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Thalamic cholinergic innervation and postural sensory integration function in Parkinson's disease

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The pathophysiology of postural instability in Parkinson's disease remains poorly understood. Normal postural function depends in part on the ability of the postural control system to integrate visual, proprioceptive, and vestibular sensory information. Degeneration of cholinergic neurons in the brainstem pedunculopontine nucleus complex and their thalamic efferent terminals has been implicated in postural control deficits in Parkinson's disease. Our aim was to investigate the relationship of cholinergic terminal loss in thalamus and cortex, and nigrostriatal dopaminergic denervation, on postural sensory integration function in Parkinson's disease. We studied 124 subjects with Parkinson's disease (32 female/92 male; 65.5 ± 7.4 years old; 6.0 ± 4.2 years motor disease duration; modified Hoehn and Yahr mean stage 2.4 ± 0.5) and 25 control subjects (10 female/15 male, 66.8 ± 10.1 years old). All subjects underwent ^{11}C-dihydrotetrabenazine vesicular monoaminergic transporter type 2 and ^{11}C-methylpiperidin-4-yl propionate acetylcholinesterase positron emission tomography and the sensory organization test balance platform protocol. Measures of dopaminergic and cholinergic terminal integrity were obtained, i.e. striatal vesicular monoaminergic transporter type 2 binding (distribution volume ratio) and thalamic and cortical acetylcholinesterase hydrolysis rate per minute (k_3), respectively. Total centre of pressure excursion (speed), a measure of total sway, and sway variability were determined for individual sensory organization test conditions. Based on normative data, principal component analysis was performed to reduce postural sensory organization functions to robust factors for regression analysis with the dopaminergic and cholinergic terminal data. Factor analysis demonstrated two factors with eigenvalues >2 that explained 52.2% of the variance, mainly reflecting postural sway during sensory organization test Conditions 1–3 and 5, respectively. Regression analysis of the Conditions 1–3 postural sway-related factor [$R^2_{adj} = 0.123$, $F(5,109) = 4.2$, $P = 0.002$] showed that decreased thalamic cholinergic innervation was associated with increased centre of pressure sway speed ($\beta = -0.389$, $t = -3.4$, $P = 0.001$) while controlling for covariate effects of cognitive capacity and parkinsonian motor impairments. There was no significant effect of cortical cholinergic terminal deficits or striatal dopaminergic terminal deficits. This effect could only be found for the subjects with Parkinson's disease. We conclude that postural sensory integration function of subjects with Parkinson's disease is modulated by pedunculopontine nucleus-thalamic but not cortical cholinergic innervation. Impaired integrity of pedunculopontine nucleus cholinergic neurons and their thalamic efferents play a role in postural control in patients with Parkinson's disease, possibly by participating in integration of multimodal sensory input information.

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Abbreviations: ^{11}C-DTBZ = ^{11}C-dihydrotetrabenazine; ^{11}C-PMP = ^{11}C-methylpiperidin-4-yl propionate; MDS-UPDRS = Movement Disorder Society revised Unified Parkinson's Disease Rating Scale

Introduction

Postural instability is a common and disabling feature of Parkinson's disease but the underlying pathophysiology of impaired postural control in Parkinson's disease remains unclear. Postural functions depend in part on integration of sensory information from visual, proprioceptive, and vestibular systems. Previous studies indicate that integration of relevant sensory information may be affected in patients with Parkinson's disease (Chong *et al.*, 1999; Frenklach *et al.*, 2009; Rossi *et al.*, 2009; Colnat-Coulbois *et al.*, 2011).

Parkinson's disease is a multisystem neurodegeneration syndrome. Nigrostriatal dopaminergic denervation is a key pathobiological marker of Parkinson's disease; however, degeneration of major cholinergic projection systems can occur early in Parkinson's disease (Bohnen and Albin, 2009, 2011; Bohnen *et al.*, 2012). The brainstem pedunculopontine complex provides cholinergic inputs to the thalamus, cerebellum, several brainstem nuclei, basal ganglia and the spinal cord (Heckers *et al.*, 1992; Martinez-Gonzalez *et al.*, 2011). Pathological studies of Parkinson's disease report significant degeneration of large cholinergic neurons of the lateral part of the pedunculopontine nucleus, pars compacta (Hirsch *et al.*, 1987; Jellinger, 1988; Zweig *et al.*, 1989; Gai *et al.*, 1991; Rinne *et al.*, 2008). The basal forebrain corticopetal complex, including the nucleus basalis of Meynert, provides the principal cholinergic input to the entire cortical mantle (Mesulam and Geula, 1988). Degeneration of basal forebrain cholinergic neurons is described well in post-mortem Parkinson's disease brains (Arendt *et al.*, 1983; Candy *et al.*, 1983).

There is accumulating evidence that postural instability in Parkinson's disease cannot be attributed to striatal dopaminergic denervation alone. Recent findings support a putative role for cholinergic system degeneration, in particular pedunculopontine cholinergic neuron loss, in the pathophysiology of postural imbalance in Parkinson's disease. Our previous *in vivo* PET studies showed that Parkinson's disease fallers had significantly decreased thalamic (i.e. pedunculopontine nucleus) cholinergic innervation compared with Parkinson's disease non-fallers, whereas there was no difference in the degree of nigrostriatal dopaminergic denervation between these two groups (Bohnen *et al.*, 2009, 2012). These findings were corroborated by recent post-mortem results (Karachi *et al.*, 2010). Treatment with the acetylcholinesterase inhibitor donepezil produced reductions in the number of falls in frequently falling patients with Parkinson's disease (Chung *et al.*, 2010). Degeneration of the cholinergic basal forebrain corticopetal complex may contribute also to postural impairments in Parkinson's disease. This system is associated with executive function and attentional capacity; both important for normal postural function (Woollacott and Shumway-Cook, 2002). Loss of cortical cholinergic innervation is associated with impairments in these and other cognitive functions (Bohnen *et al.*, 2006b), which may affect postural control in Parkinson's disease (Bohnen and Albin, 2011; Yarnall *et al.*, 2011). Basal forebrain cholinergic deficits, however, would not be expected to directly affect sensory integration. Cholinergic pedunculopontine neurons innervate virtually the whole thalamus, including sensory afferent and cerebellar relay nuclei that may mediate important aspects of sensory integration for postural control (Martinez-Gonzalez *et al.*, 2011).

The purpose of this study was to examine the relationship between cholinergic system deficits and postural sensory integration function in Parkinson's disease while taking into account the effects of other disease-specific features including striatal dopaminergic denervation. Striatal dopaminergic terminal, and thalamic and neocortical cholinergic terminal integrity were assessed with ^{11}C-dihydrotetrabenazine (a vesicular monoaminergic transporter type 2 ligand; ^{11}C-DTBZ) and ^{11}C-methylpiperidin-4-yl propionate (an acetylcholinesterase ligand; ^{11}C-PMP) PET imaging, respectively. To quantify sensory integration function in Parkinson's disease, we used the sensory organization test moving platform protocol (also known as computerized dynamic posturography) to specifically identify difficulties with the integration of postural sensory feedback into the maintenance of balance. We hypothesized that decreased thalamic cholinergic innervation would be associated with decreased postural sensory integration function in Parkinson's disease.

Materials and methods

Subjects

This cross-sectional study included 124 subjects with Parkinson's disease [32 female, 92 male, mean age: 65.5 ± 7.4 standard deviation (SD) years]. Subjects met the United Kingdom Parkinson's Disease Society Brain Bank Research Centre clinical diagnostic criteria for Parkinson's disease (Hughes *et al.*, 1992). The diagnosis of Parkinson's disease was confirmed also by the presence of a typical pattern of nigrostriatal dopaminergic denervation on ^{11}C-dihydrotetrabenazine PET imaging (Bohnen *et al.*, 2006a). Most subjects had mild-to-moderate disease severity (modified Hoehn and Yahr mean stage: 2.4 ± 0.5, median stage 2.5) (Hoehn and Yahr, 1967; Goetz *et al.*, 2004) with mean motor disease duration of 6.0 ± 4.2 years. Most subjects were on some form of dopamine replacement therapy (drug naïve: $n = 7$; carbidopa-levodopa only: $n = 52$; combination of carbidopa-levodopa and dopamine agonist only: $n = 21$; variable combinations of carbidopa-levodopa, dopamine-agonist, monoamine oxidase inhibitor, or catechol-*O*-methyl transferase inhibitor medications: $n = 44$; mean levodopa equivalent dose 674.2 ± 501.8 mg) (Tomlinson *et al.*, 2010). Neurological and clinical examinations, posturography, and dopaminergic PET imaging was performed after withholding dopaminergic medications to obtain a clinically defined OFF state.

The study also included 25 control subjects (10 female, 15 male, mean age: 66.8 ± 10.1 years). These control subjects were selectively pooled from other studies, had identical PET procedures, postural sensory integration function assessment (not reported elsewhere), and comparable clinical and cognitive assessments as the subjects with Parkinson's disease. Neurological exam reports of the control subjects showed no evidence of focal deficits, other neurological disease, or significant balance impairment. None of the control subjects had abnormal cognitive functioning, i.e. the Mini-Mental State Examination (Folstein *et al.*, 1975) or Montreal Cognitive Assessment (Nasreddine *et al.*, 2005) scores were > 26.

No subjects used anti-cholinergic or cholinesterase inhibitor drugs.

Written informed consent was obtained from all subjects before research procedures. The University of Michigan Medical School Institutional Review Board for human studies approved the study.

Clinical assessment

Clinical evaluations of the subjects with Parkinson's disease included a standardized neurological exam, collection of demographic and general clinical information, assessment of peripheral lateral malleolar vibration sensitivity (right and left averaged) with a biothesiometer (Bio-Medical Instrument Company), and assessment of overall Parkinson's disease feature severity with the Movement Disorder Society revised Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (Goetz *et al.*, 2007, 2008). Cognitive capacity was assessed with the Montreal Cognitive Assessment test (Nasreddine *et al.*, 2005) and the Dementia Rating Scale (Mattis, 1998). Based on a Dementia Rating Scale cut-off score of 138 (Villeneuve *et al.*, 2011), 31.5% of the subjects with Parkinson's disease had evidence of cognitive impairment.

Assessment of postural sensory integration function

The sensory organization test protocol of the EquiTest balance platform (NeuroCom, a division of Natus) was used for testing postural sensory integration functions. Subjects were asked to stand quietly on the platform with their arms crossed. Each of the six sensory organization test conditions was only performed once, unless the trial was incomplete due to imbalance resulting in a corrective step taken by the subject ('sensory organization test fall') (Frenklach *et al.*, 2009). In that case the sensory organization test condition was repeated with a maximum of three attempts total. For each of the sensory organization test conditions, total centre of pressure excursion over the trial duration (centre of pressure-speed) and centre of pressure variability (centre of pressure-root mean square) were determined (Prieto *et al.*, 1996). We recorded also the total number of 'sensory organization test falls' for each subject.

Imaging

Magnetic resonance imaging

All subjects underwent brain MRI for anatomical co-registration with PET. MRI was performed on a 3T Philips Achieva system (Philips) using an eight-channel head coil. A standard T_1-weighted series of a 3D inversion recovery-prepared turbo field echo was performed in the sagittal plane using repetition time/echo time/inversion time = 9.8/4.6/1041 ms; turbo factor = 200; single average; field of view = 240 × 200 × 160 mm; acquired matrix = 240 × 200. Approximately 160 slices were reconstructed to 1 mm isotropic spatial resolution.

Positron emission tomography

PET imaging was performed in 3D imaging mode using an ECAT Exact HR+ tomograph (Siemens Molecular Imaging, Inc.), which acquires 63 transaxial slices (slice thickness = 2.4 mm; intrinsic in-plane resolution = 4.1 mm full-width at half-maximum over a 15.2 cm axial field of view). A NeuroShield (Scanwell Systems) head-holder/shielding unit was attached to the patient bed to reduce the contribution of detected photon events originating from outside the imaging field of view. Before the radioligand injections, a 5 min transmission scan using rotating ^{68}Ge rods was acquired for attenuation correction of emission data using the standard vendor-supplied segmentation and re-projection routines.

^{11}C-DTBZ [no-carrier-added (+)-α-^{11}C-dihydrotetrabenazine] was prepared using high-specific activity ^{11}C-methyl iodide as reported previously (Jewett *et al.*, 1997; Shao *et al.*, 2011). ^{11}C-DTBZ PET scans were performed using a bolus/infusion protocol acquiring 15 emission scans over 60 min (4 × 30 s; 3 × 1 min; 2 × 2.5 min; 2 × 5 min; 4 × 10 min), with a priming bolus of 55% followed by continuous infusion of the remaining 45% over the study duration using a dose of 555 MBq.

^{11}C-PMP was synthesized by *N*-^{11}C-methylation of piperidin-4-yl propionate using high specific activity ^{11}C-methyl triflate as described previously (Snyder *et al.*, 1998; Shao *et al.*, 2011). Dynamic ^{11}C-PMP PET scans were performed using a bolus injection protocol acquiring 16 emission scans over 70 min (same as ^{11}C-DTBZ protocol plus an additional 10 min scan) with a dose of 666 MBq.

All subjects were studied supine, with eyes and ears unoccluded, resting quietly in a dimly lit room.

Positron emission tomography analysis

All dynamic PET imaging frames were spatially co-registered within subjects with a rigid body transformation to reduce the effects of subject motion during the imaging session (Minoshima *et al.*, 1995). These motion-corrected PET frames were spatially co-registered to the MRI using SPM8 software (Wellcome Trust Centre for Neuroimaging). IDL image analysis software (Research systems, Inc) was used to manually trace volumes of interest on the MRI scan. Traced volumes of interest included the bilateral striatum, thalamus, and neocortex. Neocortical volume of interest definition used semi-automated threshold delineation of the neocortical grey matter signal on the MRI images.

Time activity curves for each volume of interest were generated from the spatially aligned PET frames. Striatal ^{11}C-DTBZ PET distribution volume ratio (which is equal to 1 + the binding potential relative to non-displaceable uptake), a measure of dopaminergic binding, was estimated by using the Logan *et al.* (1996) graphical analysis method with the striatal time-activity curves as the target regions and neocortex as the reference input. Thalamic and neocortical ^{11}C-PMP acetylcholinesterase hydrolysis rates per minute (k_3), a measure of cholinergic activity, were estimated using reference tissue-based linear least squares analysis as described by Nagatsuka *et al.* (2001), with the striatum as the reference tissue.

Statistical analysis

Based on the normative sensory organization test data of the control subjects, principal component analysis of the covariance matrix was performed first to reduce the 12 centre of pressure variables (centre of pressure-speed and centre of pressure-root mean square for each of the six sensory organization test conditions) to varimax rotated factors with a robust eigenvalue >2. Two factors were extracted with

eigenvalues of 3.5 and 2.7, respectively. Together these factors explained 52.2% of the variance. The first factor reflected mainly centre of pressure-speed for the first three sensory organization test conditions and the second factor reflected centre of pressure-speed for sensory organization test Condition 5 (Table 1).

To test the main hypothesis, multiple linear regression was performed to assess the independent effects of striatal ^{11}C-DTBZ distribution volume ratio and thalamic and cortical ^{11}C-PMP k_3 on the centre of pressure-speed factor scores of the subjects with Parkinson's disease while taking into account the Parkinson's disease-specific confounding variables of overall motor impairment (MDS-UPDRS motor examination total score) and cognitive capacity (Dementia Rating Scale total score). Given that there were nine subjects with Parkinson's disease who were unable to complete the entire sensory organization test procedure, this analysis was limited to 115 subjects with Parkinson's disease. Variables were rank-order transformed for the regression analyses as normal distributions could not be assumed for most variables. Exploratory *post hoc* regression analysis was performed to examine possible associations between the total number of 'sensory organization test falls' and dopaminergic and cholinergic system degenerations.

All analyses were performed using IBM SPSS Statistics 20 (IBM) with assumed $\alpha < 0.05$.

Table 1 Factor loadings for centre of pressure speed and centre of pressure-root mean square for each of the six sensory organization test conditions for the two varimax rotated factors with eigenvalues of 3.5 and 2.7, respectively

		Factor 1	Factor 2
COP-speed	SOT1	**0.864**	0.017
	SOT2	**0.890**	0.066
	SOT3	**0.858**	0.413
	SOT4	0.467	0.304
	SOT5	0.077	**0.972**
	SOT6	0.137	0.593
COP-RMS	SOT1	0.398	−0.013
	SOT2	0.748	0.239
	SOT3	0.526	0.645
	SOT4	0.185	0.25
	SOT5	0.046	0.781
	SOT6	−0.032	0.070

Factor loadings > 0.8 are indicated in **bold**.
COP = centre of pressure; RMS = root mean square; SOT = sensory organization test.

Results

Mean scores for the MDS-UPDRS, the cognitive capacity scales, and PET ligands are presented in Table 2. Striatal dopaminergic innervation as well as thalamic and cortical cholinergic innervation were significantly lower in subjects with Parkinson's disease compared with control subjects (Table 2). The control subjects were not significantly different from the subjects with Parkinson's disease with regard to age (Mann-Whitney $U = 1392.5$, $P = 0.423$), gender ($\chi^2 = 2.07$, $P = 0.150$), body mass index ($U = 1824.0$, $P = 0.164$), or distal vibratory sensation detection ($U = 1601.5$, $P = 0.794$).

To test our main hypothesis, linear regression analysis was performed to assess for the independent effects of striatal dopaminergic binding and thalamic and cortical cholinergic terminal integrity on centre of pressure factor scores of the subjects with Parkinson's disease while taking into account MDS-UPDRS motor examination and Dementia Rating Scale total scores. The overall significant model of the first centre of pressure factor [$R^2_{adj} = 0.123$, $F(5,109) = 4.2$, $P = 0.002$] showed that increased centre of pressure factor score was independently associated with both decreased thalamic cholinergic innervation ($\beta = -0.389$, $t = -3.4$, $P = 0.001$; Fig. 1) and increased MDS-UPDRS motor examination total score ($\beta = 0.221$, $t = 2.3$, $P = 0.022$). There was no significant effect of cortical ^{11}C-PMP k_3 or striatal ^{11}C-DTBZ distribution volume ratio. There was no significant overall model effect for the second factor [$R^2_{adj} = -0.009$, $F(5,109) = 0.8$, $P = 0.558$].

We performed a *post hoc* linear regression analysis in the cognitively normal control subjects to examine whether the thalamic cholinergic effect on sensory integration functions for the first factor could also be found in the control subjects. The results showed a non-significant overall regression model [$R^2_{adj} = -0.110$,

Table 2 Mean scores ± standard deviation for the cognitive capacity rating scales, the MDS-UPDRS subscores, and PET ligands for the subjects with Parkinson's disease

Cognition	
Montreal Cognitive Assessment:	25.9 ± 2.6
Dementia Rating Scale:	139.5 ± 4.3
MDS-UPDRS	
Non-Motor Aspects of Experiences of Daily Living:	6.5 ± 4.6
Motor Aspects of Experiences of Daily Living:	7.9 ± 5.5
Motor Examination:	32.0 ± 13.7
^{11}C-DBTZ and ^{11}C-PMP PET	
Putamen ^{11}C-DBTZ DVR:	1.80 ± 0.28 (46.2 ± 8.4%; U = 3.0, $P < 0.001$)
Caudate ^{11}C-DBTZ DVR:	2.11 ± 0.30 (19.2 ± 11.6%; U = 299.0, $P < 0.001$)
Thalamus ^{11}C-PMP k_3 (/min):	0.0545 ± 0.0055 (10.1 ± 9.0%; U = 726.5, $P < 0.001$)
Cortex ^{11}C-PMP k_3 (/min):	0.0237 ± 0.0029 (11.4 ± 10.7%; U = 735.0, $P < 0.001$)

For the PET ligands, the percentage reduction relative to control subjects is indicated in parenthesis, followed by the Mann-Whitney U test to test for statistical difference from the control subjects.

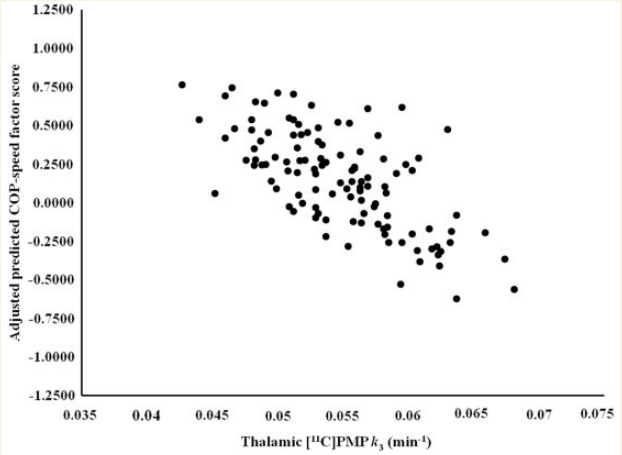

Figure 1 Scatterplot of thalamic ^{11}C-PMP activity versus adjusted predicted centre of pressure-speed factor scores in subjects with Parkinson's disease. COP = centre of pressure.

$F(6,18) = 0.6$, $P = 0.724$] and no independent effects of striatal dopaminergic binding or thalamic and cortical cholinergic activity on centre of pressure factor scores of the first factor while taking into account age, gender, and distal vibration sensitivity.

Overall, there were 21 subjects with Parkinson's disease who had to take a corrective step ('sensory organization test fall') to retain balance during one or more of the sensory organization test Conditions 2–6. There were nine subjects with Parkinson's disease who were not able to fully complete all six sensory organization test conditions, of which one subject could only complete Condition 1. Most 'sensory organization test falls' occurred during Conditions 5 and 6 (74.4%). The proportion of 'sensory organization test 'fallers' versus 'non-fallers' was not significantly different between subjects with Parkinson's disease and control subjects ($\chi^2 = 0.14$, $P = 0.713$). Significant exploratory *post hoc* linear regression analysis [$R^2_{adj} = 0.076$, $F(5,118) = 3.0$, $P = 0.013$] revealed that increased sensory organization test falls of subjects with Parkinson's disease was associated with increased MDS-UPDRS motor examination total score ($\beta = 0.242$, $t = 2.5$, $P = 0.013$). There was no independent effect of Dementia Rating Scale total score on sensory organization test falls ($\beta = 0.147$, $t = 1.6$, $P = 0.118$), neither were there any independent striatal dopaminergic ($\beta = -0.144$, $t = -1.5$, $P = 0.144$), thalamic ($\beta = -0.006$, $t = -0.05$, $P = 0.961$) or cortical ($\beta = -0.097$, $t = -0.8$, $P = 0.405$) cholinergic effects.

Discussion

The results of this study indicate a role for the pedunculopontine-thalamic cholinergic projections in postural sensory integration functions in Parkinson's disease. Increased postural sway, especially for sensory organization test Conditions 1–3, was associated with lower thalamic acetylcholinesterase activity. Increased postural sway is typically a risk factor for falling in patients with Parkinson's disease (Matinolli *et al.*, 2007; Latt *et al.*, 2009; Kerr *et al.*, 2010; Menant *et al.*, 2011). There was no observed association of neocortical cholinergic innervation changes with postural sensory integration functions. These results suggest that sensory integration functions that especially rely on somatosensory information (Condition 2) or the ability to integrate inaccurate visual information (Condition 3) are affected by thalamic cholinergic denervation. This was a Parkinson's disease-specific effect as there were no observed cholinergic associations with sensory integration functions in control subjects.

The distinct pathophysiology of progressive supranuclear palsy provides further evidence of pedunculopontine cholinergic involvement in static postural control. Progressive supranuclear palsy is an atypical parkinsonian syndrome with severe balance impairments and a higher incidence of falls compared with Parkinson's disease, already prevalent at the early stages of disease (Wenning *et al.*, 1999). Cholinergic *in vivo* PET imaging shows greater loss of thalamic cholinergic activity in patients with progressive supranuclear palsy compared with Parkinson's disease, reflecting significant loss of brainstem cholinergic pedunculopontine neurons (Hirsch *et al.*, 1987; Jellinger, 1988). Cortical cholinergic innervation is relatively spared in progressive supranuclear palsy (Shinotoh *et al.*, 1999; Gilman *et al.*, 2010). This natural model of relatively isolated pedunculopontine complex cholinergic degeneration provides further evidence for the putative role of the cholinergic pedunculopontine-thalamus projection in postural control.

Cortical cholinergic innervation is an important component of postural control functions, and is likely impaired in Parkinson's disease. However, falling in Parkinson's disease mostly occurs during more dynamic situations such as at gait initiation, walking (trips and slips), stops and turns, and while reaching, but rarely while standing quietly (Ashburn *et al.*, 2008). The sensory organization test protocol, however, assesses mostly static balance. The processing demands on the postural control system of dynamic postural tasks are more complex, attention demanding, and probably require 'higher order' cognitive feedback processes that depend on cortical cholinergic system function. Impaired balance control during more dynamic balance activities may reflect a separate impairment of 'higher order' cortical dysfunction; one that might be distinct from 'lower level' pedunculopontine postural sensory integration functions. We found no independent associations with Dementia Rating Scale score with postural sensory integration functions. Patients with Parkinson's disease with degeneration of both basal forebrain and pedunculopontine cholinergic projection systems may be particularly prone to postural control abnormalities and falls because of multiple compromised systems. By the same token, other cortical pathologies such as neocortical amyloid-β may also affect these higher order processes and further increase postural control impairments through distinct pathogenic mechanisms (Muller *et al.*, 2013).

Vestibulo-ocular dysfunction may also play a role in postural sensory integration functions of patients with Parkinson's disease. There were nine subjects in our study who were unable to complete the full sensory organization test protocol, mainly because of the inability to complete Conditions 5 and 6. Most of the corrective steps ('falls') were taken during these two conditions. Conditions 5 and 6 rely mostly on sensory inputs from the vestibular system. Vestibular dysfunction is likely more common in patients with Parkinson's disease than in normal control subjects

(Reichert *et al.*, 1982). We did not, however, directly test for vestibulo-ocular function using nystagmography or caloric tests. Nonetheless, the relative difficulties that subjects with Parkinson's disease experienced with these conditions may indicate that vestibulo-ocular dysfunction may play a role in postural control in Parkinson's disease. Further research is, however, needed to elucidate the relationship between the cholinergic and dopaminergic systems and vestibular function in Parkinson's disease.

The cerebellum likely plays an important role in sensorimotor integration of postural control. For example, a functional MRI study of imagined stance by Jahn *et al*. (2004) showed significant activation in the cerebellar vermis. Cerebellar vermis lesions typically cause severe postural deficits (Grimaldi and Manto, 2012). A primate study showed a large projection from the deep cerebellar nuclei to the pedunculopontine nucleus, suggesting that a cerebellotegmental projection may exist, whereby the cerebellum may influence the thalamus through a relay in the pedunculopontine nucleus (Hazrati and Parent, 1992). These findings are corroborated by recent diffusion tensor MRI connectivity studies, which showed connectivity of the pedunculopontine nucleus with the mid-cerebellum (Aravamuthan *et al.*, 2007, 2008). A particular limitation of the ^{11}C-PMP cholinergic radiotracer used in this study is that it cannot reliably estimate high acetylcholinesterase activity levels in the cerebellum without using invasive analysis techniques. Our recent human PET study of the vesicular acetylcholine transporter with the ^{18}F-FEOBV radiotracer showed high activity of the superior cerebellar vermis (Petrou *et al.*, 2012). Further studies are needed to delineate the independent and shared contributions of the pedunculopontine nucleus and cerebellum to postural sensory integration functions in Parkinson's disease.

Our study shows that in the presence of severe nigrostriatal dopaminergic denervation, decreased thalamic cholinergic innervation is associated with increased postural sway. This implies a role for the pedunculopontine-thalamic complex in postural sensory integration functions in Parkinson's disease. Caution should be exercised however, as the cross-sectional nature of this study does not allow for causal assessment. Nonetheless, this finding opens up a window for treatment of balance problems in Parkinson's disease. The current class of cholinergic augmentation medications, i.e. acetylcholinesterase inhibitors, have limited brain uptake (Shinotoh *et al.*, 2001; Bohnen *et al.*, 2005) and variable clinical impact (Rolinski *et al.*, 2012). An alternative therapeutic target of the cholinergic system would be the $\alpha 4\beta 2$ nicotinic acetylcholine receptor. These nicotinic receptors are strongly expressed, especially in the thalamic terminal fields of the pedunculopontine nucleus cholinergic projections (Gallezot *et al.*, 2005). Recent studies with varenicline, an $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist, have shown improvement of gait in patients with ataxic syndromes (Zesiewicz and Sullivan, 2008; Zesiewicz *et al.*, 2009, 2012).

We previously reported heterogeneity of cholinergic denervation in patients with Parkinson's disease in the absence of dementia (Bohnen *et al.*, 2012). The results of this study suggest that cholinergic treatment of patients with Parkinson's disease, especially of those with more severe cholinergic denervation, may deserve further clinical trials to investigate whether such drugs may ameliorate disabling postural instability features through improved postural sensory integration functions.

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