Health of the Cholinergic System and Risk for Alzheimer's Disease in Postmenopausal Women

Protocol Short Title:
Cognitive Health After Menopause
(CHAMP)

Protocol Version: 10

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PROTOCOL SYNOPSIS

PROTOCOL TITLE	Health of the Cholinergic System and Risk for Alzheimer's Disease in Postmenopausal Women
PRINCIPAL INVESTIGATORS	Julie Dumas, Ph.D. and Paul Newhouse, M.D.
STUDY SPONSOR	National Institute on Aging, National Institute of Health
STUDY DESIGN	Randomized, placebo controlled non-treatment study
DURATION OF STUDY PARTICIPATION	Screening visit followed by 4 study days to be completed in two months.
SAMPLE SIZE	120 total participants will be enrolled across 2 sites with 60 at the University of Vermont and 60 at Vanderbilt University Medical Center
SUMMARY OF KEY ELIGIBILITY CRITERIA	Women aged 50-70 (inclusive) years, medically healthy, willing and able to undergo test procedures that include neuroimaging and lumbar puncture.
SPECIFIC AIM 1 **SPECIFIC AIM 2**	Examine cholinergic functional “integrity” by measuring working memory performance, functional brain activation, and BFCS structure in postmenopausal women. Examine whether individual differences in menopause-relevant symptoms and known AD biomarkers are related to cognition and brain activation after anticholinergic challenge
OUTCOME MEASURES / STUDY PROCEDURES	Working memory performance, functional brain activation, and BFCS structure; volumetric and structural MRI measures; AD biomarker relationships with cognitive and imaging outcome measures. biomarkers.

1.0 INTRODUCTION

Women are at increased risk for Alzheimer's disease (AD). Notably at menopause, some women experience a change in cognition. However, not all women experience negative effects of menopause on cognition. The cognitive changes that occur at menopause have not yet been connected to late life risk for pathological aging including AD. Thus, understanding the neurobiological factors related to individual differences in cognition at menopause is critical for understanding normal cognitive aging and for determining risk for pathological aging. The challenge in understanding the role of estrogen loss on the risk for AD is the long lag time between the hormonal changes at menopause and the clinical manifestations of AD. Thus, identifying how the hormone changes after menopause are related to AD risk will alter the risk calculus for postmenopausal women in the future.

The novel study proposed here will examine an established AD-related neurotransmitter-based mechanism that may also underlie cognitive changes after menopause. We propose that the change in the hormonal milieu at menopause interacts with the cholinergic system and other brain pathologies to influence a woman's risk for cognitive decline. Preclinical studies have shown that estrogen is necessary for normal cholinergic functioning and its withdrawal leads to cholinergic dysfunction and cognitive impairment. It is important to determine whether menopause-related cognitive changes correlate with both cholinergic functional integrity and established AD biomarkers that portend increased risk for late-life cognitive impairment or dementia. This study will examine brain functioning following cholinergic blockade to separate individuals into those who are able to compensate for the hormone change after menopause and those who are not. We hypothesize women with poor compensation have increased sensitivity to cholinergic blockade by showing poor performance on a cognitive task, altered brain activation, and decreased basal forebrain cholinergic system (BFCS) volume. These cholinergic markers will be related to menopausal factors associated with poor cognition and biomarkers of AD.

The public health significance of this study is that it will identify individual difference factors that are associated with cognitive performance changes after menopause and their relationship to structural, functional, and biomarker evidence of risk for later life cognitive dysfunction. Knowledge of these factors will serve to advance personalized future risk-mitigation strategies for women including hormonal, medication, cognitive remediation, etc. that will be the subject of further research.

1.1 Objectives

Specific Aim 1. Specific Aim 1 is to examine cholinergic functional "integrity" by measuring working memory performance, functional brain activation, and BFCS structure in postmenopausal women.

Specific Aim 2.

Specific Aim 2 will examine whether individual differences in menopause-relevant symptoms and known AD biomarkers are related to cognition and

brain activation after anticholinergic challenge.

2.0 STUDY DESIGN AND SAMPLE SIZE

This study is a randomized, placebo-controlled trial of the effects of cholinergic blockade on working memory performance and brain activation in healthy postmenopausal women. To ensure the scientific rigor of our design, the order of the medication or placebo will be randomized across participants and stratified by age in 5 year age brackets in our age range from 50-70.

Performance Sites One-half of the participants will be recruited and tested at the University of Vermont and one-half will be recruited and tested at Vanderbilt University Medical Center.

Participants The participants will be 120 non-smoking (quit >2 year ago) healthy postmenopausal women (60/site) aged 50-70 years. Women who are postmenopausal will have not have had a period in the last 12 months, have FSH>30 IU/L, and estradiol (E2) <50 pg/ml. They will not be taking any kind of postmenopausal hormone treatment currently or within the past 12 months.

3.0 STUDY POPULATION

The study will enroll women aged 50-70 years who are medically healthy, willing and healthy as specified in the entry criteria below.

3.1 Inclusion Criteria

All participants must meet the following criteria:

- Women aged 50-70 years
- Postmenopausal
- Nonsmokers (quit > 2 years ago)
- Not taking systemic hormone therapy, phytoestrogens, SERMS, or antiestrogen medications and will be at least one year without such treatment
- Physically healthy
- No cardiovascular disease other than mild hypertension. Subjects will also not have current untreated or unremitted Axis I or II psychiatric or cognitive disorders (see screening below).
- IQ in the normal range >80
- Normal neuropsychological test performance

3.2 Exclusion Criteria

All participants must not meet the following criteria:

- MCI or dementia – Montreal Cognitive Assessment <26, Mattis Dementia Rating Scale <130, and Global Deterioration Scale >2
- History (< 5 years) of cancer treatment with cytotoxic and/or ongoing (current) maintenance targeted chemotherapy
- Blood pressure > 160/100 (untreated)

- Narrow angle glaucoma
- Untreated thyroid disease
- Significant cardiovascular disease
- Active uncontrolled Asthma or COPD
- Active peptic ulcer
- Active hyperthyroidism
- Active epilepsy
- Current untreated or unremitted Axis I psychiatric disorders
- Use of medications on the Prohibited medications (see list)

Subjects with major concomitant illnesses will be excluded on the basis of history, physical exam, and laboratory tests assessing hematopoietic, renal, hepatic and hormonal function. (CBC, CMP, TSH, U/A, ECG). A minimum of 14 days will elapse after discontinuing prohibited medications and the commencement of this study.

3.2.2 Exclusion Criteria related to the MRI

- Claustrophobia
- Having any of the following movable metal objects in the body: cardiac pacemakers or implantable defibrillators, aneurysm clips, neural stimulators, artificial heart valves, ear implants, implanted devices such as an insulin pump or drug infusion device, IUDs, magnetic dental appliances, metal fragments or foreign objects in the eyes, skin or body, metal plates, screws and prosthetics, non-removable metal piercings, tattoos on the head and neck, other certain older tattoos with metal containing inks, and permanent makeup (eyeliner).
- Metal jewelry, watches, and any other removable metal will need to be removed before entering the MRI room. Medication patches will also be removed before entering the MRI room.

4.0 DESCRIPTION OF STUDY VISITS

Telephone Screen

Potential participants will complete a telephone screen to determine eligibility. We will ask for verbal consent to take part in a screening procedure over the telephone. We will ask questions about medical history and medication use to ensure they are not taking any medications on the prohibited medications list that will help us determine eligibility. We will tell the participant that all of the information obtained on the telephone screen will be kept confidential, stored in a locked office in our laboratory, and on a secure computer server. Identifiers obtained during the telephone screening will be kept until the study is completed and closed at the IRB. If we determine that the person is ineligible, this phone screen with be kept with other ineligible phone screens in our locked offices. We will maintain this information so we can track how many women have a certain medical history that would deem them ineligible to participate. We will tell the person on the phone that they have the option to ask for their information to be destroyed or discontinue this study at any point. If the woman passes the telephone screening she will be scheduled for a Screening Visit.

In response to the COVID-19 restrictions related to conducting in person assessments for research studies we will create an option for the screening visit to take place in two parts. One part will be conducted over a password protected video conferencing system. The study staff member at each site will explain the study verbally and the subject will be allowed to ask questions. Using REDCap the subject will be able to view the consent form and can print it out if she prefers. If the subject is interested in enrolling in the study and continuing participation and we will obtain Electronic Informed Consent (eIC). The subject will be able to view or print out the signed consent form as well.

Study Visits

1a. Screening Visit – eIC, cognitive and neuropsychological screenings that can be completed by video conferencing.
1.b Screening Visit – medical screening and in person neuropsychological tasks that require responses from the subject that cannot be converted to an electronic system (like the trail making section of the DKEFS).
2. MRI visit
3. PET visit
4. Lumbar puncture and blood biomarker visit
5. Study day 1 – Cholinergic antagonist medication/placebo challenge and MRI
6. Study day 2 – Cholinergic antagonist medication/placebo challenge and MRI

Subjects will be encouraged to undergo all procedures. The ordered preferred priority above is provided as a guide to the staff and the participants.

Order of Visits

The MRI will be conducted prior to the LP to rule out intracranial mass for safety. In instances where this is not possible, the MRI can be performed 72 hours after the LP. Other than these constraints, the order of the visits can be flexible and will depend on the subjects' and facilities' availability. The drug Study days need to be at least 48 hours apart and study day 2 always follows study day 1 because of the drug randomization procedures.

Recruitment

Electronic and paper media will be used to advertise for this study in the Burlington, VT and Nashville, TN areas. Once a potential participant indicates she is interested in this study, she may either contact the lab by telephone or email or she may complete an online pre screening by accessing a link provided on the advertising. The goal of the prescreening is to gather initial eligibility information. Once a potential participant meets intial critera, she will then be contacted by the study staff to complete the telephone screening. After a woman is deemed eligible she will be scheduled for the screening visit.

4.1 Screening Visit

Informed Consent Potential subjects will be in a quiet room either in their homes or at the at the CRC. The experimenter will be with them on a video conferencing system. They experimenter will explain the procedures that will take place in the study and the subject can also read the written document if she likes. The subject will sign and date electronically using REDCap with either a mouse or typing directly into the signature box. We will document this process on the consent process documentation REDCap form submitted with this application. The subject, the CRC, and the research team will all get copies of the form for their records.

Medical Screening Women will be physically healthy nonsmokers with no cardiovascular disease other than mild hypertension. Participants will be assessed by history, physical exam, and laboratory tests assessing cardiac, hematopoietic, renal, hepatic, and hormonal function (CBC, CMP, TSH, FSH, E2, PT, PTT, INR, ECG) and will have a medical history review and physical examination to establish general physical health.

We will examine the following hormone levels: follicle stimulating hormone (FSH), estradiol (E2), estrone (E1), catecholestradiol, testosterone (T), and progesterone (P4). The FSH will be used to confirm menopausal status. E1, E2, catecholestradiol, T and P4 will be used in the data analyses. Hormone assays will be performed by the Stanczyk Reproductive Endocrinology Laboratory using a radioimmunoassay (RIA) method as previously described[1].

Subjects will be carefully questioned about the history of their perimenopausal and postmenopausal autonomic and vasomotor symptoms utilizing symptom review and structured menopausal symptom checklists. We will use our Reproductive History Questionnaire to gather these data. In addition, subjects will be asked about any

family history of severe perimenopausal symptoms. Such information will be recorded for use in data analysis.

Cognitive/Behavioral Screening All women will be cognitively and behaviorally assessed using standard tests designed to exclude subjects with significant cognitive or behavioral impairment including the Montreal Cognitive Assessment (MoCA[3]), Dementia Rating Scale 2 (DRS2[4]) Brief Cognitive Rating Scale[5], and the Global Deterioration Scale (GDS[6]). Subjects will be required to have a GDS score of 2, a DRS2 score of greater than 131, and a MoCA score of greater than or equal to 26. All subjects will perform the Test of Premorbid Functioning (TOPF) to estimate IQ and will be required to score above 80.

All women will undergo a behavioral screening consisting of a Structured Clinical Interview for DSM-IV (SCID) to establish the presence/absence of psychiatric disorders, and the Beck Depression Inventory-II (score < 10)[7]. Participants will be excluded if they have a current psychiatric disorder. Participants will complete the Pittsburgh Sleep Quality Index (PSQI;[8]) to assess sleep quality during the past month and a menopause symptom checklist to assess physical symptoms associated with menopause used in our prior studies[9,10].

Neuropsychological Screening To assess general neuropsychological functioning, all subjects will be screened with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS;[11]). The RBANS assesses five cognitive domains including immediate memory, language, visuospatial/constructional ability, attention, and delayed memory and provides a global total measure. We will also include selected measures from the Delis-Kaplan Executive Function System (D-KEFS;[12]) to compliment the RBANS. Specifically we will include the Trail Making Test and Verbal Fluency subtests. All participants will be required to score within one standard deviation of the mean for their age.

Subjective Cognition We will also use the Saykin (2013) Cognitive Change Index – Self and – Informant Ratings. We will also use the self and informant versions of the Everday Cognition scale (ECog)[13].

Use of Electronic Informed Consent and Screening After EIC is obtained, the screening visit will continue on the video conference platform. The majority of the assessments can be completed by video conference tools with some exceptions. Those exceptions are the trail making portions of the DKEFS and the symbol digit matching on the RBANS. We will complete these tasks when the subject is seen for the medical screening visit.

4.2 Study Days

Once participants have completed and passed all screening procedures, they will be scheduled for the MRI, PET scan, and the LP and blood biomarker visit. Depending on the availability of the medication, we may include a baseline MRI first so that we can proceed with the LP visit. We will not complete this additional visit if the medication is available. We will arrange the sleep assessment in between the study

visits. Finally, participants will be scheduled for the lumbar puncture for CSF and blood based biomarkers. These procedures are described below.

Sleep Assessment For one week after screening, subjects will place an Actigraph device on their wrist and wear it continuously for seven days. This measurement will provide an objective index of exercise and sleep behavior. All data are coded with the study identifiers and participants will not see their data during the week the Actigraph is worn or after the study.

4.2. Blood-based Biomarker Procedures

Blood/plasma will be drawn at the Lumbar puncture visit or at a visit with the medications and MRI. Samples will be banked for measurement of neural filament light (NfL), a blood-based biomarker linked to neurodegeneration[27,28] and plasma Aβ and tau levels[29,30] to be performed by the Alzheimer's Therapeutic Research Institute biomarker core (Robert Rissman, PhD). Evaluation of the baseline levels of these markers will be conducted as well as correlations with cognitive performance after anticholinergic challenge and neuroimaging results. Blood will also be taken for a fasting glucose measurement, hemaglobin A1C measurement, and a lipid panel.

4.3 Pharmacological Challenge and MRI Procedures

Subjects will be scheduled for two medication study days. Depending on the time between the screening and these study visits some measures maybe repeated according to the following schedule. If the study days are within three months of the screening visit, they will be scheduled as usual. If the study days are between three and six months after the screening, we will obtain an interval medical history and updates to current medication. If the study days are between six and 12 months ater the screening, we will obtain new safety labs and updated medical history and current medication. Any study days that are greater than one year past screening will require a full repeat of the medical screening and the neuropsychological screening. Additional screening at any time point is at the discression of the PIs and Dr. Boyd for the UVM site.

If available, the nicotinic cholinergic antagonist mecamylamine (MECA) will be administered at a dose of 20 mg orally as the hydrochloride salt. Placebos will consist of identical capsules filled with microcrystalline cellulose.

Alternatively, the muscarinic antagonist scopolamine (SCOP) will be administered. Scopolamine hydrobromide will be administered intravenously (as a push) in a dose of 2.5 µg/kg (calculated as the base) of body weight rounded to the nearest half-milligram. For example, a 50 kg subject will receive 0.125 mg for the 2.5 µg/kg dose. Subjects will be weighed within 30 days of the drug dosing date. Based on our prior work, this is a dose that produces subtle cognitive impairment but does not grossly impair functioning. These effects usually are fully dissipated by 3-4 hours following administration. Placebo will consist of normal saline. The intravenous scopolamine hydrobromide will be administered under IND# 126534 held by the Principal

Investigator, Paul Newhouse, M.D. We will utilize UVMMC and VUMC Investigational Drug Services (IDS) for the handling, storage, compounding, and dispensing of the drug.

When the participant arrives for the study an IV will be inserted. On Study day 1 blood will be taken for a genetic analysis of APOE and COMT, a fasting glucose measurement, a lipid panel, and for AD biomarkers to be described below.

Particpants also complete the Brief Trauma Questionnaire and the Connor-Davidson Resillience scales to assess trauma history and resilience on the first challenge day prior to the MRI scan.

The two drug challenge days for each participant will be spread over no more than one month. No study day will follow a previous one by less than 48 hours. This time line has proven to be acceptable to participants for their time commitment to the study and does not negatively impact recruitment. The drug sequence will be determined by a random order procedure. An example of the timing of a study day is detailed next and the specific timing will depend on the participant schedule and the availability of the facilities. Following an overnight fast, participants will report to the CRC between 0700 and 0900 depending on the subject's preference and the availability of the facilities. The study day will beging with baseline testing and evaluation. MECA/SCOP or matching placebo will be administered 2 hours (MECA) or 90 minutes (SCOP) prior to the MRI session. Vital signs and pupil diameter will be recorded throughout the study day. Cognitive testing during functional neuroimaging will be conducted at 120 (MECA) or 90 (SCOP) minutes after drug/placebo dose. This time reflect previous data from our group[14-16] on when central nervous system effects appear to be maximal following administration of these agents. Participants will be transported by a nurse and the experimenter to the MRI suite for the fMRI session that will begin with the functional scans to maximize the drug dose timing. After scanning, participants will return to the CRC, complete cognition, mood, and physical symptom assessment questionnaires and will receive lunch. Each participant will be followed until at least 6 hours after the drug/PLC dosing, by which time the effects of the medications will generally have dissipated. Participants will be checked for safety using a standard field sobriety test and if their vital signs are within normal limits, will be discharged.

The participant performs a baseline test before the medication adminsitraton at the beginnig of the day and performs another test before discharge. The nurse will report to the covering the physician the results of the results for both tests at the end of the day. We anticipate that the participant will perform similarly at the end of the study day to the beginning and it is the covering physician's discression about whether the woman can be discharged. If the woman is symptomatic and does not pass the sobiety test, she is asked to remain relaxing in bed until the side effects of the medication have dissipated. She will likely continue receiving IV fluids as indicated by the results of her vital signs assessments and the decision of the physician. Once the participant is feeling well, is not symptomatic, and passes the sobriety test, she is free to leave the research center.

If there is a delay in obtaining the medications, the participant will undergo an MRI visit with no medication challenge. At this visit, the MRI procedure will occur wihtout any of the medication or IV procedures and will take approximately 1 hour in the MRI scanner.

4.4 PET Visit

PET Imaging Procedures We will use commercially available [^{18}F]florbetapir for Aβ-PET imaging procedures at both sites. The VUMC PET scans will be conducted at Vanderbilt University Institute of Imaging Science (VUIIS), which hosts a PET Center dedicated to research studies with the Philips Vereos digital PET/CT. UVM also has a Philips Vereos digital PET/CT. Because both centers use identical PET scanner models, we do not expect significant inter-scanner differences across the two participating cites. For validation purposes, reconstructed images of 3-D Hoffman brain phantom scans acquired at both sites will be compared[17,18].

Human PET Procedures We will closely follow the ADNI [^{18}F]florbetapir PET procedures. This procedure may take as long as 2 hours. 370 MBq (10 mCi ± 10%) bolus injection will be administered. A 20-minute brain PET scan will begin approximately 50 minutes post-injection.

The images will be reconstructed immediately after the 20-minute scan, and if motion artifact is detected, another 20 minute scan will be acquired. We will use subjects' native-space T1-weighted MRI scans for the segmentation and parcellation of cortical grey matter regions of interests. Freesurfer (version 5.3.0) will be used for the MRI pre-processing steps at both sites. We will coregister each [^{18}F]florbetapir scan to the corresponding MRI volume and calculate the mean standardized uptake value rations (SUVRs) from several regions, including the medial frontal, posterior cingulate, and precuneus, which are known to be affected by earliest accumulation of Aβ[19-22]. Farrell and colleagues showed subclinical regional (posterior cingulate, precuneus, lateral parietal) Aβ-related decline in episodic memory as early as middle age (30-59). These results corroborate other recent findings[21] of a relationship between accumulating Aβ plaques and declining memory in initially amyloid negative subjects. Together, these studies support the evidence of subtle but reliable Aβ-related decline in memory below traditional thresholds for Aβ-positivity (1.11 for florbetapir SUVR from a composite region of interest including cingulate cortex, lateral temporal, lateral parietal, and frontal lobes[23]). In addition to the standard methods of PET analysis, we will utilize several novel topographic image analysis techniques, that were developed by our lab[24,25]. These tools will serve as exploratory approaches for estimating early Aβ pathology by capturing textural changes in [^{18}F]florbetapir PET images.

4.5 Lumbar Puncture

Cerebral-Spinal Fluid (CSF) Lumbar puncture will be performed after the participant is enrolled in the study and will follow standard procedures. CSF samples will be collected at each site in the morning after a 12-hour overnight fast, aliquoted

in sterile polypropylene collection tubes, and stored in a -80°C freezer. Approximately 2 ml of CSF will be sent to the clinical laboratory of the Site PI for routine cell count, protein and glucose analyses. Once the study is completed, values will be batch analyzed for T-Tau, P-Tau181, and Aβ42 to enable calculation of the Aβ/tau ratio using established methods[26].

5.0 INSTRUMENTS

5.1 Cognitive/Behavioral Evaluations

5.1.1 Montreal Cognitive Assessment (MoCA)

The Montreal Cognitive Assessment test (MoCA) is, similar to the MMSE, a brief, 30-point cognitive assessment designed to detect participants at the MCI stage of cognitive dysfunction26. This instrument has been shown to have adequate sensitivity and specificity in clinical settings to detect suspected MCI.

5.1.2 Cognitive Change Index (CCI)

The Cognitive Change Index is a 20 item self-report and study partner (informant)-report instrument. Items assess primarily memory with some coverage of executive and language changes. A CCI-Memory Total Score obtained by combining both the self and informant forms can yield a total score for memory or overall cognition.

5.1.3 Brief Cognitive Rating Scale (BCRS)

A scale used to assess functional and cognitive abilities in both normal aging and dementing disorders .

5.1.4 Global Deterioration Scale (GDS)

A scale used to assess the level of deficits in cognition and function in cognitively impaired patients.

5.1.5 Beck Depression Inventory Short Form (BDI-13)

The Beck Depression Inventory Short form is a 13-item self-reporting questionnaire that is used to evaluate the severity of depression in normal and psychiatric populations.

5.1.6 Beck Anxiety Inventory (BAI)

The BAI is a self-report inventory that assesses anxiety symptoms and symptom severity. It contains 21 questions, with answers scored on a scale value of 0 (not at all) to 3 (severely).

5.1.7 Pittsburgh Sleep Quality Index (PSQI)

The PSQI is a self-report rating form used to assess sleep quality of older adults during the past month.

5.1.8 Structured Clinical Interview for DSM-IV (SCID)

This is a brief clinical interview designed to establish the presence/absence of Axis I

psychiatric disorders.

5.1.9 Menopause Symptom Checklist

This is a checklist to assess physical symptoms associated with menopause used in our prior studies[9,10].

5.1.10 Brief Trauma Questionnaire (BTQ)

The BTQ is a brief self-report questionnaire that is derived from the Brief Trauma Interview[31]. The BTQ was originally designed to assess traumatic exposure according to *DSM-IV* but specifically asked only about Criterion A.1 (life threat/serious injury) because of the difficulty of accurately assessing A.2 (subjective response) in a brief self-report format. Criterion A.2 has been eliminated from the PTSD diagnostic criteria in DSM-5, so the BTQ provides a complete assessment of Criterion A.

5.1.11 Connor-Davidson Resilience Scale (CD-RISC)

This is a brief subject-completed form assessing resilience[32].

5.1.12 Physical Symptom Checklist

The form is a self-report inventory that assesses 21 physical symptoms and the severity of the symptom.

5.1.13 Stanford Sleepiness Scale

This is a one-item self-report questionnaire measuring levels of sleepiness.

5.1.14 Subjective Visual Analog Scale (SVAS)

Subject completed visual analog scale assessing physical symptoms.

5.1.15 Objective Visual Analog Inventory (OVAI)

Experimenter completed visual analog scale assessing subjects during study day.

5.1.16 Brief Psychiatric Rating Scale (BPRS)

The BPRS is a rating scale used to measure psychiatric symptoms such as anxiety, depression, hallucinations and unusual behavior. Each of the 24 symptoms is rated on a scale of 1 (not present) to 7 (extremely severe).

5.1.17 Profile of Mood States (POMS)

The POMS is a psychological rating scale that contains 65 self-report items using the 5-point Likert scale. Participants can choose from 0 (not at all) to 4 (extremely).

5.2 Neuropsychological Evaluations

5.2.1 Mattis Dementia Rating Scale-2 (DRS)

A battery-style assessment of overall level of cognitive functioning. The tasks are grouped into five subscales, each one evaluating different cognitive areas, including: Attention, Initiation/Perseveration (I/P), Construction, Conceptualization and

Memory.

5.2.2 Test of Premorbid Functioning(TOPF)

The TOPF is an assessment tool used to provide a measure of premorbid intelligence, the degree of intellectual function prior to the onset of illness or disease.

5.2.3 Letter Number Sequencing (LNS)

The LNS is a measure of working memory. The examinee is read a sequence of numbers and letters and asked to recall the numbers in ascending order and the letters in alphabetical order. The task involves attention, concentration, mental manipulation, sequential processing, memory span, and short-term auditory memory.

5.2.4 Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

The RBANS is a brief test designed to evaluate neuropsychological status of adults, ages 12-89. The test assesses five cognitive domains including immediate memory, language, visuospatial/constructional ability, attention, and delayed memory and provides a global total measure.

5.2.5 Delis-Kaplan Executive Function System (D-KEFS)

The D-KEFS is used to measure a variety of verbal and non-verbal executive functions. The Trail Making and Verbal Fluency subtests will be used in this study. The Trail Making Test is a visual-motor sequencing task that measures cognitive flexibility. The Verbal Fluency Test measures letter and category fluency.

5.2.6 Buschke Selective Reminding Test

The Buschke is a test designed to measure verbal learning and memory through the use of a list-learning procedure over multiple trials. The examinee is read a list of 16 unrelated words and then asked to immediately recall as many of the 16 words as possible. Every trial after the first involved selectively presenting only the words which the examinee did not recall on the immediately preceding trial. The trials proceed in this manner until 8 trials have been completed or until the examinee can correctly recall all 16 words on three consecutive trials.

5.2.7 Symbol Digit Modalities Test (SDMT)

The Symbol Digit Modalities Test (SDMT) examines cerebral dysfunction, and specifically processing speed, in adults. Using a reference key, the participant has 90 seconds to pair specific numbers with meaningless figures. They are then scored based on the correct number of substitutions that were made.

5.2.8 The Measurement of Everyday Cognition (ECog)

The ECog has a self and informant version assessing everyday cognition. Prior studies have shown good discrimination between cognitive normal older adults and those with mild dementia using this measure.

6.0 PROCEDURES

6.1 Magnetic Resonance Imaging (MRI)

fMRI Working Memory Task The N-Back Test will be used as the measure of verbal working memory. In this task, the participant sees a string of consonant letters (except L, W, and Y), one every three seconds during 27-second blocks. Three conditions are presented: 0-back, 1-back, 2-back, and 3-back. In each condition, the task is to decide whether the letter currently on the screen matches the target in the 0-back or the letter that had been presented 1, 2, or 3 back in the sequence. The N-back task is ideal for use during fMRI with populations who are expected to perform differently on the task because of the parametric manipulation of working memory load to allow for an examination of activation at different and similar levels of accuracy. The N-back task reliably activates a bilateral frontal, parietal, and cerebellar working memory network[33] and we have used it in a number of studies with postmenopausal women[14,16,34,35]. The total task time is 8.15 minutes.

The **Face-Name Encoding Task**[36] will be used as fMRI measure of episodic memory encoding. Subjects see novel faces and names appear on the computer screen one at a time during a 40 second block. There is a rest break followed by 40 seconds of two repeating face name pairs. The Novel and Familiar blocks alternate four times each during one run of the task. Each run of this task takes eight minutes to complete and volunteers will complete two runs on each study day.

We have two validated comparable versions each of the N-back and Face-Name Tasks and they will be counterbalanced across study days.

Resting State Scan Participants will be instructed to close their eyes but try to remain awake during the resting state scan. Participants will undergo two five minute resting state scans for a total of 10 minutes of rest data.

MRI Acquisition Both UVM and VUMC have 3T Philips Achieva dStream scanners and 32-channel head coils. The imaging protocol is based on that developed for the multicenter NIH-funded Adolescent Brain Cognitive Development (ABCD) study, which itself is derived from large studies such as the Human Connectome Project (HCP) and the Lifespan Connectome Project. The protocols make extensive use of simultaneous multislice imaging[37-39] (multiband SENSE) to accelerate functional and diffusion MRI acquisitions.

Structural MRI Acquisition will consist of T1- and T2-weighted images at 0.8mm isotropic resolution, along with T2-FLAIR at 1.0mm isotropic resolution. The high resolution T1- and T2-weighted images are well suited to the HCP processing streams, including the generation of cortical myelin maps [40,41], while the T2-FLAIR provides excellent sensitivity to intra-cranial pathology. Task and Resting state fMRI parameters are TR 800ms, TE 35ms, flip angle 52°, 2.4mm isotropic imaging resolution with a 216×216×144mm^3 field of view using a multiband acceleration factor of 6 (60 slices, no gap).

6.2 Magnetic Resonance Imaging (MRI) Analysis

Structural and fMRI Preprocessing will use the pipelines developed as part of the HCP[42], thus contributing to the rigor and reproducibility of our project. The HCP structural pipelines are based on FreeSurfer and include subcortical segmentation, reconstruction of white- and pial-cortical surfaces, and folding-based surface registration to an atlas. As these techniques are further developed, we will investigate the use of multimodal registration to further improve cross-subject alignment[43] using cortical myelin content[40,41] and functional imaging features. The HCP functional pipeline corrects for EPI spatial distortions using magnetic field maps, realigns volumes to account for subject motion and registers to the structural data. The functional data are then resampled into CIFTI grayordinate standard space, consisting of 91,282 data points, including ~30,000 surface vertices per hemisphere and a further ~30,000 subcortical gray matter voxels (with vertex and voxel spacing of 2mm). The HCP task fMRI pipelines will be used for first- and second-level analysis of task fMRI data. These pipelines are based on FSL FEAT (FMRIB's Expert Analysis Tool) and FLAME (FMRIB's Local Analysis of Mixed Effects), and incorporate high-pass filtering and application of general linear models (GLMs)[44,45].

Structural scans will be used to rule out incidental pathology. FLAIR images will also be used to assess white matter damage associated with aging. White matter hyperintensity (WMH) volumes will be measured using the Lesion Segmentation Toolbox[46]. Briefly, each voxel on the T1 image is assigned as gray matter, white matter, or CSF. After bias-correction the FLAIR is co-registered to the T1 image. The toolbox creates a conservative binary WMH map based on outlier values across the T1 and FLAIR images. Next, a lesion-growth algorithm using Markov Random Fields modeling extends this conservative map to define the extent of the WMH. This lesion map is then used to calculate total cerebral WMH volume which can be used as a covariate in the analyses described below.

Basal Forebrain Cholinergic System (BFCS) Volume Measures[47,48]: Brain scans will be manually reoriented, and the stereotaxic origin of each image will be set to the anterior commissure at the level of the interhemispheric plane. MRI scans will be segmented into gray matter (GM), white matter, and cerebrospinal fluid partitions of 1.5 mm isotropic voxel size using the segmentation routine of the VBM8 toolbox. The resulting GM and white matter partitions of each subject in native space will be registered to a reference template using the DARTEL algorithm[49]. Individual flow fields obtained from the DARTEL registration to the reference template will be used to warp the GM segments. Individual GM volumes of BFCS regions of interest (ROIs) will be extracted automatically from the warped GM segments by summing up the modulated GM voxel values within the respective ROI masks.

Given that the BFCS nuclei lack clear anatomical borders that could serve manual delineation on MRI scans, definition of the BFCS mask will be based on recently published cytoarchitectonic maps of BFCS nuclei in the MNI space[50]. The cytoarchitectonic BFCS map includes different cholinergic subdivisions within the

BFCS. We will consider both the entire volume of the BFCS, as well as specific sub-regions that have showed reduced volume in clinically normal elderly adults with subjective cognitive decline[51]. These include the medial septal nucleus (Ch1), the vertical nucleus of the diagonal band of Broca (Ch2), the horizontal limb of the diagonal band of Broca (Ch3), and the nucleus basalis of basal Meynert (NBM, Ch4 sector according to Mesulam's nomenclature[52]).

The Structural MRI Variable for the outcome analyses is mean left and right hippocampal volume and gray matter density (GMD) adjusted for total intracranial volume and age. A comprehensive set of brain regions of interest for volume, cortical thickness, and gray matter density are available for additional exploratory analyses.

Exploratory Functional Connectivity Analysis: Regions of the brain found to be differentially affected by nicotinic blockade will be used to examine functional connectivity in our sample. Data will be analyzed using a seed-based method similar to that of Fox et al.[53] to examine how functional connectivity is modulated by nicotinic blockade. We will utilize the 1000 Functional Connectomes Project scripts available at https://www.nitrc.org/projects/fcon_1000/. Specifically, after standard preprocessing steps including motion correction, temporal and spatial smoothing, and slice timing correction, the following will be included as nuisance regressors: global signal, white matter, cerebrospinal fluid and motion parameters. Correlations will be produced by extracting the BOLD time course from *a priori* seed regions and computing the correlation coefficients between the time course for each seed region and the time courses from all the other brain voxels. The resulting correlational maps will then be submitted to a group-level statistical analysis using the ANOVA models described above. Normal aging results in decreased posterior-frontal connectivity [54] and we will utilize this pattern to guide our interpretation of effects of nicotinic blockade in postmenopausal women.

6.3 Statistical Analysis Plan

Data Analysis for Specific Aim 1: Specific Aim 1 will examine the relationship between working memory-related performance and brain activation during the anticholinergic challenge and BFCS volume using correlations. For **performance measures**, accuracy and reaction time on hit trials will be examined at each level of N on the N-back task. For the **task-based fMRI,** the critical significance level for group-level analyses will be based on clusters of activated vertices with the probability threshold set at $p_{corr} < 0.001$. To begin with, whole brain analyses will be used since the working memory network is widely distributed across frontal to parietal lobes as well as lateral and medial regions. In addition to examining the effect of working memory performance or functional brain activation when the independent variables are recorded as continuous measures, we will also categorize women as to whether they can compensate for the cholinergic blockade based on their performance on the cognitive tasks and the level of functional brain activation. We will then examine if BFCS volume differs depending upon whether the women can compensate during the cholinergic blockade. The biostatistician Co-Investigator will assist with these analyses.

Data Analysis for Specific Aim 2: Specific Aim 2 is to examine whether individual differences in menopause-relevant symptoms and known biomarkers of increased AD risk are related to cognition and brain activation after anticholinergic challenge. We will develop a predictive model of cholinergic integrity that includes cognitive, neural, and phenotypic variables. Analytically, we will compare women who are impaired on the working memory task during the cholinergic blockade to women who are not impaired during the blockade. A voxelwise logistic regression first identifies brain regions that discriminate impaired from intact women. These brain measures are then combined with the AD biomarkers, menopause symptom measures, hormone levels, genetics, and demographic factors in a logistic regression to examine which factors may predict impairment. This initial analyses will model independent variables that are continuous in nature as such, in order to examine whether such variables are associated with impairment in a linear fashion.

We will employ a classification and regression tree (CART) analysis[55] to supplement the logistic regression analyses. CART is a non-parametric procedure that identifies mutually exclusive subgroups of a population based on shared common characteristics that might influence an outcome of interest (in this case, the ability to compensate for the cholinergic blockade). In the process, CART analysis identifies the independent variable(s) with the most explanatory power in accounting for the outcome. The process begins with one "node" containing the entire sample, referred to as a parent node. This is followed by an examination of all possible independent variables and selection of the one that produces two groups that are the most different from each other with respect to the dependent variable. These subsequent groups are the child nodes. Within each of the child nodes, the remaining independent variables are assessed to determine which results in the best additional split; thus, each child node potentially becomes a parent node to subsequent child nodes. The process continues until a stopping rule is reached and no further split can be made.

Sample Size and Power Estimate: In our proposed study, we will evaluate the effect of working memory performance on functional brain activation. Our sample size estimates for Specific Aim 1 are based on our ability to detect differences in BFCS volume between women who are and are not able to compensate for cholinergic blockade. Wolf et al.[56] estimated total BFCS volume to be 336.18 mm^3 (SD=22.61). In addition, the CH2, CH3, and CH4 volumes were 91.15 mm^3 (SD=7.51), 58.17 mm^3 (SD=7.51), and 70.36 mm^3 (SD=6.05) respectively. The sample size is calculated assuming that half of the women will be able to compensate for the cholinergic blockade while half will not. We further assume a type I error rate of 0.05. A sample size of 100 women will achieve 96% power to detect differences between these groups in total BFCS volume of 5%, consistent with our pilot data. In addition, this sample size will provide 85% power to detect similar differences in CH2, 90% power to detect this difference in CH3, 82% power to detect this difference in CH4. To evaluate the relationships between brain activation and BFCS volume when both variables are measured on a continuous scale, the sample size of 100 subjects gives us greater than 85% power to detect correlations of 0.30 as statistically significant when controlling for up to four covariates.

Sample size estimates for Specific Aim 2 are calculated based on our ability to

identify brain regions that discriminate impaired from intact women. The initial analyses will take the form of a logistic regression analysis. For these analyses, the sample size of 100 women will provide greater than 90% power to detect a statistically significant odds ratio of 1.25, with a type I error rate of 0.05. With respect to the CART analysis, Gini improvement measure used to determine node impurity is not affected by sample size[57]. In addition, while the size of the terminal nodes may be limited by the overall sample size, CART analyses are generally thought to be valuable for exploring complex data, even with small sample sizes[58]; thus, the 100 women proposed in this study should be sufficient to implement this analysis.

6.4 Quality Control

Quality Control Procedures To ensure accurate, reliable, and valid data collection, a variety of quality control procedures will be instituted including real time and batched edit checks, and manual listings review. The use of the REDCap System for all data entry (eCRF) allows for data quality checks to be run during the course of data entry. The data entry operator will receive immediate feedback (on submission of completed form) when entered data are out of range or illogical based on relationships between questions such as dependent fields or total scores. For incoming data, the project manager will be primarily responsible for coordinating quality control activities including reconciliation of the data received against the data reported in the medical record or recorded on paper forms. This reconciliation between various sources of data (e.g., eCRF data, biospecimen sample inventories, MRI and PET files, analysis datasets) will ensure that no data are lost or misrepresented. MRI and PET data will be sent to Vanderbilt for quality assurance and preprocessing using their well-developed system.

7.0 POTENTIAL RISKS

7.1 Drug Challenge Medications

Mecamylamine

Mecamylamine is a centrally and peripherally active non-competitive antagonist of nicotine (and presumably acetylcholine) at C6 (ganglionic) type nicotinic receptors. Mecamylamine was in clinical use as an oral antihypertensive agent in the 1950's and is occasionally used today for autonomic disinhibition following such conditions as spinal cord injury. Peak cognitive and physiologic effects appear to occur by 2-3 hours and dissipate by 4-6 hours after oral administration. Mecamylamine (administered alone) can cause hypotension, changes in cardiac rate, decreased salivary secretions, decreased gastrointestinal tone, constipation, and urinary hesitancy. Drs. Dumas, Potter, Boyd, and Newhouse have used mecamylamine extensively in clinical studies in identical doses to those proposed here. We have occasionally observed prolonged (>6 hrs) asymptomatic orthostatic hypotension that they have treated conservatively by keeping the subject in bed and giving fluids. No sequelae have been observed and no other significant adverse events have occurred. There have been no clinically significant behavioral changes observed with this drug, and no long-lasting cognitive changes.

Scopolamine

Scopolamine, or 1-hyoscine, is a centrally active antimuscarinic anticholinergic compound. It is the organic ester or tropic acid of scopine and much like atropine is a competitive antagonist of the effects of acetylcholine on postganglionic cholinergic nerves. Much like the other major anti-muscarinic compound, atropine, scopolamine has both peripheral and central nervous system effects, but its central actions are somewhat more pronounced and thus make it a more logical choice in a memory experiment. At the dose to be used in this study, the expected physiologic effects of scopolamine include cycloplegia, mydriasis, drowsiness, partial amnesia, decreased bowel motility, tachycardia, and decreased salivation.

After intravenous administration, the early effects of scopolamine include tachycardia, dryness of mouth, and inhibition of lacrimation and sweating. Memory and attention changes are maximal between 90 and 150 minutes after an i.v. dose. Subjects will be instructed not to operate machinery or drive after the study for 24 hours if visual difficulties are clinically significant. The drug is distributed throughout the body with a serum half-life of approximately 2-3 hours. In high doses, anti-cholinergic toxicity can produce hallucinosis or delirium. Based on our experience with the doses proposed here from our previous scopolamine studies we do not expect such toxicity. However, subjects that are impaired will be retained on the GCRC with full nursing support until their mental status is normal. Also, physostigmine will be available for administration if necessary to counteract the effects of scopolamine although this has not been found to be necessary in the past.

7.2 MRI Risks

There is no known risk in exposure to the amount of magnetism used in an MRI brain scan. Any known risks are associated with the presence of ferrous metal in

the body of a research subject. However, subjects will be screened for metallic implants or any magnetic devices on or in their bodies prior to inclusion in the study. The MRI scanner produces a loud banging noise that may be unpleasant and may be uncomfortable for some people who have claustrophobic-like reactions in confined spaces. Some degree of fatigue or anxiety could occur while undergoing testing. Individuals will be allowed to terminate their participation should strong reactions occur. The long term risks of MRI and the risks of repeated MRI are unknown but there have been no health complications traceable to MRI in over 20 years of heavy clinical use.

7.3 MRI Safety and the Potential for Hearing Loss

One potential safety issue in the administration of MR scans is the level of noise. The rapid alternations of currents within the gradient coils causes the coil assemblies to vibrate against their mountings, thus generating a loud resonant noise. The noise may sound like a banging in most cases or even a high pitched whistle with some EPI sequences. Routine clinical sequences generate acoustic noise levels of typically 65-95 dB, but when faster sequences are run the gradients tend to vibrate harder and the noise tends to get louder.

For clinical MRI systems, the FDA currently accepts acoustic noise levels established by the Occupational Safety and Health Administration (OSHA), which indicates that the average noise must remain below 105 dB and the peak acoustic noise must remain below 140 dB. It is also important to consider the duration of noise exposure as this affects the potential for hearing damage. The following table, taken from the OSHA website provides guidelines for maximum decibel levels according to duration of exposure:

Continuous[1] noise in db (A) measured on slow response	OSHA[2] maximum exposure per day (hrs)
85	16
90	8
95	4
100	2
105	1
110	0.5
115	0.25
[1]If the variations in noise level involve maxima at intervals ≤ 1 sec, it is considered continuous.	
[2]Adapted from Table G-16a OSHA Regulations for Noise Exposure 1910.95	

Our average research MRI is 90 minutes in duration, making the maximum acoustic noise level 105 decibels. Previous investigation has established that MRI acoustic noise can cause temporary hearing loss but that this hearing loss can be prevented with earplugs or similar interventions. In fact, the study found that threshold changes of 14 patients who received MRIs without hearing protection had

returned to within 10dB of baseline within 15 minutes. Those imaged with hearing protection rarely suffered hearing loss and never to the same extent.

A study conducted in 2001 found that at 1.5T-3T, the highest mean sound pressure levels varied from 103 to 115dB. It is important to note, however, that at UVM and VUMC we follow strict guidelines for the protection of subject hearing. Subjects are required to use both foam earplugs that block 29 dB of sound pressure, and noise attenuating headphones which block 30dB of sound pressure. Wearing headphones and earplugs together do not produce additive effects in hearing protection, but can increase the NRR by an additional 5-6 dB (equivalent to approximately half the sound intensity).

Therefore, the maximum level of sound pressure emitted by a 3T scanner would be 115 dB, reduced by either 30 dB attenuating earphones or 29 dB attenuating foam earplugs, resulting in either 85 or 86 dB of sound pressure. Decibel levels of 85-86 per OSHA guidelines would need to be presented at a continuous duration of more than 8 hours to endanger human hearing (see chart above). Our MRI sessions are generally 90 minutes in length, with frequent breaks: one following each 5-10 minute fMRI task or structural scan. Therefore, it is unlikely that participants under our care would suffer any form of hearing loss, but in rare instances, patients who are particularly susceptible to the damaging effects of loud noises may experience mild or temporary hearing loss.

7.4 Risk of Amyloid PET Scans: Florbetapir

The primary risk related to PET is that of radiation exposure associated with the injected radiotracers and accompanying CT. There is also a minor risk associated with the venipuncture, placement of an intravenous catheter, and radioisotope injection (pain and bruising or painful infiltration of a failed injection).

In this study each subject will receive one dose of Florbetapir that will be 7.03 mSv of radiation. The organ that receives the maximum exposure is the gallbladder. This dose of florbetapir is well below the 21 CFR 361.1 guidelines for RDRC approved studies. The radiation dose for the one PET scan is not itself expected to produce any harmful effects, although there is no known minimum level of radiation exposure considered to be totally free of risk of causing genetic defects or cancer. The risk associated with the minimum amount of radiation exposure participants will receive in this study is considered low and comparable to everyday risks. No PET studies will be performed on pregnant or potentially pregnant women, as the protocol requires that participants are postmenopausal.

The risks of radiation exposure are very small. Annual normal background dose of radiation is 3 mSv (mSV is a unit of radiation called a millisievert). The whole-body dose from PET/CT of the head in this study is about 8 mSv for PET and 3 mSv for CT which is equal to 2.7 years of background radiation exposure. The level of radiation that would cause concern for a person who works with radiation is above 50 mSv for a whole-body dose. The dose of radiation in this study is well below the safe limit for exposure to radiation during a year.

Organ dosimetry for a participant receiving 10 mCi Florbetapir

Organ	mrad/10 mCi Florbetapir
Adrenals	519
Brain	370
Breasts	222
Gallbladder wall	5296
Lower Large Intestine Wall	1037
Small Intestine	2444
Stomach	444
Upper Large Intestine wall	2741
Heart wall	481
Kidneys	519
Liver	2370
Lungs	333
Muscle	333
Ovaries	667
Pancreas	519
Red marrow	519
Osteogenic Cells	1037
Skin	222
Spleen	333
Testes	259
Thymus	259
Urinary bladder wall	1000
Uterus	593
Total Body	444

The most common side effects reported in studies using florbetapir lasted only a short time and included: headache, muscle or bone pain, increased blood pressure, nausea, fatigue, injection site reaction (bleeding, irritation, pain), anxiety, back pain, claustrophobia (fear of being in closed or narrow spaces), dizziness, feeling cold, insomnia (inability to sleep) and neck pain. Less common side effects reported were: infusion site rash, altered taste in the mouth, itchiness, rash (hives), and flushing, but participants never experienced all of these side effects simultaneously.

You should drink plenty of fluids and empty your bladder as often as possible for at least the first six hours after you have a PET/CT scano protect your bladder fromany potential effects of the radiation. Other risks associated with PET scanning include fatigue and discomfort at having to remain in the scanner for up to 30 minutes, and the discomfort and possible bruising associated with intravenous injections. To minimize these risks, this study uses the lowest possible dose of radioactivity needed

to obtain a clear image. All IV catheters are placed by medical professionals with extensive training and experience. If you experience anxiety or discomfort at any time while in the PET scanner, you can communicate via intercom with the technician at any time during the scan. Specific risks related to the radiotracer for amyloid imaging called Florbetapir are: allergic reaction, blurred vision, headache, nausea and dizziness, nervousness, and palpitations.

7.5 Lumbar Puncture

Lumbar puncture may be associated with pain during the performance of the procedure. This is usually temporary and confined to the lower back. Headache may occur in about 5% of elderly people who undergo lumbar puncture. Less commonly, in about 1-4% of subjects, a persistent low-pressure headache may develop, probably due to leakage of CSF. Lower rates of post-LP headache have been noted in elderly patients, and when atraumatic (Sprotte) needles are used. If a post-LP headache persists it may need additional treatment, e.g. additional fluids and analgesics. Uncommonly a blood patch (injection of some of the participant's blood to patch the CSF leak) may be needed. Potential but rare risks of lumbar puncture include infection, damage to nerves in the back, and bleeding into the CSF space. The risk of these is much less than 1%. In an effort to mitigate these risks, an experienced clinician must perform the LP.

Although very rare, it is possible that participants may have an allergic reaction to the local anesthetic, like lidocaine, used for the lumbar puncture. An allergic reaction would cause swelling and a rash on the skin where the anesthetic was injected.

Potential but rare risks of lumbar puncture include infection, damage to nerves in the back, and bleeding into the CSF space. The risk of these events occurring is much less than 1%. To minimize these risks, the lumbar puncture will be performed by a specialist specifically trained in the procedure.

7.6 Psychological Testing

There is a risk that subjects may have thoughts and feelings that make them uncomfortable as they reflect upon relationships and well-being. If a participant endorses self-harm on the Beck Depression Inventory we will immediately assess their intent. If the participant has a plan for suicide we will notify adult crisis services. If there is not an immediate emergency we will provide a form with information about who to talk to about study concerns (Julie Dumas at UVM and Paul Newhouse at VUMC), or mental health concerns (primary physician and mental health resources). The participant will not be discharged from the CRC/VICTR until a plan has been established and they are deemed safe for discharge to self.

7.7 Blood Draw and IV insertion

The risks of blood draw and IV insertion include pain from the needle, bruising or infection at the site of venipuncture, or fainting as a response to the needle stick.

7.8 Incidental Findings

There is a possibility that while reviewing MRI, PET scan, cerebral spinal fluid,

blood tests, and other screening measures that the investigators may detect an abnormality that may have health implications that they did not expect to see. This is what is called an “incidental finding.” The investigators will inform participants if an incidental finding is discovered and provide the participant with information about the incidental finding and inform the paticipant’s health care provider or an appropriate doctor for further evaluation.

This study is not intended to detect health problems. The imaging done as a part of this study does not substitute for a medical examination. The participant may feel anxious after learning about an incidental finding. If further tests are completed, those results could be in the participant’s medical record and could affect health or life insurance. The costs for diagnosing and/or treating an incidental finding will not be paid for by this research study. These costs would be the responsibility of the participant.

7.9 Loss of Privacy

This project collects a great deal of information about participant health status. Individuals at the study sites will be collecting personal protected health information such as name, date of birth, social security number, address, phone number, and emails. Images from MRI and PET scans will be collected as well as blood for genetic analysis. All participants will be given a subject code number and all data will be associated with the code number. The study site will maintain the personal protected health information (such as name, date of birth, social security number, address, phone number, and emails) in a secure and locked location. The data, associated with the code number will be distributed widely, but it will not be possible to identify an individual subject from the data. However, there is a very unlikely possibility that there will be a security failure, and that somehow the protected health information will be no longer protected. This is an extremely unlikely, but possible occurrence, and is a risk of this study (and almost all other medical research studies).

8.0 PROTECTION OF HUMAN SUBJECTS

8.1 Consent

Informed consent will be obtained from each participant. Each participant will receive and oral and written explanation of the purpose, procedures, and potential hazards of participating in this study. A record of the communication of this information and the participant's informed consent will be entered into her research record.

8.2 Loss of Privacy

This project collects a great deal of information about participant health status. Individuals at the study sites will be collecting personal protected health information such as name, date of birth, social security number, address, phone number, and emails. All participants will be given a subject code number and all data will be associated with the code number. The study site will maintain the personal protected health information (such as name, date of birth, social security number, address, phone number, and emails) in a secure and locked location. The data, associated with the code number will be distributed widely, but it will not be possible to identify an individual subject from the data. However, there is a very unlikely possibility that there will be a security failure, and that somehow the protected health information will be no longer protected. This is an extremely unlikely, but possible occurrence, and is a risk of this study (and almost all other medical research studies).

8.3 Adverse Events

All participants will be evaluated for adverse events at each clinical visit.

8.4 Definition of an Adverse Event (AE)

An Adverse Event is any adverse change from the participant's baseline condition including clinical or laboratory tests, or abnormalities that occur during the course of the study after consent.

8.5 Following up and reporting on Adverse Events

The investigator is obliged to follow participants with AE's until the events have subsided, the conditions are considered medically stable, or the participants are no longer available for follow up. Participants who discontinue due to Adverse Events will be treated and followed according to established medical practice. All pertinent information will be entered into the research record.

8.6 Definition of a Serious Adverse Event (SAE)

Serious Adverse Events include any event that is fatal, life threatening, significantly or persistently disabling or incapacitating, results in hospitalization, prolongs a hospital stay, or is associated with a congenital abnormality or birth defect. In addition, any experience which the investigator regards as serious, or which would suggest significant hazard, contraindication, side effect, or precaution associated with participation in the study should be reported as a Serious Adverse Event. Medical and scientific judgment should be exercised in deciding whether reporting a serious adverse event is appropriate. All Serious Adverse Events (SAEs) will be

reported to the Independent Safety Monitor.

8.7 Reporting Serious Adverse Events (SAEs)

Any such experience due to any cause, which occurs during the course of the investigation or within 30 days of the last study visit, must be reported to the UVM IRB within 24 hours after learning of the event. Sites will report SAEs based on local IRB requirements.

A serious adverse event (SAE) reported to have occurred within 24 hours of amyloid PET tracer administration (Florbetapi) will be reported, regardless of the investigator's opinion of causation. Thereafter, sites must continue to report any serious or life-threatening adverse event whether or not it is related to study procedures. A subset of those SAEs may then get reported to Avid for events related to Florbetapir.

8.8 Data and Safety Monitoring Plan

This study will have an Independent Safety Monitor review the safety of all participants enrolled in an ongoing basis. The safety monitor review study reports twice a year.

8.9 Local Data and Safety Monitoring Plans

Data and Safety Monitoring Plan: Adverse event reports will be carefully collected. Participants will be encouraged to report any and all adverse events/side effects. Adverse event reports will be summarized on a monthly basis by site study managers and forwarded to the Principal Investigators. Significant or serious adverse events that occur at each site will be immediately reported to the local IRB. The study manager will promptly forward those significant adverse reports to the principal investigators.

Reporting Mechanisms at UVM: Adverse events and protocol deviations will be reported by one of 3 mechanisms:

1) The University of Vermont Committee for Human Subject research adverse event reporting document. These reports will be forwarded to the office of the Committee for Human Research in the Medical Science (CHRMS, Waterman Building, UVM) within 5 days of the event with copies forwarded to the Research Subject Advocate (RSA) office within the Clinical Research Center (CRC) within 15 days. This will be the responsibility of the principal investigator. The CHRMS will make a determination as to whether additional reporting requirements are indicated.

2) The Safety Alert for Events Reporting Form (SAFE) may be initiated by CRC nursing staff or study personnel. These forms will be forwarded within 3 days through the nurse manager, or designee, on Shephardson 2 to the CRC RSA office where further distribution to; A) protocol principal investigator, B) University of Vermont Medical Center Risk Management Office, C) CHRMS, D) CRC Program Director, E) CRC Administrative Director, and other appropriate agencies as indicated by the nature of the report.

3) Email. An email will be sent to the Research Compliance Specialist in which the email will include the protocol number and title, the date of the event or deviation, subject

initials or number, and a description of the event or deviation.

All local serious and non-serious events that are documented by study personnel must and will be reported to the RSA office. For multi-center studies in which UVM is participating, only serious adverse events and protocol deviations that will be reported to the IRB will be forwarded to the RSA Office.

All adverse event reports will be reviewed for severity and frequency on their presentation to the RSA office and will be examined within the overall context of both protocol specific adverse events and more general CRC processes. Reviews of protocol specific adverse events will be performed no less than annually. Findings of the RSA office will be forwarded to the Scientific Advisory Committee for review and action when viewed as appropriate by the RSA office based on the emergence of protocol specific or CRC unit patterns in adverse event reports.

The PI and the Medical Director of this study will meet weekly to discuss issues related to subject enrollment. We will review protocols and side effects observed during the challenge days. Drs. Dumas and Boyd have been working together for five years in this capacity where they discuss study day progress and side effect management in studies of cholinergic drugs.

Reporting Mechanisms at Vanderbilt University: All significant adverse events will be reported by one or more of 3 mechanisms: 1) Vanderbilt Committee for Human Subject Research adverse event reporting document. These reports will be forwarded to the office of the Vanderbilt IRB, known as the within 5 days of the event. This will be the responsibility of the Principal Investigator. The IRB will make a determination as to whether additional reporting requirements are indicated.

All adverse event reports will be reviewed on their presentation to the IRB office for severity and frequency and examined within the overall context of both protocol-specific adverse events and more general processes. Reviews of protocol-specific adverse events will be performed no less than annually.

The IRB also requires reporting of all serious adverse events. All serious adverse events, regardless of the relationship, and all unexpected adverse events involving risks to subjects will be reported to the Committee. Any such problems, including those related to recruitment or the consent process, will be reported. The investigator is required to notify the Committee utilizing the “Report of Serious or Unexpected Adverse Event” form for those events occurring at this institution within 5 working days. A separate form will be filed for each event. Any local death while on active treatment will be reported to the IRB immediately. Once forwarded to the IRB, The IRB Compliance Specialist does a preliminary analysis and obtains additional information as needed. A sub-committee of the IRB provides a more in-depth review of adverse events that have been identified as requiring an additional review and analysis. The Chairperson, his/her designee or a Subcommittee will review and determine any additional actions needed. All reports, supporting documentation, and correspondence will be reviewed during the regular continuing (annual) review cycle.

In addition, the IRB is engaged in ongoing protocol monitoring. The IRB includes the following elements in the monitoring plan: 1) site visits with researcher; 2) review of protocol documents; 3) review of the consent process; and 4) review of other processes as appropriate, e.g. adverse event reporting.

The above detailed plan incorporates two major elements: the Office of Research Subject Advocacy which monitors adverse events occurring in protocols performed at the CRC, and the adverse event reporting and monitoring system of the Institutional Review Board at Vanderbilt. As this application will involve protocols performed at the CRC, safety issues in adverse events will be monitored by both systems and will converge at the IRB. This will also apply to the performance sites that will feed adverse event reports to the PI. In addition, their local adverse event reporting systems will also be used. The Institutional Review Boards require the PI to review each adverse event in terms of its relationship to the study and to address possible changes in the risk-benefit ratio that necessitate changes in the protocol and/or consent form. Generally, only adverse events that the PI determines to be serious, unanticipated, and related or possibly related to the research must be reported to the IRB.

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APPENDIX 1 Table of Events

Visit Name	Screening	Sleep Assessment	MRI and Blood Draw	Drug Challenge 1 and 2	PET Scan	Lumbar Puncture
Informed Consent	X					
Blood Draw (Standard Clinical, Hormones)	X					
Blood Draw for NfL, APOE and COMT genes, postmenopausal hormones, fasting glucose, lipid panel			X			
Physical Exam	X					
Medical History	X					
ECG	X					
Vital Signs	X		X	X		
Montreal Cognitive Assessment (MoCA)	X					
Cognitive Change Index (CCI)	X					
Everyday Cognition (ECog)	X					
Brief Cognitive Rating Scale (BCRS)	X					
Global Deterioration Scale (GDS)	X					
Mattis Dementia Rating Scale-2 (MDRS)	X					
Structured Clinical Interview for DSM 5 (SCID)	X					
Beck Depression Inventory (BDI)	X					
Beck Anxiety Inventory (BAI)	X					
Pittsburgh Sleep Quality Index (PSQI)	X					
Menopause Symptom Checklist	X					
Weschler Test of Adult Reading (WTAR)	X					
Repeatable Battery for the Assessment of Neuropsych Status (RBANS)	X					
Delis-Kaplan Executive Function System (D-KEFS) Trails & Verbal Fluency	X					
Letter Number Sequencing (LNS)	X					
Actigraph Monitoring		X				
MRI Scan (Nback, Faces, Resting State, structural scans)			X	X		
CSF collection						X
PET Procedures					X	

Brief Psychiatric Rating Scale (BPRS)				X		
Buschke Selective Reminding Test			X	X		
Profile of Mood States (POMS)			X	X		
Connor-Davidson Resilience Scale (CD-RISC)			X			
Brief Trauma Questionnaire (BTQ)			X			
Mood and Physical Symptom Questionaires (Phys Symp, Stan Sleep, SVAS, OVAI)				X		