CLINICAL STUDY PROTOCOL

Understanding effects of cannabis use and cessation on neural glutamate homeostasis

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Confidentiality Statement:

Study Personnel

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SYNOPSIS

Study Description

PROJECT SUMMARY/ABSTRACT

Cannabis use and availability continue to rise significantly in the US. It is critical to expand our knowledge of the negative and positive effects of cannabis to "catch up" to the current reality of widespread and growing use. Cannabis and tetrahydrocannabinol (THC), its primary psychoactive chemical, have widespread effects on neural glutamate homeostasis, and specifically metabotropic glutamate receptor 5 (mGluR5). mGluR5 regulates transmission of glutamate and plays a critical role in neural plasticity (i.e., long-term potentiation; LTP), memory, learning, mood, and addiction. Specifically, it is thought that mGluR5 activation by glutamate initiates production of endocannabinoids (i.e., 2-AG) that bind retrograde to presynaptic cannabinoid receptor 1 (CB1). This pathway inhibits further glutamate release and modulates synaptic plasticity diffusely in the brain. However, cannabis binding to CB1 disrupts this normal mechanism of glutamate homeostasis. While the relationship between cannabis use and glutamate regulation has been explored in preclinical models, it has not been well-characterized in humans, and particularly in people with cannabis use disorder (CUD). Our preliminary data have demonstrated for the first time that mGluR5 availability in the fronto-limbic circuit, as measured by [^{18}F]FPEB and positron emission tomography (PET), is higher in current cannabis users as compared with healthy controls (HC). Our electroencephalography (EEG) data show that chronic exposure to cannabis is associated with disruptions in theta band (4-7 Hz) neural oscillations, which are important for memory processes, and dependent on intact CB1 and mGluR5 function. Furthermore, a strong correlation linking impulsivity (an important clinical feature of CUD) with mGluR5 availability was observed in people who use cannabis. We therefore propose to conduct the first *in vivo* human multimodal neuroimaging study exploring the relationship between mGluR5 availability (PET), neural oscillations (EEG), and cognitive function in people with CUD. The goal is to test the overall hypothesis that mGluR5 availability is higher in people with CUD compared with HC. This study will advance our understanding of cannabis effects on the neural glutamate system in humans and may lead to the development of novel therapeutics and biomarkers to treat people with CUD. In Aim 1, we will determine differences in mGluR5 availability between people with CUD and HC in the fronto-limbic brain circuit. Aim 2 examines the associations between mGluR5 availability, CUD severity, neural oscillations, and cognitive function in CUD subjects. Aim 3 will determine how prolonged abstinence from chronic cannabis use affects mGluR5 availability, neural oscillations, and cognitive function in CUD subjects. The results from this innovative multimodal imaging study will improve our understanding of how chronic cannabis use and abstinence impacts neural glutamate receptors (mGluR5), and how this neurochemical mechanism relates to neural function (EEG) and clinically relevant functional measures of CUD (i.e., disease severity and impulsivity).

Objectives

SPECIFIC AIMS

Significance and Rationale: Cannabis is an addictive drug that is now widely available. Approximately 30% of cannabis users may have cannabis use disorder (CUD), and nearly 12 million young adults used cannabis in 2019.[5] Of note, there are currently no effective treatments for CUD. There is an urgent need to better understand the consequences, and potential underlying causes, of CUD. Glutamate is the brain's primary excitatory neurotransmitter, and is strongly implicated in addictive disorders.[6] Preclinical research indicates that chronic cannabinoid exposure disrupts glutamate homeostasis.[7] However, the underlying mechanisms of this effect are incompletely understood in humans. Therefore, the goal of this proposal is to advance our mechanistic understanding of the consequences of CUD, with focus on **metabotropic glutamate receptor 5 (mGluR5)**. This proposal has the potential to develop novel therapeutic targets for individuals with CUD.

mGluR5 in chronic cannabis use: mGluR5s are located mostly post-synaptically and on glia, and play a critical role in neural plasticity (i.e., long-term potentiation; LTP), memory, learning, mood, and addiction.[8,9] Using [^{18}F]FPEB and positron emission tomography (PET) neuroimaging, we can measure mGluR5 availability *in vivo* in people. Cannabis is known to significantly alter the same neurobehavioral functions mediated by mGluR5.[7,10] Using electroencephalography (EEG), we have the capacity to relate mGluR5 availability with neural functions underlying memory and synaptic plasticity in people *in vivo.*

Preclinical models have revealed an intimate relationship between mGluR5 and the cannabinoid system.[11] Specifically, mGluR5s on post-synaptic neurons regulate glutamate transmission through the synthesis of (natural) endocannabinoids (i.e. 2-AG). 2-AG binds to the presynaptic cannabinoid receptor 1 (CB1), which inhibits excessive glutamate release via negative feedback. Chronic cannabis use disrupts this homeostatic process, most notably leading to reduced synaptic glutamate release from pre-synaptic terminals via alterations in CB1 function.[12-15] These *pre-synaptic* changes likely reflect compensatory mechanisms related to chronic cannabis exposure. However, no studies have examined the effect of chronic cannabis use on ***post-synaptic*** function in CUD. Given the central role of *post-synaptic mGluR5* in the endocannabinoid system, **it is critical to examine mGluR5 in people with CUD**.

Preliminary Data, Approach, and Aims: Our preliminary data demonstrate for the first time that mGluR5 availability in the fronto-limbic circuit (orbitofrontal, anterior cingulate, ventromedial prefrontal, and dorsolateral prefrontal cortices, hippocampus, and amygdala), as measured by [^{18}F]FPEB PET neuroimaging, is higher in current cannabis users as compared with non-users (adjacent figure). In parallel, our preliminary EEG data show that chronic exposure to cannabis is associated with disruptions in theta band (4-7 Hz) neural oscillations, which are important for memory processes,[16] and dependent on intact mGluR5 and CB1

function.[17-21] Further, strong correlations linking neurocognition with mGluR5 availability were observed in cannabis users. Thus, we propose to conduct the first ever *in vivo* human multimodal neuroimaging study (PET/EEG) examining the relationships between mGluR5 availability, neural oscillations, and neurocognitive function in people with CUD. Outcome measures are focused on clinically relevant markers of CUD severity.

Aim 1: To determine differences in fronto-limbic mGluR5 availability between people with cannabis use disorder (CUD; n = 30) and healthy controls (HC; n = 30). Subjects will undergo [^{18}F]FPEB PET neuroimaging to determine mGluR5 availability. Hypothesis 1: Fronto-limbic mGluR5 availability will be higher in individuals with CUD compared with HC.

Aim 2: Within CUD subjects, to examine the associations between mGluR5 availability and: (a) CUD severity; (b) impulsivity; (c) EEG; and (d) neurocognition. CUD severity and impulsivity will be determined by validated instruments. EEG outcomes (e.g., theta power, LTP) known to be sensitive to both cannabis and glutamate will be collected during tasks of verbal memory[22,23] and neural plasticity (long term potentiation; LTP).[24] Hypothesis 2: Higher mGluR5 availability will be associated with: (a) greater CUD severity (by DSM-5); (b) higher impulsivity; (c) lower theta-band power and LTP, and (d) worse performance on verbal memory.

Aim 3: Within CUD subjects, to determine changes in mGluR5 availability, EEG, and neurocognition following cannabis abstinence. CUD subjects from Aim 1 will repeat PET scanning, EEG, and cognitive testing following a 28-day period of confirmed abstinence aided by contingency management. Hypothesis 3: Following cannabis abstinence: (a) mGluR5 will decrease; (b) theta power/LTP will be increased; (c) memory will improve.

Impact: The results from this innovative multimodal imaging study will improve our understanding of how chronic cannabis use impacts glutamate homeostasis via measures of mGluR5, and how this neurochemical mechanism relates to clinically relevant measures of brain function (CUD severity, impulsivity, EEG, and cognitive function). It will advance our mechanistic understanding of cannabis addiction and may lead to the development of novel therapeutics and biomarkers for people with CUD.

Endpoints

The primary endpoint is mGluR5 availability, as measured by FPEB, in cannabis-maintained and abstinent states.
Secondary endpoints are EEG outcomes and cognitive performance.

Study Population

(1) Individuals with cannabis use disorder
(2) Healthy controls

Accrual Ceiling

Our target enrollment for study completion is 60 subjects: 30 cannabis use disorder (CUD) subjects and 30 healthy controls.

Phase

N/A

Description of Sites/Facilities Enrolling Participants

Yale PET Center, Yale MR Center

Description of Study Intervention

Research Design and Methods:

Research Subjects & Recruitment (Aims 1, 2, and 3)

Subjects: A total of **60 subjects** will be recruited: 30 with **cannabis use disorder (CUD)** and 30 healthy controls (HC) who do not use cannabis. Each HC will be carefully matched to each CUD subject by age, sex, socioeconomic status, education, nicotine use, and other relevant variables. CUD subjects will undergo PET, an optional EEG, and cognitive testing at Day 0 and Day 28. HC will undergo these same measurements at Day 0 only.

Some study participants will be also enrolled in HIC # 2000032181 ("SV2A Density Cannabis Use Disorder"), since that study has shared goals and study personnel with our study. We have staff that are common to both studies. If a subject is also enrolled in HIC# 2000032181, common procedures except for PET scanning will be performed once between the two studies (i.e., MRI and cannabis abstinence procedures will not be duplicated). Whichever study the subject first signs up for will be where the abstinence data will be tracked. The subject would sign a release of information to share the information with the other study.

Recruitment and Screening: Subjects will be recruited with clinicians at Yale and in the greater Connecticut community with whom we have a long history of successful collaboration, including long-standing relations with the Yale Psychiatric Hospital inpatient and outpatient services and mental health clinics in the Greater New Haven area, as well as through flyers, public advertisement (newspaper, radio, internet postings), social media, and word of mouth. Subjects will be appropriately compensated for their participation in the study and retained during the cannabis abstinence period using our **validated contingency management paradigm**.

Study Schedule: Day 0: CUD subjects and healthy controls (HC) undergo PET scanning, an optional EEG, and cognitive testing. Days 1-27: CUD subjects abstain from cannabis use, aided by our validated contingency management strategy. Day 28: CUD subjects who are abstinent from cannabis use undergo repeat PET scanning, an optional EEG, and cognitive testing. Study Procedures: Eligible subjects will undergo an MRI scan for coregistration of PET data and for individualized source analysis of EEG. CUD subjects will be instructed not to use cannabis after midnight of Day 0, and last use of cannabis will be recorded. On Day 0, CUD subjects and HC will be scanned via PET, administered EEGs, and undergo cognitive testing. The same CUD subjects scanned on Day 0 will then be aided with cannabis abstinence and monitored over the next 4 weeks. On a weekly basis between Days 1-27, CUD subjects will be administered (1) the Withdrawal Discomfort score of the Cannabis Withdrawal Checklist, (2) the Cannabis Withdrawal Scale, which has subjective and objective (vital signs, weight) items, (3) Visual Analog Scale for Mood States, and (4) Pittsburgh Sleep Quality Index. CUD subjects will receive motivational enhancement and contingency management (CM) to promote and maintain abstinence. The escalating CM weekly payment schedule for cannabis abstinence is as follows: $50, $75, $125, $200, $650 (four-week abstinence bonus). If a participant is already getting paid to abstain from cannabis as part of their participation in another study (i.e. HIC# 2000032181), we will not pay them the usual amount ($1100) to

abstain for 28 days in our study. Instead, we will pay them a bonus of $400 which when combined with what they will get paid for abstaining for the other study will amount to more ($400+1100) than what they would get if they abstained for just one study. If the subject is asked to come in CUD subjects will be required to come to the clinic two days a week for (1) motivational enhancement, (2) escalating contingency management, (3) verbal confirmation of abstinence, (4) urinary drug testing to detect cannabis exposure in the past 1-24 hours, (5) supervised collection of urine samples to assay THC-COOH, the principal metabolite of THC. To account for dilution, urinary THC-COOH:creatinine (T:C) ratio will be calculated. Abstinence will be confirmed if the weekly means of urinary T:C ratio decreases over time and does not increase by more than 50% relative to the prior specimen.[113-115] On Day 28, CUD subjects will undergo a second PET scan, an optional EEG, and cognitive testing. The CUD subjects who relapse between the first and second scans (expected to be 10% of CUD subjects) will be: 1) considered dropouts and 2) referred for treatment if so desired. We will replace dropouts with new CUD subjects to ensure maintenance of adequate statistical power for hypothesis testing. We have accounted for dropouts in the analytic plan, study timeline, and budget. Cannabis Assessments: CUD severity is assessed using DSM-5 criteria.[51] Cannabis withdrawal is assessed by the Cannabis Withdrawal Scale.[116] Cannabis craving is assessed using a 7-point scale. The Marijuana Problems Scale[117] will assess psychosocial impact of cannabis use. Multiple other clinically relevant features of CUD are described in the section below (Cognitive Testing and Behavioral Symptom Assessment). Detailed information on cannabis use patterns will be obtained using a time-line follow back approach: levels of cannabis consumption (estimated number of joints or edibles consumed) will be determined via interview for lifetime, the past six months, three months, one month, and then for the week prior to the test session as has been described previously [12,66,112,118,119]. Participants will be instructed to consider each day of the week and indicate, for an average week, how much they consumed per drug-use occasion for each length of time assessed. Cognitive Testing and Behavioral Symptom Assessment: The BIS/BAS will be used to assess trait impulsiveness and reward responsiveness.[120,121] Urgency, Premeditation (lack of), Perseverance (lack of), Sensation Seeking, Positive Urgency, Impulsive Behavior Scale will assess five impulsive personality traits. The Eriksen flanker task will supplement the assessment of the ability to suppress responses that are inappropriate to a particular context.[122] In addition, the Probabilistic Reward Task (PRT) will be used to provide an objective endophenotypic measure of reward responsiveness. On each of the scan days, CUD and controls subjects will also be administered neuropsychological tests from the CogState Cognitive Battery[123,124], which we previously showed to be sensitive to detecting cognitive deficits in mood disorders[125]: executive control (Groton Maze Learning task, GMLT; Set-Shifting task, SST and Go No-Go, GNG)[126-134] and working memory (One Back task, ONB, and Two Back task, TWB)[135-137]. Further, the battery has been well-validated (studies using CogState batteries have been published in over 100 peer-reviewed journals). Most important for the current study, this battery is ideal for within-subject designs (i.e., it was specifically developed for repeated administration with minimal practice or learning effects). Finally, the CogState has been shown to have high test-retest reliability, thorough coverage of cognitive domains, comparable alternative forms, strong internal consistency, is well established in the general population, and has proven tolerability and acceptability. The entire battery takes approximately 30 minutes. For mood assessment, the

Hamilton Depression Rating Scale (HAM-D) and Montgomery-Asberg Depression Rating Scale (MADRS) will be administered by highly trained and reliable raters. These assessments will be administered at screening and at the time of PET scans.

C.1.d. Receptor Quantification Methods (Aim 1): (1) Magnetic Resonance Imaging Methods: MRI will be performed on a 3-Tesla Siemens Trio MR scanner (Siemens, Erlangen, Germany). A high resolution, 3-dimensional Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) T1-weighted sequence will be used to acquire sagittal images for anatomical determinations and co-registration (TR=1500ms, TE=2.83ms, FOV=256x256mm^2, matrix=256x 256mm^2, slice thickness=1.0mm without gap, 160 slices, voxel size 1.0 x 1.0 x 1.0 mm^3). The structural MRI data will serve to guide placement of ROIs for PET and for conducting GMM/partial volume correction (PVC) analyses. **We will acquire rs-*f*MRI and DTI** at both imaging time points, and these will be examined on an exploratory basis. A member of the research staff will accompany the study participant to the MR scans and stay for the duration of the session.

(2) Positron Emission Tomography Methods: Data Acquisition: CUD subjects will participate in two [^{18}F]FPEB scans, while controls will participate in one. Given we and others recently showed a diurnal variation in mGluR5 availability[87,88], all mGluR5 scan collection will be at the same time of day. [^{18}F]FPEB will be synthesized in the Radiochemistry Laboratory at the Yale PET Center.[79] The HRRT, or a PET/CT, will be used to image subjects. [^{18}F]FPEB will be administered as either a single bolus lasting up to one minute or bolus plus constant infusion lasting up to 2 hours. In either case, [^{18}F]FPEB will be prepared in a single syringe and administered via software-controlled pump to achieve the desired radiotracer administration schedule not to exceed 5mCi. Syringe and lines will be assayed after scanning to correct for residual activity. **Subjects are infused outside the scanner for the first 0-90 minutes during the pre-equilibrium period. Then, they will be scanned for 30 minutes in list-mode during the time the radiotracer reaches steady levels in the brain (90-120 minutes).**[79] At the beginning or end of each scan, a transmission scan, or low dose CT, will be obtained and used for attenuation correction. The emission list-mode data will then be binned into 5-min frames, and images will be reconstructed, correcting for attenuation, scatter, randoms, deadtime, and motion (using data from the Polaris Vicra using the MOLAR algorithm[89]). Pulse and blood pressure will be obtained before, during, and after scanning. Input Function Measurement: Venous concentrations of [^{18}F]FPEB reach equilibrium by 60 minutes post injection. Therefore, V_T can be determined from the ratio of activity in tissue to that in venous blood samples. Blood samples will be acquired manually before and during the equilibrium period (90-120 minutes). If the individuals from CUD group are also participating in HIC# 2000032181 and already has an has an arterial line placed, blood may be drawn through the arterial line if intravenous access is unobtainable. Radioactivity concentration in whole blood and plasma will be measured using a gamma counter (Wizard 1480; Perkin-Elmer, Waltham, MA, USA). Up to 6 tablespoons of blood will be drawn per PET scan. High-performance liquid chromatography (HPLC) will be used to establish unmetabolized parent compound levels.[79] The input function is calculated as the product of the interpolated parent fraction and the plasma concentration values. PET Image Analysis: To compute regional values, a summed PET image will be registered to each subject's T1- weighted MR image

using a six-parameter mutual information algorithm (FLIRT, FSL 3.2, Analysis Group, FMRIB, Oxford, UK), which in turn will be registered to an MR template image using nonrigid registration methods (Bioimage Suite, Yale University). Bioimage Suite atlas will be used to identify the primary regions of interest (ROIs) (Fronto-limbic circuit - hippocampus, amygdala, cingulate, dlPFC, vmPFC, and OFC). Outcome Measure Calculation: In humans, there is not a region that is completely devoid of mGluR5[81,90] — we and other have shown there is specific binding in the cerebellum.[91,92] Therefore, we plan to examine V_T.[93] The V_T images will be spatially normalized to a standard template (MNI, Montreal Neurological Institute) and analyses will be performed to assess ROI differences between groups ROIs. Gray Matter Masking: To partially account for partial volume effects, binary GMM will be employed. Individual MR images will be segmented with FAST (FMRIB's Automated Segmentation Tool, v3.1; The Analysis Group, FMRIB, Oxford, UK) to obtain gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) masks. The individual GMM images will be used to mask the candidate ROIs to obtain the mean regional values limited to GM voxels. GMM V_T will be the primary outcome measure. Partial Volume Correction (PVC): We will also examine the effect of full partial volume correction on between-group differences in mGluR5 availability. PVC[94,95] will be applied to the PET data on a frame-by-frame basis. The MR images will be segmented into GM, WM and CSF using FAST. Masked images will then be blurred by convolving the image with a Gaussian point spread function to achieve the resolution of the PET scanner (~3 mm). GM pixels will be corrected for spill-in and spill-out of activity from WM. Activity in CSF is assumed to be zero and activity in WM is assumed to be uniform and is estimated from each image.[95] This method creates a set of PVC-corrected PET dynamic images, which are then used to create PVC V_T images. Given the high resolution of the HRRT, we do not expect PVC to produce a substantial change in the data compared to the GMM analysis described above.

C.2.c Electroencephalography (EEG) Methods:

General EEG Data Acquisition, Recording, and Preprocessing*:* EEG recording and preprocessing will be performed as described previously.[21,101] Briefly, EEG data will be collected in an acoustically and electrically shielded booth, and recording will be done with the commercially available Active Two acquisition system (Biosemi, the Netherlands). A sampling rate of 1024 Hz will be utilized, with on-line low-pass filter of 256 Hz to prevent aliasing of high frequencies. A 64-channel electrode cap according to the extended 10-20 system will be used, along with additional electrodes to record the vertical and horizontal electro-oculogram. All electrodes will be referenced during recording to a common-mode signal (CMS) electrode between POz and PO3 and then subsequently re-referenced to the nose offline. EEG data will be first bandpass filtered from 0.1 — 100 Hz (24 dB/oct) and notch filtered at 60 Hz. Depending upon the particular stimulus parameters of each experiment (see below), the recorded EEG will be segmented into epochs consisting of the time period during stimulus presentation, along with a 100-300 ms baseline period. Any trial with a voltage greater than ± 100 μV will be considered an artifact (i.e., movement, etc.), and will be excluded from the analysis. Ocular movement correction will be applied using Gratton's algorithm.[102] All EEG data preprocessing will be performed using the software package Analyzer 2.0 (Brain Products GmbH, Germany). **Time-Frequency Analyses: Induced/Evoked Power and Intertrial Coherence (Phase Locking Factor):** Time-frequency analyses will be the primary dependent measures in the proposed studies, along

with event-related potentials (ERPs), and will be performed as described previously.[21,103] Baseline normalized evoked/Induced power and Interelectrode Coherence (ITC; also termed phase locking factor) will be determined using a wavelet-based time-frequency spectrogram via Analyzer 2.0. **EEG Source Analysis: Standardized Low Resolution Brain Electromagnetic Tomography (sLORETA):** Based on the scalp-recorded electric potential distribution, standardized low resolution brain electromagnetic tomography (sLORETA) will be used to compute the cortical three-dimensional distribution of current density. This will allow us to examine mGluR5 availability in ROIs in relation to EEG sources. The particular form of standardization used in sLORETA endows the tomography with the property of exact localization to test point sources, yielding images of standardized current density with exact localization, albeit with low spatial resolution (i.e. neighboring neuronal sources will be highly correlated).[104,105] sLORETA is the method of choice since it has no localization bias even in the presence of measurement and biological noise.[104] Furthermore, sLORETA has been validated in several simultaneous EEG/fMRI studies,[106,107] and in an EEG localization study for epilepsy.[108] In the current implementation of sLORETA, computations will be made both in a realistic head model, [109] using the MNI152 template,[110] and using each subject's structural MRI, with the three-dimensional solution space restricted to cortical gray matter, as determined by the probabilistic Talairach atlas.[111] The intracerebral volume is partitioned in 6239 voxels at 5 mm spatial resolution. **C.2.d. EEG Paradigms: RAVLT Task:** A modified computer version of the Rey Auditory Verbal Learning Test (RAVLT) similar to Babiloni et al. (2009) will be utilized.[22] The RAVLT will be administered while EEG data are collected in three separate conditions. In the *passive listening condition*, subjects will passively listen to a list of 15 words (presented one at a time) six times in a row. In the *memory encoding condition*, subjects will be administered a list of 15 words played one at a time, five times, and will be told to try and remember the list and to repeat as many words as possible after each list. A sixth distractor list will also be administered after the primary list as in the standard RAVLT. The inclusion of separate passive listening (which is not part of the standard RAVLT) and memory/encoding conditions will be included in order to compare neural signatures for both basic linguistic processing and processes related to memory encoding. Twenty minutes after the end of the encoding phase, subjects will be asked to repeat as many words as they can from the original list of words (the free recall phase). EEG will not be collected during the free recall phase. Following free recall, subjects will engage in a computerized recognition condition of the RAVLT. Subjects will hear words from the (1) original memorized list, (2) the distractor list, and (3) novel words not heard that day. Subjects will respond with a three-choice button box to indicate from which list each word originated. Words in the recognition condition will be presented every 4 seconds. This EEG version of the RAVLT allows separate examinations of neural oscillations during passive listening, memory encoding, and recognition. In addition, the words will be post-hoc sorted based on correct performance to examine activity during encoding for words that were accurately recalled (the "subsequent memory effect"). **LTP Task:** We induce and measure LTP using EEG with repetitive sensory stimulation.[100] This paradigm has been shown to have the properties of LTP including (1) frequency dependency, (2) temporal stability, (3) input specificity, and (4) de-potentiation by low frequency stimulation.[100] Tone pips (tone frequency 1000 Hz, presented for 50 msec) will be used in all stages of the task (i.e., for baseline, tetanus, and post-tetanic recording). During the baseline recording, 120 tone pips will be

presented with a variable inter-stimulus interval between 1800 — 2600 msec. After the baseline recording, LTP will be induced using auditory tetanus. The tetanus will consist of two minutes of the tone pips presented at 13 Hz. Subsequently, the test phase will commence, which will consist of another presentation of 120 tone pips as in the baseline period. The primary outcome measure will be N100-P200 amplitude after the auditory tetanus versus before the tetanus. The N100-P200 complex will be determined via automated algorithms and verified manually as described previously.[112]

Study Duration

5 years

Participant Duration

1 month

Schema

Schedule of Activities (SoA)

OUTCOMES	**SOURCE/MEASURE**	**HC**	**CUD (baseline)** Day 0	**CUD (abstinence)** Day 28
Neural Receptors				
mGluR5 Availability	PET ROI's V_T: OFC, anterior cingulate, vmPFC, dlPFC, hippocampus, amygdala	X	X	X
Cannabis Use Disorder — Clinically Relevant Functional Measurements				
CUD Severity	DSM-5		X	
Cannabis Use Patterns	Timeline Follow Back		X	X
Cannabis Withdrawal Severity	Cannabis Withdrawal Scale		X	X
Cannabis Craving	7-point Scale		X	X
Cannabis-Related Problems	Marijuana Problems Scale		X	
Impulsivity	BIS/BAS	X	X	

Reward Processing	Probabilistic Reward Task	X	X	X
Mood	HAM-D, MADRS, Visual Analog Scale for Mood States	X	X	X
Sleep	Pittsburgh Sleep Quality Index	X	X	X
Cannabis Biomarkers				
Cannabis Use Status	Urinary THC-COOH*	X	X	X
Neural Function				
Verbal Memory	EEG: Theta band power/coherence	X	X	X
Synaptic Plasticity / LTP	EEG: N100-P200 amplitude increase after auditory tetanus	X	X	X
Neurocognitive Function	CogState Cognitive Battery	X	X	X

End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.
The end of the study is defined as completion of the last visit or procedure shown in the SoA in the trial globally.

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1 - Statement of Compliance

1.1 Statement of Compliance

The trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

2 - Introduction

2.1 Study Rationale

Significance and Rationale: Cannabis is an addictive drug that is now widely available. Approximately 30% of cannabis users may have cannabis use disorder (CUD), and nearly 12 million young adults used cannabis in 2019.[5] Of note, there are currently no effective treatments for CUD. There is an urgent need to better understand the consequences, and potential underlying causes, of CUD. Glutamate is the brain's primary excitatory neurotransmitter, and is strongly implicated in addictive disorders.[6] Preclinical research indicates that chronic cannabinoid exposure disrupts glutamate homeostasis.[7] However, the underlying mechanisms of this effect are incompletely understood in humans. Therefore, the goal of this proposal is to advance our mechanistic understanding of the consequences of CUD, with focus on **metabotropic glutamate receptor 5 (mGluR5)**. This proposal has the potential to develop novel therapeutic targets for individuals with CUD.

2.2 Background

1. A.1. **Overview: How does cannabis affect glutamate receptors in the human brain?**
   Cannabis has a wide range of health and behavioral effects, many of which remain incompletely understood.[10] Yet, both recreational and medicinal use of the drug are widespread and continuing to increase in the US. Cannabis is addictive. Nearly 30% of users may have cannabis use disorder (CUD), and nearly 12 million young adults used cannabis in 2019.[5] Recreational use is now legal in 15 states, and medical use is legal in 35 states. As more states continue to legalize use for recreational purposes, cannabis use will likely continue to increase.[25] This could result in an increasing number of people experiencing negative effects, including addiction.[25]Cannabis use impairs cognition, memory, and motor coordination. With more prolonged or heavy use, it increases the risk of developing schizophrenia or other psychoses.[26] On the other hand, it is hypothesized that cannabis may have therapeutic effects in numerous neurologic conditions such as chronic pain, neurodegenerative diseases, and some psychiatric conditions (i.e. PTSD, anxiety, and sleep disorders). It is critical to more fully understand the neural mechanisms of cannabis to minimize harmful consequences and maximize therapeutic benefit. Tetrahydrocannabinol (THC) is the primary psychoactive component of cannabis. It is well-established that many of the psychoactive and addictive properties of cannabis are mediated by the neural circuit involving THC binding to the cannabinoid receptor 1 (CB1), a *presynaptic* receptor that is widely distributed throughout the central nervous system. Less well-understood, however, is the impact of CB1-THC binding on *postsynaptic* receptors such as **metabotropic glutamate receptor 5 (mGluR5), a key regulator of glutamate homeostasis and endocannabinoid synthesis**.[11] There is strong evidence from the preclinical literature that **cannabinoid exposure alters glutamate homeostasis via mGluR5 and CB1**, which may mediate many important neural effects. Indeed, there is a close relationship between the cannabinoid and glutamatergic systems.[19] However, this relationship has not been adequately studied in humans, and the degree to which disturbances in the glutamate system influences the potential harms and therapeutic benefits from cannabis use is unclear. It is critical to advance our understanding of how chronic cannabis exposure affects the brain, including the

consequences of these changes on neural function. Importantly, we need to better understand whether neural dysregulation normalizes when cannabis use stops. This innovative multimodal (PET/EEG/neurocognitive) study will expand our knowledge and thus advance our understanding of the neurobehavioral mechanisms underlying cannabis addiction, with a focus on the metabotropic glutamate receptor 5 (mGluR5), in human subjects. A.2. **Metabotropic Glutamate Receptor 5 (mGluR5): Importance in addiction and related behavioral phenotypes:** Glutamate is the brain's primary excitatory neurotransmitter, and is critical for optimal neuronal function.[27] Glutamate transmission between neural circuits linking key limbic and cortical brain regions maintains a balance between the reward-seeking behaviors necessary for survival and higher executive functions.[28]**mGluR5, a key regulator of glutamate homeostasis,** is a G protein-coupled receptor located mostly on post-synaptic neurons diffusely throughout the brain, especially key areas involved in addiction such as hippocampus, amygdala, striatum, prefrontal cortex, and cingulate.[29-31] It regulates synaptic plasticity of glutamatergic neurons, which is fundamental to learning and neural transmission of critical information. Alterations in mGluR5 receptor availability have been implicated in addiction[32] and its related endophenotypes such as executive function impairment,[33] mood disorders,[34] and impulsivity.[35] Preclinical studies of mGluR5 knockout mice and/or the use of mGluR5 antagonists have revealed several key findings pointing to the likely involvement of mGluR5 in addictive disorders including: (1) reduced drug self-administration and reduced drug-seeking behavior;[3637] (2) attenuation of drug-induced rewarding effects[38] and conditioned place preference[39]; and (3) reduced withdrawal.[40,41] The precise mechanisms whereby these processes occur remain unclear. These data, combined with observations of mGluR5 effects on drugs of abuse in general,[42,43] make **mGluR5 a potentially important molecular target in individuals with cannabis use disorder (CUD).**A.3. **mGluR5: Importance in synaptic plasticity:** Synaptic plasticity is critical for neuronal adaptations to a wide range of environmental conditions. Local changes in synaptic strength [i.e., long-term potentiation (LTP) or depression (LTD)] underlie much of the brain's ability to change its physical structure in response to changes in the environment, particularly during learning and memory. **mGluR5 regulates synaptic plasticity of glutamatergic neurons**, which is fundamental to learning and neural transmission of critical information.[44,45] Activation of mGluR5 induces LTD in hippocampal area CA1 and cerebellar Purkinje neurons.[46,47] Other preclinical studies have shown that antagonism of mGluR5 impaired LTP, spatial memory, and suppressed theta (5-10 Hz) activity in the dentate gyrus.[17,18] Interestingly, the cannabinoid system is also implicated in learning, memory, and synaptic plasticity, indicating a potential convergence zone wherein mGluR5 and CB1 may interact functionally.[48] Given the overlap between cannabis use, synaptic plasticity, learning, and cognition, **it is critical to study mGluR5 in the context of cannabis use**.

a. **SIGNIFICANCE**

Figure 1: **mGluR5-CB1 relationship**. mGluR5 is a key regulator of glutamate homeostasis via cannabinoid receptor 1 (CB1). The consumption of exogenous CB1 ligands via cannabis use may profoundly alter this process. The focus of this grant is on how chronic cannabis use and abstinence specifically affects mGluR5 availability in humans with cannabis use disorder. Figure Source: Cristino, et.al., 2019 (See ref 11).

A.4. **mGluR5: Key regulator of normal glutamate homeostasis via endocannabinoid synthesis:** Under normal physiological conditions, preclinical models suggest that **mGluR5 is a key regulator of glutamate homeostasis via CB1** at both excitatory (glutamatergic) and inhibitory (GABAergic) synapses, as shown in **Figure 1** and described here.[11] The focus of this grant is on understanding how chronic cannabis use and abstinence affect mGluR5 availability in humans with CUD. MGluR5 regulates glutamate primarily by triggering endocannabinoid (ECB) production. ECBs (e.g., 2-AG) are naturally occurring molecules that regulate neurotransmitter release and synaptic plasticity by binding to presynaptic CB1. Under normal physiological conditions, stimulation of presynaptic excitatory neurons leads to glutamate release into the synaptic cleft and peri-synaptically.[49] Glutamate binding to post-synaptic mGluR5 triggers the "on-demand" synthesis of the endocannabinoid 2-AG. 2-AG then binds in a retrograde manner to CB1, leading to synaptic depression and inhibition of further glutamate release. Thus, under normal conditions, **glutamate homeostasis is maintained via a negative feedback loop mediated by mGluR5 and endocannabinoid binding to presynaptic CB1**. This process may be disrupted by the consumption of exogenous cannabinoids.[50]

Thus, mGluR5 is a critical, widespread molecular target involved in neural glutamate homeostasis that interacts directly with the cannabinoid system and is significantly involved in neural plasticity, memory, addiction, executive function, cognition, and emotional processing. It is therefore **critically important to study the relationship between CUD and mGluR5, and to relate this to neural function and behavioral outcomes related to addiction.**

A.5. **Cannabis Use Disorder (CUD): Disruption of glutamate homeostasis via mGluR5 and CB1 (Aim 1):** CUD is characterized clinically as a syndrome involving impaired control over cannabis use, problems occurring as a result of cannabis use, and signs of physiologic dependence (i.e. tolerance or withdrawal upon abstinence).[51] Mechanistically, CUD is mediated primarily by recurrent and chronic THC binding to CB1.[52] Cannabis and THC have widespread effects on neural glutamate transmission.[50] Specifically, preclinical models have demonstrated that THC binding to CB1 inhibits glutamate release and leads to long-term synaptic depression of glutamatergic neurotransmission.[53,54] Animal studies have demonstrated that after chronic exposure to THC and other CB1 agonists, there is a reduction in the number and function of CB1 receptors.[55-60] Furthermore, CB1 downregulation and desensitization has a distinct regional and temporal course, and is related to the duration and magnitude of exposure, with greater downregulation in cortical compared with subcortical regions.[55,61,62] With prolonged abstinence, there is normalization in the number and function of *presynaptic* CB1 over 2 weeks, with faster recovery in subcortical compared to cortical regions.[59] However, **it is unknown what happens to *postsynaptic* mGluR5 from chronic cannabis use and following cannabis abstinence**. We plan to fill this knowledge gap through the proposed innovative and rigorous study. As we illustrate in our robust preliminary data below (Sections C.1a., C.2.a., and C.3.a.), our **central hypothesis is that mGluR5 upregulation occurs as a result of chronic cannabis use and may reverse upon cannabis abstinence**.

A.6. **Imaging cannabis use in humans (Aim 1):** Imaging studies in humans have demonstrated two key findings regarding chronic cannabis use: (1) lower neural glutamate release[14,15] and (2) CB1 downregulation,[63,64] which has been shown to normalize after 4 weeks of abstinence. These findings suggest changes in both CB1 number and function from chronic cannabis use. In the first *in vivo* study of CB1 in cannabis users, Hirvonen et al., using positron emission tomography (PET) imaging and [^{18}F]FMPEP-d2, demonstrated that chronic, heavy cannabis users showed ~20% lower CB1 availability relative to controls.[63] Consistent with animal studies, this reduction

occurred in cortical but not in subcortical brain regions. In addition to showing lower CB1 availability among people with chronic cannabis use, emerging data suggest that with abstinence of cannabis use, there is some recovery of CB1 receptor availability. We found that whereas there was a statistically significant reduction (~-15%) in CB1 availability in cannabis users at baseline relative to controls, these deficits persisted but were no longer statistically significant after 2 and 28 days of abstinence. This suggests that there is some recovery of CB1 availability over time following cannabis abstinence.[12] Collectively, these findings were interpreted as evidence of CB1 downregulation with chronic cannabis use that reversed with abstinence.

A more recent study found lower mGluR5 availability in youth (ages 18-20 years) who were at-risk for substance use disorders (i.e., based on high impulsivity and aggression), including cannabis.[65] No main effects of cannabis use alone on mGluR5 were observed. However, the study was limited by small subgroups (i.e., n = 3 CUD subjects) and uncertain timing and pattern of cannabis use relative to PET scanning. Furthermore, the cross-sectional nature of the study limited the ability to understand dynamic changes in mGluR5 over time based on cannabis use status. Our proposed study design, with scans performed longitudinally during cannabis-maintained and cannabis-abstinent states in the same subjects, **will help more definitively determine the relationship between chronic cannabis use and mGluR5**.

A.7. **Central Hypothesis: Chronic Cannabis Use Increases mGluR5 Availability (Aim 1):** The observation that chronic cannabis use leads to both lower synaptic glutamate release and CB1 downregulation raises a critical question: How do cannabis-induced changes in CB1 and glutamate release alter glutamate homeostasis? We propose it occurs via **increased mGluR5 availability**. Normally, CB1 on presynaptic glutamatergic terminals are activated by retrograde endocannabinoids (i.e., 2-AG) that reduce Ca^{2+} influx, thus playing a modulatory role by inhibiting glutamate release (**Figure 2, Left**). While chronic cannabis exposure decreases the number of CB1 receptors (downregulation), constant heavy use of cannabis leads to tonically activated CB1 via THC (despite the known downregulation) (**Figure 2, Right**). This would result in decreased and unmodulated firing in glutamatergic pyramidal cell networks, thus leading to disruptions in functionally relevant neural activity (i.e., theta oscillations).[19] We therefore propose that chronic cannabis use leads to less pre-synaptic glutamate release, less glutamate binding to mGluR5, and hence compensatory upregulation of mGluR5 (i.e., higher mGluR5 availability). Thus, it is clear that chronic exposure to THC from cannabis likely leads to profound disruption of glutamate homeostasis. **PET is the best imaging modality for studying changes in neuroreceptors *in vivo*** and is the focus of **Aim 1** of this proposal. **Using [^{18}F]FPEB PET neuroimaging, we will measure mGluR5 availability *in vivo* in people with cannabis use disorder (CUD) and compare with healthy controls.**

A.8. **Cannabis Use Disorder: Insights from Electroencephalography (EEG) (Aim 2):** While PET is the best imaging modality for studying changes in neuroreceptors *in-vivo*, this method is unable to provide information regarding how receptor changes affect neural function. By contrast, **EEG** is one of the few available neuroimaging methodologies that can **directly measure neural events** (postsynaptic potentials) with high temporal precision in humans. Thus, **combining PET and EEG** can be a powerful tool with which to examine the relationship between receptor dynamics and neural function. EEG effects from chronic cannabis use, along with validated cognitive assessments, provide **highly relevant clinical correlates to mGluR5 availability**.

The fact that CUD has disruptive effects across broad domains of brain function suggests a common neurophysiological mechanism. One potential mechanism may involve the role of CB1

in modulating glutamate function, and hence the inhibitory-excitatory (I-E) balance of neural circuits. Skosnik *et al*. (2006) hypothesized that the neurobehavioral effects of cannabis use may involve **alterations in neural synchronization**.[66] The impetus for this idea stems from the fact that interactions between glutamatergic pyramidal cells and GABAergic interneurons are involved in generating and maintaining neural oscillations in the theta and gamma (31-80 Hz) range.[19,67-73] This may be particularly germane to the effects of cannabis, as synchronized neural oscillations are thought to be involved in **memory and learning** (domains of function that are known to be disrupted by cannabinoid agonists). The effect of altered I-E balance may be particularly relevant to theta oscillations, as theta has been shown to be mediated in part by both CB1 and mGluR5 (as discussed above). From a functional standpoint, it has been established that theta may represent a "neural code" of learning and memory, and is related to synaptic plasticity.[16,74,75] The relationships between mGluR5 availability, CUD severity, neural oscillations, and other clinically relevant domains (i.e., impulsivity, cognitive function) in people with CUD are the focus of **Aim 2**. **Using EEG, we now have the capacity to measure neural function underlying memory and synaptic plasticity in human subjects *in-vivo* through theta band (4-7 Hz) neural oscillations and tetanic sensory stimulation, respectively.**

A.9. **Cannabis Use Disorder: Abstinence and the need for treatments (Aim 3):** There is a substantial amount of misinformation regarding the addictive potential of cannabis (particularly on social media), leading to a general underappreciation of the issue among the public.[76] Estimates suggest that nearly 30% of cannabis users may have cannabis use disorder, and 10% will develop addiction (severe use disorder).[7] Aim 3 focuses on the neural effects of prolonged (i.e., 4 weeks) cannabis abstinence in people with CUD. It is critical to study CUD in human subjects *longitudinally* to advance our mechanistic understanding of disease pathogenesis and identify novel treatment targets. Studying mGluR5, cognitive function, and EEG at two distinct timepoints in the addiction process (cannabis use and abstinence) will allow us to determine the long-lasting neurologic changes that might perpetuate CUD and influence disease severity, as well as changes occurring as a result of prolonged cannabis abstinence.

Lastly, it is worth noting that there are **no current FDA-approved treatments for cannabis use disorder**, despite the availability of several cannabinoid receptor agonists. Aside from the known changes in CB1 number and function, the **mechanisms underlying cannabis use disorder are still not fully understood and need further study.** Alterations in glutamate homeostasis are a likely contributing disease factor, given glutamate's central nature in endocannabinoid signaling. **mGluR5 likely plays an important role in this process**. Developing our understanding of effects on glutamate homeostasis resulting from chronic use of cannabis and subsequent abstinence from the drug will advance our ability to develop new treatment targets and therapeutics for this population. Collectively, these issues underscore the urgent need to understand the effects of chronic cannabis exposure on the brain, and importantly, whether these effects reverse when cannabis use stops.

A.10. **Summary: Significance of the current proposal:** Research efforts have been unable to keep pace with rapidly increased public demand for cannabis products, progressive legalization, and widespread use. The preclinical literature provides a biologic basis for studying the effects of chronic cannabis use on the neural glutamate system. However, it is critical to advance our understanding of cannabis use effects in human subjects. Our preliminary data, described in further detail below (Sections C.1.a., C.2.a., and C.3.a.), indicate for the first time that **mGluR5 availability is significantly higher in cannabis users** as compared with healthy controls. Among subjects who use cannabis chronically, mGluR5 availability may normalize following a

period of cannabis abstinence. **Neural oscillations link directly with glutamatergic transmission and are disrupted** from chronic cannabis use. Finally, **mGluR5 availability is strongly correlated with psychiatric symptoms and may predict disease severity** in this population, indicating that **mGluR5 is a clinically relevant biomarker** of mental health in people with cannabis use disorder. It is therefore both timely and innovative to study the effects of cannabis use on mGluR5 in humans with CUD using **multimodal imaging (PET/EEG)**. This study has the potential to increase our understanding of the mechanisms by which cannabis use affects the brain and is addictive. It may lead to novel treatment targets among people who suffer from CUD.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Potential risks: Potential risks and discomforts from this study include 1) risks associated with venous catheter insertion and with blood drawing, 2) risks associated with radiation exposure, 3) risks associated with MR, 4) risks associated with cannabis withdrawal.

1. **Risks Associated with Blood Drawing and IV line Insertion:** Drawing blood and inserting an intravenous line (IV) into an arm vein are safe and standard medical procedures. Sometimes a bruise will occur at the puncture site and rarely a blood clot or infection will occur in the vein. Certain individuals may feel light-headed during venipuncture. The volume of blood collected during this study, include screening laboratories, MRI and PET scans, will be approximately 16 tablespoons. The combined amount of blood between the two study visits on a given study day will not exceed 100 mL, and the timeframe between each visit day is over 2 weeks apart. This is not expected to have any serious negative effects on a study participant.

2. **Risks Associated with Radiation:** The Yale University Radioactive Investigational Drug Committee (RIDC) and Radiation Safety Committee (RSC) have reviewed and approved the use of radiation in this research study. This research study involves exposure to radiation from [^{18}F]FPEB PET scanning. This radiation exposure is not necessary for medical care and is for research purposes only.

   The targeted amount of radiation an individual subject can receive in this study is from up to 2 injections (HC) with a total of ≤10 mCi (up to 5mCi per injection) or up to 3 injections (CUD) with a total of ≤15 mCi (up to 5mCi per injection) from [^{18}F]FPEB, plus transmission scans, or low dose CT scans of the brain. The maximum amount of radiation exposure subjects will receive in this study is equal to an effective dose of .714 rem (HC) or 1.071 rem (CUD).

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

3. **Risks associated with magnetic resonance imaging:** Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines.

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

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

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

1. **Cannabis Abstinence / Withdrawal:**

Cannabis withdrawal: There is a risk that cannabis-dependent subjects will experience discomfort related to withdrawal from cannabis. The typical withdrawal symptoms include irritability, anxiety, anger, aggression, appetite change, weight loss, restlessness, altered sleep, strange dreams and physical discomfort.[24,25] Less common symptoms include chills, depressed mood, stomach pain, and sweating. The severity of cannabis withdrawal has been equated with the level of withdrawal from tobacco and caffeine. Cannabis withdrawal is not life threatening

and resolves spontaneously without pharmacological intervention. Most symptoms appear within 1 day of abstinence, peak within 2—3 days, and resolve within 1—2 weeks.

Resumption of cannabis use in CUD subjects who achieve abstinence: We expect that some of the CUD subjects will relapse to using cannabis after completing the study. To maximize the likelihood of continued abstinence, at the outset, subjects will be offered a referral to a cannabis treatment program at Yale.

2.3.2 Known Potential Benefits

Potential Benefits of the Proposed Research to the Subjects and Others
All study subjects with cannabis use will be referred for treatment at local treatment centers regardless of whether they choose to participate in and/or complete our research study. All participants in this study may derive subjective benefit from volunteering to take part in a study for the advancement of scientific knowledge.

Importance of the Knowledge to Be Gained
The knowledge to be gained regarding how cannabis use affects the brain is of extreme importance to better understand the negative and positive effects of cannabis. The study will produce data specific to cannabis use disorder and may guide the development of new treatments.

2.3.3 Assessment of Potential Risks and Benefits

Adequacy of Protection against Risk

Recruitment and informed consent All research subjects will be recruited under guidelines of the Yale University Institutional Review Board (Human Investigation Committee). The nature of the procedures, the risks, and financial remuneration for participation in the study will be discussed with each individual prior to obtaining informed written consent by a trained research assistant, postdoctoral fellow and/or the principal investigator of the study. The principal investigator is always available to address any questions that subjects may have during the consent procedure. Subjects are also "tested" on their knowledge about the study prior to study participation.

Protection against risk

1. Effective screening to exclude subjects who would be placed at a greater risk. This includes a medical history, the physical examination, and the screening studies (blood, urine and ECG) performed before starting studies. All screening procedures will be conducted by trained staff under the supervision of the PI. Screening procedures may take place under HIC# 2000032181, in which case they will not be repeated if the participant is also enrolled in that study and has signed a release of information.
2. The investigator or a person designated by the investigator will explain the benefits and risks of participation in the study to each subject. Subjects will then be asked to explain the study in their own words to ensure their understanding of the study procedures prior to obtaining informed consent.
3. To further prevent the risk of an adverse event and to minimize the harm incurred by such, all scans will be done in the presence of medical supervision and trained nursing

staff at the Yale PET center. In the event of serious medical complications, the PET center has immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital.

4. All subject information will be kept confidential and only members of the investigative team with appropriate IRB/HIC and HIPAA training will have access to the study data. Data will be maintained and secured in locked file cabinets or password protected electronic media. A numbering code will be used to assign a unique identifier to each subject. This information is available to study investigators and, in as far as the agents represent experimental pharmaceuticals, to representatives of the FDA and Nuclear Regulatory Commission for authorized inspection.
5. The dose of radiation is approved by the Yale University Radiation Safety Committee (RSC) and Radioactive Investigational Drug Committee (RIDC). All scans will be done in the presence of medical supervision and trained nursing staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the PET center staff have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiopharmaceuticals and performance of PET scans will be by radiochemists, physicians, and technologists of the Department of Radiology and Biomedical Imaging, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radiopharmaceuticals. Subjects will be asked about their previous radiation exposure and those who have had research exposure within the past year will be excluded if their cumulative annual exposure (including the present study) exceeds FDA limits. The information on the previous radiation exposure of study subjects will be notified to the study doctor.
6. No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing during evaluation and on each scan day before initiation of any scan procedure If subjects are breastfeeding they will not be able to participate in this research study.
7. The risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained and experienced personnel using aseptic technique to avoid injury due to fainting, the antecubital vein catheter will be inserted when the subjects are recumbent. The blood draws during PET scanning sessions will be obtained from the already inserted catheter, to minimize discomfort.
8. All subjects will be screened for any metallic objects that they may be holding or have implanted in their bodies using a questionnaire and all potential subjects with contraindications for MR will be exclude This questionnaire will be repeated immediately before each measurement to ensure that no metallic materials are brought into close proximity of the magnet, where they might be pulled toward the magnet. For additional security, subjects will be taken through a ferromagnetic metal detector immediately before going to the scan room.
9. The PI will review safety data, after every test day, during weekly research team meetings, and will suspend or modify the study (with IRB approval) if indicated. The IRB will be duly informed if there are any reasons to warrant “holding” the study. A review of the study will be submitted to the IRB annually.

3 - Objectives and Endpoints

3.1 Objectives and Endpoints

Aim 1: To determine differences in fronto-limbic mGluR5 availability between people with cannabis use disorder (CUD; n = 30) and healthy controls (HC; n = 30). Subjects will undergo [^{18}F]FPEB PET neuroimaging to determine mGluR5 availability. Hypothesis 1: Fronto-limbic mGluR5 availability will be higher in individuals with CUD compared with HC.
Aim 2: Within CUD subjects, to examine the associations between mGluR5 availability and: (a) CUD severity; (b) impulsivity; (c) EEG; and (d) neurocognition. CUD severity and impulsivity will be determined by validated instruments. EEG outcomes (e.g., theta power, LTP) known to be sensitive to both cannabis and glutamate will be collected during tasks of verbal memory[22,23] and neural plasticity (long term potentiation; LTP).[24] Hypothesis 2: Higher mGluR5 availability will be associated with: (a) greater CUD severity (by DSM-5); (b) higher impulsivity; (c) lower theta-band power and LTP, and (d) worse performance on verbal memory.
Aim 3: Within CUD subjects, to determine changes in mGluR5 availability, EEG, and neurocognition following cannabis abstinence. CUD subjects from Aim 1 will repeat PET scanning, EEG, and cognitive testing following a 28-day period of confirmed abstinence aided by contingency management. Hypothesis 3: Following cannabis abstinence: (a) mGluR5 will decrease; (b) theta power/LTP will be increased; (c) memory will improve.

4 - Study Design

4.1 Overall Design

4.2 Scientific Rationale for Study Design

A. SIGNIFICANCE

A.1. **Overview: How does cannabis affect glutamate receptors in the human brain?**

Cannabis has a wide range of health and behavioral effects, many of which remain incompletely understood.[10] Yet, both recreational and medicinal use of the drug are widespread and continuing to increase in the US. Cannabis is addictive. Nearly 30% of users may have cannabis use disorder (CUD), and nearly 12 million young adults used cannabis in 2019.[5] Recreational use is now legal in 15 states, and medical use is legal in 35 states. As more states continue to legalize use for recreational purposes, cannabis use will likely continue to increase.[25] This could result in an increasing number of people experiencing negative effects, including addiction.[25] Cannabis use impairs cognition, memory, and motor coordination. With more prolonged or heavy use, it increases the risk of developing schizophrenia or other psychoses.[26] On the other hand, it is hypothesized that cannabis may have therapeutic effects in numerous neurologic conditions such as chronic pain, neurodegenerative diseases, and some psychiatric conditions (i.e. PTSD, anxiety, and sleep disorders). It is critical to more fully understand the neural mechanisms of cannabis to minimize harmful consequences and maximize therapeutic benefit. Tetrahydrocannabinol (THC) is the primary psychoactive component of cannabis. It is well-established that many of the psychoactive and addictive properties of cannabis are mediated by the neural circuit involving THC binding to the cannabinoid receptor 1 (CB1), a *presynaptic* receptor that is widely distributed throughout the central nervous system. Less well-understood, however, is the impact of CB1-THC binding on *postsynaptic* receptors such as **metabotropic glutamate receptor 5 (mGluR5), a key regulator of glutamate homeostasis and endocannabinoid synthesis**.[11] There is strong evidence from the preclinical literature that **cannabinoid exposure alters glutamate homeostasis via mGluR5 and CB1**, which may mediate many important neural effects. Indeed, there is a close relationship between the cannabinoid and glutamatergic systems.[19] However, this relationship has not been adequately studied in humans, and the degree to which disturbances in the glutamate system influences the potential harms and therapeutic benefits from cannabis use is unclear. It is critical to advance our understanding of how chronic cannabis exposure affects the brain, including the consequences of these changes on neural function. Importantly, we need to better understand whether neural dysregulation normalizes when cannabis use stops. This innovative multimodal (PET/EEG/neurocognitive) study will expand our knowledge and thus advance our understanding of the neurobehavioral mechanisms underlying cannabis addiction, with a focus on the metabotropic glutamate receptor 5 (mGluR5), in human subjects.

A.2. **Metabotropic Glutamate Receptor 5 (mGluR5): Importance in addiction and related behavioral phenotypes:** Glutamate is the brain's primary excitatory neurotransmitter, and is critical for optimal neuronal function.[27] Glutamate transmission between neural circuits linking key limbic and cortical brain regions maintains a balance between the reward-seeking behaviors necessary for survival and higher executive functions.[28]**mGluR5, a key regulator of glutamate homeostasis,** is a G protein-coupled receptor located mostly on post-synaptic neurons diffusely throughout the brain, especially key areas involved in addiction such as hippocampus, amygdala,

striatum, prefrontal cortex, and cingulate.[29-31] It regulates synaptic plasticity of glutamatergic neurons, which is fundamental to learning and neural transmission of critical information. Alterations in mGluR5 receptor availability have been implicated in addiction[32] and its related endophenotypes such as executive function impairment,[33] mood disorders,[34] and impulsivity.[35] Preclinical studies of mGluR5 knockout mice and/or the use of mGluR5 antagonists have revealed several key findings pointing to the likely involvement of mGluR5 in addictive disorders including: (1) reduced drug self-administration and reduced drug-seeking behavior;[3637] (2) attenuation of drug-induced rewarding effects[38] and conditioned place preference[39]; and (3) reduced withdrawal.[40,41] The precise mechanisms whereby these processes occur remain unclear. These data, combined with observations of mGluR5 effects on drugs of abuse in general,[42,43] make **mGluR5 a potentially important molecular target in individuals with cannabis use disorder (CUD).**

A.3. **mGluR5: Importance in synaptic plasticity:** Synaptic plasticity is critical for neuronal adaptations to a wide range of environmental conditions. Local changes in synaptic strength [i.e., long-term potentiation (LTP) or depression (LTD)] underlie much of the brain's ability to change its physical structure in response to changes in the environment, particularly during learning and memory. **mGluR5 regulates synaptic plasticity of glutamatergic neurons**, which is fundamental to learning and neural transmission of critical information.[44,45] Activation of mGluR5 induces LTD in hippocampal area CA1 and cerebellar Purkinje neurons.[46,47] Other preclinical studies have shown that antagonism of mGluR5 impaired LTP, spatial memory, and suppressed theta (5-10 Hz) activity in the dentate gyrus.[17,18] Interestingly, the cannabinoid system is also implicated in learning, memory, and synaptic plasticity, indicating a potential convergence zone wherein mGluR5 and CB1 may interact functionally.[48] Given the overlap between cannabis use, synaptic plasticity, learning, and cognition, **it is critical to study mGluR5 in the context of cannabis use**.

Figure 1: **mGluR5-CB1 relationship**. mGluR5 is a key regulator of glutamate homeostasis via cannabinoid receptor 1 (CB1). The consumption of exogenous CB1 ligands via cannabis use may profoundly alter this process. The focus of this grant is on how chronic cannabis use and abstinence specifically affects mGluR5 availability in humans with cannabis use disorder. Figure Source: Cristino, et.al., 2019 (See ref 11).

A.4. **mGluR5: Key regulator of normal glutamate homeostasis via endocannabinoid synthesis:** Under normal physiological conditions, preclinical models suggest that **mGluR5 is a key regulator of glutamate homeostasis via CB1** at both excitatory (glutamatergic) and inhibitory (GABAergic) synapses, as shown in **Figure 1** and described here.[11] The focus of this grant is on understanding how chronic cannabis use and abstinence affect mGluR5 availability in humans with CUD. MGluR5 regulates glutamate primarily by triggering endocannabinoid (ECB) production. ECBs (e.g., 2-AG) are naturally occurring molecules that regulate neurotransmitter release and synaptic plasticity by binding to presynaptic CB1. Under normal physiological conditions, stimulation of presynaptic excitatory neurons leads to glutamate release into the synaptic cleft and peri-synaptically.[49] Glutamate binding to post-synaptic mGluR5 triggers the "on-demand" synthesis of the endocannabinoid 2-AG. 2-AG then binds in a retrograde manner to CB1, leading to synaptic depression and inhibition of further glutamate release. Thus, under normal conditions, **glutamate homeostasis is maintained via a negative feedback loop mediated by mGluR5 and endocannabinoid binding to presynaptic CB1**. This process may be disrupted by the consumption of exogenous cannabinoids.[50]

Thus, mGluR5 is a critical, widespread molecular target involved in neural glutamate homeostasis that interacts directly with the cannabinoid system and is significantly involved in neural plasticity, memory, addiction, executive function, cognition, and emotional processing. It is therefore **critically important to study the relationship between CUD and mGluR5, and to relate this to neural function and behavioral outcomes related to addiction.**

A.5. **Cannabis Use Disorder (CUD): Disruption of glutamate homeostasis via mGluR5 and CB1 (Aim 1):** CUD is characterized clinically as a syndrome involving impaired control over cannabis use, problems occurring as a result of cannabis use, and signs of physiologic dependence (i.e. tolerance or withdrawal upon abstinence).[51] Mechanistically, CUD is mediated primarily by recurrent and chronic THC binding to CB1.[52] Cannabis and THC have widespread effects on neural glutamate transmission.[50] Specifically, preclinical models have demonstrated that THC binding to CB1 inhibits glutamate release and leads to long-term synaptic depression of glutamatergic neurotransmission.[53,54] Animal studies have demonstrated that after chronic exposure to THC and other CB1 agonists, there is a reduction in the number and function of CB1 receptors.[55-60] Furthermore, CB1 downregulation and desensitization has a distinct regional and temporal course, and is related to the duration and magnitude of exposure, with greater downregulation in cortical compared with subcortical regions.[55,61,62] With prolonged abstinence, there is normalization in the number and function of *presynaptic* CB1 over 2 weeks, with faster recovery in subcortical compared to cortical regions.[59] However, **it is unknown what happens to *postsynaptic* mGluR5 from chronic cannabis use and following cannabis abstinence**. We plan to fill this knowledge gap through the proposed innovative and rigorous study. As we illustrate in our robust preliminary data below (Sections C.1a., C.2.a., and C.3.a.), our **central hypothesis is that mGluR5 upregulation occurs as a result of chronic cannabis use and may reverse upon cannabis abstinence**.

A.6. **Imaging cannabis use in humans (Aim 1):** Imaging studies in humans have demonstrated two key findings regarding chronic cannabis use: (1) lower neural glutamate release[14,15] and (2) CB1 downregulation,[63,64] which has been shown to normalize after 4 weeks of abstinence. These findings suggest changes in both CB1 number and function from chronic cannabis use. In the first *in vivo* study of CB1 in cannabis users, Hirvonen et al., using positron emission tomography (PET) imaging and [^{18}F]FMPEP-d2, demonstrated that chronic, heavy cannabis users showed ~20% lower CB1 availability relative to controls.[63] Consistent with animal studies, this reduction occurred in cortical but not in subcortical brain regions. In addition to showing lower CB1 availability among people with chronic cannabis use, emerging data suggest that with abstinence of cannabis use, there is some recovery of CB1 receptor availability. We found that whereas there was a statistically significant reduction (~-15%) in CB1 availability in cannabis users at baseline relative to controls, these deficits persisted but were no longer statistically significant after 2 and 28 days of abstinence. This suggests that there is some recovery of CB1 availability over time following cannabis abstinence.[12] Collectively, these findings were interpreted as evidence of CB1 downregulation with chronic cannabis use that reversed with abstinence.

A more recent study found lower mGluR5 availability in youth (ages 18-20 years) who were at-risk for substance use disorders (i.e., based on high impulsivity and aggression), including cannabis.[65] No main effects of cannabis use alone on mGluR5 were observed. However, the study was limited by small subgroups (i.e., n = 3 CUD subjects) and uncertain timing and pattern of cannabis use relative to PET scanning. Furthermore, the cross-sectional nature of the study limited the ability to understand dynamic changes in mGluR5 over time based on cannabis use status. Our proposed study design, with scans performed longitudinally during

cannabis-maintained and cannabis-abstinent states in the same subjects, **will help more definitively determine the relationship between chronic cannabis use and mGluR5**.

A.7. **Central Hypothesis: Chronic Cannabis Use Increases mGluR5 Availability (Aim 1):** The observation that chronic cannabis use leads to both lower synaptic glutamate release and CB1 downregulation raises a critical question: How do cannabis-induced changes in CB1 and glutamate release alter glutamate homeostasis? We propose it occurs via **increased mGluR5 availability**. Normally, CB1 on presynaptic glutamatergic terminals are activated by retrograde endocannabinoids (i.e., 2-AG) that reduce Ca^{2+} influx, thus playing a modulatory role by inhibiting glutamate release (**Figure 2, Left**). While chronic cannabis exposure decreases the number of CB1 receptors (downregulation), constant heavy use of cannabis leads to tonically activated CB1 via THC (despite the known downregulation) (**Figure 2, Right**). This would result in decreased and unmodulated firing in glutamatergic pyramidal cell networks, thus leading to disruptions in functionally relevant neural activity (i.e., theta oscillations).[19] We therefore propose that chronic cannabis use leads to less pre-synaptic glutamate release, less glutamate binding to mGluR5, and hence compensatory upregulation of mGluR5 (i.e., higher mGluR5 availability). Thus, it is clear that chronic exposure to THC from cannabis likely leads to profound disruption of glutamate homeostasis. **PET is the best imaging modality for studying changes in neuroreceptors *in vivo*** and is the focus of **Aim 1** of this proposal. **Using [^{18}F]FPEB PET neuroimaging, we will measure mGluR5 availability *in vivo* in people with cannabis use disorder (CUD) and compare with healthy controls.**

A.8. **Cannabis Use Disorder: Insights from Electroencephalography (EEG) (Aim 2):** While PET is the best imaging modality for studying changes in neuroreceptors *in-vivo*, this method is unable to provide information regarding how receptor changes affect neural function. By contrast, **EEG** is one of the few available neuroimaging methodologies that can **directly measure neural events** (postsynaptic potentials) with high temporal precision in humans. Thus, **combining PET and EEG** can be a powerful tool with which to examine the relationship between receptor dynamics and neural function. EEG effects from chronic cannabis use, along with validated cognitive assessments, provide **highly relevant clinical correlates to mGluR5 availability**.

The fact that CUD has disruptive effects across broad domains of brain function suggests a common neurophysiological mechanism. One potential mechanism may involve the role of CB1 in modulating glutamate function, and hence the inhibitory-excitatory (I-E) balance of neural circuits. Skosnik *et al*. (2006) hypothesized that the neurobehavioral effects of cannabis use may involve **alterations in neural synchronization**.[66] The impetus for this idea stems from the fact that interactions between glutamatergic pyramidal cells and GABAergic interneurons are involved in generating and maintaining neural oscillations in the theta and gamma (31-80 Hz) range.[19,67-73] This may be particularly germane to the effects of cannabis, as synchronized neural oscillations are thought to be involved in **memory and learning** (domains of function that are known to be disrupted by cannabinoid agonists). The effect of altered I-E balance may be particularly relevant to theta oscillations, as theta has been shown to be mediated in part by both CB1 and mGluR5 (as discussed above). From a functional standpoint, it has been established that theta may represent a "neural code" of learning and memory, and is related to synaptic plasticity.[16,74,75] The relationships between mGluR5 availability, CUD severity, neural oscillations, and other clinically relevant domains (i.e., impulsivity, cognitive function) in people with CUD are the focus of **Aim 2**. **Using EEG, we now have the capacity to measure neural**

function underlying memory and synaptic plasticity in human subjects *in-vivo* through theta band (4-7 Hz) neural oscillations and tetanic sensory stimulation, respectively.

A.9. **Cannabis Use Disorder: Abstinence and the need for treatments (Aim 3):** There is a substantial amount of misinformation regarding the addictive potential of cannabis (particularly on social media), leading to a general underappreciation of the issue among the public.[76] Estimates suggest that nearly 30% of cannabis users may have cannabis use disorder, and 10% will develop addiction (severe use disorder).[7] Aim 3 focuses on the neural effects of prolonged (i.e., 4 weeks) cannabis abstinence in people with CUD. It is critical to study CUD in human subjects *longitudinally* to advance our mechanistic understanding of disease pathogenesis and identify novel treatment targets. Studying mGluR5, cognitive function, and EEG at two distinct timepoints in the addiction process (cannabis use and abstinence) will allow us to determine the long-lasting neurologic changes that might perpetuate CUD and influence disease severity, as well as changes occurring as a result of prolonged cannabis abstinence.

Lastly, it is worth noting that there are **no current FDA-approved treatments for cannabis use disorder**, despite the availability of several cannabinoid receptor agonists. Aside from the known changes in CB1 number and function, the **mechanisms underlying cannabis use disorder are still not fully understood and need further study.** Alterations in glutamate homeostasis are a likely contributing disease factor, given glutamate's central nature in endocannabinoid signaling. **mGluR5 likely plays an important role in this process**. Developing our understanding of effects on glutamate homeostasis resulting from chronic use of cannabis and subsequent abstinence from the drug will advance our ability to develop new treatment targets and therapeutics for this population. Collectively, these issues underscore the urgent need to understand the effects of chronic cannabis exposure on the brain, and importantly, whether these effects reverse when cannabis use stops.

A.10. **Summary: Significance of the current proposal:** Research efforts have been unable to keep pace with rapidly increased public demand for cannabis products, progressive legalization, and widespread use. The preclinical literature provides a biologic basis for studying the effects of chronic cannabis use on the neural glutamate system. However, it is critical to advance our understanding of cannabis use effects in human subjects. Our preliminary data, described in further detail below (Sections C.1.a., C.2.a., and C.3.a.), indicate for the first time that **mGluR5 availability is significantly higher in cannabis users** as compared with healthy controls. Among subjects who use cannabis chronically, mGluR5 availability may normalize following a period of cannabis abstinence. **Neural oscillations link directly with glutamatergic transmission and are disrupted** from chronic cannabis use. Finally, **mGluR5 availability is strongly correlated with psychiatric symptoms and may predict disease severity** in this population, indicating that **mGluR5 is a clinically relevant biomarker** of mental health in people with cannabis use disorder. It is therefore both timely and innovative to study the effects of cannabis use on mGluR5 in humans with CUD using **multimodal imaging (PET/EEG)**. This study has the potential to increase our understanding of the mechanisms by which cannabis use affects the brain and is addictive. It may lead to novel treatment targets among people who suffer from CUD.

B. **INNOVATION**

This proposal is highly innovative. We propose the first known to us multimodal imaging study (combining PET, EEG, and cognitive testing) to measure the temporal course of changes in both the number and function of glutamate receptors in individuals with CUD at baseline and following a 4-week period of confirmed abstinence. By combining neuroreceptor PET imaging

(which is informative about changes in receptor availability) and EEG (which directly measures neural events with high temporal precision in humans), we will be able to examine the relationship between mGluR5 availability and neural function. In addition, examining correlations between changes in mGluR5 availability, CUD severity and related clinical features (i.e., impulsivity, craving, reward processing, etc.), electrophysiological indices sensitive to cannabinoids, and cognitive test performance in humans is highly novel. The proposed study may therefore uncover previously unknown consequences of cannabis use, and the data accrued from it will inform a large cross-section of researchers, including those studying cannabinoid dynamics, neural networks, addiction, and medicinal cannabis. Insights from this study may shift treatment paradigms and lead to more targeted treatments for individuals with CUD.

4.3 Justification for Dose

N/A

4.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.
The end of the study is defined as completion of the last visit or procedure shown in the SoA in the trial globally.

5 - Study Population

5.1 Inclusion Criteria

A total of 60 subjects will be recruited: 30 with cannabis use disorder (CUD) and 30 healthy controls (HC). Inclusion criteria for all subjects: 1) Age 18-55 years; 2) Voluntary, written, informed consent; 3) Physically healthy by medical history, physical, neurological, ECG and laboratory exams; 5) No personal or first-degree relative history of psychiatric disorders (outside of cannabis use for CUD group); 6) Full scale and verbal IQs > 80 (Wechsler Adult Intelligence Scale, Fourth Edition; WAIS-IV). CUD group: 1) Cannabis use disorder as determined by DSM-5 structured interviews; 2) Urine toxicology evidence of cannabinoid use. Healthy control group: 1) lifetime cannabis exposure less than 20 times, 2) no cannabis use in the past 2 years by self-report, 3) a negative urine drug screen. Controls will be matched to the CUD group by age, sex, socioeconomic status, education, nicotine use, and other relevant variables.

5.2 Exclusion Criteria

Subjects will be excluded for the following reasons: 1) Other substance use disorder within 1 year, except for nicotine; 2) Another primary DSM-5 Axis I major psychiatric disorder (e.g., schizophrenia, bipolar disorder, major depression, etc.) per SCID-5; 3) Urine toxicology results positive for other drugs such as opiates / opiate metabolites (e.g., methadone, buprenorphine, etc.); 4) A history of significant medical (cardiac, infectious, metabolic) or neurological illness (e.g., cerebrovascular disease, traumatic brain injury); 5) A history of seizures/epilepsy; 6) Medical contraindications to MRI imaging (e.g., ferromagnetic implants/foreign bodies, claustrophobia, etc.); 7) Pregnancy or breastfeeding (women). After completing the informed consent process, subjects will have physical and neurological examinations and an electrocardiogram (ECG). Laboratory tests to be performed at screening to exclude medical illnesses include complete blood count (CBC) and differential, chemistries, kidney function tests [creatinine, blood urea nitrogen (BUN), urinalysis], liver function tests, sex hormones and thyroid stimulating hormone (TSH). Subjects will be excluded for major medical or neurological illness or laboratories consistent with these illnesses or suggesting contraindication to PET or MR imaging. A urine drug screen and a pregnancy test (for women) will be performed before both PET and MRI scans, and subjects excluded for positive screens. Exclusion is subject to PI discretion.

5.3 Lifestyle Considerations

Subjects will be required to undergo abstinence from cannabis for up to 4 weeks. Subjects will receive motivational enhancement and contingency management (see payment structure in Human Studies Section) to promote and maintain abstinence. CUD subjects will undergo a second PET scan and EEG after 4 weeks of supervised abstinence. CUD subjects will be required to come to the clinic two days a week for 1) motivational enhancement, 2) escalating contingency management (see Human Subjects section), 3) verbal confirmation of abstinence, 4) urinary drug testing to detect cannabis exposure in the past 1-24 hours, 4) supervised collection of urine samples to assay THC-COOH principal metabolite of THC. To account for dilution, urinary THC-COOH:creatinine (T:C) ratio will be calculated. Abstinence will be confirmed if the weekly means of urinary T:C ratio decreases over time and does not increase by more than 50% relative to the prior specimen.[87-89]

5.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a modifiable factor may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 Strategies for Recruitment and Retention

RECRUITMENT AND RETENTION PLAN

Recruitment

All research subjects will be recruited under guidelines of the Yale University Institutional Review Board (Human Investigation Committee). Subjects will be recruited through a variety of advertising methods (study flyers, posters, internet/web postings, social media, radio, newspaper, and public access TV) as well as through referrals from other research groups/clinicians at the Yale-New Haven Hospital, Connecticut Mental Health Center, the CT VA, and/or the APT Foundation. Potential subjects will be encouraged to contact our study recruitment line for inclusion in our study. Subjects are recruited through our recruitment phone line. A member of our research staff will describe the study to participants who call, answer any questions the potential subjects have, and then complete a phone screening questionnaire to determine the subject's eligibility for an in-person screening visit. If an individual appears to meet enrollment criteria and is interested in participating, a face-to- face interview is conducted by the study staff and study physician. A release of information is obtained for review of any available historical and clinical data. A written authorization form is also obtained from each subject, permitting the research team to use, create, or disclose the subject's PHI for research purposes. The nature of the project, procedures, relative risks and benefits, and alternatives to participation in the project are discussed with the individual. Following this discussion, the individual is given a copy of the consent form to review, and any questions are answered. The process of informed consent will be obtained in accordance with local IRB standards by study personnel who have participated in institutionally approved training in human subject protection. Upon obtaining voluntary, written, informed consent, medical and psychiatric screening procedures will be used to confirm study eligibility. Subjects are free to discontinue their participation in the research at any time by requesting this verbally or in writing.

Retention

Retention of subjects with cannabis use disorder (CUD) will be maximized by our well-established contingency management strategies and close contact with our study subjects through phone, e-mail, and in-person. Using a combination of contingency management with escalating monetary rewards, counseling, and the use of frequent creatinine-adjusted quantification of urinary THC-COOH, we have been successfully able to have subjects remain abstinent for up to 28 days with a relatively low drop-out rate (10%).

Subjects will also be compensated for their time as both an inducement to participate and complete study procedures (by amounts / methods deemed appropriate and approved by our local IRB).

6 - Study Intervention

6.1 Study Intervention(s) Administration

6.1.1 Study Intervention Description

Study Schedule:
Day 0: CUD subjects and healthy controls (HC) undergo PET scanning, an optional EEG, and cognitive testing.
Days 1-27: CUD subjects abstain from cannabis use, aided by our validated contingency management strategy.
Day 28: CUD subjects who are abstinent from cannabis use undergo repeat PET scanning, an optional EEG, and cognitive testing.

Study Procedures: Eligible subjects will undergo an MRI scan for coregistration of PET data and for individualized source analysis of EEG. CUD subjects will be instructed not to use cannabis after midnight of Day 0, and last use of cannabis will be recorded. On Day 0, CUD subjects and HC will be scanned via PET, administered EEGs, and undergo cognitive testing. The same CUD subjects scanned on Day 0 will then be aided with cannabis abstinence and monitored over the next 4 weeks. Participants will be paid after completion of each of the study procedures they complete. Payments include $450 for each PET scan, $50 for each MRI scan, $50 for each set of cognitive tests performed on or around Day 0 and 28, $50 for each optional EEG, and up to $1,100 if abstinence is successfully completed as confirmed by urine samples and report.

Magnetic Resonance Imaging Session
MRIs will take place at the Magnetic Resonance Research Center (MRRC) at The Anlyan Center (TAC) for Medical Research & Education, 300 Cedar Street, in New Haven. A member of the research staff will accompany the study participant to the MR scans and stay for the duration of the session.

A high resolution, 3-dimensional Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) T1-weighted sequence will be used to acquire sagittal images for anatomical determinations and co-registration (TR=1500ms, TE=2.83ms, FOV=256x256mm2, matrix=256x 256mm2, slice thickness=1.0mm without gap, 160 slices, voxel size 1.0 x 1.0 x 1.0 mm3). The structural MRI data will serve to guide placement of ROIs for PET and for conducting GMM/partial volume correction (PVC) analyses.

We will also acquire rs-fMRI and DTI at both imaging time points, and these will be examined on an exploratory basis.

If a recent MRI scan is available due to participation in another Yale HIC approved protocol,
the MRI scan session may not need to be completed for healthy controls.

Positron Emission Tomography (PET) Sessions
PET procedures will be conducted at the Yale University PET Center. Female subjects of child bearing potential will be given a urine pregnancy test prior to the initiation of any imaging procedures. If the test is positive, the scan session will be canceled. HCs will complete a single PET scan session. CUD subjects will complete up to 2 PET scan sessions, one at baseline and one following cannabis abstinence.

PET scans are acquired as subjects lie supine on the scanner bed. Vital signs (blood pressure, pulse, respiration) will be obtained before and after radiotracer administration. A venous catheter will be used for IV administration of the radiotracer and for venous blood sampling. PET scans will be acquired using bolus plus constant infusion administration of ≤5 mCi of [^{18}F]FPEB. Subjects are infused outside the scanner for the first 0-90 minutes during the pre-equilibrium period. Then, they will be scanned for 30 minutes in list-mode during the time the radiotracer reaches steady levels in the brain (90-120 minutes).

In the event that scans are not able to be completed as scheduled, subjects may be asked to return on another day to complete the scan. If a scan is canceled following radiotracer injection, subjects may receive an additional injection during their return visit, for a maximum of up to 2 injections (HC) or 3 injections (CUD).

Cannabis Abstinence
On a weekly basis between Days 1-27, CUD subjects will be administered (1) the Withdrawal Discomfort score of the Cannabis Withdrawal Checklist, (2) the Cannabis Withdrawal Scale, which has subjective and objective (vital signs, weight) items, (3) Visual Analog Scale for Mood States, and (4) Pittsburgh Sleep Quality Index. CUD subjects will receive motivational enhancement and contingency management (CM) to promote and maintain abstinence. The escalating CM weekly payment schedule for cannabis abstinence is as follows: $50, $75, $125, $200, $650 (four-week abstinence bonus). If scheduling constraints extend the necessary abstinence period, an additional $200 per week of abstinence beyond 28 days will be paid. CUD subjects will be required to come to the clinic two days a week for (1) motivational enhancement, (2) escalating contingency management, (3) verbal confirmation of abstinence, (4) urinary drug testing to detect cannabis exposure in the past 1-24 hours, (5) supervised collection of urine samples to assay THC-COOH, the principal metabolite of THC. To account for dilution, urinary THC-COOH:creatinine (T:C) ratio will be calculated. Abstinence will be confirmed if the weekly means of urinary T:C ratio decreases over time and does not increase by more than 50% relative to the prior specimen.[113-115] On Day 28, CUD subjects will undergo a second PET scan, EEG, and cognitive testing.

The CUD subjects who relapse between the first and second scans (expected to be 10% of CUD subjects) will be: 1) considered dropouts and 2) referred for treatment if so desired. We will replace dropouts with new CUD subjects to ensure maintenance of adequate statistical power for hypothesis testing. We have accounted for dropouts in the analytic plan, study timeline, and budget.

Cannabis Assessments: CUD severity is assessed using DSM-5 criteria.[51] Cannabis withdrawal is assessed by the Cannabis Withdrawal Scale.[116] Cannabis craving is assessed

using a 7-point scale. The Marijuana Problems Scale[117] will assess psychosocial impact of cannabis use. Multiple other clinically relevant features of CUD are described in the section below (Cognitive Testing and Behavioral Symptom Assessment). Detailed information on cannabis use patterns will be obtained using a time-line follow back approach: levels of cannabis consumption (estimated number of joints or edibles consumed) will be determined via interview for lifetime, the past six months, three months, one month, and then for the week prior to the test session as has been described previously [12,66,112,118,119]. Participants will be instructed to consider each day of the week and indicate, for an average week, how much they consumed per drug-use occasion for each length of time assessed. Cognitive Testing and Behavioral Symptom Assessment: The BIS/BAS will be used to assess trait impulsiveness and reward responsiveness.[120,121] Urgency, Premeditation (lack of), Perseverance (lack of), Sensation Seeking, Positive Urgency, Impulsive Behavior Scale will assess five impulsive personality traits. The Eriksen flanker task will supplement the assessment of the ability to suppress responses that are inappropriate to a particular context.[122] In addition, the Probabilistic Reward Task (PRT) will be used to provide an objective endophenotypic measure of reward responsiveness.

On each of the scan days, CUD and controls subjects will also be administered neuropsychological tests from the CogState Cognitive Battery[123,124], which we previously showed to be sensitive to detecting cognitive deficits in mood disorders[125]: executive control (Groton Maze Learning task, GMLT; Set-Shifting task, SST and Go No-Go, GNG)[126-134] and working memory (One Back task, ONB, and Two Back task, TWB)[135-137]. Further, the battery has been well-validated (studies using CogState batteries have been published in over 100 peer-reviewed journals). Most important for the current study, this battery is ideal for within-subject designs (i.e., it was specifically developed for repeated administration with minimal practice or learning effects). Finally, the CogState has been shown to have high test-retest reliability, thorough coverage of cognitive domains, comparable alternative forms, strong internal consistency, is well established in the general population, and has proven tolerability and acceptability. The entire battery takes approximately 30 minutes. For mood assessment, the Hamilton Depression Rating Scale (HAM-D) and Montgomery-Asberg Depression Rating Scale (MADRS) will be administered by highly trained and reliable raters. These assessments will be administered at screening and at the time of PET scans.

Additional Visits

In some situations, participants may be asked to come in for one or more additional visits. These visits may consist of one or more of the following, including repeat blood draw, repeat urine toxicology, assessments including for cannabis use, repeat MD evaluation/physical, repeat ECG, and other procedures listed in the screening visit. Repeat MRI and PET scans will not be performed at these visits. Participants will be compensated an additional $25 for each additional visit.

6.1.2 Dosing and Administration

The PET drug [^{18}F]FPEB will be administered intravenously at a dose of no more than 5 mCi per scan day with a maximum of 1 scan for HC subjects and 2 scans for CUD.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

[^{18}F]FPEB is provided by the Yale PET Center on the scheduled scan day. The radiochemistry laboratory is responsible for its preparation and release for administration. The remaining un-injected radioactivity is returned to the radiochemistry quality control lab where it is stored in a lead-shielded pig until the sample completely decays (~10 half-lives) before it is discarded.

6.2.2 Formulation, Appearance, Packaging, and Labeling

[^{18}F]FPEB is formulated in saline for injection containing 0.9% ethanol and packaged in a sterile, clear, aluminum sealed top single dose glass (USP type I) vial with synthetic rubber stopper. The dose vial is labeled as described in the FDA approved Yale [^{18}F]FPEB Drug Master File filed under IND application No. 155250. The final PET drug product solution is clear and colorless, with no visual evidence of cloudiness or particulate matter.

6.2.3 Product Storage and Stability

[^{18}F]FPEB is stored at room temperature in its respective sterile and nonpyrogenic dose vial and is stable for at least 8 hours after its preparation.

6.2.4 Preparation

Under the supervisions of Dr. Henry Huang and Dr. Nabeel Nabulsi, [^{18}F]FPEB is prepared by GMP trained radiochemistry production lab staff at the Yale PET Center immediately before administration.

6.3 Measures to Minimize Bias: Randomization and Blinding

N/A

6.4 Study Intervention Compliance

CUD subjects will receive motivational enhancement and contingency management (CM) to promote and maintain abstinence. The escalating CM weekly payment schedule for cannabis abstinence is as follows: $50, $75, $125, $200, $650 (four-week abstinence bonus). We will collect urine samples to assay THC-COOH principal metabolite of THC. To account for dilution, urinary THC-COOH:creatinine (T:C) ratio will be calculated. Abstinence will be confirmed if the weekly means of urinary T:C ratio decreases over time and does not increase by more than 50% relative to the prior specimen.[113-115]
The CUD subjects who drop out (estimated 10%) will be replaced with new CUD subjects to ensure maintenance of adequate statistical power for hypothesis testing. We have accounted for dropouts in the analytic plan, study timeline, and budget.

6.5 Concomitant Therapy

N/A

6.5.1 Rescue Medicine

N/A

7 - Study Intervention Discontinuation and Participant Discontinuation/Withdrawal

7.1 Discontinuation of Study Intervention

Study participants may withdraw from the study at any time.

7.2 Participant Discontinuation/Withdrawal from the Study

N/A

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for their scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 2 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 - Study Assessments and Procedures

8.1 Efficacy Assessments

OUTCOMES	**SOURCE/ MEASURE**	**HC**	**CUD (baseline)** Day 0	**CUD (abstinence)** Day 28
Neural Receptors				
mGluR5 Availability	PET ROI's V_T: OFC, anterior cingulate, vmPFC, dlPFC, hippocampus, amygdala	X	X	X
Cannabis Use Disorder — Clinically Relevant Functional Measurements				
CUD Severity	DSM-5		X	
Cannabis Use Patterns	Timeline Follow Back		X	X
Cannabis Withdrawal Severity	Cannabis Withdrawal Scale		X	X
Cannabis Craving	7-point Scale		X	X
Cannabis-Related Problems	Marijuana Problems Scale		X	
Impulsivity	BIS/BAS	X	X	
Reward Processing	Probabilistic Reward Task	X	X	X
Mood	HAM-D, MADRS, Visual Analog Scale	X	X	X

	for Mood States			
Sleep	Pittsburgh Sleep Quality Index	X	X	X
Cannabis Biomarkers				
Cannabis Use Status	Urinary THC-COOH*	X	X	X
Neural Function				
Verbal Memory	EEG: Theta band power/coherence	X	X	X
Synaptic Plasticity / LTP	EEG: N100-P200 amplitude increase after auditory tetanus	X	X	X
Neurocognitive Function	CogState Cognitive Battery	X	X	X

8.2 Safety and Other Assessments

Protection against risk

1. Effective screening to exclude subjects who would be placed at a greater risk. This includes a medical history, the physical examination, and the screening studies (blood, urine and ECG) performed before starting studies. All screening procedures will be conducted by trained staff under the supervision of the PI. Screening procedures may take place under HIC# 2000032181.
2. The investigator or a person designated by the investigator will explain the benefits and risks of participation in the study to each subject. Subjects will be asked to complete a questionnaire to determine their understanding of the study procedures and risks, prior to obtaining written informed consent. The questionnaire consists of questions about the key risks (radiation, drug withdrawal, etc) and benefits of the study.
3. To further prevent the risk of an adverse event and to minimize the harm incurred by such, all scans will be done in the presence of medical supervision and trained nursing staff at the Yale PET center. In the event of serious medical complications, the PET

center has immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital.

4. All subject information will be kept confidential and only members of the investigative team with appropriate IRB/HIC and HIPAA training will have access to the study data. Data will be maintained and secured in locked file cabinets or password protected electronic media. A numbering code will be used to assign a unique identifier to each subject. This information is available to study investigators and, in as far as the agents represent experimental pharmaceuticals, to representatives of the FDA and Nuclear Regulatory Commission for authorized inspection.
5. The dose of radiation is approved by the Yale University Radiation Safety Committee (YU RSC). All scans will be done in the presence of medical supervision and trained nursing staff in an institution specifically designed to support imaging studi In the event of serious medical complications, the PET center staff have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiopharmaceuticals and performance of PET scans will be by radiochemists, physicians, and technologists of the Department of Diagnostic Radiology, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radiopharmaceuticals. Subjects will be asked about their previous radiation exposure and those who have had research exposure within the past year will be excluded if their cumulative annual exposure (including the present study) exceeds FDA limits. The information on the previous radiation exposure of study subjects will be notified to the study doctor.
6. No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing during evaluation and on each scan day before initiation of any scan procedur If subjects are breastfeeding, they will not be able to participate in this research study.
7. The risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained and experienced personnel using aseptic techni To avoid injury due to fainting, the antecubital vein catheter will be inserted when the subjects are recumbent. The blood draws during PET scanning sessions will be obtained from the already inserted catheter, to minimize discomfort.
8. All subjects will be screened for any metallic objects that they may be holding or have implanted in their bodies using a questionnaire and all potential subjects with contraindications for MR will be exclude This questionnaire will be repeated immediately before each measurement to ensure that no metallic materials are brought into close proximity of the magnet, where they might be pulled toward the magnet. For additional security, subjects will be taken through a ferromagnetic metal detector immediately before going to the scan room.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 Classification of an Adverse Event

8.3.3.1 Severity of Event

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild — Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate — Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe — Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** — The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** — There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

OR

- **Definitely Related** — There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

- **Probably Related** — There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** — There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** — A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** — The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.3.3 Expectedness

The Study PI will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The Study RA or PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence

of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 Adverse Event Reporting

Plans for Reporting Serious Anticipated and Unanticipated Adverse Events
Adverse events > level 3 will be reported to the IRB within 24 hours. Other adverse events will be reported to the IRB in a timely manner, using the following predefined causal relationships:

Attribution of Risk Categories:

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

Monitoring for data integrity and safety will be the responsibility of the Principal Investigator, the Yale University IRB, and a Data and Safety Monitoring Board (DSMB). The DSMB will meet twice per year to review study progress. A DSMB will be used to provide additional protections for participants and to monitor progress on the study.

8.3.6 Serious Adverse Event Reporting

The study clinician will immediately report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center (DCC)/study sponsor and should be provided as soon as possible.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible,

but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

8.3.7 Reporting Events to Participants

Adverse events will be reported to participants individually.

8.3.8 Events of Special Interest

N/A

8.3.9 Reporting of Pregnancy (if applicable)

Positive pregnancy results will be reported to the study participant.

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Additional example text, applicable for device protocols:
This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.4.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

UPs will be promptly reported to the IRB of record and to the DCC/study sponsor in accordance with policy and regulatory requirements. When Children's National serves as the IRB of record, UPs should be reported to the IRB within 7 business days of the investigator becoming aware of the UP.

8.4.3 Reporting Unanticipated Problems to Participants

Unanticipated problems will be reported to participants individually.

9 - Statistical Considerations

9.1 Statistical Hypotheses

Aim 1: To determine differences in fronto-limbic mGluR5 availability between people with cannabis use disorder (CUD; n = 30) and healthy controls (HC; n = 30). Subjects will undergo [^{18}F]FPEB PET neuroimaging to determine mGluR5 availability. Hypothesis 1: Fronto-limbic mGluR5 availability will be higher in individuals with CUD compared with HC.
Aim 2: Within CUD subjects, to examine the associations between mGluR5 availability and: (a) CUD severity; (b) impulsivity; (c) EEG; and (d) neurocognition. CUD severity and impulsivity will be determined by validated instruments. EEG outcomes (e.g., theta power, LTP) known to be sensitive to both cannabis and glutamate will be collected during tasks of verbal memory[22,23] and neural plasticity (long term potentiation; LTP).[24] Hypothesis 2: Higher mGluR5 availability will be associated with: (a) greater CUD severity (by DSM-5); (b) higher impulsivity; (c) lower theta-band power and LTP, and (d) worse performance on verbal memory.
Aim 3: Within CUD subjects, to determine changes in mGluR5 availability, EEG, and neurocognition following cannabis abstinence. CUD subjects from Aim 1 will repeat PET scanning, EEG, and cognitive testing following a 28-day period of confirmed abstinence aided by contingency management. Hypothesis 3: Following cannabis abstinence: (a) mGluR5 will decrease; (b) theta power/LTP will be increased; (c) memory will improve.

9.2 Sample Size Considerations

STATISTICAL DESIGN AND POWER

Frequency distributions and descriptive statistics for all variables will be computed prior to conducting analyses. If Shapiro-Wilk normality tests indicate non-normality for any continuous variable, we will compute necessary data transformations (e.g., logarithmic base 10) prior to conducting analyses. Of note, to conserve power, we will compute an average V_T value across the 6 study ROIs to be used in all PET analyses. This choice is supported by the fact that preliminary data revealed large magnitude (i.e., r's>0.80-0.95) correlations between OFC, dlPFC, amygdala and hippocampus [^{18}F]FPEB V_T. Thus, we plan to treat this set of regions as a circuit and compute a mean composite score of [^{18}F]FPEB V_T for use in analyses.

Aim 1: To determine differences in fronto-limbic mGluR5 availability between people with cannabis use disorder (CUD; n = 30) and healthy controls (HC; n = 30). Hypothesis 1: Fronto-limbic mGluR5 availability will be higher in individuals with CUD compared with HC.
Power Calculations for Hypothesis 1: We approached power calculations by considering both our preliminary data *and* relevant and reliable literature (i.e., conducted with rigorous scientific methodologies, published in competitive, peer reviewed journals, with sufficient sample sizes) to determine the magnitudes of effect sizes that we might reasonably expect to observe in this study. No studies outside of our preliminary ones presented above (Research Strategy, Section C.1.a) have examined this topic of study specifically. However, we identified studies which examined differences in receptor availability *in vivo* between individuals who did and did not report use of cannabis using PET.[64,138-140] For example, a recent examination of CB1 receptor availability in cannabis users vs. non-users showed a mean V_T 15% lower (d=-1.11)[64]. Another study found 20% lower CB1 in chronic daily cannabis users relative to non-users. Based on the

information provided in each manuscript, we calculated effect sizes for group differences. Observed effect sizes for group comparisons of cannabis users vs. non-users ranged from d= -0.68 to -1.77, with a median of d=-1.11. These effect sizes are consistent with those observed in our preliminary data, which showed a large effect size associated with differences in mGluR5 availability in CB relative to healthy control individuals in the fronto-limbic circuit (d= 1.72). We will evaluate hypothesis 1 (higher mGluR5 availability in cannabis users) using univariate analysis of variance (ANOVA). Assuming α=0.05 (two-tailed test) and two groups (cannabis vs. control), our proposed sample size of 60 (30 per group) will provide 81.07% statistical power to detect group differences as small as d= 0.82, which is smaller than the median effect size observed in previous literature and that observed in our preliminary data. Data Analysis for Hypothesis 1: As stated, we will conduct a univariate ANOVA to compare mean fronto-limbic [^{18}F]FPEB V_T between the HC and cannabis groups. Groups will be carefully matched on demographic variables. However, any demographic variables that differ significantly between groups, including **notably age and sex, will be considered for entry as additional fixed factors (categorical variables) or covariates (continuous variables).** A significant group difference suggestive of higher mGluR5 availability in CUD subjects will be considered supportive of Hypothesis 1. We will also conduct exploratory analyses to examine between-group differences in mGluR5 in other regions, including those involved in affective and cognitive dysregulation (e.g., thalamus, striatum, insula), at the same significance level.

Aim 2: Within CUD subjects, to examine the associations between mGluR5 availability and: (a) CUD severity; (b) impulsivity; (c) EEG; and (d) neurocognition. Hypothesis 2: Higher mGluR5 availability will be associated with: (a) greater CUD severity (by DSM-5); (b) higher impulsivity; (c) lower theta-band power and LTP, and (d) worse performance on verbal memory. Power Calculations for Hypothesis 2: Using the same standards applied for hypothesis 1, we considered our preliminary data *and* conducted a literature review to determine what magnitude of correlation we could reasonably expect to see in this study between relevant variables of interest. We included studies with similar methodologies, which reported correlations between similar variables (i.e. similar EEG measures and cognitive/behavioral constructs, and an *in vivo* measurement of neuroreceptor or transporter availability in humans using PET)[141-145]. Examples include an unpublished study from our research group in which the correlation between mGluR5 availability and impulsivity in BPD and PTSD individuals ranged from 0.46. to 0.80 in frontal and limbic brain regions of interest (n= 56). Observed correlations ranged in magnitude from 0.21 to 0.80 (positive or negative) with a median r = 0.57. Magnitude of relevant correlations observed in our preliminary data are comparable (i.e., correlation between impulsivity and fronto-limbic mGluR5 availability, r = 0.896). We will evaluate aim 2 using correlational analyses. Using a correlation estimate of 0.55 (based on both review of the literature and our pilot data) and a two-tailed α =0.05, a priori power analysis suggested we will require a minimum n = 24 to achieve more than 80% power for the planned analysis. As we plan to conduct correlational analyses within the CUD group (n = 30), we will be adequately powered to test this hypothesis. Data Analysis for Hypothesis 2: Correlational analysis (Spearman or Pearson, as appropriate based on observed data distributions) will be used to test for associations between fronto-limbic mGluR5 availability, CUD severity, impulsivity, θ- band power and LTP, and working memory performance. Multiple regression analyses will also be considered to control for potential confounding variables. α will be set at 0.05 for all planned correlational analyses. We will also assess for associations between mGluR5 availability and performance on

other domains as secondary/exploratory analyses, keeping significance at the same level. Significant positive correlations between fronto-limbic mGluR5, CUD severity, and impulsivity will be supportive of Hypothesis 2. Likewise, significant negative correlations between fronto-limbic mGluR5, θ- band power and LTP, and working memory performance will be considered supportive of hypothesis 2. Of note, the relationship between other relevant and clinically meaningful variables pertaining to CUD (e.g. craving, withdrawal, duration/intensity of use), and mGluR5 availability will also be examined in exploratory analyses. The influence of potentially relevant demographic variables including **age and sex will be examined.**

Aim 3: Within CUD subjects, to determine changes in mGluR5 availability, EEG, and neurocognition following cannabis abstinence. Hypothesis 3: Following cannabis abstinence: (a) mGluR5 will decrease; (b) theta power/LTP will be increased; (c) memory will improve. Power Calculations for Hypothesis 3: Using the same standards applied for Aims 1 and 2, we considered our preliminary data *and* conducted a literature review to determine the magnitude of effect sizes we might reasonably expect to observe with respect to change in mGluR5, EEG, and cognition as a function of abstinence from cannabis[64,140,146,147]. Values necessary to calculate associated effect sizes were not provided in the reviewed literature. For example, Hirvonen and colleagues (2012) observed a significant increase in CB1 receptor availability in cannabis users after abstinence in both the PFC and amygdala. While percent change (pre-post abstinence) could be roughly estimated from the figure provided, exact values are not available, preventing effect size estimation. We do not have preliminary data examining participants pre- and post-cannabis abstinence. However, we believe that comparison of available PET data showing *in vivo* mGluR5 availability in current cannabis users compared with former users may be indicative of the magnitude of change we can expect to see following abstinence. Comparison of mGluR5 availability in current and former cannabis users in our preliminary sample (n= 5 per group) showed a large effect (d = -1.2). We will evaluate Hypothesis 3 using repeated measures ANOVA. Using a moderate effect size estimate of d = 0.58 at α = 0.05 (highly conservative relative to the noted relevant finding in our preliminary data), a priori power analysis suggested we will require n = 30 to achieve 80.13% power for the planned analysis. Data analysis for Hypothesis 3: To test Hypotheses 3, we will conduct 3 repeated measures ANOVAs to examine changes pre- and post-cannabis abstinence in n = 30 CUD subjects. Specifically, models will evaluate: (1) changes in mGluR5 availability; (2) changes in θ-band power and coherence; (3) changes in cognition pre-post abstinence from cannabis in the CUD group. Variables that differ significantly between groups, including **age and sex, will be considered for entry into the models**. A statistically significant result indicative of lower mGluR5 availability following abstinence (model 1) or higher θ- band power and coherence (model 2) and improved cognition (model 3) will be considered supportive of Hypothesis 3.

9.3 Populations for Analyses

N/A

9.4 Statistical Analyses

9.4.1 General Approach

See 9.2 above.

9.4.2 Analysis of the Primary Efficacy Endpoint(s)

See 9.2 above.

9.4.3 Analysis of the Secondary Endpoint(s)

See 9.2 above.

9.4.4 Safety Analyses

9.4.5 Baseline Descriptive Analyses (if applicable)

9.4.6 Planned Interim Analyses (if applicable)

9.4.7 Sub-Group Analyses

9.4.8 Tabulation of Individual Participant Data

9.4.9 Exploratory Analyses

10 - Supporting Documentation and Operational Considerations

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Informed Consent Process

An IRB approved consent form describing the study procedures and risks will be given to the participant and written documentation of informed consent will be required prior to starting the study.

10.1.1.1 Consent/Assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

10.1.1.2 Consent Procedures and Documentation

Consent forms will be Institutional Review Board (IRB)-approved and the participant/legally authorized representative (LAR) will be asked to read and review the document. The research assistant, principal investigator, or co-investigator will explain the research study to the participant and answer any questions that may arise. This conversation will take place in a private room. Assent will not be conducted. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants/families/LAR will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants/family/LAR should have the opportunity to discuss the study with their family or surrogates, or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants/families/LAR must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants/families/LAR for their records.

10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met

- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).]

10.1.3 Data Confidentiality and Participant Policy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.
All research activities will be conducted in as private a setting as possible.
The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.
The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.
Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored in a secure electronic database such as REDCap. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.
Certificate of Confidentiality (if applicable)
To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 Future Use of Stored Human Specimens and Data

Data collected for this study will be analyzed and stored in a secure electronic database such as REDCap. After the study is completed, the de-identified, archived data will be transmitted to and stored in the electronic database for use by other researchers including those outside of the study. Permission to store data will be included in the informed consent.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the database.

10.1.5 Safety Oversight

Data and Safety Monitoring Board (DSMB)

We will establish a DSMB to provide the highest protection for study participants. We will ensure that DSMB members collectively have expertise in neuroimaging (specifically PET and MRI) and cannabis/cannabinoids, which are most pertinent to subject risk for the proposed study.

We will prepare twice yearly DSMB reports which include information on enrollment, participant retention rates, adverse events, and preliminary analyses as appropriate. The DSMB oversees an initial protocol review and then ongoing reviews. These reports are supplemented by rapid notification of all studies' serious adverse events. The PI will meet with the DSMB to review the study. The DSMB meets initially in an open session, attended by the PI and co-Is. Then, a closed session is held in which the DSMB Chair conducts a review of all issues and puts these to vote. The purview of the DSMB includes, but is not limited to, assessments of data quality and timeliness, participant recruitment, subject retention, safety and efficacy data, and protocol compliance. The DSMB also considers advances occurring elsewhere and their impact on the potential risks and benefits of the study. Following the DSMB meeting, a report of the meeting, including recommendations will be prepared and submitted to the PI, to the IRB, and to the funding agency.

10.1.6 Key Roles and Study Governance

10.1.7 Clinical Monitoring

10.1.8 Quality Assurance and Quality Control

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 Data Handling and Record Keeping

10.1.9.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.
All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.
Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.
Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into an a secure electronic database such as REDCap, a 21 CFR Part 11-compliant data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 Study Records Retention

HHS funded study documents should be retained for a minimum of 3 years after the termination of the study. Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.
These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 14 working days of identification of the protocol deviation, or within 30 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to NIDA Program Official and NIH. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.1.11 Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 10 years after the completion of the primary endpoint by contacting the study investigators.

10.1.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIDA has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 Additional Considerations

10.3 Protocol Amendment History

10.4 Abbreviations

LIST OF TABLES