Protocol Title: Dopaminergic Modulation of Brain Activation using Simultaneous PET/pharmacological MRI
Abbreviated Title: Simultaneous PET/phMRI
Protocol Number: 17-AA-0178
Date of This Submission/Version: 23FEB2022 / Version 10 – AM 12

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Total requested accrual: 40 Healthy controls, for an estimated completion of 25

Project Uses Ionizing Radiation: ☐No ☒Yes

IND/IDE ☐No ☒Yes
Drug/Device/# 54,135 ([^{11}C]raclopride)
Sponsor: NIH CC
Drug/Device/# 124,912 (IV Methylphenidate)
Sponsor: NIAAA

Durable Power of Attorney ☒No ☐Yes

Multi-institutional Project ☒No ☐Yes

Data and Safety Monitoring Board ☒No ☐Yes

Technology Transfer Agreement ☐No ☒Yes

Samples are being stored ☐No ☒Yes

Flesch-Kincaid reading level of consent form: Healthy Volunteers = 8.1

Précis:

- ***Objectives**:* The overarching goal of this study is to assess the dynamic association between dopamine (DA) D2 receptor (D2R) occupancy measured by positron emission tomography (PET) with [^{11}C]raclopride and brain activity inferred by pharmacological magnetic resonance imaging (phMRI) in the human brain, and to assess the relative sensitivity and specificity of the neurovascular coupling for slow (oral) versus rapid (intravenous, IV) stimulant methylphenidate (MP) delivery. Secondary objectives are to assess the associations between behavioral measures (heart and respiration rates and blood pressure, motor and sleep parameters, and neuropsychological testing variables), D2R occupancy and fMRI signals.
- ***Study population**:* 10 healthy males and 10 healthy females 18-55 years old will be included. Test-retest reproducibility studies will be carried out in 5 participants.
- ***Design**: Double-blind.* Participants will undergo simultaneous PET/phMRI, to evaluate dynamic changes in D2R occupancy by DA with [^{11}C]raclopride and in blood-oxygenation-level dependent (BOLD) signals, under MP or placebo (PL). The participants will be scanned on 3 different occasions: 1) oral-MP (60 mg) and iv PL (3 cc saline), 2) oral-PL and iv-MP (0.25 mg/kg in 3 cc sterile water) and 3) oral PL and iv PL, which will be carried in different study days with at least 48 hours between them and their order will be randomized across subjects. Participants and researchers will be blind to the nature of the stimulant drug (MP/PL).
- ***Outcome parameters:*** The scale factor between the distribution volume ratio (DVR) and the BOLD signal in the dorsal and ventral striatum for the slow and fast MP challenges.

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List of Abbreviations

ADHD	attention deficit hyperactivity disorder
BOLD	blood-oxygenation-level dependent
CANTAB	Cambridge Neuropsychological Test Automated Battery
CBF	cerebral blood flow
CBV	cerebral blood volume
D2R	dopamine receptor
DA	dopamine
DAT	dopamine transporter occupancy
DTI	diffusion tensor imaging
DVR	distribution volume ratio
fMRI	functional magnetic resonance imaging
iv	intravenous
MP	methylphenidate
MSN	medium spiny neurons
NAc	nucleus accumbens
PET	positron emission tomography
phMRI	pharmacological magnetic resonance imaging
PK	pharmacokinetics
PL	placebo
SN	substantia nigra
TAC	time activity curves

1. Introduction and Background

Over the last 2 decades, studies using positron emission tomography (PET) have demonstrated a relationship between methylphenidate (MP) pharmacokinetics (PK), dopamine transporter occupancy (DAT) and the behavioral effects of MP, as well as its effects at the synaptic level [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. MP is used in the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy and its uptake in the brain has similar regional distribution as that of cocaine [7]. Like cocaine, MP blocks the DAT, and increases synaptic DA in the striatum, including the nucleus accumbens [20]. PET studies in humans have demonstrated that cocaine's and MP's rapid uptake in the striatum parallels the euphoria [21, 22] whereas their brain clearance, which is much faster for cocaine (half-life 20 minutes) than for MP (90 minutes) does not. However, whereas the fast brain clearance of cocaine allows for frequently repeated administration [23], the slow clearance of MP results in target saturation, which may underlie its lower abuse liability compared to cocaine [7]. PET studies also showed that cocaine [24] and MP decrease cerebral blood flow (CBF) in striatum and cerebellum [25, 26]. More recently, functional magnetic resonance imaging (fMRI) has been used to investigate the effect of MP on brain activity. These studies have shown that oral administration of MP produced regional increases in the blood oxygenation level-dependent (BOLD) response in rodents [27], healthy subjects [28, 29, 30, 31, 32, 33, 34, 35, 36] and ADHD patients [31, 37, 38, 39, 40, 41]. However, no changes or reductions in brain activation were also observed during motor (finger tapping), reward, and reversal learning tasks [42, 43, 44], suggesting that MP's effects on brain activation depend on the nature of the fMRI task. MP has also been shown to alter 'resting-state' functional connectivity (FC), as captured with fMRI, to reduce FC in ventral striatum, and to normalize hypo FC in ADHD patients [45, 46, 47, 48, 49, 50, 51]. Likely, MP's PK and its regional effects on brain activity play significant roles in its therapeutic and behavioral effects. However, little is known about how the synaptic and molecular specific actions of MP captured with PET translate into BOLD-fMRI responses, because the BOLD-fMRI signal depends complexly on blood oxygenation, CBF, and volume (CBV; see below Neurovascular Coupling). Moreover, the functional effects triggered by MP-induced increases in DA will depend on whether there is a predominance of signaling from D1 (D1R) or from D2 receptors (D2R); whereas DA binding to D1R is stimulatory to medium spiny neurons (MSN) in striatum, DA binding to D2R is inhibitory. In turn DA's binding to D1R versus D2R will be affected by the route of administration since DA will bind to D1R, which are low affinity, only when there are large DA increases, as achieved with intravenous (iv) MP; whereas it will bind to D2R, which are high affinity, even with relatively low DA increases as achieved by oral but also iv MP. We hypothesize that BOLD-phMRI signal changes will be correlated with DA displacement in the striatum and that this effects will differ for oral than for iv MP. This study will provide novel PET/phMRI methodology for investigating neurovascular coupling in health and disease conditions.

Dopaminergic effects of stimulant drugs

DA neurons located in the ventral tegmental area (VTA) and substantia nigra (SN) of midbrain project to the ventral and the dorsal striatum via the mesolimbic and nigrostriatal pathways, respectively. The rewarding and conditioning effects of drugs

seem to be predominantly driven by transient and pronounced increases in DA cell firing that stimulate D1R in the nucleus accumbens (NAc) [52, 53]. In contrast, DA stimulation of D2R in NAc appears to be implicated in inhibiting aversive responses and in sustaining motivation [54]. In humans, PET studies that take advantage of radiotracers that bind to D2R/D3R that are sensitive to binding competition from endogenous DA, such as [^{11}C]raclopride, have shown that stimulants, such as MP, increase DA in dorsal striatum and in ventral striatum (where NAc is located) and that these increases are associated with the subjective rewarding effects of the drugs when given iv [3, 55, 56] but not when given orally [57].

The blood oxygenation level-dependent (BOLD) contrast is the most commonly used technique to non-invasively study brain function. For activated regions, the local increases of CBF and CBV largely exceed the local increase in oxygen consumption, producing a decrease in paramagnetic deoxyhemoglobin concentration, which increases the homogeneity of the local magnetic field, and consequently the local fMRI signal [58, 59, 60, 61]. This dynamic signal increase is the basis of the BOLD contrast that is widely used to study the effects of pharmacological challenges such as MP, on brain function in humans. Pharmacological MRI (phMRI) is a technique that allows researchers to noninvasively map brain function in response to the hemodynamic changes brought on by the introduction of a pharmaceutical challenge. phMRI is analogous to fMRI but employing pharmacological methods of stimulation, phMRI maps the time course and neurological response to specific pharmacological challenges and gives insight into the pharmacokinetic and pharmacodynamic properties of drugs aimed at brain diseases. It is unclear, however, how peripheral and cardiovascular changes induced by acute MP administration affect the BOLD signal. Oral MP, which is the route used for therapeutic effects, only triggers mild increases in blood pressure [62]. In contrast, iv MP (0.5 mg/kg) triggers significant increases in systolic blood pressure and heart rate (HR), which parallel closely MP's PK in the brain, and induces "high" and changes in diastolic blood pressure, which decrease rapidly despite long-lasting binding of the drug in the brain [1]. We have been doing studies with iv MP for 20 years and have not experienced any serious adverse events when using MP doses of 0.5 mg/kg iv. Nonetheless, considering that the study will be carried in the more challenging PET/phMRI environment we propose to use a lower dose of iv MP (0.25 mg/kg), which is a dose we have also shown induced significant increases in DA [17], in order to minimize the chances of adverse cardiovascular (HR and BP increases) or behavioral effects (i.e. anxiety, panic). Quantitatively, an MP dose of 0.25 mg/kg of MP corresponded to about 50% occupancy and that of 1 mg/kg to about 80% occupancy. We feel confident the 0.25 mg/kg dose will be sufficient to elicit similar responses to those of the 0.5 mg/kg dose. Compared to the 0.25mg/kg dose the 0.5 mg/kg dose has stronger cardiovascular effects which we wish to minimize considering that the study will be carried in the more challenging PET/phMRI environment. Thus, we propose to use the 0.25 mg/kg MP dose in order to minimize the chances of adverse cardiovascular (HR and BP increases) or behavioral effects (i.e. anxiety, panic). For oral MP, we will use a 60 mg dose, which we have shown induced significant increases in DA [12]. This dose is within the high range of doses used therapeutically in adults with ADHD [63]. We have not experienced any serious adverse events when using MP

doses of 60 mg po.

The neurovascular coupling is the relationship between neural activity and hemodynamic signals. The interactions between these variables are complex and involve a number of interrelated factors [64]. The basal ganglia have been studied widely in humans and nonhuman primates using PET radiotracers and are a natural target for PET/phMRI studies on the neurovascular coupling. Using simultaneous PET and MRI with pharmacological doses of a DA D2R antagonist (raclopride), Sander et al. [65] demonstrated that phMRI can provide a good surrogate for the D2R measures traditionally obtained with PET. The coupling between D2R blockade and the consequent cerebrovascular responses in the striatum presumably reflected changes in neuronal/glial activity [66]. Specifically, Sander et al found increased striatal CBV after the raclopride challenge, consistent with the CBF striatal increases/decreases in primates after administration of D2R antagonists/agonists [67, 68], and with the notion that antagonism of D2R signaling would disinhibit D2R mediated inhibition of striatal D2R-expressing MSNs [69]. Taken together with prior results, these findings indicate that antagonism of D2R signaling in the primate brain leads to striatal activation. In the striatum, D2R are predominantly expressed in postsynaptic GABAergic neurons (MSN), which project to the external globus pallidus, where DA binding to D2R inhibits MSN activity. D2R are also expressed in DA terminals (autoreceptors) and in cortico-striatal glutamatergic terminals, where DA binding, respectively, inhibits DA and glutamate release [70]. Thus, increased CBV in striatum with D2R blockade could reflect not only disinhibition of D2R-expressing MSN but also enhanced cortico-striatal glutamatergic signaling, as well as increased DA release with a resultant increase in stimulatory D1R signaling. In addition, D2R are also expressed in acetylcholine and GABA interneurons (2–4% of the total neuronal population in striatum), which could also contribute to striatal activation/deactivation signals. Hypothesizing that 1) fast- and short-lasting DA increases triggered by i.v. MP will engage stimulatory D1R in ventral striatum, and 2) slow- and long-lasting DA increased triggered by oral MP will engage D2R in dorsal striatum, we predict that the neurovascular coupling between the dynamic PET and MRI signals will be opposite for i.v. (positive linear scale factor) than for oral (negative linear scale factor) MP. Detection of phMRI changes produced by MP is challenging using the endogenous BOLD contrast because small CBV changes (<10%) are expected to cause even smaller BOLD signals (<1%) [71]. To enhance the CBV signals, Sander et al. [65] injected coated superparamagnetic iron oxide nanoparticles in the blood stream of the anesthetized primates [72], an approach not currently feasible in humans.

Simultaneous PET/phMRI

PET and phMRI are highly complementary and their combination is a powerful scientific tool to understand mechanisms underlying the BOLD signal and its relationship to a drug's PK. While PET uses radioactive tracers and labeled drugs to map synaptic elements involved in neurotransmission (receptor, transporters, etc.) with high sensitivity and chemical specificity, it suffers from low spatial and temporal resolution (2-10mm; 1 minute). In contrast, BOLD-phMRI does not use ionizing radiation and can map brain function with high spatial and temporal resolution (0.3-

3.0mm; 1 second) but suffers from low sensitivity and specificity [73]. Thus, the simultaneous PET/phMRI acquisition aims to overcome the limitations of each individual technique and advance understanding the dynamic actions of drugs on BOLD responses in the human brain. Together, PET and MRI measures have the potential to help clarify the neurochemical basis of BOLD signal changes induced by MP and other stimulant drugs. Cutting-edge PET/MRI technology, such as the 3-T Siemens Biograph mMR or General Electric SIGNA PET/MR scanners installed at the NIH Clinical Center, features PET detectors that are installed within the MRI bore [74] that can operate both modalities simultaneously without significant performance impact [74]. However, the accuracy of attenuation correction in a combined PET/MRI examination remains unclear. Some suggest that the attenuation correction problem is potentially solved for the brain by predicting bone information from anatomic MR images [75, 76, 77] while others think that attenuation correction based on the MRI Dixon sequence is not sufficiently accurate [78]. Whereas attenuation correction remains a potential concern for quantitative PET, the present approach is based on a dynamic response change (comparisons with itself) that is resilient to attenuation correction problems [65, 79].

2. Study Objectives

a. Primary objectives

To quantify the dynamic association between dopamine D2R occupancy measured by PET and brain activity inferred by phMRI in the human brain (aim 1); and to assess the relative sensitivity and specificity of the neurovascular coupling for slow (oral) versus rapid (intravenous, IV) stimulant MP delivery (aim 2).

b. Secondary objectives

To assess the associations between physiological and behavioral measures (BP, HR, and subjective behavioral effects), D2R occupancy by DA, and phMRI signals, and the reproducibility of the signals across PET/MRI instruments.

3. Subjects

a. Description of study populations

A total of 20 healthy volunteers (10 males and 10 females) will be studied. The accrual number for the study will be 40, which is the estimated necessary number to complete studies in 20 subjects and the reproducibility studies in five ($n=5$) subjects. We expect that 1/2 of the subjects that undergo screening will not complete the study or don't fulfill criteria. NIH employees may participate in this study.

b. Inclusion criteria

Healthy Volunteer Participants

1. Males or females between 18 and 55 years of age.
2. Ability to provide written informed consent.
3. Willing to abstain from drug use on scheduled testing days.

4. Have or had any prior experience with alcohol use or stimulant drugs including cocaine, methylphenidate, amphetamine or methamphetamine, diet pills (prescription or over the counter), caffeine, and others but did not have a substance use disorder.

c. Exclusion criteria

1. Pregnant or breastfeeding. Females of childbearing potential must have negative urine pregnancy test and not be currently breastfeeding. Post-menopausal or surgically sterile (tubal ligation or hysterectomy) females satisfy these criteria.
2. Unwilling or unable to refrain from use within 24 hours of scheduled study procedures: psychoactive medications or medication that may affect study results (e.g., antibiotics (must finish course at least 24 hours prior to a scheduled procedure), antidiarrheal preparations, anti-inflammatory drugs [systemic corticosteroids are exclusionary], anti-nausea, cough/cold preparations) (self-report, medical history). The following medications are allowable for entry on this study: analgesics (non-narcotic); antacids; antiasthma agents that are not systemic corticosteroids; antifungal agents for topical use; antihistamines (non-sedating); H2-Blockers/PPI (proton pump inhibitors); laxatives. The use of antihyperlipidemics and/or diuretics are permitted if they have been taken for at least 1 month before procedure visits and dose has been stabilized. The episodic use of benzodiazepines such as alprazolam (™Xanax), diazepam (™Valium) and lorazepam (™Ativan), will not exclude participants from this study unless they have been taken within the last 24 hours prior to the study.
3. The following current chronically used medications are exclusionary from the study: stimulant or stimulant-like medications (amphetamine, methylphenidate, modafinil); analgesics containing narcotics; anorexics (sibuteramine); antianginal agents; antiarrhythmics; antiasthma agents that are systemic corticosteroids; antibiotics; anticholinergics; anticoagulants; anticonvulsants; antidepressants; antidiarrheal preparations; antifungal agents (systemic); antihistamines (sedating); antihypertensives; anti-inflammatory drugs (systemic); antineoplastics; antiobesity; antipsychotics; antivirals (except for treatment of HSV with agents without CNS activity, e.g. acyclovir, ganciclovir, famciclovir, valacyclovir); anxiolytics (benzodiazepine or barbiturates); hormones (exceptions: thyroid hormone replacement, oral contraceptives, and estrogen replacement therapy); insulin; lithium; muscle relaxants; psychotropic drugs not otherwise specified (nos) including herbal products (no drugs with psychomotor effects or with anxiolytics, stimulant, antipsychotic, or sedative properties); sedatives/hypnotics. Note that nicotine and/or caffeine use will not exclude participants.
4. Current or past DSM-IV or DSM-5 diagnosis of a psychiatric disorder as determined by history and clinical exam including substance use disorder (except for nicotine/caffeine), alcohol use disorder (or alcohol dependence if assessed using DSM-IV), anxiety disorder or panic attacks. Past history

of a mental disorder as defined by DSM-IV or DSM-5 will be excluded only if it required hospitalization (any length), or chronic medication management (more than 4 weeks), and that could impact brain function at the time of the study.

5. Those with a binge drinking history every month continuously for the last 10 years will also be excluded. Binge drinkers are those who being female consume 4 or more drinks and males consume 5 or more drinks in one occasion at least once a month.
6. Major medical problems that can impact brain function at the time of the scan (including but not limited to HIV; glaucoma, central nervous system including seizures and psychosis; cardiovascular including hypertension and arrhythmias; metabolic, autoimmune, endocrine) as determined by history and clinical exam.
7. Any clinically significant laboratory finding as determined during the screening procedures.
8. Have had previous radiation exposure (from X-rays, PET scans, or other exposure) that, with the exposure from this study, would exceed NIH annual research limits.
9. Head trauma with loss of consciousness for more than 30 minutes.
10. Presence of ferromagnetic objects in the body that are contraindicated for PET/phMRI of the head (pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips, metallic prostheses, permanent eyeliner, implanted delivery pump, or shrapnel fragments), fear of enclosed spaces, or other standard contraindication to MRI/MRS (self-report checklist).
11. Cannot lie comfortably flat on back for up to 2 hours in the PET/phMRI scanner.
12. Body weight > 204 kg (> 450 lbs).
13. Allergy to methylphenidate.
14. Clinically significant EKG abnormalities. Clinically significant findings on EKG will be assessed by the Medical Advisory Investigator on the study or through a cardiology consult. EKG reviews are documented in CRIS.
15. History of glaucoma as determined by medical history.
16. NIH employees who are study investigators, as well as their superiors, subordinates and immediate family members (adult children, spouses, parents, siblings).
17. Non-English speakers (must also be able to read and comprehend English).

***** Subjects will not be excluded from enrollment in this study if their urine test is positive for drugs. However, if they test positive on scheduled study procedure days involving study imaging and neuropsychological testing, the procedures will be postponed and rescheduled. We will allow for up to 3 rescheduled study days that were the result of positive urine drug screens. If the drug test is positive on the third rescheduled visit, the participant will be withdrawn from the study. The intent of the research has no prospect of direct benefit to the subject. Therefore, we are excluding

non-English speakers in this research study since it includes the administration of questionnaires, surveys and assessments that are validated for English, although some are available in Spanish. In addition, our fMRI paradigms (particularly the Delay Discounting task) require that the subject be able to speak, read and comprehend English.

4. Study Design and Methods

a. Study overview

Participants will be tested with simultaneous [^{11}C]raclopride-PET/phMRI to map the dynamics of DA receptor occupancy (reflecting extracellular DA levels) and BOLD signals using the MP challenge (oral and iv). During the study, participants will also undergo a neuropsychological battery and personality tests, which are detailed below. Time points are targets, and the order can be adjusted for practical considerations. The studies will be conducted as outpatients.

b. Recruitment

Participants will be recruited through referrals from the NIH Volunteer Office, the Patient Recruitment and Public Liaison (PRPL) Office, and through ResearchMatch.org.

Participants (including healthy volunteers who are NIH employees) will also be recruited by word of mouth and through IRB-approved advertisements. These will include: 1) Flyers posted on NIH campus, campuses of universities and colleges, public places and public transportation services in the greater Washington DC/Baltimore metro areas; 2) Advertisements in electronic and printed local media (newsletters, websites, newspapers, craigslist.org, NIH and other local email distribution lists), following IRB approval. There will be no direct solicitation of employees by supervisors or coworkers.

ClinicalTrials.gov may also represent a source of recruitment.

Participants will also be recruited via the 14-AA-0181 Natural History protocol.

c. Telephone Pre-Screening

Initial contact with potential subjects will be done through use of a telephone pre-screening questionnaire. Subjects will be briefly interviewed over the telephone to determine that they report meeting inclusion and not meeting exclusion criteria as described above. Subjects who appear to meet criteria will be scheduled for a screening visit. Subjects who do not meet criteria based on this telephone interview will not be scheduled for a screening visit and their telephone screening form will be shredded in a cross-cut shredder. A waiver of consent for these screening activities has been requested in Section **13.**

d. Screening

1. **Screening Procedures performed under present study or Protocol #14-AA-0181 (NIAAA Natural History protocol):** Consent will be obtained prior to any study procedures are done. As of 12/14/2021, we will not screen under 14-AA-0181 since initial eligibility was done in all subjects prior to this date. Procedures will include diagnostic assessments for psychiatric disorders, patient medical history and physical examination including EKG and standard laboratory tests (Chem Screen; CBC; TSH; PT; PTT; cholesterol levels, high sensitivity C-reactive protein, homocysteine & Urinalysis) and, for female participants of childbearing potential, a urine pregnancy test. STAT urine tests to identify drug abuse (cocaine, methamphetamine, amphetamines, opioids, cannabinoids, benzodiazepines and barbiturates). Urine tests will be repeated on each day of study at least once to ensure lack of drug use. We will not repeat the screening tests that have already been done under the NIAAA Natural History protocol (14-AA-0181). Clinical or non-clinical tests performed under 14-AA-0181 are valid for one year. Missing or outdated screening tests will be done under the present study. The following information and test results, which may be collected under the present study or the Natural History protocol #14-AA-0181, will be shared with this study:
   - *Demographics*
   - *Vital signs*: heart rate, blood pressure, pulse, temperature
   - *Measurements*: height, weight, BMI
   - *Bloodlabs*: Blood test panels to assess physiological functions and screen for clinically significant disease presence. No more than 30 mL will be drawn. The blood tests include:
     - Complete blood count with differential (CBC with diff) PT, PTT.
     - Chem 20 Panel (Chem 20): Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid, amylase.
   - *Urine test*
     - Urinalysis this is a series of tests done on urine to screen for abnormalities – results received next morning and reviewed.
     - Urine drug screens: The Qualitative (DLM) tests for benzodiazepines, cocaine, methamphetamines, opiates and tetrahydrocannabinol (THC). It provides a result within hours. Urine drug screens will be performed on subsequent days of study – STAT.
     - Pregnancy test. This is required by Clinical Center regulations prior to MRI and PET scanning – STAT. If sample is processed by DLM, result is provided within hours.
   - Electrocardiogram (EKG)
   - MR Safety Questionnaire – this will be obtained during initial screening and on each day that PET/phMRI is done – takes 5 min.

- *Questionnaires, Assessments, Interviews (**all subjects**):* If any of the following are obtained through the Natural History protocol, we will only repeat if too much time has passed since initially done. Some of these questionnaires, assessments and interviews do not require repeating since the answers don't change over time. Drug History interview, rating scales, diagnostic interview and self-report questionnaires are as follows:
  - MR Safety Questionnaire.
  - Edinburgh Handedness Scale [80].
  - Smoking History Questionnaire [81] – takes 10 min.
  - Fagerström Test for Nicotine Dependence (FTND) [82] – takes 5 min.
  - Adult ADHD Self-Report Scale (ASRS-v1.1) - The Symptom Checklist is an instrument consisting of the eighteen DSM-IV-TR criteria. Six of the eighteen questions were found to be the most predictive of symptoms consistent with ADHD. These six questions are the basis for the ASRS v1.1 Screener and are also Part A of the Symptom Checklist. Part B of the Symptom Checklist contains the remaining twelve questions [83].
  - The Timeline Follow-Back (TLFB) technique collects drinking and smoking information using personal historical events recounted over a fixed time period [84, 85]. It is a commonly used technique to assess alcohol drinking patterns and quantification in treatment programs. This questionnaire may need an update if more than a year has passed since last completed by the participant who will return for scans on the GE PET/MR scanner.
  - Alcohol Use Disorders Identification Test (AUDIT) [86].
  - Structured Clinical Interview (SCID): The SCID is a widely-used, standard clinical interview to establish criteria for psychiatric diagnoses [87]. It is a structured interview consisting of 11 modules and up to as many as 292 questions that take between 60 and 180 minutes to complete.

2. **Study Evaluations and Procedures:** Consent for the current study will be obtained prior to starting study procedures under this study. All information and results of screening tests described in section 4.d.1 *Procedures performed under NIAAA Natural History protocol #14-AA-0181* above will be shared with this study and reviewed for eligibility criteria discussed in section 3. *Subjects* above. If any of the lab tests done under the NIAAA Natural History protocol are missing or outdated (performed more than 1 year prior to current date), the tests will be done under this study for ongoing eligibility. All tests obtained under NIAAA Natural History protocol #14-AA-0181 will be reimbursed under that protocol. Clinical and non-clinical tests performed under this protocol will be reimbursed under this protocol.

   The following procedures will be done on each day of study once consent has been obtained:
   - *Vital signs - daily*: heart rate, blood pressure, pulse, temperature

- *Measurements - daily*: height, weight, BMI
- *Brief Medical/Physical HX - daily*
- *Breath alcohol test*
- *Urine tests - daily*
  - Urine drug screens: The Qualitative (DLM) tests for benzodiazepines, cocaine, methamphetamines, opiates and tetrahydrocannabinol (THC). It provides a result within hours. Urine drug screens will be performed on each day of study – STAT.
  - Pregnancy test: This is required by Clinical Center regulations prior to MRI and PET scanning – STAT. Urine pregnancy test will be performed on any day of study involving imaging scans and/or drug dosing.
- Electrocardiogram (EKG) – this will be done on each day that involves drug dosing followed by PET/phMRI. This will be started prior to drug dosing through to end of the imaging scan.
- MR Safety Questionnaire – this will be obtained and reviewed on each day that PET/phMRI is done prior to participation in PET/phMRI – takes 5 min.
- Drug History (HX) Parts I & II – takes about 15 min.
  - Drug HX Part II will be obtained on each subsequent visit – takes about 5 min. Only Drug HX Part II will be repeated in participants returning to repeat the scans on the new GE PET/MR scanner.

Note: Bulleted arrow items in sections 4.d.1 & 4.d.2 above that are shaded in pale yellow will be done to confirm ongoing eligibility for subjects returning to repeat the scans on the new GE PET/MR scanner. The bulleted items starting from Edinburg Handedness Scale down through the SCID do not have to be repeated for participants since they were obtained in these subjects previously. The exception to this list is the Timeline Follow Back which may need an update to satisfy exclusion #5 which deals with alcohol drinking history.

Once it has been determined that the subject meets/maintains the criteria and all standard laboratory test results have been received/reviewed and they indicate the subject has no clinically significant lab results that would interfere with study participation, they will be considered enrolled and subsequently scheduled to start imaging procedures. If a subject has visited the NIH CC recently and had some clinical and/or non-clinical tests performed, we plan to use the existing results for determining eligibility. If a subject's most recent visit is greater than a year, we will order new lab tests if needed for ongoing eligibility. As standard operating procedure (SOP), the NIH Clinical Center requires that a clinical MRI scan of the brain, which is a minimal risk procedure and may include any standard clinical MR sequence as well as Diffusion Tensor Imaging (DTI) sequences, must be done on all enrolled subjects. This clinical scan can be done at any time prior to completion of the study. In general, we plan to obtain this clinical MRI scan on the day that PET/phMRI scanning is scheduled, but it could be done on another day too.

e. Study Timeline: This research study will take place over four or more days including screening. After initial screening, subjects will be expected to come to the Clinical center 3 or more days for the PET/phMRI scans, and NPT and personality tests. If the imaging scans and NP tests cannot be completed on the days they are scheduled, then the subject might have to come on another day to finish the procedures. All PET/phMRI scans, DNA from blood, NP testing results and personality measures obtained will be for research purpose only and the results will not be shared with the participants. Participants who return for repeat scans on the new GE scanner will not repeat NP tests, personality measures genotyping or GENEActiv activity monitoring. Study visits will be planned as follows:

- Initial Study Screening
- *Study Procedures Day A: PET/phMRI Scanning Session
- *Study Procedures Day B: PET/phMRI Scanning Session
- *Study Procedures Day C: PET/phMRI Scanning Session

*Note: The order of Days A, B and C will be randomized. In addition, the NP testing can occur in any of the 3 study days that subjects are scheduled for PET/phMRI, or on another day.

1. *Screening Visit* (Day 1 for all): First, informed consent is obtained followed by initial study screening. Participants will be screened for eligibility as delineated in the inclusion and exclusion criteria for the study. Once eligibility has been confirmed, and the subject agrees to continue participation, he/she will be scheduled for the PET/phMRI scanning sessions and NP testing as outpatients.

The orders for these three days of scanning will be randomized

2. PET/phMRI Scans: PET/phMRI scanning will be carried out on a 3Tesla (3T) Siemens Biograph mMR or General Electric SIGNA PET/MR scanner installed in the Clinical Center. MRI evaluations of the brain are being performed at 3T. These MRI scans are performed on FDA-approved scanners with approved radiofrequency coils, and their use conforms to the corresponding FDA labels. The MR scanner is being used in normal mode (not research mode) using the following FDA magnetization-prepared rapid gradient-echo (MPRAGE) pulse sequences and echo-planar imaging (EPI).

In preparation for the PET/phMRI scans, a catheter will be placed into the subject's antecubital vein (venous catheter) for tracer injection. Tracer administration of [^{11}C]raclopride will be injected over a period of approximately 1-2 minutes using an FDA approved infusion pump with an approximate delivery range of 9-16 mCi of [^{11}C]raclopride. At the start of [^{11}C]raclopride injection, a dynamic PET scan will be initiated (list mode). Ultrashort (UTE) or zero (ZTE) echo-time imaging will be used to map

structures with short T2 relaxation times such as bone [88]and used for attenuation correction during PET retrospective image reconstruction.

- **Study Procedures DAY A:** Oral MP (60 mg) will be given 30 minutes prior to [^{11}C]raclopride injection followed by iv placebo (PL) given 30 minutes post injection of [^{11}C]raclopride.
- **Study Procedures DAY B:** Oral PL will be given 30 minutes prior to [^{11}C]raclopride injection followed by iv MP (0.25 mg/kg) given 30 minutes post injection of [^{11}C]raclopride.
- **Study Procedures DAY C:** Oral PL will be given 30 minutes prior to [^{11}C]raclopride injection followed by iv PL given 30 minutes post injection of [^{11}C]raclopride.

A high resolution T1-weighted magnetization-prepared rapid gradient-echo pulse sequence will be used to map brain structure with high spatial resolution and excellent gray-white contrast. Single-shot echo planar imaging (3mm isotropic resolution; TE/TR= 30/3000ms) will be used to collect functional images continuously during 100 minutes to measure brain activation and to map functional connectivity using a single-shot T2*-weighted EPI pulse sequence. MRI and PET acquisitions will start at the same time. The PET/phMRI scan will be done under dim illumination. We will monitor heart rate and blood pressure using an MRI compatible non-invasive blood pressure monitor. In the event of an adverse event we will immediately contact the cardiologist on call from the clinical center or initiate the emergency response as clinically indicated.

Rating Scales: To assess the effects of MP before and after PET/phMRI scans we will use self-rating indicators of drug effects (Drug Evaluation questionnaire) and the Profile of mood Scale (POMS), which is adapted from the Profile of Mood States ([89]).

Food control: A standard small meal will be provided for the subjects about 3 hours prior to the scheduled PET/phMRI scans. We will provide a 300-500 calories snack (choices salad or sandwich). Subjects will be asked to fast from the time s/he wakes up in the morning until we provide this standard meal. After PET/MRI is complete, subjects may be provided with a meal if they are hungry.

3. **Genetic Testing (Genotyping – collected on any Day of study):**
Since the inter-subject variability of the FC partially reflects genetic variability, the genetic profile of the individuals could help us understand the mechanism underlying diversity in DA signaling and in brain functional connectivity. As information on new genes involved with DA signaling and brain function emerge we can then assess the role of relevant genetic polymorphisms on brain dopamine, functional connectivity and energetic efficiency.

All participants will be asked to give a total of 10 ml of blood split into two vials containing 5 ml each (1 teaspoon) for genetic research purposes. The genetic material, DNA, will be extracted from one of the samples and analyzed in order to assess the role of relevant genetic polymorphisms on brain dopamine, BOLD signals and energetic efficiency. Leftover DNA and the other 5 ml sample will be stored at -80C^{o} for future use. Genetic samples will be analyzed at NIH. Subjects will consent to keep their sample so that in the future we can analyze them for yet-to-be-discovered polymorphisms associated with behavioral and imaging data. The results from this research study will be preliminary. Further research may be necessary before they are fully understood. The genetic testing that will be done as part of this study is for research purposes only and results will not be placed in the medical record. Genetic testing is optional.

Genetic testing will not be done in participants who return to repeat scans on the new GE PET/MR scanner.

4. **Neuropsychological Tests:** NP tests will be done either on the day of the PET/phMRI scans (before the scans) or asked to come on a separate day. These tests will not be done following the oral MP administration. We will use the Cambridge Neuropsychological Test Automated Battery (CANTAB), which is a computerized battery of tests [90]. The CANTAB has normative data and has good reliability [91, 92] and is sensitive to drug effects [93]. We will obtain the following CANTAB tests:
    - Reaction Time –Multiple choices reaction time test.
    - Stop Signal- it's a measure of response inhibition
    - Intra/Extra-Dimensional Set Shift –Attention test, similar to the Wisconsin Card Sorting Test.
    - Pattern Recognition Memory –Stockings of Cambridge – Visuospatial planning test that is on the 'Tower of London' test.
    - Spatial Span –Spatial working memory span test.
    - Spatial Working Memory –This is a test of a subject's ability to retain and to manipulate spatial information in working memory.
    - Cambridge Gamble Task, test of decision making
    - Affective Go/No-go – test for emotional bias
    - Emotion Recognition Task –test to assess social cognition

    Additional NP measures not included in CANTAB:
    - Barratt's Impulsiveness scale will be used to assess impulsivity [94]. If this has been collected under the Natural History protocol, it will not be repeated.
    - The Beck Depression Inventory Second Edition (BDI-II) is a 21-question multiple-choice self-report inventory for measuring the severity of symptoms of depression as listed in the DSM-IV [95]. In case BDI-II scores are 20 or higher, indicating moderate to severe

depression, or in case of suicidal thoughts (that is, a scoring of 2 "I would like to kill myself" or 3 "I would like to kill myself if I had the chance" on item 9), the participant will be seen by a psychiatrist or nurse immediately for follow-up and appropriate clinical referrals. Please note, however, that before the BDI-II is filled out, all participants have been screened with the SCID, and with MADRS scores of 20 or higher.

- The Wechsler Abbreviated Scale of Intelligence-Second Edition (WASI-II) is a widely used brief measure of cognitive abilities [96] and has excellent reliability and validity. The two subscales "Vocabulary" and "Matrix Reasoning" will be used, which yield a full scale IQ score. It takes about 20 minutes to complete.

  http://www.cambridgecognition.com/?gclid=CNH9qq-96roCFWrNOgodvHsA8Q

Personality Measures:

- Morningness-Eveningness Questionnaire (MEQ), which provides a self-report circadian typology that is based on the preferred timing for activity, sleep/wake preferences, and optimal timing for mental alertness [97]. Responses to these items are considered to represent underlying circadian function.
- The NEO-PI Five Factor Inventory (FFI) organizes personality traits into five domains: Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness [98].
- Multidimensional personality questionnaire (MPQ), which three main personality dimensions: Positive Emotionality or PEM (or extraversion), Negative emotionality or NEM (or neuroticism) and constraint [99]. These measures tap into characteristics associated with vulnerability to substance use disorders and correlate with specific patterns of brain activity [100].

NP testing and Personality tests will not be done in participants who return to repeat scans on the new GE PET/MR scanner.

5. **Motor Activity and Sleeping Behavior measured with ™GENEActiv:** To assess spontaneous motor activity we will ask participants to wear a GENEActiv accelerometer [101] for one week either prior or after completion of the scans. The recording from the GENEActiv will also allow us to determine the average number of hours slept per day and their levels of spontaneous motor activity. The GENEActiv measures can be obtained prior to or after completion of the PET/phMRI scans.

   GENEActiv procedures will not be done in participants who return to repeat scans on the new GE PET/MR scanner.

f. End of participation

Clinically relevant information may be shared with receiving health care providers if in clinical assessment this is for the benefit of the patient, and the patient consents to this.

Potential Future Contact: While all steps are always taken to ensure quality data from all participants, we may ask participants who generate unusable data (or if there is an equipment failure) on one of their experimental sessions to repeat that session. This will have the effect of maximizing the risk/benefit ratio as it will prevent having to discard usable data from one session and will minimize the number of participants recruited overall to obtain sufficient data to meet power requirements. We may ask the participant to return at a later date or to repeat study procedures for genotyping, PET/phMRI scans. There may only be one repeat scan per participant. Subjects will receive additional compensation if experimental sessions are repeated; compensation for this *as needed* research procedures may vary based on the inconvenience units and will be based on NIAAA recommendations.

5. Management of Data and Samples

a. Storage

Data will be stored on an NIH data server, under the management of the ORIT, NIAAA. The existence and types of information contained in the data management system have been publicly reported as required by the FOIA.

Subject data will be stored in one of two locations. Fully identifiable data will be stored in the NIH Clinical Center CRIS system, where clinical patient data are routinely stored and adequate privacy protections are implemented by design. Additional data will be stored in the B2L124 NIAAA/LNI space in Building 10 on an access controlled NIAAA server located in secured space to prevent physical theft of storage media. We will feed some of the data residing in CRIS and on the NIAAA servers to the NIH Biomedical Translational Research Information System (BTRIS). Data on the NIAAA server will be kept in a coded form. The code key will be kept separately by the trial leader or designee.

Biological specimens obtained under this protocol will be stored in coded form (protocol identifier plus randomization number), in freezers located in Bldg 10 CRC Room 15281 area of NIAAA. We will collect 10 ml of venous blood from each participant, which will be divided into 2 parts; one of which will be frozen and stored at -80ºC and the other to be used for DNA isolation and subsequent genotyping tests.

We will collect DNA so that in the future when new findings emerge with respect to genes implicated in brain diseases so we can assess across integrated data from various brain imaging studies if they are associated with changes in brain connectivity. However, DNA collection will be optional and will not exclude

subjects from participating in this study if they do not want to give blood for genotyping.

b. Data including genomic data and sample sharing plan

Samples and data, including genomic data may be shared with collaborators.

Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

6. Additional Considerations

a. Research with investigational drugs or devices

[^{11}C]raclopride is injected into the subject's bloodstream to produce the images that are captured by the PET scan. The radiotracer [^{11}C]raclopride is used for imaging D2R, under the IND 54,135.

IV methylphenidate (MP), used under IND #124,912, is injected into the subject's bloodstream to be used in conjunction with PET imaging to show displacement of [^{11}C]raclopride. We received approval from the FDA for the use of iv methylphenidate under the IND #124,912.

7. Risks and Discomforts

Potential risks from this study include the following:

a. *Medical examination and Laboratory testing*: Medical examinations are associated with minimal risks. We will first explain and familiarize the subjects with the laboratory testing to minimize discomfort, if any, during testing. Tests may disclose that the potential subject's health is at risk. If there are abnormal tests uncovered as part of the physical examination, this information will be

given to the subject and explained by a physician who will encourage them to seek medical evaluation with their primary care physician.

b. *PET/phMRI Risks*: People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. Subjects will be screened for these conditions before having any scan, and if he/she has any, he/she will not receive the PET/phMRI scan. If a subject has a question about any metal objects being present in his/her body, he/she should inform the staff. In addition, all magnetic objects (for example, watches, coins, jewelry, and credit cards) must be removed before entering the PET/phMRI scan room.

It is not known if MRI is completely safe for a developing fetus. Therefore, all women of childbearing potential will have a pregnancy test performed no more than 24 hours before each PET/phMRI scan. The scan will not be done if the pregnancy test is positive.

People with fear of confined spaces may become anxious during a PET/phMRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research PET/phMRI scan will be fitted with hearing protection. If the hearing protection comes loose during the scan, the subject should let a member of the staff know right away. The subject will be asked to notify the investigators if he/she has hearing or ear problems. The subject will be asked to complete an MRI screening form for each MRI scan he/she has. There are no known long-term risks of MRI scans.

PET detects injected radioactivity within the body. PET is associated with no known physical hazards to the subject lying on the table. We routinely use a series of procedures to minimize the risk for discomfort during scanning sessions. Namely, the procedures are conducted in the presence of trained health professionals to whom subjects will have ready access, should they experience any problems. Subjects can communicate with the trained health professionals while in the scanner and can be removed from the scanner or withdraw from the study at any time if they wish to do so. Occasionally subjects become anxious during the scan. In that case, subjects can request the operator of the PET/phMRI to stop the scan.

c. *Radiation exposure risks*: The consent form uses wording approved by the NIH Radiation Safety Committee to communicate to the subject the risk associated

with this radiation exposure. Radiation exposure will be from a maximum of 64 mCi of [^{11}C]raclopride. This amount includes a contingency plan for an additional scan if we need the subject to repeat a day of PET/phMRI study. This is expected to happen infrequently. In subjects who complete the PET/phMRI scan protocol, the effective dose from emission scans on a maximum of 4 days of study will be **1.3 rem.** No harmful effects to humans have been observed from the levels of radiation used in this study. Additional measures taken to ensure subject safety will be to obtain a careful history of exposure to radiation from other sources or medical procedures to ensure that total exposure remains within an acceptable range. Pregnancy and breastfeeding are exclusion criteria for women of child-bearing potential. Using the standard way of describing radiation dose, from participating in this study, subjects will receive a maximum of 7.5 rem to their gallbladder wall, 6.1 rem to their small intestine and 4.2 rem to their liver. All other organs will receive smaller amounts of radiation.

d. <u>*Venous line placements*</u>: In preparation for the PET/phMRI scan, one catheter is placed into the subject in an antecubital vein (venous catheter) in one arm for radiotracer injection. A second catheter is placed in the other arm on the day the subject receives study drug (either placebo or oral MP 60 mg, or iv methylphenidate 0.25 mg/kg) for blood sampling levels of placebo or MP in plasma before, during and at the end of study. There is a slight risk of bruising or, rarely, infection from insertion of indwelling peripheral venous catheters for infusion. Risks from IV catheterization are minimized by experienced medical personnel who will perform catheter placement using sterile technique and following universal precautions. Participants will be seated or supine when catheters are placed.

e. <u>*Oral MP exposure*</u>: MP (brand name Ritalin™) is a widely used stimulant drug used for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) and Narcolepsy. For treatment of ADHD, and when given as immediate release tablets (as per the current study) the typical doses correspond to 10-20 milligrams (mg) two to three times a day (typical oral doses of 60 mg/day up to maximal oral doses of 1g/day). Methylphenidate hydrochloride (HCl) is available as a generic or by the brand name Ritalin™ and is FDA approved. Methylphenidate is a Class II controlled substance. MP will be purchased from a commercial vendor and will be stored in the NIH Pharmacy. Inventory and accountability records are maintained by the Pharmacy as per DEA regulations.

Administration of the active drug (methylphenidate HCl): The active drug will be administered as a commercial tablet(s) over-encapsulated in a hard gelatin capsule, with common inactive excipients added to protect the blind.

Placebo formulation: A hard gelatin capsule, identical to that used for over-encapsulation of the active drug tablet(s), containing inactive ingredients, primarily anhydrous lactose. The inactive ingredients will be the same as those used in the active drug formulation.

Adverse Events related to oral MP administration: The most common adverse reactions of MP are: Hypersensitivity; skin rash, urticaria (hives, an allergic skin eruption), fever; Central Nervous System (CNS): dizziness, headache, dyskinesia (impairment of voluntary movement), drowsiness; Cardiovascular: blood pressure and pulse changes; tachycardia; angina; cardiac arrhythmias; palpitations; Gastro-Intestinal (GI): nausea, anorexia (loss of appetite); and Miscellaneous: nervousness and insomnia. The side effect that is most common is increased heart rate and blood pressure. Once consent has been obtained for this study he/she will received an EKG to exclude subjects who may have heart abnormalities. This initial EKG may have been obtained through the NIAAA Natural History Protocol #14-AA-0181. In addition, if the subject has a history of heart trouble or hypertension requiring medication, he/she will be excluded from participation. Heart rate and blood pressure will be monitored during MP administration (prior to receiving study drug through to the end of imaging study) as a procedure on PET/phMRI study days. Clinically significant findings on any EKG, heart rate or blood pressure will be assessed by the MAI on the study or through cardiology consult. A physician/CRNP with current certification in advanced cardiac life support (ACLS) will be present during the study. Other Warnings: As stated in the methylphenidate monograph in "Facts and Comparisons" 2004: Hypersensitivity - arthralgia; exfoliate dermatitis; erythema multiforme with histopathological findings of necrotizing vasculitis; thrombocytopenia purpura. Less common Leukopenia and/or anemia; scalp hair loss; abnormal liver function, ranging from transaminase elevation to hepatic coma; cerebral arteritis and/or occlusion; transient depressed mood; neuroleptic malignant syndrome (very rare). *Contraindications to MP (MicroMedex)*: MP should not be given to individuals using monoamine oxidase inhibitors (MAOI's, e.g., phenelzine and tranylcypromine) or to those with a history of seizures, tics, agitation, psychotic disorders, glaucoma, cardiovascular disorders, hypertension, and arrhythmia since it can exacerbate those symptoms. To minimize the possibility of an untoward reaction we will exclude subjects with past or present histories of these conditions.

f. *INTRAVENOUS (iv) MP Exposure (0.25 mg/kg)*: In this study, participants will be given an iv MP dose of 0.25 mg/kg through the catheter in the arm on study Day B. This is a route of administration that results in stronger behavioral and cardiovascular effects that when given orally; and participants may feel "high" and excited. Participants may even experience anxiety or become overexcited and restless after iv MP. Some participants describe this as unpleasant because it makes them feel anxious and out of control, and rarely, it may result in paranoid ideas. In addition, the same side effects described above for oral MP can occur with iv MP. Participants will be asked not to exercise for the next 24 hours (including dancing or partying) following oral or iv MP or the only placebo day session (Study Procedures Day 3) and not to take any drugs, including alcohol, after the studies. Sudden increases in BP may be potentially harmful. Participants may feel the effects of iv or oral MP for about 3 to 4 hours

after its administration. If a participant has a history of seizures, anxiety, panic attacks or psychosis or glaucoma, MP could make these conditions worse. If a participant has any of these conditions, he/she will be excluded from this study. Also since MP typically increases HR and BP, he/she will be excluded from this study if he/she has a history of heart disease including arrhythmias or hypertension or if clinically significant abnormalities in a participant's EKG or in their BP are detected prior to the PET/phMRI study. HR and BP will be monitored during the scanning. A physician with certification in advanced cardiac life support (ACLS) will be present in the PET/phMRI laboratory at all times during the study. After injection with MP, the physician or CRNP will remain in the scanning room with the participant for at least 30 minutes; this will not be necessary for oral MP since the cardiovascular effects are much milder. If a participants BP increases and remains above 180 or falls below 60 for 10 minutes, or his/her HR increases above 180 beats/minute or becomes irregular, or he/she shows abnormal clinical signs of discomfort, the study will be stopped, symptoms will be treated immediately, and he/she will not be permitted to continue in the study. Participants will be monitored and will not be allowed to leave the NIH Clinical Center until vital signs have stabilized. If a participant has an unexpected adverse reaction to MP, the study will be stopped immediately and he/she will not be permitted to complete any remaining parts of the study.

Contraindications: MP should not be given to individuals with a history of seizures, psychotic disorders, glaucoma, cardiovascular disorders, hypertension, and arrhythmia since it can exacerbate those symptoms. To minimize the possibility of an untoward reaction we will exclude subjects with past or present histories of these conditions.

g. *Genotyping (genetic tests)*: The genetic testing that will be done as part of this study is for research purposes only and results will not be placed in the medical record. It will not provide any information about the participant's health or ancestry. It is our policy to not provide the results of such genetic testing to study participants. Problems, such as with insurance or employment discrimination, may occur if a participant discloses information about this genetic testing or if he/she agrees to have his/her medical records released. We will not release any information about a participant to any physician, insurance company or employer unless he/she signs a document allowing release of the information.

h. *NP and personality measures*: These tests are not harmful, but may be frustrating or stressful. Subjects may refuse to answer any question that makes them uncomfortable and may stop a test at any time and for any reason.

i. *Blood Banking for Future Genetic Studies*: This will allow us to repeat our genetic tests, thus to ensure and validate the genotyping results. In addition, we will be able to conduct analyses of new variations in the brain related genes that

are yet to be discovered. Blood samples will be stored in secured freezers on the NIH campus. The participants name and identifying information will not be on the samples; we will assign them a code. The key to the code will be kept in a separate, secure area. The samples may be used for other research projects. If participants do not want his/her sample used for other projects, he/she should not participate in this study. If a participant withdraws from this research project before it is complete, any remaining samples he/she have contributed will be discarded. Results obtained before he/she withdraws will be kept and the participant's privacy will be protected.

8. Subject Safety Monitoring

Subjects' safety will be monitored by the medical advisory investigator (MAI).

Parameters to be monitored for all participants before, during and at the end of the study sessions will include vital sign (heart rate, blood pressure and temperature).

If any participant reports an adverse reaction to the experiment or demonstrates adverse responses during the post-experimental assessment of adverse events (AE), or appears to be in distress at any point during the experiment, the procedures will stop and the patient will meet with the medical advisory investigator to assess suitability for continuation in the study. Additionally, procedures will be stopped for any participant who asks to stop them at any point. The reasons for participants' discontinuation from the study will be logged and changes to procedures necessary to prevent future adverse reactions will be made.

Any individual may withdraw from the study at any time and with no penalty. This option is clearly stated in the consent form and will be emphasized to participants.

NCI Common Terminology Criteria for Adverse Events v. 3.0 will be used for grading and reporting adverse events (CTCAE, 2007).

Criteria for individual subject withdrawal

- Significant AE, including clinically significant changes in mood or behavior such as aggression, agitation, depression or suicidal ideation or suicidal behavior or clinically significant changes in heart rate (>180), BP (Systolic >180 < 60) or arrhythmias (2 consecutive readings of blood pressure parameters).
- Three positive drug screens.
- Pregnancy.
- At the discretion of the Principal Investigator (PI) and Medical Investigator, based on adverse event (AE) severity.
- Non-compliance with protocol procedures or investigator request(s).
- Patient request.
- Repeated positive drug screens.

9. Outcome Measures

a. Primary outcome measures:

To assess:

- The distribution volume ratios (DVR).
- The %BOLD signal change after MP (oral and iv).
- The %DVR change after MP (oral and iv).
- The temporal correlation between BOLD(t) and DVR(t).
- The test-retest reproducibility of MP-related changes in %BOLD and DVR.

b. Secondary outcome measures

To assess:

- The correlation between DVR(t) and behavioral measures (heart rate, BP, and respiration rate).
- The correlation between BOLD(t) and behavioral measures (pupil size, eye blinks, heart rate, blood pressure, and respiration rate).

10. Statistical Analysis

a. Analysis of data/ study outcomes

The PET and phMRI reconstructed images will be spatially normalized to match the stereotactic space of the Montreal Neurological Institute (MNI) with 3-mm isotropic voxels framing intervals of 1 min. The PET data will be analyzed using the cerebellum as a reference region, where D2R concentration is 10^3 times smaller than in the striatum. Thus, [^{11}C]raclopride has negligible specific binding in the cerebellum. A graphical method that does not require blood sampling [^{102}]will be used for determining DVR(t). Linear trends will be removed to minimize first-order scanner signal drifts. Then the signal change from baseline will be used to compute the combined PK/PD responses [%BOLD(t) and DVR(t)]. The PK/PD response to oral MP should be an inverted U-shape, peaking ~90 minutes after ingestion (i.e. ~40 minutes after the start of EPI acquisition and ~60 minutes after the start of the dynamic PET scan). We will also quantify the area-under-the-curve (AUC), and the peak values for the %BOLD(t) and DVR(t) signals. The effect of Oral MP will be computed by contrasting estimates from Days A and C; the effect of IV MP will be computed by contrasting estimates from Days B and C. For instance, for the effect of oral MP on BOLD signals we will contrast BOLD estimates from Day A versus those from Day C; for the effect of iv MP on DVR signals we will contrast DVR estimates from Day B versus those from Day C. Paired t-test (with BP, HR and respiration frequency covariates to control for potential effects of PaCO2 on BOLD responses) will be used for statistical testing on these intermediate measures. One-sample t-test (fixed effects) will be used to test *Hypothesis 1A*: "The %BOLD(t) signal change will increase in proportion to %DVR(t) change in the striatum". Statistical significance will be set by $P_{FWE} < 0.05$, corrected for multiple comparisons using the random field theory with a family-wise error correction. One sample t-test (random effects) will be used to test Hypothesis 1B

"The scale factor between %BOLD(t) signal changes and %DVR(t) changes in the striatum will be statistically significant across subjects". Statistical significance will be set by $P_{FWE} < 0.05$. We expect that the DA increases triggered by i.v. MP will engage stimulatory D1R in ventral striatum, and that those triggered by oral MP will engage D2R in dorsal striatum. This would result in opposing scale factors for i.v. (positive linear scale factor) than for oral (negative linear scale factor) MP. Paired t-test (random effects) will be used to test *Hypothesis 2*: "The scale factor for oral MP will differ from that for iv MP". Specifically, whereas iv MP will trigger short lasting and immediate increases in %BOLD(t) signal in ventral striatum (where NAc is located) after injection, oral MP will trigger slow and long lasting decreases in %BOLD(t) signals in striatum. Correlation analysis will be used to assess the reproducibility of PET and MRI signals collected with different PET/MRI instruments (the Siemens Biograph mMR and General Electric SIGNA PET/MR).

b. Power analysis

Within-subjects: The statistical power of the proposed sample size will allow us to detect effect sizes > 0.63 (Hypothesis 1A), 0.66 (Hypothesis 1B) and 0.62 (Hypothesis 2) which are significantly lower than the correlations between CBV(t) and the time activity curves (TAC) previously reported for the striatum (R > 0.6) for different pharmacological doses of raclopride in macaques [65].

Our effect size estimations (based on data from 2 macaques) for the reported correlations between CBV(t) and the time activity curves (TAC) by Sander et al [65] is ~ 2. Different from Sander et al [65], superparamagnetic nanoparticles will not be used to boost the MRI signal in this study. Thus, our conservative power analysis includes a smaller effect size that accounts for the expected noisier BOLD(t) signals.

11. Human Subjects Protection

a. Subject selection

Adults (age 18-55) who fulfill the eligibility criteria will be included, regardless of race, ethnicity, sex, or religious affiliation. We predict the racial composition is approximately 60% Caucasian, 35% African American, and remaining 5% proportion of Asian or other. We predict the Hispanic ethnicity to be approximately 25%. On the same basis, the expected breakdown is 50% male and 50% female. The upper age limit of 55 years of age was chosen to minimize cardiovascular side effects from the administration of iv MP, which are more common in older subjects.

b. Justification for exclusion of children

Children are excluded because the study is more than minimal risk without benefit to the individual child, thus making the risks outweigh the benefits of the study.

c. Justification for exclusion of other vulnerable subjects

Subjects that are mentally ill may have severe impairment of cognitive function, which would interfere with those individuals' ability to give consent. Their ability to consent may also change over time. Subjects that are mentally ill may also require hospitalization. . . Subjects with body weight > 204 kg (>450 lbs) are excluded since this is the weight limit of the PET/phMRI scanner.

d. Justification for sensitive procedures

This study employs [^{11}C]raclopride and oral and iv MP to evaluate D2R occupancy as a function of increases in extracellular DA.

e. Safeguards for vulnerable populations and sensitive procedures

Vulnerable populations such as pregnant women will not be studied due to the potential unknown risks of radiation and of MP exposure to this population. Urine Pregnancy tests will ensure women who may be pregnant are excluded from the study. We will rely on self-report for women who are breast feeding and they too will be excluded.

This study is open to NIH staff to participate as healthy volunteers. NIH OHSRP policy 404 permits the participation of NIH employees to participate in NIH studies unless prohibited by their IC. There is no such policy in our institute. We will follow Policy 404 Research Involving NIH Staff as Subjects. Prior to enrollment, we will request that the NIH staff member review the "*Leave Policy for NIH Employees Participating in NIH Medical Research Studies (NIH Policy Manual 2300-630-3*", and we will provide a copy of the "*NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research* for their review. Although NIH employees may participate, study investigators and their superiors, subordinates and immediate family members (adult children, spouses, parents, siblings) will be excluded. Protections for employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "*NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research*" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff. Co-workers will not obtain consent.

This study collects sensitive information on drug/alcohol use, medical, neurologic and psychiatric histories. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures. In addition, all staff will have completed the "Just in time" human subject's protection training course on "Biomedical- Vulnerable Subjects-Workers/Employees" through CITI prior to engaging in recruitment of NIH employees. Privacy and confidentiality for NIH Employees will be adhered to the same as for other subjects. The certificate of confidentiality (CoC) that will be applied for this study will be used to resist demands for subject information as allowed by law.

12. Anticipated Benefit

There is no direct benefit to the subjects participating in the study. The results of the study may improve our understanding of brain function in healthy conditions. Thus, the research is likely to yield generalizable knowledge.

13. Consent Documents and Process

a. Consent procedures

Participants will be consented on this protocol before any procedures commence. They will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing. Either the PI or an AI will conduct the consent process in accordance with NIH policy. Only subjects that can give informed consent will be included in this study. The PI and/or AI's will determine that the subjects are able to give informed consent based on their clinical assessment of the subject's ability to understand the nature of the study and the risks. If the subject's capacity to consent remains questionable, the Ability to Consent Assessment team (ACAT) will be consulted. Consent may not be obtained by a co-worker.

Since consenting is an ongoing process, the protocol will be repeatedly re-explained to participants as needed during the study to reassess their understanding of the nature of the protocol.

b. Consent documents

The consent form contains all required elements. The original consent is filed with their NIH medical record and a digital copy is uploaded into the CRIS system. A copy of the signed consent (with subject signature redacted) is also made for the subject's research binder for regulatory purposes.

c. Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study, screening activities listed in Section **4c.** may be performed. We request a waiver of consent for these activities because based on the nature of the telephone script and questionnaire and/or review of the same data available from participation in other research protocols at NIAAA, these activities present no more than minimal risk of harm. The script informs prospective subjects about the types of questions that will be asked and that there are no plans to ask sensitive questions. This research cannot be carried out unless we are able to brief the participant, verify their age and participation in other NIAAA studies, and gather information such as pregnancy status, presence of non-removable metal in their body and verify their ability to speak, read and understand English before consenting them in this study. It would not be practical to recruit subjects without a preliminary screening to reduce the number of potential disqualifications based on the information already available

at NIAAA. There are robust confidentiality practices involving limiting access to the information obtained during this telephone pre-screening process. Prospective subjects will indicate willingness to participate during telephone pre-screening by providing verbal consent. Normally, there is no requirement for written consent for telephone screening outside the research setting. This waiver will not adversely affect the rights and welfare of the subjects. We will not collect and store any information from these individuals once their qualification is found implausible or they express lack of desire to participate in the study.

14. Data and Safety Monitoring

a. Data and safety monitor

Data and safety monitoring will be done by an Independent Safety Monitor (ISM) Sarah Lisanby, MD, who will review all safety data for each participant yearly. Dr. Lisanby has agreed to serve as ISM for this protocol. Her qualifications include that she is a board certified physician licensed in the states of NY and NC and she has over 20 years experience conducting clinical research. Her experience includes conducting radiotracer PET studies and studies using pharmacological challenge, in which she was responsible for placing arterial lines, injecting radiotracers, prescribing medications, performing medical assessments on the subjects, identifying and reporting adverse events, and clinically managing the patients. In addition, the Medical Associate Investigator (MAI) has over 30 years of experience in PET and MRI imaging and the administration of oral and iv methylphenidate. The PI will consult with the ISM and the MAI should any concerns about safety arise.

b. Data and safety monitoring plan

This study will be monitored on a yearly basis at the time of continuing review. We will look at possible trends in AE's and UP's to determine if a change to the protocol/consent/study procedures becomes necessary via study amendment. In addition, individual subject case report forms will be monitored on an ongoing basis upon completion of study visits. Frequency of monitoring may increase if the PI determines it is necessary.

c. Criteria for stopping the study or suspending enrollment or procedures

This study will be stopped and enrollment suspended if any serious adverse events occur that is determined to potentially be related to one of the study procedures.

15. Quality Assurance (QA)

a. Quality assurance monitor

The Principal Investigator, Lead Investigator and the MAI will be monitoring data collection and the study yearly or more often. Quality assurance (QA) will be performed by NIDA QA and NIAAA QA staff as indicated in the NIDA and NIAAA Quality Assurance Monitoring Plan on a schedule determined by the Clinical Director.

b. Quality assurance plan

Quality assurance (QA) will be performed by the PI, as well as by an independent QA monitor, which according to established practice is considered sufficient for small, single-site trials, like the present project. The QA monitor for this study will have considerable expertise in clinical trials in drug dependence, but s/he will not work in the same section of the PI. Therefore, his/her role as QA monitoring will assure expertise and independence in the same time.

In addition to yearly monitoring of data collection, the Laboratory of Neuroimaging (LNI) utilizes a multi-layer study monitoring program. As a general practice, LNI lab staff records study procedures using a case report form (CRF) checklist on the day of each study. The study procedure checklist is completed on the day of study as well as within a few business days after the procedures were performed. Once a subject has completed all procedures per the protocol, a final study CRF monitoring log is completed for each participant. The CRF monitoring log captures that procedures were verified, data has been pushed to appropriate databases if necessary, questionnaire data entry verified if necessary, and the participant CRF folder monitoring log is signed by the LNI lab staff who verified the data. Following that process, each folder is monitored independently by other lab staff (i.e., post bac IRTA, clinical protocol coordinator, CRNP) as a final independent check that procedures were performed per protocol followed by signoff by the Principal Investigator.

16. Reportable Events

Unanticipated problems, non-compliance, and other reportable events will be reported to the NIH IRB as per Policy 801.

It is both the Principal Investigator's (PI) and the Sponsor's responsibility to ensure the safety of those on the clinical trial. The PI is responsible for tracking adverse events during the study and providing adverse events lists to the Sponsor at regular intervals per request. In addition, the PI is responsible for updating the Sponsor about known risks from the drug, as discovered from literature searches or other means.

In accordance with the requirements of 21 CFR 312.32, the PI or designee will report all SAEs, whether or not these are considered related to the investigational drug or study intervention, that occur throughout the study to the Sponsor, including those events listed in the protocol or Investigator's Brochure as anticipated to occur, as follows:

Deaths: within 24 hours of the investigator's awareness
All other SAEs: within 48 hours of the investigator's awareness
All AEs will be sent to the Sponsor quarterly, or at minimum annually, for submission to the FDA in the IND Annual Report.

The PI will immediately report all deaths and SAEs to the Sponsor by disclosing all event-related information in a completed MedWatch Form 3500A. This form should include the IND number, protocol number, PI name, and an assessment on the reasonable possibility of a relationship between the event and the study drug or intervention. The completed MedWatch Form 3500A will be sent to the Clinical Director/CEO and/or designated medical monitor with a copy to the NIH Office of Research Support & Compliance (ORSC) Regulatory Support Section.

The CEO and/or designee will be responsible for determining whether the event is reportable to the FDA as an IND Safety Report if it is a serious, unexpected, and suspected adverse reaction (SUSAR). If the sponsor determines the SAE meets the criteria of a SUSAR, the ORSC will submit an Initial IND Safety Report to the FDA no later than 15 calendar days after the PI's notification of the event to the Sponsor. Deaths or life-threatening events will be reported to the FDA no later than 7 calendar days after the PI's notification of the event to the Sponsor. The Sponsor will submit any relevant additional information in a Follow-up IND Safety Report no later than 15 calendar days after receiving the information. All SAEs will be monitored until satisfactory resolution. All AEs and SAEs will be documented on appropriate study records.

17. Alternatives to Participation

Participants neither receive nor forego treatment in order to participate in this study. The alternative, therefore, is not to participate.

18. Privacy

All research activities will be conducted in as private a setting as possible.

19. Confidentiality

a. For research data and investigator medical records

Medical records will be handled according to standard NIH Clinical Center policies, designed to prevent breach of privacy at a level considered sufficient for sensitive health care data. Investigators will be trained to respect the privacy and confidentiality of NIH employees and staff. Confidentiality and information technology standards are in place at the NIAAA and NIDA intramural programs to protect electronic repositories of patient data as well as other clinical patient related material. It is reasonably expected that these safeguards will protect participants' medical and personal health information, ensuring their privacy.

Information obtained while being screened for or participating in this protocol will become part of the patient's NIH medical record. This includes potentially sensitive information, such as results of urine tests that are positive for illicit drugs. Access to this information may be requested by third parties. Such access will not be granted without the explicit, written consent of the subjects. However,

failure on the part of the subject to provide access to the information may in itself be to the disadvantage of the subject, e.g. in the case of a potential employer or insurer requesting the information. This situation will be made clear in the consent.

b. For stored samples

Samples and data will be stored using codes that we assign. Data will be kept in password protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.

c. Special precautions

The Investigators are applying for a Certificate of Confidentiality (CoC) issued by NIH to further protect subject confidentiality.

20. Conflict of Interest

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

There are no conflicts of interest to report.

c. Role of a commercial company or sponsor

There is no drug company involved.

21. Technology Transfer

This study has a human material transfer agreement (MTA) in place under docket number #AA-MTAOUT-21-11005. This MTA is between the National Institute on Alcohol Abuse and Alcoholism (NIAAA) ("Provider") and Virginia Commonwealth University (VCU) ("Recipient") for the transfer of material isolated from individuals who have participated in clinical research (each a "Human Subject"), with or without accompanying data, to be used for research purposes and analysis of drug levels and endogenous and exogenous compounds (e.g., sex hormones) in plasma. VCU will receive coded samples and no access to the code. VCU will have no role or participation in the research protocol other than analyzing research blood samples for a fee.

22. Research and Travel Compensation

Volunteers will be compensated for time and research-related inconveniences, to the extent to which they complete them, as follows:

Procedures	Inconvenience Units	$ Payment	Frequency	Total
Screening				
Time involved	3	30	1	30

Brief History & PE	1	10	1	10
			Screening – Total Compensation	**$40**
Screening Update: (If any clinical/non-clinical tests listed in this section are out of date (not performed within 1 year of enrollment) for maintaining eligibility, they will be obtained under this study and paid under this study.				
Routine Urinalysis	1	10	1	10
Routine Bloodlabs	4	40	1	40
update Interview/ Questionnaires	2	20	1	20
Time Involved	2	20	1	20
			Screening Update – Total Compensation up to	**$90**
GE PET/MR repeat scanning **Eligibility**				
Consent with GE icf time involved, TLFB update	2	20	1	20
Routine Urinalysis	1	10	1	10
Routine Bloodlabs & EKG	4	40	1	40
			GE Eligibility – Total Compensation up to	**$70**
Study Procedures DAY A				
Urine Drug Screen	1	10	1	10
Urine pregnancy if female who can become pregnant	1	10	1	10
Brief History & PE, Drug HX Part II	1	10	1	10
Intravenous Line	4	40	1	40
PET/phMRI	20	200	1	200
MP (60 mg po)	5	50	1	50
Placebo (IV)	5	50	1	50
EKG – continuous	5	50	1	50
			DAY A – Total Compensation up to	**$ 420**
Study Procedures DAY B				
Urine Drug Screen	1	10	1	10
Urine pregnancy if female who can become pregnant	1	10	1	10
Brief History & PE, Drug HX Part II	1	10	1	10
Intravenous Line Placement(s)	4	40	1	40

PET/phMRI	20	200	1	200
Placebo (po)	5	50	1	50
MP (0.25 mg/kg IV)	5	50	1	50
EKG – continuous	5	50	1	50
			DAY B – Total Compensation up to	**$ 420**
Study Procedures DAY C				
Urine Drug Screen	1	10	1	10
Urine pregnancy if female who can become pregnant	1	10	1	10
Brief History & PE, Drug HX Part II	1	10	1	10
Intravenous Line Placement(s)	4	40	1	40
PET/phMRI	20	200	1	200
Placebo (po)	5	50	1	50
Placebo (IV)	5	50	1	50
EKG – continuous	5	50	1	50
			DAY C – Total Compensation up to	**$ 420**
Genotyping				
Blood Draw Genetic	2	20	1	20
			Genotyping – Total Compensation	**20**
NP and Behavioral				
NP Test Battery	3	30	1	30
CANTAB –test	3	30	1	30
NEO-FFI	1	10	1	10
MPQ276	3	30	1	30
Paper questionnaires	3	30	1	30
			NP – Total Compensation	**130**
GENEActiv – 1 week monitoring				
Actinometer	10	100	1	100
			GENEActiv – Total Compensation	**100**
PET/phMRI Make-up Day (contingency plan to make-up Day A, B or C)				
Urine Drug Screen	1	10	1	10
Urine pregnancy if female who can become pregnant	1	10	1	10
Brief History & PE, Drug HX Part II	1	10	1	10

Intravenous Line Placement(s)	4	40	1	40
PET/phMRI	20	200	1	200
Oral drug (MP or PL)	5	50	1	50
IV drug (MP 0.25 mg/kg or PL)	5	50	1	50
EKG – continuous	5	50	1	50
PET/phMRI Make-up Day (contingency plan) up to				**$420**
Cancellation – short notice due to fault of NIH				
Cancellation fee	20	200	up to 3X	Max $600
Cancellation – compensation				**Max $600**
Total Compensation to all subjects (consent #1) up to				**$2660**
Total Compensation to subjects who sign consent #2 (GE PET/MR) and repeat the study on the new scanner will earn an additional amount up to				**$2350**

Compensation will be prorated for parts completed if subjects do not complete the study. We have added the potential for up to three short notice cancellation payments of $200 each, for a maximum total of $600 if we cancel. We don't anticipate cancelling the study frequently. This cancellation payment will not be paid when a subject cancels on us. If needed, subjects will be provided with a taxi paid for by NIH or if they drive, they will be reimbursed for their mileage. Just in case any day of study needs to be repeated, we have added that day into the above table and subjects could earn a total of up to $2660.

For the five subjects (*n=5*) that we bring back to participate in repeating the study on the new GE PET/MR scanner in the PET Dept., we will aim to repeat everything except for Genotyping, GENEActiv and NP & Behavioral tests. Compensation to these subjects will be up to a total of $2350 (see sections shaded in pale yellow). We added a section to the chart above to cover consenting for the GE PET/MR scans and repeating lab tests that are outdated by over 1 year since last done. This amount takes into account the potential for repeating a day of scanning and up to 3 cancellations. We don't anticipate cancelling the study frequently. This cancellation payment will not be paid when a subject cancels on us or fails to meet criteria on the day of study.

NIH employees or staff who participate during work hours must have permission from their supervisor. NIH employees or staff must either participate outside of work hours or take leave in order to receive compensation.

23. References

See end of document.

24. Attachments/ Appendices

a. Eligibility checklists
Uploaded to iRIS for healthy volunteers.

b. Recruiting advertisements
Uploaded to iRIS.

c. Eligibility Exclusion Medications checklist
Uploaded to iRIS for IRB review.

d. Study Day Medications checklist
Uploaded to iRIS for IRB review

25. Consent Forms

Healthy adult volunteer uploaded to iRIS.

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