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Olfactory dysfunction, central cholinergic integrity and cognitive impairment in Parkinson's disease

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Olfactory dysfunction is common in subjects with Parkinson's disease. The pathophysiology of such dysfunction, however, remains poorly understood. Neurodegeneration within central regions involved in odour perception may contribute to olfactory dysfunction in Parkinson's disease. Central cholinergic deficits occur in Parkinson's disease and cholinergic neurons innervate regions, such as the limbic archicortex, involved in odour perception. We investigated the relationship between performance on an odour identification task and forebrain cholinergic denervation in Parkinson's disease subjects without dementia. Fifty-eight patients with Parkinson's disease (mean Hoehn and Yahr stage 2.5 ± 0.5) without dementia (mean Mini-Mental State Examination, 29.0 ± 1.4) underwent a clinical assessment, [^{11}C]methyl-4-piperidinyl propionate acetylcholinesterase brain positron emission tomography and olfactory testing with the University of Pennsylvania Smell Identification Test. The diagnosis of Parkinson's disease was confirmed by [^{11}C]dihydrotetrabenazine vesicular monoamine transporter type 2 positron emission tomography. We found that odour identification test scores correlated positively with acetylcholinesterase activity in the hippocampal formation ($r = 0.56$, $P < 0.0001$), amygdala ($r = 0.50$, $P < 0.0001$) and neocortex ($r = 0.46$, $P = 0.0003$). Striatal monoaminergic activity correlated positively with odour identification scores ($r = 0.30$, $P < 0.05$). Multiple regression analysis including limbic (hippocampal and amygdala) and neocortical acetylcholinesterase activity as well as striatal monoaminergic activity, using odour identification scores as the dependent variable, demonstrated a significant regressor effect for limbic acetylcholinesterase activity ($F = 10.1$, $P < 0.0001$), borderline for striatal monoaminergic activity ($F = 1.6$, $P = 0.13$), but not significant for cortical acetylcholinesterase activity ($F = 0.3$, $P = 0.75$). Odour identification scores correlated positively with scores on cognitive measures of episodic verbal learning ($r = 0.30$, $P < 0.05$). These findings indicate that cholinergic denervation of the limbic archicortex is a more robust determinant of hyposmia than nigrostriatal dopaminergic denervation in subjects with moderately severe Parkinson's disease. Greater deficits in odour identification may identify patients with Parkinson's disease at risk for clinically significant cognitive impairment.

Keywords: acetylcholinesterase; cognitive impairment; Parkinson's disease; positron emission tomography; smell

Abbreviations: AChE = acetylcholinesterase; UPSIT = University of Pennsylvania Smell Identification Test; VMAT2 = vesicular monoamine transporter type 2

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Introduction

The pathophysiology of hyposmia in Parkinson's disease is poorly understood. Neuronal degeneration with deposition of α-synuclein within the olfactory bulb and anterior olfactory nucleus occurs early in Parkinson's disease (Braak *et al.*, 2002; Hawkes *et al.*, 2009). There is evidence also of α-synuclein pathology within the limbic rhinencephalon (Silveira-Moriyama *et al.*, 2009). Deficits in olfactory function in Parkinson's disease are described in odour identification, odour discrimination, threshold detection and odour recognition memory (Mesholam *et al.*, 1998). While there is some evidence that odour identification and discrimination may be impaired independently of olfactory detection threshold sensitivity in Parkinson's disease (e.g. Boesveldt *et al.*, 2009), effect sizes are large in Parkinson's disease for all three of these nominally distinct domains (Mesholam *et al.*, 1998), and tests of odour identification, detection and discrimination typically share considerable variance (Doty *et al.*, 1994). Odour discrimination tasks may preferentially recruit the hippocampus, possibly reflecting its role in the working memory element of such tasks (Kareken *et al.*, 2003). We reported previously that impaired odour identification in early Parkinson's disease is correlated with hippocampal more than amygdala or striatal dopaminergic denervation (Bohnen *et al.*, 2008). These findings suggest that hippocampal dopaminergic denervation and/or dysfunction may contribute to olfactory dysfunction in early Parkinson's disease. Olfactory impairments, however, are not affected by dopaminergic medications, and correlate poorly, if at all, with disease stage or duration of motor features (Doty *et al.*, 1988; Tissingh *et al.*, 2001; Herting *et al.*, 2008), suggesting a 'floor' phenomenon in hyposmia with respect to dopaminergic degeneration in advancing Parkinson's disease. Diminished olfactory performance in non-demented subjects with Parkinson's disease, however, may indicate increased risk of visual hallucinations, implying higher risk for developing dementia 2–6 years later, features that cannot be attributed easily to dopaminergic neuron degeneration (Stephenson *et al.*, 2008).

Hyposmia occurs also in Alzheimer's disease (Doty *et al.*, 1987) and increases with severity of dementia (Murphy *et al.*, 1990; Serby *et al.*, 1991; Wilson *et al.*, 2009). Higher density of entorhinal cortex and hippocampal neurofibrillary tangles correlate with greater deficits of odour identification, suggesting a role for hippocampal dysfunction in Alzheimer's disease hyposmia (Wilson *et al.*, 2007). Loss of basal forebrain cholinergic neurons is an important feature of Alzheimer's disease (Yan and Feng, 2004), with early involvement of septohippocampal projections (Lehericy *et al.*, 1993). Cholinergic system degeneration is also an early feature of Parkinson's disease and worsens with the appearance of dementia (Ruberg *et al.*, 1986; Shimada *et al.*, 2009). It is possible that limbic cholinergic denervation may also be a contributory factor to Parkinson's disease hyposmia. If so, hyposmia would be most marked in those subjects with evidence of cognitive dysfunction. The goal of this study was to test the hypothesis that deficits in odour identification would correlate with central cholinergic denervation, especially of the hippocampus, and that odour identification scores correlate with cognitive performance in Parkinson's disease.

Materials and methods

Subjects and clinical test battery

This cross-sectional study involved 58 subjects with Parkinson's disease (49 males and 9 females), mean age 69.0 ± 7.6 (range 51–83) years and 26 control non-Parkinson's disease subjects (17 males and 9 females), mean age 67.2 ± 10.5 (range 50–84) years. Patients with Parkinson's disease met the UK Parkinson's Disease Society Brain Bank Research Centre clinical diagnostic criteria (Hughes *et al.*, 1992). The diagnosis of Parkinson's disease was also confirmed by the presence of nigrostriatal dopaminergic denervation on (+)-[^{11}C]dihydrotetrabenazine vesicular monoamine type 2 (VMAT2) positron emission tomography (PET). Dihydrotetrabenazine binding is a good marker of nigrostriatal dopaminergic denervation, as over 95% of the striatal signal is attributable to dopaminergic vesicular binding and shows excellent correlation with tyrosine hydroxylase-positive neuron density in the substantia nigra pars compacta (Vander Borght *et al.*, 1995). Thirty-one Parkinson's disease subjects were taking a combination of dopamine agonist and carbidopa-levodopa medications, 18 were using carbidopa-levodopa alone, 7 were taking dopamine agonists alone and 2 were not on dopaminergic drugs. No subjects were on anti-cholinergic or cholinesterase inhibitor drugs. Most subjects had moderate severity of disease: 1 patient in Stage 1, 2 in Stage 1.5, 11 in Stage 2, 24 in Stage 2.5, 19 in Stage 3 and 1 in Stage 4 of the modified Hoehn and Yahr classification (Hoehn and Yahr, 1967). Mean duration of disease was 7.0 ± 3.8 (standard deviation) years (range 0.5–17). Subjects with a Mini-Mental State Examination score of 24 or less were not eligible for the study (Folstein *et al.*, 1975).

The Unified Parkinson's Disease Rating Scale was performed (Fahn and Elton, 1987). Subjects on dopaminergic drugs were examined and imaged in the morning after withholding dopaminergic drugs overnight. Patient and control subjects underwent olfactory testing using the University of Pennsylvania Smell Identification Test (UPSIT, Sensonics, Inc. Haddon Heights, NJ, USA; (Doty *et al.*, 1984), administered by a trained examiner. The UPSIT incorporates microencapsulation technology and consists of encapsulated odours, one per page (Doty *et al.*, 1984). There are 40 different odours and a forced choice is made from four possible answers. Each subject underwent a neuropsychological examination. Neuropsychological tests evaluating different cognitive domains were used for analysis following an approach as previously reported for cognitive impairment in Parkinson's disease (Aarsland *et al.*, 2009). These tests included: measures of verbal and non-verbal memory—California Verbal Learning Test (Delis *et al.*, 2000) and Benton Visual Retention Test (Benton, 1974); and executive/reasoning functions—Wechsler Adult Intelligence Scale-III Picture Arrangement test (Wechsler, 1997) and Stroop Colour Word Interference test (Stroop, 1935), together with a switching version of the Stroop 3 test in which subjects name the ink, unless the word is surrounded by a box, in which case, they read the word itself (Stroop 4). This measure makes an additional demand on cognitive flexibility (Bohnen *et al.*, 1992). Stroop Colour Word Interference Test scores were calculated as the time difference for completion of the interference measures minus the non-interference tasks; attention/psychomotor speed as absolute times on the Stroop 1 and 2 subtests (Stroop, 1935). Visuospatial function was also measured, with the Benton Judgment of Line Orientation test (Benton *et al.*, 1975). Composite *z*-scores were calculated for these different cognitive domains (memory, executive, attention and visuospatial functions) based on normative data from the control subjects.

The study was approved by the Institutional Review Boards of the University of Michigan and Ann Arbor Veterans Affairs Medical Centre for studies involving human subjects. Written informed consent was obtained from all subjects.

Imaging techniques

All subjects underwent brain magnetic resonance imaging and acetylcholinesterase (AChE) and VMAT2 PET. Magnetic resonance imaging was performed on a 3 Tesla Philips Achieva system (Philips, Best, The Netherlands) utilizing an eight-channel head coil and the 'ISOVOX' exam card protocol primarily designed to yield isotropic spatial resolution. 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. One hundred and sixty slices were reconstructed to 1 mm isotropic resolution. This sequence maximizes contrast among grey matter, white matter and cerebrospinal fluid and provides high-resolution delineation of cortical and subcortical structures.

AChE and VMAT2 PET imaging were performed in 3D imaging mode using an ECAT HR+ tomograph (Siemens Molecular Imaging, Inc., Knoxville, TN), 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, Montreal, Canada) head-holder/shielding unit was attached to the patient bed to reduce the contribution of detected photon events originating from the body outside the scanner field of view (Thompson *et al.*, 2001). Prior to the dihydrotetrabenazine and methyl-4-piperidinyl propionate injections, a 5 min transmission scan was acquired using rotating ^{68}Ge rods for attenuation correction of emission data using the standard vendor-supplied segmentation and re-projection routines.

[^{11}C]methyl-4-piperidinyl propionate was prepared in high radiochemical purity (>95%) by N-[^{11}C]methylation of piperidin-4-yl propionate using a previously described method (Snyder *et al.*, 1998). Dynamic PET scanning was performed for 70 min immediately following a bolus intravenous injection of 666 mega-Becquerel (18 milliCuries) of [^{11}C]methyl-4-piperidinyl propionate. The dose contained less than 200 μg cold methyl-4-piperidinyl propionate mass. Emission data were collected in 16 sequential emission scans: four × 30 s; three × 1 min; two × 2.5 min; two × 5 min; and five × 10 min.

No-carrier-added (+)-[^{11}C]dihydrotetrabenazine (250–1000 Ci/mmol at the time of injection) was prepared as reported previously (Jewett *et al.*, 1997). Dynamic PET scanning was performed for 60 min immediately following a bolus injection of 55% of 666 mega-Becquerel (18 milliCuries) of (+)-[^{11}C]dihydrotetrabenazine dose (containing less than 50 μg of cold dihydrotetrabenazine mass) over the first 15–30 s of the study, while the remaining 45% of the dose was continuously infused over the next 60 min, resulting in stable arterial tracer levels and equilibrium with brain tracer levels after 30 min (Koeppe *et al.*, 1997). A series of 15 frame sequence of scans over 60 min were obtained as following: four × 30 s; three × 1 min; two × 2.5 min; two × 5 min; and four × 10 min. All subjects were studied supine, with eyes and ears unoccluded, resting quietly in a dimly lit room.

Analysis

All image 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.*, 1993). IDL image analysis software (Research systems, Inc., Boulder, CO) was used to trace volumes of interest manually on the magnetic resonance imaging scan, representing the hippocampus, amygdala and the striatum. Total cortical volumes of interest were defined using semi-automated thresholding delineation of cortical grey matter signal on the magnetic resonance imaging scan.

AChE hydrolysis rates (k_3) were estimated using the striatal volume of interest (defined by manual tracing on the magnetic resonance imaging scan of the putamen and caudate nucleus) as the reference input tissue (Nagatsuka *et al.*, 2001).

Dihydrotetrabenazine images were analysed using equilibrium modelling to estimate the non-displaceable binding potential (BP_{ND}), which is equivalent to the ratio of specific (V_S) to-non-displaceable (V_{ND}) binding in each imaged voxel or target volume of interest (Koeppe *et al.*, 1997). We estimated specific dihydrotetrabenazine binding by subtraction of the global neocortex value, a reference region very low in VMAT2 binding sites, with the assumption that the non-displaceable distribution is uniform across the brain at equilibrium (Koeppe *et al.*, 1999).

Standard pooled-variance t or Satterthwaite's method of approximate t-tests (t_{approx}) were used for group comparisons (SAS version 9.1, SAS institute, Cary, North Carolina). Pearson correlation coefficients were calculated for correlation between clinical or PET variables.

Results

Olfactory and cognitive findings

There were no significant differences in age or education between the groups (Table 1). Patients with Parkinson's disease had significantly reduced UPSIT scores compared to controls (Table 1). Parkinson's disease subjects had significantly worse performance on most cognitive measures compared to the control subjects (Table 1).

Acetylcholine PET imaging findings in Parkinson's disease and control subjects

Analysis of the AChE PET data demonstrated reduced mean neocortical, hippocampal and amygdala AChE activity in the Parkinson's disease group (Table 2). There were no significant left-right hemispheric differences for AChE activity in the patients with Parkinson's disease.

Relationship between olfaction, *in vivo* imaging and cognitive findings

UPSIT scores correlated positively with AChE activity in the hippocampus ($r = 0.56$, $P < 0.0001$; Fig. 1), amygdala ($r = 0.50$, $P < 0.0001$) and neocortex ($r = 0.46$, $P = 0.0003$). Thus, individuals with higher UPSIT scores had higher AChE activity, and those with lower UPSIT scores had lower AChE activity, in these brain regions. Striatal VMAT2 activity correlated positively with UPSIT scores ($r = 0.30$, $P = 0.022$).

Multiple regression analysis including limbic (hippocampal and amygdala) and neocortical AChE activity and striatal VMAT2 activity using UPSIT scores as the dependent variable demonstrated

Table 1 Demographic, olfactory and cognitive measures in the Parkinson's disease and control subjects

	Parkinson's disease ($n=58$)	Controls ($n=26$)	Statistical significance
Age (year)	69.0 ± 7.6	67.2 ± 10.5	$t=0.8$; $P=0.44$
Education (year)	15.0 ± 3.1	16.0 ± 2.8	$t=1.4$; $P=0.16$
Mini-Mental State Examination	29.0 ± 1.4	29.8 ± 0.5	$t_{approx}=4.0$; $P=0.0001$
University of Pennsylvania Smell Identification Test	16.7 ± 9.0	30.3 ± 8.3	$t=6.6$; $P<0.0001$
California Verbal Learning Test – Immediate Memory	8.3 ± 2.1	10.2 ± 2.2	$t=3.8$; $P=0.0003$
California Verbal Learning Test – Short Term Memory	8.2 ± 3.0	11.2 ± 2.7	$t=4.4$; $P<0.0001$
California Verbal Learning Test – Long Term Memory	9.4 ± 3.5	11.1 ± 3.0	$t=4.6$; $P<0.0001$
Benton Visual Retention Test	6.1 ± 2.0	7.7 ± 1.0	$t_{approx}=4.6$; $P<0.0001$
Stroop Colour Word Test 1 (s)	62.4 ± 17.7	51.6 ± 12.6	$t=2.8$; $P=0.007$
Stroop Colour Word Test 2 (s)	80.2 ± 16.7	66.4 ± 19.1	$t=3.4$; $P=0.001$
Stroop Colour Word Test 3 (s)	155.5 ± 47.1	121.1 ± 41.1	$t=3.2$; $P=0.001$
Stroop Colour Word Test 4 (s)	173.9 ± 58.8	141.2 ± 43.2	$t=2.5$; $P=0.013$
Picture Arrangement Test	11.5 ± 5.0	13.0 ± 3.5	$t_{approx}=1.5$; $P=0.13$
Judgment of Line Orientation Test	23.8 ± 4.2	24.5 ± 3.8	$t=0.6$; $P=0.52$

Data are mean (±SD).

Table 2 Limbic and neocortical AChE hydrolysis rates (k_3; $\min^{-1}$) and striatal VMAT2 binding potential (BP_{ND}) in the Parkinson's disease and control subjects

	Parkinson's disease ($n=58$)	Controls ($n=26$)	Statistical significance
Neocortical AChE k_3	0.0272 ± 0.0026	0.0303 ± 0.0035	$t=4.6$; $P<0.0001$
Hippocampal AChE k_3	0.0426 ± 0.0058	0.0472 ± 0.0091	$t_{approx}=2.3$; $P=0.025$
Amygdala AChE k_3	0.0596 ± 0.0096	0.0681 ± 0.0156	$t_{approx}=2.5$; $P=0.015$
Striatal VMAT2 BP_{ND}	0.96 ± 0.32	1.98 ± 0.32	$t=13.4$; $P<0.0001$

Data are mean (±SD).

an overall significant regression model ($F=10.0$, $P<0.0001$) with significant regressor effect for limbic AChE activity ($F=10.1$, $P<0.0001$), borderline for striatal VMAT2 activity ($F=1.6$, $P=0.13$), but no longer significant for cortical AChE activity ($F=0.3$, $P=0.75$).

Multiple regression analysis was performed to control for potential confounders (age, disease duration and Unified Parkinson's disease Rating Scale motor severity) to evaluate the main regression effect between UPSIT scores and limbic AChE activity. The overall regression model was significant ($F=8.1$, $P<0.0001$) with a significant regressor effect for limbic AChE activity ($F=19.2$, $P<0.0001$) independent of significant effects for motor severity ($F=5.0$, $P=0.03$). Effects for age ($F=0.2$, $P=0.70$) or duration of motor disease ($F=0.7$, $P=0.40$) were not significant in the model.

Higher UPSIT scores were associated with better scores on cognitive measures of memory (composite memory z-score, $r=0.26$, $P<0.05$). Comparison between verbal versus non-verbal learning demonstrates a significant effect for episodic verbal learning ($r=0.30$, $P=0.023$) but not for visual non-verbal memory ($r=0.18$, $P=0.17$). There was a borderline positive correlation between the UPSIT and the Mini-Mental State Examination scores ($r=0.25$, $P=0.055$). There were no significant correlations between the UPSIT and visuospatial function ($r=0.001$, $P=0.99$), attention ($r=-0.05$, $P=0.80$) or executive ($r=0.1$, $P=0.46$) function composite z-scores.

Limbic AChE activity correlated positively with executive cognitive ($r=0.36$, $P=0.006$), memory ($r=0.29$, $P=0.03$), borderline with attention ($r=-0.26$, $P=0.054$) but not with visuospatial function ($r=-0.04$, $P=0.76$) composite z-scores.

Post hoc analysis

A *post hoc* analysis was performed to evaluate a possible nigrostriatial dopaminergic denervation statistical 'floor' effect with more advanced disease relative to its correlation with the olfactory measures. Analysis limited to Parkinson's disease subjects stage Hoehn and Yahr 2 or below ($n=14$) demonstrated significant correlation between striatal VMAT2 activity and UPSIT ($r=0.61$, $P=0.02$). In contrast, analysis limited to Parkinson's disease subjects Hoehn and Yahr stage 2.5 and higher demonstrated a borderline trend between striatal VMAT2 activity and UPSIT scores ($r=0.25$, $P=0.10$). Significant correlations between limbic and neocortical AChE activity and UPSIT scores were present in both Parkinson's disease severity subgroups. Analysis limited to Parkinson's disease subjects in stage Hoehn and Yahr 2 or below demonstrated positive correlations between UPSIT scores and limbic ($r=0.55$, $P=0.04$) and cortical ($r=0.60$, $P=0.024$) AChE hydrolysis rates. Analysis limited to Parkinson's disease subjects Hoehn and Yahr stage 2.5 and higher demonstrated positive correlations between UPSIT scores and limbic ($r=0.60$, $P<0.0001$) and cortical AChE hydrolysis rates ($r=0.42$, $P=0.0045$).

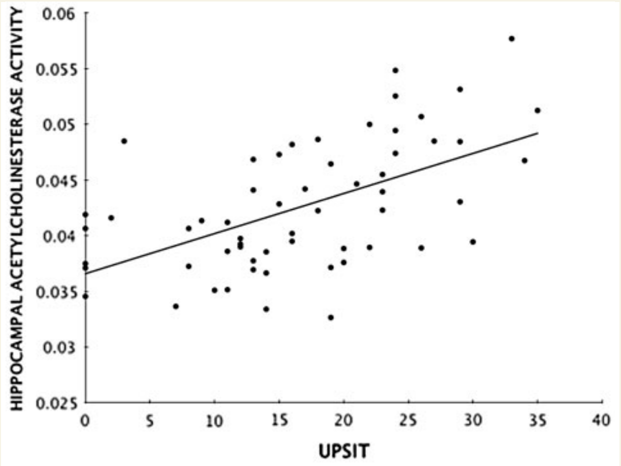

Figure 1 Scatter plot of the relationship between hippocampal AChE k_3 hydrolysis (min^{-1}) rates and UPSIT score in the Parkinson's disease subjects ($r = 0.56$, $P < 0.0001$).

Discussion

We found a positive association between odour identification performance and a measure of forebrain cholinergic pathway integrity in Parkinson's disease. Diminished cholinergic innervation of the limbic archicortex, in particular, was associated with olfactory dysfunction. This finding suggests that impaired cholinergic function may contribute to the pathophysiology of Parkinson's disease olfactory dysfunction.

Olfactory deficits in Parkinson's disease are described in odour identification, odour discrimination, odour threshold detection and odour recognition memory (Mesholam *et al.*, 1998; Haehner *et al.*, 2009), and these tests share considerable variance (Doty *et al.*, 1994). Proper interpretation of olfactory dysfunction in these different domains requires a distinction between the cognitive processes involved in an olfactory task and the physiological olfactory element of the task. For example, odour discrimination tasks may preferentially recruit the hippocampus, possibly reflecting its role in the working memory element of such tasks (Kareken *et al.*, 2003). Odour identification requires recognizing or naming the odour, a long-term memory function, whereas forced-choice threshold tests recruit short-term memory processes. These operational processes in olfactory tests will in part depend on the integrity of structures involved in higher order cognitive or memory processing, such as the limbic cortex (Larsson *et al.*, 2004; Wang *et al.*, 2005). For example, regional grey matter atrophy of the paralimbic cortex has been found to correlate with the presence of olfactory dysfunction in early Parkinson's disease whereas atrophy of the limbic cortex correlated with olfactory deficits in patients with moderately advanced Parkinson's disease (Wattendorf *et al.*, 2009). We found a robust positive association between limbic AChE activity and performance on an odour identification task in a population of non-demented subjects with Parkinson's disease (mean Mini-Mental State Examination score of 29.0). Furthermore, the association between limbic AChE activity and memory performance was only modest, implying that our findings cannot be explained on the basis of memory deficits alone.

Hippocampal and entorhinal pathology is an early feature of Alzheimer's disease and odour identification deficits occur early in the disease course. Alzheimer disease olfactory defects reflect deficits of odour cognitive processing rather than odour detection, which is not altered until later in Alzheimer's disease (Serby *et al.*, 1991). We found that the UPSIT had more selective association with performance on episodic verbal learning. This is likely to reflect the specific verbal cognitive functions involved in odour identification. Episodic memory impairment is a hallmark of Alzheimer's disease, and loss of basal forebrain cholinergic neurons that innervate the hippocampus and neocortex is an early and key feature of Alzheimer's disease (Yan and Feng, 2004). Post-mortem studies describe marked regional variations in the extent of cholinergic losses with the temporal lobe displaying the most significant losses of cholinergic fibers (Geula and Mesulam, 1996). These findings provide evidence for selective loss of the cholinergic septohippocampal pathway and subregions within the nucleus basalis of Meynert, namely the posterior portion that innervate the temporal lobe, including the amygdala and the hippocampal formation (Emre *et al.*, 1993; Lehericy *et al.*, 1993; Geula and Mesulam, 1996). Our findings of more robust correlation between deficits in odour identification and limbic than neocortical AChE activity are concordant with these observations in Alzheimer's disease.

Involvement of the limbic cholinergic system in olfactory processing is also supported by animal studies. For example, disruption of normal cholinergic function alters odour memory, including both habituation to a familiar odour and odour-based social recognition (Hunter and Murray, 1989; Paolini and McKenzie, 1993, 1996; Berger-Sweeney *et al.*, 2000), short-term memory for odours (Ravel *et al.*, 1992, 1993, 1994) and acquisition of a complex odour discrimination task (De Rosa *et al.*, 2001; Mandairon *et al.*, 2006). Systemic physostigmine enhances discrimination between extremely similar odours in rats (Doty *et al.*, 1999). Conversely, systemic scopolamine impairs olfactory perceptual learning (Fletcher and Wilson, 2002). Local injection of scopolamine in the hippocampal formation and prelimbic cortices impairs memory for socially transmitted food preference in rats (Carballo-Marquez *et al.*, 2009). Selective lesioning of basal forebrain cholinergic neurons is associated with anterograde and retrograde deficits in a social transmission of food preference task in rats (Vale-Martinez *et al.*, 2002), and lesions of the horizontal limb of the diagonal band of Broca impair olfactory memory (Linster and Hasselmo, 2000). There is a single human study showing that the AChE inhibitor drug donepezil improves odour identification in patients with Alzheimer's disease, providing preliminary clinical support for a cholinergic component of olfactory processing in neurodegeneration (Velayudhan and Lovestone, 2009).

There is increasing evidence that cholinergic denervation occurs early in Parkinson's disease and progressive cholinergic denervation is associated with the presence of dementia in this disorder (Shimada *et al.*, 2009). Lewy first identified the eponymous Lewy body in neurons of the nucleus basalis of Meynert (Lewy, 1913), the source of cholinergic innervation of the cerebral cortex. In the Braak *et al*. (2003) staging scheme of Parkinson's disease pathology, nigral and magnocellular basal forebrain pathology occur simultaneously. A recent AChE PET study found evidence of significant cholinergic denervation in early drug naïve Parkinson's disease subjects without cognitive symptoms (Shimada *et al.*, 2009). We found evidence of cholinergic denervation that correlated with cognitive performance on memory and executive tasks in patients with Parkinson's disease without dementia. *In vivo* imaging studies have also shown that the presence of dementia in Parkinson's disease is associated with more severe and widespread cholinergic denervation compared to Parkinson's disease without dementia (Kuhl *et al.*, 1996; Shinotoh *et al.*, 1999; Bohnen *et al.*, 2003; Hilker *et al.*, 2005). These imaging results are consistent with post-mortem evidence that basal forebrain cholinergic system degeneration appears early in Parkinson's disease and worsens with the appearance of dementia (Ruberg *et al.*, 1986). A post-mortem study found greater reductions of AChE in the frontal cortex of demented (−68%) compared with non-demented (−35%) patients with Parkinson's disease (Ruberg *et al.*, 1986). Furthermore, cognitive impairment has been found to correlate with cortical choline acetyltransferase levels, but not with the extent of plaque or tangle formation in Parkinson's disease (Perry *et al.*, 1985; Mattila *et al.*, 2001). Similarly, Mattila *et al*. (2001) found that cognitive decline in Parkinson's disease was associated with lower cortical choline acetyltransferase levels, even in the absence of Alzheimer's disease pathology. Therefore, impairment or degeneration of the cholinergic system may play a significant role in the cognitive decline in Parkinson's disease (Perry *et al.*, 1985).

We previously reported significant correlations between UPSIT scores and hippocampal, amygdala and striatal dopaminergic activity using a highly specific dopamine transporter PET radioligand, [^{11}C](−)-2-β-Carbomethoxy-3-β-(4-fluorophenyl)tropane, in (very) early Parkinson's disease, including drug-naïve subjects (Bohnen *et al.*, 2007, 2008). A limitation of the present study is that the VMAT2 ligand cannot be used for specific assessment of extra-striatal dopaminergic innervation as this ligand fails to discriminate between dopaminergic and serotonergic or adrenergic innervation of the hippocampus and amygdala. Furthermore, the Parkinson's disease subjects enrolled in the present study had lower smell performance and approximately twice the duration of disease and clinical severity of motor impairment than patients with Parkinson's disease from our previous study. As there may be a 'floor' effect underlying the relationship between dopaminergic denervation and odour identification, we performed a subgroup analysis stratified for severity of disease and found significant correlations between striatal VMAT2 activity and UPSIT scores in the subjects with mild disease but not in those with more advancing disease. These data provide supportive evidence for the presence of a 'floor' effect with respect to hyposmia and nigrostriatal dopaminergic denervation in advancing Parkinson's disease. In addition, multiple regression analysis demonstrated significant regression for odour identification scores and limbic AChE activity while regression with striatal VMAT2 activity was only borderline significant in the present study. We did not find evidence of a cholinergic denervation 'floor' effect relative to odour identification in the present study. Thus, cholinergic denervation may be a more important determinant of odour identification deficits than nigrostriatal dopaminergic denervation in patients with advancing Parkinson's disease.

Parkinson's disease is now being recognized as a multi-system neurodegeneration syndrome (Langston, 2006). It is plausible that hyposmia in Parkinson's disease may have multiple components including olfactory bulb and primary olfactory cortex components, and limbic dysfunction secondary to dopaminergic, cholinergic and other denervations. Olfactory impairments have been shown in an animal model with reduced monoaminergic storage capacity (Taylor *et al.*, 2009). Dopaminergic effects may be more significant in hyposmia of early Parkinson's disease because dopaminergic, unlike cholinergic, denervation is uniformly and severely present in all subjects with early prototypical Parkinson's disease. In contrast, cholinergic denervation is more heterogeneous and more severe loss of smell may reflect cholinergic denervation in the subset of patients with Parkinson's disease and incipient cognitive impairment. Our findings cannot be explained by tandem multi-system neurodegeneration as the cholinergic denervation hyposmia finding was independent from the effects of age, duration of disease, severity of motor disease or degree of nigrostriatal dopaminergic denervation.

We conclude that cholinergic denervation, especially of the limbic archicortex, is a more robust determinant of odour identification deficits than nigrostriatal dopaminergic denervation in subjects with moderately severe Parkinson's disease. Future longitudinal research is needed to determine whether lower smell performance predicts development of dementia in Parkinson's disease.

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