaine.* Lidocaine, a local anesthetic, is administered prior to arterial catheter placement. There is the possibility of an allergic reaction following the topical placement or the injection of the lidocaine. Lidocaine will not be administered to participants with known allergy to lidocaine. Lidocaine may cause drowsiness, tremors, and decreased respirations for a few hours after administration. The maximal amount of lidocaine the participant will receive in this study is 10% of the maximal recommended dose and 5% of the dose at which any side effects (such as drowsiness or tremors) would be expected. Therefore, side effects from lidocaine are unlikely.
3. *Radiotracer.* Only high specific activity ^{18}F-ASEM is being administered in this study. Each participant will receive approximately 15 mCi of ^{18}F-ASEM during the PET scanning procedure as a bolus injection. This is a tracer dose of drug; therefore, no behavioral or adverse effects of the radiotracer are anticipated. There is a low risk of an allergic reaction following the injection of the radiotracer. Dr. Wong has extensive experience with ^{18}F-ASEM under IRB approved protocol (NA_00091295). ^{18}F-ASEM will be used at very small doses, well below levels with any known pharmacologic activity. No adverse events are expected. Subjects are carefully monitored for any side effects after receiving the radiotracer. There is currently no information on the effects ^{18}F-ASEM on an embryo or fetus; however, it is known that higher levels of radiation can cause damage to an embryo or fetus.
4. *Radiation exposure.* ^{18}FASEM is an imaging agent that includes radioactivity. A single dose of 15 mCi of ^{18}F-ASEM will be administered to each participant during the PET scanning procedure intravenously bolus administration, exposing participants to 0.95 rem. From the PET transmission scan, participants will receive an additional 0.0026 rem of external radiation exposure. If participants are required to have the skull x-rays performed to verify it is safe for the participant to enter the MRI area, the radiation exposure will increase by 0.01 rem. Thus, the total possible maximum amount of radiation exposure from the combined internal and external sources of radiation in this study will be about 0.9626 rem, which is below the allowable annual radiation exposure limit of 5 rem per calendar year set by JHU. The amount of radiation exposure is also within the limits of allowable occupational exposure in the state of Maryland (21CFR361.1). Overall, the radiation exposure in this study conforms to the "ALARA" (as low as reasonably achievable) principle for radiation exposure.
5. *Women for child bearing potential:* To avoid unknown risks for the fetus, women of child bearing potential must use an effective form of birth control and will have urine pregnancy tests at screening and prior to all procedures; only women with a negative pregnancy test may participate.

6. *Other risks.* Several additional risks occur throughout the course of the PET study. PET risks include a risk of pain, bleeding or infection from the catheters used for PET scan procedures. In rare cases, fainting or infection could occur.

Nicotine/tobacco withdrawal: Abstinence after chronic daily use of tobacco cigarettes is associated with nicotine/tobacco withdrawal discomfort that may include increases in symptoms such as anxiety, irritability, craving, sweating, and difficulty concentrating[68-71]. These symptoms are time limited, not associated with severe health consequences, not medically dangerous, and typically resolve on their own within 2 weeks.

Treatment Diversion: Individuals who are attempting to quit smoking using another treatment method (e.g., NRT) or who made a quit attempt in the last 3 months, using smoking cessation medications are also are not eligible to participate. Some tobacco users could delay seeking other treatment in order to participate.

Blood collection: Blood collected during the PET scan will be less than the amount taken for routine blood donation. Blood draw procedures involve minimal risks, such as a slight risk of discomfort at the intravenous site. A small amount of bleeding under the skin will produce a bruise in about 5% of cases. The risk of temporary clotting of the vein is about 1%. The risk of infection or significant blood loss is less than 1 in 1000. In rare cases, fainting could occur. To minimize risks associated with venipuncture, experienced medical personal using sterile equipment, will perform the blood draws.

b. Steps taken to minimize the risks.

Recruitment and Informed Consent: Participants will be identified through the media using IRB-approved advertisements displayed on forums such as social media, newspaper/magazine, and radio. At the initial contact, the research coordinator will discuss the study purpose and requirements with the participants. Prior to the start of the screening, subjects provide written informed consent using a document approved by the Johns Hopkins IRB. The Project Coordinator will read and, as necessary, explain the consent form to the volunteers before they are asked to sign it. Volunteers will receive a copy of the signed consent form to keep. The consent form describes the experimental procedures and their associated risks. It provides an assurance that volunteers may ask and will receive answers to questions, assures volunteers that their participation is voluntary and may be terminated by them at any point if they wish. The consent form also gives the conditions for investigator termination of research participation, and provides names and numbers to contact in the event of questions or concerns. Prior to completing any assessment materials, subjects are breathalyzed and must provide a 0mg% reading to participate in the interview. Subjects also must provide a urine sample and test negative for illicit drug use and for pregnancy. Subjects with any contraindications are excluded from participation. Subjects are permitted to discontinue their participation at any time. Subjects are carefully and continually monitored throughout their participation. In case of an adverse event, a physician or nurse practitioner is on call for assistance.

Insuring protocol comprehension: We exclude potential subjects with literacy below the 5th grade reading level as determined using the Shipley-2 because of concerns about their ability to adequately participate in the study procedures. Many of our behavioral/subjective measures are self-administered and require basic literacy and language skills. If subjects are not at a 5th grade level, they have difficulty responding accurately to the study questionnaires.

Psychosocial assessments: The risk of distress or personal discomfort elicited during testing is minimized by the use of standardized assessment procedures widely used in research settings. In addition, all study staff are trained in nonjudgmental interview techniques and crisis intervention procedures.

ECG Screening. A 12-lead ECG will be conducted in those that appear to meet all other study inclusion/exclusion criteria as an additional screening procedure. There is a small risk of some skin irritation from the pads used to do the ECG. Any irritation is usually mild and will resolve on its own. Sometimes a small amount of your body hair where the ECG pads will be placed must be shaved to help pads stick to the skin. Only a small amount of hair would be shaved, but may cause some temporary skin irritation. There is a risk the screening ECG results may identify a potentially clinically significant cardiac abnormality (i.e., incidental finding). The screening ECG only identifies an "incidental finding" and is not a full diagnostic ECG. One of the study physicians will contact the participant and discuss the nature of the ECG findings, and if results are clinically significant, the physician will recommend that the participant see his/her physician for diagnostic evaluations, or if needed, refer the participant to an appropriate physician. Disclosure of these incidental findings to the participant may be distressing to the participant. We will provide a copy of the ECG report. Participants that have clinically relevant ECG abnormalities will be excluded from further study participation.

MRI: The risk of pain associated with metal in the body will be reduced by taking the following steps: 1) Subjects will be instructed to inform the investigator if they have metal objects on or in their body that cannot be removed (e.g., bone pin, skull plate, braces). 2) Subjects will only be allowed to receive an MRI scan if the implant or device is made of non-magnetic materials. 3) Skull x-rays will be used to determine if at risk subjects (e.g., metalworkers) have metal in their eye prior to the MRI exam. If they are found to have metal in their eye, they will not be allowed to continue in the study. Subjects are also informed that the MRI scanner produces banging sounds, and that some people find these conditions unpleasant or claustrophobic. In addition, if the MRI scan identifies a significant brain abnormality (e.g., enlarged ventricles, brain lesions) subjects will be terminated from the protocol (exclusion criteria). One of the study Physicians will contact the participant and discuss the nature of the findings (e.g. benign, congenital abnormalities etc.) if it is not exclusionary, and if clinically significant, will recommend that the participant see his/her physician. Participant notification of incidental findings from the MRI are required by the JHU IRB. We will provide a copy of the radiology report of the abnormality. If the participant does not have a physician, contact information for one will be provided.

PET and Radiologic Procedures: We have safely studied research subjects with a wide variety of psychiatric, drug use and medical disorders under similar protocols. A physician is present throughout the PET procedure and monitors the subject to insure the comfort and well-being, and respond appropriately to remedy and adverse effects. Vital signs will be taken prior to the tracer injection, every 5 minutes for the first 20 minutes after the injection, then every 15 minutes for the next 30 minutes, then every 30 minutes until the end of the PET scan, and once just after the scan is completed. During the PET scan, continuous ECG monitoring will be conducted. ECG monitoring of subjects during the scan is utilized as a safety precaution. If any clinically relevant abnormalities are detected, a printout is obtained and appropriate intervention in consultation with the staff cardiologist will be done.

IV and arterial catheters. Participants will receive lidocaine hydrochloride intradermally (2 mL 1%; 20 mg or 0.3 mg/kg for most adults) before the insertion of the arterial line to provide local anesthesia. If participants experience discomfort at the site of insertion of the arterial line, then they will be administered subcutaneous injections of 2 mL 1% lidocaine hydrochloride. This

dose corresponds to 10% of the maximal recommended dose and 5% of the dose associated with effects on the central nervous system. The arterial line will be monitored by a physician and nuclear medicine technologist staff throughout the entire procedure. The arterial line will be removed by a study physician who will ensure there is no residual bleeding and will advise the subjects on the monitoring and precautions for the next 24 hours following its removal. An arterial line is necessary for analysis of PET imaging data; if one cannot be placed, then the PET scan will be cancelled.

Radiation: Participants that have had research related exposure to ionizing radiation that in combination with the study's estimated radiation exposure would result in a cumulative exposure that exceeds recommended exposure limits of 5 rem per year would be excluded from participation. The amount of radiation exposure in the current protocol is within the limits set forth by 21CFR361.1.

Women of child bearing potential and risk to the fetus: Females who are pregnant, planning to become pregnant or nursing will be excluded from study participation. All females must have a negative pregnancy test at screening and prior to PET procedures. A positive pregnancy test at any point during the study results in study termination. Women of child bearing potential must also use a medically acceptable method of contraception for the duration of the study.

Nicotine/Tobacco withdrawal: Symptoms of nicotine/tobacco withdrawal are time limited and typically resolve without clinically-significant problems. In case of an emerging or adverse event, a study physician is available via pager for assistance.

Treatment Diversion: The informed consent process will clearly indicate that this research is not a substitute for other treatments. As previously described, participants will be instructed that should they withdraw from the study at any point to pursue treatment elsewhere; they will still be compensated for their participation up until that point in the study. At the end of the last study visit, smokers will be offered a standard smoking cessation counseling session, guided by the NCI booklet "Clearing the Air: Quit Smoking Today" and will receive a referral card to a tobacco quit line[72]. Participants can decline this session.

Blood collection: To minimize risks associated with venipuncture, experienced medical personnel, using sterile equipment, will perform the blood draws. The total volume of blood drawn in the protocol is under the Johns Hopkins IRB ceiling of 500 ml and is not expected to lead to discomfort or health concerns.

Confidentiality: Our staff is well trained in the matters of confidentiality. Participants' names will be recorded only on the screening, informed consent, and necessary medical and payment forms. All medical information obtained will be handled in accordance with HIPAA regulations. Anonymous participant identification numbers will be used on all other forms and labeling of biological fluids and test results. Subject numbers will be used to code all data forms for computer entry and storage. All information gathered will be kept in locked research staff offices or file cabinets. Only research staff will have access to participant research records. Study findings are reported using group data only. No information about subjects will be provided to anyone outside of the study including family members, third persons or organizations. Historical healthy control data we will access will also be entirely de-identified. Experimental sessions will take place in the BPRU, Johns Hopkins 550 Building and the Johns Hopkins Clinical Research Unit. The limits of confidentiality (e.g. suspected child abuse or neglect, or harm to self or others) will be discussed in detail with the participants during the

informed consent process. To reduce the likelihood of patient records disclosure we have a Certificate of Confidentiality for this study.

Images specific to the protocol will be shared with collaborator Dean Wong at Washington University in St. Louis. The JHU study team will use re-coded image IDs to label the images when in transit to Washington University in St. Louis. Dr. Weerts will track the accession numbers or, for those images lacking accession numbers, MRN with scan date and time for requested images specific to each protocol. Dr. Weerts will use the Washington University in St. Louis Central Neuroimaging Data Archive (CNDA) XNAT Desktop Uploader to share images and recoded data with Washington University in St. Louis. The CNDA was established in 2004 at Washington University, as the Neuro informatics Research Group flagship installation of XNAT (a database widely used and developed at Washington University in St. Louis). Dr. Wong (Washington University in St. Louis Joint PI) will ensure the CNDA will share a report of all the files uploaded with their upload date and file size along with the csv log files for the uploads with Johns Hopkins. Only copies of the data will be transferred to the Washington University Institute of Radiology in Dr Wong's account by secure electronic transfer . All original data will remain at JHM or JHU. A data dictionary of data that will be shared is provided in the eIRB protocol.

c. Plan for reporting unanticipated problems or study deviations.

All adverse events, protocol deviations, and other unanticipated problems are required to be reported to the PI, and other applicable study team members assisting with the protocol, as soon as possible. The PI and study team members will review the information pertaining to the event. For adverse events, the review will include a determination of severity, relationship to investigational drugs and/or protocol procedures, and reportability of the adverse event as outlined in our data safety and monitoring plan document. For protocol deviations, the review will include a determination as to why it occurred, the significance of the deviation, and any corrective action to be taken (e.g., amending the protocol). All other unanticipated problems that cannot be categorized as an adverse event or protocol deviation will be reviewed by the investigators and study MDs to determine the reportability of the problem.

All events will be reported to the IRB, FDA, and other applicable reviewing committees per the policies, regulations, and/or guidelines of these entities. If any of the events require revisions to the consent form(s) and/ or the protocol, amendments will be submitted to IRB prior to implementation, with the exception of the implementation of protocol procedures that are required to protect human participants. In the latter case, these protocol revisions will be submitted to the IRB for review within 1 to 5 working days after their implementation.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

This study involves questions about drug use and dangerous or illegal behavior, psychiatric history. If there is a breach of confidentiality, then there may be a legal risk of release of sensitive information. To reduce the likelihood of patient records disclosure we will obtain a Certificate of Confidentiality.

e. Financial risks to the participants.
This study includes healthy volunteers for research and the financial risks are minimal.

9. Benefits

a. Description of the probable benefits for the participant and for society.

There is no direct benefit to the participants from being in this study. If they participate, they may help others in the future. The primary benefit of the proposed research is in the knowledge gained regarding the relative biological, subjective and behavioral effects of chronic exposure to tobacco and the tobacco abstinence syndrome. Because we anticipate relatively minor risks to these tobacco-experienced study participants, we feel that the proposed research has a positive risk benefit ratio.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

All participants will be compensated $40 for completing the screening visit. Participants will be paid in cash for the initial screening procedures. Should participants need to complete an additional study visit, such as a second visit to complete additional screening procedures/assays, participants would receive $25 for this additional visit, although additional visits will likely be rare.

Participant compensation is as detailed in Table 1 below. The balance of the payments for study procedures will be made after each study visit. Payments of $50 or greater will be via check. Compensation during the 8-day quit attempt is partially contingent on successfully providing exhaled breath CO and/or urine cotinine samples indicative of tobacco abstinence Participants failing to abstain for 24 hrs prior to the scheduled PET (quit day 1) based on targeted CO and cotinine thresholds for these outcomes will be given one additional chance to complete PET procedures (i.e., these individuals would be invited to re-start their quit attempt from day 1 during a subsequent week) and will also be compensated $25for a visit in which they provide a sample over the specified CO and urine cotinine thresholds. After the PET, study participants will continue in the study whether or not they maintain CO and cotinine criteria levels, and are eligible for the bonus ($50) for study completion.

Procedures for cigarette smokers:

STUDY VISITS	Procedure	Payment	Incentive bonus (not smoking)	Maximum visit payment
Visit 1 Smoking as Usual	Assessments	$40	not applicable	$125
	MRI	$85		
Visit 2 - Practice Quit Day 1	Assessments	$40	$75	$315
	PET scan	$200		
Visit 3- Practice Quit Day 8	Assessments	$40	$75	$115

Completion Bonus		$50
Subtotal		$605
Typical Total compensation with Screening visit		**$645**
Max Compensation with extra visit	$25	$670

Participants who drop out or do not complete all procedures will be paid the money earned for completed procedures prior to drop out, but will forfeit future possible earnings. If study investigators decide that it is not safe for a participant to continue the study, or are excluded after the ECG or MRI incidental findings, participants will be paid the money earned for completed procedures.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

Participants are not responsible for the cost of the study procedures or drugs. The only direct costs to the participants will be their transportation to and from our research facilities for study visits. That cost has been factored into the compensation for participating.

References

1. CDC. Current Cigarette Smoking Among Adults—United States, 2011. . Morbidity and Mortality Weekly Report Centers for Disease Control and Prevention; 2012. p. 889–94.
2. Hughes JR, Keely J, Naud S. Shape of the relapse curve and long-term abstinence among untreated smokers. Addiction. 2004;99(1):29-38. PubMed PMID: 14678060.
3. Herd N, Borland R, Hyland A. Predictors of smoking relapse by duration of abstinence: findings from the International Tobacco Control (ITC) Four Country Survey. Addiction. 2009;104(12):2088-99. doi: 10.1111/j.1360-0443.2009.02732.x. PubMed PMID: 19922574; PMCID: PMC4517970.
4. Hughes JR. Effects of abstinence from tobacco: valid symptoms and time course. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco. 2007;9(3):315-27. Epub 2007/03/17. doi: 10.1080/14622200701188919. PubMed PMID: 17365764.
5. Chang Q, Hanania T, Mash DC, Maillet EL. Noribogaine reduces nicotine self-administration in rats. Journal of psychopharmacology. 2015;29(6):704-11. doi: 10.1177/0269881115584461. PubMed PMID: 25995321; PMCID: PMC4456428.
6. Rahman S. Nicotinic receptors as therapeutic targets for drug addictive disorders. CNS & neurological disorders drug targets. 2013;12(5):633-40. PubMed PMID: 23574176.
7. Ortells MO, Barrantes GE. Tobacco addiction: a biochemical model of nicotine dependence. Medical hypotheses. 2010;74(5):884-94. Epub 2009/12/08. doi: 10.1016/j.mehy.2009.11.004. PubMed PMID: 19962246.
8. Ray R, Schnoll RA, Lerman C. Nicotine dependence: biology, behavior, and treatment. Annual review of medicine. 2009;60:247-60. Epub 2009/07/28. doi: 10.1146/annurev.med.60.041707.160511. PubMed PMID: 19630572.
9. Francis MM, Cheng EY, Weiland GA, Oswald RE. Specific activation of the alpha 7 nicotinic acetylcholine receptor by a quaternary analog of cocaine. Molecular pharmacology. 2001;60(1):71-9. PubMed PMID: 11408602.
10. Brunzell DH, McIntosh JM. Alpha7 nicotinic acetylcholine receptors modulate motivation to self-administer nicotine: implications for smoking and schizophrenia. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2012;37(5):1134-43. doi: 10.1038/npp.2011.299. PubMed PMID: 22169946; PMCID: PMC3306875.
11. Paradiso KG, Steinbach JH. Nicotine is highly effective at producing desensitization of rat alpha4beta2 neuronal nicotinic receptors. J Physiol. 2003;553(Pt 3):857-71. doi: 10.1113/jphysiol.2003.053447. PubMed PMID: 14555718; PMCID: PMC2343639.
12. Fenster CP, Rains MF, Noerager B, Quick MW, Lester RA. Influence of subunit composition on desensitization of neuronal acetylcholine receptors at low concentrations of nicotine. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1997;17(15):5747-59. PubMed PMID: 9221773.
13. Papke RL, Porter Papke JK. Comparative pharmacology of rat and human alpha7 nAChR conducted with net charge analysis. British journal of pharmacology. 2002;137(1):49-61. doi: 10.1038/sj.bjp.0704833. PubMed PMID: 12183330; PMCID: PMC1573461.
14. Briggs CA, McKenna DG. Activation and inhibition of the human alpha7 nicotinic acetylcholine receptor by agonists. Neuropharmacology. 1998;37(9):1095-102. PubMed PMID: 9833639.
15. Levin ED, Petro A, Rezvani AH, Pollard N, Christopher NC, Strauss M, Avery J, Nicholson J, Rose JE. Nicotinic alpha7- or beta2-containing receptor knockout: effects on radial-arm maze learning and long-term nicotine consumption in mice. Behavioural brain research. 2009;196(2):207-13. doi: 10.1016/j.bbr.2008.08.048. PubMed PMID: 18831991; PMCID: PMC2638590.
16. Brody AL, Mandelkern MA, London ED, Khan A, Kozman D, Costello MR, Vellios EE, Archie MM, Bascom R, Mukhin AG. Effect of secondhand smoke on occupancy of nicotinic acetylcholine receptors in brain. Archives of general psychiatry. 2011;68(9):953-60. doi: 10.1001/archgenpsychiatry.2011.51. PubMed PMID: 21536968; PMCID: PMC3380615.
17. Brody AL, Mandelkern MA, London ED, Olmstead RE, Farahi J, Scheibal D, Jou J, Allen V, Tiongson E, Chefer SI, Koren AO, Mukhin AG. Cigarette smoking saturates brain alpha 4 beta 2 nicotinic acetylcholine receptors. Archives of general psychiatry. 2006;63(8):907-15. Epub 2006/08/09. doi: 10.1001/archpsyc.63.8.907. PubMed PMID: 16894067; PMCID: 2773659.
18. Wullner U, Gundisch D, Herzog H, Minnerop M, Joe A, Warnecke M, Jessen F, Schutz C, Reinhardt M, Eschner W, Klockgether T, Schmaljohann J. Smoking upregulates alpha4beta2* nicotinic

acetylcholine receptors in the human brain. Neuroscience letters. 2008;430(1):34-7. doi: 10.1016/j.neulet.2007.10.011. PubMed PMID: 17997038.
19. Staley JK, Krishnan-Sarin S, Cosgrove KP, Krantzler E, Frohlich E, Perry E, Dubin JA, Estok K, Brenner E, Baldwin RM, Tamagnan GD, Seibyl JP, Jatlow P, Picciotto MR, London ED, O'Malley S, van Dyck CH. Human tobacco smokers in early abstinence have higher levels of beta2* nicotinic acetylcholine receptors than nonsmokers. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2006;26(34):8707-14. doi: 10.1523/JNEUROSCI.0546-06.2006. PubMed PMID: 16928859.
20. Brody AL, Mukhin AG, La Charite J, Ta K, Farahi J, Sugar CA, Mamoun MS, Vellios E, Archie M, Kozman M, Phuong J, Arlorio F, Mandelkern MA. Up-regulation of nicotinic acetylcholine receptors in menthol cigarette smokers. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum. 2013;16(5):957-66. doi: 10.1017/S1461145712001022. PubMed PMID: 23171716; PMCID: PMC3758251.
21. Cosgrove KP, Batis J, Bois F, Maciejewski PK, Esterlis I, Kloczynski T, Stiklus S, Krishnan-Sarin S, O'Malley S, Perry E, Tamagnan G, Seibyl JP, Staley JK. beta2-Nicotinic acetylcholine receptor availability during acute and prolonged abstinence from tobacco smoking. Archives of general psychiatry. 2009;66(6):666-76. doi: 10.1001/archgenpsychiatry.2009.41. PubMed PMID: 19487632; PMCID: PMC2796827.
22. Rotering S, Scheunemann M, Fischer S, Hiller A, Peters D, Deuther-Conrad W, Brust P. Radiosynthesis and first evaluation in mice of [(18)F]NS14490 for molecular imaging of alpha7 nicotinic acetylcholine receptors. Bioorg Med Chem. 2013;21(9):2635-42. doi: 10.1016/j.bmc.2013.02.018. PubMed PMID: 23507153.
23. Ravert HT, Dorff P, Foss CA, Mease RC, Fan H, Holmquist CR, Phillips E, McCarthy DJ, Heys JR, Holt DP, Wang Y, Endres CJ, Dannals RF, Pomper MG. Radiochemical synthesis and in vivo evaluation of [18F]AZ11637326: an agonist probe for the alpha7 nicotinic acetylcholine receptor. Nuclear medicine and biology. 2013;40(6):731-9. doi: 10.1016/j.nucmedbio.2013.04.005. PubMed PMID: 23680470.
24. Ettrup A, Mikkelsen JD, Lehel S, Madsen J, Nielsen EO, Palner M, Timmermann DB, Peters D, Knudsen GM. 11C-NS14492 as a novel PET radioligand for imaging cerebral alpha7 nicotinic acetylcholine receptors: in vivo evaluation and drug occupancy measurements. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2011;52(9):1449-56. doi: 10.2967/jnumed.111.088815. PubMed PMID: 21828113.
25. Gao Y, Ravert HT, Valentine H, Scheffel U, Finley P, Wong DF, Dannals RF, Horti AG. 5-(5-(6-[(11)C]methyl-3,6-diazabicyclo[3.2.0]heptan-3-yl)pyridin-2-yl)-1H-indole as a potential PET radioligand for imaging cerebral alpha7-nAChR in mice. Bioorg Med Chem. 2012;20(12):3698-702. doi: 10.1016/j.bmc.2012.04.056. PubMed PMID: 22608919; PMCID: PMC3527104.
26. Dolle F, Valette H, Hinnen F, Vaufrey F, Demphel S, Coulon C, Ottaviani M, Bottlaender M, Crouzel C. Synthesis and preliminary evaluation of a carbon-11-labelled agonist of the alpha 7 nicotinic acetylcholine receptor. Journal of labelled compounds & radiopharmaceuticals. 2001;44(11):785-95. doi: DOI 10.1002/jlcr.504. PubMed PMID: WOS:000171348700004.
27. Hashimoto K, Nishiyama S, Ohba H, Matsuo M, Kobashi T, Takahagi M, Iyo M, Kitashoji T, Tsukada H. [11C]CHIBA-1001 as a novel PET ligand for alpha7 nicotinic receptors in the brain: a PET study in conscious monkeys. PloS one. 2008;3(9):e3231. doi: 10.1371/journal.pone.0003231. PubMed PMID: 18800169; PMCID: PMC2529405.
28. Toyohara J, Sakata M, Wu J, Ishikawa M, Oda K, Ishii K, Iyo M, Hashimoto K, Ishiwata K. Preclinical and the first clinical studies on [11C]CHIBA-1001 for mapping alpha7 nicotinic receptors by positron emission tomography. Ann Nucl Med. 2009;23(3):301-9. doi: 10.1007/s12149-009-0240-x. PubMed PMID: 19337782.
29. Toyohara J, Ishiwata K, Sakata M, Wu J, Nishiyama S, Tsukada H, Hashimoto K. In vivo evaluation of alpha7 nicotinic acetylcholine receptor agonists [11C]A-582941 and [11C]A-844606 in mice and conscious monkeys. PloS one. 2010;5(2):e8961. doi: 10.1371/journal.pone.0008961. PubMed PMID: 20126539; PMCID: PMC2813863.
30. Ogawa M, Nishiyama S, Tsukada H, Hatano K, Fuchigami T, Yamaguchi H, Matsushima Y, Ito K, Magata Y. Synthesis and evaluation of new imaging agent for central nicotinic acetylcholine receptor alpha7 subtype. Nuclear medicine and biology. 2010;37(3):347-55. doi: 10.1016/j.nucmedbio.2009.11.007. PubMed PMID: 20346874.
31. Pomper MG, Phillips E, Fan H, McCarthy DJ, Keith RA, Gordon JC, Scheffel U, Dannals RF, Musachio JL. Synthesis and biodistribution of radiolabeled alpha 7 nicotinic acetylcholine receptor ligands.

Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2005;46(2):326-34. PubMed PMID: 15695794.
32. Gao Y, Kellar KJ, Yasuda RP, Tran T, Xiao Y, Dannals RF, Horti AG. Derivatives of dibenzothiophene for positron emission tomography imaging of alpha7-nicotinic acetylcholine receptors. J Med Chem. 2013;56(19):7574-89. doi: 10.1021/jm401184f. PubMed PMID: 24050653; PMCID: PMC3866913.
33. Horti AG, Gao Y, Kuwabara H, Wang Y, Abazyan S, Yasuda RP, Tran T, Xiao Y, Sahibzada N, Holt DP, Kellar KJ, Pletnikov MV, Pomper MG, Wong DF, Dannals RF. 18F-ASEM, a radiolabeled antagonist for imaging the alpha7-nicotinic acetylcholine receptor with PET. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2014;55(4):672-7. doi: 10.2967/jnumed.113.132068. PubMed PMID: 24556591; PMCID: PMC4112566.
34. Wong DF, Kuwabara H, Pomper M, Holt DP, Brasic JR, George N, Frolov B, Willis W, Gao Y, Valentine H, Nandi A, Gapasin L, Dannals RF, Horti AG. Human brain imaging of alpha7 nAChR with [(18)F]ASEM: a new PET radiotracer for neuropsychiatry and determination of drug occupancy. Mol Imaging Biol. 2014;16(5):730-8. doi: 10.1007/s11307-014-0779-3. PubMed PMID: 25145965.
35. Cosgrove KP, Esterlis I, McKee SA, Bois F, Seibyl JP, Mazure CM, Krishnan-Sarin S, Staley JK, Picciotto MR, O'Malley SS. Sex differences in availability of beta2*-nicotinic acetylcholine receptors in recently abstinent tobacco smokers. Archives of general psychiatry. 2012;69(4):418-27. Epub 2012/04/05. doi: 10.1001/archgenpsychiatry.2011.1465. PubMed PMID: 22474108; PMCID: 3508698.
36. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. The Journal of clinical psychiatry. 1998;59 Suppl 20:22-33;quiz 4-57. Epub 1999/01/09. PubMed PMID: 9881538.
37. Babson KA, Boden MT, Bonn-Miller MO. The impact of perceived sleep quality and sleep efficiency/duration on cannabis use during a self-guided quit attempt. Addictive behaviors. 2013;38(11):2707-13. Epub 2013/08/03. doi: 10.1016/j.addbeh.2013.06.012. PubMed PMID: 23906725.
38. Shipley WC. A self-administering scale for measuring intellectual impairment and deterioration. . The Journal of Psychology. 1940;9:371-7.
39. Stitzer ML, Bigelow GE. Contingent reinforcement for reduced carbon monoxide levels in cigarette smokers. Addictive behaviors. 1982;7(4):403-12. PubMed PMID: 7183194.
40. Stitzer ML, Bigelow GE. Contingent reinforcement for carbon monoxide reduction: within-subject effects of pay amount. J Appl Behav Anal. 1984;17(4):477-83. doi: 10.1901/jaba.1984.17-477. PubMed PMID: 6526768; PMCID: PMC1307969.
41. Stitzer ML, Bigelow GE. Contingent reinforcement for reduced breath carbon monoxide levels: target-specific effects on cigarette smoking. Addictive behaviors. 1985;10(4):345-9. PubMed PMID: 4091067.
42. Deveci SE, Deveci F, Acik Y, Ozan AT. The measurement of exhaled carbon monoxide in healthy smokers and non-smokers. Respir Med. 2004;98(6):551-6. PubMed PMID: 15191041.
43. Bernert JT, Harmon TL, Sosnoff CS, McGuffey JE. Use of continine immunoassay test strips for preclassifying urine samples from smokers and nonsmokers prior to analysis by LC-MS-MS. Journal of analytical toxicology. 2005;29(8):814-8. PubMed PMID: 16374940.
44. Perkins KA, Karelitz JL, Jao NC. Optimal carbon monoxide criteria to confirm 24-hr smoking abstinence. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco. 2013;15(5):978-82. doi: 10.1093/ntr/nts205. PubMed PMID: 22990219; PMCID: PMC3733388.
45. Tidey JW, Rohsenow DJ, Kaplan GB, Swift RM, Reid N. Effects of contingency management and bupropion on cigarette smoking in smokers with schizophrenia. Psychopharmacology. 2011;217(2):279-87. Epub 2011/04/09. doi: 10.1007/s00213-011-2282-8. PubMed PMID: 21475970; PMCID: PMC3685403.
46. Drummond MB, Astemborski J, Lambert AA, Goldberg S, Stitzer ML, Merlo CA, Rand CS, Wise RA, Kirk GD. A randomized study of contingency management and spirometric lung age for motivating smoking cessation among injection drug users. BMC Public Health. 2014;14:761. doi: 10.1186/1471-2458-14-761. PubMed PMID: 25074396; PMCID: PMC4132916.
47. Mueller ET, Landes RD, Kowal BP, Yi R, Stitzer ML, Burnett CA, Bickel WK. Delay of smoking gratification as a laboratory model of relapse: effects of incentives for not smoking, and relationship with measures of executive function. Behavioural pharmacology. 2009;20(5-6):461-73. doi: 10.1097/FBP.0b013e3283305ec7. PubMed PMID: 19741301; PMCID: PMC2886581.

48. Dunn KE, Sigmon SC, Thomas CS, Heil SH, Higgins ST. Voucher-based contingent reinforcement of smoking abstinence among methadone-maintained patients: a pilot study. J Appl Behav Anal. 2008;41(4):527-38. PubMed PMID: 19192857; PMCID: PMC2606604.
49. Dunn KE, Sigmon SC, Reimann EF, Badger GJ, Heil SH, Higgins ST. A contingency-management intervention to promote initial smoking cessation among opioid-maintained patients. Experimental and clinical psychopharmacology. 2010;18(1):37-50. doi: 10.1037/a0018649. PubMed PMID: 20158293; PMCID: PMC3605744.
50. Dunn KE, Saulsgiver KA, Sigmon SC. Contingency management for behavior change: applications to promote brief smoking cessation among opioid-maintained patients. Experimental and clinical psychopharmacology. 2011;19(1):20-30. doi: 10.1037/a0022039. PubMed PMID: 21341920; PMCID: PMC3131670.
51. Tidey JW, Colby SM, Xavier EM. Effects of smoking abstinence on cigarette craving, nicotine withdrawal, and nicotine reinforcement in smokers with and without schizophrenia. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco. 2014;16(3):326-34. Epub 2013/10/12. doi: 10.1093/ntr/ntt152. PubMed PMID: 24113929; PMCID: PMC3920337.
52. Fan H, Kotsikorou E, Hoffman AF, Ravert HT, Holt D, Hurst DP, Lupica CR, Reggio PH, Dannals RF, Horti AG. Analogs of JHU75528, a PET ligand for imaging of cerebral cannabinoid receptors (CB1): development of ligands with optimized lipophilicity and binding affinity. European journal of medicinal chemistry. 2009;44(2):593-608. Epub 2008/05/31. doi: 10.1016/j.ejmech.2008.03.040. PubMed PMID: 18511157; PMCID: 2728551.
53. Horti AG, Fan H, Kuwabara H, Hilton J, Ravert HT, Holt DP, Alexander M, Kumar A, Rahmim A, Scheffel U, Wong DF, Dannals RF. 11C-JHU75528: a radiotracer for PET imaging of CB1 cannabinoid receptors. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2006;47(10):1689-96. Epub 2006/10/04. PubMed PMID: 17015906.
54. Wong DF, Kuwabara H, Horti AG, Raymont V, Brasic J, Guevara M, Ye W, Dannals RF, Ravert HT, Nandi A, Rahmim A, Ming JE, Grachev I, Roy C, Cascella N. Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [11C]OMAR. NeuroImage. 2010;52(4):1505-13. Epub 2010/04/22. doi: 10.1016/j.neuroimage.2010.04.034. PubMed PMID: 20406692.
55. Normandin MD, Zheng MQ, Lin KS, Mason NS, Lin SF, Ropchan J, Labaree D, Henry S, Williams WA, Carson RE, Neumeister A, Huang Y. Imaging the cannabinoid CB1 receptor in humans with [11C]OMAR: assessment of kinetic analysis methods, test-retest reproducibility, and gender differences. J Cereb Blood Flow Metab. 2015;35(8):1313-22. doi: 10.1038/jcbfm.2015.46. PubMed PMID: 25833345; PMCID: PMC4528005.
56. Ashburner J, Friston KJ. Spatial normalization using basis functions. In: Frackowiak RSJ, Friston KJ, Frith C, Dolan KJ, Price CJ, Zeki S, Ashburner J, Penny WD, editors. Human Brain Function, 2nd Edition: Academic Press; 2003. p. 655-72.
57. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage. 2002;15(1):273-89. Epub 2002/01/05. doi: 10.1006/nimg.2001.0978. PubMed PMID: 11771995.
58. Friston KJ, Frith CD, Liddle PF, Frackowiak RS. Comparing functional (PET) images: the assessment of significant change. J Cereb Blood Flow Metab. 1991;11(4):690-9. Epub 1991/07/01. doi: 10.1038/jcbfm.1991.122. PubMed PMID: 2050758.
59. Calhoun VD, Adali T, Pearlson GD, Pekar JJ. A method for making group inferences from functional MRI data using independent component analysis. Hum Brain Mapp. 2001;14(3):140-51. doi: doi:10.1002/hbm.1048. PubMed PMID: 11559959.
60. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. NeuroImage. 2012;59(3):2142-54. doi: 10.1016/j.neuroimage.2011.10.018. PubMed PMID: 22019881; PMCID: PMC3254728.
61. Behzadi Y, Restom K, Liau J, Liu TT. A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. NeuroImage. 2007;37(1):90-101. doi: 10.1016/j.neuroimage.2007.04.042. PubMed PMID: 17560126; PMCID: PMC2214855.
62. Satterthwaite TD, Elliott MA, Gerraty RT, Ruparel K, Loughead J, Calkins ME, Eickhoff SB, Hakonarson H, Gur RC, Gur RE, Wolf DH. An improved framework for confound regression and filtering

for control of motion artifact in the preprocessing of resting-state functional connectivity data. NeuroImage. 2013;64:240-56. doi: 10.1016/j.neuroimage.2012.08.052. PubMed PMID: 22926292; PMCID: PMC3811142.
63. Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. Brain Connect. 2012;2(3):125-41. doi: 10.1089/brain.2012.0073. PubMed PMID: 22642651.
64. Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. NeuroImage. 2010;52(3):1059-69. doi: 10.1016/j.neuroimage.2009.10.003. PubMed PMID: 19819337.
65. Atasoy S, Donnelly I, Pearson J. Human brain networks function in connectome-specific harmonic waves. Nat Commun. 2016;7:10340. doi: 10.1038/ncomms10340. PubMed PMID: 26792267; PMCID: PMC4735826.
66. Yeo BT, Krienen FM, Sepulcre J, Sabuncu MR, Lashkari D, Hollinshead M, Roffman JL, Smoller JW, Zollei L, Polimeni JR, Fischl B, Liu H, Buckner RL. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. J Neurophysiol. 2011;106(3):1125-65. doi: 10.1152/jn.00338.2011. PubMed PMID: 21653723; PMCID: PMC3174820.
67. Shirer WR, Ryali S, Rykhlevskaia E, Menon V, Greicius MD. Decoding subject-driven cognitive states with whole-brain connectivity patterns. Cereb Cortex. 2012;22(1):158-65. doi: 10.1093/cercor/bhr099. PubMed PMID: 21616982; PMCID: PMC3236795.
68. Haney M, Ward AS, Comer SD, Foltin RW, Fischman MW. Abstinence symptoms following smoked marijuana in humans. Psychopharmacology. 1999;141(4):395-404. Epub 1999/03/25. PubMed PMID: 10090647.
69. Budney AJ, Moore BA, Vandrey RG, Hughes JR. The time course and significance of cannabis withdrawal. Journal of abnormal psychology. 2003;112(3):393-402. Epub 2003/08/29. PubMed PMID: 12943018.
70. Budney AJ, Hughes JR, Moore BA, Vandrey R. Review of the validity and significance of cannabis withdrawal syndrome. The American journal of psychiatry. 2004;161(11):1967-77. Epub 2004/10/30. doi: 10.1176/appi.ajp.161.11.1967. PubMed PMID: 15514394.
71. Haney M. The marijuana withdrawal syndrome: diagnosis and treatment. Current psychiatry reports. 2005;7(5):360-6. Epub 2005/10/12. PubMed PMID: 16216154.
72. Update Panel L, and Staff. A Clinical Practice Guideline for Treating Tobacco Use and Dependence: 2008 Update A U.S. Public Health Service Report. American Journal of Preventive Medicine. 2008;35(2):158-76. doi: https://doi.org/10.1016/j.amepre.2008.04.009.