Human Subjects Research Protocol

The Common Human Subjects Protocol Cover Form **must** be completed and **attached** to the front of this form. This Protocol form should be completed for any human subjects research proposal that does not have a specific "protocol," such as a grant application. This form must be submitted along with a copy of the complete grant proposal and all the information in this form **must** be consistent with that proposal. This protocol form, once IRB approved, will be the working protocol for that research. **When completing this document, do not refer to page numbers within your grant**. If revisions are necessary during the course of the research, amendments should refer to this protocol form, not the grant proposal. Enter responses for all sections. Check N/A if the section does not apply.

PROTOCOL SUMMARY

Project Title:	Protocol Version Date:
The Nicotinic Cholinergic System and Cognitive Aging	

Principal Investigator: Julie Dumas, Ph.D.

Grant Sponsor: NIH/NIA Grant Number: **30282**

(For grants routed through UVM, indicate the OSP Proposal ID # located at the top of the OSP Routing Form)

Lay Language Summary: *(Please use non-technical language that would be understood by nonscientific IRB members to summarize the proposed research project. The information must include: (1) a brief statement of the problem and related theory supporting the intent of the study, and (2) a brief but specific description of the procedure(s) involving the human subjects. Please do not exceed one single-spaced 8 ½ X 11" page.)*

Prior research has shown that a chemical system in the brain called the cholinergic system is the primarily responsible for cognitive symptoms in seen in people with dementia. While therapeutic benefits are clear in dementia, what remains uncertain is the role that the cholinergic system in general and a subset of receptors called the nicotinic system plays in cognition in healthy non-demented older adults (referred to as normal cognitive aging). This is critical because the ever growing healthy aging population will show declines in cognition that fall short of dementia but still impact functional abilities and independence. In dementia structural changes in the nicotinic system appear to be related to cognitive dysfunction. However, in normal aging there is no evidence for structural changes in the nicotinic system but age-related functional differences exist. The preservation of structural aspects of the nicotinic system in normal aging implies that a manipulation to enhance nicotinic functioning may lessen the cognitive symptoms of aging. Understanding the effects of age-related functional changes on the nicotinic system will elucidate a critical neurobiological mechanism that may be responsible for age-related changes in cognition and will shed light on how nicotinic dysfunction precedes cognitive dysfunction in normal aging. As the search continues for safe and effective cognitive enhancers, it will be important to understand the role of the nicotinic system in cognitive aging and whether nicotinic mechanisms have the potential to benefit cognition in healthy adults.

We propose that the functioning of the nicotinic system is affected by normal aging, is involved in the increase in frontal activation seen for older compared to younger adults, and therefore can be manipulated to model "older" and "younger" patterns of performance and brain activation.

Older adults (aged 65-75 years) and younger adults (aged 21-30 years) will participate in a screening visit and then three (3) nicotinic challenge study days which include performing a working memory task during functional magnetic resonance imaging (fMRI). The nicotinic challenges will consist of 1) 20 mg oral mecamylamine, 2) 7 mg transdermal nicotine, and 3) matching placebos. After an extensive medical, behavioral, and cognitive screening we will enroll and complete 48 older adults and 48 younger adults.

PURPOSE AND OBJECTIVES

Purpose: *The importance of the research and the potential knowledge to be gained should be explained in detail. Give background information.*

Prior research has shown that the cholinergic system is the primary neurotransmitter system responsible for cognitive symptoms in dementia (Bartus et al. 1982). The cholinergic hypothesis of geriatric memory dysfunction (Bartus et al. 1982) hypothesizes that functional disturbances in cholinergic activity occur in the brains of demented patients and that these disturbances play a role in memory loss and related cognitive problems. This hypothesis has been supported by the finding that cholinesterase inhibitors show positive effects on cognition in Alzheimer's disease (AD) patients (e.g., Hansen et al. 2008) and nicotine administration improves attention and memory in patients with Mild Cognitive Impairment (MCI; Newhouse et al. 2012). Much clinical development research on cholinergic agents has followed since the initial proposal and although the overall clinical effects are limited, the cognitive enhancers that modulate cholinergic functioning remain the most widely used medications that have been approved for use in AD.

While therapeutic benefits are clear in dementia, what remains uncertain is the role that the cholinergic system in general and the nicotinic system specifically plays in cognition in healthy non-demented older adults (referred to as normal cognitive aging in this application). This is critical because the ever growing healthy aging population will nonetheless show declines in cognition that fall short of dementia but still impact functional abilities and independence (Blazer et al. 2015). The goal of successful aging is to maintain intact cognitive functioning all the way until death. Since Bartus' original proposal there have been significant advances in understanding how changes in the nicotinic cholinergic system occur in pathological aging and to a lesser extent with normal aging to affect cognition. Specifically, nicotinic systems are involved in attention and other cognitive processes to the extent that they require effortful processing like working memory (e.g. Warburton & Rusted 1993). In dementia structural changes in the nicotinic system appear to be related to cognitive dysfunction (Picciotto & Zoli 2002). However, in normal aging there is no evidence for structural changes in the nicotinic system but age-related functional differences exist (Decker 1987*). The preservation of structural aspects of the nicotinic system in normal aging implies that a manipulation to enhance nicotinic functioning may lessen the cognitive symptoms of aging.* More recently, with the increased use of brain functional magnetic resonance imaging (fMRI) in combination with psychopharmacological probes, data patterns have emerged that further define the role of the nicotinic system in cognition, aging, and dementia. Understanding the effects of age-related functional changes on the nicotinic system will elucidate a critical neurobiological mechanism that may be responsible for age-related changes in cognition and will shed light on how nicotinic dysfunction precedes cognitive dysfunction in normal aging. *As the search continues for safe and effective cognitive enhancers, it will be important to understand the role of the nicotinic system in cognitive aging and whether nicotinic mechanisms have the potential to benefit cognition in healthy adults.*

References*. Include references to prior human or animal research and references that are relevant to the design and conduct of the study.*

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Objectives: *Clearly state the primary and secondary objective(s) of the study.*

The primary objectives will be examined through the following specific aims:

Specific Aim 1 is to examine the effects of **nAChR blockade** compared to placebo on working memory performance and brain activation using fMRI in older and younger adults. nAChR blockade will model age-related declines in nicotinic system functioning. **Hypothesis 1a** is that nAChR blockade compared to placebo will impair performance and this impairment will be greater for older adults than younger adults. **Hypothesis 1b** is that nAChR blockade compared to placebo will increase activation in frontal regions of the working memory network and in the MFG in particular. This increase will be greater for older adults than younger adults.

Specific Aim 2 is to examine the effects of **nAChR stimulation** compared to placebo on working memory performance and brain activation using fMRI in older and younger adults. nAChR stimulation will model "younger adult" nicotinic system function. **Hypothesis 2a** is that nAChR stimulation compared to placebo will improve working memory performance and this improvement will be greater for older adults than younger adults. **Hypothesis 2b** is that nAChR stimulation compared to placebo will decrease activation in frontal regions of the working memory network and in the MFG in particular. This decrease will be greater for older adults than younger adults.

METHODS AND PROCEDURES

Study Design: *Describe the research design, including a description of any new methodology and its advantage over existing methodologies.*

Design: Older adults (aged 65-75 years) and younger adults (aged 21-30 years) will participate in a screening visit and then three (3) nicotinic challenge study days which include performing a working memory task during fMRI. The nicotinic challenges will consist of 1) 20 mg oral mecamylamine, 2) 7 mg transdermal nicotine, and 3) matching placebos. After an extensive medical, behavioral, and cognitive screening we will enroll and complete 48 older adults and 48 younger adults.

Procedures: *Describe all procedures (sequentially) to which human participants will be subjected. Identify all procedures that are considered experimental and/or procedures performed exclusively for research purposes. Describe the types, frequency and duration of tests, study visits, interviews, questionnaires, etc. Include required screening procedures performed before enrollment and while on study. Please provide in table, list or outline format for ease of review. (describe and attach all instruments)*

Note: A clinical research protocol may involve interventions that are strictly experimental or it may involve some aspect of research (e.g., randomization among standard treatments for collection and analysis of routine clinical data for research purposes). It is important for this section to distinguish between interventions that are experimental and/or carried out for research purposes versus those procedures that are considered standard therapy. In addition, routine procedures performed solely for research purposes (e.g., additional diagnostic/follow-up tests) should be identified.

Cognitive/Behavioral Screening: All **older participants** will be cognitively and behaviorally assessed using standard tests designed to exclude participants with significant cognitive or behavioral impairment including the Mini Mental State Exam (MMSE) (Folstein et al. 1975), Brief Cognitive Rating Scale (Reisberg et al. 1993b), the Mattis Dementia Rating Scale (Jurica et al. 2001), and the Global Deterioration Scale (GDS) (Reisberg et al. 1993a). Participants will be required to have a GDS score of 1-2, a MMSE score of greater than or equal to 27, and score above 130 on the Mattis scale. Older participants will have an informant complete the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE; Jorm 2004) and have scores of 3 or less indicating no cognitive change.

All older and younger participants will undergo the following behavioral screening measures: a Structured Clinical Interview for DSM-IV-TR (SCID) (First et al. 2001) to establish the presence/absence of Axis I psychiatric disorders, and the Beck Depression Rating Scale (BDI, score < 10) (Beck et al. 1961). Participants will be excluded if they have a current Axis 1 disorder. All participants will perform the Wechsler Abbreviated Scale of Intelligence (WASI) to assess IQ and will be required to score above 80.

Neuropsychological Screening: In an effort to ensure the older adults are not at the beginning stages of MCI or dementia and to assess general neuropsychological functioning, **older and younger participants** will be screened with the **Repeatable Battery for the Assessment of Neuropsychological Status** (**RBANS**; Randolph 1998). RBANS was developed for the dual purposes of identifying and characterizing abnormal cognitive decline in older adults and as a neuropsychological screening battery for younger adults. The RBANS assesses five cognitive domains including immediate memory, language, visuospatial/constructional ability, attention, and delayed memory and provides a global total measure. Each participant will need to score within one standard deviation (*SD*) of the mean for her/his age. A measure of executive functioning is missing from the RBANS so we will include selected measures from the **Delis-Kaplan Executive Function System** (**D-KEFS**). Specifically we will include the Trail Making Test and Verbal Fluency subtests. All participants will be required to score within one *SD* of the mean for their age.

Medical Screening: Participants will be physically healthy **nonsmokers** with a BMI ≤ 32 kg/m^2, and 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, U/A, ECG), and will have a medical history review and physical examination to establish general physical health. Blood will also be obtained for an **exploratory genetic analysis** to examine APOE and COMT genotypes as these genes have been shown to be important in cognition in older adults. APOE is important for cognitive decline in older adults and associated with cholinergic function (Espeseth et al, 2006). The COMT Val^{158}Met polymorphism modulates working memory performance in psychiatric populations (e.g. Meyer-Lindenberg & Weinberger 2006) but not in healthy subjects (Blanchard et al. 2011). However, it affects working memory-related brain activation (Jacobs & D'Esposito 2011).

Depending on the distribution of genotypes we will use this information as a covariate in the imaging analysis described below.

Once a participant passes the screening, s/he will be scheduled for ***3 challenge and fMRI study days***.

Pharmacological Challenges: These procedures used for the nAChR challenges are similar to the procedures used in our prior studies (Dumas et al. 2008; 2010; 2012; Carstens et al. 2011; Potter et al. 2009; 2012). The nAChR 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 lactose. This dose of MECA is the same dose used in our prior studies in older women (Dumas et al. 2008; 2012). Nicotine (**NIC**) will be administered with a 7 mg patch placed on the back of the shoulder for 45 minutes. Matching placebo patches will be used. This dosing procedure has utilized at UVM for many years to study the acute effects of nicotine in nonsmokers (Carstens et al. 2011; Potter et al. 2009; 2012). One dose of MECA, NIC, and placebo will require three challenge days for each participant.

Drug Challenge Procedures: The three 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 and will be balanced across the older and younger participant groups. Following an overnight fast, participants will report to the CRC at 0800 when the study will begin with baseline testing and evaluation. MECA or matching placebo will be administered at 0900. NIC or placebo patch will be administered at 1015 and removed immediately before scanning. fMRI scanning will begin at 1100. A double blind, double placebo system will be used. On the days that the MECA pill is active, the NIC patch is placebo and vice versa. Vital signs and pupil diameter will be recorded at 30 min intervals. Cognitive testing during functional neuroimaging will be conducted at 120 minutes after MECA dose and 45 minutes after NIC administration (1100). These times reflect previous data from our group (Dumas et al. 2008, 2010; 2012; Carstens et al. 2011; Potter et al. 2009; 2012) on when CNS effects appear to be maximal following administration of nAChR 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 mood assessment questionnaires (described below), and will receive lunch. Each participant will be followed until at least 1400, by which time the effects of the nAChR drugs will 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.

Figure 7. Study Day Time Line

Working Memory Task during fMRI: Participants will complete a visual verbal **N-back Test** of working memory during the fMRI. This N-back task has three equivalent versions so that a different version will be administered on each study day. The specific version of the task will be counterbalanced across study days for each participant. These versions have been pilot tested with younger and older adults to ensure comparability of different versions and to ensure there is neither ceiling nor floor effects for performance for any age group.

*In this **N-back task** participants see a string of consonants (except L, W, and Y), presented in upper case letters, one every three seconds. Four conditions are presented: 0-back, 1-back, 2-back, and 3-back. The 0-back control condition had a minimal working memory load; participants are asked to decide if the current letter matches a single target letter that was specified before the epoch begins. In the 1-, 2-, and 3-back conditions, participants indicate whether the current letter on the screen matches a letter that is either 1, 2 or 3 back.*

The 0-, 1-, 2-, and 3-back conditions are repeated three times in a counterbalanced order such that the same condition is not repeated two times in a row. In this block design task, participants respond to nine items in each block that takes 27 seconds. A rest break follows with a plus sign (+) fixation for 12

seconds. The total time of the task is 8 minutes 12 seconds. Participants will practice the N-back task before drug dosing on each study day to ensure that they understood task instructions.

Behavioral Measures: After the drug challenge and fMRI session, behavioral measures will be completed by the participant and experimenter that have been shown to be sensitive to nicotinic modulation of physical symptoms. Participants will complete the **Profile of Mood States** (POMS) (McNair et al. 1971), a visual analogue battery of items (Newhouse et al. 1994), a physical symptom checklist, and the **Stanford Sleepiness Scale** (Hoddes et al. 1973), which correlates highly with objective measures of decreased arousal (Newhouse et al. 1989). The experimenter will complete the **Brief Psychiatric Rating Scale** (BPRS) (Overall and Gorham 1993), a sensitive measure of psychopathologic behaviors that appears to be sensitive to the behavioral changes induced by drugs in normals, and a visual analogue scale (Newhouse et al. 1994). If group differences are found for these measures, they will be used as covariates in the analyses described below.

For research involving survey, questionnaires, etc.: *Describe the setting and the mode of administering the instrument and the provisions for maintaining privacy and confidentiality. Include the duration, intervals of administration, and overall length of participation. (describe and attach all instruments)*

☐ ***Not applicable***

All questionnaires will be administered by the experimenter in a private room at the CRC. The experimenter will explain any instructions for each questionnaire and will be available to answer any questions a subject may have. The questionnaires at screening will take approximately 1 hour to complete. The questionnaires on each study day will take about 15 minutes to complete. See attached forms.

Statistical Considerations: *Delineate the precise outcomes to be measured and analyzed. Describe how these results will be measured and statistically analyzed. Delineate methods used to estimate the required number of subjects. Describe power calculations if the study involves comparisons. Perform this analysis on each of the primary and secondary objectives, if possible.*

Statistical Analysis

Analyses for Working Memory Performance Hypothesis Testing (H1a and H2a): Comparisons of **MECA to placebo** will be conducted in support of **Hypothesis 1a**, while similar analyses comparing **NIC to placebo** will be used to test the hypotheses associated with **Hypothesis 2a**. The overall statistical model is: $Y_{ijk}=\mu+s_{ik}+\pi_j+\tau_{t[i,j]}+\lambda_{d[i,j-1]}+x_k+\varepsilon_{ijk,}$ where Y_{ijk} is the value of the outcome of interest (***derived from the performance data described here and imaging data described below***) in subject k in sequence i at period j, μ is the overall mean of the outcome of interest; s_{ik} is the effect of subject k in sequence i, i=1, 6; π_j is the effect of period j, j=1, 3; $\tau_{t[i,j]}$ is the effect of treatment t, t=1,2 in period j of sequence i; $\lambda_{d[i,j-1]}$ is the carryover effect of the treatment administered in period j_1 of sequence i, where $\lambda_{[i,0]}=0$; x_k is the effect of baseline covariates for subject k; and ε_{ijk} is the random error (Jones and Kenward 2015). Because of the possibility of missing values in some of the measurements, we will employ a mixed effects model for these analyses, relying on the SAS proc mixed procedure for these analyses (Brown and Prescott, 2006). ***For this model, the effects of s_{ik} (subject) and ε_{ijk} (error) are considered random; all other effects are fixed.***

We will directly examine the effects of the drugs in older adults compared to younger adults. The mixed effects model will be adapted by the inclusion of additional variables to account for the effect of age group (g_{il} the effect of subject i in age group l; l=younger, older) and the treatment-by-age group interaction ($g_{il}*\tau_{t[i,j]}$). The effect of interest in these analyses will be the interaction, which will test whether the effects of MECA or NIC differ with respect to the age of the subject. We predict there will be a significant interaction and will examine the age differences in response to the challenge drugs. For **performance analyses, $p<0.05$** will be used for statistical significance. *We have used similar models in our prior work (Dumas et al. 2012; Potter et al. 2009).*

Analyses for fMRI Hypothesis Testing (H1b and H2b): Preprocessing and analysis of the functional data will be performed with Brain Voyager QX software (Brain Innovation, Maastricht, The Netherlands). The volumes will be realigned to the first volume to minimize the effects of head movements. Further data preprocessing comprises spatial (6 mm full-width half-maximum isotropic Gaussian kernel) as well as

temporal (high pass filter: 2 cycles/run) smoothing to remove aliased signal correlated with background respiration and heart rate. Anatomical and functional images are co-registered and normalized to Talairach space. Statistical analysis will be performed by multiple linear regression of the signal time course at each voxel. The expected BOLD signal change for each different type will be modeled by a canonical hemodynamic response function. One mean image per individual for the task-based contrast of interest (e.g. 3-back > 0-back) will be created. These contrast images will then be used for second level multi-subject/between group analyses using the standard ANOVA procedures described above.

The critical significance level for the **fMRI** will be p_{corr}**< 0.001**. To begin with, **whole brain analyses** will be used *because our hypotheses are about changes in activation in the working memory network and restricting the analysis to a region of interest may miss important effects of the nAChR drugs on brain activation.*

Additional Analysis: We will examine the **correlation** between working memory performance and MFG activation by conducting a regression analysis based on the difference between the placebo and nAChR blockade or stimulation conditions. First, we will compute difference scores for the performance measures and the percent signal change for the MECA and NIC study days compared to the placebo day. The differences scores for activation will then be used as the dependent measures in the analyses, while the difference scores for performance measures will serve as independent variables.

***Sample Size Calculations*:** The sample size calculations are based on pilot data collected in our laboratory in separate studies: 1) older women with MECA and placebo challenges 2) younger adults during NIC and placebo challenges, and 3) older and younger adults with no challenge. We assume that, while the **fMRI** changes in younger adults will be in the same direction as older adults, the magnitude of the changes will be smaller. fMRI performed in younger adults during a 3-back condition showed a mean beta value of -1.12 in the **MFG**, compared to a mean of -0.54 in older adults. We assume that the MECA challenge in the younger adults will increase activation to a mean beta value mimicking that of the older adults on placebo (-0.54), while older adults will have an even greater activation increase to a mean beta of 1.00. Assuming a standard deviation of 0.40, a correlation of 0.35, both calculated from our pilot data, and a Type I error rate of 0.001, 13 subjects will provide 90% power to detect differences between younger and older adults in this region after MECA compared to placebo. A sample size of 19 younger adults and seven older adults will provide greater than 90% power to detect the within-group differences as statistically significant.

For **working memory performance**, sample size calculations are based on a Type I error rate of 0.05. The mean proportion correct for the 3-back performance in older adults was 0.75 compared to a mean of 0.88 in the younger adults, with a standard deviation of 0.13. We predict that the effect of the **MECA** challenge will be to reduce this accuracy measure in both the younger and older adults, with the older adults proportion correct decreasing to 0.60 while the decrease in younger adults will be less severe, decreasing to 0.83. Assuming a correlation of 0.35 a total of **48 subjects** will provide 90% power to detect differences on this test with MECA compared to placebo. This sample size will be sufficient to provide 90% power to detect a statistically significant decrease in performance among the older adults.

Consistent with our results of younger adults following **NIC** challenge, we assume that the effects of this stimulation will be in the opposite direction but similar in magnitude to those outlined above. We assume approximately a 20% drop-out rate; therefore, we will recruit 56 subjects/group, with the goal of **48** subjects/age group completing the study.

For the correlational analyses, 48 subjects in each group will provide 90% power to detect a correlation of 0.46 as statistically significant and 80% power if the statistically significant correlation coefficient is 0.41.

Risks/Benefits: *Describe any potential or known risks. This includes physical, psychological, social, legal or other risks. Estimate the probability that given risk may occur, its severity and potential reversibility. If the study involves a placebo or washout period, the risks related to these must be addressed in both the protocol and consent. Describe the planned procedures for protecting against or minimizing potential risks and assess their likely effectiveness. Where appropriate, discuss plans for ensuring necessary medical or professional intervention in the event of adverse effects to the subjects. Discuss the potential benefits of the research to the subjects and others. Discuss why the risks to the subjects are reasonable in relation to the anticipated benefits to subjects and others. Discuss the importance of the knowledge gained or to be gained as a result of*

the proposed research and why the risks are reasonable in relation to the knowledge that reasonably may result. If there are no benefits state so.

POTENTIAL BENEFITS

Participants will not necessarily derive any direct benefits from participation in these studies other than the monetary remuneration for their time. It has been the experience of the CNRU that participants may derive psychological benefit and satisfaction from their participation in these types of studies because of the knowledge that their efforts may help future patients. The benefit to society is twofold: the knowledge gained about the nature of the cognitive changes associated with normal aging role of nicotinic cholinergic system in normal aging.

Importance of the Knowledge to be Gained

The benefits to society of greater knowledge about the etiology of the normal cognitive changes in aging and their possible amelioration would appear great. The risks include temporary side effects of the drugs, and as mentioned above, can be monitored and treated appropriately when this kind of study is conducted in a medical environment. When considering increasing older adult population and the increasing cost of care for those not able to live on their own because of debilitating changes in cognitive functioning, the overall the risk/benefit ratio appears to be in favor of conducting these studies.

POTENTIAL RISKS

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 and urinary hesitancy. Drs. Dumas, Potter, Naylor, 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.

NICOTINE TRANSDERMAL PATCH: At the nicotine dose proposed in this study, the major peripheral action of nicotine is facilitation of impulses through all autonomic ganglia, stimulation of the adrenal medulla and stimulation of sensory receptors including chemoreceptors in the carotid body. Ganglionic depression occurs at higher nicotine levels. In cardiovascular systems, mild increases in heart rate and blood pressure from sympathetic ganglion stimulation, catecholamine release from adrenal medulla, and aortic and carotid body chemoreceptor stimulation may occur. A mild parasympathetic response may be seen in the gastrointestinal tract and bladder (increased tone and motor activity), with increased secretion of exocrine glands. Nausea and vomiting can occur from peripheral (bowel activity and vagal efferent nerve stimulation) and central (medullary emetic chemoreceptor trigger zone stimulation) causes. Low dose stimulation of the CNS could in theory produce tremors and respiratory stimulation, although this is rarely seen except in patients with tremor disorders. Toxic nicotinic doses result in CNS depression. The most common adverse side effect of the nicotine transdermal patch is skin irritation and accounted for approximately 25 percent of adverse event reports regarding the nicotine transdermal patch to the FDA (Spyker et al. 1998). These effects consist of erythema, pruritus, edema, and rash. The PI and co-investigators have experience in administering transdermal nicotine for over ten years to non-smoking, healthy younger adults, healthy premenopausal women, and older patients with Mild Cognitive impairment. As the participants to be enrolled in this study will be selected for the absence of significant cardiovascular, cerebrovascular disease, or diabetes, we believe that the cardiovascular and cerebrovascular risk profile of transdermal nicotine in such participants is minimal.

fMRI RISKS: MRI has no known risks. Participants 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 and may be uncomfortable for some people who have claustrophobic-like reactions in confined spaces. Individuals will be allowed to terminate their participation should strong reactions occur. The long-term risks of functional MRI are unknown.

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 alternation of currents within the gradient coils causes the coil assemblies to vibrate against their mountings 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 60 minutes (or less) 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 (Brummet et al. 1988). 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 by Price et al. (Price et al. 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/FAHC we follow strict guidelines for the protection of subject hearing. Subjects are required to use both foam earplugs which 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 60 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.

***PROTECTIONS AGINST RISK*:** In addition to the exclusion criteria outlined above, the following steps will be taken to minimize risk. To enhance the confidentiality of participant data, subjects will be assigned a study number. All data will be kept in locked offices and suites and computerized data will be kept on a secure server.

All participants will be given medication under the close supervision of a physician and research nurse. Participants will be assessed by a research nurse and physician at the completion of each experimental session for residual effects of study medications on cognitive, motor, or cardiovascular functions. A standard field sobriety test will be used to assess motor and/or cardiovascular difficulties. This standardized test is completed at the beginning and end of protocol day and consists of three subtests including visual gaze nystagmus assessment, heel-to-toe walking, and standing on one leg. The participant's score on this test battery must be the same at discharge as at admission. Any participant having residual effects or impairments will be asked to remain in the hospital for observation until such effects dissipate. No prolonged residual effects have been seen in previous studies conducted by the CNRU.

Therapeutic Alternatives: *List the therapeutic alternatives that are reasonably available that may be of benefit to the potential subject and include in the consent form as well.*

x	***Not Applicable***

Data Safety and Monitoring: *The specific design of a Data and Safety Monitoring Plan (DSMP) for a protocol may vary extensively depending on the potential risks, size, and complexity of the research study. For a minimal risk study, a DSMP could be as simple as a description of the Principal Investigator's plan for monitoring the data and performance of safety reviews or it could be as complex as the initiation of an external, independent Data Safety and Monitoring Board (DSMB). The UVM/UVM Medical Center process for review of adverse events should be included in the DSMP.*

DATA AND SAFETY MONITORING PLAN:

Adverse events and protocol deviations will be reported by one of 3 mechanisms:

1) The University of Vermont/Fletcher Allen 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 245 South Park Suite 900, UVM) within 5 days of the event with copies forwarded to the Research Subject Advocate (RSA) office within the Clinical Research Center (CRC) on Shephardson 2 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 Baird 7 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.

Adverse Event and Unanticipated Problem (UAP) Reporting: *Describe how events and UAPs will be evaluated and reported to the IRB. All protocols should specify that, in the absence of more stringent reporting requirements, the guidelines established in the Committees on Human Research "Adverse Event and Unanticipated Problems Reporting Policy" will be followed. The UVM/UVM Medical Center process for review of adverse events and UAPs to subjects or others should be included in the DSMP.*

Adverse events reported to or noted by the study personnel will be reported to the PI within 24 hours. The PI will follow the guidelines established in the Committees on Human Research "Adverse Event and Unanticipated Problems Reporting Policy". The PI will review all adverse events and other safety information annually. Any unanticipated problems (protocol deviations, issues with confidentiality, or problems reported by the subject that do not meet the definition of an adverse event that occur during this study will be reported by the study personnel to the PI within 24 hours. The PI will report the unanticipated problem to the appropriate university officials (IRB) as per protocol.

Withdrawal Procedures: *Define the precise criteria for withdrawing subjects from the study. Include a description of study requirements for when a subject withdraws him or herself from the study (if applicable).*

Participants may be discontinued from this study at any time. Specific reasons for discontinuing a subject from this study are:

1) Voluntary discontinuation by the participant at any time
2) Safety reasons as judged by the investigators
3) Subject non-compliance with study requirements
4) Other reasons as determined by the investigator

Sources of Materials: *Identify sources of research material obtained from individually identifiable human subjects in the form of specimens, records or data. Indicate whether the material or data will be obtained specifically for research purposes or whether use will be made of existing specimens, records or data.*

Data obtained from the subjects will be scores on the neuropsychological screening measures, accuracy and response time measures on the cognitive tests, and brain imaging information. Blood will be collected for medical screening, genetic and hormone analyses. The data will be collected solely for research purposes.

DRUG AND DEVICE INFORMATION

Investigators are encouraged to consult the UVM Medical Center Investigational Pharmacy Drug Service (847-4863) prior to finalizing study drug/substance procedures.

Drug (s) ☐ ***Not applicable***

Drug name – generic followed by brand name and common abbreviations. Availability – Source and pharmacology; vial or product sizes and supplier. If a placebo will be used, identify its contents and source. (attach investigational drug brochure)

Nicotine and Mecamylamine, oral placebo, placebo patch.

Preparation: Reconstitution instructions; preparation of a sterile product, compounded dosage form; mixing guidelines, including fluid and volume required. Identify who will prepare.

oral mecamylamine and commercially available 7 mg nicotine patch.

Storage and stability – for both intact and mixed products.

These medications will be stored at the investigational pharmacy as per packaging instructions. Mecamylamine will be created from commercially available capsules compounded by the Investigational Pharmacists with lactose.

Administration – Describe acceptable routes and methods of administration and any associated risks of administration.

Patches will be applied to the subject's upper back. Mecamylamine will be an oral capsule.

Toxicity – Accurate but concise listings of major toxicities. Rare toxicities, which may be severe, should be included by indicated incidence. Also adverse interactions with other drugs used in the protocol regimen as well as specific foods should be noted. Address significant drug or drug/food interactions in the consent form as well. List all with above details.

Drug Safety:

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 and urinary hesitancy. Drs. Dumas, Potter, Naylor, 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.

NICOTINE TRANSDERMAL PATCH

Nicotine Side Effects: At the nicotine dose proposed in this study, the major peripheral action of nicotine is facilitation of impulses through all autonomic ganglia, stimulation of the adrenal medulla and stimulation of sensory receptors including chemoreceptors in the carotid body. Ganglionic depression occurs at higher nicotine levels. In cardiovascular systems, mild increases in heart rate and blood pressure from sympathetic ganglion stimulation, catecholamine release from adrenal medulla, and aortic and carotid body chemoreceptor stimulation may occur. A mild parasympathetic response may be seen in the gastrointestinal tract and bladder (increased tone and motor activity), with increased secretion of exocrine glands. Nausea and vomiting can occur from peripheral (bowel activity and vagal efferent nerve stimulation) and central (medullary emetic chemoreceptor trigger zone stimulation) causes. Low dose stimulation of the CNS could in theory produce tremors and respiratory stimulation, although this is rarely seen except in patients with tremor disorders. Toxic nicotinic doses result in CNS depression. With use, tolerance develops to virtually all acute adverse effects.

General Safety Experience with the Nicotine Transdermal Patch: A large meta-analysis was conducted examining data from 35 clinical trials utilizing the transdermal nicotine patch in over 5500 individuals (Greenland et al. 1998). Few adverse cardiovascular outcomes were reported and no excess of these outcomes was detected among patients assigned to nicotine patch use compared to placebo patch users. Minor adverse effects such as sleep disturbances, nausea, localized skin irritation, and respiratory symptoms were elevated in patch users compared to placebo users.

Dermatologic Safety: The most common adverse side effect of the nicotine transdermal patch is skin irritation and accounted for approximately 25 percent of adverse event reports regarding the nicotine transdermal patch to the FDA (Spyker et al. 1998). These effects consist of erythema, pruritus, edema, and rash. Mild skin irritation is common and generally occurs after three weeks of continuous use. Mild to moderate reddening of the skin is seen in 25% of subjects and transient itching in 29%. More severe reactions requiring modification of treatment have been reported in up to 12% of users (DrugDex Drug Evaluation Monograph). Management of the symptoms is usually straightforward and is accomplished by patch rotation, local treatments, and instructing the patient to remove the patch prior to going to bed. The PI has experience in administering transdermal nicotine for over five years to a non-smoking patient with Huntington's disease for movement disorder control. This long-term exposure has been extremely well tolerated with only minor skin irritation seen.

Cardiovascular Safety: There are a number of mechanisms whereby nicotine could potentially cause or aggravate cardiovascular disease (Benowitz 1998a). Nicotine stimulates CNS sympathetic systems and increases release of catecholamines from both the adrenal and vascular nerve endings. While tolerance appears to develop to these cardiac stimulatory effects, the tolerance developed is only partial (Benowitz 1998a). While there may be a small chronic cardio-stimulatory effect (approximately seven beats per minute), the dose response curve appears to be flat (Benowitz 1998a).

However, studies have not demonstrated that nicotine replacement therapies are associated with increased cardiovascular risk or increased incidence of cardiovascular adverse events (DrugDex Drug Evaluation Monograph). The largest and longest such study was the Lung Health Study that enrolled almost 6000 individuals in a study over 5 years involving nicotine replacement therapies for smoking cessation. In this group with chronic lung disease, nicotine use was found to be marginally protective of cardiovascular health compared to non-use of nicotine (Murray and Daniels 1998). This protective effect persisted even when adjusted for smoking status. Even within the ex-smoking sub-group in the same study, nicotine users had substantially lower rates of hospitalization then non-users. Nicotine also showed a marginally protective effect against peptic ulcer disease in the same subjects. In a long-term maintenance study of non-smoking patients with ulcerative colitis, there were no increased cardiovascular events and markers of cardiovascular risk either did not change or actually decreased (e.g. fibrinogen) (Rhodes et al. 1998). A recent investigation of the effects of 26 weeks of chronic oral nicotine showed improved cardiovascular risk parameters (e.g. capillary flow, fibrinogen) after smoking cessation with no negative effects of nicotine (Haustein et al. 2002). Nicotine does not appear to promote thrombosis or platelet aggregation nor does nicotine replacement therapy increase the risk of acute myocardial infarction (DrugDex Drug Evaluation Monograph).

Studies of patients with known cardiovascular disease have similarly not shown an increase in cardiovascular events or toxicity secondary to nicotine therapy. Two large studies of men with documented coronary artery disease with up to 10 weeks of nicotine therapy showed lower rates of cardiovascular endpoints and events in the nicotine-treated group(Rennard et al. 1998). A study of myocardial perfusion in men with coronary artery disease showed that cigarette smoking was associated with significantly greater myocardial perfusion deficits than nicotine therapy alone, suggesting that such a perfusion defect is due to factors from tobacco other than nicotine. In reviewing the available clinical trial literature and data reported to the FDA as of 1998, Rennard and colleagues concluded: "the available clinical trial and the clinical experience reported to date are consistent with the relative safety of transdermal nicotine in stable patients with cardiac disease."

Cerebrovascular Safety: Smoking is a preventable risk factor for ischemic stroke and some preclinical studies have suggested potential mechanisms by which smoking and/or nicotine might increase the risk of ischemic stroke (Gerzanich et al. 2001; Hawkins et al. 2002; Zidovetzki et al. 1999). However, a large meta-analysis of 35 smoking cessation trials did not find any increased incidence of stroke in nicotine replacement therapy users(Greenland et al. 1998).

Conclusion regarding cardio- and cerebrovascular safety: As the subjects to be enrolled in this study will be selected for the absence of significant cardiovascular, cerebrovascular disease, or diabetes, we believe that the cardiovascular and cerebrovascular risk profile of transdermal nicotine in such patients is excellent.

Insulin Sensitivity: There have been some epidemiologic studies suggesting a positive relationship between smoking and insulin resistance (Mikhailidis et al. 1998) although some studies are contradictory (Henkin et al. 1999). Some investigations have suggested that changes in insulin sensitivity may be restricted to smokers who are also diabetic (Targher et al. 1997). One study that did examine long-term nicotine use without cigarette smoking showed that nicotine gum users had higher circulating leptin levels that were negatively correlated with the degree of insulin sensitivity (Eliasson and Smith 1999). However, the subjects were also recent ex-smokers, complicating interpretation of these results and acute administration of nicotine during the study did not change circulating leptin levels. Contradictory results were also seen in studies of smokeless tobacco use on cardiovascular risk factors and insulin levels with one study of heavy users finding impaired measures of glucose tolerance (Persson et al. 2000) while another study did not (Eliasson et al. 1995). At this point, it is not clear that nicotine use alone in nonsmokers is associated with changes in insulin sensitivity.

Carcinogenesis: Nicotine has not been shown to be carcinogenic in animals. Long-term epidemiologic studies of oral tobacco use suggest that the nitrosamine content of tobacco is critical to determining the cancer risk from non- smoke related tobacco use, rather than nicotine (Benowitz 1998b). Whether nicotine can act as a permissive agent to encourage the development of cancer is unclear, but it does not seem to have any effect unless co-administered with tobacco (Benowitz 1998a).

Abuse Potential: We believe that the probability that subjects in this study might be prompted by their participation to begin to use nicotine containing products or tobacco is extremely low because:

1) Nicotine replacement therapies have an extremely low abuse liability (Hughes et al. 1989). A recent study showed that adolescents are extremely unlikely to be interested in taking up nicotine replacement therapies because of the easier availability of cigarettes as opposed to over-the-counter nicotine replacement therapies (Adams et al. 2001). Investigators from this study concluded that the prevalence of the recreational use of nicotine replacement therapy products was so low that the hypothesis that the nicotine replacement patch or spray might act as in "gateway drug" did not seem tenable.

2) The duration of exposure to nicotine is short in this study (60 minutes) and is a single administration. The onset of psychoactive effects is relatively slow. Nicotine patches have some unpleasant side effects and therefore are unlikely to be reinforcing.

3) A study by Fertig et al (1986) showed that experimental administration of tobacco did not induce ex-smokers to relapse into smoking. In another study (Hughes et al. 1989b), when non-smokers and ex-smokers were followed after participating in a study of nicotine gum administration, no subjects were found to be smoking or using other nicotine products three months following completion of the study.

4) An important characteristic of all drugs which produce dependency is the pharmacokinetic parameters associated with the route and form of administration (Farre and Cami 1991). With respect to nicotine, researchers of the NIDA Addiction Research Center (Henningfield and Keenan 1993), as well as others in the field (Marks et al. 1987; Pomerleau 1992; Rose et al. 1985)(Rose et al. 1996) (Shytle et al. 1996), have reported that the slower absorption of nicotine offered by the transdermal patch relative to tobacco

products substantially reduces the likelihood of nicotine dependence in users of the patch. This was supported by a study describing a double-blind placebo-controlled study investigating the therapeutic potential of the transdermal nicotine patch for patients suffering from ulcerative colitis (Thomas GA et al. 1995). Although all of the subjects were adults and many former tobacco users, despite 26 weeks of daily applications of 15 mg nicotine patches, no withdrawal symptoms were reported from these patients following discontinuation of the patch. In addition, a crossover trial evaluating the "liking" rating for the patch (22mg or 44 mg/24hr) in adults found no difference in scores between the active and placebo systems (Bunker et al. 1992).

5) We have administered intravenous and/or transdermal nicotine and structurally related nicotinic agonists over the past 17 years to several hundred non-smoking subjects including young and elderly normal volunteers, patients with Alzheimer's disease, and patients with Parkinson's disease. We have not had a single subject take up tobacco use as a consequence of study participation.

6) In studies treating adolescents with Tourette's Syndrome with nicotine patches, researchers reported no known cases of participants craving nicotine or beginning to smoke following exposure to transdermal nicotine as part of a research treatment protocol. Specifically, none of the children with Tourette's Syndrome who had been treated repeatedly (19 days) with the transdermal nicotine patch reported that they felt "addicted to" or "craved" the nicotine patch when it was discontinued (Shytle et al 1996)
The safeguards that we have instituted in conjunction with data documenting that the administration of nicotine via non-tobacco routes is not associated with abuse liability, suggests that the incremental additional risk to subjects for tobacco use over and above their background use rate is likely to be very small.

Is it FDA approved: (include FDA IND Number)

1. *in the dosage form specified? If no, provide justification for proposed use and source of the study drug in that form.*
   See above.
2. *for the route of administration specified? If no, provide justification for route and describe the method to accomplish.*
   See above.
3. *for the intended action?*
   See above.

Device (s) [x] ***Not applicable***

Device name and indications (attach investigational device brochure)

Is it FDA approved: (include FDA IDE Number)

1. *for indication specified? If no, provide justification for proposed use and source of the device.*

Risk assessment (non-significant/significant risk) - PI or sponsor needs to assess risk of a device based upon the use of the device with human subjects in a research environment.

SUBJECT CHARACTERISTICS, IDENTIFICATION AND RECRUITMENT

Subject Selection: *Provide rationale for subject selection in terms of the scientific objectives and proposed study design.*

Participants: In order to complete testing on 48 **non-smoking** healthy older adults, aged 65-75 years and 48 **non-smoking** healthy younger adults, aged 21-30 years, we will screen 58 potential participants in each age group for a total of 116. An equal number of men and women will be recruited. During the drug challenge phase of the study younger female participants will be tested in the follicular phase of their menstrual cycles and will have a negative pregnancy test before each session. The older adult group will be carefully screened using a neuropsychological assessment (below) to ensure they do not have MCI or pre-MCI. In both age groups, we anticipate a dropout rate of approximately 20%, as has been our experience in our prior studies. We aim to have 48 older and 48 younger participants complete the study for a total of 96 participants.

Cognitive/Behavioral Screening: All **older participants** will be cognitively and behaviorally assessed using standard tests designed to exclude participants with significant cognitive or behavioral impairment including the Mini Mental State Exam (MMSE) (Folstein et al. 1975), Brief Cognitive Rating Scale (Reisberg et al. 1993b), the Mattis Dementia Rating Scale (Jurica et al. 2001), and the Global Deterioration Scale (GDS) (Reisberg et al. 1993a). Participants will be required to have a GDS score of 1-2, a MMSE score of greater than or equal to 27, and score above 130 on the Mattis scale. Older participants will have an informant complete the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE; Jorm 2004) and have scores of 3 or less indicating no cognitive change.

All older and younger participants will undergo the following behavioral screening measures: a Structured Clinical Interview for DSM-IV-TR (SCID) (First et al. 2001) to establish the presence/absence of Axis I psychiatric disorders, and the Beck Depression Rating Scale (BDI, score < 10) (Beck et al. 1961). Participants will be excluded if they have a current Axis 1 disorder. All participants will perform the Wechsler Abbreviated Scale of Intelligence (WASI) to assess IQ and will be required to score above 80.

Neuropsychological Screening: In an effort to ensure the older adults are not at the beginning stages of MCI or dementia and to assess general neuropsychological functioning, **older and younger participants** will be screened with the **Repeatable Battery for the Assessment of Neuropsychological Status** (**RBANS**; Randolph 1998). RBANS was developed for the dual purposes of identifying and characterizing abnormal cognitive decline in older adults and as a neuropsychological screening battery for younger adults. The RBANS assesses five cognitive domains including immediate memory, language, visuospatial/constructional ability, attention, and delayed memory and provides a global total measure. Each participant will need to score within one standard deviation (*SD*) of the mean for her/his age. A measure of executive functioning is missing from the RBANS so we will include selected measures from the **Delis-Kaplan Executive Function System** (**D-KEFS**). Specifically we will include the Trail Making Test and Verbal Fluency subtests. All participants will be required to score within one *SD* of the mean for their age.

Medical Screening: Participants will be physically healthy **nonsmokers** with a BMI ≤ 32 kg/m^2, and 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, U/A, ECG), and will have a medical history review and physical examination to establish general physical health. Blood will also be obtained for an **exploratory genetic analysis** to examine APOE and COMT genotypes as these genes have been shown to be important in cognition in older adults. APOE is important for cognitive decline in older adults and associated with cholinergic function (Espeseth et al, 2006). The COMT Val^{158}Met polymorphism modulates working memory performance in psychiatric populations (e.g. Meyer-Lindenberg & Weinberger 2006) but not in healthy subjects (Blanchard et al. 2011). However, it affects working memory-related brain activation (Jacobs & D'Esposito 2011). Depending on the distribution of genotypes we will use this information as a covariate in the imaging analysis described below

Vulnerable Populations: *Explain the rationale for involvement of special classes of subjects, if any. Discuss what procedures or practices will be used in the protocol to minimize their susceptibility to undue influences and unnecessary risk (physical, psychological, etc.).*

x ***Not applicable***

Number of Subjects: *What is the anticipated number of subjects to be enrolled at UVM/UVM Medical Center and in the case of a multi-center study, with UVM/UVM Medical Center as the lead, the total number of subjects for the entire study.*

Participants: In order to complete testing on 48 **non-smoking** healthy older adults, aged 65-75 years and 48 **non-smoking** healthy younger adults, aged 21-30 years, we will screen 58 potential participants in each age group for a total of 116. An equal number of men and women will be recruited. During the drug challenge phase of the study younger female participants will be tested in the follicular phase of their menstrual cycles and will have a negative pregnancy test before each session. The older adult group will be carefully screened using a neuropsychological assessment (below) to ensure they do not have MCI or pre-MCI. In both age groups, we anticipate a dropout rate of approximately 20%, as has been our experience in our prior studies. We aim to have 48 older and 48 younger participants complete the study for a total of 96 participants.

Inclusion/Exclusion Criteria: *Eligibility and ineligibility criteria should be specific. Describe how eligibility will be determined and by whom. Changes to the eligibility criteria at a later phase of the research have the potential to invalidate the research.*

Cognitive/Behavioral Screening: All **older participants** will be cognitively and behaviorally assessed using standard tests designed to exclude participants with significant cognitive or behavioral impairment including the Mini Mental State Exam (MMSE) (Folstein et al. 1975), Brief Cognitive Rating Scale (Reisberg et al. 1993b), the Mattis Dementia Rating Scale (Jurica et al. 2001), and the Global Deterioration Scale (GDS) (Reisberg et al. 1993a). Participants will be required to have a GDS score of 1-2, a MMSE score of greater than or equal to 27, and score above 130 on the Mattis scale. Older participants will have an informant complete the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE; Jorm 2004) and have scores of 3 or less indicating no cognitive change.

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Inclusion of Minorities and Women: *Describe efforts to include minorities and women. If either minorities or women are excluded, include a justification for the exclusion.*

INCLUSION OF WOMEN

The focus of this proposal is on cognitive changes in aging of the brain nicotinic systems. One half of the subjects will be women in this study. Dr. Dumas has a proven track record of recruitment of women into studies such as the one proposed in this application as her research in the past has focused on cognition and menopause that occurs in older women. We also believe that the role of the nicotinic system in cognition does not differ by gender and thus we will recruit and equal number of men and women into the proposed study.

INCLUSION OF MINORITIES

We are unaware of data to suggest differential performance on pharmacological challenge and cognition studies of racial/ethnic minorities when equated for cognitive level. Prior studies of the cognitive effects

of cholinergic agents have not found an ethnic/racial effect, but the studies have overwhelmingly been in Caucasians and most studies have not looked for such an effect. However, there is no a priori reason to suspect that the results from this study will not be generalizable to all populations. Our recruited patients for studies to date have been overwhelmingly majority, as reflects the very low presence of minority individuals in the areas that we recruit from (Northern New England, see Tables 2 & 3). Nonetheless, assisted by the CRC effort to recruit minority subjects for clinical research studies, we will be actively soliciting minority patients from area practitioners. In sum, while questions remain about ethnic/racial issues in studies of cholinergic and dopaminergic effects on cognition, we expect the results to be generalizable in spite of low minority participation. This study will make every attempt to recruit members of all racial/ethnic groups within the limits of our available population.

Table 1. National Demographics for Normal Volunteer Populations (% of total population; male not reported)

	American Indian or Alaskan Native %	Asian or Pacific Islander %	Black, not of Hispanic Origin %	Hispanic %	White, not of Hispanic Origin %	Other or Unknown %	Total %
Female	0.4	1.4	6.3	4.3	38.7	0	51

Table 2. Local Racial Demographics for Normal Volunteer Populations (% of total population, based on 2000 Census)

	American Indian or Alaskan Native %	Asian or Pacific Islander %	Black, not of Hispanic Origin %	White, not of Hispanic Origin %	2 or more Races %	Other or Unknown %	Total %
Vermont	0.4	0.9	0.5	96.8	1.2	0.2	100

Table 3. Local Ethnic Demographics for Normal Volunteer Populations (% of total population, based on 2000 Census)

	Caucasian%	Hispanic %	Total %
Vermont	99.1	0.9	100

Outreach Program for Increasing Minority Participation: We are able to draw on the CRC Minority Outreach Program to try to increase minority participation in our research studies. The CRC assisted information dissemination is an outreach to the small minority communities that do exist in our region. A description of this program is as follows:

The State of Vermont has a population of 606,000 people and 98% of them are Caucasian. Despite two programs to relocate Vietnamese and Laotian refugees in the State of Vermont, the number of minorities, both in absolute numbers and as a percentage of the total population, remains unusually low.

In some studies, certain investigators have achieved adequate numbers of minorities in their studies. Achieving this goal, however, has remained problematic and elusive for most other investigators. These efforts are further hampered by the increasing suspicion amongst some minority constituents that the research community is sometimes more interested in filling a quota than in contributing to their present and future well-being. This problem is obviously compounded by the extremely low percentages and absolute numbers of individuals of a given minority, which can often result in the same individual being repetitively polled to participate in the studies of different investigators.

The Administrative Manager of the CRC has held a series of meetings and discussions to try and develop a workable policy to address the issues of minority recruitment. Input was solicited from the

following individuals or organizations:

The National Institute of Health Guidelines on Inclusion of Women and Minorities
The University of Vermont Commission on Racial Equality and Multicultural Education
The Office of Minority Health, The Vermont State Department of Health
The African/Latino/Asian/Native American Health Summit Report from Burlington, Vermont (ALANA)
The Office of Research on Minority Health of the National Institutes of Health
The Vice-Provost for Research of the University of Vermont
Dean of the College of Medicine of the University of Vermont
Vermont Senators, The Honorable Patrick Leahy and The Honorable Bernard Sanders
Vermont Congressman The Honorable Peter Welch
American Indian Law Center, Albuquerque, New Mexico
The Director of Office for the Protection of Research Risks (OPRR)
CRC Scientific Advisory Committee

These discussions resulted in the formulation and acceptance of formal CRC goals and guidelines for minority research. The guiding principle accepted from that document was that "As the CRC becomes involved in studies investigating questions about health and disease issues in communities as well as individuals, the implications for the communities involved in studies need to be taken into account. Perspectives gained from members of the community during the conception and design of any research will enhance understanding of the focus of the research, encourage community participation and ensure that cultural, religious, spiritual and community values will not be undermined or ignored during the study."

The CRC Outreach Program is, consequently, focused primarily on the dissemination of information about opportunities for participation in clinical research. Once approved by the IRB and CRC Scientific Advisory Committee, this protocol will be eligible to be processed through the Outreach Program. The components of this program are as follows:

1. Incorporation of a brief description of the study, the study population, and the contact person and telephone number into a news sheet produced from CRC administration and entitled Study Highlights. This news sheet is not only posted prominently throughout the University of Vermont Medical Center and the University of Vermont campus, but is made available to publicity officers both within and outside the institution.
2. Dissemination of individual announcements, or the Study Highlights document described above, through newspaper or radio or television announcements. Although individual investigators are often required to pay for these services, the Program has been successful in many cases in reducing or entirely abolishing the normal fees associated with such advertising processes.
3. Dissemination of individual information or the Study Highlights document to the Office of Minority Health at the Vermont State Department of Health. Lauren Corbett, MSW, coordinates this office which has formed extensive ties to many community-based and professional organizations serving communities of color and minorities in Vermont. These organizations include Abenaki Self Help, ALANA Community Organization, Austin School for the Deaf, Burlington Community Health Center, Burlington Council on Refugees and Immigrants, Dawnland Native American Center, Vermont Refugee Assistance Program, Refugee Resettlement Program, Tibetan Resettlement Project, Vietnamese Association, Vermont Red Cross Language Bank, Africans in Motion for Communities of Color, Vermont Human Rights Commission, Burlington Community Economic Development Office, Green Mountain Lao Association, Missisquoi Health Center, Vermont Coalition for Lesbian and Gay Rights, Vermont Aids Council, American Civil Liberties Union, Racial Justice and Equity Project, Multicultural Council of Vermont, Medical Student Alana Group and Community Health Awareness Project. Many of these organizations have either informal, or increasingly, formal information networks often including newsletters. The Office of Minority Health also keeps an up-to- date listing of interpreters who are available throughout the State of Vermont to translate consent forms and dialogues between investigators and subjects into any of the numerous languages and dialects spoken amongst these communities.

Inclusion of Children: *Describe efforts to include children. Inclusion is required unless a clear and compelling rationale shows that inclusion is inappropriate with respect to the health of the subjects or that inclusion is inappropriate for the purpose of the study. If children are included, the description of the plan should include a rationale for selecting or excluding a specific age range of children. When included, the plan must also describe the expertise of the investigative team in working with children, the appropriateness of the available facilities to accommodate children, and the inclusion of a sufficient number of children to contribute to a meaningful analysis relative to the purpose of the study.* ***If children are excluded*** *then provide appropriate justification. Provide target accrual for this population.*

INCLUSION OF CHILDREN

Children will not be included in this study. The topic of this application concerns the effects of aging on brain nicotinic systems. The type of studies described in this application would generally be precluded in children because the clinical interventions envisioned, including non-therapeutic drug administration (nicotinic antagonist and agonists) are generally not permitted in children.

For protocols including the use of an investigational drug, indicate whether women of childbearing potential have been included and, if not, include appropriate justification.

All pre menopausal women will be required to have a negative pregnancy test at screening and before each study day begins.

If HIV testing is included specifically for research purposes explain how the test results will be protected against unauthorized disclosure. Include if the subjects are to be informed of the test results. If yes, include the process and provision for counseling. If no, a rationale for not informing the subjects should be included.

x ***Not applicable***

Recruitment: *Describe plans for identifying and recruitment of subjects. All recruitment materials (flyers, ads, letters, etc) need to be IRB approved prior to use.*

Participants will be recruited through print and electronic advertisements around the greater Burlington and Northern New York region.

FINANCIAL CONSIDERATIONS

Expense to Subject: *If the investigation involves the possibility of added expense to the subject (longer hospitalization, extra studies, etc.) indicate in detail how this will be handled. In cases where the FDA has authorized the drug or device company to charge the patient for the experimental drug or device,* ***a copy of the authorization letter from the FDA or sponsor must accompany the application. Final approval will not be granted until the IRB receives this documentation.***
There are very limited circumstances under which study participants may be responsible (either directly or via their insurance) for covering some study-related expenses. If the study participant or their insurer(s) will be billed for any portion of the research study, provide a justification as to why this is appropriate and acceptable. For example, if the study involves treatment that is documented standard of care and not investigational, state so. In these cases, the protocol and the consent should clearly define what is standard of care and what is research.

There will be no expenses to the participant in this study.

Payment for participation: *Describe all plans to pay subjects, either in cash, a gift or gift certificate. Please note that all payments must be prorated throughout the life of the study. The IRB will not approve a study where there is only a lump sum payment at the end of the study because this can be considered coercive. The amount of payment must be justified. Clarify if subjects will be reimbursed for travel or other expenses.*

☐ ***Not applicable***

Participants will receive monetary compensation ($400) for the time commitment involved in this study. Participants who withdraw prior to the completion compensation will be pro-rated.

Collaborating Sites*. When research involving human subjects will take place at collaborating sites or other performance sites when UVM/UVM Medical Center is the lead site, the principal investigator must provide in this section a list of the collaborating sites and their Federalwide Assurance numbers when applicable. (agreements may be necessary)*

x ***Not applicable***

INFORMED CONSENT

Consent Procedures*: Describe the consent procedures to be followed, including the circumstances under which consent will be obtained, who will seek it, and the methods of documenting consent. Specify the form(s) that will be used e.g. consent (if multiple forms explain and place identifier on each form), assent form and/or HIPAA authorization (if PHI is included). These form(s) must accompany the protocol as an appendix or attachment.*

Note: Only those individuals authorized to solicit consent may sign the consent form confirming that the prospective subject was provided the necessary information and that any questions asked were answered.

Informed consent will be obtained from all participants. Each participant will receive an oral and a written explanation of the

purposes, procedures and potential hazards of these studies. A copy of the informed consent will be given to the participant and one will be entered into her/his CRC research record.

Information Withheld From Subjects: *Will any information about the research purpose and design be withheld from potential or participating subjects? If so, explain and justify the non-disclosure and describe plans for post-study debriefing.*

x	***Not applicable***

Attach full grant application, including budget information and/or any contract or draft contract associated with this application.