Brain imaging of cannabinoid type 1 (CB1) receptors in women with cannabis use disorder and male and female healthy controls

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Brain imaging of cannabinoid receptors

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1. Abstract

The primary goals of this developmental project are to examine whether heavy cannabis use alters brain cannabinoid type 1 receptor (CB1R) availability in females, and if severity of cannabis withdrawal is correlated with CB1 receptor availability. It is well established that dependence-producing effects of cannabis (marijuana, MJ) are due primarily to the actions of Δ-9-tetrahydrocannabinol (THC) on cannabinoid type 1 receptor (CB1R)[1, 2]. Why is it important to study female MJ users? The scientific premise is that women have greater sensitivity to the abuse-related effects of MJ and with chronic use may show neuroadaptive changes in CB1R that might be similar or differ from men[3]. Women show an accelerated trajectory from first MJ use to onset of CUD and first treatment admission[4, 5]. It is also established that women entering treatment present with more severe medical, psychological, and social problems, and have a higher rate of relapse than men[6, 7]. The causes of 'telescoping' and greater relapse risk in women are unknown, but sex differences in neurobiology are proposed[4-6]. CB1R are widely distributed in the human brain and can be quantified using positron emission tomography (PET) with the radiotracer ^{11}C-OMAR[8]. Our group and others have found CB1R availability is higher in female than male healthy non-users [9]. Such innate differences in CB1R may contribute to sex-differences in MJ effects and the development of cannabis use disorder (CUD). Surprisingly, the consequences of chronic MJ use on brain CB1R have not been studied in females. Toward this goal, we will enroll 10 female MJ users with CUD and 10 matched controls in an inpatient protocol that includes administration of smoked MJ, followed by monitored abstinence with daily behavioral assessments, and PET imaging with ^{11}C-OMAR. The proposed study is an important first step to determine whether localized CB1R changes in female MJ users help explain, and provide a neurobiological target for intervention. Results will increase knowledge of cannabinoid mechanisms of CUD in females, an understudied population.

2. Objectives (include all primary and secondary objectives)

The proposed exploratory studies will help fill gaps in existing research by determining if female MJ users show changes in CB1R availability when compared to matched controls, and if severity of MJ withdrawal, craving and negative mood during abstinence in MJ users are correlated with CB1R availability. CB1R availability (distribution volume, VT) will be determined for regions associated with mood (amygdala and hippocampus), reward (ventral striatum), cognition (frontal cortex), motor function and learning (cingulate, putamen, and globus pallidus).

Primary objective 1: To examine CB1R availability (VT) in female MJ users and matched controls.

Hypotheses1: When compared to controls, female MJ users will have lower VT, and the magnitude of downregulation will be correlated with greater severity of CUD.

Primary objective 2: To evaluate whether severity of withdrawal during MJ abstinence is negatively associated with CB1R VT in female MJ users.

Hypotheses 2: Greater severity of withdrawal symptoms, negative mood and craving will be correlated with lower VT, particularly in the amygdala and hippocampus.

Secondary objective 3: To examine subjective effects before and after acute MJ administration in MJ users and explore the relationship of these measures to CB1R VT.

Hypothesis 3: Ratings increases in subjective ratings of 'good' and 'take again' for MJ will be inversely correlated with VT in ventral striatum, cingulate, and putamen.

Secondary objective 4: To examine cognitive performance before and after acute MJ administration in MJ users and explore the relationship of these measures to CB1R VT.

Hypothesis 4: MJ will produce impairment of working memory, sustained attention, executive function and planning in MJ users, when compared to their own baseline and when compared to non-drug conditions in controls. We will examine if cognitive performance deficits in response to MJ are correlated with CB1R VT in frontal cortex, cingulate, and putamen.

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. Cannabis (Marijuana, MJ) is the most widely used illicit drug in the world. An estimated 5.4 million individuals in the U.S. use MJ daily, and about 4.3 million meet criteria for current Cannabis Use Disorder (CUD)[10]. CUD treatment admissions have been steadily increasing, but most patients entering treatment fail to achieve sustained MJ abstinence[11]. Chronic MJ use has been linked with anxiety and mood disorders[12, 13], and women are at greater risk for these disorders than men[5]. At the same time, MJ and related compounds are under investigation for medical use[14]. There are currently 25 U.S. states and the District of Columbia that enacted laws to legalize medical use of MJ, and 4 states legalized non-medical use of MJ; two states have pending legislation in 2016. Coincident with this, rates of daily MJ use have increased and perceptions of harm associated with use have decreased[15-17]. As a result, there is growing need for improved understanding of the consequences of MJ use to the individual and society.

The endocannabinoid system. The endocannabinoid system includes two naturally occurring ligands arachindonoylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG), which bind to and activate 2 receptors, cannabinoid type 1 (CB1R) and type 2 (CB2R). CB1R are expressed preferentially in the human brain with high density in regions involved in drug reward (nucleus accumbens), mood regulation (amygdala and hippocampus), motor function and learning (caudate, putamen, globus pallidus) and cognition (frontal cortex)[8]. Studies in rodents indicate that repeated cannabinoid exposure produces down-regulation of CB1R in key brain regions[18-21], which was concurrent with development of cannabinoid tolerance[18-20].

Sex differences in cannabinoid sensitivity. Studies in humans and animals indicate females are more sensitive than males to many effects of MJ[7, 22, 23], and females reported more severe MJ withdrawal, compared with males matched for MJ use[24]. Thus, innate sex differences in CB1R and MJ sensitivity, combined with possible sex-specific neuroadaptive changes associated with chronic

MJ use may contribute to greater withdrawal severity and relapse in females. Preclinical research indicates that females show more cannabinoid-induced motor impairment, catalepsy and antinociception than males[25, 26], and develop tolerance to these effects more rapidly under the same dosing regimen as males[27]. When compared to males, female rats acquire cannabinoid self-administration more quickly, show more persistent responding during extinction, and are more sensitive to cannabinoid-associated cues[28, 29]. Discontinuation of chronic THC produced more anxiety-related behaviors in female rats than males[30]. Surprisingly few human laboratory studies have examined sex differences in MJ sensitivity, but those to date indicate women are more sensitive to the abuse-related subjective effects of MJ[23, 31]. Studies in our laboratory and others have found that women experience greater withdrawal distress than men, but these studies were retrospective[24, 32, 33].

When measured prospectively, abrupt abstinence after heavy MJ use results in a withdrawal syndrome that includes increases in anxiety, depression, irritability, anger, sleep problems, decreased food intake and weight loss[34-37]. Symptoms are observed within 1-3 days, peak at 3-6 days, then slowly return to baseline over days to weeks[35, 38]. The fact that symptoms are reversed by resumption of MJ use or oral THC indicates that withdrawal is pharmacologically specific to THC[39, 40]. Prospective studies that characterized withdrawal symptoms during verified MJ abstinence were conducted in largely male samples. We know far less about MJ withdrawal in women or the mechanisms involved. Clearly, this area of research needs to be addressed.

Quantification of CB1R via PET: There are a number of CB1R radiotracers available for human use[41]. Only a few human PET studies have evaluated CB1R in MJ users; two studies were in all male MJ smokers[42, 43], and a third was in almost all male MJ users (only 2 women)[44]. Still, these studies demonstrated CB1R downregulation in male MJ users, and that CB1R binding increased with continued abstinence[42, 43]. This suggests that CB1R downregulation may reflect a state rather than trait condition, and thus may be a primary mechanism of MJ withdrawal. However, prior studies have not controlled for time since last MJ use and studies were in almost entirely male subejcts. Potential changes in CB1R in women are unknown.

[^{11}C]-OMAR (JHU75528), which is an analog of the CB1R antagonist/inverse agonist rimonabant, was developed, synthesized and first tested in humans at the JHU PET center[8, 45, 46], and will be used in the proposed studies under IND76,698 (PI: Wong). Our [^{11}C]-OMAR data in rodents, baboons and humans are highly consistent with the density and distribution of CB1R in human autoradiography studies[47, 48]. It has excellent test-retest reliability (mean ΔVT -3%, SD10%)[49], and specific to non-specific binding is in the order of other PET CB1 tracers[41, 50]. CB1R selectivity of [^{11}C]-OMAR is supported by PET data in CB1R knockout and wild type mice[51], as well as in our in vivo occupancy studies in humans, which showed a CB1R antagonist inhibited [^{11}C]-OMAR binding (¬90%)[52]. In mice, we verified acute THC (10-40 mg/kg, IP) did not displace [^{11}C]-OMAR (Wong, unpublished findings), and are unaware of any studies showing THC displacement of any of the CB1 PET ligands. Because [^{11}C]-OMAR functions as an antagonist/inverse agonist at CB1R, it is less affected by CB1R agonists like THC (unpublished data Wong lab). Indeed, for all PET ligands developed across the different receptor systems, the majority are antagonists for this reason. Thus, we believe [^{11}C]-OMAR binding will not be impacted by residual THC. Rodent studies support this interpretation as chronic cannabinoid administration decreased CB1R density, and not CB1R affinity[53-55]. [^{11}C]-OMAR has been successfully used to demonstrate 16-22.9% increases in VT in multiple VOIs in alcohol dependent subjects[56], and 15% decreases in male MJ

users[43], when compared to controls. Thus, given meaningful group differences detected by us and others, we believe [^{11}C]-OMAR is the appropriate radiopharmaceutical for our studies.

Sex differences in CB1R. Sex-related differences in CB1R density have been identified in autoradiography studies in rodents, although the direction of sex effects differ depending on the brain region, age and procedures used[53, 57]. In one study, intact female rats had higher in vitro densities of CB1/CB2 receptors in the amygdala, but lower CB1R density in the hypothalamus than males[54]; this effect was sex steroid dependent, as ovariectomy resulted in CB1R upregulation in females. When estrus cycle was included in the analyses, females had higher endocannabinoid levels in the hypothalamus, pituitary, striatum, midbrain, and hippocampus than males[58, 59]. A human PET study using [18F]MK-9470 reported lower Patlak slope Ki binding in women than men[60] (It is important to note that this measure does not separate flow and binding per se so decreased Ki could be a combination of both). In contrast, in our studies and others[9, 61] using 11C-OMAR PET in healthy volunteers, women had higher mean composite VT than men. The reason for the differences across studies is unclear, but highlights the need for research to understand cannabinoid neurobiology. We need to examine mechanisms underlying more rapid progression to CUD in women, and prospectively examine their withdrawal symptom presentation. Given that the primary pharmacological treatments for CUD are those that act via the CB1R, and that women make up almost half of the population seeking treatment, research on MJ effects on the brain and behavior collected under scientifically rigorous, must be extended to studies in women. The public health importance of these data include identification of sex-specific symptoms, and improved treatment approaches based on CB1R differences.

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 research study will be conducted at the Johns Hopkins Behavioral Pharmacology Research Unit (BPRU), the Johns Hopkins Bayview Clinical Research Unit (CRU) and the Johns Hopkins Hospital (JHH) PET Imaging Center. All participants will be healthy volunteers and all procedures will be for research purposes.

Female MJ users will be enrolled in a protocol that includes an outpatient drug administration session and a 4-day/3-night inpatient stay on the Johns Hopkins Bayview Clinical Research Unit (CRU). During the outpatient visit, MJ users will have an MRI conducted and they will complete a placebo MJ self-administration session. Subsequent to this, a residential study visit will be completed. MJ users will arrive at the CRU early in the morning of Day 1 to complete a smoked MJ self-administration session, and be admitted to the CRU. MJ users will reside on the CRU, abstinent from MJ, and complete an MRI, and daily assessments of MJ craving, withdrawal and mood. On Day 4, [^{11}C]-OMAR PET scan will be completed to measure CB1R VT. Controls (matched with MJ users for age, race, cigarette use, and BMI) will complete MRI, PET and cognitive testing under an outpatient protocol (no MJ administration).

Human Subjects Involvement, Characteristics, and Design

Recruitment will include newspaper, radio and other advertisements in the local Baltimore area. Female subjects aged 18-45 will be recruited based on demographics of MJ smokers as in our prior studies and as represented in samples of the community. Based on a recent census, the greater Baltimore area population is mostly Caucasian (61%) or African American (30%); the remaining

9% is mostly Asian (6%) and persons reporting other/ two or more races (2%). About 8% of the population report Hispanic or Latino origin. For Baltimore City, where JHU is located, the population is mostly African American (64%) or Caucasian (29%). MJ smokers in our prior studies were mostly African American (>80%) and non-Hispanic (96%); we anticipate a similar distribution for the current study sample; we will recruit non-users to match demographic characteristics of MJ users.

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 3 persons for each subject who completes study procedures. This is necessary to have a sufficient pool of subjects who meet inclusion/exclusion criteria and matching rules to achieve balanced subject groups.

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 Behavioral Pharmacology Research Unit (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)[62] for DSM-5 will be used to assess CUD, other SUD, and psychological disorders. Alcohol and MJ use will be characterized using the 90-day Time Line Follow Back (TLFB)[63]. The MINI and Fagerström Test for Nicotine Dependence (FTND)[64] will be used to determine presence of tobacco use disorder and severity of nicotine dependence. The Marijuana Quit Questionnaire[65] and a retrospective Marijuana Withdrawal Checklist (MWC)[35] will be used to obtain history of MJ use, quit attempts, and presence of MJ withdrawal symptoms during prior periods of abstinence. Nonusers will be recruited to match MJ users for age, rage, cigarette use and BMI. Only healthy volunteers are eligible. Volunteers who appear eligible for study participation at the end of the screening visit will complete training on the battery of computer assessments, and 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. Although cannabinoids in general do not have an ECG indicated for their prescription, 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.

Outpatient Session 1

Cannabis users and Controls who meet eligibility criteria will come to the BPRU for and complete an MRI in the morning. In the afternoon, Controls will complete the cognitive performance tasks, and MJ users will complete an outpatient session that includes cannabis self-administration and cognitive performance tasks (see below).

MRI Procedures.

Eligible participants will have a 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 40 min. 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 anteriorposterior (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.

Clinical Research Unit (CRU) Stay

MJ users will complete a 4-day protocol that includes a stay on the Bayview CRU for 3 nights. MJ users will complete procedures as part of an inpatient stay. Throughout the CRU stay participants can use a telephone, have reading material and watch television. No visitors will be allowed during the CRU stay. Throughout the CRU stay, cannabis users will complete daily assessments three times daily (7am, noon, 7pm). Assessments include:

- Vital signs (heart rate, blood pressure)
- Breath CO levels
- Profile of Mood State (POMS) [66, 67]
- Beck Anxiety Inventory (BAI)[68]
- Marijuana Withdrawal Checklist (MWC) [35]
- Marijuana Craving Questionnaire Short Form (MCQ-SF)[69].

Laboratory assessment of MJ withdrawal.

The 14-item MCQ-SF[69] will be used to assess MJ craving along 4 dimensions: compulsivity, emotionality, expectancy, and purposefulness. In prior laboratory studies using the MCQ-SF we demonstrated craving is increased during abstinence[70, 71]. The MWC lists 32 symptoms and includes non-specific items to minimize response bias and is labeled the Behavior Checklist to minimize expectancy. Participants indicate severity on a 4-pt scale ranging from 0 (not at all) to 3 (severe) which yields a composite Withdrawal Discomfort Score (WDS) for symptoms reliably observed in MJ withdrawal that is sensitive to changes during abstinence and to oral THC (dronabinol)[35, 39, 40, 72, 73]. In heavy MJ users (>25 days/month)[35], WDS scores for MJ users increase from baseline at 1-3 days, remained high over days 4-6, then slowly declined. Thus, imaging after 3 days abstinence will target peak withdrawal.

Cannabis Self-administration Session Procedures.

MJ users will complete two self-administration sessions. One session will be completed during an outpatient visit (OP Visit 1) and the second session will be completed on the day of inpatient admission to the CRU.

MJ users will be scheduled to arrive to the BPRU early in the morning for cannabis self-administration session. All participants will complete a breath alcohol test on arrival. Participants with a positive BAL will be immediately discharged from the study. Urine drug and pregnancy testing will then be conducted for all participants to test for evidence of recent illicit drug use and pregnancy. Volunteers must have negative urine drug screens (except for MJ for MJ users) on the day of the cannabis exposure session to participate. Participants will be fed a standardized low fat breakfast (e.g. toast and jam) each morning prior to cannabis administration. An intravenous catheter will be placed in one arm for blood sampling.

Dried cannabis will be smoked using a small commercial pipe. Placebo (<1% THC) and active (approximately 10-13% THC cannabis will be prepared by the BPRU pharmacy such that the same volume of cannabis will deliver 0 mg, or 25 mg of THC. This procedure has been demonstrated to reliably deliver doses of THC from dried cannabis and adequately blinds study conditions. The pipe will be filled by the pharmacy with the appropriate THC dose, and then dispensed to study participants for self-administration.

The THC doses will be administered in fixed order during the OP visit 1 (placebo), and IP visit Day 1 (active). Study participants and research staff administering the cannabis will be blinded to THC dose administered. Participants will be provided with cannabis and drinking water approximately one hour after finishing their standardized low fat breakfast, and will be instructed that they need to self-administer the cannabis within 5 minutes. The conclusion of cannabis consumption will be considered the "0 hour" by which remaining protocol assessments will be scheduled. Cannabis smoking will occur in a specially ventilated room in the BPRU designed for the conduct of research with smoked/inhaled drugs that minimizes staff exposure to second-hand smoke.

Assessments during active and placebo MJ administration sessions will include:

- Vital signs (heart rate, blood pressure)
- Drug Effect Questionnaire (DEQ)[39, 40]
- Marijuana Rating Form (MRF)[74]
- Marijuana Craving Questionnaire Short Form (MCQ-SF)[69].
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Assessment of circulating levels of THC and its metabolites.

At least 30 min before the session, a catheter will be inserted into a vein of one arm for withdrawal of blood specimens during the Cannabis Self-administration Session . Blood samples will be collected immediately after subjective and cardiovascular measures -15 (baseline), immediately after smoking (0) and then +5, +15, +30, +45, +60, +90, +120, +180 min after the start of smoking, and plasma levels of THC and its metabolites (11-OH-THC and THC-COOH) will be determined. Times are based on prior studies[75]. Samples will be frozen (−20 C) until analysis, then analyzed for quantitative levels using GC/MS as in our prior studies[76, 77].

Performance Session.

We will test domains of cognitive and psychomotor functioning relevant to daily functioning in both cannabis users and match healthy control non-users using the following computerized tasks:

- Digit Symbol Substitution Task (DSST), a measure of psychomotor ability and short-term memory
- Paced serial addition task (PASAT), a measure of working memory and sustained attention
- Divided Attention Task (DAT), a measure of the ability to divide attention on more than one task

During the assessment visit, subjects will complete training and practice tasks until performance is stable (+20%) for 3 consecutive trials. These tasks are sensitive to MJ-induced impairment[78-81].

PET Scan Procedures:
Controls will complete PET procedures during an outpatient visit (OP Visit 2), and MJ users will complete the PET procedures on day 4 of the CRU impatient stay.

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) Laboratory Assessments:

On the day of the PET scan, prior to starting the PET procedures, all participants will be required to provide a urine sample for urine drug screen. The urine drug screen must also be negative (except for cannabis in cannabis users) for the participant to continue with the study. Participants will also take breathalyzer tests for exhaled carbon oxide (CO) and alcohol. The breathalyzer test must also be negative for alcohol the participant to continue with the study. CO levels must be lower than admission CO (verified smoking abstinence)

(iv)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.

(v)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 approximately 20 mCi of [^{11}C]-OMAR will be administered. Dynamic PET data will be acquired during scanning for approximately 90 minutes following tracer injection.

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

Participants will have up to 6 months 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 (inpatient vs. outpatient) for each group are as follows:

. **Cannabis Users:**

Outpatient Visits

OP Visit 1: MRI, MJ administration & performance session 1 at BPRU
Completion of self-report measures

Inpatient (IP) Stay (4 days, 3-nights)

IP Day 1: MJ administration & performance session 2 at BPRU
Admission to Bayview CRU

IP Day 1-4: Completion of daily self-report measures &
Monitored MJ abstinence

IP Day 4: Completion of self-report measures
C11-OMAR PET scan
CRU discharge

Controls:

Outpatient Visits

Visit 1: MRI & performance session 1 at BPRU

Completion of self-report measures

Visit 2: Performance session 2 at BPRU
Completion of self-report measures
C11-OMAR PET scan

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

Cannabis dose order of active vs. placebo will be blinded for participants in this study. Research assistants administering assessment forms will also be blinded. It is standard procedure for appropriate scientific control in studies evaluating dose effects of psychoactive drugs to blind dose assignment.

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

This is a non-treatment study. Participants in this study will be healthy volunteers. Routine care for any medical illness that may arise during participation will not be affected. Participants seeking treatment for CUD are not eligible.

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

Cannabis dose order (active vs. placebo) will be blinded for participants in this study. Research assistants administering assessment forms will also be blinded. It is standard procedure for appropriate scientific control in studies evaluating dose effects of psychoactive drugs to blind dose assignment. Medical personnel and PIs will not be blind to the dose of cannabis.

e. Definition of treatment failure or participant removal criteria.

This is not a treatment study. Participants may withdraw at any time for any reason. The study investigators will discharge study participants for failing to attend their scheduled session, failure to follow the protocol requirements, or for other reasons not known at this time. Participants can also be removed from the study for a positive toxicology test (except for MJ in MJ users), or pregnancy test. Participants will be removed if they are unable to complete all protocol procedures. Participants will also be removed from the study if the participant experiences an event making it unsafe for the participant to continue to participate in the study. Participants may also be removed if the principal investigator feels it is in the best interest of the participant.

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

This is not a treatment trial; there is no direct course of therapy related to the participant population being targeted. We are recruiting healthy adults with experience using cannabis and who are not seeking treatment for substance use problems. Should any report the desire for

treatment they will be discontinued and referred to appropriate community service centers. 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; unable to have an arterial line placed for the PET scan) or where continuation may increase risk as determined by study physicians. Participants will be compensated for their participation in the number of procedures up the point of the premature termination of their participation.

Premature termination of participation may result in the need to recruit additional research volunteers, but should have no impact on the study volunteer directly.

5. Inclusion/Exclusion Criteria

Inclusion criteria.

- Female, healthy adult volunteers who are either MJ users and nonusers (controls)
- 18-45 years of age
- Laboratory parameters within the normal range, unless the Investigator considers an abnormality to be clinically irrelevant for healthy participants; however serum creatinine and hepatic enzymes (AST, ALT) must be within the normal limits/.
- 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 meet one of the following three criteria:
    - negative pregnancy test by serum pregnancy test
    - Following a reliable method of birth control (hormonal, intrauterine device, barrier, or abstinence) and
    - agreeing to follow a reliable method of birth control during the study and for one month following all study procedures

Additional inclusion criteria for MJ users

- self-reported MJ use at least 25 days/month for the prior 3 months,
- present MJ positive urine sample at screening and admission,
- meet DSM-5 criteria for moderate to severe CUD
- report at least 2 MJ withdrawal symptoms in previous period of abstinence

Additional inclusion non-users

- report no MJ use in the past 12 months
- Report MJ use of no more than 5 times in lifetime
- present a MJ-negative urine sample at screening and prior to study procedures.

Exclusion Criteria: Women will be excluded if pregnant, breast feeding, or planning pregnancy; have amenorrhea, or menstrual cycle dysfunction. Other exclusion criteria are:

- 5th grade reading level as determined by the Shipley-2 vocabulary score < 18 or by Rapid Estimate of Adult Literacy in Medicine (REALM Health Literacy Test),
- Current DSM-5 mood or anxiety disorder;
- Current DSM-5 alcohol or substance use disorder (excluding MJ or nicotine)
- Illicit drug use in last 30 days or positive urine test for illicit drugs (excluding MJ for MJ users)
- Using MJ under the guidance of MD;
- History of seizures, closed head trauma;
- BMI <18 or >35;
- Clinically-significant abnormality on ECG;
- unstable hypertension (BP > 150/90);
- conditions preventing MRI (implanted metal, claustrophobia)
- significant anatomical abnormalities on MRI (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
- 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.
- Presence or history of drug allergy, or allergic disease diagnosed and treated by a physician.
- any serious medical condition in whom participation is contraindicated.

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

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.

Marijuana (MJ).

MJ is a DEA Schedule I substance. All MJ (active and placebo) will be obtained specifically for use in this study from the Federal Drug Supply System, and will be stored and dispensed by the BPRU pharmacy. Dr. Vandrey has DEA and Maryland Schedule I Researcher licenses for MJ research. MJ will be administered under Dr. Vandrey's supervision in his laboratory in the BPRU. We are cross-referencing his IND (103,211). This protocol will be conducted under his DEA license. Placebo MJ contains the plant material from which the THC has been removed via ethanol extraction. The active MJ will contain 250mg cannabis with 10-13% THC (25mg THC dose). This dose was selected to ensure participant safety and tolerability (based on our previous experience) while maximizing the likelihood that we will administer doses that approximate current use patterns. In a previously published study, Abrams and colleagues administered THC doses up to

30.6 mg via smoking[82]. Participants in that study were current cannabis users (at least once in the past 30 days), but not heavy/daily users (maximum of 10 cannabis cigarettes or equivalent amount of plant material in the prior 30 days). All doses in that study were well tolerated. Thus, we believe that most study volunteers will be able to tolerate the proposed doses in the present study.

A potential risk of smoking MJ is burning or irritation of the throat/lungs. Acute administration of MJ can produce tachycardia, intoxication, altered mood, impaired coordination, and cognitive deficits. Given the experience of the participants with smoking MJ and the high tolerance to THC demonstrated by prior research participants in our lab and others, the risk of serious adverse events related to smoking MJ in the laboratory setting is minimal. There is no known risk of overdose or death related to MJ/THC. In cases where a participant experiences panic and or paranoid reactions, research staff will engage the person in relaxation exercises and will suspend research procedures until the volunteer has regained comfort. These types of effects are typically of short duration. In the case of an extreme adverse reaction, participants will be taken to the Johns Hopkins Bayview ER for treatment.

The effects of MJ on an unborn fetus are unknown but are potentially harmful. Women who are pregnant, planning to become pregnant or nursing will be excluded from study participation. Women of child bearing potential must use an effective non-hormonal 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.

PET radiotracer:

^{11}C-OMAR (4-cyano-1-(2,4-dichlorophenyl)-5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1Hpyrazole-3-carboxamide), previously JHU75528, is a CB1R PET radiotracer. It is an analog of the CB1R antagonist/inverse agonist rimonabant. ^{11}C-OMAR was developed, synthesized and first tested in humans at the JHU PET center[8, 45, 46], and will be used in the proposed studies under IND 76,698 (effective date: 4/20/07), which is held by Dr. Dean F. Wong (Co-I on this protocol). A single dose of approximately 370-1110 MBq of [^{11}C]-OMAR (approximate mass dose of 5 to 14 micrograms) will be administered during the PET scanning procedure by intravenous bolus administration. Each participant will receive a single intravenous injection of [^{11}C]-OMAR.

^{11}C-OMAR is the appropriate radiopharmaceutical for our studies. It has excellent test-retest reproducibility[9], and has been used successfully and safely in PET studies in alcohol dependent subjects[56], post-traumatic stress disorder patients[61], schizophrenic patients[8] and more recently in male cannabis users[43].

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.
   N/A

Derivation of outcome variables. There is no appropriate, reliable reference region for ^{11}C-OMAR due to the widespread distribution of CB1R in 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[83] a standard VOI template[84] to individual

subjects on Spoiled Grass (SPGR) sequenced MRI volumes. VOIs are: ventral striatum, amygdala, putamen, cingulate, globus pallidus, insula, frontal cortex, and hippocampus to target regions associated with drug reward, mood, cognition, motor function and habit learning (see Aims). 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[8]. V_T is a standard outcome for many receptor binding radiopharmaceuticals.

Sample size and Power Analysis: We propose a use of 10 subjects per group (total n=20) to reliably detect CB1R differences in MJ users vs. controls. We completed power analyses with SAS v3.2.2 using our mean and SD ^{11}C-OMAR V_T data, and assuming equal variance per group, we have 95% power for amygdala and globus pallidus, and 80-89% power for putamen, cingulate, insula, frontal cortex, and hippocampus to detect a significant ($p<0.05$) group difference in V_T of 10% or greater. We will be underpowered to detect 10% change for the ventral striatum (63% power, $p<0.05$), but this VOI is not one of those hypothesized to be downregulated in withdrawal. Thus, we have sufficient power ($\geq$80%, $p<0.05$) for our study aims.

Statistical Analysis Plan: Baseline participant characteristics will be summarized and compared between groups (female MJ users vs. controls). For each outcome variable descriptive statistics will be generated; continuous variables will be reported as means (SD) and categorical variables as frequencies and percentages. Outcome variables will undergo data cleansing and evaluated for 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. Groups will be matched for age, race, BMI and nicotine use, and we will use propensity score analysis[85] to evaluate group differences. To compare the groups across different VOIs, we will use a general linear model, where V_T will be the dependent variable with group status (encoded as a dummy variable) as the independent variable, along with covariates for age, race or any other variables indicated by propensity analyses. All VOI-based statistical analyses will be done with SPSS (IBM) statistical software. After the initial VOI-based analyses, we will also use Statistical Parametric Mapping (SPM)[83, 86] to identify changes of V_T across groups without restrictions of VOIs. The same linear 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). Using the SPM package for MATlab, our model will also include fixed and random effects[87].

Aim 1: To examine the relationship between V_T and severity of CUD, our model will have V_T as the dependent variable, and severity of CUD (number of symptoms on the MINI) as the independent variables.

Aim 2: To determine if VT will be associated with greater withdrawal severity, greater craving and increased negative mood during cannabis abstinence, our model will have V_T as the dependent variable, and assessment instrument scores for mood and MJ withdrawal (MWC)[35] and craving (MCQ-SF)[69] as the independent variables (Hypothesis 2).

Exploratory Aim: For cognitive tasks and subjective effects, we will use ANOVA and linear regression to compare the groups. These pilot data in females will be pooled with our historic samples to generate effect sizes and power analysis for future sex differences research. For all analyses we will add covariates as based on propensity analyses. Levels of estrogen/progesterone will be included for analyses of V_T.

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. Although cannabinoids in general do not have an ECG indicated for their prescription, 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 not 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 CB1R radiotracer ^{11}C-OMAR. Use of ^{11}C-OMAR PET in current protocol will be under IND# 76,698 (effective date: 4/20/07), which is held by one of the PIs, Dr. Dean F. Wong. 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 ^{11}C-OMAR (20 mCi) is being administered in this study. Each participant will receive approximately 740 MBq of ^{11}C-OMAR (mass dose of 0.14 micrograms/kg) 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 ^{11}C-OMAR under IRB approved protocols NA_00009703,NA_00026434, and NA_00086242. There were no significant side effects or adverse experiences reported during prior studies in healthy control subjects, subjects with schizophrenia, PTSD or alcohol dependence[8, 56, 61]. However, the most common side effects reported with these types of drugs—such as Rimonabant—may include nausea, altered mood, depression, anxiety, dizziness, headache, euphoria, fatigue, and insomnia.
4. *Radiation exposure.* A tracer dose of 20 mCi (~740 MBq) is delivered in the PET scan and results in a maximum of 0.227 rem effective dose radiation exposure. From the PET transmission scan, participants receive an additional 0.0026 rem of external radiation exposure. Thus the total radiation exposure from the one PET scan will be about 0.23 rem. 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 0.24 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 set forth by 21CFR361.1.
5. *Women for child bearing potential:* To avoid unknown risks for the fetus, women of child bearing potential must use an effective non-hormonal 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.

For MJ users only:

MJ withdrawal:

Abstinence after chronic daily use or near daily use of MJ is associated with MJ withdrawal discomfort that includes increases in anxiety, depression, irritability, anger, sleep problems, and decreased food intake and weight loss[34-37]. In a subset of MJ users, blood pressure can significantly increase during MJ abstinence [88]. Since this is a focus of the study, and we will be selecting individuals that have shown 2 or more symptoms in the past, it is likely participants will experience some of these symptoms. These symptoms are time delimited, not associated with severe health consequences, and typically resolve on their own within 2 weeks.

Controlled MJ administration.

MJ is a DEA Schedule I substance. All MJ (active and placebo) will be obtained specifically for use in this study from the Drug Supply System of the National Institute on Drug Abuse (NIDA) and will be stored and dispensed by the BPRU pharmacy. Dr. Vandrey has DEA and Maryland Schedule I Researcher licenses for MJ research. This protocol will be conducted under his DEA license and we are cross-referencing his IND (103,211).

Active MJ will contain MJ (potency approximately 10-13% THC by volume). Placebo MJ contains the plant material from which the THC has been removed via ethanol extraction. A potential risk of smoking MJ is burning or irritation of the throat/lungs. Acute administration of MJ can produce tachycardia, intoxication, altered mood, impaired coordination, and cognitive deficits. Only healthy volunteers who are current MJ users and are fully eligible based on the study inclusion/exclusion criteria will receive MJ. Given the experience of the participants with smoking MJ and the high tolerance to THC demonstrated by prior research participants in our lab, the risk of serious adverse events related to smoking MJ in the laboratory setting is minimal. There is no known risk of overdose or death related to MJ/THC. The effects of MJ on an unborn fetus are unknown but are potentially harmful. Women who are pregnant, planning to become pregnant or breast feeding will be excluded from study participation. Women of childbearing potential must use an effective non-hormonal 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.

Treatment Diversion:

Despite clear efforts to indicate otherwise, participants who are cannabis users may mistake the proposed studies as treatment or may delay treatment seeking in order to participate.

Blood collection: Blood is collected at baseline, at the time of admission to the CRU, during the cannabis self-administration session, and during the PET scan. A maximum of approximately 300 mL of blood will be taken during the entire study. This is 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.

b. Steps taken to minimize the risks.

Recruitment and Informed Consent: Participants will be identified through the media. To date, we have used newspaper, radio and social media advertisements. All ads are reviewed and approved by the IRB prior to use. 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 (excluding THC) 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 Rapid Estimate of Adult Literacy in Medicine (REALM Health Literacy Test) or 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. Although cannabinoids in general do not have an ECG indicated for their prescription, 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 these 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. An ECG will be performed at least an hour before the tracer injection. 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.

MJ withdrawal. MJ users will complete MJ abstinence and withdrawal on the CRU and are closely monitored. They will report withdrawal symptoms on standard forms and CRU nurses take vital signs three times daily. Symptoms of MJ 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.

MJ administration: Only frequent MJ users with a positive urine test for MJ will receive MJ in the current study. MJ will be provided by the NIDA federal drug supply and is standardized for THC content. The amount of THC consumed during the session is effective at inducing MJ intoxication, but is lower than what is typically acutely self-administered in the natural environment by most research volunteers for this type of study. Prior to study participation, overall health is evaluated via

history and physical exams, with the study physician or the nurse practitioner. Only healthy MJ users may participate in the study. To reduce the risk associated with MJ-induced tachycardia, subjects will have an EKG during the health screen; subjects with a clinically significant ECG abnormality will be excluded. Subjects with uncontrolled hypertension (blood pressure > 150/90) are also excluded from study participation. To further reduce the likelihood of adverse reactions to administration of smoked MJ during the study, nursing staff will be instructed to withhold administration for any participant with heart rate above 120, systolic blood pressure above 160, or diastolic blood pressure above 100. Throughout the MJ administration sessions, subjects are monitored by the research nurse. In case of an emerging or adverse event, the study physician is available via pager for assistance.

Women of child bearing potential and risk to the fetus: The effects of MJ and radiation exposure on an unborn fetus are unknown. 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, prior to CRU admission and prior to study 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.

Treatment Diversion: All advertisements and the informed consent process will clearly indicate that this research is designated only for those not seeking treatment, that participation is not a substitute for treatment, and that participation offers no clinical benefit. They will be clearly informed that they will be asked to smoke MJ during their participation. Any participant who expresses an interest in receiving immediate treatment for MJ or other substance use will be referred to a community treatment clinic. If this occurs during the study, their participation in the study will be terminated. As previously described, participants will be instructed that should they withdraw from the study at any point to pursue treatment they will still be compensated for their participation up until that point in the study.

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. 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 will obtain a Certificate of Confidentiality from the FDA.

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. It does not involve patients receiving treatment; therefore, the financial risks are minimal.

Participants will be fairly compensated for their time and effort in complying with the study protocol.

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 cannabis and the cannabis abstinence syndrome. The study will also extend the extant literature investigating the acute dose effects of cannabis in women, including subjective effects, cognitive performance, and their correlation with brain CB1R availability. Because we anticipate relatively minor risks to

these cannabis 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 $30 for completing the screening visit. Occasionally a second visit is needed to complete screening procedures; participants would receive $25 for this additional visit, if needed. Participants will be paid in cash for the initial screening procedures.

Participants who are eligible will complete procedures while staying in the CRU (marijuana users) or at outpatient visits (healthy volunteers without marijuana use). Payments for marijuana users and healthy volunteers who do not use marijuana are different since only MJ users complete the inpatient phase and MJ administration procedures. Payments are as detailed below. The balance of the payments for study procedures will be made by check after study completion.

Payments for visits for marijuana users

MRI & performance tests	$50
Outpatient visit to BPRU	$50
PET scan	$150
3-night CRU stay	$300
Completion Bonus	$100
Subtotal for procedures	**$650**

Typical Total Compensation (including $30 screening visit) $680
Maximum Total Compensation with extra visit) = $705

For healthy volunteers (no marijuana use):

MRI & performance tests	$50
PET scan	$150
Completion Bonus	$50
Subtotal for procedures	**$250**

Typical Total Compensation (including screening visit) $280
Maximum Total compensation with extra visit = $305

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.

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