CLINICAL STUDY PROTOCOL

Interventional Drug or Biologic

Novel in vivo synaptic imaging in experienced meditators

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Protocol Version

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REVISION HISTORY:

Revision #	Version Date
V2 – add remote screening	July 25, 2022
V3 –update inclusion criteria to increase maximum age from 70 to 80	September 20, 2022
V4 – update advertising methods to include the YCCI database and social media	November 8, 2022
V5 – update inclusion criteria to decrease minimum hours of meditation required from 5,000 to 2,500	December 7, 2022
V6 – add the ability to re-contact prior healthy controls to collect meditation related information	June 6, 2023
V7- update healthy control recontact to increase data set	April 7, 2024

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Synopsis

Primary Objectives To utilize positron emission tomography (PET) imaging to characterize the distribution of [^{11}C]UCB-J (aka [^{11}C]APP311at the Yale PET Center) in cortical and subcortical areas in experienced meditators compared to non-meditating controls.
Secondary Objective Examine relationships between synaptic density and structural and functional MRI
Study Duration 5 years
Study Design We will conduct a PET study of the novel SV2A imaging tracer [^{11}C]APP311 in humans, to characterize the distribution of [^{11}C]APP311 in cortical and subcortical areas in experienced meditators compared to non-meditating controls. A total of up to 20 subjects will complete the study. Subjects will undergo one PET scan with [^{11}C]APP311. Each subject will also undergo one MRI scan for anatomical identification of brain regions.
Number of Study Sites This protocol will be conducted at Yale University.
Study Population Up to 20 adult male and female experienced meditator subjects, aged 28-80, who meet the inclusion criteria, and who do not meet any of the exclusion criteria will be enrolled into the study.
Number of Participants Up to 40 experienced meditator subjects may be consented/screened in order to enroll 20, to complete a total of 20 [^{11}C]APP311 PET scans.
Primary Outcome Variables [^{11}C]APP311 BP_{ND} and V_T
Secondary Outcome Variables meditation assessments, MRI and rs-fMRI

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Abbreviations

Abbreviation	Explanation
AE	adverse event
ECG	electrocardiogram
ED	effective dose
EM	experienced meditators
MRI	magnetic resonance imaging
PET	positron emission tomography
SAE	serious adverse event
SV2A	synaptic vesicle glycoprotein 2
BPND	binding potential
VT	volume of distribution

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1 Introduction

1.1 Introductory Statement

This document is a protocol for a human research study. The purpose of this protocol is to ensure that this study is to be conducted according to ICH GCP guidelines, and according to CFR 21 Part 312, other applicable government regulations and Institutional research policies and procedures.

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2 Background

2.1.1 Preclinical Experience

N/A, [^{11}C]-APP311 has been used in human studies.

2.1.2 Clinical Experience

We recently developed ^{11}C-UCB-J (known as ^{11}C-APP311 at the Yale PET Center), a promising Positron Emission Tomography (PET) tracer for quantitative imaging of SV2A *in vivo [1]*. Initial first-in-human PET studies were carried out in healthy subjects and two epilepsy patients. ^{11}C-APP311 was found to have high brain uptake (peak SUV: ~10), good plasma free fraction (~30%), and moderate peripheral metabolism (parent fraction: ~30% at 30 min). The kinetic profile was also excellent with compartment modeling showing gray matter V_T of ~20 mL·cm^{-3}, and ~6 mL·cm^{-3} for white matter. To date, based on more than 500 human administrations at Yale, ^{11}C-APP311 has been found to be an excellent tracer for quantitative imaging of SV2A in the human brain.

2.2 Background/prevalence of research topic

Mindfulness meditation training focuses a non-judgmental attention to experiences in the present moment to improve psychological capabilities, such as attentional and emotional self-regulation [2-4]. It has become popular in the US with a subsequent increase in the amount of mindfulness meditation (shortened to meditation hereto) research (Fig 1). A large portion of this research has focused on the effectiveness of meditation in treating various health conditions such as chronic stress, coping with physical illness and as adjunctive treatments for psychiatric disorders [5, 6].

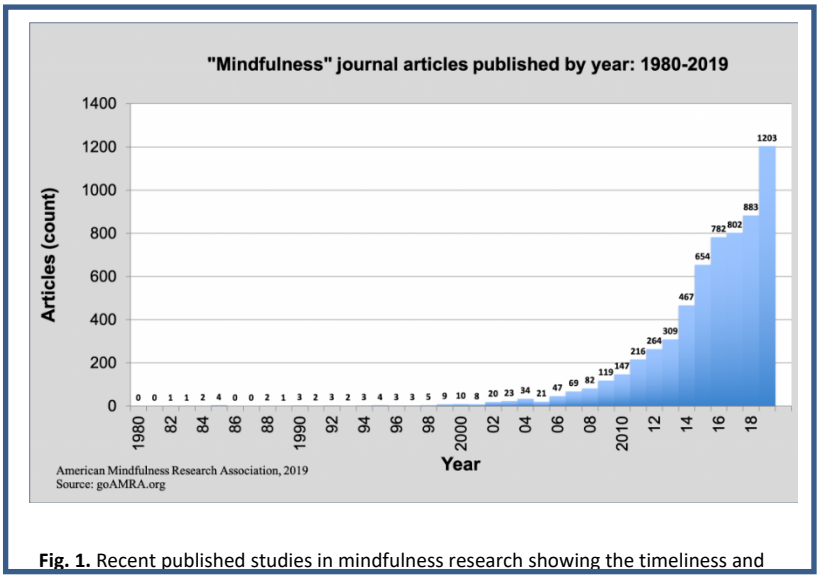


Fig. 1. Recent published studies in mindfulness research showing the timeliness and

More recently, attempts to understand the underlying neural effects of meditation practice have been investigated. This has been almost exclusively with varying magnetic resonance imaging (MRI) modalities. A recent review highlighted structural imaging studies with significant increases in gray matter with experienced meditators compared to controls in parts of the brain including the insula, considered to relate to body awareness, and the amygdala, middle and superior frontal sulci and the prefrontal cortex, considered to relate to self and emotion regulation [5, 7]. Different outcomes such as cortical thickness, gray matter volume and fractional anisotropy has been a strength of these studies and provides preliminary evidence that meditation can produce changes in underlying structure.

Activation and connectivity changes identified with functional MRI (fMRI) [8] also provide evidence that meditation has underlying effects on brain function. A major line of work has shown changes in

the default mode network (DMN), which is involved in mind wandering and self-referential processing [9], cognitive processes that are considered to be counter to meditation. The DMN includes areas of the brain such as the medial prefrontal cortex, posterior cingulate cortex (PCC), anterior precuneus and inferior parietal lobule [10]. Functional connectivity analysis revealed stronger coupling in experienced meditators in these areas both at baseline and during meditation, which was interpreted as demonstrating increased cognitive control [8]. Thus, considerable progress has been made in understanding the underlying brain areas involved in meditation. *What underlies these changes* remains a challenge, however. It is hypothesized that meditation practice induces changes via underlying synaptic plasticity or neuronal preservation [2, 7, 11], but given the impossibility of animal models it has been difficult to empirically test synaptic differences until recently.

Thus, despite an increase in high quality studies the lack of clear evidence of underlying mechanisms by which meditation changes the brain have left some in the scientific community with skepticism [12]. For instance, structural MRI is an indirect measure of synaptic and neuronal changes as it cannot differentiate between underlying gray matter structures (e.g., glial cells, capillaries, neuropil) and potential confounds in the technique have recently been critiqued as not necessarily reflecting underlining brain differences but rather "differences in MRI measurements" [13]. Likewise, fMRI results are influenced by cardiovascular and blood effects. This is likely salient in this field as differential enlargement of blood vessels, which could occur in meditation, may lead to measurement artifacts [12, 14, 15]. In addition, slower breathing patterns in meditators may influence motion artifacts and lead to biases [16].

Another imaging modality, positron emission tomography (PET), has been underutilized in meditation research. PET can provide *in vivo* molecular information of underlying biological tissues, and despite this advantage, only eight PET studies have been published on meditation as indexed in PubMed. Perhaps even more surprising, seven of the studies involved cerebral blood flow (with ^{18}fluorodeoxyglucose (^{18}F-FDG) or ^{15}O-H_20 PET) to investigate functional differences, leaving a sole paper that investigated underlying molecular differences in meditation. In that study,^{11}C-raclopride was used to show nearly 8% reductions of dopamine D2/3 binding in the ventral striatum during a meditative state (N=8), consistent with increased dopaminergic activity [17], and providing evidence that PET can be effectively used to measure brain changes in molecular targets in meditation research.

This study aims to be the first to directly measure an underlying neural mechanism of meditation via synaptic density PET imaging. We will study long-term experienced meditators and, compared to non-meditators, test whether meditation has the potential to increase synaptic density in specific brain areas and circuits already attributed to meditation. This has the potential to make inroads into a knowledge of a mechanism that is "still in its infancy" [2], but clearly important to elucidate for meditation to achieve its full clinical potential as there is no current established biomarker for the possible beneficial effects of meditation practice.

3 Rationale/Significance

3.1 Problem Statement

The purpose of this study is to perform an evaluation of [^{11}C]APP311 (aka [^{11}C] UCB-J), a SV2A radiotracer labeled with carbon-11 (11C), to characterize the distribution of [^{11}C]APP311 in cortical and subcortical areas in experienced meditators (EM) compared to non-meditating controls.

3.2 Purpose of Study/Potential Impact

Measuring the pattern of potential deficits in the brain of EM patients in comparison to controls could lead to advancements in the scientific knowledge of the relationship between synaptic density changes and meditation.

3.2.1 Potential Risks

Risks Associated with Evaluation: The process of eligibility screening may be stressful and tiring for the participant. Breaks will be offered frequently, and the participant may discontinue at any time. During the evaluation we might uncover unanticipated psychiatric and medical information. In that case we will discuss the findings with the participant and will recommend appropriate follow-up.

Stress and fatigue will be minimized by offering frequent breaks and allowing the subject to discontinue the evaluation at any time.

Risks Associated with Unanticipated Events: The subject's health and safety will always be the primary concern of the doctors and staff performing the study. In the event of an unanticipated event, all necessary medical action will be taken. Medication might be administered as needed, per the Yale PET Center standard operating procedure for medical emergencies, in order to treat complications.

Risks Associated with IV Insertion and Blood Drawing: Drawing blood and inserting an intravenous line (IV) are safe and standard medical procedures. Sometimes a bruise will occur at the puncture site and, rarely, a blood clot or infection may occur in the vein. Certain individuals may feel light-headed during venipuncture.

For EM subjects (if not recently completed as part of the participants clinical care), blood samples will be drawn for routine labs at screening (up to 50mL) and blood may be drawn to repeat labs (40mL), if needed. Blood will be taken during the PET scan (up to 90 mL). A total of up to 180 mL will be collected during the study. In the event of a PET scan cancellation that occurs after blood has been collected on the scan day, up to an additional 90mL may be collected on the rescheduled PET scan day, for a maximum of 270 mL.

The risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained and experienced personnel using aseptic technique. To avoid injury due to fainting, the vein catheters will be inserted when the subjects are recumbent. The blood draws during PET scanning sessions will be obtained from an already inserted catheter, to minimize discomfort. Subjects who have donated blood within 8 weeks of the present study will be excluded. Participants will be told that they should not give blood for at least 8 weeks.

Risks Associated with MRI: MR carries a risk for subjects who are claustrophobia or have pacemakers, metal pieces, aneurysm clips, large colored tattoos, or any other contraindications for MR.

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

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

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

We want subjects read and answer very carefully the questions on the MR Safety Questionnaire related to your personal safety. We will be sure that subjects have read the MR Safety Questionnaire and tell us any information they think might be important.

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

To minimize risks, all subjects will be screened for any metallic objects or other MR contraindications that they may be holding or have implanted in their bodies using a questionnaire. All potential subjects with contraindications for MR will be excluded. This

questionnaire will be repeated immediately before scanning to ensure no metallic materials are brought into close proximity of the magnet, where they may be pulled toward the magnet. For additional security, subjects will be taken through a ferromagnetic metal detector immediately before going to the scan room.

Risks Associated with Radiation: The Yale University Radioactive Drug Research Committee (YU RDRC) and Yale University Radiation Safety Committee (YU RSC) have reviewed and approved the use of radiation in this research study. This research study involves exposure to radiation from [^{11}C]APP311 scanning and transmission or low dose head CT scans. This radiation exposure is not necessary for medical care and is for research purposes only.

The targeted amount of radiation an individual subject will receive from participating in this study is from up to 1 injection of ⩽20 mCi of [^{11}C]APP311, plus transmission scans. However, in situations where a PET scan is not successful following a [^{11}C]APP311 injection (e.g., problems with the PET camera), the subject may receive an additional injection, up to a total of 2 injections of [^{11}C]APP311, and additional transmission scans, if deemed appropriate. *The total ED would be 1.219 rem (0.607 rem x 2 injections plus 0.0056 rem from up to 4 transmission scans)*

Targeted and maximum doses are listed in the table below.

Targeted # of [^{11}C]APP311 injections	Targeted ED (rem)	ED (rem) w/ HRRT transmission scans (2 per PET)	Maximum # of [^{11}C]APP311 injections	Maximum ED (rem)	ED (rem) with HRRT transmission scans (2 per PET)
1	0.607	0.610	2	1.214	**1.219**

All scans will be done in the presence of medical supervision and nursing staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the Yale University PET scan facilities have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiopharmaceuticals and execution of PET scans will be performed by radiochemists, health care providers, and technologists of the Department of Radiology and Biomedical Imaging, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radionuclides. Subjects will be asked about their previous radiation exposure, and those who have had research exposure within the past year will be excluded if their cumulative annual exposure (including the present study) exceeds FDA limits.

No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing during evaluation and on each scan day before initiation of any scan procedures. If subjects are breastfeeding they will not be able to participate in this research study

3.2.2 Potential Benefits

This is not a therapeutic treatment study. Therefore, there is no direct benefit to the research participants other than contributing to the knowledge of [^{11}C]APP311.

4 Study Objectives

4.1 Hypothesis

We predict a higher distribution of [^{11}C]APP311 in the insula, amygdala, posterior cingulate cortex, middle and superior frontal sulci and the prefrontal cortex in EM compared to controls.

4.2 Primary Objectives

The primary objectives of this study are:

1. Characterize the distribution of ^{11}C-UCB-J in cortical and subcortical areas in long-term meditators compared to non-meditating controls.
2. Examine relationships between synaptic density and structural and functional MRI.

4.3 Secondary Objective

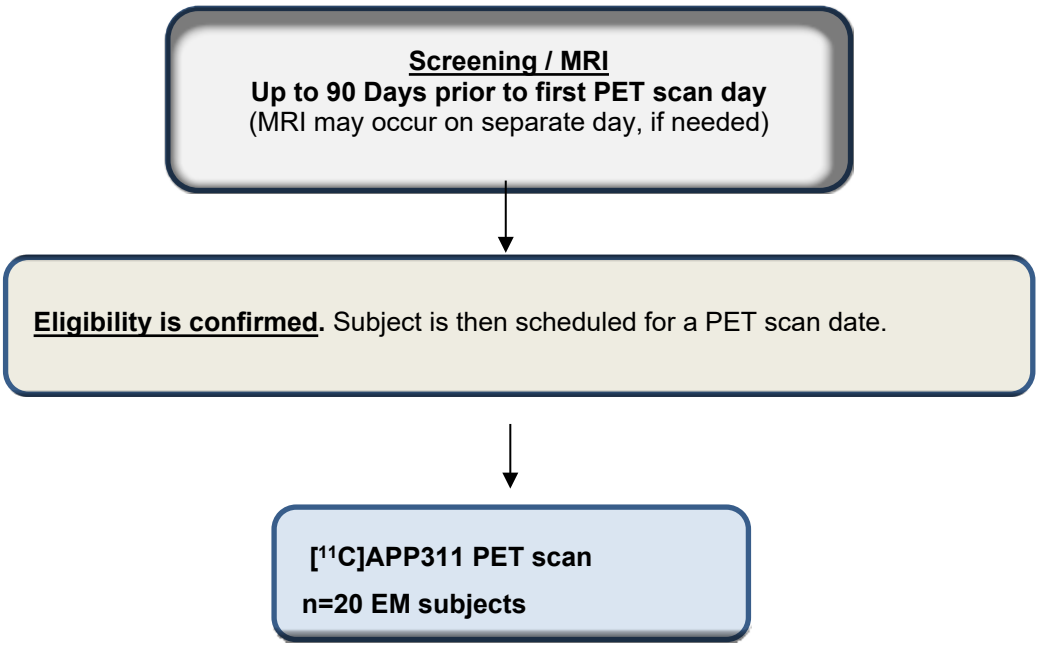
An exploratory aim will utilize assessments for correlations between SV2A binding in a priori regions of interest with measures of frequency of mindfulness states and attentional ability (e.g., the Mindful Attention Awareness Scale).

5 Study Design

5.1 General Design Description

In this study, we propose to use PET imaging with our recently developed synaptic density tracer, [^{11}C]APP311 (aka [^{11}C] UCB-J), to study EM. This tracer binds to a protein in the vesicles of presynaptic nerve terminals (synaptic vesicle glycoprotein 2A or SV2A), and based on our pilot data and human study experience (over 500 human administrations of [^{11}C]APP311 at Yale), we believe it is an excellent tracer for quantitative imaging of SV2A and the first in vivo imaging marker of synaptic density suitable for human studies.

This study will include up to 20 completed EM participants. For the primary Aim, one scan with [^{11}C]APP311 will be performed on one day. In the event that scans are canceled, for any reason, subjects may be scheduled to return on another day to complete the scan. Each subject will also undergo one MRI scan for anatomical identification of brain regions.



General Information

EM subjects will be recruited for this study via flyers, email, word of mouth, the YCCI database, and social media.

The repository protocol (HIC# 1412015027) will be employed for control group data. Controls will have the same tracer and be matched as closely as possible to age, gender and BMI. Data will be de-identified.

In addition, healthy controls that have previously completed an APP311 PET study with Dr. Matuskey (as a PI or Co-I), and have agreed to be contacted for future studies, may be contacted to answer some questions about any current or past meditation history, and will be asked if they will allow their PET data to be shared, for use in this study. This is to ensure there is a control group that have been confirmed to be non-meditators.

This study requires three types of visits: Screening Visit, MRI Visit, and PET Visit. Subjects will report to the Yale University PET Center for initial screening (by Yale PET Center clinicians), MRI visit, and for PET Scan Visits. If needed, some screening procedures may be done remotely.

For the primary Aim, one scan with [^{11}C]APP311 will be performed on one day. In the event that scans are canceled, for any reason, subjects may be scheduled to return on another day to complete the scans.

In situations where one PET scan is not successful following tracer administration (e.g., problems with the PET scanner), subjects may receive one additional tracer administration. Thus, the maximum number of tracer administrations per subject is 2.

We will utilize data from HC subjects who have previously completed [^{11}C]APP311 scans at the Yale University PET Center with appropriate PI approval via the Yale PET Center Data Repository process.

Medical Coverage during study procedures

The PI for this study, Dr. Matuskey, will be the primary contact point for any medical questions that arise from participants during this study. If Dr. Matuskey is unavailable, a responsible study physician will be contacted.

On the screening/MRI day, a Yale PET Center study physician may be consulted. On the PET scan day, if Dr. Matuskey is unable to be reached, the covering physician will be consulted for guidance regarding medical questions. The Yale University PET Center has a chain of command in place that includes a designated physician responsible for medical issues on the imaging floor.

5.1.1 Study Date Range and Duration

This study is expected to last up to five years, including recruitment and data analysis.

5.1.2 Number of Study Sites

This study will be conducted at Yale University.

5.2 Outcome Variables

Primary Outcome Variables

Data Analysis

Dynamic images are reconstructed with corrections for motion, attenuation, scatter, randoms, and deadtime. Using the MR image, PET images are registered into a standard space for analysis and comparison to matched controls.

For analysis of imaging data, kinetic modeling approaches will be used to quantitate total tracer binding, V_T and BP_{ND}, specifically voxel-by-voxel compartment model fitting with the arterial input function, or a reference model. Summed PET images will be registered to the subject's T1-weighted MR images, which, in turn, will be registered to an MR template. Gray matter regions of interest are determined by combining a predefined set of regions, defined on the template (Anatomical Automatic Labeling (AAL) for SPM2) with the gray matter segmentation mask (FAST algorithm in FSL). This process will permit direct, automatic determination of outcome values. Primary regions-of-interest (ROIs) are in the striatum (caudate and putamen), substantia nigra and prefrontal and motor cortices. Partial volume correction will also be applied to account for atrophy. MRI analyses will be done by standardized methods.

5.2.1 Secondary Outcome Variables

Meditation assessments with mindfulness and attention measures will be correlated with SV2A binding.

5.3 Study Population

EM Subjects

Adult male and female subjects, aged 28-80, who are in general good health, meet the inclusion criteria, and who do not meet any of the exclusion criteria will be eligible for enrollment into the study.

5.3.1 Number of Participants

Up to 40 subjects will be screened in order to enroll/complete 20 subjects.

5.3.2 Eligibility Criteria/Vulnerable Populations

Eligibility will be determined by the PI and investigators. We will not recruit vulnerable populations.

Inclusion/exclusion criteria include:

Inclusion Criteria:

1) Age 28-80 years

2) Voluntary, written, informed consent

3) Physically healthy by medical history, physical, ECG and laboratory examinations

4) At least 10 years and/or 2,500 hours of regular meditation practice

5.) For females, non-lactating, no longer of child-bearing potential or agree to practice effective contraception during the study, as well as a negative serum pregnancy (β-HCG) test at screening, and negative urine pregnancy on PET scanning days.

Exclusion Criteria:

1) A history of significant psychiatric, medical (e.g., cardiovascular, renal) or neurological (e.g., cerebrovascular, seizure, traumatic brain injury) illness that is unstable and/or might affect the study objectives.

2) Current or history of substance dependence (e.g., alcohol, nicotine, opiates, sedative hypnotics, etc.)

3) Subjects with history of prior radiation exposure for research purposes within the past year such that participation in this study would place them over FDA limits for annual radiation exposure. This guideline is an effective dose of 5 rem received per year.

4) Subjects with current, past or anticipated exposure to radiation in the work place within one year of proposed research PET scans that in combination with the study tracer would result in a cumulative exposure that exceeds recommended exposure limits.

5) Medical contraindications to participation in a magnetic resonance imaging procedure (e.g., ferromagnetic implants/foreign bodies, claustrophobia, cardiac pacemaker, prosthetic valve, otologic implant, etc.)

6) History of a bleeding disorder or are currently taking anticoagulants (such as Coumadin, Heparin, Pradaxa, Xarelto).

7.) Medications that effect SV2A binding (e.g., levetiracetam).

6 Methods

6.1 Treatment

6.1.1 Identity of Investigational Product

The investigational drug to be used in this study is the PET drug [^{11}C]APP311 (aka [11C]UCB-J) which is a radiotracer specific for imaging synaptic density. This product is not FDA approved and is being administered under the auspices of the Yale University Radioactive Drug Research Committee (YU RDRC).

To date, more than 500 PET measurements have been conducted with [^{11}C]APP311. There have been no adverse events associated with the administration of the radiopharmaceutical. [^{11}C]APP311 exhibits very attractive characteristics as a PET imaging tracer in human subjects.

6.1.2 Dosage, Administration, Schedule

All subjects will be administered approximately the same dose. For each PET scan, up to 20 mCi of [^{11}C]APP311 i.v. will be administered.

6.1.3 Method of Assignment/Randomization

Not Applicable

6.1.4 Blinding and Procedures for Unblinding

Not Applicable

6.1.5 Packaging/Labelling

[^{11}C]APP311, will be prepared at the Yale PET Center under the supervision of Drs. Henry Huang and Nabeel Nabulsi in accordance with local Chemistry Manufacturing & Control (CMC) procedures and quality specifications described in our FDA approved local Drug Master File (DMF, IND 123271), which has also been approved by the Yale University Radioactive Drug Research Committee (YU RDRC).

6.1.6 Storage Conditions, Preparation and Use

Due to the short half-life, PET drugs are prepared *ex tempore* and formulated immediately before administration, and therefore there are no issues with storage or stability. PET drug products are stored at room temperature and are stable for at least 60 min after preparation.

The preparation of sterile PET drug products is validated prior to human use. Sterility is achieved by passing the PET drug product through a sterile 0.22 micron membrane filter during the last step of the formulation process. Prior to release for administration, a bubble point test is performed on the membrane filter used for terminal sterilization in order to validate and verify its integrity during the filtration process. Due to the short half-life, a sample of the PET drug product is tested for sterility *ex post facto* for further confirmation.

The level of endotoxin in each batch of the final PET drug product is determined quantitatively prior to release for administration using the FDA approved Charles River Laboratory's Portable Testing System (Endosafe®-PTS).

6.1.7 Concomitant therapy

There are no restrictions.

6.1.8 Restrictions

The Yale PET Center will monitor the dosimetry history of each subject, to ensure that radiation exposure received by participating in this research study would not result in exceeding the radioactive drug dose limits set by the FDA for research participants (21 CFR 361.1). Depending on this information; participants could be excluded from being enrolled in the study.

6.2 Assessments

6.2.1 Efficacy

Not Applicable

6.2.2 Safety and Pregnancy-related policy

Pregnancy

All female participants will undergo pregnancy tests as part of screening as well as before any PET imaging procedures. Positive pregnancy will exclude participant from further participation in the study.

Description of Safety Assessments

Physical Examination: A routine physical exam will be performed at screening. The routine physical examination will include, but is not limited to, assessments of the following: General Appearance, H.E.E.N.T (Head, Eyes, Ears, Nose, and Throat), Cardiovascular, Respiratory, Gastrointestinal/Digestive, Endocrine, Musculoskeletal, Lymphatic, Dermatologic.

Neurological Examination: A neurological exam will be performed at screening. The routine examination will include, but it not limited to, assessments of the following: Mental status, cranial nerves, sensory and motor system, coordination and gait, and reflexes.

Height/Weight: Height will be measured at the screening visit only. Body weight will be measured at screening and upon arrival at each PET visit.

Body Temperature: To be collected at screening and on the PET scan day.

Vital Signs: Vital Signs will be collected after at least a 5 minute rest period in a supine position at screening and on PET scan days. They will include supine respiration rate, systolic and diastolic blood pressure (BP) and pulse rate. It is expected that the values will generally be within normal ranges, however minor deviations, where they are not considered to be clinically significant, per the study physician, are acceptable.

Electrocardiogram: 12-lead ECGs will be collected at screening and on each PET scan day. The subject must rest in a supine position for ≥ 5 minutes before the ECG is obtained. Electrocardiogram tracings (paper or electronic) will be reviewed by the study physician.

Laboratory Tests: Blood and urine samples will be obtained at time points specified in Table 1. Laboratory results that are out of range and deeming clinically significant at screening may be repeated. Additional tests may be ordered for safety purposes, if needed. Laboratory Tests include the following:

- *Hematology:* CBC with Differential *(*Hemoglobin, Hematocrit, MCHC, MCH, MCV, RBC, WBC, RDW, Platelet Count, MPV, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, ANC (absolute neutrophil count)
- *Coagulation:* Prothrombin Time/INR (PTINR) and Partial Thromboplastin Time (PTT)
- *Chemistry:* Anion Gap*,* Sodium, Potassium, Magnesium, Calcium, Chloride, CO2, Glucose, Creatinine, ALP, AST, ALT, BUN, TBIL, Total Cholesterol, Total Protein, Albumin, LDL, HDL, Triglycerides
- *Urinalysis:* Opacity, Color, Specific Gravity, pH, Protein, Glucose, Ketones, Blood, Bilirubin, Leukocyte Esterase, Nitrites, Urobilinogen (additionally, a microscopic exam is performed when the macroscopic exam is abnormal)
- *Urine Drug screen:* Urine drug screens will be completed via dipstick. The following controlled substances will be tested for: Amphetamine, Barbiturates, Benzodiazepines, Cocaine, Methadone, Opiates, Oxycodone, Cannabinoids, and PCP.

6.2.3 Adverse Events Definition and Reporting

Definitions

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

An AE or suspected adverse reaction is considered "serious" (SAE) if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

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

Severity

Adverse events will be assessed by a health care provider and severity categorized as follows:

- Mild – An event that is easily tolerated by the participant, requires minimal or no treatment, and does not interfere with the participant’s daily activities.

- Moderate – An event that causes a low level of discomfort and interferes with normal everyday activities.
- Severe – Events that interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. [Of note, the term "severe" does not necessarily equate to "serious".]

Assessment of Causality (relationship to radiotracer)

All AEs must have their relationship to the study drug, in this case the radiotracer, assessed by the health care provider who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

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

Expectedness

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

Time Period for Collecting AE Information

The collection will of adverse event information will begin at the screening appointment (following signing of the consent) and conclude at the time of the final follow-up call (for subjects participating in more than one PET scan sessions, there will be multiple follow-up calls).

Adverse events may be reported by the subject spontaneously, or determined during an examination, assessment or phone call conducted by study personnel.

All adverse events will be followed-up through resolution of the event.

Reporting

Serious Adverse events will be reported to the Yale IRB, Yale YU RDRC, and YU RSC, per University reporting policies, and to the NCCIH. In addition, any non-serious adverse event that may potentially be related to the radiotracer will be reported to the relevant radiation safety committees per Yale HRRP policy 940 GD.1.

6.2.4 Pharmacokinetics (if applicable)

N/A

6.2.5 Biomarkers (if applicable)

N/A

6.3 Study Procedures

6.3.1 Study Schedule

- Subjects will complete up to two visits (*Table 1*)

Table 1

Procedures	Visit 1	Visit 2
	Screening/MRI *(Up to 90 days before first PET Scan Day)*	PET Scan Day
Informed Consent	x	
Demographics	x	
Medical History/ Prior Medication History	x	
Physical and Neurological Examination	x	
Assessments for EM	x	
Height	x	
Weight	x	x

Body Temperature	x	x
Vital Signs[a]	x	x
12-lead Electrocardiogram (ECG)[b]	x	
Clinical Laboratory Tests • Hematology • Serum Chemistry • Urinalysis	x	
Urine Drug Screen	x	
Pregnancy Test (Serum at screening/urine on PET Day)	x	x
Inclusion/Exclusion Criteria	x	
MRI Scan (3T)[c]	x	
Intravenous/ Line Placement		x
[^{11}C]APP311 PET Scan		x
Adverse Event Reporting	x	x
Concomitant Medication Reporting	x	x
Discharge		x

a. Vital signs measurements include supine diastolic and systolic blood pressure, pulse rate, and respiration rate.
b. ECGs will be collected after the subject has been resting in a supine position for a minimum of 5 minutes.
c. The MRI may occur at the screening visit, or on a subsequent day prior to the PET scan day.

6.3.2 Informed Consent

Interested individuals who contact study personnel by phone in response to advertisements are told that the information they give over the phone is written down and discussed by the research team. They are advised that if they do not enroll in research, the information is destroyed, and that if they do, it becomes part of their research chart. A phone screen is completed after the potential subject gives verbal authorization. The information collected at that time is used simply to rule out any obvious exclusion criteria, such as age and substance abuse/medical history, as well as to collect some simple demographic information. If a participant is deemed ineligible or decides not to participate in the study, their information is destroyed, i.e., shredded.

The consenting process will take place at the Yale University PET Center, or remotely via videoconference (e.g. Facetime, Zoom, or Microsoft Teams). Subjects will be informed that no immediate personal medical benefits will be derived from participation in the study. The study procedures will be described as a research tool with potential to enhance our knowledge about brain function and the physiology of meditation. Subjects will also be informed of all potential risks of participation. Informed consent will be documented using specific forms that are reviewed and approved by the Yale HIC. Subjects are required to read the informed

consent form, and members of the research team will describe the risks and discomforts that may be associated with this study. Any subject who appears incapable of providing informed consent (e.g., due to cognitive impairment, etc.) will be excluded. Subjects will be informed that they can decline to participate in the study without penalty, and given the opportunity to withdraw from the study prior to analysis of their data. Following the resolution of any questions, the subjects will be asked to sign the consent form, if he/she agrees to participate.

Subjects with limited decision-making capacity will not be recruited for this study. Investigators and research personnel will ensure that the subject understands the study by asking them questions about the study procedures and the risks associated with participation. If any concern arises that the subject does not fully understand the study, the responsible personnel may decide that the subject is not suitable for participation. If the subject is still interested, after all questions have been answered, the responsible personnel will ask the subject to sign the informed consent form.

6.3.3 Screening and MRI

Screening

Subject eligibility will be evaluated at the Screening Visit, which will occur within approximately 90 days prior to the first PET scan. The purpose of this evaluation is to ensure that subjects meet study criteria.

Screening Visit

Subject eligibility will be evaluated at the screening visit, which will take place at the Yale University PET Center and will occur within 90 days prior to first PET scan. The purpose of this evaluation is to ensure that subjects meet study criteria. After informed consent is obtained, a medical history, complete physical examination, neurological examination, and vital signs and an ECG will be performed. Blood will be drawn at this visit (50 mL, or up to ~3.5 tablespoons) in order to run several laboratory tests, including a complete blood count (CBC) and chemistry profile. Urinalysis and a serum pregnancy test (for women of childbearing potential) will be done at screening and a urine pregnancy test before radiotracer administration. Results of these tests are confidential. A qualified health care provider will inform the subjects of any clinically significant abnormal results. Lab abnormalities that indicate the presence of an unstable medical condition, a medical condition that may put the patient at risk if s/he participates in the study, or a medical condition that may interfere with the outcomes measured, will be exclusionary. This decision will be made by the PI and the team of co-investigators.

Neurocognitive and meditation assessments that have been used in meditation research previously will be collected and include the Five Facet Mindfulness Questionnaire (FFMQ) that examines daily mindful awareness and is comprised of five facets: observing, describing, acting with awareness, nonjudging, and nonreactivity [18]; the Attention Network Test (ANT) that activates a frontal brain network and has been used to measure skill in the resolution of mental conflict induced by competing stimuli [19, 20]; and the Mindful Attention Awareness Scale (MAAS) that assesses individual differences in the frequency of mindful states over time [21]. The ANT can be accessed via the Science of Behavior Change website:

https://scienceofbehaviorchange.org/measures/attentional-network-task/ and http://expfactory.org/experiments/attention_network_task/preview.

Clinical ratings, including behavioral measures and computerized cognitive testing (CogState) may also be obtained. Assessments may take place during the screening session or on the same day as the MRI or PET scan(s).

Remote Visits

In order to reduce subject burden, the option to complete the informed consent process, neurocognitive and meditation assessments, and review of medical and psychiatric history, will be offered remotely. This would be done by videoconference (e.g. Facetime, Zoom, or Microsoft Teams).

Remote visits will be conducted after completion of the initial phone screen, to determine if potential participants meet initial study criteria. A copy of the consent form will be e-mailed to the participant, so they may walk through the document with a member of the research team. After verbally confirming understanding of all study procedures, and agreeing to participate, subjects will be asked to sign, date, and return the consent form to research staff electronically. If preferred, subjects may also print, sign, and mail the document. Once received, the consenting personnel will then also sign and provide a copy of the completed informed consent document to the participant.

Once the consent process is complete, the neurocognitive and meditation assessments, and review of medical and psychiatric history may proceed. The entire remote visit may take up to 2 hours to complete

Remote Visits for Prior Healthy Controls

For the healthy control (HC) population, we would like to contact prior research participants, to ask their permission to share previously collected PET data with this study, and also collect some information on any prior meditation history. To collect this information, subjects that have signed a consent for an APP311 IRB approved protocol where Dr. Matuskey serves as the PI or a Co-I, will be contacted. These studies include: HIC#1603017469 (app311_hcep), HIC#1507016243 (app311_to1), HIC#2000021652 (app311_pd), HIC#1404013781 (app311_fih), and HIC#1503015567 (app311_mpg). Healthy Control Subjects that have participated in HIC#2000029552 (app311_cud, PI: Angarita-Africano), and HIC #2000021592 (app311_schiz, PI: Radhakrishnan) may also be contacted. Dr. Angarita-Africano and Dr. Radhakrishnan have given their permission to utilize this data, if subjects agree to participation.

We will only call HC subjects who have given permission to be contacted for participation in future research. During the phone call, subjects will be asked if they are interested in participating in a new study that will involve use of their previously collected PET data (no new scans), and also answer some questions. The primary goal of this call is to ask if subjects if they would like to allow their prior data to be shared, and also ask if they have meditated, and if so, how much. This is to verify they are not experienced meditators. Subjects will also be asked about exercise, and the MAAS and SES (BSMSS) assessments will be provided for

subjects to complete and return. As there are no procedures involved, verbal consent will be requested to collect this information. Subjects will be paid $20 for their participation.

Re-Screening

In the event that a PET scanning session is postponed and is scheduled to occur more than 90 days after the screening visit, subjects may be asked to return for an additional screening visit. This is to ensure that subjects still meet eligibility criteria. This visit may include additional blood work and a physical exam.

If any values are out of range during the initial screening visit (laboratory test results, vital signs, ECG) subjects may be invited to return to repeat these procedures up to two times.

Magnetic Resonance Imaging (MRI)

Anatomical Magnetic resonance imaging (MRI) scans (3T) will be collected in each ET and HC subject to co-register PET and MRI for image analysis, unless a recent MRI scan is available due to participation in another Yale HIC approved protocol. Structural MRI, resting state MRI, diffusion tensor imaging (DTI), neurite orientation dispersion and density imaging (NODDI) will be completed. The total scan time will be approximately 30 minutes.

MRIs will take place at the Magnetic Resonance Research Center (MRRC) at The Anlyan Center (TAC) for Medical Research & Education, 300 Cedar Street, in New Haven. A member of the research team will accompany the subject to the MRRC and will stay for the MRI study. If the MRI is schedule on a day that is separate from the screening, a member of the research team will meet the participant at the MRRC. In either case, study personnel will remain at the MRRC with the participant during the MRI scan.

The following acquisition sequences will be collected:

Localizer

Sag 3d_mprage: 256fov; 1mm thick slices; 176 slices; TE 2.44ms; TR2530; TI 900; FA 9; tfl

t1_fl2d_tra_2.0mm: 216FOV; FIX Voxel size: 1.0×1.0×2.0; 60 slices; TE:2.6; TR400; FA:45; fl

sms_ep2d_bold: 216FOV; 2.4mm thick slices; 60 slices; TE:30; TR:1000; FA:52; epfSM

sms_dMRI: 216FOV; 2.4mm thick slices; 76 slices; TE:77; TR4000; FA1:90; FA2:180; epse

NODDI_64: 240FOV; 1.7mm thick slices; 81 slices; TE:88; TR4100; FA1:90; FA2:180; epse

NODDI_32: 240FOV, 1.7mm thick slices; 81 slices;TE:88; TR:4100; FA1:90; FA2:180; epse

t1_tse_r_tra_2.5mm: 220FOV; 2.5mm thick slices; 13 slices total; TE:9.3; TR:600; FA:150; tse_rs

If a recent MRI scan is available due to participation in another Yale HIC approved protocol, the MRI scan session may not need to be completed. In this case, permission to use the MRI data already located on the PET server may be sought from the subject and PI (if different from this protocol). Recent is defined as within one year of the first PET date.

6.3.4 Enrollment

Eligibility of subjects will be determined by the PI and co-investigators. We will not recruit vulnerable populations. Subjects are considered enrolled after they have consented, completed screening procedures, and met eligibility criteria.

6.3.5 On Study Visits

PET Visits

PET procedures will be conducted at the Yale University PET Center. The following will be completed on the PET scan day (also listed in the schedule of assessment tables):

- Female subjects of child bearing potential will be given a urine pregnancy test prior to the initiation of any imaging procedures. If the test is positive, the scans will be canceled.
- PET scans are acquired as subjects lie supine on the scanner bed. Venous catheters will be used for intravenous administration of the radiotracer and possibly venous blood sampling.
- PET scans will be performed with the Siemens HRRT scanner. An attenuation correction scan is obtained immediately before or after each emission scan.
- Subjects are connected to a cardiac monitor throughout the study and vital signs (e.g., blood pressure, pulse) will be obtained before and after radiotracer administration.
- For each scan, a bolus injection of up to 20mCi of [^{11}C]APP311 will be administered by infusion pump, followed by up to 120 minutes of dynamic PET data acquisition.
- Subjects will be monitored for approximately 1 hour after completion of the PET scanning day. This includes explanation of discharge instructions, and providing the subject with a light meal.

PET Scan Cancellations/Rescheduling

- In the event that scans are not able to be completed as scheduled, subjects may be asked to return on another day to complete the scan.
- In situations where a PET scan is not successful following the radiotracer infusion (e.g., problems with the PET camera) the subject may receive an additional radiotracer infusion, if deemed appropriate. For each subject, radiation exposure received at the Yale PET Center, within the past year, will be checked, and any additional exposure discussed with the subject prior to PET scanning. This process ensures that radiation exposure received by participating in this research study would not result in radiation exposure over the FDA annual limit.

6.3.6 End of Study and Follow-up

Subjects will be contacted by phone 1-3 days after completion of each PET scanning session in order to assess them for adverse events. Subjects will be considered off study upon completion of the last follow-up call, unless there is an unresolved AE. Subjects will be followed until all adverse events have resolved.

6.3.7 Removal of subjects

Research participants may change their mind at any time, and withdraw from the study. However, the information collected prior to withdrawal will continue to be used. Data will not be discarded or destroyed once it is collected.

If a subject withdraws from the study early, the PI may still ask subjects to agree to complete discharge procedures, in order to ensure safety. A study team member would also follow-up by phone within 1-3 days after study withdrawal, to inquire about any adverse events. This is also to ensure subject safety. This does not apply if withdrawal occurs after only completing the screening procedures.

The PI may withdraw subjects from participating in the research, if necessary. Conditions under which subject may be withdrawn from the research include development of serious side effects, or non-compliance.

The YU RDRC, YU RSC, or the Yale Human Investigation Committee may also stop the study at any time for any reason.

6.4 Statistical Method

6.4.1 Statistical Design

The number of subjects is not based on statistical power considerations. Based on previous experience it is expected that 40 participants (20 EM subjects; 20 controls) will be adequate to characterize the distribution of [^{11}C]APP311 in brain areas involved in meditation and to evaluate the magnitude and regional pattern of potential differences.

Sample Size Considerations

Based on models with pilot ^{11}C-UCB-J PET work, we expect substantial increases in ^{11}C-UCB-J availability in the primary regions for the EM group. In Aims 1 and 2, assuming two-sided tests with alpha=0.05, the proposed sample size of n=20 EM and n=20 control subjects will provide 80% statistical power to detect large between-group effects (d=0.909). This compares favorably with the robust effects observed for differences of ^{11}C-UCB-J in Alzheimer's disease and Parkinson's disease (d=1.44 and 2.3) in ROIs, and is in line with the sample size of previous MRI and PET meditation studies [11, 17, 22] . Additional power will be gained for the primary global binding comparison between groups (i.e., main effect), as the appropriate covariance of the data across several regions will be modeled [49]. For the exploratory aim, correlations between SV2A binding in a priori regions of interest with measures of frequency of mindfulness states and attentional ability (e.g., the Mindful Attention Awareness Scale), our sample size will also allow for exploratory detection of measures correlations as low as r=0.58 within cohorts

6.5 Planned Analyses

6.5.1 Primary Objective Analysis

Dynamic Images are reconstructed with corrections for motion, attenuation, scatter, randoms, and deadtime. Using the MR image, PET images are registered into a standard space for analysis and comparison to matched controls.

For analysis of imaging data, kinetic modeling approaches will be used to quantitate total tracer binding, V_T and BP_{ND}, specifically voxel-by-voxel compartment model fitting with the

arterial input function, or a reference model. Summed PET images will be registered to the subject's T1-weighted MR images, which, in turn, will be registered to an MR template. Gray matter regions of interest are determined by combining a predefined set of regions, defined on the template (Anatomical Automatic Labeling (AAL) for SPM2) with the gray matter segmentation mask (FAST algorithm in FSL). This process will permit direct, automatic determination of outcome values. Primary regions-of-interest (ROIs) are in the insula, amygdala, PCC, middle and superior frontal sulci and prefrontal cortex. Partial volume correction will also be applied to account for atrophy. MRI analyses will be done by standardized methods.

6.5.2 Secondary Objectives Analyses

Standard MRI based software (e.g., SPM12)

6.5.3 Exploratory Objectives Analyses (if applicable)

Not applicable

6.5.4 Safety

Vital signs and laboratory results will be collected and reviewed.

ECG readings will be evaluated by the investigator and abnormalities, if present, will be categorized as either clinically significant or non-clinically significant.

Adverse events will be reviewed on an ongoing basis throughout the study

6.5.5 Analysis of Subject Characteristics

The following characteristics will be documented for inclusion in data analysis: age, sex, race, ethnicity, handedness, years of education, alcohol use, nicotine use, and any past or present drug use.

6.5.6 Interim Analysis

No formal interim analysis will be conducted for this study.

6.5.7 Health economic evaluation

Not Applicable

6.5.8 Subsets and Covariates

Not applicable.

6.5.9 Handling of Missing Data

In the event of subject withdrawal or if PET data is unusable, replacement subjects may be enrolled to complete the data set.

APPROVED BY THE YALE UNIVERSITY IRB 5/31/2024

7 Trial Administration

7.1 Ethical Considerations: Informed Consent/Assent and HIPAA Authorization

Qualified and trained Yale University PET Center research personnel will ensure participants are clearly and fully informed about the purpose, potential risks, and all other critical information regarding this study in which they are volunteering to participate.

The informed consent document utilized in this study will adhere to the ethical principles that have their origin in the Declaration of Helsinki.

Required private identifiable information about individuals, such as their medical history, current medications, clinical laboratory and EKG results, physical exam information, psychiatric problems, and family history will be collected by the research staff and be used for research purposes and charting after consent is obtained. Yale PET Center staff will collect required research data through study procedures as outlined in this protocol, and record it in confidential research records and protected computer files.

Identifiable research data, including recruitment and screening information and code keys, are stored on a secure database located on the internal PET Center Network. The PET network is protected by a firewall operated by ITS. All research data are backed up nightly to a Dell PV-136T library wit 4 IBM Ultrium-4 tape drives using the backup software Legato Networker 8.2 from EMC. [Note: The software may be updated throughout the study in the event upgrades are scheduled. This will not impact the process of how the data is saved, which is backed up nightly to a tape library]

Human subjects enrolled in the study are assigned a subject-specific random identifier. Subject identifiers and the means to link the subject names and codes with the research data are stored in separate locations within the database. The software of the database limits the ability to connect the random identifier to the actual subject identification information to research team members only. Access to the database is password protected and each research team member is required to have a unique ID and password to gain access to the database. Authorized users employ their netid and authentication is performed using Yale's central authentication server. Users always access research data through the random identifier only.

Research participants will be compensated for their time and reasonable transportation costs will be reimbursed. This is consistent with all other current Yale University PET Center research protocols in healthy control populations.

7.2 Institutional Review Board (IRB) Review

The protocol will be submitted to the IRB for review and approval. Approval of the protocol must be obtained before initiating any research activity. Any change to the protocol or study team will require an approved IRB amendment before implementation. The IRB will determine whether informed consent and HIPAA authorization are required.

The IRB will conduct continuing review at intervals appropriate to the degree of risk, but not less than once per year.

A study closure report will be submitted to the IRB after all research activities have been completed.

Other study events (e.g. data breaches, protocol deviations) will be submitted per the Yale University IRB's policies.

7.3 Subject Confidentiality

Subject confidentiality is held in strict trust by the research team. Procedures to ensure confidentiality follow the regulations and policies of the Yale University School of Medicine. Subject medical record review will be limited to the just the elements needed to complete the study. Only authorized HIPAA and GCP trained study team members will be allowed to extract research data from medical records and enter it into study related spreadsheets.

Each subject will be assigned a unique study number. This is the identifier that will be used in all data analyses. A master list linking the unique study number to the human subject is maintained on the PET Center server.

7.4 Deviations/Unanticipated Problems

If the study team becomes aware of an anticipated problem (e.g. data breach, protocol deviation), the event will be reported to the IRB as soon as possible. Where applicable, the YU RDRC and YU RSC will also be notified.

Additional details are included in section 7.12 (Data Safety Monitoring Plan).

7.5 Data Collection

Only authorized HIPAA and GCP trained study team members will be allowed to access the source data. The following source data will be collected:

Screening/MRI

- Informed Consent
- General screening form including: demographic information, vital signs, confirmation of blood collection, urine drug screen results, timing of ECG and physical/neurological exam, verification of any adverse events.
- Medical History and Physical/Neurological Exam Form, including medication history
- ECG Tracings
- Clinical Laboratory Report
- Subject Payment Form (including current address)
- Questionnaires
- MRI questionnaire
- Data from MRI scan

PET Scan Days

- Data from the PET Scan (including electronic image data and paper source documenting blood sampling and vital signs)
- Medical Progress Note
- Adverse Event Form, if applicable
- Discharge Form

Follow-up

- Record of phone calls made 1-3 days post PET scan day

Data will be retained as described in section 7.10 (Retention of Records).

7.6 Data Quality Assurance

A standardized set of source data collection forms will be utilized and PET Center Procedures will be conducted according to SOPs. The study team will attend a kick-off meeting to review study procedures prior to study initiation.

Study documents will be reviewed for accuracy on an ongoing basis to check that good source documentation is being applied.

7.7 Study Records

Study records include:

- All regulatory documents, including the protocol and consent
- All source documentation, including information collected at screening and on MRI, and PET scan days.
- Laboratory reports and EKGs
- Rapid test results (urine pregnancy and urine drug tests)
- Case Report Form (CSF)
- Cognitive and Behavioral Assessments

7.8 Access to Source Documents

Screening source documents will be paper source that are entered into an excel database.

MRI and PET data will be electronic and maintained on the PET Center secured server. Access to this data will be restricted to the study team. Upon study closure, the de-identified data may be made available to other Investigators through the PET Center Data repository.

7.9 Data or Specimen Storage/Security

Hard copies will be stored in locked filing cabinets within a locked PET Center research office. Electronic data will be stored on a secured server.

Identifiable research data, including recruitment and screening information and code keys, are stored on a secure database located on the internal PET Center Network. The PET network is protected by a firewall operated by ITS. All research data are backed up nightly to a Dell PV-136T library with 4 IBM Ultrium-4 tape drives using the backup software Legato Networker 8.2 from EMC. Human subjects enrolled in the study are assigned a subject-specific random identifier. Subject identifiers and the means to link the subject names and codes with the research data are stored in separate locations within the database. The software of the database limits the ability to connect the random identifier to the actual subject identification information to research team members only. Access to the database is password protected and each research team member is required to have a unique ID and password to gain access to the database. Authorized users employ their netid and authentication is performed using Yale's central authentication server. Users always access research data through the random identifier only.

De-identified PET data may be used in the future. PET data is not anonymized as a link to the subject is needed in order to track radiation exposure if the subject participates in multiple studies. The amount of radiation exposure each subject receives from PET research must be monitored on an on-going basis, to ensure subject safety and compliance with federal regulations. The link between the subject and their coded information will be kept secure with access limited to PI and members of the study research team.

7.10 Retention of Records

The PI will retain all paper study records for a minimum of 2 years after the study is discontinued. After 2 years records may remain onsite or be transferred to a secure offsite storage facility, such as Iron Mountain. Records may be stored offsite indefinitely, but the minimum storage retention total, including the initial 2 years, will be 7 years.

Electronic records, such as MRI and PET data, will be retained indefinitely.

7.11 Study Monitoring

Quality assurance reviews will be conducted on an ongoing basis on all source documents by members of the study team (project manager and coordinators). These reviews will include screening, eligibility, MRI, and PET scan documentation. Once the study is complete, all study records will be reviewed by the study team for accuracy and completion.

7.12 Data Safety Monitoring Plan

This study represents a greater than minimal risk to the participants. Children will not be recruited to participate.

1. Personnel responsible for the safety review and its frequency:

The clinical study data consisting of all required observations, adverse events (AEs), and imaging and laboratory data are entered into a computerized database in a timely manner. Dr. Matuskey will review adverse events on an ongoing basis. On an annual basis, status reports of all AEs and SAEs are reviewed by the PI and Co-I's to view composite data across subjects. SAEs will be reported, as appropriate, per HIC and YU RDRC/YU RSC policy.

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews. During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment.

Either the principal investigator, the HIC, the Yale University Radioactive Drug Research Committee (YU RDRC), or the Yale University RSC (YU RSC), or the National Center for Complementary and Integrative Health (NCCIH), have the authority to stop or suspend the study or require modifications.

2. The risks associated with the current study are deemed greater than minimal for the following reasons:

1. Based on experience we view the risks associated with radiation exposure as greater than minimal.

2. Given the established safety of PET radiotracers in prior work, we view the proposed studies as greater than minimal risk.

Although we have assessed the proposed study as one of greater than minimal risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator, Dr. Matuskey, according to the following categories:

1.) Related

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).

b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).

c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).

2.) Not related

d.) Unlikely: Likely not to be related to the investigational procedures(s)/agent(s).

e.) Unrelated: Clearly not related to investigational procedures(s)/agent(s).

4. Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

1. *Mild:* no intervention required; no impact on activities of daily living (ADL)
2. *Moderate*: minimal, local, or non-invasive intervention indicated;

moderate impact on ADL

3. *Severe*: significant symptoms requiring invasive intervention; subject seeks medical attention, needs major assistance with ADL

5. Plan for Determining Seriousness of Adverse Events:

Serious Adverse Events:

In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it results in any of the following outcomes:

1. Death;
2. A life-threatening experience in-patient hospitalization or prolongation of existing hospitalization;
3. A persistent or significant disability or incapacity;
4. A congenital anomaly or birth defect; OR
5. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

6. Plan for reporting UPIRSOs (including Adverse Events) to the IRB

The principal investigator will report the following types of events to the IRB:

Any incident, experience or outcome that meets ALL 3 of the following criteria:

1.Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-

approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND

2. Is related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND

3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include – but are not limited to – serious, unexpected, and related adverse events and unanticipated adverse device effects. Please note that adverse events are reportable to the IRB as UPIRSOs only if they meet all 3 criteria listed above.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. All related events involving risk but not meeting the prompt reporting requirements described in IRB Policy 710 should be reported to the IRB in summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented. In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

7. Plan for reporting adverse events to co-investigators on the study, and regulatory and decision-making bodies.

For the current study, the following individuals, funding, and/or regulatory agencies will be notified (choose those that apply):

- All Co-Investigators listed on the protocol.
- The HIC
- Yale University Radiation Safety Committee (YU-RSC)
- Yale University Radioactive Drug Research Committee (YU-RDRC)
- National Center for Complementary and Integrative Health (NCCIH)

The principal investigator, David Matuskey, MD, will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

Adverse events will be submitted according to the current HIC and YU-RDRC/YU RSC reporting policies.

NCCIH Reporting

SAEs that are unanticipated, serious, and possibly related to the study procedures will be reported to the Independent Safety Monitor(s) and NCCIH as noted below:

- Unexpected fatal or life-threatening AEs related to the study procedures will be reported to the NCCIH Program Officer, and Independent Safety Monitor(s) within 3

days of the investigator becoming aware of the event. Other serious and unexpected AEs related to study procedures will be reported within 7 days.

- Anticipated or unrelated SAEs will be handled in a less urgent manner but will be reported to the Independent Safety Monitor(s), IRB, and other oversight organizations in accordance with their requirements, and will be reported to NCCIH on an annual basis.
- All other AEs documented during the course of the trial will be reported to NCCIH on an annual basis by way of inclusion in the annual report and in the annual AE summary which will be provided to NCCIH and to the Independent Monitors. The Independent Safety Monitor(s) Report will state that all AEs have been reviewed.

7.13 Study Modification

The study will be conducted in compliance with the protocol. Protocol modifications will be drafted and submitted to the IRB, and any applicable ancillary committees, for review/approval. Once approved, the changes will be immediately implemented.

If the modification substantially alters the study design or increases the potential risk to the participant: (1) the consent form will be revised and submitted to the IRB and ancillary committees (if applicable); (2) the revised form will be used to obtain consent from participants currently enrolled in the study if they are affected by the amendment; and (3) the new form will be used to obtain consent from new participants.

7.14 Study Discontinuation

If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and the Yale University PET Center will provide the reason(s) for the termination or suspension, as applicable.

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Difficulty in study recruitment or retention that significantly impacts the ability to evaluate the study endpoints
- Any new information becomes available during the trial that necessitates stopping the trial
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met

7.15 Study Completion

Study completion is defined as the date when all data has been collected and the study is closed to enrollment. Data analysis and review may continue beyond this date. The IRB will be notified when enrollment has been completed.

7.16 Conflict of Interest Policy

All investigators will follow the applicable Yale University conflict of interest policies, which includes completion of a Conflict of Interest (COI) disclosure annually. Investigators are not permitted to remain on the study if their COI is not up-to-date.

7.17 Funding Source

NIH 1 R21 AT011575-01

7.18 Publication Plan

The Principal Investigator, Co-Investigators, and study team are responsible for publishing study results. All publications and associated results will also be made available online for public use.

7.19 References

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8 List of Tables