Cholinergic Mechanisms of Gait Dysfunction in Parkinson's Disease

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Project III: Specific Aims

Falls and other abnormalities of gait and balance are common in PD, are largely levodopa unresponsive, and are major contributors to morbidity and mortality. The central theme of the U-M Udall Center is that degeneration of basal forebrain (BF) and pedunculopontine (PPN) cholinergic projections, in the context of degraded striatal motor control, contributes significantly to PD gait and balance deficits (3 Hit model; detailed in **Overview**). Our preclinical work (**Project I**) identifies attentional deficits, secondary to loss of cortical cholinergic afferents, as a key mechanism through which cholinergic deficits contribute to impaired gait and balance. The goal of this pilot target engagement/pharmacodynamic clinical study is to assess $\alpha4\beta2^*$ nicotinic cholinergic receptors (nAChRs) as a therapeutic target for improving gait, balance, and attentional capacity in PD. This proposal is directly responsive to NINDS PD 2014 Research Report "highest priority recommendations" calling for studies aimed at understanding mechanisms of and developing treatments for balance and gait disorders in PD (Clinical recommendations 2 & 3; Basic recommendation 3; Translational recommendation 6) and the Udall RFA request for pilot target engagement/pharmacodynamics studies.

PD exhibits heterogeneity of cholinergic degeneration (**Overview**, **Project II**), indicating that cholinergic interventions should be targeted selectively to the subgroup of patients with cholinergic deficits. $\alpha4\beta2^*$ nAChRs are key mediators of CNS cholinergic neurotransmission. Our preclinical data (**Project I**) indicates that $\alpha4\beta2^*$ nAChR agonists reduce fall risk by improving attentional function in our model of combined dopaminergic-cholinergic degeneration. *The central hypothesis of this clinical target engagement/pharmacodynamic study is that deficient activation of $\alpha4\beta2^*$ nAChRs is a key contributor to gait and balance abnormalities in PD, and that restoring activity of this target will reduce these debilitating levodopa unresponsive symptoms.*

To demonstrate that $\alpha4\beta2^*$ nAChRs are appropriate therapeutic targets in PD, it is necessary to study key pharmacokinetic-pharmacodynamic features of $\alpha4\beta2^*$ nAChR *in the context of the degenerating, hypocholinergic PD brain*, a pathologic environment in which they may exhibit unique features. This *personalized medicine* approach focuses our studies on the subgroup of hypocholinergic PD subjects identified by **Project II** and the **Clinical Resource Core**. We will assess $\alpha4\beta2^*$ nAChR features using PET imaging with the $\alpha4\beta2^*$ nAChR ligand [^{18}F]flubatine, subacute administration of the $\alpha4\beta2^*$ nAChR partial agonist varenicline (VCN), and laboratory measures of gait, balance, and attention. We will use [^{18}F]flubatine PET to assess VCN occupancy of brain $\alpha4\beta2^*$ nAChRs. Using this PET data to select an appropriate VCN dose, we will perform a pharmacodynamic study with subacute VCN administration to determine if $\alpha4\beta2^*$ nAChR stimulation improves laboratory measures of gait function, postural control, and attentional function in hypocholinergic PD subjects.

Specific Aim 1: To use [^{18}F]flubatine PET to assess VCN occupancy of brain $\alpha4\beta2^*$ nAChRs after subacute VCN administration to the subgroup of PD subjects with cholinergic deficits in comparison with normal control subjects.
Hypothesis 1a: Low dose, subacute VCN administration produces high $\alpha4\beta2^*$ nAChR occupancy in PD subjects with cholinergic deficits.
Hypothesis 1b: Low dose, subacute VCN administration produces equivalent $\alpha4\beta2^*$ nAChR occupancy in PD subjects with cholinergic deficits and normal control subjects.
Specific Aim 2: To assess the effects of pharmacologic $\alpha4\beta2^*$ nAChR activation on laboratory measures of gait function and postural control in PD subjects with cholinergic deficits.
Hypothesis 2: Pharmacologic $\alpha4\beta2^*$ nAChR activation in PD subjects with cholinergic deficits will improve laboratory measures of gait function and postural control.
Specific Aim 3: To assess the effects of pharmacologic $\alpha4\beta2^*$ nAChR activation on a measure of attentional function (SAT; sustained attention test) in PD subjects with cholinergic deficits.
Hypothesis 3: Pharmacologic $\alpha4\beta2^*$ nAChR activation in PD subjects with cholinergic deficits will improve attentional performance on the SAT. The improvement in SAT performance will correlate with improvement in laboratory measures of gait function and postural control assessed in Specific Aim 2.

Our group possesses unique expertise in preclinical models, nAChR pharmacology, cholinergic imaging, and physiological measures of gait function, postural control, and attention. This project interacts directly with **Project II**, depends on the **Clinical Resource Core**, and is strongly connected to **Project I** preclinical experiments. The potential **impact** of the proposed experiments is considerable. This work would provide strong support for our *central hypothesis*, establish therapeutic validity of the novel animal model employed in **Project I**, and implement new methodologies for the evaluation of treatments to improve gait and balance deficits in PD. These experiments would identify $\alpha4\beta2^*$ nAChRs as a therapeutic target in a clinically definable PD subgroup, setting the stage for trials of $\alpha4\beta2^*$ nAChR agonists as adjunctive treatments to mitigate balance and gait abnormalities in these patients.

Project III: Research Strategy

Significance

While the major clinical features of PD are associated historically with dopaminergic nigrostriatal projection degeneration, several important and disabling clinical features of PD are caused by the combined degeneration of dopaminergic and non-dopaminergic systems and are resistant to dopamine replacement therapy. Among the most troubling dopamine resistant phenomena are falls and associated gait abnormalities (1-7). Of the cardinal features of PD, gait and balance disorders are the least responsive to dopaminergic replacement, even in subjects with early disease (8-10). In longitudinal studies, dopamine non-responsive features, including postural control deficits, falls, and gait disorders, dominate the clinical picture (11-13). Hoehn and Yahr stage 3 (HY3) marks increased risk for fall-related disability in PD, as up to 40% of HY3 patients experience multiple falls, often with injury, including a high fracture incidence (6). In one PD cohort study, approximately one-third experienced a new hip fracture over a 10 year period (14). Even when falling is avoided by self-imposed activity reductions, fear of falling impairs function significantly and degrades quality of life. There are no effective therapies to prevent falls and only limited understanding of the non-dopaminergic systems that impair gait and postural control in PD.

Project III will tackle this critical clinical problem by pursuing pilot target engagement studies to identify a novel treatment strategy in PD. Our selected target, $\alpha4\beta2^*$ nicotinic acetylcholine receptors (nAChRs), is based on compelling preliminary data implicating degeneration of cholinergic systems in postural control and gait dysfunction in PD, and the critical role of $\alpha4\beta2^*$ nAChRs in mediating relevant aspects of cholinergic neurotransmission (see below, **Overview, Project I**, **Project II**). Beyond its potential clinical impact, this work will improve scientific knowledge of gait and postural control dysfunction in PD. Together with the other projects in the U-M Udall Center, **Project III** will contribute to testing the 3 Hit Model of gait dysfunction in PD (**Overview**). This model posits the PD-defining posterior putaminal dopaminergic deficit as the first "hit" disrupting execution of habitual motor actions such as gait, placing increased demand for compensation on other brain systems. The model hypothesizes that degeneration of basal forebrain (BF) and pedunculopontine (PPN) cholinergic projections (second and third "hits") are significant causes of the progressive levodopa- resistant gait abnormalities in PD. Consistent with this model, degeneration of cholinergic terminals/neurons of the BF and the PPN are documented well in PD (15,16). PD patients with a history of falls, moreover, have significantly reduced cholinergic projections compared to non-falling patients, whereas these groups do not differ in the degree of dopaminergic denervation (see **Approach** below; **Overview** and **Project II**) (17,18).

$\alpha4\beta2^*$ nAChRs are expressed highly in the terminal fields of BF and PPN cholinergic projections (cortex and thalamus/cerebellar cortex/striatum, respectively), highlighting their potential utility as a pharmacologic target for gait and balance abnormalities in PD. These receptors exhibit complex pharmacology, however, necessitating pharmacokinetic-pharmacodynamic studies, as proposed here, to determine their value as a therapeutic target. It was believed previously, for example, that $\alpha4\beta2^*$ nAChRs upregulated by chronic agonist treatment are desensitized, but recent *in vivo* experiments indicate that chronic nicotininc agonist exposure induces functional $\alpha4\beta2^*$ nAChRs (see **Project I** for further discussion) (19-25). **Critically, $\alpha4\beta2^*$ nAChRs might also show unique alterations in the context of the degenerating, hypocholinergic PD brain, necessitating assessment of this target in this particular environment**. For example, in PD there are significant post-transcriptional alterations in receptor subunit composition and function for N-methyl-D-aspartate receptors, another important ionotropic receptor family (reviewed by Picconi et al. [26]). Regions with little intrinsic pathology could be affected by changes in cholinergic afferents and PD pathologies could induce significant changes in $\alpha4\beta2^*$ nAChR composition and function. Our studies are highly significant because they will assess the function of $\alpha4\beta2^*$ nAChRs in the hypocholinergic degenerating PD brain, establishing the potential of these receptors as a therapeutic target.

Innovation

The proposed experiments are innovative in pursuing *in vivo* pharmacokinetic-pharmacodynamic studies to evaluate engagement of a specific molecular target in the subgroup of hypocholinergic PD subjects – defined by PET imaging in **Project II**. These innovative experiments will use PET methods to assess a key pharmacokinetic variable, receptor occupancy, to select the dose for pharmacodynamic studies. Our PET studies will employ a novel nAChR ligand, [^{18}F]flubatine, with favorable kinetic features.

Our approach differs considerably from previous work and the unique design proposed will overcome key limitations of those studies. A pilot study by Dr. Fay Horak (consultant for this Center) and colleagues explored the acetylcholinesterase inhibitor (AChEI) donepezil for PD-related falls, with modest positive results (27). But, AChEIs are non-specific activators of nAChRs and muscarinic AChRs, including presynaptic autoreceptors inhibiting ACh release. Several features of cholinergic neurotransmission suggest that such non-specific agonism is unlikely to be effective (28). In addition, imaging data indicates that AChEI treatment produces at most 20% inhibition of brain cholinesterase, with many subjects not achieving significant cholinesterase inhibition (29). One $\alpha4\beta2^*$ nAChR agonist, SIB-1508Y, was tested in PD (30). In contrast to several aspects of our proposed work, this study did not identify a hypocholinergic subgroup, did not specifically assess gait and postural control, and all subjects were levodopa naive. Our 3 hit model and preliminary data (**Project I**) suggests that an effect of cholinergic agonists may require simultaneous treatment with levodopa. The $\alpha4\beta2^*$ nAChR partial agonist varenicline (VCN) has not been tested in PD, but there is relevant experience in Spinocerebellar Ataxia Type 3 (SCA3) (31). Zesiewicz et al. reported that VCN was tolerated well and improved axial but not appendicular function in SCA3 subjects (31). This finding is intriguing in view of our [^{18}F]FEOBV data demonstrating significant cholinergic innervation of the cerebellar vermis and archicerebellum, but not the cerebellar hemispheres (**Project II** Preliminary Data).

Results confirming our predictions would strongly support our central hypothesis that $\alpha4\beta2^*$ nAChRs possess pharmacokinetic-pharmacodynamic characteristics appropriate for future trials aimed at ameliorating gait and postural control dysfunction secondary to cholinergic deficits. These experiments will expand the clinical pharmacology of $\alpha4\beta2^*$ nAChRs in the context of PD and implement new methods for evaluating potential new therapeutic targets in PD. This work will also help to establish therapeutic validity of the novel rodent model further developed in **Project I** (32). Validation of the rodent model will greatly enhance the future research on treatments to improve gait and postural control disorders in PD.

Interaction with Other Projects and Cores

The experiments proposed here are strong analogues of critical **Project I** experiments. Subjects studied in this Project will undergo characterization of brain cholinergic pathways in **Project II**. Subject clinical characterization, general cognitive assessments, assessment of gait function and postural control, attention, and other relevant assessments will be performed in the Clinical Resource Core (**Core B**). Data management and biostatistical support for data analyses will be provided by the Biostatistics and Data Management Core (**Core C**). This project will be assisted indirectly by Education and Outreach Core (**Core D**) for subject recruitment, and relies on the Administrative Core (**Core A**) for budgetary oversight and project management.

Approach

Background & Preliminary Data

Cholinergic Neurotransmission & PD Gait Dysfunction: Numerous studies indicate that cognitive dysfunction affects gait and balance functions (reviewed by **33**, **34**, and in **Overview**), implicating BF cholinergic degeneration in gait dysfunction in PD. Impaired attention and executive control are risk factors for falling and simultaneous cognitive task (dual-task) demands affect biomechanical parameters of balance and gait in PD patients (35-38). In controls and PD subjects, individuals with attentional/executive deficits are more prone to falls and gait abnormalities (see **Project II**) (35-39). Degeneration of BF corticopetal projections is well established in PD and the magnitude of BF terminal loss is greater in PD than in AD (15,16,40,41). BF cholinergic projections are crucial for

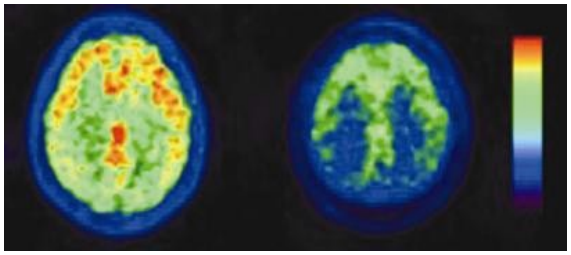

Figure 1: [^{11}C]PMP-PET and heterogeneity of cholinergic deficits in PD. [^{11}C]PMP-PET imaging of cortical cholinergic terminals in PD. Left image; PD subject with normal cortical cholinergic innervation. Right image; PD subject with diminished cortical cholinergic innervation.

neocortical modulation of attention by enhancing detection of relevant cues and filtering distractors (28). As described in considerable work by one participant in this Udall Center application, Martin Sarter, BF mediated cholinergic neurotransmission regulates attention via modulation of thalamocortical afferent effects on cholinergic presynaptic heteroreceptors (see **Project I**) (28,42-45). Dr. Sarter's and Dr. Albin's recent studies in a novel rodent model of combined dopaminergic and cholinergic pathway lesions indicate that this dual lesion paradigm, mimicking the pathology of many PD patients, produces gait and balance abnormalities analogous to those found in PD (**Project I** Preliminary Data and below) (32,46).

The mesopontine PPN complex has wide projections to many regions, including other brainstem structures, the cerebellum, and dense connections with the basal ganglia and thalamus (47-50). The PPN

contains heterogeneous cholinergic, glutamatergic, and GABAergic neuron subpopulations with important cholinergic projections to the basal ganglia, thalamus, and brainstem/cerebellar locomotor regions (49). BF and PPN cholinergic projections may overlap in the mediodorsal and reticular thalamic nuclei (51). A recent post-mortem study correlated PD fall propensity with magnitude of PPN pathology (52). PD fallers had greater loss of PPN cholinergic neurons. A parallel "experiment of nature" supports a crucial role for PPN cholinergic neuron integrity for maintenance of normal posture. Progressive Supranuclear Palsy is a neurodegenerative tauopathy with profound loss of PPN cholinergic neurons (see **Project II**)(53), and falls are an early, prominent feature of that illness. Recent non-human primate experiments further highlight the importance of PPN function in the maintenance of postural control and gait. Karachi et al demonstrated that isolated cholinergic PPN lesions produce dopamine non-responsive gait slowing and postural control abnormalities (52,54). Consistent with our "3 Hit" model of PD gait and balance deficits, impairments of postural control and gait were more pronounced in older MPTP lesioned primates with PPN cholinergic neuron loss.

Our previously published work associates decrements in cholinergic BF and PPN projections with fall propensity, and provides a strong rationale for exploring cholinergic neurotransmission as a potential target to ameliorate falls in PD (17,18). These PET imaging studies demonstrate considerable heterogeneity of cholinergic system changes in PD subjects (**Figure 1**, previous page), allowing for correlation with important clinical features (17,18,55). These data suggest the presence of PD subgroups that may exhibit unique responses to pharmacologic interventions. Using the cholinergic terminal

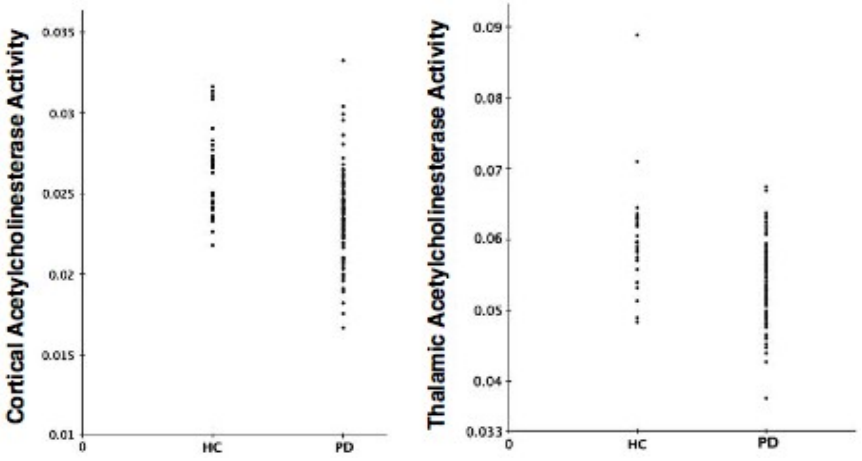

Figure 2: Cortical and thalamic cholinergic innervation in healthy elderly controls (HC) and non-demented PD subjects (from reference 17). PD subjects exhibit significant but widely variable cortical cholinergic denervation and a smaller fraction of PD subjects exhibit thalamic denervation. Y-axis scales different between cortical and thalamic regions of interest.

ligand [^{11}C]PMP, we quantified neocortical (index of BF system integrity) and thalamic (index primarily of PPN integrity) cholinergic terminal density in PD subjects with and without fall histories (18). PD fallers had thalamic deficits, while those without fall histories were comparable to controls. PD fallers exhibited cortical cholinergic deficits of significantly greater magnitude than those without fall histories. In contrast, falling and non-falling PD subjects did not differ in striatal dopaminergic deficits. In a subsequent large (N=101) [^{11}C]PMP study, we correlated cholinergic projection changes with measures of gait function, cognition, and postural control (17). We identified significant decreases in cortical cholinergic innervation in the PD group with substantial overlap with controls (**Figure 2**). One third of the PD group exhibited cortical cholinergic denervation (5th percentile or below of controls) and approximately one sixth of the PD group exhibited equivalent thalamic cholinergic denervation. PD subjects with cortical cholinergic deficits exhibited significantly slower gait speeds, even after adjustment for striatal dopaminergic deficits (17,56). These subjects exhibited significant impairments of executive-attentional function. PD subjects with thalamic cholinergic deficits were significantly more likely to have a fall history (**Project II** Preliminary Data). Striatal dopaminergic terminal changes were comparable between PD subjects with and without cholinergic deficits. Considered together, our results implicate both BF and PPN deficits in abnormal postural control and gait in PD, and associate these problems with cognitive, particularly attentional, dysfunction.

Nicotinic Cholinergic Receptors (nAChRs) as a Therapeutic Target in PD: The great majority of CNS nAChRs are $\alpha4\beta2^*$ heteropentamers (* for potential presence of other subunits), a significant minority are 〈7 homopentamers, and small populations of other subtypes are expressed (57). $\alpha4\beta2^*$ nAChRs may be pre- and/or post-synaptic. Presynaptic nAChRs are located on both cholinergic (homosynaptic) and non-cholinergic (heterosynaptic) terminals. $\alpha4\beta2^*$ nAChRs are expressed in the terminal fields of BF (cortex) and PPN (thalamus, cerebellar cortex) cholinergic projections. Considerable evidence indicates that BF cholinergic projections modulate attention by activating presynaptic thalamocortical terminal $\alpha4\beta2^*$ nAChRs (heteroreceptors; **Project I**) (28,46). In the primate MPTP model, Schneider and colleagues demonstrated that a $\alpha4\beta2^*$ nAChR agonist, SIB-1508Y, improves attention and executive function (58). There is little evidence of thalamic pathology in PD and thalamocortical projections are not known to degenerate in PD. These facts open the door to selective modulation of attention via neocortical mechanisms by $\alpha4\beta2^*$ nAChR agents; enhancing attention by activating these receptors with $\alpha4\beta2^*$ nAChR agonists may improve gait and postural dysfunctions in PD patients with cholinergic deficits.

Postmortem and imaging studies demonstrate reduced $\alpha4\beta2^*$ nAChR expression in several regions in PD brain and likely reflect reduced expression of both presynaptic homoreceptors and postsynaptic receptors (59,60,61). One recent study, for example, of mildly to moderately affected PD subjects with the $\alpha4\beta2^*$ nAChR PET ligand [^{18}F]FA-85380 described varying (20%-40%) reductions of $\alpha4\beta2^*$ nAChR binding in many regions (61). Our understanding, however, of the expression of $\alpha4\beta2^*$ nAChRs in PD is limited. Some prior studies used less resolute SPECT imaging, and [^{18}F]FA-85350 $\alpha4\beta2^*$ nAChR imaging has disadvantageous kinetics, with an up to 7 hour scan acquisition time – quite difficult for PD subjects. Moreover, in contrast to our proposed study *focused on hypocholinergic subjects*, prior work was in unselected cohorts that certainly examined PD subjects with varying cholinergic deficits, probably increasing variation in estimates of regional $\alpha4\beta2^*$ nAChRs.

We will overcome existing shortcomings by imaging $\alpha4\beta2^*$ nAChRs in two ways. First, we will use (−)-[^{18}F]norchloro-fluoro-homoepibatidine (NCFHEB; flubatine), a newly developed and highly specific $\alpha4\beta2^*$ nAChR ligand with favorable binding kinetics, which allows scanning in 90 minutes (62-66). Our PET Center is now performing human [^{18}F]flubatine imaging routinely (see **SA1) (**66). Second, we will only assess PD subjects with documented loss of cholinergic projections, as determined by [^{18}F]FEOBV imaging, a ligand for the vesicular acetylcholine transporter (VAChT) that provides previously unattainable resolution of cholinergic terminals (**Project II**). This innovative approach is likely to provide information on $\alpha4\beta2^*$ nAChRs in PD uniquely valuable for the assessment of this target in PD-related gait abnormalities.

$\alpha4\beta2^*$ nAChR Partial Agonists: A number of nAChR partial agonists are under development and one, varenicline (VCN; "Chantix"), is in clinical use for smoking cessation (67,68). High levels of receptor occupancy can be achieved with partial agonists. This feature is useful for pharmacokinetic imaging experiments, as proposed in **SA1**, as a large percentage of the total receptor population can be imaged. VCN is a potent $\alpha4\beta2^*$ nAChR partial agonist (K_i of 0.4 nM) with good brain penetration, high oral bioavailability, an elimination $t_{1/2}$ of ~24 hours, low plasma protein binding, and primarily renal excretion without metabolism (69). Low plasma protein binding and minimal hepatic metabolism reduce adverse drug interactions. VCN has some 〈7 nAChR partial agonist activity, though at 200-fold higher concentrations (K_i > 84 nM). Recent PET data indicates that VCN binds human CNS $\alpha4\beta2^*$ nAChRs robustly at relevant and relatively low oral doses. Lotfipour et al. used [^{18}F]FA-85350 PET to show that a single, low oral VCN dose (0.5 mg; usual treatment dose = 1 mg po bid) produced near

total occupancy of brain α4β2* nAChRs in acutely abstinent smokers (70). α4β2* nAChR partial agonists have additional properties that are reassuring in the context of PD. α4β2* nAChRs are located on dopaminergic nigrostriatal perikarya and terminals. These compounds may promote dopamine release from nigrostriatal terminals (67). The contribution of stimulating these dopamine neuron located α4β2* nAChRs to their beneficial effects on cognition is unclear (see **Project I**), but there is speculation that use of α4β2* nAChR agonists may allow lower L-dopa doses. Zhang et al. studied the effects of the α4β2* nAChR partial agonist ABT-089 (to be evaluated in **Project I**) in the non-human primate MPTP model (71). ABT-089 treatment reduced levodopa related peak dose dyskinesias without altering parkinsonism or cognition. The dopamine releasing properties of nigrostriatal α4β2* nAChRs will not be a confounding factor in our studies as we are assessing dopamine non-responsive features of PD in subjects on L-dopa. There is speculation also that nAChR stimulation may have neuroprotective effects as nicotinic agonists reduce dopaminergic neuron death in PD toxin models (72). There is no reason to think that α4β2* nAChR partial agonists will interfere with dopaminergic treatment for PD, impair cognition, or worsen underlying neurodegenerative processes.

Safety is a critical issue. Following reports of suicidal behavior and suicide associated with VCN, the FDA issued a "black box warning". Confounding assessment of possible side effects is the fact that these problems occur during nicotine withdrawal and tobacco abusers have a high prevalence of relevant psychiatric comorbidities. Recent comprehensive reviews of VCN trial experience and other large datasets, such as the UK General Practice Research Database, and a recently completed trial specifically examining this issue, **do not** support a connection between worsening mood disorders, suicidality, and VCN (73-78). In a recent subacute VCN treatment study in normal adults, there was no change in mood, impulsivity, or emotional processing related to depressive features, though VCN did enhance cognitive performance (79). Concerns have been raised about VCN and rare but serious cardiovascular events. Recent analyses of trial data did not show a significant relationship and a recent very large cohort study of VCN use in Denmark did not find evidence of increased cardiovascular events (80,81). Indeed, in a recent preventive cardiology trial, VCN use was associated with better control of cardiovascular risk factors (82). Considered together, these data argue strongly that VCN is safe, and suggest that earlier concerns, to the extent they are accurate, apply to the subgroup of acutely abstinent smokers. This subgroup will be excluded from our studies (see **Clinical Resource Core)**. In a very large (~8000 participants), recently published trial examing psychiatric symptoms accompanying VCN use for smoking cessation, there was no increased risk of mood disorder or suicidality (Athenelli et al., Lancet,2016 Jun 18;387(10037):2507-20. doi: 10.1016/S0140-6736(16)30272-0). Moreover, we emphasize that these studies seek to establish α4β2* nAChR as a viable target, not to promote a specific compound as a therapeutic - which would require a clinical trial. In this context, we employ VCN as a representative agonist to explore the pharmacodynamics of α4β2* nAChR stimulation in the context of the hypocholinergic degenerating PD brain. Regardless of whether VCN is ultimately tested in a clinical trial - a real possibility considering the considerable new data indicating it's safety and tolerability (73 - 81) - these data are critical for the evaluation α4β2* nAChR as a therapeutic target in PD-related gait disturbances.

The FDA recently (3/9/15) updated the labeling for VCN (http://www.fda.gov/Drugs/DrugSafety/ucm436494.htm). There are some reports of seizures following VCN use. In addition, there are some reports of alcohol intolerance in individuals using VCN. Again, these have occurred in acutely abstinent smokers. These are isolated reports and whether VCN is actually associated with seizures and alcohol intolerance is unknown. We will take precautions, however, to guard against these potential side effects (see below).

Preclinical Data: nAChR Partial Agonists and PD-related Falls: In experiments described in **Project I**, we present a new model of combined dopaminergic-cholinergic deficits that recapitulates key features of gait and balance disorders in PD (32). In this model, Dr. Sarter piloted adjunctive treatment with ABT-089, an α4β2* nAChR partial agonist. ABT-089 produced attentional improvements and showed excellent tolerability in phase II clinical trials for AD and ADHD (83-89). Preliminary studies show that ABT-089 administration improves balance in the dual lesion model (**Project I** Preliminary Data). Moreover, there is a strong correlation between fall propensity and performance on a measure of attention capacity, the sustained attention test (SAT; $r = 0.61$ [$p < 0.05$]). We will perform parallel experiments with VCN in hypocholinergic PD subjects and Dr. Sarter will examine VCN in the novel rodent model in **Project I**. This parallel design will help to establish therapeutic validity of the rodent model and, in turn, provide mechanistic insight into the pharmacodynamic properties of α4β2* nAChR explored in these clinical studies.

Summary of Preliminary Data: The foregoing discussion establishes our capacity to recruit and characterize appropriate types and numbers of PD subjects, demonstrates a link between cholinergic system changes in gait and balance dysfunction in PD – including preclinical data (**Project I**) supporting our central hypothesis – and shows

capacity to use multi-tracer PET imaging. Our group has also developed a novel, specific cholinergic terminal tracer ([^{18}F]FEOBV; **Project II** Preliminary Data) that will facilitate PD participant selection.

Overall Rationale and Organization

These clinical pharmacokinetic-pharmacodynamic experiments aim to establish α4β2* nAChRs as appropriate targets for treatment of levodopa resistant gait and balance deficits in hypocholinergic PD subjects. Subjects will be drawn from **Project II** and the **Clinical Resource Core**. In **SA1**, we will use [^{18}F]flubatine PET imaging to determine the lowest VCN dose producing high α4β2* nAChR occupancy. In **SA2**, we will use this dose to perform pharmacodynamic studies of VCN effects on laboratory measures of gait and postural control. These studies will determine if α4β2* nAChR stimulation produces the predicted effects on laboratory measures of balance and gait – directly addressing our central hypothesis. In **SA3**, we will study the effects of subacute VCN administration on a measure of attentional capacity sensitive to cholinergic deficits, testing our hypothesis that improvement in attention capacity will correlate with improvements in gait and postural control. These data will evaluate a likely physiological mechanism mediating the effects of stimulating α4β2* nAChRs in the context of the hypocholinergic PD brain, and provide important validation data for the rodent model used in **Project I**.

We propose subacute (days) VCN administration and focus on hypocholinergic PD subjects. Because repeated α4β2* nAChR stimulation, as would be experienced by chronically treated patients, induces functional receptor upregulation, assessing α4β2* nAChR occupancy with a single VCN dose in VCN naïve subjects will be misleading. Moreover, a VCN dose that produces high percent receptor occupancy in a VCN naïve subject may exhibit considerably less α4β2* nAChR occupancy in subjects exposed repeatedly to VCN and with α4β2* nAChR upregulation. It might be scientifically interesting to include normocholinergic PD subjects, but safety concerns hinder this approach. In our model (**Overview**; **Project I** data), dopaminergic striatal deficits result in compensatory "loading" of other brain systems. Normocholinergic PD subjects depend on increased cholinergic neurotransmission to compensate for striatal dysfunction, placing them at increased risk of adverse events resulting from reduced cholinergic signaling (32,46). VCN is a partial agonist with an efficacy of approximately 40% of ACh. In normal individuals, VCN produces measurable improvements on cognitive tests (79,88), but in normocholinergic PD subjects, VCN may interfere sufficiently with α4β2* nAChR mediated compensation to produce functional impairments, including cognitive impairment and/or diminished balance, including damaging falls. It is not feasible to recruit sufficient subjects to do these experiments in an inpatient setting, which would be required to mitigate these potential side effects. For these reasons, we will not expose normocholinergic PD subjects to VCN. Beyond these important safety concerns, adding study groups to the proposed experiments would reduce statistical power for a fixed sample size, increasing the Ns required for a valid study beyond that which can be supported by funds available for Udall Center studies.

We will investigate the possibility that regulation of α4β2* nAChRsmight be different in PD brain versus brains of normal individuals. We will investigate this possibility with a focused study of α4β2* nAChR receptor occupancy in a small group of normal subjects. Following the conclusion of receptor occupancy studies in hypocholinergic PD subjects, we will perform a more limited, parallel, dose-receptor occupancy study to determine if there are major differences in VCN receptor occupancy between PD and control subjects.

Subject Selection: PD subjects will be drawn from **Project II** subjects who have undergone [^{18}F]FEOBV imaging to identify cholinergic deficits.

We will study subjects with cortical innervation deficits below the 5th percentile cutoff of controls as defined previously (17). General inclusion and exclusion criteria are specified in the **Clinical Resource Core** and **Project II**. Current or previous (within last 6 months) use of any product or medication containing nicotinic agents, including use of tobacco products such as cigarettes, cigars, pipes, chewing tobacco, etc., e- cigarettes, OTC nicotine patches, chewing gum containing nicotine, or varenicline is an exclusion. While most data does not indicate that VCN causes suicidality or cardiovascular problems (73 - 81), we will exclude subjects with depression, active cardiovascular disease, history of seizures, or alcohol use disorders. (see **Human Subject Protection**).

The **Clinical Resource Core** and **Project II** project initial enrollment of 95 PD subjects with longitudinal follow-up of 75 PD subjects. Based on prior results, we project that 30% (32) of the initially enrolled subjects will be hypocholinergic. Based on preliminary data (**Project II**), 25% (20) of the longitudinally followed PD subjects will convert from normo- to hypocholinergic during the project period. This gives a total of 52 eligible subjects (Table 1). Our long-standing experience is that a significant fraction of subjects participate in multiple experiments, so we anticipate little difficulty in recruiting the projected 55 PD subject-equivalents required. In our recently concluded Program Project Grant (NS15655), we studied 151 PD subjects. All underwent at least 2 PET studies with 112

undergoing 3 or more scans (69 - 3 studies; 43 - 4 studies). We are basing our estimates on a stringent 5th percentile cut-off relative to our normal control database. We will carefully monitor subject recruitment to enroll the target number of hypocholinergic PD subjects. If needed, **Core B** will preferentially recruit an enriched sample of PD subjects with longer duration disease and/or mild cognitive changes, subjects more likely to exhibit cholinergic deficits. **Core B** will be aided in recruitment by the **Core D** (Education and Outreach Core). **Appendix 1** contains a detailed schedule of study activities.

IND Exemption: We obtained an IND exemption from the FDA.

Specific Aim 1: To use [^{18}F]flubatine PET to assess VCN occupancy of brain α4β2* nAChRs after subacute VCN administration to the subgroup of PD subjects with cholinergic deficits.

Rationale and Predictions: We will use [^{18}F]flubatine PET to select an appropriate VCN dose for **SA2** and **SA3** studies. Application of [^{18}F]flubatine PET will allow selection of the lowest dose producing high (>90%) receptor occupancy. Measuring VCN displacement of [^{18}F]flubatine obviates the need for clinical dose escalation studies in hypocholinergic PD subjects. α4β2* nAChRs are complex heteromeric proteins with highly regulated expression and function. These studies are essential, as the PD brain is a very different environment from a normal brain. We predict that that these studies will identify a VCN dose at the lower end of the clinically used range producing high (>90%) α4β2* nAChR occupancy. A recent acute VCN dose study in acutely abstinent smokers (upregulated α4β2* nAChRs) with [^{18}F]FA-85380 reported that a single 0.5 mg

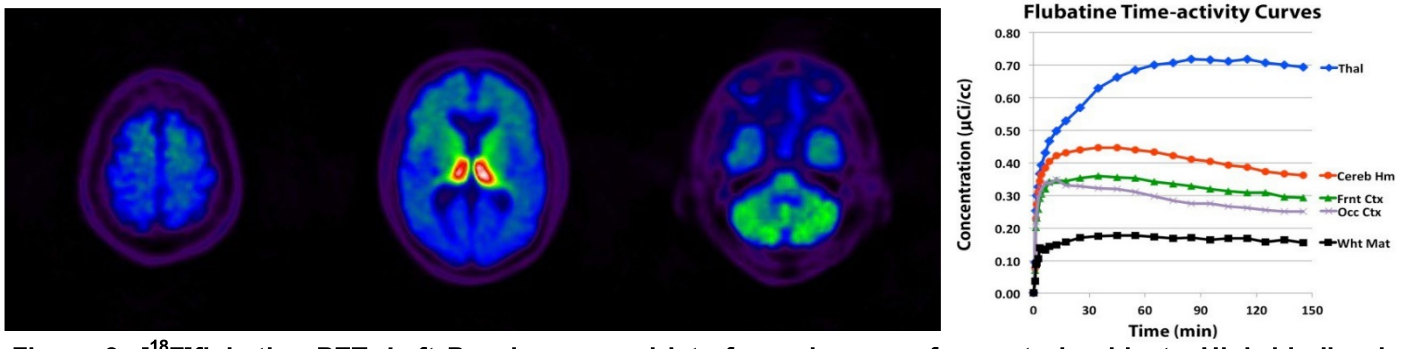

Figure 3: [^{18}F]flubatine PET. Left Panel: averaged late frame images of a control subject. High binding in thalamus, striatum, cerebellum, and frontal cortex. Right Panel: Typical time-activity curve for a control

oral VCN dose (typical treatment dose = 1 mg bid) produced ~95% receptor occupancy with imaging several hours after ingestion (70). This result suggests strongly that lower clinical VCN doses will produce high (>90%) receptor occupancy. All subjects will undergo baseline [^{18}F]flubatine scanning after subacute VCN treatment to induce upregulation and following VCN washout to allow measurement of per cent VCN occupancy of upregulated α4β2* nAChRs.

Preliminary Data: Our PET Center is performing [^{18}F]flubatine imaging on a routine basis (**Figure 3, Table 2**). [^{18}F]Flubatine PET has regional distribution expected for α4β2* nAChRs, excellent signal to noise properties, and imaging can be completed in 90 minutes. Notably, regional binding values are highly reproducible across subjects with low coefficients of variation (**Table 2**). *Procedures:* α4β2* nAChR expression will be measured in vivo with [^{18}F]flubatine. Subjects will receive varying VCN doses. [^{18}F]Flubatine imaging will be done 10 days after initiation of VCN administration and 5 days after VCN discontinuation (**Figure 4**). The difference between [^{18}F]flubatine binding before and after VCN discontinuation will be measured to assess α4β2* nAChR occupancy. Receptor occupancy will be assessed in regions exhibiting high α4β2* nAChR expression; thalamus, striatum, and cerebellum, as results are likely to be more variable in regions with lower α4β2* nAChR expression. Based on prior animal and human studies, 7-10 days is adequate to induce receptor upregulation (91-95). VCN has a $t_{1/2}$ of ~24 hours and 10 days will be adequate to attain equilibrium and induce upregulation. VCN pharmacokinetics (PK) do not change with age and absorption is unaffected by food (96). Five days is adequate to clear VCN. T_{max} is 2-4 hours post oral administration. The first scan will occur in the afternoon of day 10 after the morning VCN dose to avoid peak dose effects which could increase variance.

Table 2: [^{18}F]Flubatine binding values for regions expressing high levels of α4β2* nAChRs. N = 3 controls. DVR = Distribution Volume Ratio; SD = Standard Deviation; COV = Coefficient of Variation.

Region	Mean DVR	SD	COV
Thalamus	1.57	0.11	6.9%
Striatum	0.90	0.02	2.1%

The recommended clinical VCN dose schedule is 0.5 mg per day for 3 days, followed by 0.5 mg bid for 4 days, followed by 1 mg bid. Mocking et al. recently reported positive neuropsychological results of subacute VCN

treatment (0.5 mg/day for 3 days, then 1 mg/day for 4 days) in normal controls (79). Because we are administering VCN to a clinical population and not young, healthy controls, we propose lower initial doses than Mocking et al. We will start with 0.25 mg/day on a subacute treatment schedule of 10 treatment days. A potential acute side effect of VCN is nausea, though this is unusual in non-smokers with doses below 1 mg per day. To reduce the chance of nausea or other side-effects, we will use a series of escalating dose schedules (**Appendix 2**). VCN will be prepared by the U-M Health System Research Pharmacy. VCN pills are small, easily encapsulated in gelatin capsules, and will be dispensed in blister packs to facilitate administration and compliance monitoring. The initial maximum dose studied will be 0.25 mg/day. Subsequent, projected maximum doses are 0.25 po bid, 0.5 mg po bid, and 1 mg po bid (if needed). Twice daily dosing is used to minimize peak dose effects. We anticipate 3 dose points, each consisting of 3 participants (total of 9

Figure 4: Timeline of SA1 Experiment

participants), to establish the required oral dose for **SA2** and **SA3** experiments. The results of the initial (0.25 mg/day maximum dose) study will be used to select the next dose point. If there is a substantial effect on [^{18}F]flubatine binding (>50% reduction), we will use the 0.25 mg bid maximum dose schedule for the next experiment. If the effect on [^{18}F]flubatine binding is <50% reduction, we will use 0.5 mg bid maximum dose schedule for the next experiment. Selection of the subsequent dose schedule will be based on the results of
the 0.5 mg bid maximum dose experiment. We will increment dose schedules until there is no additional reduction in [^{18}F]flubatine binding, i.e., remaining binding is non-specific binding, and the penultimate maximum dose defines our **SA2-SA3** study dose. Based on the work of Lotfipour et al., who found that a single oral 0.5
mg VCN dose produced ~100% receptor occupancy (70), we don't anticipate using the highest (1 mg po bid) dose schedule). We will measure plasma VCN levels at the time of the initial scan (Pharmacokinetics Core, U- M College of Pharmacy; see attached letter from Dr. Duxin Sun, Core Director). These measurements are not for formal PK studies as VCN PK is well established, but rather to confirm study drug compliance. We aim to establish the lowest dose producing high ~100%) receptor occupancy and if we achieve this goal after studying 2 dose schedules (6 participants total), we will stop and proceed to **SA2** experiments.

PD Subjects will continue their customary PD treatments. Subjects will be evaluated in the University of Michigan Functional Neuroimaging, Cognitive and Mobility Laboratory (see **Facilities & Resources**). All subjects will undergo a standard clinical and brief psychometric evaluation immediately prior to VCN treatment initiation, at the time of initial scan and at the time of the second scan. Subjects will undergo a MDS-UPDRS, Geriatric Depression Scale, Montreal Cognitive Assessment, WAIS Digit Span and Digit Symbol coding tests, and D-KEFS executive function/working memory tests. Subjects will be screened for suicidality with the Columbia Suicide Severity Rating Scale (C-SSRS; http://www.cssrs.columbia.edu/) before VCN treatment and at the time of each PET study. Dr. Albin is certified in use of the C-SSRS (**Appendix 3**) and Dr. Dauer and other relevant members of the Clinical Resource Core will also become certified. Subjects will be directly observed for 3 hours after their initial VCN dose, with phone follow-up the next day and weekly thereafter. Subjects will be screened for history of seizures and alcohol abuse. The latter will be assessed with the WHO Alcohol Use Disorder Identification Test (AUDIT). Per the WHO guidelines, individuals with scores >8 (ages 18-65) or >7 (ages >65) will be excluded. Individuals with a history of seizures will be excluded. Participants will be advised to refrain from alcohol use during study participation. An investigator (Dr. Albin or Dr. Dauer) will be on-call for subject concerns at all times. Data will be recorded on standard CRFs and entered into the database for management and analysis by the **Biostatistics and Data Management Core**. Adverse events will be classified by organ system and assessed for causality. All potential possible adverse events will be discussed by the Udall Center Executive Committee acting as DSMB, and reported promptly to the IRB and NINDS.\

Following completion of the PD subject study experiment 1, we will perform a limited experiment to assess $\alpha4\beta2^*$ nAChR occupancy in age-matched controls. Controls will receive VCN (no placebo) for 10 days. We will select a subset of the doses used for the PD subjects in experiment 1 to assess receptor occupancy in normal controls. Assuming we don't detect very high (>90%) occupancy with our initial dose (0.25 mg/day), we will expose controls to one of three different dosages of varenicline to construct a dose-occupancy relationship for comparison with the results in PD subjects. Based on the results of experiment 1, experiment 2 will include the following dosages

with a goal of 3 patients per cohort: .25mg QD, .25mg BID and .5mg BID. All procedures will be identical to experiment 1. Control subjects will be drawn from the normal control pool characterized concurrently in the Clinical Core of the Udall project, much as the PD subjects are drawn from the pool of PD subjects. Control subjects will also be recruited from the UM Health Research website if needed. We anticipate studying 9 subjects, 3 at each dosage point. To adjust for potential dropouts, we will budget 12 control subjects.

Potential Problems and Alternatives: The results of Loftipour et al. suggest that low doses will produce high $\alpha4\beta2^*$ nAChR occupancy (70). Commercially available VCN pills are small and division beyond bisection is likely to be inaccurate. 0.25 mg/day is the lowest feasible dose and if we find this dose produces high per cent receptor occupancy, no further testing will be necessary. VCN tolerability may be a problem as this agent has not been tried specifically in PD subjects previously. To avoid any problems, we are starting with low doses and using an incrementing schedule. Nausea was infrequent in a recent trial in AD which used significantly higher doses (89). If tolerability is an issue, then we will alter the incrementing schedule to more gradually "ramp" up to the desired dose.

Specific Aim 2: To assess the effects of pharmacologic $\alpha4\beta2^*$ nAChR activation on laboratory measures of gait function and postural control in PD subjects with cholinergic deficits.

Rationale and Predictions: The central hypothesis of this project is that deficient activation of $\alpha4\beta2^*$ nAChRs consequent to the degeneration of cholinergic projections causes balance and gait abnormalities in PD. Consequently, restoration of $\alpha4\beta2^*$ nAChR stimulation should improve these deficits. In this target engagement pharmacodynamic experiment, we will evaluate the effects of the $\alpha4\beta2^*$ nAChR partial agonist VCN on laboratory measures of gait function and postural control in hypocholinergic PD subjects. We predict that subacute VCN administration will improve gait function and postural control in hypocholinergic PD subjects. We chose a pair of relevant physiologic endpoints; gait speed and Horak's JERK (97,98,99). Slow gait is a cardinal sign of PD and we demonstrated recently that slowed gait is strongly associated with neocortical cholinergic denervation (56). Similarly, Rochester et al. found that gait speed in PD correlated with an indirect clinical electrophysiologic measure of cortical cholinergic function (100). Slowed gait is also a feature of our combined dopaminergic-cholinergic rat model (32). We will apply the Instrumented test of postural Sway (ISway; APDM Mobility Lab; http://apdm.com/gait-and-posture/Mobility-Lab/index.html), a relatively simple and robust method for evaluating postural control with a belt-worn inertial sensor (97,98,99). ISway was developed specifically to assess postural control in PD subjects and is validated against more complex methods. Abnormalities of these types of measures are associated in both cross-sectional and prospective studies with increased fall risk in the elderly, and measure relevant postural control phenomena in PD (101-104). JERK is one of several parameters measured by ISway, captures postural sway irregularity, and possesses strong validity, good test-retest behavior, and good correlation with UPDRS III PIGD scores (97).

Preliminary Data: Our group implemented the ISway system in the Functional Neuroimaging, Cognitive and Mobility Laboratory (**Table 2**). We studied 11 similar PD subjects, 6 with a history of falls and 5 without any fall history. ISway measures of postural control differentiated fallers and non-fallers (**Table 2**).

Table 3: ISway: data in non-fallers (n=5) and falling PD (n=6). Means + SD.

	Non-Faller	Faller	t-test (p-value)
Jerk	0.082 ± 0.080	0.213 ± 0.125	t = -2.0 (0.072)
RMS	0.058 ± 0.021	0.109 ± 0.045	t = -2.3 (0.045)
Velocity	0.114 ± 0.050	0.200 ± 0.067	t =-2.3 (0.049)
Centroid Freq (AP)	0.734 ± 0.160	0.617 ± 0.140	t=2.7, (0.026)

Procedures: The **SA1** experiment will select the VCN dose. Based on discussions with the proposed **Biostatistical and Data Management** co-Core leaders, we will employ a randomized double- blind, placebo-controlled, 2-period, 2-sequence crossover design to assess the effects of subacute VCN administration on gait function, postural control, and attention in PD subjects with cholinergic deficits (**Figure 5**). PD participants will be on stable doses of dopaminergic therapy for 3 months prior to enrollment and continue on their usual schedule of dopaminergic medications for the duration of this study. Individuals with depression and unstable cardiac disease will be excluded. Within-subjects crossover designs were employed successfully in recent VCN trials for AD and mood disorders during smoking cessation (89,90).

We will administer VCN or placebo for 3 weeks, followed by a 3 week washout period, and crossover to placebo or VCN for the 3 week second treatment period. Study visits will be completed within a five business day window before or after the target date. The duration of the treatment and washout periods is based partly on prior human experimental and trial experience (89-95). Mocking et al. detected improved cognitive effects of VCN with a week of treatment (79). We chose a longer period to ensure stable receptor upregulation and because initiation of treatment may require gradual dose incrementing. The washout period is based partly on work by Zhang et al. with another $\alpha4\beta2^*$ nAChR partial agonist in MPTP lesioned non-human primates (71). This group reported an effect of

ABT-089 on levodopa induced dyskinesias close to 2 weeks after dose cessation, well beyond the $t_{1/2}$ of this agent. This wash-out interval was used also in the recent VCN AD trial (89). The randomization schedule and process will be prepared by the **Biostatistics and Data Management Core.** Blinded, encapsulated study drug (VCN and placebo) will be supplied by the U-M Research Pharmacy to the study coordinator. The latter will dispense the study medication. Subjects will be evaluated in the University of Michigan Functional Neuroimaging, Cognitive and Mobility Laboratory (see **Facilities and Resources**). Subjects will be directly observed for 3 hours after initial VCN dose and with phone follow-up calls 1-2 business days after starting the medication treatment period, and weekly thereafter. Follow-up phone calls will be completed within a two business day window before or after the target date. VCN plasma levels will be evaluated at end of both treatment periods on days 22 and 64 through a blood draw to monitor compliance. Blood samples will be stored at the University of Michigan Functional Neuroimaging, Cognitive & Mobility Laboratory until the study is finished. Data will be recorded on standard eCRFs and entered into the database for management and analysis by the **Biostatistics and Data Management Core.** Adverse events will be classified by organ system and assessed for causality. All potential possible adverse events will be discussed by the Executive Committee, and reported promptly to the IRB and NINDS.

Gait speed and postural control will be assessed at baseline, at the end of the first VCN/placebo administration period, at the end of the washout period, and at the end of the second VCN/placebo administration period. Gait and postural control will assessed by the **Clinical Resource Core (Core B)** using the Mobility Lab system that is already established (see Table 3; methodology for Mobility Lab will be as described in detail in Project II). MDS-UPDRS, Geriatric Depression Scale, Montreal Cognitive Assessment, WAIS Digit Span and Digit Symbol coding tests, and D-KEFS executive function/working memory tests will be administered as part of the evaluations. Subjects will be screened for suicidality with the C-SSRS before VCN treatment, weekly during phone follow-up, and at the end of treatment-washout periods.

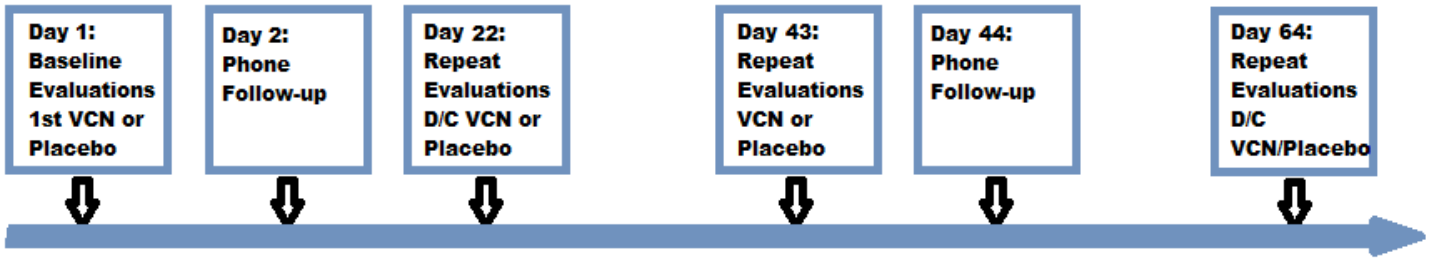

Figure 5: Schematic Timeline for Experiment 3

Sample Size: The main basis for the sample size for this 2-sequence, 2-period, 2-treatment crossover study is based on gait speed and the postural control parameter, ACC JERK. Both endpoints are considered primary and so a Bonferroni adjustment will be made to control the Type I error for the study at the one-sided
1.5 level (i.e., each endpoint will be tested at the 0.025 level). Published (56) and preliminary data in PD subjects (**Table 2**) provide estimates of effect and variability for these parameters in the placebo group. We powered our study based on conservative estimates derived from these data (i.e., using the 95% upper confidence bound of the SD as the estimate of variability in the sample size calculations) and the desire to detect at least a 10% relative treatment effect in gait speed and 60% relative treatment effect in ACC
JERK. With a total of 33 subjects, we have at least 80% power to detect a treatment difference between VCN and placebo of 0.112 m/s and -0.131 m^2/s^5 with a Bonferroni-adjusted two-sided Type I error of 2.5%, assuming within-subject correlation of at least 0.64 and 0.72, respectively (a conservative estimate given that
test-retest correlation of ACC JERK in PD subjects was 0.86[97]). We will enroll 42 subjects to allow for a 10% attrition rate. We will examine some pre-defined secondary endpoints; RMS (root mean square of center of pressure variability), gait speed variability, and anterior-posterial sway velocity. These secondary endpoints are chosen on the basis of published data with ISway (97), and preliminary data (**Table 2**), and additional preliminary data suggesting association of gait speed variability and anterior-posterior sway velocity with deficits of specific cholinergic pathways (**Project II** preliminary data). We will use a gatekeeper testing strategy to control the familywise error for the secondary endpoints that play a supportive role in interpreting the effects of VCN (105,106). If at least one of the primary endpoints is significant (at least one of the hypotheses is rejected), hypotheses for the secondary endpoints will be tested using Hochberg's step-up method (107). This approach ensures the overall Type I error rate is no greater than 0.05.

Analysis Method. For all aims, we will conduct exploratory analyses to examine the distributions of outcomes under each treatment, as well as individual and mean profiles over time. Graphical approaches such as boxplots and scatterplots with linear or non-linear (e.g., loess) methods will be used, allowing identification of outliers, linearity, and correlation of measurements within person and across time. If outcome data do not appear normally distributed, transformations will be employed. Because participants' outcomes will measured at more than one time point per

person, our analyses will be based on linear mixed effects models (108-110). We expect no carryover since treatments will be spaced three weeks apart. Tests to compare treatments will be based on Wald and chi-squared tests based on maximum likelihood or restricted maximum likelihood methods. Alternative covariance structures (other than compound symmetry) will also be explored and model fit examined using the Akaike Information Criteria (AIC).

Potential Problems and Alternatives: All measures proposed for this experiment are well established in the University of Michigan Functional Neuroimaging, Cognitive and Mobility Laboratory and the **Clinical Resource Core**. The major foreseeable difficulties are subject recruitment and retention. We've had considerable success recruiting and retaining subjects in protocols involving up to 4 PET studies, serial evaluations, long-term follow-up (months to years), and subject clinical-psychometric-gait & balance evaluations as intensive as those proposed. We budget a 10% drop out rate in this study, which is typical of our experience in prior prospective studies and identical to the dropout rate in the recent AD study (89). A related concern would be unexpected difficulty with VCN tolerability, leading to excessive dropouts. The goal of the SA1 experiments is to find the lowest dose providing high $\alpha4\beta2^*$ nAChR occupancy, which provides protection against this possibility. In the recent AD study, VCN was tolerated well at higher doses than we expect to employ. If tolerability is a problem, we would use a slower dose escalation regimen.

It is possible that there will be differential effects of VCN on gait speed and postural control. We will be drawing these subjects from **Project II**, where all participants will receive anatomically resolute [^{18}F]FEOBV imaging. As discussed in **Project II**, there may be correlates between specific pathway deficits, such as BF projection versus PPN projection degeneration, or even more specific pathway changes such as PPN to cerebellar vermis projection deficits, and specific gait or postural control measures (see **Project II** Preliminary Data). We will have a rich dataset to examine these possibilities, including not only imaging data but also the considerable gait and balance data generated by the Mobility Laboratory system for exploratory analyses. It is possible that VCN will have no effect on gait, balance, or cognition in these subjects. This would be disappointing but important as it would steer the field towards other types of cholinergic receptors as therapeutic targets. Comparison of these results with parallel **Project I** experiments will also be important for evaluating the novel model developed in that Project.

Specific Aim 3: To assess the effects of pharmacologic $\alpha4\beta2^*$ nAChR activation on a measure of attentional function (SAT; sustained attention test) in PD subjects with cholinergic deficits.

Rationale and Predictions: Our preclinical work with a novel rodent model of cholinergic-dopaminergic gait deficits indicates that diminished attentional function following loss of corticopetal BF afferents is a key mediator of the effects of cholinergic system degeneration on gait and balance (**Overview**; **Project 1**). A large body of prior research indicates attentional function impairments contribute significantly to impaired gait and postural control, including falls, in normal elderly and PD patients (33-39). In a recent large prospective study, for example, attentional function deficits predicted falls in older adults (39). Dr. Sarter's extensive prior work indicates that $\alpha4\beta2^*$ nAChRs are crucial actors in cholinergic modulation of attention (**Project 1**) (42-46). Consistent with this work, our preliminary preclinical data suggests that stimulation of $\alpha4\beta2^*$ nAChRs improves gait and balance in the novel dual lesion model (**Project 1**). These combined results lead to an important prediction: subacute $\alpha4\beta2^*$ nAChR administration to hypocholinergic PD subjects will improve attentional performance and improved attentional performance will correlate with improved gait and postural control induced by $\alpha4\beta2^*$ nAChR stimulation. Evaluating this prediction is important for assessing the mechanisms by which cholinergic projection system degeneration impairs gait and balance and is important also for assessing the validity of the novel rodent model presented in **Project 1**.

Preliminary Data: Dr. Sarter's group developed a robust test of attentional function, the Sustained Attention Test (SAT), for use in rodent experiments, and subsequently adapted and validated the SAT for use in humans (**Project 1**) (111-113). Briefly the SAT consists of a randomized sequence of cued and non-cued (or signal and nonsignal) trials. Responses are hits and misses, and correct rejections and false alarms, respectively. Hits and correct rejections are rewarded. SAT performance reflects frontal cortical cholinergic signaling through $\alpha4\beta2^*$ nAChRs. We evaluated a particularly relevant version of the SAT including a distractor component (dSAT), in PD subjects who had previously undergone [^{11}C]PMP imaging to assess cholinergic projection systems integrity. There was considerable variation in dSAT performance, which was a linear function of cortical cholinergic terminal integrity (**Figure 6**). These results indicate that dSAT performance in PD directly reflects cortical cholinergic signaling, likely mediated by $\alpha4\beta2^*$ nAChRs.

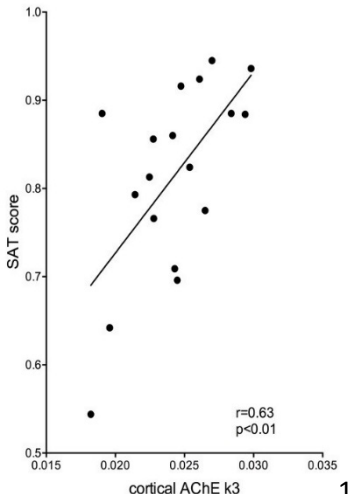

Figure 6: Correlation of cortical cholinesterase

Procedures: This experiment will be conducted in parallel with those for SA2. Attention will be evaluated with the dSAT at all evaluation points in the double-blind crossover design VCN intervention study described above. We will correlate dSAT changes in response to VCN with gait speed and postural control changes in response to VCN. The sample size is determined from the goals of **SA2**. With this sample size, an effect size of 0.4 can be detected with at least 80% power and a two-sided Type I error of 0.05 assuming a within-subject correlation of 0.69. Similar statistical methods as those used for SA2 will be employed to test the impact of VCN on dSAT. An exploratory analysis will be conducted to examine medication of treatment effects on gait and other postural outcomes by dSAT. In the presence of a treatment effect, a variable is declared a mediator if it complies with three things: it is affected by the treatment, it predicts the endpoint of interest, and the treatment effect is attenuated when the potential mediating factor is adjusted for. Mediation analysis will be conducted by including mediating factors in the mixed model regression analysis outlined above. Structural equation models will be used to estimate the direct effect of VCN and the indirect effects of the VCN due to mediators, as has been recommended for intervention studies of disease prevention (114,115).

Potential Problems and Alternatives: All procedures, including the dSAT, are well established in our center. Most of the potential problems are discussed above (**SA2** *Potential Problems and Alternatives*). Our prediction is that improved dSAT performance will correlate with improved gait speed and postural control measures. There may be dissociations between results of dSAT testing and gait speed-postural control outcome measures. Attentional performance may improve but not gait speed and postural control. Gait speed and postural control may improve without commensurate improvements in dSAT performance. These outcomes would be important for evaluating our understanding of how cholinergic deficits degrade gait and postural control. Lack of correlation would direct future research away from attentional mechanisms and towards alternative mechanisms, such as PPN projection roles in sensorimotor integration. We will have [^{18}F]FEOBV data on cholinergic projection systems integrity for all these subjects and secondary analyses looking at specific functional deficits and specific pathway changes may be revealing. Whether or not dSAT performance response correlates with improvements in gait speed and postural control measures is also important for evaluating the validity of the dual lesion model. It is crucial to know the limitations of this interesting model prior to using it extensively for preclinical evaluations of potential therapies.

Standard Methods:

MRI and VMAT2 PET Imaging, Standard PET Methods and Image Processing: See **Clinical Resource Core**. *Flubatine PET Imaging: [18F]Flubatine will be prepared in high radiochemical purity (>95%) with a method developed by our group (68).* PET scans will be acquired using a protocol consisting of 18 frames acquired over 90 min following bolus injections of 8 mCi [^{18}F]flubatine. Individual image frames within each scan will be co-registered to frame 10 (10-15 min post-injection) to account for patient motion.Full kinetic modeling requires arterial blood sampling and >two hours of imaging, impractical for this patient population and we will use simpler analyses. Our primary analysis will use an average of the 70-90 min scan data, when tissue TACs are relatively constant, approaching equilibrium (**Figure 3**). Venous blood samples will be drawn at 70 and 90 min, plasma extracted and counted in a NaI well-counter. Plasma metabolites will be assessed to determine the radioactivity concentration of authentic flubatine. Radiotracer binding measures will be obtained by normalizing the PET tissue concentrations to the authentic venous plasma radiotracer concentration averaged over the 70 and 90 min samples. This measure of tracer binding approximates the total tissue volume of distribution relative to plasma, V_T. This method is well suited to quantification of flubatine binding as flubatine undergoes minimal catabolism, has only moderate plasma protein binding, and equilibrates instantaneously between blood and plasma (116). A secondary analysis will use the simplified reference tissue model (sRTM) (117). SRTM assumes a reference tissue devoid of specific binding sites, used in lieu of an arterial plasma input function to estimate tissue binding potentials. Since no gray matter tissue is devoid of specific binding sites for flubatine, we will test white matter as a reference tissue. White matter reference regions will be defined on co-registered MR volumes, and segmented with FreeSurfer to produce white matter masks. Binary masks, smoothed to PET resolution with white matter defined by all brain voxels with a mask value >0.9 after smoothing, will assure that reference region voxels have >90% contribution from white matter.

Gait Speed and Postural Control Methods: Gait and postural control will be assessed in the Functional Neuroimaging, Cognitive and Mobility Laboratory with Mobility Lab (APDM, Portland, OR) systems now standard in our center (see **Project II**, **SA2 Preliminary Data**). The postural control and gait assessments are usually completed in approximately 45 minutes. These studies will be performed in the **Clinical Resource Core** using methodology described in detail in **Project II.**

dSAT: The dSAT will be administered as described previously (108-110). A PC with E-Prime software is used to present either a visual signal or no cue in the middle of the screen followed after a constant delay by an auditory signal to indicate via key pushing whether or not a visual signal was presented. Using different keys, participants indicate both signal presentation and non-presentations. Participants receive feedback on response accuracy with a small financial reward for correct responses on each trial. Signal durations and inter- trial intervals are varied. In the distractor condition, the background screen alternates between silver and black at 10 Hz. This task requires minimal training and reliable data is obtained in a single session. The SAT score is calculated from all outcomes.

Future Directions: These experiments are likely to lead in several useful directions. Demonstration that $\alpha4\beta2^*$ nAChR stimulation improves physiological measures of gait function and postural control in hypocholinergic PD subjects will spur investigation of $\alpha4\beta2^*$ nAChR agonists for treatment of gait and postural control problems in PD. nAChR pharmacology is an active area of industrial R&D with compounds going to phase II trials and coming to market. With the preclinical methods exemplified by the novel model featured in **Project I** and methods used in this project, we would be well positioned to evaluate other $\alpha4\beta2^*$ nAChR agonists or other cholinergic agents as adjunctive treatments in PD. We would aggressively evaluate additional promising compounds. Our group would move an $\alpha4\beta2^*$ nAChR agonist into clinical trials for PD, preferably in phase IIa trials. Tthe development and validation of convenient methods to identify the subset of PD patients with cholinergic deficits (see **Project II** and **Clinical Resource Core**), and the relatively non-invasive physiologic methods used to evaluate $\alpha4\beta2^*$ nAChR stimulation in this study would be valuable components of such studies. Our group, with large clinic populations, strong clinical expertise, an institutional CTSA (Michigan Institute for Clinical & Health Research; MICHR), and strong expertise in both the basic and clinical pharmacology of cholinergic agents would be well positioned to lead the development of novel cholinergic therapies for PD. We would pursue these studies outside the Udall Center mechanism with complementary R01, U01, translational R21, or NeuroNext applications.

Project III: Protection of Human Subjects

i) Human Subjects

Description of subject populations: Subjects will include 57 PD subjects and 12 control subjects.

Recruitment: Subjects will be recruited from the UMHS and VAAAHS Movement Disorders Clinics, the **Clinical Resource Core** and with the assistance of the **Education and Outreach Core**. The UMHS Movement Disorders clinic follows a population of approximately 2,000 clinically well-defined patients with PD and/or parkinsonism and will be the main source of recruitment of patients for this study. Dr. Albin, Co-Director of the UMHS Movement Disorders Clinic, will be in charge of UM patient recruitment. In addition, subjects will be recruited from the VAAAHS Movement Disorders clinic, directed by Dr. Bohnen, and currently follows approximately 250 well-defined subjects with PD. At present, we recruit 4-6 subjects with PD for imaging studies at UM per month. We anticipate that about some participants for this study can be recruited from the concluding P01 NS15655 (Kirk Frey, PI) that studied about 4 subjects with PD per month, most of whom have longer duration of disease. Further information about recruitment and retention are described in the **Clinical Resource Core** and the **Education and Outreach Core**.

Inclusion Criteria:

1. PD diagnosis will be based on the United Kingdom Parkinson's Disease Society Brain Bank Research Center (UKPDSBRC) clinical diagnostic criteria. We will enrich the cohort using the following strategies: recruiting subjects at modified Hoehn and Yahr stages 2 or higher (from data collected in a medication "OFF" state in UDALL project 2), recruiting subjects with a duration of motor disease 5 years or longer, recruiting subjects that are age >65 years, or recruiting subjects with the PIGD phenotype. Duration of motor disease will be defined as the time between onset of motor symptoms and time of entry into the study. The PIGD phenotype is defined as described previously. PD subjects with defined cholinergic deficits will be recruited as described in **Project II**. PD subjects will have cortical cholinergic deficits based on 5^{th} percentile cutoff of the normal controls as defined previously.
2. PD Patients with stable dopaminergic replacement therapy for 3 months prior to enrollment and expected to maintain stable dopaminergic therapy for duration of study participation.

OR

3. Normal healthy control individuals age 45 years and older without any history of neurological disease.

Exclusion Criteria:

1. Other disorders which may resemble PD with or without dementia, such as vascular dementia, normal pressure hydrocephalus, progressive supranuclear palsy, multiple system atrophy, corticobasal ganglionic degeneration, or toxic causes of parkinsonism. Prototypical cases have distinctive clinical profiles, like vertical supranuclear gaze palsy, early and severe dysautonomia or appendicular apraxia, which may differentiate them from idiopathic PD. The use of the UKPDSBRC clinical diagnostic criteria for PD will mitigate the inclusion of subjects with atypical parkinsonism and all participants will undergo [^{11}C]DTBZ PET to confirm striatal dopaminergic denervation.
2. Subjects on neuroleptic, anticholinergic (trihexiphenidyl, benztropine), or cholinesterase inhibitor drugs.
3. Current or previous (within last 6 months) use of any product or medication containing nicotinic agents, including use of tobacco products such as cigarettes, cigars, pipes, chewing tobacco, etc., e- cigarettes, OTC nicotine patches, chewing gum containing nicotine, or varenicline.
4. Evidence of a stroke or mass lesion on structural brain imaging (MRI).
5. Participants in whom magnetic resonance imaging (MRI) is contraindicated including, but not limited to, those with a pacemaker, presence of metallic fragments near the eyes or spinal cord, or cochlear implant.
6. Severe claustrophobia precluding MR or PET imaging
7. Subjects limited by participation in research procedures involving ionizing radiation.
8. Pregnancy (test within 48 hours of each PET session) or breastfeeding.
9. Significant risk of cardiovascular event.
10. Active, significant mood disorder.
11. History of seizures.
12. Active alcohol abuse.

ii) Sources of Materials

Information gathered specifically for this research project includes test results from clinical test scales, standardized neuropsychological measures and from experimental cognitive tests, neurobehavioral rating scales, clinical motor data, questionnaires, standardized interview, and data derived from the PET studies that reflect the amount of dopaminergic nerve terminals, cholinergic nerve terminals, and nicotinic receptors in the brain, and from structural MRI.

iii) Potential Risks

Confidentiality of Research Information: The research data to be collected from subjects will consist of confidential information relating to clinical, neuropsychological, mood, partial genotype, neuroanatomical, and neurochemical functions. These research data are not intended for entry into the subjects' clinical medical records. However, the data remain potentially discoverable. This may lead to violation of privacy and embarrassment of subjects.

Clinical, neuropsychological and Behavioral Testing: Risks in regard to the neuropsychological, behavioral, and motor assessments are limited to fatigue, frustration and momentary embarrassment that may occur when one experiences difficulty performing a task or learning a new skill.

PET scans & Venipuncture: Insertion of a catheter for intravenous injection of the PET radiopharmaceutical may be commonly associated with slight pain or bruising at the puncture site and rare chance of infection. Participation in this research study will involve exposure to radiation associated with the PET transmission scans and the administration of the radioactive drugs. Subjects participating in experiments involving PET imaging will undergo 2 [^{18}F]Flubatine PET studies. These subjects will have previously undergone [^{11}C]DTBZ and [^{18}F]FEOBV PET and in some subjects undergoing [^{18}F]Flubatine PET may be studied in the same year as they undergo DTBZ and FEOBV PET, for a total of 4 PET studies in 1 year. Maximum total dosimetry for any subject would be 15 mCi for DTBZ, and 8 mCi for each FEOBV and flubatine study. Total exposure will be well below the annual radiation exposure (5 rem) permitted to radiation workers by federal regulations. Participants will be instructed to void two hours post injection to minimize bladder exposure. This radiation dose is not expected to produce any harmful effects, although there is no known minimum level of radiation exposure for non-radiation workers considered to be totally free of the risk of causing genetic defects or cancer. The risk associated with the amount of radiation exposure participants receive in this study is considered low and comparable to every day risks. In the event of incomplete imaging or technical failure, it may be necessary to repeat the [^{18}F]Flubatine

PET scan. We will not exceed radiation dose limits. No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing. The use of these PET tracers is considered to be generally safe and effective as approved by the University of Michigan Radioactive Drug Research Committee in accordance with Food and Drug Administration regulations (21 CFR 361.1). Adverse reactions to tracers used in this study have not been reported. However, the possibility exists for a rare reaction to any of the drugs or procedures to which the participant will be exposed. ACLS-certified staff will be in attendance at all times during the study and an emergency cart will be in close proximity. Other physical risks involve possible muscle aches from lying still. Medical intervention will be furnished by UMHS if serious adverse effects would occur.

Participants will be asked to provide a blood sample on two separate visits. There is an infrequent risk of bruising, bleeding, infection or soreness associated with the venipuncture, similar to the risks associated with routine blood testing. Participants may feel dizzy or lightheaded or may rarely even faint when the needle is put in or taken out.

Mood Disorder: In anecdotal reports, VCN has been associated with increased risk of significant mood disorder, including worsening mood, suicidal ideation, suicide attempts, and completed suicide. The FDA issued a "Black Box" warning for VCN because of concerns about these possible psychiatric side-effects of VCN. Though present data do not substantiate a connection between VCN administration and serious mood problems (see **Approach**), we will exclude patients with active mood disorders.

Cardiovascular Risk: In anecdotal reports, VCN has been associated with increased risk of serious cardiovascular events, such as myocardial infarction or stroke. Though systematically collected data do not substantiate a connection, subjects with considerable risk for cardiovascular events will be excluded.

Seizures: In anecdotal reports, VCN has been associated with seizures. Potential subjects with a history of seizures will be excluded.

Alcohol Intolerance: In anecdotal reports, VCN has been associated with intolerance of alcohol use. Potential subjects with active alcohol abuse will be excluded.

Sleep Disorders: VCN use is associated with sleep disorders, including insomnia and vivid dreams. Participants will be counseled about these potential side effects and instructed to contact the investigators for any concerns.

Gastrointestinal Symptoms: The primary known side effect of VCN is gastrointestinal symptoms. Nausea, vomiting, and constipation are reported with VCN use.

Risk of Multiple Study Participation: Being in more than one research study at the same time, or even at different times, may increase the risks to you. It may also affect the results of the studies. You should not take part in more than one study without approval from the researchers involved in each study.

Other Symptoms: Lightheadedness has been reported with VCN use.

iv) Adequacy of protection against risks

Recruitment and Informed Consent:

Subjects will be recruited from the UM and VAAAHS Movement Disorders clinics. Individuals willing to participate will be scheduled for the research procedures at which time the nature and risks of the procedures will again be reviewed with the subjects and a written informed consent form will be obtained by one of the study investigators.

One copy of the signed consent form will be given to the subject, one will be placed in the patient's medical record and a third will be kept in the patient's study binder kept at the patient's research site. Study visits will be conducted in the UM Functional Neuroimaging, Cognitive & Mobility Laboratory. Imaging procedures will be performed at the University of Michigan Health System.

Protection Against Risk:

Confidentiality of Research Information: The possibility of unintended disclosure of medical or research data is minimal, but not entirely impossible. We will employ stringent safeguards against unintended and inappropriate discovery and dissemination of personal medical and research data in our subjects by a multi-layered approach. All data bearing potential subject identifiers will reside solely in locked files in the offices of the study

investigators or study coordinator. Original data collection documents will be maintained in secure files under the control of the investigators or study coordinator. Entries regarding details of the research project and its results will not be submitted to clinical medical databases. Electronic databases in the project will employ subject codes that cannot be linked directly to participants without a "key", possessed only by the study investigators in a secure location, and maintained separately from the databases. Primary databases will not be housed on systems with Internet access, preventing unauthorized intrusions. Personal information that would directly identify study subjects will not be used in any publications or presentations resulting from this research study, unless separate written permission is given by the subject (or proxy). Any superfluous records will be shredded.

Clinical, neuropsychological and Behavioral Testing: Care will be taken to minimize distress. In addition to a lunch and afternoon rest break, subjects are given multiple short breaks during the clinical testing day when needed. Subjects will be addressed in a courteous manner that does not infringe the patient's dignity. Physicians and trained personnel with considerable experience working with older individuals and patients with PD will perform all evaluations and administer tests. These individuals are prepared to respond to patient anxiety, concern and other behavioral changes as appropriate to the situation. Offering breaks and reassuring subjects will further minimize risks when necessary. If needed for some subjects, the clinical testing protocol can be split over 2 days. The UM Functional Neuroimaging, Cognitive and Mobility laboratory has facilities to allow rest breaks when needed. The investigators presently have similar ongoing studies with 1-day clinical testing and 1-day imaging protocols in similar PD populations. Excepts when subjects are severely demented, our PD participants who are typical septuagenarians or octogenarians have no difficulties completing these protocols.

PET Scan & Venipuncture: A nuclear medicine technologist will perform the PET studies under the supervision of a physician who is a specialist in nuclear medicine. A physician or nurse will be available at all times during the study, and any adverse reactions will be treated immediately. A fully equipped medical cart is located in the Nuclear Medicine suite. We will use aseptic techniques and highly trained personnel to minimize the risks associated with venipuncture. No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing within 48 hours prior to each PET imaging session. In order to lessen the pain and fatigue of lying still, the subject will be given a break outside of the scanner midway through the procedure.

Mood Disorders: All potential participants will be screened formally for mood disorder and suicidal ideation with the Geriatric Depression Scale (GDS) and Columbia Suicide Severity Rating Scale (C-SSRS). Individuals with GDS scores > 5 will be excluded at the discretion of the PI. Similarly, any potential participants describing significant suicidal ideation (scores of 4 or 5 on the relevant questions of the C-SSRS) will be excluded. Mood and suicidality will be evaluated regularly during the study (see Approach; and Schedule of Activities, **Appendix**) with the GDS and C-SSRS. Individuals with worsening GDS scores will be evaluated by the investigators promptly and referred for psychiatric evaluation if clinical interview substantiates concerns about significantly worsening mood disorder. Significant suicidal ideation will result in emergent psychiatric evaluation. VCN administration will be stopped immediately in cases of worsening mood or suicidal ideation. Mood disorder risks will be discussed with potential subjects and the "Black Box" labeling disclosed explicitly. All subjects will be instructed to page the investigators with concerns at any time.

Cardiovascular Disease Events: Individuals with history of stroke (excluded under general exclusion criteria) or myocardial infarction within 1 year, unstable angina, or active PVOD will be excluded. Subjects will be counseled about risk of cardiovascular events and instructed to page the investigators with any concerns. Any suggestions of cardiovascular disease – chest pain, claudication type pain, TIA – will result in VCN discontinuation and emergent evaluation.

Seizures: Individuals with a history of seizures will be excluded. Participants will be counseled about possible seizure risk and instructed to page the investigators with any concerns. Any suggestions of seizures will result in VCN discontinuation and emergent evaluation.

Alcohol Interactions: Individuals with active alcohol abuse will be excluded. The Alcohol Use Disorders Identification Test (AUDIT) will be used to screen for alcohol abuse. Individuals scoring over the recommended cutoffs (>7 for >65 years; >8 for 18-65 years) will be excluded. Participants will be counseled to refrain from alcohol during study participation.

Sleep Disorders: VCN use is associated with sleep disorders, including insomnia and vivid dreams. Participants will be counseled about these potential side effects and instructed to contact the investigators for any concerns.

Gastrointestinal Symptoms: VCN will be initiated at a very low dose and the dose increased over 2-3 days. Individuals who are not able to tolerate the full dose of VCN will be reduced to the next lowest dose as needed. If they are still not able to tolerate the VCN then the VCN will be stopped and the patient will be withdrawn from the study.

Study Monitoring Plan: (please see section vii below).

v) Potential Benefits of the Proposed Research to the Subjects and Others

In the course of this study patients will receive clinical evaluations including neuropsychiatric testing and imaging studies. No direct immediate benefit of these studies is anticipated. Although generally the results of these examinations will not be made available to their treating physician, if a significant, unexpected abnormality is detected this will be reported to the patient and his/her physician.

vi) Importance of the Knowledge to be Gained

Data obtained from this research may become important for the development of improved methods of reducing gait and balance impairments in PD and other disorders. Such mechanisms would provide a rational basis for targeted intervention of gait and balance impairments in PD and other disorders.

vii) Study Monitoring Plan

We obtained an IND exemption from the FDA. Study safety will be monitored in 2 complementary ways. A study monitor from the MICHR IND/IDE Investigator Assistance Program (MIAP) will monitor the study according to standard procedures once experiment 2 begins. We impaneled an independent DSMB board; Dr. Stephan Taylor (Dept. of Psychiatry), Dr. William Gehring (Dept. of Psychology), and Dr. Wen Ye (Dept. of Biostatistics). The DSMB will review the study annually during experiment one, then every 6 months during experiment two. The PIs will be responsible for monitoring any break in confidentiality and for reporting any adverse events following University of Michigan IRB guidelines. For purposes of this study, an AE is defined as any unfavorable or unintended change in structure, function, signs, or symptoms temporally associated with participation in this study, whether or not a causal relationship with the study has been established. Clinically significant abnormalities may be considered an AE if deemed appropriate by the PIs. Unexpected worsening of a pre- existing condition is also considered an AE, as is the discovery of an abnormal finding during physical exam that was not included in the medical history. Breaches of confidentiality will be considered related to the research whenever they occur and will be reported. Withdrawals from the study and the reason for these withdrawals will also be reported. The PIs are in daily contact with the Project research staff testing the participants, scoring and entering data, and will monitor their procedures to ensure that confidentiality is maintained. The PIs will ensure that the DSMB and IRB are notified of any adverse event following the IRB guidelines. Expected and unexpected serious (including fatal) adverse reactions and major unresolved disputes between the research investigator(s) and the research participant or between research investigator(s) will be expeditiously reported to the IRB of the University of Michigan. At the time of renewal, the IRB will be provided with a summary indicating the frequency of the monitoring, cumulative adverse event data, information regarding participant safety or ethics changes, confidentiality issues, benefit-to-risk changes and recommendations on continuing, changing or terminating the study. The sponsor, NINDS, will also be notified of any adverse events simultaneously with the DSMB and IRB.

Costs and Payments: Subjects will not be charged for their participation in this study. Subjects will be compensated for time and effort with $200 for completing the initial clinical evaluations and $400 upon completion of the imaging or VCN administration procedures. Subjects will receive $100 to defray housing and meal costs (for subject and caregiver) as we anticipate a number of subjects will have at least one overnight stay, and $50 to defray travel costs (fuel and parking costs).

Project III: Inclusion of Women and Minorities

Inclusion of Women:

Women will be included in this research project. Males and females will be given equal priority in recruitment. However, because PD affects males more than women we expect to recruit a higher percentage of males. The PI will monitor recruitment of women to this project throughout the study, and institute procedures to

enhance the enrollment of women, if numbers are not adequate. See the Targeted/Planned Enrollment Table.

Inclusion of Minorities:

See the **Targeted/Planned Enrollment Table** for this project. Enrollment targets are based on population estimates of the 2010 US census for the state of Michigan and Washtenaw County (location of UMHS) with some modifications. For Michigan; 78.9% White, 14.2% African-American, 0.6% Native American, 2.4% Asian, 0% Hawaiian & Pacific Islander, 1.5% Other Race, 2.3% Two or More Races; 4.4% Hispanic/Latino, 95.6% Not Hispanic or Latino. For Washtenaw County; 74.5% White, 12.7% African- American, 0.3% Native American, 7.8% Asian, 0% Hawaiian & Pacific Islander, 1.5% Other Race, 2.3% Two or More Races; 4.0% Hispanic/Latino, 96% Not Hispanic or Latino. Hispanic/Latino residents tend to be recent immigrants and younger than the population average, so we project enrollment of fewer Hispanic/Latino participants than the Hispanic/Latino proportion of state/county population. Similar considerations apply to Asians. Some epidemiologic evidence suggests that PD is less prevalent among African-Americans, so we project fewer African-American enrollees than the proportion of African-Americans in our state/county. The Education and Outreach Core will assist with Minority recruitment.

Project III: Inclusion of Children

Justification for Exclusion of Children:

No children will be included. PD occurs predominantly in late-life and, indeed, the risk of developing PD increases with increasing age. In rare cases with clear familial inheritance, the onset of the disease may occur in the 30's and 40's, but there is no evidence of clinical PD in children. Therefore, the research topic to be studied is not relevant to children.

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Project III: a4b2* nAChRs, Gait, and Balance in PD

Domestic/Foreign: Domestic

Comments:

Racial Categories	Ethnic Categories: Not Hispanic or Latino		Ethnic Categories: Hispanic or Latino		Total
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	1	0	0	0	**1**
White	28	38	1	1	**68**
More than One Race	0	0	0	0	**0**
Total	**29**	**38**	**1**	**1**	**69**

Study 1 of 1

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