**Protocol Title:** PET Imaging of the Dopaminergic and Serotonergic Systems in Treated HIV Positive Subjects

**Protocol Number:** 18CC0117

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**Abbreviated Title:** Brain PET in Treated HIV

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Principal Investigator: Dima Hammoud, MD
Radiology & Imaging Sciences Department, CC
Building 10, Room. 1C368
10 Center Dr.
Bethesda MD 20814
Phone: 301-402-3041
Email: hammoudd@cc.nih.gov

**Investigational Agents:**

| | |
|---|---|
| Drug Name: | 18F-FDOPA |
| IND Number: | 35513 |
| Sponsor: | NIH CC |
| Manufacturer: | CC PET Department |

# Précis:

Background: An extensive body of literature points towards neuronal brain injury in human immunodeficiency virus positive (HIV-positive) subjects despite virological suppression of the virus in the periphery under the effect of antiretroviral therapies (ART). Existing evidence suggests that the central nervous system (CNS) could be an important reservoir for human immunodeficiency virus (HIV) regardless of cumulative time on treatment. This results in progressive neurocognitive dysfunction despite optimal treatment and peripheral control of the infection. Even though structural imaging studies have described abnormalities in optimally-treated HIV-positive subject population, there has been only a few attempts at deciphering the cellular levels of brain damage in those subjects using in vivo molecular imaging biomarkers. As part of CNS involvement, specific neurotransmitter systems including the dopaminergic and serotonergic systems are thought to be affected by the infection with distinct neurological, cognitive and psychological manifestations, even in optimally-treated subjects.

Objective: This protocol aims at identifying aspects of dopaminergic and serotonergic dysfunction in optimally-treated HIV-positive subjects using high resolution positron emission tomography (PET) of the brain and radioligands targeted against the dopaminergic (18F-FDOPA) and serotonergic (11C-DASB) systems.

Study population: We will identify 25 eligible HIV-infected individuals and 50 eligible HIV-negative (HIV-) individuals for the dopaminergic arm, and 20 HIV-infected individuals and 20 HIV-negative individuals for the serotonergic arm. Subjects will be selected from IRB approved NIH protocols, self-referred or will be referred form outside providers/institutions and those who meet eligibility criteria will be offered enrollment in our study.

Design: Subjects will undergo either a one-time 18F-FDOPA PET scan or a one-time 11C-DASB PET scan or both, if eligible. HIV-positive subjects and HIV-negative individuals will be included in the study.

Outcome Measures: Influx constant (Ki) for 18F-FDOPA PET and Binding potential relative to non-displaceable binding ($BP_{ND}$) values for 11C-DASB PET

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# List of Abbreviations

| | |
|---|---|
| 11C-DASB | 11C-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile |
| 18F-FDOPA | 18F-3,4-dihydroxy-6-18F-fluoro-l-phenylalanine |
| AIDS | acquired immune deficiency syndrome |
| ACC | American College of Cardiology |
| AE | adverse event |
| AHA | American Heart Association |
| ART | antiretroviral therapy |
| ASCVD | atherosclerotic cardiovascular disease |
| BBB | blood-brain barrier |
| BLD | below level of detection |
| CNS | central nervous system |
| CSF | Cerebrospinal fluid |
| DAT | dopamine reuptake transporter |
| FDA | Food and Drug Administration |
| FDG-PET | fluorodeoxyglucose-positron emission tomograph |
| HAART | highly active antiretroviral therapy |
| HAND | HIV-associated neurocognitive disorders |
| HC | healthy controls |
| HCV | hepatitis C virus |
| HBV | hepatitis B virus |
| HIV | human immunodeficiency virus |
| HRRT | high resolution research tomograph |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| MRI | magnetic resonance imaging |
| MAO | Monoamine oxidase |
| NIH | National Institutes of Health |
| OSEM | ordered-subsets expectation maximum |
| PBMCs | peripheral blood mononuclear cells |
| PET | positron emission tomography |
| PI | Principal Investigator |
| ROS | reactive oxygen species |
| SAE | serious adverse event |
| SERT | serotonin transporter |
| SIV | Simian immunodeficiency virus |
| SLE | systemic lupus erythematosus |
| SSRIs | selective serotonin reuptake inhibitors |
| SUVmean | mean standardized uptake value |
| Tat | transactivator of transcription |
| Tg rat | HIV-1 transgenic rat |

TH tyrosine hydroxylase

# 1 Introduction and Background

## 1.1 Background information:

Similar to what has been recently described in simian immunodeficiency virus (SIV) infected monkeys [1], it is our hypothesis that the central nervous system (CNS) harbors latent HIV provirus despite long-term viral suppression by ART. HIV DNA, measured by quantitative and droplet digital PCR, was identified in > 50% of brain tissues obtained from subjects with low or undetectable viral load in the periphery prior to death [2]. In the setting of appropriate viral suppression, HIV reservoirs in the brain include latently infected cells such as perivascular macrophages, microglial cells, and astrocytes [3]. Integrated HIV provirus within those cells results in the production of HIV viral proteins as has been shown with detectable levels of transactivator of transcription (Tat) in the cerebrospinal fluid (CSF) of those subjects [4]. In theory, the incomplete penetration of antiretroviral drugs across the blood-brain barrier (BBB) allows for low level production of those viral proteins which results in a subtle but continuous pattern of brain injury and neurotoxicity, eventually leading to cognitive and/or psychiatric dysfunction despite peripheral control of the infection.

An extensive body of literature points towards neuronal brain injury in HIV positive subjects despite virological suppression of the virus in the periphery under the effect of antiretroviral therapies (ART). Even though structural imaging studies have described abnormalities in optimally-treated HIV-positive population, there has been only a few attempts at deciphering the cellular levels of brain damage in those subjects using in vivo molecular imaging biomarkers of specific neurotransmitter systems. Two systems that are known to be affected by HIV are the dopaminergic and serotonergic systems.

## 1.2 Dopaminergic System Involvement:

Dopaminergic dysfunction manifested as parkinsonian symptomatology is one of the facets of HIV-associated neurocognitive disorders (HAND) [5-8]. In fact, HIV infection is associated with especially marked vulnerability of the dopaminergic system to the effect of the virus which seems to target the basal ganglia resulting in loss of dopaminergic neurons [9]. The mechanism of neurotoxicity involving the dopaminergic system is thought to be related to the neurotoxic effects of viral proteins, namely Tat and envelope glycoprotein 120 (gp120). The neurotoxicity of Tat was shown in primary hippocampal rat cell cultures in which Tat 1-72 triggered mitochondrial depolarization, increased intracellular production of reactive oxygen species (ROS) and protein oxidation, and caused neuronal degeneration [10]. Similarly, the neurotoxic effect of gp120 was demonstrated in mesencephalic neuronal/glial culture model, with gp120-induced reduced function (decreased dopamine uptake), morphological changes, and reduced viability of the dopaminergic neurons [11]. In another study, gp120 produced loss of nigrostriatal neurons, as shown both by histochemical analysis of brain sections for apoptosis and biochemical determination of dopamine concentration [12].

In vivo, dopaminergic loss was documented in SIV infected monkeys, found to have decreased neuronal number and neuronal density in the globus pallidus and substantia nigra compared to controls [13]. Dopamine defects actually seem to precede neurologic

deficits in SIV-infected monkeys, thus implicating dysfunction of the dopaminergic system in the etiopathogenesis of HIV dementia [14]. Similarly, in brain sections of subjects with known history of HIV encephalitis, striatal dopaminergic markers were abnormal: decreased presynaptic tyrosine hydroxylase (TH) protein and phosphorylated TH, increased presynaptic dopamine reuptake transporter (DAT), decreased postsynaptic dopamine receptor type 2 (D2R) and increased postsynaptic dopamine receptor type 3 (D3R) [15]. More interestingly, even in treated HIV-positive subjects, postmortem specimen revealed significant decrease in dopamine in the caudate nucleus, putamen, globus pallidus, and substantia nigra [16].

The neurotoxicity of HIV viral proteins is also synergistic with abuse drugs such as methamphetamine and cocaine that also act on the dopaminergic system [9,17,18]. Drug abuse in this subject population is a common problem and as such can severely increase dopaminergic dysfunction in already predisposed individuals. Considering the poor penetration of the BBB by a large number of highly active antiretroviral therapy (HAART) drugs, and taking into account the high prevalence of drug abuse in HIV-positive subjects, it appears that the need for neuroprotective agents in this subject population is great. The endpoints in studies of neuroprotective approaches however are variable and not easily quantifiable which results in uncertainty about the efficacy of the drugs [19,20]. Having a quantifiable secondary endpoint in these situations can significantly improve the power of the study and might provide prognostic information not otherwise available through current clinical endpoints. One such quantitative technique is positron emission tomography (PET) of the dopaminergic system.

In the literature, only two studies have been performed using PET targeting different components of the dopaminergic system. In the first study, PET imaging of DAT in HIV subjects with associated dementia (but not those without dementia) showed significantly lower DAT availability in putamen ($P = 0.009$) and ventral striatum ($P = 0.03$) compared with seronegative controls. Higher plasma viral load in the HIV dementia subjects also correlated with lower DAT in the caudate and putamen [21]. In the second study, HIV-positive subjects with (HIV-positive Coc) and without history of cocaine dependence had lower DAT in putamen while only HIV-positive Coc showed lower DAT in caudate compared to HIV-negative controls [22]. Some of the subjects in those studies however were still viremic. To our knowledge, there has been no evaluation yet of presynaptic dopamine metabolism in the setting of HIV and no evaluation of optimally treated subjects with any dopaminergic ligand.

### 1.3 Serotonergic System Involvement

Depression remains an important facet of HAND [23-26], despite significant advances in treatment of peripheral disease and control of the infection. There is however limited literature about the contribution of the serotonergic system to depressive disorders in HIV. Using real-time (RT)-PCR, the levels of serotonin transporter (SERT) mRNA in the peripheral blood mononuclear cells (PBMCs) of SHIV infected rhesus macaques was significantly reduced in comparison to control animals, suggesting SERT expression might be affected in HIV/acquired immune deficiency syndrome (AIDS) [27]. Tryptophan metabolism dysregulation is also suspected to play a role in HIV-1 associated depression [28,29]. One PET study targeting SERT in the setting of HIV depression showed lower 11C-DASB (a PET ligand that specifically targets SERT in the brain) binding in HIV-positive subjects compared to healthy controls (HC), however with depressed subjects showing higher 11C-DASB uptake compared to non-depressed subjects [30]. No other cross-

sectional or longitudinal PET studies have been performed to confirm this observation. All the findings above suggest a possible role for dysregulated serotonergic transmission in HIV-associated depression.

### 1.4 Summary of Relevant Preliminary Studies:

In our human studies using fluorodeoxyglucose positron emission tomography (FDG-PET) scanning, there was generalized hypometabolism in the HIV-positive subjects compared to age-matched HC. We found statistically significant lower whole brain maximum standardized uptake value (SUVmax) values in HIV-positive compared to HC but not to HIV-negative (HIV-) subjects with similar co-morbidities to the HIV-positive group. Among regions, mean standardized uptake (SUVmean) values in the thalamus, caudate and cerebellum were significantly lower than HC but not compared to HIV-negative subjects. Among the relative SUVmean measurements however, only the thalamic values were significantly lower in HIV-positive compared to both HC and to HIV-negative subjects. Using a mixed-effect statistical model, the most predictive clinical variables for thalamic relative SUVmean values was study group (HIV status), cardiovascular disease risk and prior drug use. Considering the HIV-positive group separately, cardiovascular disease risk predicted most of the other SUV values. Our findings suggest an HIV specific effect on hypometabolism in the brain that is separate from the associated co-morbidities.

Along the same lines, we have also evaluated HIV-1 transgenic (Tg) rats in our lab, looking for dopaminergic dysfunction. Along with decreased D2/D3R expression on PET, an interesting observation we made was significantly decreased TH staining. This suggested to us that there is a strong element of presynaptic dopaminergic loss in those animals which might be even greater than the postsynaptic deficit[31]. We then proceeded to evaluate DAT using a specific PET ligand (18F-FP-CMT). Decreased DAT expression in older Tg rats was seen, suggesting both pre and post synaptic dopaminergic deficits in this animal model of treated HIV-positive subjects[32].

Preliminary results in SIV infected monkeys: we have recently imaged SIV infected monkeys at baseline, before inoculation and at multiple time points after inoculation. Out of seven monkeys, six animals demonstrated a consistent trend of increased 11C-DASB binding especially in areas with high binding such as the midbrain and thalamus. This could reflect upregulation (increased expression) of SERT under the effect of SIV infection or could reflect increased affinity of the receptor. The first possibility is the more likely one since no allosteric modifications that would change receptor affinity have been described yet for SERT.

### 1.5 Rationale:

One issue of neurocognitive dysfunction in optimally-treated and virologically-controlled subjects is the magnitude of actual brain damage, and how much of it is related to HIV versus other commonly encountered co-morbidities in this subject population such as drug abuse, alcohol abuse, and co-infections (e.g. hepatitis C virus [HCV]). FDG-PET scans showed evidence of hypometabolism in the HIV-positive subjects however hypometabolism in some brain regions was also seen in HIV-negative individuals with co-morbidities. A few regions like the thalamus and caudate, however, demonstrated a more severe involvement in the HIV-positive group compared to the healthy and HIV-negative individuals with co-morbidities suggesting a definite role for HIV in the neuropathology. To better understand the effect of HIV on various neurotransmitter

systems beyond the effect of co-morbidities, a detailed evaluation of those systems becomes necessary. Although this has been attempted previously using different ligands, the current proposal will be the first to assess the pre-synaptic dopaminergic and serotonergic systems in a homogenous, very well characterized, optimally-treated HIV-positive subject population.

# 2 Study Objectives

## 2.1 Primary Objectives

To understand the pathophysiology of dopaminergic and serotonergic involvement in chronically infected and virologically controlled HIV-positive subjects compared to HIV-negative subjects using high resolution PET scanning with 18F-FDOPA and 11C-DASB.

## 2.2 Secondary Objectives

- To distinguish dopaminergic neurotransmitter damage related to HIV versus co-morbidities.

- To correlate 11C-DASB binding in the brain with depressive symptomatology in optimally-treated HIV-positive subjects.

- To correlate in vivo uptake of 18F-FDOPA and 11C-DASB PET with duration of infection with HIV, the duration of HIV infection prior to the initiation of antiretroviral therapy (ART), and nadir CD4.

# 3 Subjects

## 3.1 Description of study population: All Subjects (Groups A-E):

Subjects will include chronically infected HIV-positive subjects with plasma HIV-RNA <100 copies/$mm^3$ or below level of detection (BLD) for greater than one year () along with HIV-negative subjects with and without co-morbidities.

Accrual Ceiling: 155
Dopaminergic arm: 35 HIV-positive and 60 HIV-negative subjects (total 95 subjects)

Serotonergic arm: 30 HIV-positive and 30 HIV-negative subjects (total 60 subjects)

Our target sample size is:

Dopaminergic arm: 25 HIV-positive and 50 HIV-negative subjects (total 75 subjects)

Serotonergic arm: 20 HIV-positive and 20 HIV-negative subjects (total 40 subjects)

Any withdrawals/dropouts will be replaced.

NIH employees may participate if eligible. Subordinates of investigators will not be allowed to participate.

## 3.2 Inclusion criteria

**Subject groups:**

**Dopaminergic arm:**

**Group A: HIV-positive subjects with or without co- morbidities**
**Group B: HIV-negative subjects with co-morbidities**
**Group C: HIV-negative subjects without co-morbidities**

**Serotonergic arm:**

**Group D: HIV-positive subjects with or without co-morbidities**
**Group E: HIV-negative subjects with or without co-morbidities**

3.2.1 **All Subjects (Groups A-E):**

1. Men and women, 18-70 years of age
2. Ability to sign informed consent by the subject
3. Subjects may be enrolled in or have been discharged from IRB approved NIH protocols OR subjects may be referred from outside providers/institutions.
4. Has the ability to be seen by an outside medical doctor who provides care.

3.2.2 **All HIV-positive Subjects with or without co-morbidities (Groups A [dopaminergic arm, n=25)] and Group D [serotonergic arm, n=20])**

1. Known and documented HIV-1 infection
2. Plasma HIV-RNA BLD (<100 copies/$mm^3$) for greater than one year since the last available documented viral load measurement.
3. At least one year of continuous ART prior to last documented suppressed viral load measurement and no history of ART modification or interruption since then.

3.2.3 **HIV-negative Subjects WITH Co-morbidities (Group B, n=25)**

1. HIV-antibody negative
2. At least one or more of the following criteria:
    a. Hypertension, as defined by treatment with medications for hypertension or with a systolic blood pressure at screening ≥140mm Hg.
    b. Diabetes mellitus, as defined by HgbA1C ≥6.5% or known treatment for diabetes.
    c. Hepatitis C infection as documented by lab results of a positive Hepatitis C antibody and/or detectable Hepatitis C viral load. Subjects who responded to HCV treatment (SVR) will be included.
    d. History of previous but not current drug abuse
    e. History of previous but not current alcoholism (defined as alcohol intake that affect/affected the subject's work or home life).
    f. Clinical atherosclerotic cardiovascular disease (ASCVD) (e.g. history of acute coronary syndromes, or myocardial infarction, stable or unstable angina, coronary or other arterial revascularization or peripheral arterial disease of atherosclerotic origin) and/or 10-year heart disease risk score (ASCVD risk score) >7.5% (7.5 % score is the threshold for starting statin therapy as per the 2013 American College of Cardiology [ACC] / American Heart Association [AHA] guidelines [33]).

3.2.4 **HIV-negative Subjects WITHOUT co-morbidities (Group C, n=25)**

1. HIV-antibody negative
2. No history of any of the following:
    a. Hypertension, as defined by treatment with medications for hypertension or with a systolic blood pressure at screening ≥140mm Hg.
    b. Diabetes mellitus, as defined HgbA1C ≥6.5% or treatment for diabetes.
    c. Hepatitis C infection as documented by lab results of positive Hepatitis C antibody and/or detectable Hepatitis C viral load. Subjects who responded to HCV treatment (SVR) will not be included.

d. History of previous drug abuse.
e. History of previous alcoholism. Alcoholism is based on alcohol having affected the subject's work or home life.
f. Clinical ASCVD (e.g. history of acute coronary syndromes, or myocardial infarction, stable or unstable angina, coronary or other arterial revascularization or peripheral arterial disease of atherosclerotic origin) and/or 10-year heart disease risk score (ASCVD risk score) >7.5% (7.5 % score is the threshold for starting statin therapy as per the 2013 ACC/AHA guidelines [33]).
g. Any other disease entities including chronic infections (e.g. Hepatitis B, Lyme disease), neurological diseases (e.g. Multiple sclerosis, vasculitis) or systemic diseases (e.g. Sjogren's diseases, sarcoidosis, systemic lupus erythematosus [SLE]) that in the opinion of the investigator would be considered a significant co-morbidity.

3.2.5 **HIV-negative Subjects with or without co-morbidities (Group E, n=20)**

1. HIV-antibody negative

**3.3 Exclusion criteria**

3.3.1 **All Subjects (Groups A-E):**

1. Illness or other condition that, in the opinion of the PI, may interfere with study participation at the time of enrollment, including known history of significant intracranial structural damage such as previous stroke(s) or history of intracranial benign or malignant tumors.

2. Conditions other than HAND associated with cognitive impairment or dementia such as Alzheimer's, Parkinson's disease, head injury with loss of consciousness >30 minutes, or seizure disorders.

3. A positive screening result for psychiatric diseases that are known to affect the dopaminergic or serotonergic systems.

4. Current substance abuse that would interfere with PET scan results at the investigators' discretion [36-41].

5. Medications: use of any drug with known dopaminergic or serotonergic activity within 6 months prior to planned imaging date(s).

6. Pregnant or Lactating women: Women of childbearing potential must have a negative serum or urine pregnancy test within 1 week prior to study entry. Pregnancy testing will also be performed in enrolled female participants prior to any radiation exposure.

7. Prior or planned/anticipated exposure to radiation due to clinical care or participation in other research protocols, which would exceed the recommended acceptable annual limit of radiation exposure once accounting for the requirements of the current study.

3.3.2 **Additional Exclusion Criteria for the Dopaminergic Arm (Groups A, B and C):**

1. Use of any of the following drugs within 6 months from planned imaging date(s):
    a) Haloperidol (increased intracerebral dopamine turnover caused by haloperidol may result in increased accumulation of 18F-DOPA) [34]
    b) Monoamine oxidase (MAO) inhibitors (may result in increased accumulation of 18F-DOPA in the brain) [34]
    c) Reserpine (reserpine-induced depletion of the contents of intraneuronal vesicles may prevent retention of 18F-DOPA in the brain) [35]
    d) Carbidopa/LDOPA (LDOPA competes with 18F-DOPA for DOPA decarboxylase activity) [35-40])
2. Allergy to carbidopa

Note that HBV is not an exclusion criterion for groups A, B, D or E since it's not known to have direct CNS pathology in the absence of advanced liver disease and cirrhosis.

# 4 Study Design and Methods

## 4.1 Study overview

Subjects will be recruited through protocol number 13-N-0149 (ALL HANDS protocol, PI Dr. Avindra Nath), other IRB-approved intramural NIH protocols, or through referrals from outside providers/institutions or self-referred. Participants will be evaluated for eligibility to one of the groups (A-E). Once consented and found eligible, subjects will undergo PET scanning using 18F-FDOPA and/or 11C-DASB. All the visits will be on outpatient basis unless a participant is inpatient at the Clinical Center for another protocol. There will be no follow-up visits after the completion of the PET scan(s). In case the planned scans cannot be done for any reason and the subject is still eligible and willing to participate, a second visit will be scheduled to complete the study procedures.

## 4.2 Recruitment

Subjects will be recruited through protocol number 13-N-0149 (ALL HANDS protocol, PI Dr. Avindra Nath), through other IRB-approved intramural NIH protocols, through referrals from outside providers/institutions or through self-referral.

We will send an IRB approved letter to the PIs of NIH protocols targeting HIV infected subjects (Appendix B) and ask for help in identifying potential subjects in their protocols who might be interested in our study. We will also include an IRB approved flyer describing our study to give to volunteers. If the subjects express interest, we will access their medical record at NIH to evaluate if they are potentially eligible. We will then proceed to contact them for more information as below.

If we get referrals from outside providers/institutions or self-referred potential subjects contact us, we will conduct a phone interview and ask them to send us relevant medical records to evaluate if they satisfy the eligibility criteria.

When a subject (either referred from an NIH protocol, from outside providers/institutions or self-referred) is evaluated and found to be potentially eligible based on the inclusion/exclusion criteria of one or more of the groups, he/she will be re-contacted

about participating in one or more of our PET studies. If the subject is at NIH, the investigators will explain the nature of the study with all the associated risks in person. If they are not at NIH, investigators will call the subject to explain the nature of the study with all the associated risks. If the subjects are still interested in participating, they will be asked to sign the informed consent document before any study procedures are conducted and they will be scheduled for screening procedures to evaluate if they are eligible. For NIH employees, there will be no direct solicitation of employees/staff by supervisors.

### 4.3 Schedule of Activities (SOA)

| Procedures | Time Point [a] | | |
|---|---|---|---|
| | Screening | Visit 1 | Visit 2 |
| Informed consent | X | | |
| Demographics | X | | |
| Medical history | X | | |
| Physical exam and vital signs | X | | |
| Client Diagnostic Questionnaire (CDQ) | X | | |
| Laboratory Tests [b] | X | | |
| Urine toxicology screen [c] | X | X | |
| Pregnancy test [c] | | X | |
| Neuropsychological testing [d] | | | X |
| MRI (Brain) | X | | |
| PET (18F-FDOPA and/or 11C-DASB) [e] | | X | |
| Adverse event review and evaluation | | X | |

[a] If not all procedures could be completed in one visit, they could be rescheduled to another day.
[b] HIV RNA Viral Load (only for groups A and D)), CBC/diff/platelets, hepatic panel, Hemoglobin A1c (if known history of Diabetes mellitus), fasting lipid profile, Acute Care Panel, Hep C antibody and HCV RNA Quant) (No need to be performed if its performed within 3 month of planned PET scan)
[c] Urine toxicology and urine or serum pregnancy test will be performed 48 hours or less prior to the planned scan and must be checked before scanning.
[d] Neuropsychological test may include the following: WTAR; WAIS-III; TMT; HVLT-R; BVMT-R; WCST-64; COWAT; FAS and Animals; PASAT; Grooved Pegboard Test; Judgment of Line Orientation and Medical Symptom Validity Test.
[e] The PET (18F-FDOPA and/or 11C-DASB) scanning will be performed either on the same day (one visit) or on two separate visits based on the subject's preferences and scan time availability.

### 4.4 Screening

#### 4.4.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects.
- Review of existing medical records to include H&P, laboratory studies, etc.

- Review of existing brain MRI images.

Since this study was approved before revised Common Rule that did not allow pre-screening procedures without the consent, it is being requested that consent for pre-screening procedures be waived

4.4.2 Screening activities performed after a consent for screening has been signed

The evaluations will be completed at the National Institutes of Health (NIH) Clinical Center. We will obtain informed consent before any screening procedures. The following procedures will be performed for screening purposes:

- History and physical exam including vital signs (No need to be repeated if it has been performed at NIH within 3 month of planned PET scan)
- Laboratory tests: HIV RNA Viral Load (only for groups A and D), CBC/diff/platelets, hepatic panel, Hemoglobin A1c (if known history of Diabetes mellitus), fasting lipid profile, Acute Care Panel, Hep C antibody and HCV RNA Quant). (No need to be repeated if performed at NIH within 3 month of planned PET scan)
- MRI Brain (No need to be repeated if performed at NIH within one year of planned PET scan)
- Client diagnostic test (CDQ) (No need to be repeated if performed at NIH within 3 months of planned PET scan)

4.4.2.1 Client Diagnostic Test (CDQ)

Psychiatric disorders and substance use disorders will be assessed with the Client Diagnostic Questionnaire (CDQ) [41], a psychiatric screening tool that was adapted from the PRIME-MD [42]a well-validated screening tool for assessing psychiatric disorders and substance use disorders in primary care settings. The CDQ was developed and validated for persons affected by HIV. For the diagnosis of any disorder, the sensitivity, specificity, overall accuracy, and kappa coefficient of the CDQ were 91%, 78%, 85% and 70%, respectively. We will use the CDQ to screen for presence of major depressive disorder, other depressive disorder, panic disorder, generalized anxiety disorder, post-traumatic stress disorder (PTSD), psychosis, alcohol abuse and substance abuse. If during the CDQ, we identify evidence of severe depression ("I would like to kill myself" or "I would kill myself if I had a chance"), a psychiatric consult will automatically be offered to the subject with the goal to assess his or her immediate safety. The role of this psychiatry consult is only to assess and manage immediate safety concerns, and not to provide an ongoing mental health treatment. However, in each case where the psychiatrist or psychologist, following the safety assessment, recommends mental health treatment, the participant will be advised to pursue the recommended treatment outside of the clinical center and according to her/his healthcare access plan.

4.4.2.2 MRI brain

Subjects will undergo a 3 tesla MRI scan of the brain for research purposes and to evaluate anatomical abnormalities. This scan may also be used for localization of PET regions for other studies of HIV-HAND planning to use PET. MRI scanning will be performed at the NIH Clinical Center or the NMR Center, and the procedure takes about 1-2 hours. Persons requiring more than oral lorazepam (or an equivalent) as sedation for MRI will be excluded from the protocol.

The imaging protocol may include:

2. Basic anatomical imaging sequences, including but not limited to PD/T2-weighted images as well as unenhanced T1, T2*, and FLAIR images.
3. Diffusion weighted imaging.
4. 3D T1weighted images, which will be used for co-registration with the PET.

Interpretation of imaging studies: All structural MR images of the brain will be interpreted qualitatively by the neuroradiologist on call as soon as possible after the completion of the scan. During scanning, the images will be monitored for quality (e.g., motion artifacts degrading the images) and repeated as needed. Coregistration of 3D T1 weighted images with the PET images will be performed later on a specialized workstation connected to the PACS system in Radiology/nuclear medicine. The MRI imaging will be used to rule out major acute abnormalities that need immediate clinical attention, or subacute/chronic conditions that require further medical care. This information will be shared with the subject and his/her primary doctor. If needed, the subject will also be referred to his/her primary doctor for further evaluations and treatment. If the subject does not have insurance or primary care physician, the study team will assist him/her in finding medical care.

MRI scans are performed on FDA-approved scanners with FDA-approved radiofrequency coils and FDA-approved pulse sequences, and their use conforms to the corresponding FDA labels. Acquisition parameters may be modified within the permitted range. These studies may involve software modifications as allowed under 21 CFR 812.2(b)(4), which stipulates that any modification "is not for the purpose of determining safety or effectiveness and does not put subjects at risk." All research pulse sequences will not conform to the FDA's Criteria for Significant Risk Investigations of Magnetic Resonance Diagnostic Devices.

We use MR Image Reconstruction and Analysis Software, this software is provided/developed by Pmod (Zurich, Switzerland). We use Pmod to reconstruct MRI imaging and co-register MRI to PET scans acquired in this study. This software is used for research purposes only and not intended for use for clinical diagnosis or direct clinical efficacy testing. We classify the software as an NSR device under this protocol. For the purposes of this protocol, research post processing software is exempt from the IDE regulations under 21 CFR 812.2(c) because it is noninvasive, does not require an invasive sampling procedure that presents significant risk, and does not by design or intention introduce energy into a subject.

## 4.5 Study Procedures

### 4.5.1 Clinical Evaluations

An interim medical history (including antiretroviral adherence) and assessment of vital signs will be performed within 48 hours of the scan. If in the assessment of the investigator, there are interim findings that could impact selection group criteria (e.g. change in medications or new symptoms), additional screening lab work may be ordered and scanning will be not be performed until the results are back. Abnormal results will be communicated to subjects.

4.5.2 Laboratory Evaluations

For all subjects: A urine toxicology screen will be performed 48 hours or less prior to the planned scan date, and will be checked before scanning.

For women of childbearing potential: A human chorionic gonadotropin (β-hCG) test with a sensitivity of 25-50 mIU/mL using urine or serum will be performed 48 hours or less prior to the planned scan and must be confirmed as negative before scanning.

Both the urine pregnancy and toxicology screening results are required prior to proceeding to the PET scan.

All the laboratory tests performed under this protocol are solely for research purposes.

4.5.3 Neuropsychological Testing

All subjects who enroll will undergo neuropsychological testing to evaluate for presence and severity of cognitive impairment (see Appendix A).
Tests will be administered by an experienced neuropsychologist or trained psychometrician under the supervision of a neuropsychologist. These tests will be administered to establish an initial baseline of neuropsychological functioning and diagnostic classification. This battery of tests is consistent with instruments used in other HIV related studies assessing cognitive functioning, both inside and outside of the NIH (i.e., it is largely consistent with the CHARTER battery [43], but includes a few additional instruments). The battery provides a thorough evaluation of the major domains of cognitive functioning.
Neuropsychological testing results will be accepted if performed within one year of enrollment and if consistent with the NIH group of tests. If testing was performed outside of NIH, subjects will need to arrange for copies of the raw data to be sent to the NIH protocol neuropsychologist prior to initial visit so the validity of the testing and the scores can be verified. For subjects enrolled in other NIH studies the data can be made available across protocols with the subject's consent.

***Brief Description of Neuropsychological Tests***

The primary purpose of our neuropsychological battery is for classification of HAND and not for outcome scores, per se. The HAND classifications that we will use for this study to estimate the prevalence of HAND will remain consistent throughout the course of the study. We will also explore in this study how other classification methods will compare against the gold standard HAND diagnosis, but this will not be a primary outcome measure of this study. Nevertheless, the primary measures we will look at to make such classification will include demographically corrected T-scores for the following:

1. **Wechsler Test of Adult Reading (WTAR)** (The Psychological Corporation, 2001 #31) is a test of word-reading used to estimate premorbid intelligence. (Scoring: this test is administered to estimate IQ and is not used for classification, per se.)
2. **Wechsler Adult Intelligence Scale – Third Edition (WAIS-III)** subtests [44] including Coding, Symbol Search and Letter-Number Sequencing will be administered. They measure aspects of working memory and information processing speed. (Scoring: Digit Symbol/Coding, Symbol Search, and Letter-Number Sequencing: number correct or number correct minus errors, as specified in the test manual.)
3. **Trail Making Test (TMT) Parts A and B** [45] is a test of visual attention and task switching that require visual scanning, number and letter sequencing, and visual

motor speed. The test requires the individual to connect the dots of 25 consecutive targets (numbers only in Trail A and then numbers and letters in Trail B) on sheets of paper. (Scoring: completion time.)

4. **Hopkins Verbal Learning Test-Revised (HVLT-R)** [46] is a list-learning task assessing immediate and delayed recall as well as recognition. (Scoring: total recall and delayed recall.)
5. **Brief Visual Memory Test-Revised (BVMT-R)** [47] is a test of visual memory assessing immediate and delayed recall. (Scoring: total recall and delayed recall.)
6. **Wisconsin Card Sort Test (WCST-64)** [48] is an objective measure of executive dysfunction, emphasizing abstract reasoning, ability to use feedback to shift cognitive sets, and goal-directed behavior in response to changing contingencies. (Scoring: total errors and perseverative errors.)
7. **Controlled Oral Word Association Test (COWAT; FAS and Animals)** [49] measure language and executive functioning test by evaluating the spontaneous production of words beginning with a given letter or of a given class within a limited amount of time. (Scoring**:** number of words generated and semantic fluency.**)**
8. **Paced Auditory Serial Addition Test (PASAT)** [50] is a measure of cognitive function that specifically assesses auditory information processing speed and flexibility, calculation ability, and working memory. (Scoring: number correct.)
9. **Grooved Pegboard Test** [51] is an assessment of bilateral fine motor manual dexterity. (Scoring: speed for dominant and non -dominant hands.
10. **Judgment of Line Orientation** [52] is a test of visuospatial abilities. (Scoring: number correct.)
11. **Medical Symptom Validity Test** [53] is a memory-based test of symptom validity.

In addition to this list of tests, we will be screening for depression using the Beck Depression Inventory - II [54]. A high score on the BDI-II will not preclude participation in the protocol, but will be taken into consideration when attempting to classify HAND. When BDI-II item 9 is endorsed at level 2 or 3 ("I would like to kill myself" or "I would kill myself if I had a chance"), a psychiatric consult will automatically be offered to the subject with the goal to assess his or her immediate safety. If item 9 is endorsed at level 1, a determination will be made by the study psychologist or psychiatrist whether offering a full psychiatry consult is necessary. The role of this psychiatry consult is only to assess and manage immediate safety concerns, and not to provide an ongoing mental health treatment. However, in each case where the psychiatrist or psychologist, following the safety assessment, recommends mental health treatment, the participant will be advised to pursue the recommended treatment outside of the clinical center and according to her/his healthcare access plan.

We will also assess subjects' level of functional disability through questionnaire and/or tasks. Texas Functional Living Scale (Cullum, Weiner, and Saine, 2009), Patient's Assessment of Own Functioning (PAOFI; Chelune, Heaton, and Lehman, 1986), and the Lawton Activities of Daily Living (Self and Other). Additional clinical neuropsychological measures as described in Lezak, Howieson, and Loring [55] and/or Strauss, Sherman, and Spreen [56] or other similar neuropsychological test compendia may be administered as the situation warrants, such as to rule out other primary or secondary diagnoses, follow up on interesting leads for future research, and/or for more in depth description of subjects. Examples of additional measures include: subtests from the

Delis-Kaplan Executive Function System [57], California Verbal Learning Test-II [58], and the Wechsler Memory Scale-III [59].

As part of the neuropsychological testing we will be using the Clinical Trials Survey System (CTSS). Portions of this testing will be administered on a computer after the participant has signed informed consent. The forms used may include Activities of Daily Living, Neurobehavioral Medical Screen, Employment Questionnaire, and Patient's Assessment of Own Functioning.

4.5.4 Imaging Studies

The imaging studies in this protocol include radiation exposure and are performed solely for research purposes. Each PET scan will be completed over the course of one visit based on scheduling and participant convenience. We will scan subjects on the CPS/CTI high resolution research tomograph (HRRT), head-only camera.

Both the 18F-FDOPA and 11C-DASB PET scans may be completed by each participant if eligible, however each scan will be performed only once (this is a cross-sectional only PET study and there will be no longitudinal scans). If a participant is found to be eligible for both PET studies, the scanning will be performed either on the same day (one visit) or on two separate visits based on the subject's preferences and scan time availability. If performed on the same day, the 11C-DASB scan will be performed first (in the morning) and the 18F-DOPA will be performed after at least seven half-lives of the isotope (11C half-life = 20 minutes) which is equivalent to 140 minutes after injection of 11C-DASB.

4.5.4.1 18F-DOPA PET Procedure

18F-DOPA is a Radioactive Research Drug (regulated under 21 CFR 361). The drug will be used under the approved Investigational New Drug (IND) No 35513. The sponsor of the IND is the NIH clinical Center and the authorized representative of the sponsor is Dr. Peter Herscovitch, PET department.

Subjects will be asked to fast for 4-6 hours prior to scanning. The exact time to begin fasting will be determined based on the planned FDOPA scan time. If the patient is diabetic and taking oral medications or long acting insulin, we will ask him/her not to make any changes. If they are taking short acting insulin before meals, we will ask them not to take their dose until after the scan. We will make an effort to schedule them for an early scan to avoid long fasting. The maximum number of hours fasting will be seven and a half hours (including scanning time of 90 minutes). If we suspect the patient to be hypoglycemic at any point, we will check blood sugar level by finger stick.

Upon presentation to the PET department at the NIH Clinical Center, subjects will receive 200 mg of carbidopa orally (to reduce peripheral metabolism of FDOPA and increase tracer availability in the brain) one hour prior to the injection of labeled 18F-DOPA. 18F-DOPA is metabolized peripherally. Carbidopa inhibits peripheral DOPA decarboxylase and increases delivery of 18F-DOPA to the brain, improving image quality [60]. It is widely used for this purpose in conjunction with 18F-DOPA scanning. This dose (200 mg) is higher than the clinical dose (12.5-25 mg TID) however there has been no reported issues or side effects although it has been used in hundreds of PET studies at and outside NIH. The maximum daily dosage for Sinemet (carbidopa/levodopa, 25/100) as approved by the Food and Drug Administration (FDA) is eight tablets, which corresponds to 200 mg of carbidopa. A recent study has even showed no side effects when subjects took the drugs at dosages above the 800 mg threshold [61].

Scanning procedure: Subjects will be positioned in the scanner and a swimming cap with small light reflectors will be put on their head. It is used to monitor the head position and movement during the scan; the information is used in the PET image reconstruction process to reduce any blurring of the PET images. Prior to the 18F-DOPA administration, a transmission scan will be obtained with a rotating Cs-137 source for attenuation correction.

18F-DOPA administration – Approximately 10-12 mCi of 18F-DOPA will be brought by shielded carrier to the PET scanner. The radiopharmaceutical will be injected over a period of approximately 1-2 minutes using an FDA-approved infusion pump under the supervision of an authorized user of radiopharmaceuticals. Subsequently, dynamic PET imaging will be obtained in list mode for a total scan duration of 90 min.

After the completion of the scan, subjects will empty their bladder and will be instructed to drink plenty of fluids and void at approximately 2-hour intervals thereafter.

4.5.4.2 11C-DASB PET Procedure

11C-DASB is a Radioactive Research Drug (Regulated under 21 CFR 361). The drug has been approved by the Radioactive Drug Research Committee (RDRC) at NIH (Rad Authorization No.: 2675). 11C-DASB use at NIH does not require an IND.

Scanning procedure: Subjects will be positioned in the scanner and a swimming cap with small light reflectors will be put on their head. It is used to monitor the head position and movement during the scan; the information is used in the PET image reconstruction process to reduce any blurring of the PET images. Prior to the 11C-DASB administration, a transmission scan will be obtained with a rotating Cs-137 source for attenuation correction.

11C-DASB administration: Approximately 20 mCi of 11C-DASB will be brought by shielded carrier to the PET scanner. The radiopharmaceutical will be injected over a period of approximately 1-2 minutes using an FDA-approved infusion pump under the supervision of an authorized user of radiopharmaceuticals. Subsequently, dynamic PET imaging will be obtained in list mode for a total scan duration of 90 min.

After the completion of the scan, subjects will empty their bladder and will be instructed to drink plenty of fluids and void at approximately 2-hour intervals thereafter. All procedures are solely for research purposes. Radiation will be used for research purposes only. There will be no “follow-up visits” done after completion of the PET scan(s).

### 4.6 End of participation

Once protocol procedures have been completed, study participants will be considered off study, and patients can continue their participation in any other NIH protocols if they already are enrolled in those protocols. Subjects will be notified of new abnormal laboratory test results. Other laboratory results will be available to them per request, including via the patient portal. Because PET scans have low resolution and do not provide structural information like MRI, the scans will be reviewed for quality control in real time but not for incidental findings. No qualitative description of the findings will be provided to the subject.

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Death
- Screen Failure

# 5 Management of Data and Samples

## 5.1 Storage

No blood or urine samples will be stored for this study. Test results and data from the imaging studies will be stored in the archives of the NIH PET department and will be accessible only by the investigator and investigator team using a pre-allocated user name and password. The raw data will also be stored in an NIH password protected research folder (\\ccruby\GROUPS\HammoudLab) only accessible by the principle investigator's team. Only investigators or their designees will have access to the data.

## 5.2 Data and sample sharing plan

This protocol is not subject to the Genomic Data Sharing (GDS) policy.

Blood and urine test results and imaging data 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 from this protocol may be open-access or restricted access.

Blood and urine test results and imaging 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 data will be transferred in accordance with NIH and federal regulations.

# 6 Additional Considerations

## 6.1 Research with investigational drugs or devices

18F-DOPA is a Radioactive Research Drug (regulated under 21 CFR 361). The drug will be used under the approved Investigational New Drug (IND) No 35513. The sponsor of the IND is the NIH clinical Center and the authorized representative of the sponsor is Dr. Peter Herscovitch, PET department.

## 6.2 Gene therapy

There are no gene therapies used for this protocol.

# 7 Risks and Discomforts

## 7.1 Potential Risks

Potential risks from this study include those associated with: 1) Neuropsychological Testing, 2) MRI scanning 3) placement of venous lines, 4) PET scans, 5) radiation exposure from the PET ligands.

**Neuropsychological Testing**
Subjects may be uncomfortable talking about how they feel. Portions of the different tests may be somewhat challenging, and answering questions about memory, ability to learn and think, and mood may be difficult for some people. The neuropsychological tests are not harmful, but may be frustrating or stressful. We will only ask that they try their best and inform them that no one performs perfectly on these tasks. If the subject gets tired or frustrated during the evaluation, they can ask for a break, they may choose not to answer every question, or they may choose to stop the testing all together.

**Client Diagnostic Questionnaire**
Subject may feel uncomfortable talking about their feelings and remembering past experiences that made them feel sad or scared. Subjects can choose to not answer questions if they don't want to answer. Subjects can stop the questionnaire at any time.

**MRI Scanning**
The MRI images obtained are the same as routinely obtained scans for a variety of clinical and research reasons. MRI uses powerful magnetic fields and electromagnetic radiation (radio waves). Adverse Events associated with MRI are extremely rare, and have generally been associated with lapses in process, e.g. inadvertent presence of a metallic object within the suite. Very rarely, accidents related to the RF coils have caused AEs. The feeling of being isolated or confined in the scanner may cause some subjects to request that the procedure be stopped, and it may be expected that some subjects in this protocol will also be unable or unwilling to cooperate. We hope to minimize these problems by carefully explaining the procedure and maintaining voice contact with the subject at all times. The most likely complication is that a subject will be unable to hold still for the duration of the procedure. This would result in the degradation of the quality of the image obtained, but it would not be dangerous to the subject. All subjects will complete a detailed questionnaire for metal screening, which will address possible occupational or accidental exposure to metal slivers, shavings, shrapnel, etc. Those with a history suggestive of such a problem will be excluded. No subjects with surgically implanted metallic objects, which could affect the magnets used in the scanner, will be eligible to enter the study.
Once on the table, the patient's head will be secured with cushions. The table will then be slowly moved into the scanner. The subject will be informed that the scan can be stopped at any time should he/she be too uncomfortable. There is no evidence that scanning at high magnetic field strengths is dangerous, but long-term effects are not known.

**Venous catheter insertion:** Venous catheter insertion can be associated with pain, bruising, infection, or clot formation. Using proper placement techniques will minimize these risks.

**PET scanning:** There are no known physical hazards to subjects from being inside the PET scanner, which detects injected radioactivity within the body. Additionally, the scanning procedures are conducted in the presence of trained staff, should subjects experience any discomfort and require medical attention. Subjects can communicate with the staff while in the scanner and can withdraw from the study at any time, if they wish to do so. Occasionally subjects become anxious during the scanning procedure, in which case,

they can request the operator to stop the scanning and to be removed from the scanning bed.

**18F-FDOPA:** 18F-DOPA is a radioactive molecule used for dopaminergic imaging. After intravenous injection, 18F-DOPA is taken in organs proportionally to the levels of DOPA decarboxylase enzyme within those organs. Imaging begins 1 hour after administration of DOPA decarboxylase blocker, carbidopa. Carbidopa does not cross the BBB and thus only blocks enzyme activity in the periphery. This results in increased 18F-DOPA levels in the brain. Imaging begins at the same time as the administration of 18F-DOPA intravenously. The principal route of excretion of 18F-DOPA is through the urinary system. There are no known side effects associated with 18F-DOPA injection. The dose of 18F-DOPA administered is approximately 10-12 mCi per scan (per year).

**11C-DASB:** 11C-DASB is a radioactive molecule targeted against SERT, and commonly used for serotonergic imaging. After intravenous injection, 11C-DASB is taken in organs proportionally to the levels of SERT. Imaging begins at the same time as the administration of 11C-DASB intravenously. The principal route of excretion of 11C-DASB is through the urinary system. There are no known side effects associated with 11C-DASB injection. The dose of 11C-DASB administered is approximately 20 mCi per scan (per year).

**Administration of Carbidopa:** There are no reports of adverse events when carbidopa is given once at the dose used in this PET scan (200 mg).

**Radiation Exposure Risks:** PET scans require exposure to radiation. All tests requiring subject exposure to ionizing radiation are considered potentially hazardous. A detailed breakdown of the radiation exposure risks expected for the subjects enrolled in this protocol are included in the NIH 88-23(a) Application for Authorization to Use Radiation in Research Involving Human Subjects. The radiation received conforms to the NIH Radiation Safety Committee guidelines for subjects participating in research studies, defined as a 5 rem effective dose in 12 consecutive months.

The total radiation exposure from the 18F-DOPA PET scan from this protocol in one year is 0.89 rem. The total radiation exposure from the 11C-DASB PET scan from this protocol in one year is 0.52 rem. If a subject is eligible and consents to participating in both 18F-DOPA and 11C-DASB studies, the total radiation exposure from this protocol in one year is 1.41 rem.

To minimize the risks of radiation, the subject's medical records will be reviewed carefully and the subjects will be asked about any recent research radiation exposure inside and outside NIH to make sure the total yearly exposure would not exceed 5 rem effective dose in 12 consecutive months

# 8 Subject Safety Monitoring

## 8.1 Parameters to be monitored

Study personnel, overseen by the PI, will closely monitor participant safety during study procedures. If, during the course of interviewing, assessment and imaging procedures, study staff identifies a condition that should require immediate clinical intervention, all procedures will be immediately stopped and necessary steps will be taken. Parameters to be monitored for safety reasons include level of consciousness. Toxicity will be assessed

according to the most recent version of the NCI Common Terminology Criteria for Adverse Events (CTCAE v5.0).

### 8.2 Criteria for stopping procedures in an individual

A subject may be removed from study under the following conditions:

- If a subject satisfies eligibility criteria at screening but in the interval between consent and scanning events happen and the eligibility criteria are no more met.
- If the subject is unable to stay still in the PET scanner for the required study duration.

### 8.3 Criteria for individual subject withdrawal

A subject may ask to be removed from the study at any time.

A subject may be withdrawn from the study at the discretion of the principal investigator (PI).

# 9 Outcome Measures

### 9.1 Primary outcome measure

- Influx constant (Ki) for 18F-FDOPA PET.
- Binding potential relative to non-displaceable binding ($BP_{ND}$) values for 11C-DASB PET.

### 9.2 Secondary outcome measures

- Correlation of 18F-FDOPA with co-morbidities (e.g. Co-infections, atherosclerotic cardiovascular disease (ASCVD) risk scores, prior and current drug abuse).
- Correlation of 11C-DASB with Neuropsychiatric evaluation (e.g. depressive and executive function/cognitive scores).
- Correlation of 18F-FDOPA and 11C-DASB with HIV infection parameters (e.g. time since HIV diagnosis, duration of infection before treatment initiation, nadir CD4).

# 10 Statistical Analysis

### 10.1 Statistical considerations for the FDOPA study

Sample size for the FDOPA PET: There has been no previous imaging of FDOPA in HIV-positive subjects. Based on a paper [62] evaluating subjects with familial parkinsonism that were either symptomatic or asymptomatic, in comparison to healthy volunteers and subjects with Parkinson's Disease, Ki mean values and standard deviations were reported as shown in **Table 1** below:

**Table 1 Ki mean values and standard deviations in subjects with familial Parkinsonism compared to healthy volunteers**

| | | caudate | putamen |
|---|---|---|---|
| **Healthy controls** | **Avg** | **0.0113** | 0.01 |
| | ***SD*** | ***0.0017*** | *0.0013* |
| PD | Avg | 0.0073 | 0.0039 |

| | | | |
|---|---|---|---|
| | *SD* | *0.0028* | *0.0013* |
| **Familial asymptomatic** | **Avg** | **0.008833** | 0.0067 |
| | ***SD*** | ***0.001358*** | *0.000755* |
| Familial symptomatic | Avg | 0.0056 | 0.00414 |
| | *SD* | *0.001765* | *0.001324* |

We believe the HIV-positive subjects will have similar results as the familial asymptomatic PD; while our subjects do not have PD symptoms, they are expected to have defective dopaminergic systems based on the FDG-PET findings. Hence we decided to use the asymptomatic PD data as reflective of the HIV-positive subject group. Since we found the caudate nucleus to be more affected in our subject population than the putamen, the means and standard deviations of Ki values in the HC and asymptomatic familial group were used to calculate the sample size needed for our study. For the second group (HIV-negative subjects), we assumed Ki values to be at a mid-point between HC (mean 0.0113, SD 0.0017) and HIV-positive subjects (familial asymptomatic mean 0.008833, SD 0.001358), with the mid-point value being 0.0100665 (pooled SD 0.001539).

Sample size estimates were for a one-way analysis of variance (ANOVA) comparing the three groups while considering post-hoc pairwise comparisons, particularly between the HIV-positive subjects and the HIV-negative subjects with co-morbidities. Thus, we utilized a more cautious approach focused on being able to detect a difference between these two subject groups using the Ki estimates described above. Calculations yielded a sample size of 23 subjects per group, with alpha=0.05 and 80% power. Considering a 5-10% drop-out rate resulted in a final sample size of 25 per group. Given the ANOVA comparison among three groups, an additional 25 HC would be needed for this study, for a total of 75 subjects equally balanced among the three groups. Subgroup analysis by sex, age, and ethnicity will be carried out during data analysis.

### 10.2 Statistical Considerations for the DASB Study

In our previous study we were able to show higher 11C-DASB uptake in depressed subjects compared to non-depressed subjects using a sample size of 9 each. From our recent animal data, the mean $BP_{ND}$ before inoculation was 2.11 (0.51), while the mean values at the last time point was 2.69 (0.56). We expect the difference between pre- and post- inoculation monkeys (average SD of 0.53, differences in means of 0.56) to be the same in the two independent groups (HIV-positive vs HIV-negative, HC).

**Table 2. Numeric Results for Two-Sample T-Test Allowing Unequal Variance**

Alternative Hypothesis: H1: $\delta = \mu1 - \mu2 \neq 0$

| Target Power | Actual Power | N1 | N2 | N | μ1 | μ2 | δ | σ1 | σ2 | Alpha |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.80 | 0.81642 | 15 | 15 | 30 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.81 | 0.81642 | 15 | 15 | 30 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |
| 0.82 | 0.84202 | 16 | 16 | 32 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |
| 0.83 | 0.84202 | 16 | 16 | 32 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |
| 0.84 | 0.84202 | 16 | 16 | 32 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |
| 0.85 | 0.86442 | 17 | 17 | 34 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |
| 0.86 | 0.86442 | 17 | 17 | 34 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |
| 0.87 | 0.88395 | 18 | 18 | 36 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |
| 0.88 | 0.88395 | 18 | 18 | 36 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |
| 0.89 | 0.90091 | 19 | 19 | 38 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |
| 0.90 | 0.90091 | 19 | 19 | 38 | 2.1 | 2.7 | -0.6 | 0.5 | 0.6 | 0.050 |

Based on that assumption, 19 subjects are needed per group, at alpha = 0.05 and power at 90%, with 5% dropout rate resulting in N= 20 per group.

However, since monkey data might not accurately reflect human data, we are planning on reassessing our results after ten people in each group are recruited in order to re-estimate the accuracy of presumed variance and if deemed necessary increase the sample size accordingly. This would guarantee that the sample size calculation is based on the estimate of variance. We will not have an interim analysis. As part of the final data analysis, subgroup analysis by sex, age, and ethnicity will be carried out.

The total accrual ceiling for this protocol including 18F-DOPA and 11C-DASB studies is 155 subjects.

10.2.1 Data analysis:

18F-DOPA: PET scans will be reconstructed in 22 frame protocol (3×20, 2×30, 2×60, 3×120, 8×300 and 4×600 s) using the ordered-subsets expectation maximum (OSEM) algorithm, in a 31× 31-cm field of view and a 256×256 pixel matrix with a pixel size of 1.2×1.2 mm. PET frames will be corrected for radioactive decay.

The attenuated and decay corrected scan will be registered to the same subject's anatomical magnetic resonance imaging (MRI) scan (obtained under our protocol or other NIH approved protocols if applicable). The MRI scans will then get affine normalized to a brain MRI template and volumes of interest will be drawn on the template. The kinetic rate constant Ki for 18F-DOPA uptake will then be calculated using a linear fit based on the Patlak method [63] for the VOIs with a time activity curve in the occipital reference region as the input function. Ki values will then be compared across subject groups.

11C-DASB: dynamic PET imaging will be obtained in list mode for a total scan duration of 90 min. PET scans will be reconstructed in 22 frame protocol (3×20, 2×30, 2×60, 3×120, 8×300 and 4×600 s) using the OSEM algorithm, in a 31× 31-cm field of view and a 256×256 pixel matrix with a pixel size of 1.2×1.2 mm. PET frames will be corrected for radioactive decay.

The attenuated and decay corrected scan will be registered to the same subject's anatomical MRI scan (obtained under our protocol or other NIH approved protocols if applicable). The MRI scans will then get affine normalized to a brain MRI template (MNI) and volumes of interest will be drawn on the template. The binding potential values of 11C-DASB in various parts of the brain will then be calculated using 2-

parameter multilinear reference tissue parametric imaging method (MRTM2) using the cerebellum as a reference region.

10.2.2 Interpretation of 18F-FDOPA and 11C-DASB PET Imaging

Data analysis and interpretation will be done in a blinded manner. When the data is transferred from the PET server to the investigator's server, one operator (co-investigator) will make sure the images are de-identified and saved prior to detailed analysis. The same operator will eventually re-identify the data once the analysis is complete.

# 11 Human Subjects Protection

## 11.1 Subject selection

Subject selection will be equitable among eligible patients. No population will be excluded from the study on the basis of race, ethnicity, sex, disability, nationality or religion.

## 11.2 Justification for exclusion of children

Subjects younger than 18 years of age are excluded because of radiation risks to children/young adults and because the developing brain is not within the scope of this study.

## 11.3 Justification for exclusion of subjects over 70 years:

We are excluding subjects over 70 years of age because we want to avoid the potential influence of advanced age on the PET scans.

## 11.4 Justification for exclusion of non-English speakers

Non-English-speaking participants are not eligible for enrollment in in our protocol because many of the neuropsychological subtests that are necessary to our study are only validated in English.

## 11.5 Justification for inclusion or exclusion of other vulnerable subjects

Exclusion of Pregnant or Lactating Women:

Women who are pregnant or lactating are not eligible to enter the study. The study requires exposure to radiation, which could pose a risk to the fetus. Lactating females are excluded due to exposure to the radioactive compound for PET scans, which may be excreted in the breast milk and could be potentially harmful to breast-fed infants.

## 11.6 Justification for Inclusion of Neurocognitively Impaired Subjects

By definition, subjects with HAND are neurocognitively impaired. Because of the need to study this disorder, we must include subjects with neurocognitive impairment into this study. However, each such subject must have capacity to provide informed consent to participate. If subjects, at any point during the screening and study procedures, become decisionally impaired and lose their capacity to provide informed consent, they will be excluded.

## 11.7 Protection of Human subjects/protection of vulnerable subjects (employees)

NIH staff and family members of study team members may be enrolled in this study as this population meets the study entry criteria. Neither participation nor refusal to

participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.
Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner.
The NIH Information Sheet on NIH Staff Research Participation will be made available. Please see section 13.2.1 for consent of NIH Staff.

# 12 Anticipated Benefit

There are no anticipated direct benefits to the participants but the measurements performed may add information about the subjects' neurocognitive health status. This information may or may not help in the subjects' medical management.

This study will likely yield generalized knowledge that can ultimately impact our understanding of the human brain function and neurotransmitter involvement in HIV-1 infection.

# 13 Consent Documents and Process

## 13.1 Designation of those obtaining consent

Study investigators designated as able to obtain consent in study personnel page, will obtain informed consent.

## 13.2 Consent procedures

All volunteers 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 volunteers. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing. The investigator obtaining consent will document the consent process in the participant's medical record. A copy of the completed signed informed consent form will be provided to the participant.

Loss of consent capacity during participation is unlikely, so a durable power of attorney will not be required. If loss of consent capacity occurs during the study, the subject will be excluded/taken off study.

In most cases, it is clear whether subjects are competent to make their own decisions, and this can be judged by the evaluating physician. Occasionally, it is not so clear. In those cases, the subject is considered able to provide informed consent if he/she:

- Understands his or her situation/medical condition.
- Understands the risks associated with the research.
- Communicates a decision based on that understanding.

If after this assessment, there is still doubt about the subject's competency to provide informed consent, we will consult the Clinical Center Capacity Assessment Team (ACAT) before enrolling the subject. Individuals who are unable to consent are not eligible for initial enrollment.

The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the study. The rights and welfare of the subjects will be protected by emphasizing to the

subjects that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Prior to the scheduled PET scans, subjects will undergo a focused short assessment to confirm no change in eligibility criteria and to rule out the possibility of pregnancy despite a negative pregnancy test.

#### 13.2.1 Considerations for Consent of NIH staff, or family members of study team

Consent for NIH staff will be obtained as detailed above with following additional protections:
Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. Otherwise, the consent procedure will be independently monitored by the CC Department of Bioethics Consultation Service in order to minimize the risk of undue pressure on the staff member.

### 13.3 Consent documents

The attached consent form contains all required consent elements. We are attaching one unified consent form for both the DOPA and DASB studies (Unified consent all groups).

# 14 Data and Safety Monitoring

### 14.1 Data and safety monitor

This study's monitoring will be conducted according to the Clinical Center Intramural Clinical Monitoring Guidelines. Monitoring will be done by the study team (PI and associate investigators) with reviews of study enrollment, data collection status, and regulatory obligations annually.

### 14.2 Data and safety monitoring plan

Accrual and safety data will be monitored by the PI, who will provide oversight to the conduct of this study. The PI will continuously evaluate implementation of the protocol for any unusual or unpredicted complications that occur and will review the data for accuracy and completeness. The PI, in collaboration with associate investigators and clinic OP8 staff will review any cases associated with serious adverse outcomes and advise on whether any changes in the research plan are warranted.

### 14.3 Criteria for stopping the study or suspending enrollment or procedures

If, in the judgment of the PI and Lead Associate Investigator, a specific study procedure is yielding frequent unexpected or adverse outcomes, that procedure will be suspended until a review can be undertaken in consultation with the IRB. Depending on that consultation, the procedure may be dropped from the protocol via an amendment, or specific language may be added to the protocol and consent forms to reflect the changing risk level.

# 15 Quality Assurance (QA)

### 15.1 Quality assurance monitor

The Principal Investigator will ensure that:

- the protocol is being correctly followed
- changes to the protocol have been approved by the IRB

- accurate, complete, and current records are being maintained and are secure
- subject withdrawal or study failure is noted in the records and
- informed consent has been correctly documented

### 15.2 Quality assurance plan

The NIH Clinical Center's Quality Assurance Program will conduct study monitoring at least annually or more frequently as required for open studies unless studies are monitored by an outside organization or sponsor. Monitoring visits will include a review of subject consent documents and safety laboratory results and diagnostic test results will be monitored for accuracy and correct dating. All regulatory reports, reviews and amendments, adverse events and problem reports related to study, along with investigator credentials, training records, and the delegation of responsibility log will also be reviewed during monitoring visits. Any major findings will be summarized in writing and reported to the study PI. Any major findings mentioned in the monitoring report will be summarized in writing and reported to the IRB at the time of the continuing review.

# 16 FDA and NIH Reporting

### 16.1 Definition of reportable events

Please refer to definitions provided in Policy 801: Reporting Research Events.

### 16.2 Expedited reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events.

### 16.3 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events.

### 16.4 Clinical Director Reporting

Problems expeditiously reported to the IRB in iRIS will also be reported to the Clinical Director. A separate submission is not necessary, as reports in iRIS will be available to the Clinical Director.

### 16.5 For FDA-Regulated Research associated with IND # 35513 (18F-DOPA)

According to the requirements of 21 CFR 312.64(b), the PI will immediately report any SAE to the Sponsor whether or not considered drug related, including SAEs listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. The PI will record nonserious AEs and report them to the Sponsor representative according to the protocol. The sponsor will be responsible for reporting to the FDA. The Principal Investigator will also provide the Sponsor with a copy of the CC monitoring report for the clinical investigation on an annual basis.

To avoid deviation reports for anticipated missed visits/procedures, anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team.

# 17 Alternatives to Participation

Subjects do not receive any treatment in this study or forego any 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

## 19.1 For research data and investigator medical records

All the imaging data and results of the laboratory studies will remain confidential.

All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the NINDS, the OHRP, or the sponsor's designee.

For NIH employees, 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.

Data from the imaging studies will be stored in the archives of the NIH PET department and will be accessible only by the investigator and investigator team using a pre-allocated user name and password. Raw and analyzed data will also be stored in a password-protected computers in a password protected research folder (\\ccruby\GROUPS\HammoudLab ). Only investigators or their designees will have access to the data.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

# 20 Conflict of Interest

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

# 21 Technology Transfer

There are no technology transfer agreements for this protocol.

# 22 Research and Travel Compensation

All participants will be compensated for research-related discomfort and inconveniences in accord with NIH guidelines (Table 3).

Payment will be sent after each visit. Travel will not be provided or compensated for.

No escort fee will be provided. Compensation will be prorated for parts completed if subjects do not complete the 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.

**Table 3. SUBJECT REIMBURSEMENT SCHEDULE:**

| | Inconvenience units | Pay for inconvenience | Time (h) | Pay for time | Total pay |
|---|---|---|---|---|---|
| MRI with movement restriction | 11 | 110.00 | 2 | 30.00 | 140.00 |
| Neurocognitive testing | 10 | 100.00 | 4 | 50.00 | 150.00 |
| *Mental Health Questionnaire (CDQ)* | 1 | 10.00 | 1 | 20.00 | 30.00 |
| PET 18F DOPA | 15 | 150.00 | 3 | 40.00 | 190.00 |
| PET 11C DASB | 15 | 150.00 | 3 | 40.00 | 190.00 |
| Antecubital venous catheter 18F DOPA | 3 | 30.00 | | | 30.00 |
| Antecubital venous catheter 11C DASB | 3 | 30.00 | | | 30.00 |
| Blood tests first visit | 3 | 30.00 | | | 30.00 |
| Blood tests second visit (if needed) | 3 | 30.00 | | | 30.00 |
| *TOTAL:* | **Variable depending on optional procedures elected. Max compensation is $820** | | | | |

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