Depressed Mood Improvement through Nicotine Dosing (Depressed MIND Study)
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1.0 BACKGROUND

Late-life depression (LLD) is characterized both by affective symptoms and broad cognitive deficits. The co-occurrence of cognitive deficits in LLD, particularly executive dysfunction, is a clinically relevant phenotype characterized by significant disability and poor antidepressant response. Cognitive deficits can persist even with successful antidepressant treatment and increase the risk of depression relapse. Despite the clinical importance of cognitive deficits in LLD, there are no established treatments that specifically target cognition in this population. This is particularly important, as the cognitive deficits appear to directly contribute to disability and poor antidepressant treatment outcomes. T**he lack of clear pharmacologic targets and therapies aimed at improving cognitive deficits in depression is a substantial deficiency in current therapeutics**.

We propose that modulation of the cholinergic system by nicotinic receptor stimulation will improve both mood and cognition in depressed elders. Through its effects on cognitive control and default mode networks, we hypothesize that nicotine will benefit mood. As observed in smokers, nicotine's effect to increase cognitive control network activity while reducing default mode network activity will reduce depression's characteristic bias to negatively valenced stimuli and decrease rumination. Supporting this theory, nicotinic receptor activity stimulates serotonin release and protects against worsening mood with tryptophan depletion. Clinically, transdermal nicotine improves mood in smokers while a placebo-controlled pilot trial in nonsmoking depressed adults found that transdermal nicotine significantly improved mood.

In our previous trial examining Mild Cognitive Impairment, we demonstrated that transdermal nicotine safely improved cognitive function as measured by tests of attention, episodic memory, and processing speed. These data support the current NIH-funded multi-site MIND (Memory Improvement through Nicotine Dosing) study (Newhouse, PI). These same cognitive domains are impaired in LLD and we anticipate individuals with LLD will experience similar benefit. Nicotinic acetylcholine receptor stimulation appears to increase or "tune" activity in attention and cognitive control networks while improving default mode network deactivation during tasks. We hypothesize that these neural effects of nicotine will mediate nicotine's cognitive benefit in LLD.

2.0 RATIONALE AND SPECIFIC AIMS

Our central hypothesis is that in LLD, *transdermal nicotine will safely improve depression by increasing activity in cognitive control regions and decreasing activity in DMN regions*. *This will result in a decreased attentional bias to and reactivity to negative stimuli*. Secondarily, we hypothesize that transdermal nicotine will also improve *subjective and objective cognitive performance through these same network effects.* In this initial pilot

project, we will test our hypotheses in 15 nonsmoking depressed elders with subjective cognitive impairment. Following baseline neuroimaging and cognitive testing, participants will receive 12 weeks of open-label transdermal nicotine. Afterwards, participants will repeat neuroimaging and cognitive assessments.

Primary Aim 1: **To determine whether administration of transdermal nicotine over 12 weeks improves clinical symptoms in patients with LLD with SCI.**

Hypothesis 1: Transdermal nicotine administration will result in reductions in depression severity measured by the Montgomery-Asberg Depression Rating Scale (MADRS; primary outcome). It will also result in improvement in broader assessments of depressive symptomatology, including anhedonia, apathy, fatigue, sleep, and rumination (secondary outcomes).

Hypothesis 2: Transdermal nicotine administration will result in improvements in attentional performance on the Conner's Continuous Performance Task (CPT; primary outcome). It will also result in improvement in subjective and objective cognitive performance on other tasks measuring attention, episodic memory, working memory, processing speed, and executive function (secondary outcomes).

Secondary Aim 2: **To determine whether administration of transdermal nicotine over 12 weeks modulates canonical intrinsic functional network activity in LLD with SCI.**

Hypothesis 3: On repeat administration of the Posner task of external attention, transdermal nicotine administration will result in increased activity within the cognitive control network and decreased activity within the default mode network.

Hypothesis 4: Transdermal nicotine administration will result in increased functional connectivity within the cognitive control network and decreased connectivity within the default mode network at rest.

Hypothesis 5: Changes in intrinsic network activity / connectivity with transdermal nicotine administration will be associated with changes in mood symptoms and subjective and objective cognitive performance.

3.0 ANIMAL STUDIES AND PREVIOUS HUMAN STUDIES

3.1. Nicotine effects on depression: The concept that there is an association between cholinergic function and depression dates back decades. The original cholinergic hypothesis of depression by Janowsky and colleagues proposed that depression is associated with hyperactivation of the cholinergic system, resulting in decreased noradrenergic activity (1). Pharmacologically, cholinergic effects on mood must be mediated either by the nicotinic receptor (nAChR) family or the muscarinic receptor (mAChR) family, with muscarinic mechanisms being implicated in the studies of scopolamine by Furey and Drevets (2,3). The cholinergic hypothesis is supported by observations that smoking rates are high in MDD (4,5) while depressed smokers have difficulty with smoking cessation and are at risk for depression relapse during smoking cessation (6-11). However, these data do not inform about specific effects of nAChR stimulation.

Animal models support that modulation of nAChR activity is beneficial in depression (12,13). Several studies in rat models of depression demonstrate that nicotine and nAChR agonists reduce depressive behavior (14-20). Clinical studies in humans support that nAChR stimulation may have antidepressant properties. The few studies examining nonsmokers with MDD **demonstrate that transdermal nicotine administration results in significant improvement in mood** (21-23) and may have long-term efficacy comparable to that of fluoxetine (24). Although these studies examined small samples, nicotine exhibited antidepressant effects even at low doses in a placebo-controlled trial (22). In contrast, results have not been encouraging for nAChR antagonists. Despite early results suggesting the nAChR antagonist mecamylamine could have antidepressant augmentation effects in MDD (25), large-scale trials examining mecamylamine's s-enantiomer in MDD did not find antidepressant efficacy (Targacept press release). To our knowledge, there has not been a study examining either nAChR agonists or antagonists in late-life depression (LLD).

3.1.a. Nicotine and affective cognition: Nicotine also modulates affective stimuli processing. Transdermal nicotine in nondepressed smokers reduces the attentional bias to negatively valenced stimuli (26,27) and the distraction caused by negative stimuli (28). Similar effects are seen in nondepressed nonsmokers (29), although another study of nondepressed nonsmokers found increased reactivity to negative stimuli (30).

These findings have great significance in context of depression. Dating back to Beck's cognitive model

(31), depression is characterized by negative schema and dysfunctional attitudes that filter and bias affective information. Individuals with MDD exhibit greater and prolonged neural reactivity to negative stimuli (32-36), attend to negative stimuli longer (37) and show enhanced memory for negatively valenced information (38,39). *A drug that reduces attention to negative stimuli may have great benefit for depressed individuals*. Moreover, it has the potential to improve cognitive performance, a significant problem for many individuals with LLD.

3.2. Nicotine as a cognitive enhancer: The cholinergic system is the primary neurotransmitter system responsible for cognitive symptoms in dementia (40), with nicotinic receptors being particularly important (41). Neuronal nicotinic receptors (nAChRs) are found throughout the central nervous system and nicotinic innervation of the hippocampus, amygdala and frontal cortex are vital to memory function (Levin 2000)(42). In addition to direct stimulation of nAChRs, nicotine stimulates the release of a variety of transmitters involved in cognitive function, including dopamine, norepinephrine, serotonin, and glutamate (43,44).

3.2.a Cognitive Effects of Nicotinic Stimulation*:* Cognitive improvement is a well-established effect of nicotine. A recent meta-analysis of over 41 double-blind placebo-controlled studies concluded that nicotine has positive effects on attention, memory, and motor abilities which likely represent true performance enhancement (45). In smokers, nicotine improves performance on attentionally and cognitively demanding vigilance tasks (46-48) even in the absence of withdrawal effects (45,49). The nicotinic system appears to modulate controlled attentional processing when task conditions are difficult (50-52).

Experimental pharmacological approaches are illuminating. Newhouse et al (53,54) examined blockade of nAChRs with mecamylamine and found that nAChR blockade impaired cognitive performance in a way that modeled age- and disease-related learning impairments. The use of nicotinic agonists also reveals a role for the cholinergic system in attention and memory. Studies of nicotine in younger adults showed that the nicotinic system is involved in the partitioning of attentional resources, working memory, inhibition of irrelevant information, and improved performance on effortful tasks (55-57). Nicotine improves aspects of selective attention (58), spatial attention (59), and reaction time in an attention tasks (60).

3.2.b. Clinical Studies of Nicotinic Stimulation for Cognition*:* Newhouse and colleagues first showed evidence of improved memory with intravenous nicotine injection in Alzheimer Disease (AD) subjects (61). Subcutaneous nicotine injection (62,63) and nicotine skin patch treatment also significantly improves cognitive function in AD patients (64,65). In AD, nicotine improves attention and lessen errors (66). Newhouse extended this work to show that the novel nicotinic agonist ABT-418 also has positive effects on learning and memory in AD (67). M**ost recently, Newhouse and colleagues (68) showed that chronic transdermal nicotine treatment improved attention and episodic memory in patients with MCI (**see Fig 1 and 2**). This supported the current NIA-funded multi-site MIND Study (Memory Improvement through Nicotine Dosing; Newhouse, PI).**

Crucial for use in the LLD population, *nicotine also improves executive dysfunction*. Newhouse and colleagues demonstrated the positive effects of nicotinic stimulation on executive function measures in adolescents and adults with ADHD (69,70). Additional studies in ADHD also report that stimulating nAChRs can improve executive function (69-79).

3.3. Effects of Nicotinic Stimulation on Brain Function*:* Nicotinic effects on cognitive processing and brain circuitry have been examined in neuroimaging studies (80,81). With normal aging, nicotinic activity

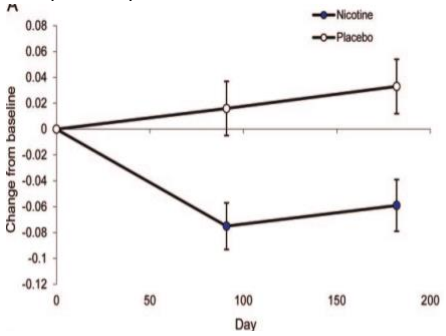


Figure 1. **Continuous Performance Task (CPT)**, change from baseline. Nicotine improved performance over placebo ($F_{1,57}$ = 14.96, p = 0.003).

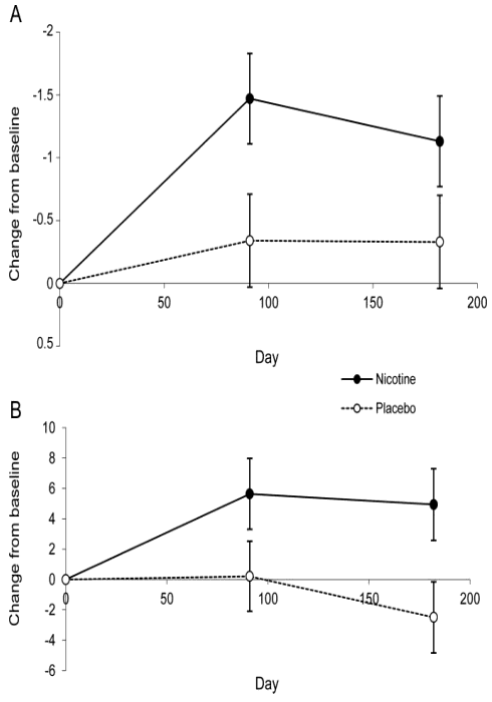


Figure 2. Episodic Memory.
A) Paragraph recall, nicotine > placebo ($F_{1,60}$=4.42, p=0.04). B) Word recall, nicotine > placebo ($F_{1,70}$=5.92, p=0.018).

increasingly engages frontally-mediated attentional processes to resolve uncertainties regarding sensory input and the contents of working memory (82). This may have the effect of increasing frontal activation, as observed when older adults exhibit task performance similar to younger adults during fMRI (83). This increase in frontal activity is necessary to maintain performance accuracy (84,85).

Across multiple studies, nAChR agonists appear to enhance activity in cognitive control networks while suppressing activity in the default mode network (DMN) (86). The DMN is a set of midline regions more active at rest that exhibit decreased activity during task performance (87). During tasks in younger nonsmokers, nicotine improved accuracy and speeded performance (88) while increasing activation in frontal, cingulate, and parietal regions (89). In contrast, during attention and memory tasks, nicotine reduces activation in default mode regions (90,91).

In summary, nicotinic receptors are widely distributed and intimately involved with several neurotransmitter systems important for cognitive function. These systems have modulatory effects on attentional and executive function and secondarily on learning and memory. Human studies support that nAChR stimulation is practical and potentially therapeutic, particularly in age-related disorders. Nicotine treatment may be more effective in older adults with less severe cognitive impairment and more nicotinic receptors, such as in MCI or LLD with cognitive complaints. Thus stimulation of nAChRs may be a promising strategy to improve cognitive function in LLD. As the cognitive impairment of LLD also involves difficulty in effortful processing, age-related changes in nicotinic functioning may be contributing to this impairment (83). *Although nicotine has beneficial effects on cognition in older adults with MCI and AD, no studies thus far have examined the effects of nicotinic stimulation on cognitive functioning in LLD. Similarly, there are no reports of nicotine's effect on neural circuits in LLD.*

3.4. Safety of transdermal nicotine patches in nonsmokers: Given the historical context of nicotine administration through tobacco, there are often concerns for effects on nicotine administration on health. However much of these data do not clearly delineate risks of nicotine from risks associated with smoking cigarettes or cigars, which contain many more components than just nicotine. We address potential risks below. Overall, data do not support that transdermal nicotine carries significant risks. Further, transdermal nicotine was well tolerated in our previous MCI study where we had no severe adverse events related to drug treatment and found no withdrawal symptoms reported following drug treatment.

4.0 INCLUSION/EXCLUSION CRITERIA

Inclusion Criteria:

1) Age $\geq$ 60 years;
2) DSM-IV-TR diagnosis of major depressive disorder, single or recurrent episode;
3) Subjective cognitive decline, defined as endorsing 20% of items on the Cognitive Complaint Index (CCI);
4) *severity*: MADRS (92) ≥ 15;
5) *cognition*: MOCA $\geq$ 24;
6) fluent in English;
7) intact hearing / vision allowing completion of study procedures;
8) for individuals on antidepressants at study entry, they must be on a stable dose for at least 8 weeks.

Exclusion Criteria:

1) Other Axis I psychiatric disorders, except for anxiety symptoms occurring in a depressive episode;
2) History of alcohol or drug dependence or abuse in the last 3 years;
3) Tobacco or nicotine use in last year;
4) History of a developmental disorder or IQ score < 70;
5) Acute suicidality;
6) Acute grief (<1 month);
7) Current or past psychosis;
8) Primary neurological disorder, including dementia, stroke, brain tumors, etc. Coexisting mild cognitive impairment is allowable;
9) Any MRI contraindication;
10) Unstable medical illness;
11) Allergy or hypersensitivity to nicotine patches;

12) Regular use of drugs with centrally acting cholinergic or anticholinergic properties in the last 4 weeks, including acetylcholinesterase inhibitors;
13) Current or planned psychotherapy;
14) Electroconvulsive therapy (ECT) or transcranial magnetic stimulation (TMS) in last two months.

5.0 **ENROLLMENT**

We will enroll patients from clinical referrals and response to advertisements. In these cases, potential participants will call our study contact number. We will describe the study to them, including a description of the study entry criteria. Those who continue to be interested will then be scheduled for an evaluation. After scheduling, a study doctor will review their electronic medical record to assure that potential subjects meet entry criteria, however no data will be recorded. Occasionally a clinician may identify a patient who may be eligible and request that we initiate contact. We will do this, but the referring clinician must first broach the idea of research and secure the patient's agreement to be contacted.

Upon presenting for evaluation, we will obtain formal written consent from all participants. Following policies of the Vanderbilt University Health System Institutional Review Board, written informed consent will be obtained and documented by the study's Research Coordinator before any study-related procedures are performed. The study coordinator will review study procedures and the consent form with each potential participant. A study doctor will be available should any consent-related questions arise. Each individual may take as much time as they like to decide if they do or do not wish to participate.

Accounting for screen failures, we anticipate consenting up to 35 subjects total. We will start study patches on only 15 participants.

6.0 **STUDY PROCEDURES**

Overview: After a screening visit to confirm eligibility and diagnosis, participants will then be scheduled to complete a baseline assessment consisting of a broad symptom assessment, memory testing, and cranial MRI. They will then complete 12 weeks of open-label transdermal nicotine patches. At the end of the 12 weeks, they will then repeat baseline assessments, have blood drawn for measurement of nicotine metabolites, and have study drug tapered over three weeks.

6.a. CLINICAL ASSESSMENTS: The clinical and cognitive batteries are similar to those used in our past LLD studies. They are not burdensome and we allow breaks. Refer to the separate "Schedule of Events" file for a timing of all procedures.
6.a.i. Diagnoses and Past History: Current and past psychiatric diagnoses will be assessed using the electronic version of the validated Mini-International Neuropsychiatric Interview Plus (**MINI+**) (93,94) & confirmed through a clinical interview which will assess **age of initial onset**. The study physician will assess prior antidepressant exposure in the current episode using a modified Antidepressant Treatment History Form (**ATHF**) (95,96) updated to include current antidepressants and antipsychotics approved for MDD. We will request medical records as needed.

6.a.ii. Mood Symptom Assessments: Depression severity will be assessed by a study physician using the **MADRS** (92) as our primary mood outcome measure. This measure will be collected at each visit. **Secondary**: We will examine in-depth a number of depressive symptoms through discrete, validated questionnaires. These will be obtained at study entry and exit and include:

- Anhedonia: Snaith-Hamilton Pleasure Scale (SHAPS)
- Anxiety: Penn State Worry Questionnaire (PSWQ)
- Apathy: Apathy Evaluation Scale
- Fatigue: Fatigue severity scale
- Negative Affect: Type D Scale (DS-14)
- Rumination: Ruminative Response Scale (97).
- Sleep: Insomnia Severity Index
- Stress: Perceived Stress Scale (PSS) (98).

Safety and thoughts of suicide will be assessed at every contact. Individuals endorsing thoughts of death will be questioned by a psychiatrist about thoughts of suicide, intention, and potential plans. When there are

concerns for safety, subjects will be withdrawn from the study and treated per clinical care.

6.a.iii. Medical Assessments: Medical history and comorbidity will be quantified using the Cumulative Illness Rating Scale (**CIRS**) (99), which covers all major systems and provides a composite medical morbidity score. Individuals with unstable or previously unrecognized medical disorders will be excluded. Concomitant medications and vital signs (blood pressure, pulse, weight) will be reviewed at each visit. We also will assess past tobacco use, including duration of use and age of cessation.

6.a.iv. Side Effects and Treatment Compliance: Side effects are assessed using the Frequency and Intensity of Side Effects Rating / Global Rating of Side Effect Burden (**FISER/GRSEB**) scales (100). Compliance is assessed with a patch count and the Medication Adherence Questionnaire (**MAQ**) (101).

6.b. COGNITIVE ASSESSMENTS:

6.b.i. Subjective Cognitive Functioning: For eligibility, participants must meet criteria for subjective cognitive decline, defined as endorsing at least 20% of all items on the **Cognitive Complaints Index (CCI)**. The CCI score is calculated from a broad battery integrating questions from multiple instruments, including: the Memory Functioning Questionnaire, Memory Self-Rating Questionnaire, the Neurobehavioral Function and Activities of Daily Living Rating Scale (ADL-self), the Informant Questionnaire on Cognitive Decline in the Elderly (IQCDE), 30 cognitive items from the Geriatric Depression Scale (GDS), 12 items from a telephone-based screening for mild cognitive impairment (MCI), and 20 items from the Memory Assessment Questionnaire adapted in part from the Functional Activities Questionnaire. The CCI score is calculated as the percentage of all items endorsed. We will use the Memory Functioning Questionnaire and the PROMIS Applied Cognition Abilities Short Form as measures of subjective cognitive function. These will be obtained every 3 weeks at clinic visits.

6.b.ii. Objective Cognitive Functioning / Neuropsychological Test Battery (Table 1): We developed this battery to obtain a wide representation across cognitive domains. We are particularly focused on domains of attention (known to be modified by nicotinic receptor stimulation), executive function (often impaired in LLD and associated with poor response to antidepressant medication), and memory. We use a number of tests obtained with the Cogstate cognitive battery to assure consistency with the larger MIND study examining transdermal nicotine in MCI. We augment this with tasks particularly relevant for depression. The Conners Continuous Performance Task (CPT) will be the primary cognitive measure given its known sensitivity to nicotinic receptor stimulation.

TABLE 1. COGNITIVE TASK

Task	Time (m)
Attention	
Continuous Performance Task	12
Choice Reaction Time	4
Identification Test (Cogstate)	3
Executive Function	
Trail Making Test (A & B)	7
Stroop Color-Word	3
Groton Maze (Cogstate)	7
Episodic Memory	
NYU Paragraph Recall	5
1-Card Learning (Cogstate)	6
Shopping List, imm (Cogstate)	5
Shopping List, delay (Cogstate)	1
Processing Speed	
Detection Test (Cogstate)	3
Trails A / Stroop Color naming	-
Working Memory	
One-back test (Cogstate)	4
Emotion Processing	
Emotional Dot Probe	11
Trait Adjectives Task	5
Trait Adjectives Recall	2
TOTAL TIME	**78**

- **Attention**: The primary measure is the **Conners Continuous Performance Test (CPT)**, measuring sustained and selective attention control (102). Secondary measures of attention and reaction time include the **Choice Reaction Time (CRT)** task that decomposes overall reaction time into recognition and motor components and also the **Identification Test (Cogstate)**.
- **Executive Function**: We will examine performance on the **Trail-Making Test** Part B (examining set-shifting) and performance on the color-word interference condition of the **Stroop** (examining response inhibition). Poorer performance on these tasks is associated with LLD and poor antidepressant response. We will augment these with the **Groton Maze Learning Task (Cogstate)** of spatial problem solving, a repeatable task sensitive to nicotinic receptor stimulation (103).
- **Episodic Memory**: The **Shopping List Task** (**Cogstate**; Immediate verbal recall and delayed verbal recall). This will be supplemented by the **NYU Paragraph Recall test** (Immediate and delayed). Both tasks have alternate forms for repeated assessments. Additionally, the **one-card learning task**

(**Cogstate**) will assess visual learning and memory.

- **Working Memory**: **1-back test (Cogstate)** wherein participants indicate if a card is identical to the one seen just before.
- **Processing Speed**: Processing speed will be assessed using tasks discussed above, including the **Stroop** (color naming), **Trail-Making Test Part A**, and the **Detection Test (Cogstate).**
- **Emotional Processing:** We will examine the effects of emotional stimuli on attentional performance using an **emotional dot-probe test**. We will examine negativity bias using the **Trait Adjectives Task**, wherein participants must quickly indicate whether positive and negative adjectives apply to them. This is followed 15 minutes later with a test of emotional memory, wherein participants must recall adjectives they viewed. As early change in performance on the Trait Adjectives Task is related to subsequent antidepressant response, we will also repeat this task at week 3 (after 2 weeks at a transdermal nicotine dose of 7mg).

6.c. STUDY DRUG ADMINISTRATION:

6.c.i. Overview: After completing the cognitive battery and MRI, eligible participants will begin a 12-week open-label trial of transdermal nicotine patch. MRI and the cognitive battery will be repeated after this blinded phase. During the study drug phase, participants will be seen in-clinic every 3 weeks (+/- 4 working days).

6.c.ii. Transdermal Nicotine: Dosing and drug administration will be concordant with the MIND study. Participants will be instructed to wear the study patch during the day and remove it at bedtime (target goal of 16 hours/day). Following our titration schedule (**Table 2**), participants will receive active 24-hour patch starting at 3.5mg daily (one-half of a 7mg patch) for 1 week, then 7mg daily for 2 weeks, then 14mg daily for 3 weeks, then 21mg daily starting at week 7. Doses can be reduced to the previous level or patches divided to reduce the dose if needed for tolerability.

6.c.iii. Duration & Dosing Rationale: We selected a 12-week duration as in MCI we detected differences in cognitive performance at 90-days (68). Thus this will be a sufficient period to detect changes in cognitive performance. Such a time period will also be sufficient to detect differences in mood as the majority of individuals remitting to current antidepressants do within 12 weeks (104).

We selected the highest available daily dose of transdermal nicotine as we have no *a priori* knowledge of the optimally tolerated dose. However, we do know the highest dose was well tolerated and effective in MCI. Given the lack of knowledge of possible benefits at lower doses, we will advance to the highest dose, providing for dose reductions as needed for tolerability.

Table 2. Dosing

Week	Days	Dose
1	0-7	3.5mg
2-3	8-21	7mg
4-6	22-42	14mg
7-12	43-84	21mg
13-14	85-98	14mg
15	99-105	7mg

6.c.iv. Compliance: We will assess compliance using the Medication Adherence Questionnaire (**MAQ**) (101) and a patch count at each study visit.

6.c.v. End of study medication procedures: Following completion of the 12-week trial, doses will be slowly lowered and study drug discontinued over 3 additional weeks (Table 2). This time will be shortened for participants who do not reach the target dose of 21mg daily. If individuals feel the study drug has provided benefit and elect to continue its use by purchasing over-the-counter patches, we will not require this discontinuation. Subjects will return for a final safety assessment. For individuals who have tolerated study patch well, this final safety visit can optionally be conducted by telephone.

6.c.vi. Concomitant medications: To enhance generalizability, most nonpsychotropic medications will be permitted. However, an exclusion criterion specifies regular use in the last 4 weeks of agents that have substantial procholinergic or anticholinergic properties. This includes acetylcholinesterase inhibitors (donepezil, rivastigmine, or galantamine) or other agents used for tremor, urinary control, or vertigo (amantadine, benzotropine, cyproheptadine, diphenhydramine, hydroxyzine, meclizine, prochlorperazine, or promethazine).

As we are examining the effects of transdermal nicotine as an augmentation agent, we will allow concomitant use of any psychotropic medication approved by the US FDA for treatment of depression, except for tricyclic antidepressants that are prohibited due to their anticholinergic properties. Concomitant psychotherapy will not be allowed as it is an effective treatment that contributes to remission through different mechanisms. We allow short-acting hypnotics (eszopicione, zolpidem, or zaleplon) for sleep and lorazepam up to 2mg daily for anxiety. Hypnotics will be discouraged the day of cognitive testing.

6.d. MRI PROCEDURES

6.d.i. MRI Screening: To assure safety for MRI, we carefully screen participants for metal. Working with the Vanderbilt University Institute for Imaging Science, we identify past medical procedures and surgeries that may include metal implants. In these cases, we request medical records to determine if metal was used, and if so, if it is safe for 3Tesla MRI. However, in some cases these records cannot be obtained. In such cases we may obtain an x-ray if there was a question whether there was or was not implanted metal.

We will only obtain an x-ray in cases where a) there is a safety concern about a potential implant or metal; b) medical records for the surgery are not available; and c) participants do not think that metal was involved in the surgery. Thus this procedure will not be needed for the majority of participants. If the x-ray shows there is implanted metal that could be a MRI contraindication, that participant will be withdrawn from the study. Radiation exposure will vary dependent on the area needing to be evaluated.

Table 3. MRI Scans	
Scan	Time
T1	4.5
FLAIR	7.5
T2/HPC	4
Rest fMRI	8
Posner	7
Emotional Stroop	13
TOTAL	44 m

6.d.ii. Image Acquisition: We will image subjects on a 3T Philips Achieva system using a protocol that acquires multi-contrast data to allow automated tissue identification and BOLD data at rest and during the cognitive tasks (Table 3). Cranial MRI will be performed over 1 hour. Similar MRI durations have been well tolerated in our past studies of LLD. This protocol will acquire proton density (PD), T1-weighted, T2-weighted, fluid-attenuated inversion recovery (FLAIR), and a specific T2-weighted hippocampal acquisition for assessment of hippocampal subfield structure.

Functional MRI is performed using echoplanar BOLD imaging. For whole brain fMRI scanning, a reference scan is obtained first. For the fMRI sequences, a single-shot, gradient-echo, echoplanar pulse sequence will be used (TR 2500 ms/TE 35 ms/flip angle 90 degrees/1 NSA). Resolution is 3.5 mm x 3.5 mm x 3.5 mm. Thirty contiguous slices of 3.5mm thickness and no gap will be obtained in the axial oblique plane parallel to the AC-PC line. Field map correction for magnetic inhomogeneities is accomplished by acquiring images with offset TE at the end of the functional series. During pre-processing, reconstruction options permit adjustment of the functional image series for bandpass asymmetry correction and unwarping to remove spatial artifact.

6.d.iii. fMRI Tasks: We will assess intrinsic resting state functional connectivity (rsFC) during an 8-min period in which participants are instructed to rest without moving and focus on a fixation cross presented in the center of the screen. This will be accompanied by a task examining nonemotional attentional performance (Posner task) and a task examining the effect of affective stimuli on executive function performance (emotional Stroop).

A version of the **Posner Task** of attentional orienting (105) will assess the ability of subjects to disengage attention and shift to a new target. Subjects will be asked to press a button corresponding to the side of the screen on which a stimulus appears. Before the stimulus, a cue will indicate the side on which the stimulus will next appear. This cue will be valid (occur on the same side as the stimulus) 90% of the time and invalid (occurring on the opposite side of the stimulus) during the remainder. This paradigm is sensitive to nicotinic stimulation effects (106-108).

We will additionally use a modified **emotional Stroop task** (109). The classic Stroop paradigm was modified and adapted to an emotional conflict task in which faces with fearful and happy expressions were presented with the words “happy” or “fear” written across them. We then asked subjects to identify the emotional expression of the faces while ignoring the words, which were either congruent or incongruent with the facial expression. Incongruent stimuli are thus associated with a response conflict that arises from an emotional incompatibility between task-relevant and task-irrelevant stimulus dimensions (e.g., a fearful expression with the word “happy”). This task has been demonstrated to dissociate neural systems involved in resolving conflict between congruent versus incongruent emotional distractors (109,110).

6.d.iv. Image Processing: Functional MRI Analysis: Preprocessing and analysis of the functional data will be performed with SPM8 (111). Volumes will be realigned to minimize head movement. Further data preprocessing includes a correction for slice x time errors and spatial (6 mm full-width half-maximum isotropic Gaussian kernel) as well as temporal (high pass filter: 1 cycles/run) smoothing to remove aliased signal correlated with background respiration and heart rate. Anatomical and functional images are co-registered and normalized to the MNI template.

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 relevant contrasts will be created. For both the Posner task and emotional Stroop task, we will examine relevant contrasts from each session. For the Posner task, we will examine change in the contrast between responses to valid and invalid cues. For the emotional Stroop task, we will examine a) change in response to congruent negative stimuli (fearful face with "fear" written across it) and b) change in the contrast between responses to congruent and incongruent stimuli, also examining for differences based on the emotional valence of the presented face. These contrast images are then used for second level analyses examining within-subject changes in brain activity over the course of the 12-week trial. The critical significance level for treatment-level analyses will be based on clusters of activated voxels with the probability threshold set at $p_{corr} < 0.05$ and cluster threshold with false discovery rate less than 0.05 corrected for multiple comparisons (with alpha simulation in REST toolbox for SPM).

6.d.v. Resting State Functional Connectivity Analysis: Resting-state scans will undergo the following preprocessing steps: slice timing correction, motion correction, coregistration to structural images, spatial normalization to the MNI152 template, and 6mm spatial smoothing. We will use the "scrubbing" procedure to guard against spurious correlations introduced by head motion. Volumes within a run with frame-wise displacement greater than 0.5mm and BOLD intensity changes between frames greater than 0.5% will be tagged and excluded from the connectivity analysis by including the tagged scans as nuisance regressors in the connectivity analysis. We will extract quality assurance metrics from scans, including signal-to-noise ratio, to include as covariates in analyses. We will us canonical network seed regions for connectivity analyses, with our analyses focused on the Default Mode Network (midline posterior cingulate gyrus seed) and the Cognitive Control Network (bilateral dorsolateral prefrontal seed). Connectivity between these seeds and whole-brain connectivity maps will be calculated using the CONN Functional Connectivity toolbox. The mean BOLD signal time-series is extracted and the ROI-to-voxel correlation matrix is created. A 2nd level whole-brain seed-to voxel analyses of all participants' baseline functional connectivity will be used to define a mask for the DMN and CCN networks. **Repeating the process before and after the 12-week trial, we will be able to assess within-participant changes in each functional network map.** We can then examine how this change is related to change in mood and cognitive performance.

6.e. MEASUREMENT OF NICOTINE METABOLITES: At the time of the 12-week MRI, participants will have 10ml of blood drawn for measurement of nicotine metabolites, including nicotine, cotinine, and 3HC/COT (a measure of nicotine clearance). This will be done at least 4 hours after the patch is applied that morning. Samples will be frozen and stored in a -70 freezer. After all sample collections are complete, they will be sent for analyses to our collaborator, Dr. Rachel Tyndale at the Center for Addiction and Mental Health at the University of Toronto.

6.f. OPTIONAL STUDY PROCEDURE: <u>Electroencephalogram /Event Related Potential Test (EEG/ERP):</u>
Participants will be asked to complete an optional EEG session at two points during the testing period. Previous research found that patients with LLD display slower sensory processing as shown in longer P300 latencies to auditory stimuli, as well as diminished inhibitory processing compared to healthy controls (112,113). LLD patients have also been observed to exhibit greater slow wave power at rest compared to age-matched controls (112). This EEG session will examine whether nicotine intervention alters the sensory and inhibitory processing of patients with LLD. This will be examined in parallel with assessments of mood, cognition, and repeated MRI.

Methodology: Each participant will be tested individually in a quiet private room at the baseline visit and then again at week 12. Visual and auditory EEG signals will be recorded using a 128-channel Geodesic sensor net (EGI, Inc., Eugene, OR) (114-116). The net is made of Ag/AgCl-coated carbon electrodes embedded in soft electrolytic sponges and arranged into a net using elastomer strings. Each electrode is connected via carbon wiring to a high-impedance (1MOhm) low-noise amplifier that provides analogue-to-digital (A/D) conversion of the EEG signals. Prior to application, the net is soaked in warm saline (KCl) solution. The electrode impedances will be kept at or below 40 kOhms. The use of high-impedance amplifiers minimizes any decrease in signal-to-noise ratio and allows collection of high quality data without having to abrade the scalp, thus

minimizing any discomfort and reducing infection risks. Tests of this system reported no significant signal loss over a range of EEG frequencies. Another advantage of this system is fast electrode application and impedance adjustments (< 10 minutes). The EEG signals will be sampled every 4ms with filters set at 0.1 Hz - 100 Hz. During data collection, all electrodes will be referred to vertex (Cz). EEG will be continuously monitored and during periods of motor activity or inattention, stimulus presentation will be suspended until behavior quiets and the researchers will use redirect the child to the task. The entire recording session will last approximately 30 minutes.

- **Resting state EEG session:**

The participants will complete a short resting state EEG session, consisting of an eyes-open block and an eyes closed block. During the eyes open block, the participants will be asked to fixate on a cross in the middle of monitor. Each block will last for 3 minutes for a total task time of 6 minutes.

- **Auditory oddball task**:

The "oddball", or P300 response, will assess attention and memory in auditory modalities. A pair of two pure tones (single formant) at 1000 and 1500 Hz will serve as stimuli. Tones will be equated in duration (300 ms) and rise/decay times. One tone (standard) will occur 70% of the time while the other (target) will be presented in 30% of the trials. Tone frequency (i.e., high vs. low) assignment to conditions will be counterbalanced across participants. Tones will be presented at 75 dB SPL (measured at the ear) through a speaker positioned 1 meter in front of the participant.
*Procedure***:** 150 trials will be presented with the inter-stimulus interval varying randomly between 1000-1300 ms to prevent habituation to stimulus onset. Each participant will be asked to press a button using their preferred hand every time they detect the target stimulus. On average, the auditory tasks will last ~8 minutes.
Variables of interest: Analyses will focus on the parietal (P300) and frontal (P3a) responses to standard and target stimuli.

- **Cued Go/Nogo Task:**

A cued go/nogo task will be used to assess response preparation and inhibition. During this task participants will be presented with a cue which will instruct them to prepare a response to with either their left or right hands. After an interval of 1500ms either a valid target (66% of trials) or a nogo target (33% of trials) will be presented. Participants will have to either respond with the indicated hand, or withhold their response. The task will consist of 180 trials with a 1000-2000ms inter-stimulus interval to prevent habituation. The task will last ~15 minutes. There will be a short practice block to familiarize participants with the task before recording commences.

7.0 LONG-TERM FOLLOWUP (SUB-STUDY)

Individuals who complete the trial will be asked to enter long-term follow-up over the next year. This will include only minimal-risk interviews and questionnaires occurring 6-months and 12-months after study exit. This will not affect clinical treatment decisions over this period. The goal of this sub-study is to gather data on long-term rates of depression relapse and subjective memory performance. For individuals who elect to continue commercially available transdermal nicotine patches, it will also obtain data on long-term tolerability.

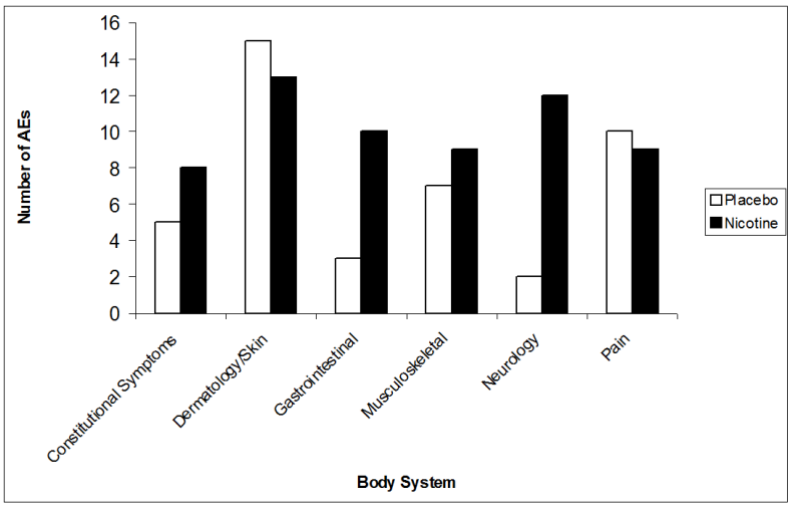
7.1. Consent: We will discuss this optional sub-study at their final study visit. Those who are agreeable will be asked to sign a separate sub-study consent form.
7.2. Procedures: This will only involve a telephone interview and completion of study questionnaires at 6- and 12-months after study completion. The telephone interview will include assessments of current medication use with the ATHF, transdermal nicotine use and tolerability, any recent smoking, and depression severity using the MADRS. The telephone interview will be followed by a completion of study questionnaires assessing depressive symptoms – these are listed in Section 7.1.d above.
7.3. Safety: For any participants who are depressed at either assessment, we will assure that they are currently receiving treatment for their depression. If so, we will encourage them to let their physician know about their symptoms. If they are not, we will provide referral recommendations, including a referral to the Vanderbilt Psychiatry Outpatient Clinics. As we make these referrals at the end of the main study, we do not anticipate that many individuals will be without treatment.

We will also assess for thoughts of death or suicide as part of measuring depression severity with the

MADRS. A study physician will then assess the safety of anyone endorsing these thoughts and act as is clinically indicated. This may include feedback to the patient's treating psychiatrist or referrals to intensive outpatient or inpatient hospitalization.

8.0 RISKS – STUDY DRUG

Side Effects of Nicotine: At the nicotine doses 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 (117). 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.

In our published MCI trial (68), total adverse events (AEs) for the double-blind treatment period were 82 for nicotine versus 52 for placebo ($p < 0.05$). However, the majority of AEs were mild (nicotine 57.3 %; placebo 54.9%) and there was no statistically significant difference in the proportion of adverse events within the different severity classifications between treatments (Mann-Whitney test $p = 0.97$). No severe AEs were classified as related to drug treatment in either treatment group. Adverse event rates by body systems reported in more than 10% of subjects (Figure) were generally comparable with the exception of gastrointestinal and neurological for which there were more AEs reported in the nicotine-treated group. Approximately 75% of AEs in both placebo and nicotine groups were judged not related or doubtfully related to treatment. More nicotine-treated subjects (N=4) discontinued treatment for adverse events than placebo-treated subjects (N=0) ($X2 (1) = 3.79$; $p = 0.05$). No withdrawal symptoms were reported by subjects or informants nor were any subjects reported to be continuing to use nicotine after the six-month study was completed.

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 (118). 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

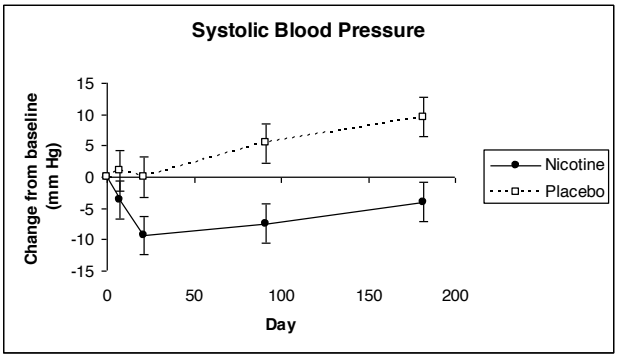
usually straightforward and is accomplished by patch rotation, local treatments, and instructing the patient to remove the patch prior to going to bed. Dr. Newhouse has experience in administering transdermal nicotine for over five years to a non-smoking patient with Huntington's disease for movement 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 (119). Nicotine stimulates CNS sympathetic systems and increases release of catecholamines from the adrenal and vascular nerve endings. While tolerance appears to develop to these cardiac stimulatory effects, the tolerance developed is only partial. While there may be a small chronic cardio-stimulatory effect (approximately seven beats per minute), the dose response curve appears to be flat (119).

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 (120). 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) (121). An 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 (122). 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 (123). 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."

In our MCI study, an examination of change in systolic blood pressure revealed a significant reduction in systolic blood pressure compared to placebo treatment (Figure below). By day 182, the placebo group showed an average increase of 9.6 mmHg in SBP compared to a reduction of 4 mmHg in the nicotine-treated group. There was a small reduction in diastolic blood pressure by day 182 in the nicotine-treated group. An examination of the change in pulse showed no overall treatment effect ($p = 0.51$).



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 (124-127). However, a large meta-analysis of 35 smoking cessation trials did not find any increased incidence of stroke in nicotine replacement therapy users (117).

Conclusion regarding cardio- and cerebrovascular safety: As the subjects to be enrolled in this study will be nonsmokers

selected for the absence of unstable cardiovascular or cerebrovascular disease, we believe that the cardiovascular and cerebrovascular risk profile of transdermal nicotine in such patients is excellent.

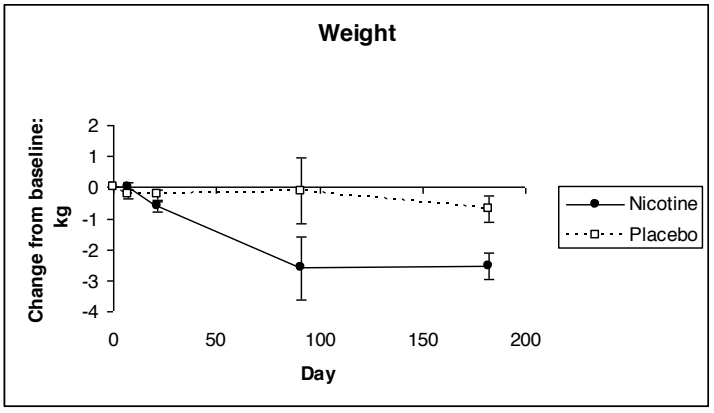
Insulin Sensitivity: There have been some epidemiologic studies suggesting a positive relationship between smoking and insulin resistance (128) although some studies are contradictory (129). Some investigations have suggested that changes in insulin sensitivity may be restricted to smokers who are also diabetic (130). 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 (131) while another study did not (132). At this point, it is not clear that nicotine use alone in nonsmokers is associated with changes in insulin sensitivity.

Carcinogenesis: Nicotine alone has not been shown to be carcinogenic. 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 (133). 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 (133).

Fetal Development: The FDA does not recommend the use of transdermal nicotine patches during pregnancy. Given how our age entry criterion (age 60 years or older) is concordant with a post-menopausal state, we will not require pregnancy testing.

Safety Experience in Older individuals: We have extensive experience in administering nicotine and novel nicotinic agonists to older patients with Alzheimer's and Parkinson's disease (134-136) . We have performed over 200 intravenous infusions of nicotine bitartrate salt to such patients with cardiac telemetry. No instances of cardiac ectopy or irritability have been seen and no other significant side effects other than nausea and/or vomiting have occurred. We have also administered nicotine by transdermal patch for four weeks to elderly patients with Parkinson's disease (137). Tolerability was excellent, even up to 22 mg nicotine patch per day. Only minor gastrointestinal upset was seen that was easily managed by dose reduction. No incidences of cardiovascular symptoms or difficulties occurred.

Chronic nicotine has been administered transdermally by other investigators to nonsmoking AD and PD patients in several studies for up to several weeks (137-141) without significant problems. No significant cardiovascular problems have been documented. Patients reported a vague feeling of lightheadedness, and some had mild behavioral changes.



Weight: In our recently published MCI study, examination of the change in body weight across visits showed that the nicotine-treated group showing a significant decline in body weight by day 91 compared to placebo (-2.6 kg versus -0.1 kg for the placebo-treated subjects). However the nicotine-treated group stabilized and no further decline in weight occurred through day 182. We will monitor weight over the course of the study.

Abuse Potential: We believe that the probability that the subjects in this study might be prompted by their participation to begin to use nicotine containing products or tobacco is extremely low. There have been no cases reported in the medical literature of primary abuse by never smokers of nicotine replacement therapies. Furthermore, there are no cases reported of ex-smokers taking up nicotine replacement therapy and becoming addicted or dependent. Additional reasons for our assertion that the risk of abuse of transdermal nicotine in this population is low include:

1) Nicotine replacement therapies have an extremely low abuse liability (142) . Nicotine patches have some unpleasant side effects and therefore are unlikely to be reinforcing.

2) Studies (143,144) show that experimental administration of tobacco does not induce ex-smokers to relapse into smoking. In another study (142), 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.

3) An important characteristic of all drugs that produce dependency is the pharmacokinetic parameters associated with the route and form of administration (145). With respect to nicotine, researchers of the NIDA Addiction Research Center (146,147), as well as others in the field (148-151), 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 (121). 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 (152).

4) We have administered intravenous and/or transdermal nicotine and structurally related nicotinic agonists over the past 20 years to several hundred non-smoking subjects including young and elderly normal volunteers, patients with Alzheimer's disease, MCI, and patients with Parkinson's disease. We have not had a single subject take up tobacco use as a consequence of study participation. Perhaps most importantly, in our recently completed MCI trial, *no withdrawal symptoms were reported by subjects or informants* nor were any subjects reported to be continuing to use nicotine after the study was completed.

There are potential unknown risks related to transdermal nicotine patches.

Experience in Late-Life Depression (3/3/2017 update): To date, the majority of adverse events are expected and concordant with what is described above. However, one participant reported a sharp increase in anxiety when increased from the 7mg patch to the 14mg patch. This anxiety resolved on lowering the dose and occurred again on rechallenge.

RISK/BENEFIT RATIO: When the safety record outlined above is considered, the risks of participation in this study are low. The risks mainly consist of temporary side effects from the nicotine and/or the transdermal patch that do not constitute a serious danger when administered within a medical environment. Long-term cardiovascular, cerebrovascular, and neurological safety of transdermal nicotine appears to be very favorable. Subjects may benefit from cognitive improvement and mood. The benefits to society of greater knowledge about the treatment of the cognitive changes in late-life depression and their possible amelioration, considering the human and economic costs of this disorder, would appear great. Overall the risk/benefit ratio appears to be in favor of conducting these studies.

9.0 RISKS – OTHER THAN STUDY DRUG

9.1 Interview, emergencies, and possible suicidal ideation. Subjects may experience discomfort during the clinical interview and evaluations when discussing symptoms, life events, and social support. The Project Coordinator is experienced and skilled in interviewing depressed subjects. Should the subject wish to stop or take a break, the coordinator will allow it. Dr. Taylor or Dr. Newhouse will be available as a backup. In addition, should the subject express suicidal ideation at any time during the interview, Dr. Taylor or Dr. Newhouse will be contacted immediately to assess the subject and to determine the appropriate course of action. Thoughts of suicide will be taken very seriously. Options for addressing this may include contacting the individual's mental health caregiver, referring for urgent evaluation and treatment, or emergent evaluation and hospitalization. Similar practices will be used for other emergencies, including but not limited to psychosis,

homicidal or violent thoughts, or an acute change in a subject's physical status.

9.2 Magnetic Resonance Imaging. Although this procedure is generally low-risk, there are particular concerns. Individuals will be screened for the presence of implanted metal (including but not limited to medical devices, shrapnel, tattoos or permanent makeup); those who screen positive will be excluded from the study. Claustrophobia is also an issue for many potential subjects. During the MRI, subjects will have voice contact with a radiology technician, and may request the scan be stopped at any time.

9.3 Incidental Findings: Magnetic Resonance Imaging: Another risk is the occurrence of incidental findings on MRI. All scans are reviewed at time of acquisition and concerning findings are discussed with an attending neuroradiologist. Should any concerning findings be seen, Dr. Taylor or Dr. Newhouse will convey these findings to the subject along with recommendations for further evaluation, and facilitate referrals for such evaluation and treatment.

9.4 Venipuncture: Risks of blood draw include pain from the needle, bruising or infection at the site of venipuncture, or fainting as a response to blood draw.

9.5 End of Study Procedures: During the course of the study we will work with study subjects to identify providers who will continue their care at the end of the study. Participants will undergo a blinded three-week taper of transdermal nicotine. They will then return for a final safety visit. If they feel they have benefited from study drug, they will be able to continue it as transdermal nicotine patches are available over-the-counter. In these cases, participants will not be required to progress through the three-week taper. However, we will extensively re-discuss potential risks and lack of knowledge of risks of long-term use.

9.6 Breach of confidentiality: There is the potential risk of breach of confidentiality of clinical, genetic, and laboratory information. Both Dr. Taylor and Dr. Newhouse have extensive experience as clinical investigators dealing with such sensitive information and have experience assuring that data is adequately protected. Safeguards to protect confidentiality include locked records and firewalls around password-protected electronic data, and all study data being coded, with the key linking the code with a subject's identity being kept in a separate, locked file.

10.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

We define an adverse event as any adverse change in health or development of a side effect occurring in a study participant after enrollment. These may be expected events (known drug effects, as detailed in the consent form, safety monitoring plan, or package insert) or unexpected events. We define a serious adverse event as any event that results in hospitalization, disability or permanent damage, is life threatening, results in death, or any other serious event that does not fit these outcomes, but require urgent medical intervention.

We will carefully monitor adverse events throughout the study. Subjects will be assessed for safety, medication tolerability, and unanticipated problems at each contact. Emergency contact information will be provided to each subject for urgent, unanticipated problems. All adverse events will be reviewed by Dr. Taylor at least weekly as they occur. All AEs, regardless of being judged as related or non-related, will be summarized and included in the annual IRB continuing review. All serious adverse events will be reported to the VUMC IRB within 7 working days. Unanticipated, non-serious AEs will be reviewed by Dr. Taylor and Dr. Newhouse. If felt to be likely related to study participation, we will consider whether they need to be added to the consent form. If so, we will submit them as an AE report and submit an amendment adding that risk to the consent form and other study documents. Dr. Taylor will have responsibility for this reporting requirement.

11.0 STUDY WITHDRAWAL/DISCONTINUATION

Participants may withdraw from the study at any time. If participants leave the study early, we will recommend a medication taper and clinical referrals for further care.

A participant will be withdrawn from the study if:

1) The participant withdraws his or her consent
2) The PI considers it is in the best interest of the patient for him or her to stop study participation
3) In the PI's judgment, the participant's depressive symptoms have worsened significantly since study drug initiation
4) The patient develops suicidal ideation where he or she should be referred for regular clinical care for safety
5) The patient is lost to follow-up

12.0 STATISTICAL CONSIDERATIONS

12.a. Data Management: All study data will be stored in a REDCap study database. The exception is for the raw and processed MRI scans that will be stored in the Vanderbilt XNAT Image Database system.

12.b. Statistical Analyses: As these data will be used to support future grant applications, these data will primarily be used to estimate variance in response and effect sizes for future power calculations. For primary analyses (*Primary Aim, Hypothesis 1*) we will examine change in MADRS over the course of study participation using a last-observation carried forward (LOCF) paired t-test approach. In secondary analyses we will use a similar approach to examine change in scores on other mood symptom questionnaires (Sect. 6.a.ii.). We will also report remission rates, defined as achieving a final MADRS <= 8. A similar approach will be used for analyses of cognitive performance (*Primary Aim, Hypothesis 2*) examining change in CPT performance, specifically change in hit reaction time, as the primary cognitive outcome. In secondary analyses we will also examine the other cognitive measures. Jointly, these data and analyses will inform our scientific model and how to focus our hypotheses in subsequent larger-scale proposals.

Our Secondary Aim focuses on functional MRI data. Analytic plans for Hypotheses 3 and 4 are discussed above, in Sections 6.d.iii. and 6.d.iv. For Hypothesis 5, we will conduct exploratory analyses examining whether change in fMRI signal (during tasks or during rest) over the study drug period is associated with change in mood or cognitive test measures. After creating the contrast from the pre- and post-study drug scans, we will extract the change in BOLD signal (during tasks) and connectivity (at rest) in the identified regions of difference. We will then examine correlations between these MRI measures and clinical measures.

12.c. Power: We calculated power for our Primary Aim / Outcome of change in depression severity with the MADRS. Using a SD of 7 based from a previous 12-week open-label antidepressant study, at N=15 and alpha=0.05, we have 79% power to detect a 5-point change in MADRS (compared with a change of 0). This change is a clinically meaningful reduction in depression severity, particularly in individuals who have not responded to a current antidepressant.

13.0 PRIVACY/CONFIDENTIALITY ISSUES

As the PI, Dr. Taylor will assure all procedures protecting study data designed to guard subject confidentiality conform to the Vanderbilt Human Research Protection Program requirements. Additionally, the Vanderbilt IRB must approve all procedures and safety precautions before the study can begin. All non-electronic data (clinical evaluations, paper assessments) will be stored securely in locked offices or laboratories accessible only by study staff. All electronic data will be stored in secured servers with limited access. RedCAP will be used for data management.

Further, all information that could potentially directly identify a subject is removed from all study data. This includes MRI data, electronic and paper assessments. Direct identifiers will be replaced with a unique four-digit code. The key to linking the code to subject identity will be kept separately from study data, in a locked file in password-protected computer in Dr. Taylor's office. Only study staff involved in clinical recruitment and assessment will have access to individually identifiable private information. All other study staff, including image analysts, will be blinded to subject identity and will only have access to the coded identifier.

Samples and specific data will be leaving Vanderbilt University Medical Center. First, we will send blood for analysis of nicotine metabolites to Dr. Rachel Tyndale at the Center for Addiction and Mental Health at the

University of Toronto. These samples will be deidentified and include only the subject's study ID number. Second, raw results from neuropsychological testing done with the Cogstate battery will be sent to Cogstate and results will be returned to the study. This allows calculation of test results and normalization by age and sex. These data will also be deidentified, including only the subject's study ID, date of administration, date of birth, and sex.

14.0 FOLLOW-UP AND RECORD RETENTION

We anticipate participant recruitment and contact with subjects will be completed within 12-18 months. Study records will be maintained for at least six years after the study is closed with the IRB. After that time, study data may be destroyed or anonymized, meaning all links to direct identifiers will be destroyed. Any study data in the medical record will be kept indefinitely.

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