Document Title: Study Protocol and Statistical Analysis Plan

Study Title: Brain Function and Connectivity in Methamphetamine Dependence: The Link to Neuroinflammation and the Effects of Ibudilast

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Methamphetamine (MA) use is a substantial health concern in veterans. This proposal will test whether MA-induced neuroinflammation results in deficits in the activation and connectivity of the mesocorticolimbic dopamine (DA) system and investigate whether ibudilast, an anti-inflammatory drug, improves those impairments. This project is based on findings supporting the hypothesis that MA use leads to neuroinflammation and renders the brain more vulnerable to disruptions in DA brain regions (1), which may exacerbate cognitive control and functional brain deficits (2). Although dysregulated DA signaling is associated with inflammation (3) and with MA use (4), the mechanistic link between MA-induced neuroinflammation and mesocorticolimbic DA deficits in humans has not been studied.

In line with evidence for aberrant DA signaling with MA use, MA users show greater mesocorticolimbic resting-state functional connectivity (RSFC) (2, 5) and ventral striatal activation during reward-related decision-making compared to controls (5). Degeneration of DA nerve terminals caused by MA, however, is preceded by activation of striatal microglia (1, 6). In animal models, MA exposure leads to reactive gliosis and increases the release of pro-inflammatory cytokines by activated microglia. In humans, MA users compared to controls show greater activated microglia in DA terminal regions including midbrain and striatum with positron emission tomography (PET) (7). MA users also exhibit higher levels of inflammatory intermediates in frontal gray matter and basal ganglia than controls in magnetic resonance spectroscopy studies (8, 9).

Inflammatory molecules disrupt DA synthesis, reuptake, and release (3), thereby contributing to DA-related behavioral deficits (3). Administration of inflammatory cytokines (10) and endotoxins (11) modifies the response to hedonic reward and alters the activation of the ventral striatum. Furthermore, cytokines and C-reactive proteins are related to abnormalities in RSFC between ventral and dorsal striatum and ventral medial prefrontal cortex (12). In light of the role of DA function in the clinical features of addiction, these studies provide strong support for the hypothesis that inflammation-induced changes in DA transmission may mediate MA-related deficits in mesocorticolimbic function, cognitive control and reward evaluation. Reducing neuroinflammation with the use of a novel anti-inflammatory treatment, therefore, is an important therapeutic goal and has potential to normalize DA function and accelerate effective treatment strategies for veterans with MA-use disorder. Indeed, a recent study showed that ibudilast has potential for treating MA symptoms (13). However, whether ibudilast reduces neuroinflammation and mediates brain function is unknown.

This proposal will test a model whereby MA-induced inflammation and its reduction with ibudilast influences brain function, RSFC and behavior. Participants with and without MA-use disorder will undergo a neuropsychiatric battery consisting of MA use and craving questionnaires and cognitive control tasks. PET imaging with [^{11}C]-N-(2-methoxybenzyl)-N-(4-phenoxypyridin-5-yl) acetamide ([^{11}C]PBR28, IND #138123) will be used to index neuroinflammation. Mesocorticolimbic RSFC will be measured with functional magnetic resonance imaging (fMRI) when participants are at rest, and the Monetary Incentive Delay task (MID), a task that reliably induces striatal activation and DA release (14), will be used to assess striatal function in response to reward. The effects of a 6-week treatment of ibudilast (IND #133175) on these measures will then be tested in a double-blind randomized placebo-controlled study in 40 MA users.

SA 1: Determine the relationship between neuroinflammation and cognitive control, craving, mesocorticolimbic RSFC and ventral striatal activation to reward on the MID.

Hypothesis 1. As MA use is linked with neuroinflammation, increased mesocorticolimbic RSFC, heightened ventral striatal activation in response to reward and impaired cognitive control, we predict that MA users will exhibit greater neuroinflammation than controls with greater positive relationships between neuroinflammation and 1) RSFC, 2) ventral striatal activation to reward, 3) craving and 4) impairments in cognitive control (Group by Neuroinflammation interaction).

SA 2: Test whether ibudilast reduces neuroinflammation, improves brain function and attenuates MA craving.

Hypothesis 2. MA users in the Ibudilast vs. Placebo group will show 1) greater reductions in neuroinflammation, 2) greater improvements in brain function and 3) less craving for MA.

SA 3: Test whether reductions in neuroinflammation improve deficits in mesocorticolimbic RSFC, ventral striatal responses to reward, and cognitive control performance and reduces craving for MA.

Hypothesis 3. A reduction in neuroinflammation will result in cognitive control improvements and less ventral striatal reactivity to reward and mesocorticolimbic RSFC, with greatest effects in the Ibudilast Group.

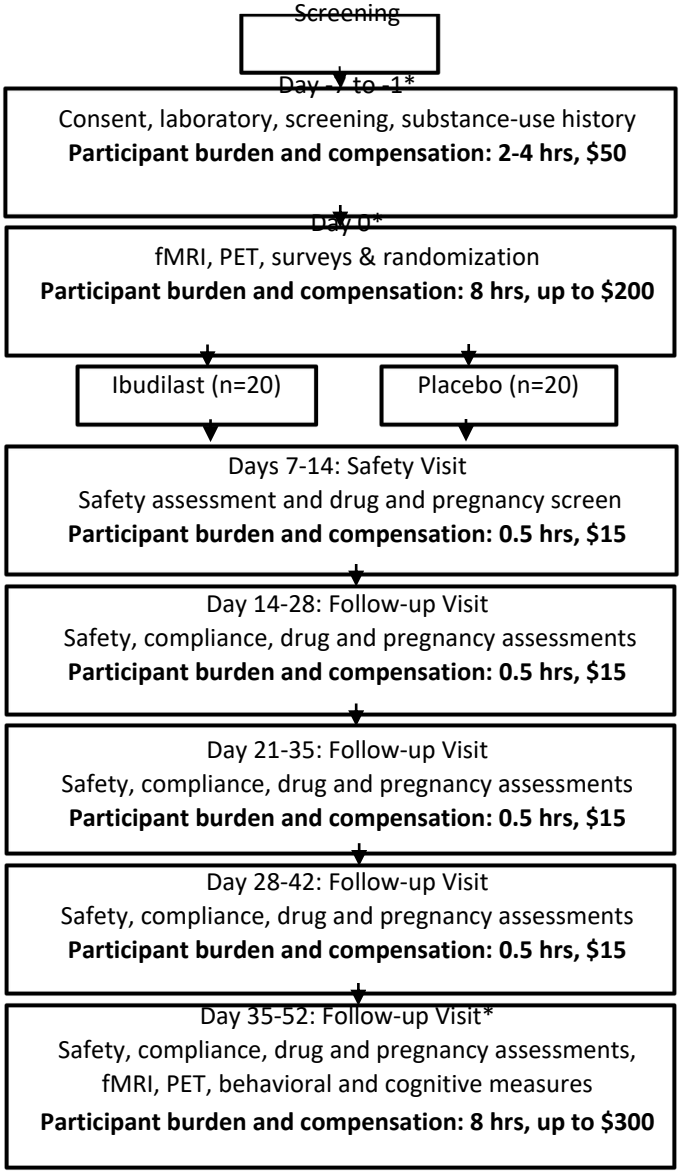
Hypothesis 4. Improvements in imaging measures will reflect the reductions in craving.

General Study Design

Overview of Study Design.

The first aim of this project proposes to examine neuroinflammation and its relationship tomesocorticolimbic RSFC and ventral striatal reactivity to reward. Participants (up to 70 MA users and 50 controls) will complete brain scans, a neurocognitive battery and questionnaires related to drug use, craving and withdrawal. Neuroinflammation will be indexed by translocator protein (TSPO) binding with [^{11}C]-N-(2-methoxybenzyl)-N-(4-phenoxypyridin-5-yl) acetamide ([^{11}C] PBR28-PET). Mesocorticolimbic RSFC and ventral striatal reactivity to reward will be assessed with functional magnetic resonance imaging (fMRI) at rest and during the MID, respectively. MA users will then participate in a randomized placebo-controlled study of ibudilast on the same imaging and behavioral measures. We will examine whether a 6-week treatment of ibudilast (20 mg of ibudilast twice daily for two weeks followed by twice daily doses of 50 mg for four weeks) reduces neuroinflammation and whether those reductions track with changes in brain function, connectivity, craving and cognitive measures. All assessments will be acquired at baseline and 6-weeks following randomization of ibudilast or placebo in MA users (20/group).

Table 1. Ibudilast Study Flow



*Study activitiesforthesevisits may takeplaceover multiplevisits

Participant Recruitment. We plan to recruit up to 200 subjects to account for attrition and have complete data for 18 healthy controls and 40 MA users. Data may be pulled from the MARC Biorepository (mIRB 3164) to supplement subjects enrolled into this study for data analysis. The data used will be similar to this protocol and will include subject demographics, drug/alcohol use history, survey data, computer task data, PET scans, MRI scans, saliva or meth metabolite data, and genetic information. Data will be coded and will not include any identifiers. A Waiver of Consent Documentation will be obtained for analysis of this data.

Subjects will be recruited through the Veterans Affairs Portland Health Care System (VAPORHCS) using posters, flyers and advertisements. In addition, the Translational Service Core (TSC) of the Methamphetamine Abuse Research Center (MARC) will assist in recruiting veteran MA users from substance abuse treatment centers, community-based 12 step programs and support groups. In addition, subjects may be recruited from the previous MARC project (mIRB 8702) where an IRB-approved member of that study will contact individuals who consented to be contacted for future studies by phone. The study team member will give a short overview of the study and give the potential participant contact information for our lab to call if they are interested.

Recruitment flyers will also be posted in addiction treatment centers and distributed to local addiction counselors. Approved flyers will be distributed throughout the community (retail locations, community assistance programs, primary care clinics, emergency departments, dental clinics, community outreach programs). Advertisements will be online using sources such as: Craigslist, the VA Facebook page, the VAPORHCS web page, and the quarterly newsletter (Veteran Connection), OHSU's Research Opportunities Page, the OHSU website (MARC page or dedicated lab page), job boards such as Indeed.com, Ziprecruiter, Google Jobs, Localwise.com, Skoll.org, and community news publications (Street Roots, Portland Mercury, Willamette Weekly and possibly others). When necessary (i.e., Craigslist listings, other job boards and social media), the email reply option will be turned off so subjects will have to contact the study team by phone. Internal

promotion will be utilized via the weekly E-news employee newsletter, and an all staff E-mail. Members of the research staff will meet regularly with clinicians at Portland-area recruitment sites, while clinical staff will identify patients who are interested in participation and give the potential subjects information about how to contact research staff. Research staff may also conduct recruitment activities on-site at the locations listed above, with appropriate site approval, including conducting IRB-approved eligibility screenings and scheduling initial appointments. Potential subjects may also be recruited from the Hoffman Lab Contact Information Data Repository (mIRB 4512).

Subjects who call in response to advertisements will be given a brief screening interview to verify age, gender, treatment status, time abstinent, current medications, concurrent medical illnesses and contraindications to MRI, PET scan and/or study drug. Contact information will be collected on the screening form for scheduling purposes. If the potential subject agrees, email will be collected to send an appointment confirmation email (Azure).

Research Match and OHSU OCTRI Research Volunteer Registry will be used to recruit potential subjects. An IRB approved description of the study will be sent to individuals by Research Match who meet criteria for the study. If the individual indicates they are interested, their contact information (i.e. name, mailing address, email, phone number) will be sent through Research Match to the Hoffman Lab Research Match account. This information will remain in the ResearchMatch system and/or be exported to the OHSU RDS server. OHSU OCTRI Research Volunteer Registry will provide contact information of individuals who meet study criteria and this information will be stored on the OHSU RDS server. Research assistants will contact these individuals using phone or email to set-up a phone screen. Subjects will be emailed via hoffmanlab@ohsu.edu to set up a phone screen.

If participants need transportation, Circulation, a fully HIPPA compliant platform, may be utilized, up to $100/visit. If necessary, a profile for the participant will be generated on Circulation's website that includes their name, phone number, and preferred address for pickup. Rider profiles are stored within Circulation until participation in the study is complete; afterwards, the participant's information is erased from their web application. This service will primarily be utilized for imaging visits and visits where the study drug is administered. All other transportation decisions will be made at the Principal Investigators discretion.

Inclusion/Exclusion Criteria. Entry criteria will be assessed on the basis of self-report questionnaires, m~~edical history, psychiatric~~ diagnoses (SCID-V) and urine toxicology tests. All subjects will be asked to abstain from all drugs and have negative urine drug screens (UDS), except for nicotine, marijuana and methamphetamine, on test days to participate. Saliva samples may be collected on test days to quantify MA levels. MA users must meet diagnosis for recent MA-use disorder (DSM-V). A diagnosis of marijuana-use disorder is permitted for all subjects. Exclusion criteria includes: 1) age < 18 years or ≥ 55 years, 2) Known sensitivity to ibudilast, 3) MRI contraindications (e.g. metal in body), 4) Clinically significant neurological, endocrine, renal, hepatic, or systemic disease (including HIV or Hepatitis C) that would compromise safe participation or confound outcomes, 5) Left-handedness, 6) any psychiatric diagnoses of primary psychotic or mood disorders (past diagnoses of depression are allowed), 7) a diagnosis of any drug use disorder other than tobacco, MA use disorder, or marijuana use disorder 8) any recreational or prescriptive use of psychotropic medications, 9) self-reported claustrophobia, 10) women who are pregnant or breastfeeding, 11) neurodegenerative diseases that present with neuroinflammation (multiple sclerosis, amyotrophic lateral sclerosis, alzheimers, parkinsons), 12) > 4 weeks of abstinence from MA 13) rs6971 genotype that confers low TSPO binding affinity to prevent unnecessary radiation exposure, 14) blood donation within eight weeks of the start of the study, 15) history of a bleeding disorder, and 16) currently taking anticoagulants (such as Coumadin, Heparin, Pradaxa, Xarelto). Due to the rare reporting (< 1%) of adverse effects of ibudilast, we will exclude participants with liver disease requiring medication or medical treatment, and/or aspartate or alanine aminotransferase levels greater than 3 times the upper limit of normal, gastrointestinal or renal disease that would significantly impair absorption, metabolism or excretion of study drug, or require medication or medical treatment and participation in an investigational drug study within the past 3 months. Because it is unknown how ibudilast could affect a fetus, sexually active men and women must maintain at least one form of contraception for the duration of the study and for 30 days after the study. This can include birth control pills, a patch, long-acting progestins, an IUD, a diaphragm, a condom with spermicide, abstinence, or sterilization.

Data pulled from the MARC biorepository will have come from subjects with similar and most likely identical relevant inclusion/exclusion criteria which will be evaluated before inclusion. Additional questions will be asked to screen for recent exposure and symptoms of COVID-19. If recent exposure or symptoms are disclosed, and the subject otherwise qualifies, we will wait to schedule them until they have self-isolated for two weeks from time of exposure and/or when their symptoms have completely resolved. Our screening criteria already excludes those who are at increased risk of severe illness from COVID-19; however some subjects who might be at an increased risk may still qualify to participate during modified operations including smokers and those with well-controlled moderate-to-severe asthma, hypertension, and/or high blood pressure. These subjects will be informed that they may be at higher risk for COVID-19 infection and they may not want to put themselves at risk by participating in research.

Study Design. Once subjects meet entry criteria and have given informed consent, they will undergo health and physical examinations and perform baseline assessments prior to receiving ibudilast. Study procedures are listed in Table 1. MA users will complete all study procedures in Table 1 and control subjects' participation will terminate after completing the imaging portion of visit 2. Control subjects will not be randomized and will not receive study drug. Subjects may undergo all neuroimaging measures or a subset. Up to and including the first 4 subjects in both groups may not undergo neuroimaging measures as we assess safety and feasibility of ibudilast administration and neuropsych evaluations. Visits 2 and 7 may be broken into multiple appointments, depending on scheduling. As rates of subject no-shows are high for this study population (active drug users), Visits 2 and 7 may also be double-booked to minimize high PET/MRI cancellation fees. Furthermore, as PET imaging requires advanced coordinated scheduling between multiple departments at OHSU and the VA and the use of VA patient clinical resources (i.e., PET suite and Nuclear Medicine staff), double-booking will help ensure the best use of time and resources. Due to limited PET availability, MA subjects may be asked to complete the study without undergoing PET imaging. Subjects must come to the Portland VA for all imaging procedures; however, if the subject is in treatment, all other procedures may be completed at the subject's treatment facility. Subjects will be screened for COVID-19 symptoms during confirmation calls prior to study visits and again at the beginning of each in-person visit along with a temperature check. If any symptoms are revealed, the visit will be rescheduled for at least two weeks or until symptoms are resolved.

Visit 1: Consent/Screening. Consenting individuals will undergo laboratory tests, including blood chemistry panel (including liver profile), CBC, HIV and hepatitis C to determine eligibility. Laboratory tests will include a blood draw or cheek swab for genotyping performed with quantitative polymerase chain reaction (qPCR) using Thermo Fisher Scientific's QuantStudio 3 with a TaqMan SNP assay for rs6971. Participants with genotypes that encode low binding affinities for [^{11}C]PBR28 PET (rs6971 SNP A:G) will be excluded from the PET imaging portion of the study. Subjects will also provide urine samples for a UDS, cotinine (to test nicotine levels in smokers), and urine pregnancy test (for women) HCG. UDS and cotinine tests will be conducted at all visits to test for nicotine, benzodiazepine, cocaine, MDMA, MA, marijuana (THC), oxycodone, and opiate use along with pregnancy tests (for women). Subjects will complete a retention/locator form and the VA Request For and Authorization to Release Medical Records or Health Information form. The retention form will include home address, mobile and home telephone numbers, email address, physician or treatment counselors' phone number and names of ≥ 2 people to contact if there are problems reaching the participant. Participants will also complete an MRI safety screening form to identify MRI contraindications. Additionally, information about past drug and alcohol use, psychiatric and medical history, and general demographics will be collected. A journal will be given to participants to track methamphetamine craving and daily alcohol, drug, and nicotine use. Journal information will be collected at each in-person visit. Blood and/or saliva tests may be collected for female subjects to test hormone levels.

Visit 2: Baseline assessment, neuroimaging, and dispensation of ibudilast. Subjects will complete neurocognitive assessments and neuroimaging measures or a subset of these measures. For MA users, following baseline assessments and prior to receiving medication, a medical evaluation by the study physician will occur prior to drug randomization to assure subjects have no medical conditions that might preclude study participation. UDS, cotinine, and pregnancy tests will be re-administered prior to being given the study medication. A blood sample and cheek swab will be collected and stored in the biorepository. For MA users, a saliva sample may be collected and stored in the biorepository for testing MA metabolite levels. For females, an additional blood and/or saliva sample may be collected to test hormone levels. If this visit is broken into multiple visits, saliva and blood samples may be collected on each day associated with this visit. Only IRB-

approved physicians can order study medications for this study. Subjects may be provided a meal from Oregon Clinical and Translational Research Institute during this visit. Subjects will be given two weeks (14 days) worth of study drug and will be instructed to take it twice daily.

Visit 3-6: Safety assessment and compliance. One week after medication initiation, or as schedule permits, up to 14 days after initiation, an in person follow-up safety exam will be scheduled, which will take place at the VA or at the treatment center and will include an evaluation of any side effects from the study medication, a query of any new medications since last visit, a UDS, pregnancy test (for women) and cotinine test, and the remainder of the medication will be dispensed. The remaining three follow-up exams will be scheduled every 7-10 days to assess safety, adverse events and compliance. These visits may be done in-person, either at the VA or at the subject's treatment center, or over the phone. Safety assessments will also include tests for depressive symptoms, suicidality, and other adverse events. Other assessments performed will include drug craving, drug use patterns since last visit, and cigarette use. Medication compliance will be estimated using MEMS, pill count method and self-reports. The MEMS has shown to improve medication compliance in drug abuse pharmacotherapy trials (94, 95) and will be used to estimate compliance by calculating the number of days on which the MEMS cap reported the bottle was opened divided by the number of doses prescribed, expressed as a percentage (94, 95). The unused capsules in the bottle will also be counted (pill count) and reconciled with self-reports.

Visit 7: Follow Up: Surveys, biorepository blood draw and saliva samples, and brain imaging will be repeated at the 6-week follow-up visit. For females, an additional blood and/or saliva sample may be collected to test hormone levels. If this visit is broken into multiple visits, saliva and blood samples may be collected on each day associated with this visit Blood will also be drawn to repeat the CBC, chem7 and liver panel as an additional safety check on the effects of the study drug. Subjects may be provided a meal from Oregon Clinical and Translational Research Institute during this visit.

Telephone Check-ins: MA subjects may be contacted by phone on a weekly basis between Visit 2 and 7 and once more approximately one to three weeks after the final visit to assess any adverse events and/or medication compliance.

Appointment confirmations: Phone calls or emails may be sent via Azure to subjects to confirm appointments. Brief scheduling correspondence may also happen through Azure if the study team is not able to connect with the subject via phone.

Biorepository Data Use: A separate cohort of data will be pulled from the MARC biorepository and included in the analysis for this project. Data pulled will be nearly identical to the data used in this project and will include demographics (no identifiers), questionnaire data, information on drug/alcohol abuse, mental health diagnoses, computer task data, imaging data (MRI and PET), coded lab results, and data generated from specimen analyses from other protocols (which will be contributed to the MARC biorepository after analysis) including meth/meth metabolite analyses from saliva samples.

Participant reimbursement: Subjects will be paid at the end of each visit in the form of prepaid debit cards, gift cards, or other VA approved methods such as Electronic Funds Transfer (EFT), or vouchers for completing all or partial study procedures. Subject's name, address, SSN, DOB, and/or bank account information including account and routing numbers are used for EFT and Clincard payments. Clincard is a secure website that is approved by OHSU/VA as a form of payment and identifying information is input directly into the website. For Clincard payments, subjects will be registered by name in the Clincard online system and issued a loaded debit card.

Total possible payment is $250 for healthy controls and up to $610 for MA users completing the randomized placebo-controlled study of ibudilast. Subjects must complete visit 2 to be eligible for study visits 3-7. Subjects will be paid as follows for completing study visits:
Visit 1: $50
Visit 2: $140 plus an additional $60 for PET to total $200
Visit 3-6: $15/visit
Visit 7: $240 plus an additional $60 for PET to total $300

Payments are in line with similar studies at OHSU that involve surveys, lab draws, study drugs, and imaging sessions. Subjects will also receive Trimet day use tickets if they are using public transportation. If both subjects show-up to a double-booked appointment, the subject not undergoing imaging may complete other study procedures for that visit and be compensated accordingly or they will receive $50 for the inconvenience and be rescheduled. Subjects may be asked to repeat procedures if data isn't usable or if we are not able to perform the MRI and PET in close proximity to each other. If an entire visit is repeated, then they'll be paid for the whole visit at the regular amount again. They will be paid at a pro-rate of $20/hr for any other repeated procedures or partial visits.

At visit 1 subjects will complete a questionnaire about hardships. The study team will determine qualifying expenses and will provide/offer reimbursement of $15/hr for caregiving, $15 for travel/transportation if using public transportation or ride share, and/or $50 if experiencing homelessness (up to $100 per visit). Participants will be required to sign the documentation of qualifying reimbursements, which also stipulates that the amount of reimbursement is fixed throughout the course of the study unless there is a significant change in hardship later in the study. Other hardships will be considered and the PI will determine the amount of reimbursement. ***Drug packaging/handling/storage/accountability:*** The VAPORHCS research pharmacy will maintain control and accountability of the study drug (ibudilast) and placebo. The pharmacy will keep records of all activities associated with the study compound, including drug dispensed and drug returned. The research pharmacy will be notified when a subject is scheduled for study drug administration and dispense study drug to a research assistant, for delivery to study physician. Subjects will not have access to ibudilast upon study conclusion but will continue their current substance abuse treatment.

Randomization: This study will be a double-blind placebo controlled design. Randomization will be conducted and maintained by the VAPORHCS research pharmacy. Study team members involved with study procedures and analysis will remain blinded throughout the study until data lock (following completion of the last follow-up visit of the last subject enrolled). On a quarterly basis, the VAPORHCS Research Pharmacy will provide Dr. Hoffman (study physician and mentor) with a list of enrolled participants, and a research assistant will provide Dr. Hoffman with a list of adverse events, which will be used to assess differences in adverse events by study condition for reporting to the Institutional Review Board (IRB).

Assessment Instruments: Subjects will be interviewed by an experienced research assistant and asked to complete the following measures.

Demographic and drug-use history questionnaires. These questionnaires will provide information on general demographics, health and mental health history, and detailed information about substance use patterns.
Structured Clinical Interview (SCID). This structured diagnostic interview for DSM-V and ICD-10 psychiatric disorders, will be administered at intake.
Substance-use History will inquire about substance use patterns (current, lifetime, quantity of use) of stimulants, alcohol, tobacco, marijuana, sedatives, opioids, hallucinogens etc. and a 30-day track back to accurately quantify recent drug use.
Addiction Severity Index (ASI)-lite (76, 77) The scale generates severity scores for alcohol and drug dependence which exhibit high reliability and validity in diverse population (78-81).
Brief MA Craving Scale (adapted from Brief Cocaine Craving Scale). This 10-item self-report assessment of MA craving will be administered at all study visits.
Adverse Events and Concomitant Medications. Adverse events will be recorded and the study physician will determine relatedness to study interventions. Concomitant medications will be recorded at baseline to determine eligibility.
Fagerstrőm Test for Nicotine Dependence (smokers only) (82). This questionnaire evaluates severity of nicotine dependence, a potential covariate to control for.
Shiffman-Jarvik Withdrawal Scale (83). This 25-item questionnaire will be used to assess nicotine withdrawal during cognitive and brain imaging sessions. Given immediately before scans.
Beck Depression Inventory (BDI-II) (84). This self-report questionnaire, administered at baseline and at each visit, will be used for data collection and to monitor onset of clinical depression, thereby contributing to participant safety.
Menstrual Cycle Questionnaire will be used to control for potential cycle effects on the neuroimaging data.

Childhood Trauma Questionnaire will be used to determine a history of maltreatment.
Brief Trauma Questionnaire, a 10-item self-report will be used to assess traumatic exposure. With the potential of past trauma affecting neuroinflammation, these data will be used as covariates as necessary.

Neurocognitive assessments

- *National Institute of Health (NIH) Phenix Toolbox Cognitive Battery and Standard Neuropsychological Battery* Assessment of attention, memory, language, visualspatial and executive function.
- Measuresof impulsivity, mood, pain, sleep, trauma and executive functioning will be collectedusing the Barratt impulsiveness Scale, PROMIS, GAD-7, Fatigue Severity Scale, Brief Pain Index, Pittsburgh Sleep Quality Index, Multi-Attribute System for Methamphetamine Use, and the cognitive battery including a subset of the NAB, D-KEFS, and WRAT4.
- Computer tasks: Delay discounting will be administered to test for impulsivity, the Stroop Color and Word Test to determine cognitive flexibility, creativity, and reaction to cognitive pressures, and the Iowa Gambling Task (IGT) to investigate decision-making behavior.

Neuroimaging procedures

Brainimaging procedures: Healthy control participants will undergo one PET and one MRI session at baseline. MA users will each undergo two PET and MRI sessions: one before randomization and one 6-weeks post ibudilast/placebo induction. Participants will be escorted to the Oregon Health and Science University (OHSU) Advanced Imaging Research Center (AIRC) by study staff to undergo an MRI session. Scans may include all or a subset of the following: an fMRI scan, where participants will perform the MID (10-min), a resting-state fMRI scan (10-min) and two structural scans (3-min) (Table 2). Participants will also undergo a PET scan (90-min) (Table 2) at the VA Imaging Services. Participants will be required to refrain from smoking 1 hour before each scan to minimize and balance the effects of recent smoking on neurotransmitter release, acute inflammatory responses and brain function against the effects of nicotine withdrawal and craving. Nicotine withdrawal will be measured by the Shiffman-Jarvik Withdrawal Scale immediately before scanning and will be used as a covariate in analyses as needed.

Table2.Imaging Times andParameters

Modality	Key Imaging Parameters
Localizer	30 seconds
Matched Bandwidth	2.5 minutes
Structural MRI	0.8 mm^3 isotropic, 2 x 6 minutes (T1 and T2)
Resting state fMRI	1.6 mm^3 isotopic, MB8; TR = 720 ms, 10 minutes
Task-based fMRI	1.6 mm^3 isotopic, MB8; TR = 720 ms.10 minutes
PET	3D mode on a Philips Vereos digital system PET-CT after administration of 555-740 MBq [^{11}C]PBR28

MRI Acquisition: A 3-tesla Siemens Magnetom Prisma scanner with a 32-channel head coil will be used. Structural data will be acquired using a high-resolution magnetization-prepared rapid acquisition gradient echo (MPRAGE) scan for registration. Blood oxygen-level dependent (BOLD) fMRI data will be collected with echo planar imaging (EPI) pulse sequence while subjects are at rest or performing the MID.

Monetary incentive delay task (MID): The MID is a validated task to examine incentive-reward reactivity of the ventral striatum (85). The task requires little cognitive demand and participants simply respond to a brief presentation of a visual target to win or lose money. The MID task session consists of 180 6-second trials. During each trial, participants are shown a cue (cue phase, 1 second), indicating that they have the potential to win or lose money ($0.20, $1 or $5) or that no money is at stake for that trial (Control). A fixation cross is present during a variable interval (anticipatory delay phase, 2000–2500 ms), and when a target appears, participants press a button (target phase, 160–260 ms). For WIN trials, participants will be told that if they successfully press the button while the target is onscreen (a “hit”) they will win the amount of money indicated by the cue. For LOSE trials, participants will be told that no money will be won or lost for hits, but misses will lead to a loss of the amount indicated by the cue for that trial. By contrasting brain activations in the anticipatory delay phase in WIN trials vs. Control trials it is possible to examine the extent to which the anticipation of potential reward modulates ventral striatal activity during this preparatory period. A Feedback

phase (1650 ms) follows the target's disappearance. Feedback will display amount won or lost for each trial and total cumulative winnings.

Resting-state functional connectivity: Participants will undergo a 10-minute scan where they will be instructed to stare at a black screen with a white fixation cross on it. The examination of functional connectivity of neural circuits by measuring temporal correlations of activity in the absence of a task provides information of intrinsic activity in the absence of a cognitive task. It enables examination of basal levels of network activity and facilitates the examination of the relationship between network connectivity and inflammation.

Cytokine Analysis: Plasma samples (pulled from the MARC biorepository) from both pre- and post-Ibudilast treatment will be analyzed in duplicate using a customized, high-sensitivity multiplex polystyrene bead-based immunoassay. This will measure peripheral immune biomarkers associated with inflammation, specifically IL-6. Intra- and inter-assay coefficients of variations, as indices of within- and between-assay precision, respectively, will be calculated to examine the reliability of the cytokine measurements. A linear regression will be used to test for the main effects of group, age, and sex on differences in IL-6.

[11C]PBR28 PET acquisition, reconstruction and modeling: Microglia cells express TSPO, an intracellular protein formerly known as the peripheral benzodiazepine receptor (86). As a number of well-validated clinical studies show changes in TSPO binding with [^{11}C]PBR28 (90), this project will use [^{11}C]PBR28 to assess TSPO binding (IND 138123). PET scans will be acquired in 3D mode using a Philips Vereos digital PET-CT (Philips Medical System). Participants will be positioned on the scanner bed with the brain centered in the axial field of view, and will be free to open and shut their eyes. Each participant will be given [^{11}C]PBR28 at a dose of 555 to 740 MBq and less than 5 μg of PBR28 in <10 mL of injectate. Before the injection, a low-dose CT-transmission scan (0.43 mSv) will be performed to obtain data for attenuation correction. [^{11}C]PBR28 will be injected intravenously over < 20 seconds at the start of a 90-minute 3D list-mode PET acquisition and reconstructed (FBP or OSEM) with standard corrections for deadtime, attenuation, scatter and random coincidences and decay-corrected. Images will be binned into frames at 8x15 sec, 3x60 sec, 2x120 sec, 16x300 sec. PET data will be reconstructed to a 128x128 matrix with pixel sign of nominally 2x2x4 mm. To determine the arterial plasma input function, five 1 mL blood samples from the radial artery will be collected at 20 sec intervals initially and 3-4 mL samples at 3, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 70, 80, and 90 min to quantify the plasma time-activity function (AIF) corrected for the plasma fraction. About six of the blood samples will be analyzed by HPLC to measure the fraction of tracer that is free of metabolites (Owen 2014). As we gain experience with data analysis, we expect that we can reduce the number of blood samples that will be required and eventually not need to administer arterial lines on future participants. Initially, up to 10 controls will have arterial catheters placed while we build a kinetic model (MA users who are enrolled during this time will not have arterial catheters). If we are successful in building the model, no additional subjects will have the catheter. If we are not successful then we will place arterial catheters on all additional enrolled subjects, including MA users. We will continue to attempt building a reliable model throughout the study and we will stop using arterial catheters when and if we are successful. On subjects who do not have arterial catheters, we will draw blood from an IV line with the same frequency and amounts as for the arterial catheter. These blood draws will continue throughout the study, even if we have a successful kinetic model. The late PET images will be summed and co-registered with anatomic MRI and we will use FSL to outline whole brain and anatomically-defined subcortical regions of interest (ROIs), most importantly dorsal and ventral striatum. Data from bilateral regions will be the sum of right and left hemispheres.

Because we do not know what regions of the brain will experience changes in TSPO, we will initially use a conservative approach of full kinetic analysis that includes an irreversible component from the blood to vascular endothelium. See Figure 1 below. Inclusion of the vascular component has been shown to improve the fit for early times and substantially improved test-retest reliability of model parameters (Rizzo 2014; Veronese 2018). Model parameters for the time series of images from individual ROIs will be estimated using a weighted nonlinear least squares routine in PMOD v5. The relative weights for the individual data points will be defined as the inverse of the variance of the ROI measurement error, which we assume to be additive, uncorrelated and normally distributed with variance equal to the decay-corrected activity divided by the duration of the scan interval. The initial value for the vascular K_b will be set at the value for vascular trapping of an adenosine A2 receptor radioligand that has similar endothelial and tissue distribution.

This model analysis will result in several parameters: total distribution volume V_T (mL/cm^3) for each region, blood volume fraction Vb, and the microscopic K and k rate parameters. Analysis of our hypotheses will consider changes to V_T between baseline and treatment, changes in specific binding assessed as k3/k4 and non-displaceable binding assessed as K1/k2 and changes in vascular flow, K_b. Each of these will be measured for all of the ROIs and will be evaluated for affect of ibudilast.

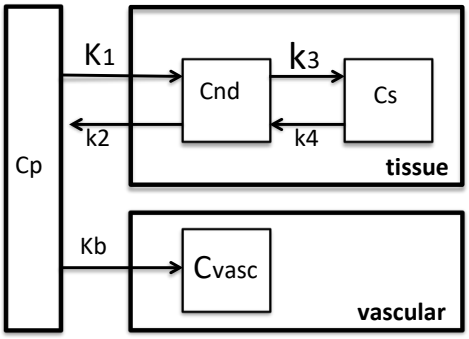


Figure 1. Compartmental model for analysis of [^{11}C]PBR28 kinetics, incorporating a vascular space to account of the endothelial component of TSPO binding. This approach reduces inter- and intra-subject variability but preserves sensitivity to changes in affinity and has been particularly useful in comparison studies before and after a drug intervention (Veronese 2018). TSPO-pos vessels account for ~30% of the vascular volume in cortical and white matter. This model accounts for non-displaceable (Cnd) and specific binding (Cs) in tissues and a rate Kb for vascular flow.

Study Drug: Ibudilast. Ibudilast (AV-411 or MN-166) is a non-specific phosphodiesterase inhibitor, which suppresses glial cell activation and has other anti-inflammatory properties. Ibudilast has been used clinically in Japan for two decades for asthma, pulmonary, and cardiovascular diseases. More recently, Ibudilast has been found to have anti-inflammatory properties in the central nervous system and has been investigated as a potential therapeutic agent for treatment of multiple sclerosis, neuropathic pain and opioid dependence. The effects of Ibudilast on MA administration and associated behavioral phenotypes and physiological responses is currently underway. Pre-clinical administration of Ibudilast following exposure to MA has been shown to reduce the acute, chronic, and sensitization effects of MA-induced locomotor activity, stress-induced MA relapse, and MA self-administration (71, 100, 101). A Phase I clinical trial to test the safety of the Ibudilast taken in combination with MA has been completed. Participants received Ibudilast (20 mg twice daily followed by 50 mg twice daily) or placebo, and then underwent intravenous MA challenges (15 and 30 mg). Results show that the rates of adverse events were similar between Ibudilast and placebo conditions, and there was no significant change in cardiovascular measures with MA. Pharmacokinetic analysis also showed no significant change in maximum concentration or half-life of MA with Ibudilast (96). Currently, the research is into its second phase, and data collection for the 12-week Phase II clinical trial was expected to be complete in June 2018 (ClinicalTrials.gov Identifier: NCT01860807).

Ibudilast pharmacokinetics and clinical safety: Ibudilast has been shown to be safe and well tolerated in the treatment of respiratory and cardiovascular diseases, neurological diseases and substance-use disorder (97). Ibudilast (AV-411 or MN-166) is a non-specific phosphodiesterase inhibitor, which suppresses glial cell activation and has other anti-inflammatory properties. Ibudilast pharmacokinetics have shown it to be dose-dependent in both single and repeat-dose trials. Although concentrations in multi-day, repeat-dosing regimens of ibudilast in rats show significant diminution within 1-2 weeks, steady-state drug levels remain stable in humans for at least 2 weeks of repeat dosing (98, 99). Plasma protein binding of ibudilast in most species is approximately 95%. Ibudilast is metabolized by numerous cytochrome P450 isozymes, with the primary metabolite being a 6,7-dihydrodiol metabolite. Because ibudilast is not predicted to be a clinically relevant inhibitor or inducer of CYP enzymes in vivo, drug–drug interactions are not anticipated (97).

The pharmacokinetics of ibudilast has been examined after single- and multiple-dose regimens of 30 mg for 14 days in healthy volunteers (99). Peak plasma concentrations after the single dose were 32 ng/ml reached at a median of 5 hours, with plasma pharmacokinetics reaching steady-state within two days. The elimination half-life was 19 hours and drug elimination in urine was negligible. Ibudilast is well tolerated at therapeutic doses with the primary adverse event being gastrointestinal upset. Other effects include dizziness, headache andelevated liver function. Tremor, insomnia, sleepiness, apathy, diarrhea, palpitation, hot flashes, bilirubin elevation and thrombocyte-paenia have also been reported but with less frequency (97).

Ibudilast dose justification: For the first 2 weeks, participants will receive 20 mg of ibudilast twice daily followed by twice daily doses of 50 mg for the remaining 4 weeks. Doses of 20-100 mg ibudilast have been used in safety clinical trials for MA dependence. Neither 20 mg nor 50 mg twice daily doses of ibudilast co- administered with IV MA (15 mg or 30 mg) produced any significant adverse effects (96). Positive effects of MA were reduced with both 40 mg and 100 mg doses of ibudilast, with the greatest effect at the 100 mg doses. Similar doses of Ibudilast have been tested to assess the safety and tolerability under conditions of morphine maintenance. Both 20 mg and 40 mg doses of Ibudilast were well tolerated and there were no significant differences between the groups in physiological outcomes, such as temperature, blood pressure and heart rate. Adverse events were mild and not significant with no differences between groups in the frequency or severity of negative side effects (Cooper 2017). The doses proposed for this study (50 mg ibudilast, after 20 mg titration) are in line with the safety and tolerability in previously published studies.

Subjects may be given additional drug if there are scheduling conflicts which prevent the final imaging visit (Visit 7) from taking place exactly 6 weeks after the subject begins drug. Subjects may remain on drug until we are able to complete all study procedures, which will happen as close to 6 weeks as possible.

Placebo formulation: Placebo will include 10 mg of mannitol as a placebo substitute for ibudilast. Light anhydrous silicic acid is used as a filler. Binding agents include, ethyl cellulose, hydroxypropyl methylcellulose phthalate and hydroxypropyl cellulose and coating agents used are ethyl cellulose and D-mannitol.

Double blind provisions

Maintenance of blinds: Study team members involved with study procedures and analysis will remain blinded throughoutthestudyuntildata lock (following completion of the last follow-up visit of the last subject enrolled).

Breaking the blind: Dr. Huffman will serve as Medical Monitor for this study. On a quarterly basis, theVAPORHCS Research Pharmacy (keeper of the study drug assignment code) will provide the Medical Monitor with a list of enrolled participants, which the Medical Monitor will use to assess differences in adverse events by study condition for reporting to the IRB and manufacturer. Team members involved in the analysis will be unblinded following data lock after all study subjects have completed all study procedures.

Subjects who develop acute medical conditions (e.g. urgent surgery, major trauma, etc.) will be unblinded as to treatment assignment and they and their treating provider will be informed of receipt of ibudilast vs. placebo. On a quarterly basis, a research assistant will provide a list of adverse events and results of laboratory tests by subject unique identifier to the Medical Monitor.

Interim analysis: An independent data monitoring committee will be assigned to conduct interim analyses to ensure that the preliminary results justify the continuation of the study. Interim analyses will be conducted with the completion of 10 subjects. The independent data monitoring committee will examine whether 1) the inclusion rate of patients is acceptable and as expected 2) adverse events are unexpectedly high 3) levels of craving for MA is comparable to previous studies and 4) a trend for significant differences between groups exist in outcome measures.

The study will be stopped if the independent data monitoring committee determines that the trial has an unexpectedly high rate of adverse events that warrants closure of the study.

Potential Risks to Subjects.

Ibudilast risk. Overall Ibudilast is well tolerated at therapeutic doses with the primary adverse event being gastrointestinal upset. Less than 5% of patients experience rash, dizziness, headache, anorexia, nausea, vomiting, abdominal pain, dyspepsia and elevated liver enzyme levels. Tremor, insomnia, sleepiness, apathy, diarrhea, palpitation, hot flashes, bilirubin elevation, gastric ulcer, anemia, leukopenia, malaise, tinnitus and thrombocytopenia have also been reported (less than 0.1% of patients).

As a medication trial, this study has a risk classification of “Significant” due to the trial use of a medication that is not approved to treat MA dependence. This study poses greater-than-minimal risks, which will be described fully in the study consent form.

Psychological/Social Risks. There are few anticipated psychological, sociological, economic, or legal risks to participation in this study. Some possible risks to participation include: unauthorized disclosure of confidential information; discomfort or embarrassment related to urine collection or questionnaires dealing with personal habits, lifestyle, drug or alcohol use; and possible unwanted encounters with friends or associates at the research site.

MR-related risks. There are no known risks associated with the MR scanning procedures; however, the magnetism of the machine does attract certain metals. Therefore, if a subject would fail to make the investigator aware of the presence of metal fragments or metal devices (e.g. cardiac pacemaker, artificial heart valve, implanted infusion pump, cochlear implant, or spinal cord stimulator) in his or her body and were to be scanned, the magnetism could affect or stop the device from working properly. Subjects will be queried as to whether they have any ferromagnetic metallic foreign bodies in their bodies, and if so, will be excluded from participation. Safety screening forms based on guidelines distributed by the International Society for Magnetic Resonance in Medicine will be used. Subjects will also be informed of the consequences that could result in undergoing an MRI scan with such an implanted device present. To minimize any potential for exposure of a fetus or child to even these low levels of magnetic activity, pregnant and lactating women will be excluded and fertile women will be required to use acceptable birth control methods as described above.

During the MR scans, subjects may experience some anxiety or claustrophobia associated with confinement in the scanner tube. The MR scanning procedures require that the subject be confined in a small, partially enclosed space. The sound of the MR scanner can be loud, but will be reduced by special earplugs. Rarely, subjects experience vertigo, a metallic taste or muscle stimulation. All efforts will be made to assist the subject in remaining calm and as comfortable as possible.

Risks of PET-CT: The PET-CT scan(s) involve lying on a table for about 1.5 hours, which may be uncomfortable. A CT scan (computed tomography scan) is a scan that uses X-rays to image the anatomy of the brain. The PET scan uses a radioactive tracer to image the sites where the tracer binds in the brain. The radiation doses received in this research study include radiation from PET and CT scans. The maximum radiation exposure that subjects may receive in this study is 4.9 ± 1.7 mSv/administration for each [^{11}C]PBR28 PET scan and 0.43 mSv from each CT scan. This amount is a little over 10% of the allowed whole body exposure to research subjects. Background radiation to the public is ~1 mSv per year. This value is well below the levels that have been demonstrated to result in a risk of harmful effects. The radioactivity disappears very quickly; [^{11}C]PBR28 will totally disappear within 24 hours. The radiation exposure from these scanning procedures will not exceed the levels that are considered safe for research participants. Participants are requested to inform the study investigators if he/she received X-rays or any radiological procedure such as CAT or CT scans, fluoroscopy, cardiac catheterization or radioactive materials as part of their medical care, or for research purposes, in the past year. If he/she participates in a research study involving radiation at the VA in the next 12 months, the investigator and/or associates will ensure, through accurate record keeping, that the total amount of radioactivity administered for research purposes will remain small and not exceed an amount that could cause any adverse effects. If he/she participates within a year in a research project outside the VA/OHSU, they should tell the researchers about this research study. If he/she has participated in research in a different institution over the past year, the researchers of this study will check on their previous absorbed dose of radiation. Study staff will also ask about any radiation exposure for diagnostic or treatment purposes over the past year. Several blood draws will occur during the PET scan and those risks are described in the section below.

Arterial Catheter Risk: On the PET scan day, a radial arterial catheter may be inserted. Certain individuals may feel light-headed during arterial catheter placement. Arterial catheter placement may be associated with mild-to-moderate pain, hematoma, inflammation, bleeding, or bruising at the puncture site. If any of these, or other, symptoms occur and do not diminish within 24 to 72 hours after the arterial line removal, or in the event that they worsen, subjects will be advised to call the study physician. In very rare instances (< 1/1000) blocking of the artery, tearing of the artery, arterial leakage, poor healing, or infection at the catheter insertion site may occur.

Blood Draw Risk. Subjects may experience discomfort or pain associated with blood draw procedures and there is a small chance that bleeding, infection or fainting may occur.

Confidentiality/Health Information Risk. There is a risk that others may see a participant's Protected Health Information (PHI). PHI is considered individually identifiable health information transmitted or maintained in any form(electronic means, on paper, online or through oral communication) that relates to the past, present, or futurephysical or mental health conditions of an individual that may be used or disclosed.

Protection Against Risk.

If subjects were to find any aspects of their involvement in the study psychologically or otherwise uncomfortable, they may ask to speak with the study physician, or principal investigator. If unable to resolve

their discomfort, subjects may discontinue from the study at any time without penalty. In the event that a subject experiences any symptom that unduly threatens his/her physical or psychological health, the subject will be removed from the study and will receive medical and psychiatric intervention as necessary.

Safety Procedure Overview: Participants will be fully informed of potential risks (described above), which we have considered and addressed in extensive previous human research. Attempts will be made to make the participant as comfortable as possible during the study.

Safety Procedures for All Participants: All studies conducted by our group use a set of procedures to ensure safe participation during the research study. The study physician assumes primary medical responsibility for each participant and personally evaluates any significant complaints and clinical adverse events. The status of each participant will be reviewed to assess medical and psychiatric safety and data completion. All staff members have received training and are MRI certified to conduct MRI scanning procedures and to follow the steps needed to respond appropriately to any complaints from a participant or adverse events.

Minimizing Psychological Risk: Careful clinical procedures will be followed to avoid any potential harm to participants from psychological/medical assessment that may induce embarrassment or other negative response. Participants will be told that questions of a sensitive nature may be addressed, but that they may choose not to answer any question that they feel uncomfortable addressing. We will ensure availability of counseling and support after study visits, if needed.

***Minimizing Risks Associated with Confidentiality*:** A potential risk in studies involving the collection of sensitive information (i.e., questions related to medical history and drug use) is breach of confidentiality. A federal Certificate of Confidentiality, which protects participants' records against subpoena, has been acquired for this study. Exceptions to confidentiality for subjects are those required by law and include suspicion of child abuse, elder abuse, and threat of imminent action on suicidal or homicidal ideation. Participants will be informed of these exceptions during the informed consent process. See below (Data and Safety Monitoring) for additional protection of confidentiality associated with study data.

***Minimizing Risks Associated with MRI*:** To minimize the potential risk of exposure to a magnetic field, participants will be queried as to whether they have any metals or devices implanted, and if so, will be excluded from all MRI procedures. Safety screening forms based on guidelines distributed by the International Society for Magnetic Resonance in Medicine will be used. Participants will also be informed of the consequences that could result in undergoing an MRI scan with such an implanted device present. To minimize any potential for exposure of a fetus to low levels of magnetic activity, pregnant women will be excluded and fertile women will be required to use acceptable birth control methods. The research team will obtain orbital x-rays if subjects have any history of welding, grinding or other exposure to metallic particles that could lodge in the eye.

***Minimizing Risks Associated with PET*:** The radiation exposure from these scanning procedures will not exceed the levels that are considered safe for research participants. Exposure to radiation will only be about 1/10th of the yearly limit allowed by law for people who work around radiation. To minimize discomfort from lying down and to minimize the risk of radiation exposure to the bladder, participants will be instructed to water load and void immediately following the scan.

Minimizing Risks Associated with Arterial Catheter: Risks of radial artery cannulation are minimized by having the procedure performed by an experienced physician. Infection is avoided by adequate cleansing of the skin prior to and after intravascular line insertion. After arterial catheter removal, bleeding is prevented by direct pressure applied to the site for a minimum of 15 minutes followed by a pressure dressing (coban) that should be kept clean and dry until evening. Subjects will have their hand and finger blood supply examined after arterial cannulation throughout the study, and again following catheter removal. Also, subjects will be asked to abstain from aspirin and other NSAIDs for 7-10 days prior to arterial line insertion and 7-10 days following arterial line removal. Subjects will be provided a 24 hour emergency physician contact number to call if they encounter pain, discoloration, numbness, tingling, coolness, hematoma, inflammation, or any other unusual symptoms in the wrist or hand, or fever, chills or drainage from the vascular puncture sites, following the procedure. In addition, if an emergency arises at the time of cannulation or scanning, 911 will be called, and the subject will be sent to the Emergency Department for evaluation and treatment. A nurse or qualified study team member will provide discharge instructions outlining general instructions in addition to post-arterial catheter precautions, problems to watch for, and procedures to follow should such problems occur.

Minimizing Risks of X-Ray: X-rays will expose the participant to a small amount of radiation. In order to prevent exposure to excessive amounts of radiation, we will ask the participant to report the amount of radiation exposure in the past year, either in other research studies, or during the course of work or clinical treatment. Although no harmful effects to a fetus from radiation levels below 10,000 mrem have been demonstrated, women must not be pregnant or nursing for the duration of the study. All women must agree to give urine samples for pregnancy testing before any scans.
Minimizing Risks of Ibudilast: Careful clinic study treatments/procedures are followed to avoid any potential harm to the participants from possible adverse medical reactions or from study conditions. We will use several measures to assess medication tolerability including: 1) medical history, 2) physical exam, 3) lab results, and 4) vital signs. At each visit, medical staff will also ask about the occurrence of adverse experiences and use of concomitant medications. Subjects will be closely monitored by clinical personnel (study physician) throughout the study, and will be withdrawn from study participation if signs of serious adverse reactions that pose a threat to physical or psychological health are observed.

All potential risks will be thoroughly discussed with each subject during the initial face-to-face interview, and will be evaluated by the study physician to screen for physical or psychiatric illnesses or medication interactions that would prevent safe study participation. All subjects will be provided with a 24-h phone number linked to an answering service operator through which the study physician or Principal Investigator may be contacted to answer questions or to provide direction in case of emergency. Our group has an aggressive set of safety procedures to ensure that subjects receive a high and consistent level of monitoring that also meets reporting requirements to the IRB. Safety issues will be reviewed weekly, during which staff discusses potential emergent issues for all subjects.
Minimizing Risks of Blood Draws: Blood draws will be performed by VA pathology lab phlebotomists, PET Center staff, or study staff who are highly qualified to perform the necessary lab draws for this study.

Deviation from the investigational plan
Theprincipal investigator will conduct this study in accordance with the 1572 and clinical protocol. The investigator shall notify the reviewing IRB in writing no later than 5 working days after any significant deviation fromthe investigational plan to protect the life or physical well-being of a subject in an emergency. Except in suchan emergency, prior approval by the sponsor is required for significant deviations from the investigational plan.Other minor deviations from the investigational plan will be reported to the sponsor on the case report formsfor that visit. If these changes or deviations affect the scientific integrity of the plan or the rights, safety, orwelfare of subject, then notification of the US Food and Drug Administration (FDA) and the reviewing IRB is alsorequired.

Subject non-compliance
Iftheparticipant has a positiveUDSonscandays for anything other than marijuana or methamphetamine (for MAusers), they will be withdrawnfromthestudy and asked to discontinue the use of the study drug. We will askparticipants to bring theirmedicationwiththem to study visits so that it may be collected from them if they arewithdrawn from the studyoriftheyhaveextra doses at the end of the study.
Individual adverse events*.*

Participantswillbequestionedateachclinicvisit about their general health. Any physical or clinical change or illness, whetherornotitisconsideredrelatedto the study drug, will be recorded on the medical record chart and an adverseeventscasereportform.Investigators will promptly report to the IRB all unanticipated problems involvingriskstohumansubjectsorothers, including adverse events that should be considered unanticipatedproblems(§§56.108(b)(1),312.53(c)(1)(vii), and 312.66). If adverse events are thought to reflect drug-relatedtoxicity,appropriateevaluationand management will occur. This may include un-blinding the individual'sstudycondition(terminatingtheirstudy participation) or early termination from the trial without un-blinding. Subjectswhoexperienceadverseevents will be monitored clinically until the adverse event is either satisfactorilyresolvedorstabilized,asdetermined by the study physician. Finally, a brief follow-up medical evaluation willbeperformedonallsubjectsafter study completion or termination. The study physician will report all adverseevents,whetherornotdeemed drug-related, within 24 hours and a written report, including a

full description of the event will follow as required. Subjects who report suicidal thoughts during any safety assessment will be discontinued from further study procedures and referred for appropriate mental health care.

Termination Protocol/Stopping rules. An investigator may terminate the study if deemed clinically appropriate for any reason, including: 1) significant side effects from the study medication; 2) serious or unexpected AEs; and/or 3) subjects showing significant inability to comply with the protocol. As stated above, subjects who develop acute medical conditions will be discontinued from the study and will be unblinded as to treatment assignment and they and their treating provider will be informed of receipt of ibudilast vs. placebo. All participants will be given a wallet card containing information and study personnel contact numbers to show to treating providers in the context of a medical emergency.

Statistical Plan. We will use graphical and numerical summaries to screen the data for outliers and violations of model assumptions and to assess the need for transformations or non-parametric methods. We will use covariates if groups differ in demographic or drug use variables and examine sex differences and interactive effects of sex. We will correlate inflammation markers and PBR binding with female hormone levels and use methamphetamine metabolite levels as covariates in the following analyses.

Craving and composite measure of cognitive control.

We will create a normed scale using independent MARC data of controls. Standard z-scores for each of the cognitive control measures will be calculated and summed to create a composite score, reflecting performance across the entire battery of cognitive control measures. Should there be significant group differences in the omnibus cognitive control score, exploratory analyses will be conducted to determine which tests are driving the overall relationship. Craving will be measured with scores on the brief MA craving scale.

Aim 1. Determine the relationship between neuroinflammation and cognitive control, mesocorticolimbic RSFC and ventralstriatal functionalactivationto rewardontheMID.

Our primary analytic tool will be the GLM. TSPO binding and MA-use status and their interaction will be used as primary predictors along with subject-level random effects for RSFC and task-based activation. The interaction between TSPO binding and group will be modeled to determine if the associations between fMRI measures vary by inflammation and MA status. Post hoc analyses will be used as necessary to estimate associations between brain imaging measures in each group separately.

Aim 2. Test whether ibudilast reduces neuroinflammation, improves brain function and attenuates MA craving.

Our primary analytic tool will be the generalized linear mixed model (GLMM). The GLMM properly accounts for correlations induced by repeated measurements within subjects and allows for both fixed and time-varying covariates. It allows use of the canonical link function if the normality assumption holds, or of other appropriate link functions (Poisson, negative binomial) if the outcome variable is non-normal. GLMMs automatically handle missing data, producing unbiased estimates if data is missing at random, which permits the use of all data and minimizes the effects of loss to follow-up and motion artifacts.

For Hypothesis 2, *MA users in the Ibudilast vs. Placebo group will show 1) greater reductions in neuroinflammation, 2) greater improvements brain function and 3) less craving for MA*, we will use the GLMM to test for a Group (Ibudilast, Placebo) by Time (baseline to completion) interaction on outcome measures of neuroinflammation and craving, separately. We expect significant decreases in TSPO binding and craving in the Ibudilast compared to Placebo group, with the greatest effect in the MA Ibudilast group. For fMRI outcome measures, we will use GLMMs to test for Group by Time interactions, here using ventral striatal activation and midbrain RSFC as the dependent variables. We expect significant interactions such that, relative to the Placebo Group, the Ibudilast Group will show greater decreases in ventral striatal reactivity to reward and mesocorticolimbic RSFC. The main effects of time and drug within and across each group will also be examined.

Aim 3. Test whether reductions in neuroinflammation improve deficits in mesocorticolimbic RSFC, ventral striatal responsesto reward, and cognitivecontrolperformance and reduces craving for MA.

For Hypothesis 3, *A reduction in neuroinflammation willresult in cognitivecontrolimprovementsandinless ventral striatalreactivitytoreward andmesocorticolimbicRSFC, with greatest effects in the IbudilastGroup,* we will add change in TSPO binding as a covariate to the GLMM models for fMRI measures. If change in microglia activation, indexed with TSPO binding is indeed the mechanism by which neuroinflammation alters

brain function, we expect Group by Time interaction parameters in these models to change significantly when TSPO binding is included. This test is an extension of the GLMM framework of the approach to mediation outlined for standard linear regression (102).

For Hypothesis 4, *Improvements in imaging measures will reflect the reductions in craving,* we will add fMRI measures as covariates to GLM models that assess the relationship between PET measures and craving. We will test the extent to which change in fMRI measures mediate the relationship between PET measures and craving (hypothesis 3), expecting regression parameters in the models would change significantly when change in fMRI measures are included.

DATA ANDSAFETY MONITORING PLAN (DSMP)

Oversight.

Dr. MilkyKohnowill be the IRB-approvedPrincipalInvestigator.ThePIisresponsibletoensureprotocoland regulatorycompliance through propermonitoringoftheinvestigation.Dr.Kohnowillberesponsiblefor conductingthestudy in accordancewiththe1572andinvestigationplan(protocol,IRBapplication, InvestigationalDrug Brochure), applicablelaws,FDAregulations,andanyconditionsofapprovalimposedby the reviewingIRB. Dr. Kohno willalsoacceptresponsibilityforallaspectsofthestudyincludingtheactionsof any co-investigators participatinginthestudyattheinvestigationalsite.

Data andSafetyMonitoring.

Study Data Monitors. Thisstudywillutilizeaninvestigatormonitor,Dr.BrandonCornejo,amedicaldoctor and part of the IRB-approvedstudystaff.Themonitorwillnotbedirectlyinvolvedintheanalysisofdata resulting from thisstudy.Thestudymonitorwillreviewstudydataandadverseeventsquarterlyandstudystaff will report any reportableeventstothemonitorwithin5businessdays.Additionalmonitorsmaybeappointed as necessary. Themonitorwillvisittheinvestigationalsiteregularlyduringthestudy.Duringtheperiodic monitoring visits,assessmentsoftheinvestigationalsitewillincludethefollowingitems:

- Completionofrequiredstudydocumentation
- Continuedacceptabilityofthefacilities
- Adherencetotheclinicalprotocol
- AdherencetotheapplicableFDAregulationsregardingtheobligationsoftheinvestigatorand maintenance of records

Medical Monitors.Dr.WilliamHoffmanwillserveasMedicalMonitorforthisstudy.Onaquarterlybasis,the VA ResearchPharmacywillprovidetheMedicalMonitorwithalistofenrolledparticipantsandaresearch assistant will provide a list of adverse events, which the Medical Monitor will use to assess differences in adverse events by study condition for reporting to the IRB and manufacturer.

Outside Oversight.Thisstudyisregisteredonclinicaltrials.gov.Dr.Kohnohasprimaryresponsibilityfor registering and updating this clinical trial. The joint VA/OHSU IRB3 will oversee this study. The VA also performs study audits every three years as an additional level of oversight and this study will also be subject to VA OfficeofResearchOversightComprehensiveProgramReviews.

Sponsor Reports.WewillstayinclosecommunicationwithMediciNovaInc.(drugmanufacturer)and according to the research agreement, MediciNova will keep us informed of any updates or relevant findings related to the study medication. These will be reported to the IRB within 5 business days as Relevant New Information. The sponsor-investigators (Dr. Hoffman, IND 133175 and Dr. Link, IND 138123) will report any adverse events to the FDA as required (expected AE's reported annually, serious/unexpected AE's within 15 calendar days and unexpected, related or possibly related deaths or potentially life-threatening events within 7 calendar days).

Collection, Management, and Protection of Data. Allsubjects'ProtectedHealthInformationwillbe confidential and will be protected according to the guidelinesestablishedbytheHealthInformationPortability and Accountability Act (HIPAA).

Collection of Data*:* The key personnel and research staff identifiedinthisapplicationwillcollectalldata.All members of the research staff have completed training on the ethicalconductofresearch,researchHIPAA and good clinical practice through the Collaborative Institutional TrainingInitiative(CITI)Program.Allstudy staff will be IRB-approved to complete study procedures and accessdata.

Management & Protection of Data: Risks to confidentiality from the proposed research will be minimized by using a coding system for all data collected, with identifying information kept in a secure locked area accessible only to the investigators and study staff. Protection procedures for specific data media are described below. Records of all data will be maintained according to the current VA research record control schedule.

Electronic Data*:* The online data collection system for this project will be a REDCap database with role based access limited to IRB-approved site staff. All personnel with access to the REDCap database will have documented Responsible Conduct of Research Training and HIPAA training at their respective institutions as well as training on the data security procedure for the Portland VA in general and specifically related to this study. All personnel will follow the Portland VA security policies including but not limited to: all computers will have automatic password protected screen savers. The REDCap database is stored on servers at the OHSU Advanced Computing Center (ACC). The ACC will provide hosting, storage and backup at their 200 square foot Data Center which provides redundant backup systems and multiple layers of security. Since REDCap is a web-based system the electronic research data is never stored on the computers at the study sites. In the unlikely event that a breach of confidentiality occurs it will be reported to the Portland VA IRB as a UP and other overseeing IRB(s) as appropriate. Only coded data will be stored in REDCap. Coded data will also be stored on OHSU's shared network drive, which will be accessible by the PI and approved research staff. No PHI will be entered into the REDCap system, or stored on the ACC or OHSU shared network drives at any time. PHI will be stored at the Portland VA in a password-protected spreadsheet behind a VA firewall.

Imaging Data: All imaging data will be identified only with the participant ID number. Our standard data interchange formats will contain no identifying information beyond the ID number and can be viewed using special-purpose computer hardware and software, limiting unauthorized viewing of the data. MRI scans are obtained at the Advanced Imaging Research Center (AIRC) at OHSU and stored on a disk partition specific to this study and only accessible by study staff. The AIRC will wipe the partition at the end of the study. The imaging data are downloaded to image analysis workstations located in the Hoffman Lab at the VA and raw images are archived on a dedicated server at the OHSU Advanced Computing Center (ACC). PET data will be stored on the VA Image Server and then transferred to the same OHSU ACC server (MARC server).

Hardcopy Data: Hardcopy data including consent forms and questionnaires will be stored at the Portland VA in a locked cabinet in a locked office and a copy of participant names will be stored at OHSU's Center for Radiochemistry Research in a locked filing cabinet. Only approved study staff will have access to the data. PHI will be stored separately from study data.

Banked Data: The banking component of the protocol involves the banking of blood samples, cheek swabs, saliva samples, coded imaging data, study data and coded demographic information. This component is required for participation in the study. The blood samples will be collected by the VA laboratory or a trained professional listed on this protocol and the cheek swab and saliva samples will be collected by the research team. The blood samples will either be stored as whole blood or spun in a centrifuge and stored as plasma by either the VA laboratory or by an approved research team member in Jennifer Loftis' lab in VA building 103, room E-141. The samples will be coded with a unique study identifier, IBU, plus a 3 digit random number (for example, PINM653) and stored in the MARC Translational Service Core Biorepository (IRB#03164) freezer located in VA building 103, room E-140 and E-141, which is accessible by prox card only. The MARC Biorepository Director is Dr. Jennifer Loftis.

Electronic Information pulled from the MARC Biorepository will be stored in excel spreadsheets and transferred from the VA research drive to the OHSU network drive (rdsmsb.ohsu.edu/MARC) by securex.ohsu secure data transfer. Imaging data will be transferred from one study folder to another on the OHSU network drive (both folders will be on rdsmsb.ohsu.edu/MARC).

Banked Contact Information*:* Subjects will be asked to have the following information collected for a contact repository: contact information, date of birth, gender, Veteran status, smoking status, substance use information, mental health status and previous participation in a Hoffman lab research study. This will be used to contact subjects who may be eligible for future studies with the Hoffman lab. Subjects will sign an addendum to the VA ICF if they consent to having their information stored for future contact, this is optional. Information collected for the contact repository will be stored on a password protected spreadsheet separate from the coded study data and will just have PHI and contact information.

Special Populations to Consider:

Women and Minorities: Thestudyteam will make every effort to include women and persons of minority race/ethnicity.

Children: No children under 18 will be included in this study.

Prisoners: We will include participants who are court-mandated to treatment, however, in previous IRB reviews it has been determined that these participants do not meet criteria to be considered prisoners. Some subjects may become incarcerated during study participation. Study participation will be suspended for periods of incarceration following study enrollment. If, however, participants are incarcerated and released within the window of study participation, we will attempt to complete remaining study procedures as long as inclusion criteria are still met. A federal Certificate of Confidentiality has also been obtained for this study.

VA Requirements:

Determining VA from Non-VA Research: This study will take place both at the Portland VA and at OHSU. VA Research will include consent, screening, questionnaires, Ibudilast dispensing from VA Research Pharmacy, blood draws, urinalyses, x-rays, physical exams, and PET scans. Non-VA Research will be only the MRI scans acquired at OHSU's AIRC.

Inclusion of Non-Veterans: We have decided to also include non-veterans in the proposed design after considering various limitations, which would prevent greater generalizability. For example, although methamphetamine is linked to neuroinflammatory markers, a growing literature suggests that traumatic events also present with neuroinflammation. The variation in exposure to trauma amongst veterans would limit statistical power and limit the ability to dissociate the effects of MA or trauma on our outcome variable. Limiting the enrollment criteria to veterans would also likely cause an imbalance in the study groups on various demographic variables such as sex

Study Completion: Control subjects' participation will end following imaging procedures on visit 2 and there will be no follow-up procedures. For MA subjects: as the package insert for ibudilast does not recommend tapering upon discontinuation, no taper-down will be performed towards study completion or early termination. Subjects will return for a final visit (approximately 6-weeks after starting study medications) to record any adverse events, complete final safety assessments and the neuroimaging and cognitive tests as well as complete a brief health check with the study physician. A telephone safety assessment will be scheduled at 30 days after their last study visit, and they will be offered referrals to community service providers. These post-medication evaluations are included solely to document safety, as required by the FDA, IND #133175. These evaluations are not intended to be intensive or to collect data for hypothesis testing. Subjects will be compensated $300 for completing the final follow-up visit. At the end of the study, the principle investigator will complete a study closure visit to collect any remaining information and review the regulatory requirements for record retention. Subjects must provide two reliable collateral informants, preferably family members with whom they have regular communication, who can be contacted to locate the subject in case they drop-out of treatment or cannot be reached using their current contact information. Subjects will complete a retention/locator form and the VA Request For and Authorization to Release Medical Records or Health Information form with the contact information for collaterals and clinicians (case managers, physicians) and collaterals will be contacted to obtain their consent to be an informant for the study and contacted if subjects drop out and are no longer reachable. Subjects will also be asked to give permission to contact clinicians (case managers, physicians) involved in their care to ensure treatment compliance.