Imaging acetylcholinesterase density in peripheral organs in Parkinson’s disease with 11C-donepezil PET

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Parkinson’s disease is associated with early parasympathetic dysfunction leading to constipation and gastroparesis. It has been suggested that pathological α-synuclein aggregations originate in the gut and ascend to the brainstem via the vagus. Our understanding of the pathogenesis and time course of parasympathetic denervation in Parkinson’s disease is limited and would benefit from a validated imaging technique to visualize the integrity of parasympathetic function. The positron emission tomography tracer 5-[11C]-methoxy-donepezil was recently validated for imaging acetylcholinesterase density in the brain and peripheral organs. Donepezil is a high-affinity ligand for acetylcholinesterase—the enzyme that catabolizes acetylcholine in cholinergic synapses. Acetylcholinesterase histology has been used for many years for visualizing cholinergic neurons. Using 5-[11C]-methoxy-donepezil positron emission tomography, we studied 12 patients with early-to-moderate Parkinson’s disease (three female; age 64 ± 9 years) and 12 age-matched control subjects (three female; age 62 ± 8 years). We collected clinical information about motor severity, constipation, gastroparesis, and other parameters. Heart rate variability measurements and gastric emptying scintigraphies were performed in all subjects to obtain objective measures of parasympathetic function. We detected significantly decreased 11C-donepezil binding in the small intestine (−35%; P = 0.003) and pancreas (−22%; P = 0.001) of the patients. No correlations were found between the 11C-donepezil signal and disease duration, severity of constipation, gastric emptying time, and heart rate variability. In Parkinson’s disease, the dorsal motor nucleus of the vagus undergoes severe degeneration and pathological α-synuclein aggregations are also seen in nerve fibres innervating the gastro-intestinal tract. In contrast, the enteric nervous system displays little or no loss of cholinergic neurons. Decreases in 11C-donepezil binding may, therefore, represent a marker of parasympathetic denervation of internal organs, but further validation studies are needed.

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Abbreviations: AChE = acetylcholinesterase; DMV = dorsal motor nucleus of the vagus; RR = distance in ms between consecutive normal R waves in QRS complexes; SUV = standardized uptake value
**Introduction**

Parkinson’s disease is the second-most common neurodegenerative disorder and has a prevalence of 1% in those over 65 years of age. It is clinically characterized by motor symptoms of tremor, slowness and rigidity, but the early involvement of the parasympathetic nervous system has become increasingly apparent. It has been suggested that the pathology of Parkinson’s disease, abnormal aggregation of α-synuclein, initially starts in the autonomic nerve endings of the gastrointestinal mucosa, perhaps triggered by an unknown toxin (Hawkes et al., 2007). The disease process then spreads in a prion-like fashion via the vagal nerve to the brainstem. This hypothesis could explain why 80% of patients have an increased colonic transit time (Jost and Schimrigk, 1991) and why 34% of patients with Parkinson’s disease are constipated more than 20 years before diagnosis (Savica et al., 2009). It also explains the observation that the dorsal vagal motor nucleus (DMV) is a primary target structure in the brainstem (Braak et al., 2003). Furthermore, de novo, untreated patients with Parkinson’s disease display impaired gastric emptying (Tanaka et al., 2011), decreased saliva production (Tumilasci et al., 2006), and decreased heart rate variability (Kallio et al., 2000)—all symptoms of a dysfunctional parasympathetic nervous system. The ability to image the sympathetic nervous system, with ¹⁸F-dopamine PET (Goldstein et al., 2002) and ¹²³I-meta-iodo-benzyl-guanidine (MIBG) SPECT (single photon emission computed tomography) has been instrumental in demonstrating cardiac sympathetic dysfunction in Parkinson’s disease (Haensch et al., 2009), even at a prodromal stage (Miyamoto et al., 2006). An equivalent imaging technique to visualize parasympathetic dysfunction is currently not available, but could significantly improve our understanding of the time course of the denervation in Parkinson’s disease. Importantly, the ability to image a parasympathetic nervous system deficit could become an important biomarker for diagnosing prodromal disease.

We have validated the novel PET tracer 5-[¹¹C]-methoxy-donepezil for the in vivo quantification of acetylcholinesterase (AChE) density in human peripheral organs (Gjerloff et al., 2014), and similar validation studies were recently carried out in rats (Watabe et al., 2014). Donepezil is a non-competitive, high affinity, reversible antagonist of AChE with low affinity for butyrylcholinesterase. ¹¹C-donepezil undergoes very little peripheral metabolism during a 60-min PET scan (Hiraoka et al., 2009; Gjerloff et al., 2014), and exhibits intense and highly specific binding to various internal organs, including the gastro-intestinal tract and pancreas (Gjerloff et al., 2014). Although AChE is not exclusively produced by neurons, histological measurements of AChE activity have been used for many years to quantify integrity of the parasympathetic and enteric nervous systems (Schmid et al., 1979), and the cardiac conduction system (Pauza et al., 2013). An early study demonstrated that sub-diaphragmatic vagotomy induced a 50% decrease of AChE activity in the upper gastrointestinal tract of guinea pigs (Schmid et al., 1979), suggesting that AChE PET imaging could provide a biomarker of vagal functional integrity.

An imaging marker of vagal function is particularly relevant to Parkinson’s disease, given that the DMV shows a ~50% neuron loss and its cholinergic preganglionic parasympathetic neurons are known to be preferentially vulnerable (Eadie, 1963; Gai et al., 1992). The DMV also has the highest number of ubiquitin-positive neurons of any brain nucleus in Parkinson’s disease, surpassing even the substantia nigra. Pathological α-synuclein aggregates (Lewy bodies and neurites) have been reported in the esophagus, stomach, pancreas and intestine (Beach et al., 2010), and accumulating evidence suggests that these pathological inclusions are present years before symptom onset (Shannon et al., 2012; Hilton et al., 2014). The vast majority (~90%) of α-synuclein pathology in the gastrointestinal tract takes the form of Lewy neurites rather than cytoplasmic Lewy bodies in enteric neurons (Wakabayashi et al., 1988; Braak et al., 2006; Greene, 2014). The α-synuclein pathology exhibits a rostral-caudal distribution, being most prevalent in the esophagus and stomach (Beach et al., 2010; Annerino et al., 2012). This distribution closely mirrors the DMV innervation of the gastro-intestinal tract (Hopkins et al., 1996). Taken together, these observations suggest that α-synuclein histopathology within vagal efferents to the gut predominates over histopathology in intrinsic enteric neurons. This viewpoint is further supported by the fact that the myenteric and submucosal plexus exhibits only a slight loss of enteric neurons in Parkinson’s disease (Lebouvier et al., 2010; Annerino et al., 2012).

In the present study, we used ¹¹C-donepezil PET to quantify AChE binding in the internal organs of patients with Parkinson’s disease and healthy control subjects. AChE is synthesized in the perikaryon of cholinergic neurons and transported to dendrites and axons (Giacobini, 2000). As a substantial fraction of efferent DMV neurons are already lost in clinical Parkinson’s disease, and given that some or even most of the remaining vagal axons are dysfunctional because of the presence of Lewy neurites, we hypothesized that AChE density would be measurably decreased in organs receiving rich vagal innervation. We performed 60-min dynamic ¹¹C-donepezil PET scans with the heart, stomach, small intestine, liver, spleen, and kidneys in the field of view. Arterial blood sampling was performed to provide an input function and radio-metabolite levels were measured to allow full PET kinetic modelling. The first aim of the study was to explore differences in the ¹¹C-donepezil PET signal between patients with Parkinson’s disease and healthy subjects. The second study aim was to interrogate correlations between organ ¹¹C-donepezil uptake in Parkinson’s disease and severity of symptoms and measures of parasympathetic function.
The severity of motor and non-motor symptoms, including hyposmia, REM sleep behaviour disorder, and constipation, was rated with standard scales. Cardiovagal dysautonomia was investigated by performing heart rate variability measurements at rest and while subjects performed deep breathing and aValsalva manoeuvre. Finally, solid meal $^{99m}$Tc-labelled colloid gastric scintigraphy was performed to assess gastric emptying time, which is known to be perturbed in patients with a parasympathetic deficit.

**Materials and methods**

The Central Denmark Region Committee on Health Research and the Danish Health and Medicines Authority approved the study protocol. The study was monitored by the GCP unit at Aarhus University Hospital and registered in the www.clinicaltrials.gov database (NCT02012595). All subjects signed a written informed consent form.

**Human study population**

Twelve patients with idiopathic Parkinson’s disease (Hoehn and Yahr stage 1–3) were recruited to the study. All were diagnosed according to UK Brain Bank criteria (Hughes et al., 2001). The patients were receiving the following anti-parkinsonian medication: MAOB inhibitor ($n = 1$), dopamine agonist ($n = 1$), levodopa + MAOB inhibitor ($n = 2$), levodopa + COMT-inhibitor ($n = 3$), levodopa + dopamine agonist ($n = 5$). The control group comprised 12 healthy age and gender matched control subjects. Exclusion criteria included systemic diseases, thoracic and abdominal cancers, previous irradiation to the thoracic and abdominal region, previous major surgery to the gastro-intestinal tract, neurological or psychiatric diseases, substance abuse disorders, or medications affecting acetylcholinesterase.

**Clinical assessment and questionnaires**

Neurological evaluations were performed on all subjects prior to inclusion. Motor symptom severity in the Parkinson’s disease group was rated with the updated MDS Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) (Goetz et al., 2007). Dementia screening was performed using the Mini-Mental State Examination. Symptoms of REM sleep behaviour disorder were assessed using the RBD behaviour questionnaire (RBDSQ) (Stiasny-Kolster et al., 2007). Constipation severity was assessed using the Constipation Scoring System (CSS) (Agachan et al., 1996) and by the sum of the seven constipation items (Questions 9 to 15) on the Rome III questionnaire (ROME3; Rome, 2006). Chronic constipation was diagnosed as defined by Rome III criteria (Rome, 2006). Gastroparesis symptoms were assessed using the ROME III nausea-vomiting module, which rates meal-related nausea, vomiting, and food regurgitation. Olfaction was measured using the Sniffin’ Sticks 16-item identification test (Hummel et al., 1997).

**Gastric scintigraphy**

All participants were examined after an overnight fast of 8 h for solids and 4 h for liquids. Patients were withdrawn from anti-parkinsonian medication for $> 12$ h. Over 10 min the subjects ingested a standardized solid meal of 120 ml egg white omelette containing 19–37 MBq $^{99m}$Tc-labelled colloid along with two slices of bread, 30 g of jam and 120 ml of water. Scintigraphy was performed with a Siemens Symbia T6 dual-head SPECT/CT gamma camera (Siemens), using a low-energy high-resolution collimator. Summed 1 min anterior and posterior images were obtained in the upright sitting position at 0, 30, 60, 90, 120 and 180 min after finishing the meal. Semi-automatic data analysis was done on a Hermes workstation (Hermes Medical Solutions). Total counts were defined on each image from 2D regions of interest delineating the stomach boundary. The mean gastric retention and the half-emptying time ($T_{1/2}$) were computed from time-activity curves.

**Heart rate variability**

Participants observed the same fasting restrictions as outlined above. An electrocardiogram from lead II (sampling rate 1000 Hz) was recorded in the supine position during 10 min rest, during two sessions of nine deep respiratory cycles (six breath per min), and during two Valsalva manoeuvres (deep breath followed by exhaling against 40 mmHg for 15 s with an air leak to ensure an open glottis). Each test was separated by a 5-min rest period. Precautions before autonomic testing are described in detail elsewhere (Terkelsen et al., 2012). QRS complexes were detected, noise or arrhythmic behaviour was manually removed and heart rate variability measures estimated as expressed elsewhere (Terkelsen et al., 2012). Autonomic measures were expressed as mean RR (the distance in ms between consecutive normal R waves in QRS complexes), RMSSD (the square root of the mean squared differences of successive RR intervals), high frequency power (0.15–0.4 Hz), total power, and the coefficient of component variance in the high frequency power bands. The Valsalva ratio was estimated as the longest RR-interval 30 s after completing the manoeuvre divided with the shortest RR-interval induced by the Valsalva manoeuvre, selecting the largest response in two tests. For each respiratory cycle the longest RR-interval was subtracted from the shortest RR-interval and mean of the five largest differences were estimated. The first difference was deleted if it was more than twice the remaining.

**PET/CT imaging**

All participants fasted for a minimum of 8 h and abstained from drinking for 4 h before PET. The synthesis of $^{11}$C-donepezil and PET/CT imaging of the thorax and abdomen has been previously described in detail (Gjerloff et al., 2014). In brief, catheters were inserted in the radial artery for blood sampling and in the contralateral cubital vein for injection of $^{11}$C-donepezil. A 60-min dynamic PET scan was performed for each subject using a standardized 60-s bolus. The PET field of view included the upper abdomen/lower chest region and covered the heart, liver, intestines, pancreas, and stomach. Low-dose CT scan was performed for attenuation correction and anatomical localization purposes. The average injected dose of $^{11}$C-donepezil was $469 \pm 54$ MBq (range 372–531) for the patient group and $419 \pm 121$ MBq (range 404–540) for the control group.
Volume of interest definition
Volumes of interest were manually defined (PMOD software) based on anatomical CT scans using different approaches. For the small intestine, two volumes of interest were defined, as previously described (Gjerløff et al., 2014). One large PET volume of interest was drawn to include all PET signal from the small intestine at a sufficient distance from neighbouring ‘hot’ organs to avoid spill-over. Another, smaller volume of interest was automatically derived by segmenting the CT scan, using the large PET volume of interest as the constraining search space. The CT scans were smoothed using a 2 mm, isotropic Gaussian filter, and a cut-off threshold of −20 Hounsfield units was used to define the small intestine. Voxels, which did not derive from these organs (e.g. vessels), were removed manually. The final time-activity curves in the small intestine were obtained by scaling the curve from the PET-volume of interest to this smaller CT volume. This procedure was chosen to compensate for frame-by-frame organ movement due to peristalsis.

In the spleen, volumes of interest were defined in the parenchyma 10 mm from the outermost tissue border. The pancreas, kidney cortex, and left myocardial ventricle were defined on six consecutive slices using a 10 mm brush size in PMOD. In the myocardium, we performed an additional subsegmentation of the volume of interest into septal and lateral wall regions of interest since a previous study, using 18F-dopamine PET, demonstrated that sympathetic denervation in Parkinson’s disease is most profound in the lateral wall (Goldstein et al., 2002). The anatomical pancreas volume was automatically derived similar to the procedure described for the small intestine. With the current PET/CT protocol, it was impossible to derive meaningful image data from the stomach wall. In many subjects, the stomach is situated adjacent to the pancreas and liver, which display the highest level of 11C-donepezil uptake (Fig. 1A). Thus, severe spill-over effects from the liver and pancreas into the stomach volume of interest were seen in some subjects. Furthermore, the stomach displays extreme peristaltic movements leading to considerable PET-to-CT misalignment, as the CT is obtained >60 min before the end of the dynamic PET scan.

Image and statistical analyses
Statistical analyses were performed using STATA 13 (StataCorp). Group comparisons of demographic and clinical parameters were performed using unpaired t-tests or the non-parametric equivalent depending on type and normality of the data. PET SUV data were analysed with multiple regression analyses with correction for age and gender. Importantly, the small intestine and pancreas SUVs were further corrected for organ volume. The pancreas is a slim organ and quite variable among subjects. Thus, in a very slim pancreas the standard 10 mm volume of interest in the centre of the pancreas would potentially suffer more from activity spill-over effects than in a corresponding larger pancreas. In the small intestine, the inverse problem is true. As the small intestine PET time-activity curve was scaled to the intestinal volume derived from the CT, any group difference in intestinal volume could proportionally bias the PET SUV values. Correlations between SUVs and the kinetic parameters (K1, k2, Vd) were explored using linear regression. Associations between PET SUV values and gastric emptying time, ECG measurements, and clinical scores were investigated with multiple regression analyses.

Figure 1 Whole body PET. (A) Whole body 11C-donepezil PET in a healthy volunteer (adapted from Gjerløff et al., 2014). (B) Representative summed abdominal PET images (55–60 min post-injection) from a healthy control (top) and a patient with Parkinson’s disease (bottom).
Results

Demographic and clinical data are summarized in Table 1. The healthy control subjects were age- and gender-matched to the patients, who had mild-to-moderate clinical disease. Table 2 summarizes data from questionnaires and clinical examinations. The Parkinson’s disease group was significantly more affected by nausea-vomiting and constipation, as rated by the Constipation Scoring System and ROME III questionnaires. Six patients and one control subject had chronic constipation, defined by ROME III criteria (Rome, 2006). Most patients were hyposmic on testing and the group had significantly more symptoms of REM sleep behaviour disorder.

The Parkinson’s disease group had a significantly faster mean gastric emptying time than controls. This group difference was mainly caused by two patients, who had very rapid emptying (T1/2 < 27 min). The mean Valsalva ratio was significantly decreased in the Parkinson’s disease group, signifying the presence of sympathetic cardiac dysautonomia. No group differences were present in any of the cardiovagal measurements.

Table 1 Demographic and clinical data

<table>
<thead>
<tr>
<th></th>
<th>Parkinson</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Gender (f/m)</td>
<td>3/9</td>
<td>3/9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68 (40–74)</td>
<td>65 (44–75)</td>
</tr>
<tr>
<td>MMSE</td>
<td>29 (24–30)</td>
<td>29.5 (27–30)</td>
</tr>
<tr>
<td>Disease duration (y)</td>
<td>4.5 (0.3–12)</td>
<td>–</td>
</tr>
<tr>
<td>MDS-UPDRS (motor)</td>
<td>25.5 (9–56)</td>
<td>–</td>
</tr>
<tr>
<td>Hoehn and Yahr (I/II/III)</td>
<td>1/8/3</td>
<td>–</td>
</tr>
</tbody>
</table>

Values are median (range).

Table 2 Paraclinical parameters and questionnaires

<table>
<thead>
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<th>Parkinson’s disease</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBDSQ</td>
<td>5 (2–12)</td>
<td>2 (0–7)</td>
<td>0.002</td>
</tr>
<tr>
<td>Olfaction score</td>
<td>7 (1–12)</td>
<td>12 (8–15)</td>
<td>0.000</td>
</tr>
<tr>
<td>CSS</td>
<td>7 (1–14)</td>
<td>1 (0–10)</td>
<td>0.005</td>
</tr>
<tr>
<td>ROME III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea-vomiting</td>
<td>5 (4–23)</td>
<td>0 (0–3)</td>
<td>0.044</td>
</tr>
<tr>
<td>Constipation (9–15)</td>
<td>5.5 (0–20)</td>
<td>0 (0–14)</td>
<td>0.003</td>
</tr>
<tr>
<td>Gastric emptying T1/2 (min)</td>
<td>55 (20–102)</td>
<td>62 (52–113)</td>
<td>0.047</td>
</tr>
<tr>
<td>Cardiovagal function</td>
<td>Deep breathing (Δ-BPM)</td>
<td>7.7 ± 3.3</td>
<td>9.4 ± 3.2</td>
</tr>
<tr>
<td>Valsalva ratio</td>
<td>1.43 ± 0.24</td>
<td>1.69 ± 0.15</td>
<td>0.006</td>
</tr>
<tr>
<td>RR interval (ms)</td>
<td>1012 ± 182</td>
<td>1037 ± 216</td>
<td>0.78</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>27.2 ± 15.0</td>
<td>29.6 ± 26.6</td>
<td>0.82</td>
</tr>
<tr>
<td>HF-power (ms²)</td>
<td>360 ± 539</td>
<td>319 ± 345</td>
<td>0.78</td>
</tr>
<tr>
<td>CCV-HF (%)</td>
<td>1.37 ± 0.77</td>
<td>1.54 ± 0.88</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Values are median (range) or mean ± SD.

PET standardized uptake value data

11C-donepezil PET showed high signal in the liver, stomach and pancreas, with more moderate tracer uptake in the brain, salivary glands, heart and small intestine (Fig. 1). Decreased 11C-donepezil uptake was visually apparent in the small intestine of most and the pancreas of several patients with Parkinson’s disease (Fig. 2).

Figure 3 displays small intestine and pancreas time–activity curves and volume-corrected SUV values for the patients with Parkinson’s disease and healthy controls. The small intestine SUV values in the Parkinson’s disease group showed a mean 35% decrease (P = 0.003), and a mean 22% decrease was seen in the pancreas (P = 0.001; Table 3). No significant difference was seen in pancreas volume (patients: 79 ± 5 cm³, controls: 72 ± 4 cm³; P = 0.29), but the patient group trended towards having a larger small intestine volume (patients: 146 ± 10 cm³, controls: 124 ± 9 cm³; P = 0.065, Mann Whitney). As outlined in the ‘Materials and methods’ section, the SUV analyses of the intestine and pancreas were corrected for organ volume removing it as a confound when assessing group differences in SUV values (Supplementary Fig. 1).

The patient group showed a 9% decrease in myocardial SUV values (P = 0.044; Table 3), and a positive correlation was seen with age in the myocardium. There was no difference in the lateral wall-to-septum ratio between the groups (P = 0.99). No significant group differences in SUV values were seen in the spleen and kidney (Table 3). No significant effects of age and gender were seen on SUVs in the intestine, pancreas, spleen, and kidney.

Kinetic PET analyses

The PET kinetic parameters are summarized in Table 3. As previously reported (Gjerløff et al., 2014), a one-tissue
compartments model yielded the most robust fits. Significantly decreased mean $^{11}$C-donepezil V$_d$ was seen in the small intestine ($P = 0.001$), and a near-significant mean V$_d$ decrease was seen in the pancreas ($P = 0.061$) (Supplementary Fig. 2). No group differences in V$_d$ were seen for the myocardium, spleen and kidney.

Across the two populations, a significant linear correlation was seen between V$_d$ and SUV in the pancreas, and a near-significant linear correlation was found in the small intestine (Fig. 4). After exclusion of a significant SUV outlier in the control group ($P < 0.05$; Grubb’s test), the intestinal V$_d$ versus SUV correlation was significant ($r^2 = 0.20$, $P = 0.03$). Significant positive V$_d$ versus SUV correlations were also seen in the myocardium ($r^2 = 0.19$, $P = 0.03$), spleen ($r^2 = 0.26$, $P = 0.016$), and kidney ($r^2 = 0.49$, $P < 0.001$). These results suggest that simple SUV values at 60 min post-injection are a reasonable approximation of the distribution volume, as previously reported (Gjerløff et al., 2014). Finally, no significant between group differences were seen in K$_1$ values, whereas the k$_2$ values tended towards being higher in patients (Table 3). Thus, the between group differences in SUV

Figure 2 Intestinal PET. $^{11}$C-donepezil uptake in small intestine at 60 min post-injection in 12 healthy controls (A) and 12 patients with Parkinson’s disease (B). All images are scaled to the same SUV threshold, and the hottest part of the small intestine is shown for each subject. Hot organs (pancreas, liver) have been masked. The considerably decreased intestinal uptake is visually apparent in most patients.

Figure 3 Time–activity curves in small intestine and pancreas. (A) Time-activity curves in the small intestine from 12 patients with Parkinson’s disease (PD) and 12 control subjects. (B) Small intestine SUV values at 60 min (grey area in A) were decreased in nearly all patients. (C and D) Corresponding time–activity curves and SUV values from the pancreas.
values seems to be related more to faster tracer wash-out (i.e. $k_2$) in the patient group, than to differences in blood flow (i.e. $K_1$).

### Correlations between PET and clinical parameters

No significant correlations were detected between intestinal or pancreatic SUV values and Constipation Scoring System scores, ROME$_{9-15}$ constipation score (Fig. 5A), ROME nausea-vomiting score, or gastric emptying $T_{1/2}$ values (Fig. 5B) (all $P$-values $> 0.3$). No significant correlations were seen between myocardial SUV values and any of the cardiac autonomic measurements, including deep breathing ($\Delta$-BPM), Valsalva ratio, RR-interval, RMSSD, HF-power, or coefficient of component variance in the high frequency power bands (all $P$-values $> 0.3$). No correlations were seen between the PET SUVs in any of the investigated organs and olfaction or REM sleep behaviour scores.

### Discussion

The present study provides the first in vivo PET imaging evidence of decreased AChE density in the gastro-intestinal...
tract of patients with Parkinson’s disease. The patients displayed a highly significant loss of $^{11}$C-donepezil signal in the small intestine. The gut is richly innervated by the vagus nerve, and the pattern of innervation displays a rostral-caudal gradient being most dense in the lower oesophagus, stomach, and upper small intestines (Hopkins et al., 1996). This innervation pattern is similar to the observed pattern of $^{11}$C-donepezil binding, which is highest in the upper gastro-intestinal tract and lower in the ileum and colon (Fig. 1A). AChE is produced in neuron cell bodies and transported axonally to nerve terminals (Giacobini, 2000). Thus, degeneration of vagal efferents in Parkinson’s disease would be expected to decrease vagus-derived AChE in the gastro-intestinal tract. In support of this viewpoint, it has been reported that subtotal vagotomy resulted in a 40–50% decrease in AChE activity in the myenteric plexus of the duodenum and upper colon of guinea pigs (Schmid et al., 1979).

It should be emphasized, however, that AChE is not a specific marker for vagal cholinergic parasympathetic nerve terminals. An abundance of enteric neurons are cholinergic and express AChE (Anlauf et al., 2003). Therefore, decreased $^{11}$C-donepezil signal (i.e. AChE density) in the gut could also be caused by loss of enteric neurons as well as vagal efferents. However, previous studies have failed to demonstrate any appreciable loss of enteric neurons in Parkinson’s disease (Lebouvier et al., 2010; Annerino et al., 2012). Moreover, the bulk of the gastrointestinal α-synuclein pathology seen in Parkinson’s disease comprises Lewy neurites distinct from the enteric neurons (Wakabayashi et al., 1988; Braak et al., 2006; Greene et al., 2014) probably originating from the vagus nerve (Beach et al., 2010). Rodent studies have reported that only a minority (13–22%) of myenteric neurons were positive for α-synuclein, whereas all preganglionic efferent vagal axons and nerve terminals were α-synuclein positive (Phillips et al., 2008). Furthermore, vagotomy dramatically decreased the presence of α-synuclein positive axons and varicosities in the myenteric plexus. Taken together, this evidence suggests that α-synuclein positive nerve fibres in the gut of patients with Parkinson’s disease are primarily of preganglionic parasympathetic origin, in line with the ~50% loss of efferent motor neurons in the DMV (Eadie, 1963; Gai et al., 1992). The preferential loss of preganglionic parasympathetic efferents from the DMV, rather than a loss of enteric neurons, seems therefore to be the most plausible explanation for our finding of decreased $^{11}$C-donepezil signal in the small intestine of patients with Parkinson’s disease. However, even though the number of cholinergic enteric neurons may remain intact in Parkinson’s disease, it is possible that AChE levels are downregulated in these enteric neurons or indeed in the vagal nerve terminals themselves. One study reported upregulation of acetylcholine production and downregulation of AChE in inflamed rat intestine subsequent to nematode parasite infection (Davis et al., 1998). Another study reported downregulated AChE in circulating blood in patients with inflammatory bowel disease (Maharshak et al., 2013). To our knowledge, the relative magnitudes of AChE expression in different cell structures of the intestine in Parkinson’s disease has not been explored in detail, and downregulation remains a competing explanation for the $^{11}$C-donepezil signal decrease seen in the small intestine. Of note, with the present PET/CT protocol, it was impossible to extract valid SUV values or time–activity curves from the stomach, due to peristalsis and it being adjacent to the liver and pancreas. Thus, we cannot comment on whether a similar between-group difference is present in the stomach.

The pancreas has received little attention in the context of Parkinson’s disease, but α-synuclein pathology has been reported in this organ (Beach et al., 2010). We found a significant decrease in the pancreas $^{11}$C-donepezil signal of patients with Parkinson’s disease. The pancreas is a mixed endocrine and exocrine organ, and receives rich parasympathetic innervation from the DMV (Fox and Powley, 1986). Immunohistochemistry studies have demonstrated intense AChE staining throughout the endocrine and exocrine pancreas (Delbro, 2012), and several studies showed that AChE is concentrated in a dense network of cholinergic nerve fibres in the pancreas, which was most likely parasympathetic in origin (Hiramatsu et al., 1993; Ushiki and Watanabe, 1997). The decrease in $^{11}$C-donepezil signal could, therefore, point to parasympathetic denervation of both the endocrine and exocrine gland. However, other pancreatic cells have recently been demonstrated to contain AChE. One study demonstrated AChE expression in the lysosomal system of acinar cells in the rat exocrine pancreas (Bendayan and Gisiger, 2001). To our knowledge, no histology studies have examined the distribution of cholinergic markers in the pancreas of patients with Parkinson’s disease, so it is unknown which cell structures exhibit decreased AChE expression. Interestingly, Parkinson’s disease may be associated with a higher prevalence of diabetes (Cereda et al., 2011), and several early studies demonstrated that >50% of patients with Parkinson’s disease show intolerance on the standard oral glucose test (Lipman et al., 1974; Sandyk, 1993). A pathological oral glucose response in Parkinson’s disease could be caused by loss of the early post prandial insulin response, which is mediated via the DMV and the vagal parasympathetic efferents (Teff, 2008). We are currently planning a second $^{11}$C-donepezil PET study of patients with Parkinson’s disease and oral glucose tolerance tests will also be performed.

The patients manifested only a 9% decrease in the myocardial $^{11}$C-donepezil SUV values, far smaller than the decreases of sympathetic function reported in $^{123}$I-MIBG SPECT and $^{18}$F-dopamine PET studies of the cardiac sympathetic innervation (Goldstein et al., 2002; Haensch et al., 2009). The exact interpretation of the cardiac $^{11}$C-donepezil signal is unclear at present. The preganglionic parasympathetic efferents primarily terminate on ganglia, which are found in connective tissue adjacent to the atrial
wall, pulmonary trunk and superior vena cava (Chow et al., 2001). Using autoradiography of porcine heart tissue, we previously demonstrated homogeneous 11C-donepezil binding throughout the left ventricular myocardium (Gjerlof et al., 2014). This is in accordance with a previous report of AChE-positive nerves being the predominant neural subtype observed throughout the endo-, epi-, and myocardium in the porcine heart (Crick et al., 1999). However, these nerves are mainly postganglionic nerves and it is not clear whether this neuronal subpopulation undergoes degeneration in Parkinson’s disease. Moreover, the cardiovascular preganglionic parasympathetic neurons arise predominantly from the nucleus ambiguus rather than the DMV (Greene, 2014) and, in contrast to the DMV, no appreciable loss of neurons is seen in the nucleus ambiguus in Parkinson’s disease (Eadie, 1963). In addition, it has recently been demonstrated that cardiomycocytes synthesize and secrete acetylcholine in a paracrine fashion (Roy et al., 2013). It seems likely that cardiomycocytes would also synthesize AChE, although this was not explored by the investigators. In summary, the decrease in cardiac 11C-donepezil SUV values was minor and not comparable to the dramatic decreases of sympathetic innervation seen with PET and SPECT imaging markers. We saw no group differences in the lateral wall-to-septum ratio values in contrast to those reported in 18F-dopamine PET studies (Goldstein et al., 2002).

We detected no between-group differences in kidney and spleen 11C-donepezil uptake. These highly perfused organs display tracer kinetics characterized by a high initial uptake and subsequent rapid wash-out (Gjerlof et al., 2014). In humans, the major route of donepezil excretion is renal and 79% of the recovered dose is eventually found in the urine (Wilkinson, 1999), with the remaining 21% found in faeces. Therefore, estimation of 11C-donepezil uptake in the renal parenchyma is difficult. In the spleen, the presence of parasympathetic innervation is controversial, and may target only the vessels (Bellinger et al., 1993). We previously demonstrated that lymphoid nodules are responsible for most of the splenic 11C-donepezil uptake, consistent with the presence of AChE in white blood cells (Gjerlof et al., 2014). In the liver, 11C-donepezil time activity curves and SUV values were similar between the groups (data not shown). As 11C-donepezil is metabolized by the liver and subsequently secreted into the bile, it is not possible to conduct meaningful estimations of AChE density in the liver. Importantly, we carefully checked that our 11C-donepezil analyses in the upper intestine was not confounded by radioactive bile metabolites (for more details, see Supplementary Fig. 3).

Small intestine SUV values did not correlate with disease duration, motor symptom severity, constipation scores, or gastric emptying time as measured by scintigraphy. Myocardial 11C-donepezil uptake did not correlate with cardiac autonomic measurements. Of note, our patient group did not have delayed gastric emptying; in fact it was rapid in some of the patients with Parkinson’s disease. It has been previously reported that patients with early Parkinson’s disease display more prolonged gastric emptying than patients at a more advanced disease stage in whom normal or rapid emptying can be seen (Hardoff et al., 2001). It has been speculated that this may be related to levodopa use, and 10 of our 12 patients were receiving levodopa. Our patients displayed significantly decreased Valsalva ratios, but showed no significant differences in cardiovascular measures. It is possible that our sample of patients with Parkinson’s disease had little myocardial parasympathetic denervation despite the complaint of constipation by six of the patients.

The reduced small intestine SUV values in the patient group showed low variance (Fig. 3B) suggestive of a ‘floor effect’. This could have reduced our power to demonstrate a correlation with clinical parameters. An observation of tight data in the patient group but a wider variance in the control values is also seen with both 123I-MIBG (Miyamoto et al., 2006) and 18F-dopamine (Goldstein et al., 2002) cardiac imaging in patients with manifest Parkinson’s disease, and these imaging measures also correlate poorly with measures of orthostatic hypotension and sympathetic heart rate variability measurements (Goldstein et al., 2002; Haensch et al., 2009). Parkinson’s disease is also characterized by degeneration of various central nuclei involved in autonomic regulation. Thus, autonomic symptoms may arise from simultaneous degeneration in both central and peripheral parts of the nervous system. Moreover, the enteric nervous system is complex and displays a great deal of plasticity. Additionally, even though the brunt of α-synuclein pathology is in the form of Lewy neurites, populations of enteric nerves do contain α-synuclein inclusions and this may influence the motility and function of the gastrointestinal tract. Chronic use of parkinsonian medication and laxatives could also affect the presence and severity of constipation. Thus, failure to find a simple relationship between autonomic symptoms and PET imaging findings is perhaps not unexpected. As pointed out above, we cannot rule out competing causes for the 11C-donepezil signal decrease, which would be another explanation for the lack of correlations.

Our kinetic analyses of the PET time–activity curves demonstrated positive correlations between SUV values and V4 estimates from a one-tissue compartment model (Fig. 4). The correlation was tight for the pancreas but only modest for the small intestine. Extracting valid time–activity curves from the small intestine was challenging because of intestinal peristalsis (Gjerlof et al., 2014), which added noise. Nevertheless, the between-group difference in small intestine V4 was highly significant. Importantly, there was no group difference in K1, which suggests that the group differences in V4 and SUV values were not caused by differences in perfusion. The small intestine and pancreas k3 estimates were larger in the patient group. Thus, tracer wash-out was in general faster in the patient group, which is consistent with decreased tissue density of
AChe. In summary, our findings demonstrate that SUV values derived from static whole-body 11C-donepezil uptake at 40–60 min post-injection yield a reasonable estimate of tracer Vd.

Several limitations of the study need to be addressed. Although AChE histology has been used extensively for demonstrating cholinergic nerves, it is not a specific marker of parasympathetic innervation. As discussed above, other factors such as AChE down regulation in enteric neurons is an alternative explanation for our findings. The validity of the present findings would also be reinforced if replicated using a tracer such as 18F-FEOBV with affinity for the vesicular acetylcholine transporter. This tracer was recently validated for human use, but to date only brain kinetics have been explored (Petrou et al., 2014). The binding affinity of 18F-FEOBV to peripheral organs in humans has not yet been investigated in detail, and rapid secretion of radioactive metabolites to the bile could pose a challenge. Moreover, 18F-FEOBV binding is also not specific for parasympathetic nerve terminals and the tracer could accumulate in enteric cholinergic neurons similar to 11C-donepezil.

In conclusion, using 11C-donepezil PET we detected markedly decreased AChE density in the small intestine and pancreas of patients with Parkinson’s disease at an early-to-moderate disease stage. Given that the enteric nervous system exhibits no appreciable neuron loss in Parkinson’s disease, it seems likely that progressive damage to parasympathetic nerve terminals and degeneration of the dorsal motor nucleus of the vagus is a major contributor to the decrease in the 11C-donepezil PET signal. However, other causes such as downregulation of AChE expression in enteric neurons cannot be ruled out by this study. Nevertheless, 11C-donepezil PET may be the first successful imaging technique to visualize systemic parasympathetic denervation, and has potential applications in other disorders, including diabetic neuropathy. Future 11C-donepezil PET studies of patients with de novo Parkinson’s disease and prodromal subjects with REM sleep behaviour disorder are warranted to determine whether similar findings are present. If so, 11C-donepezil PET could be a valuable imaging biomarker for the early diagnosis of Parkinson’s disease.

Supplementary material
Supplementary material is available at Brain online.

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References


