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The prominent role of serotonergic degeneration in apathy, anxiety and depression in *de novo* Parkinson's disease

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Apathy, which can occur separately or in combination with depression and anxiety, is one of the most frequently encountered neuropsychiatric symptoms in Parkinson's disease. Pathophysiological evidence suggests that parkinsonian apathy is primarily due to a mesolimbic dopaminergic denervation, but the role of the serotonergic alteration has never been examined, despite its well-known involvement in the pathogenesis of depression and anxiety. To fill this gap, we address here the pure model of *de novo* Parkinson's disease, without the confounding effects of antiparkinsonian treatment. Fifteen apathetic (Lille Apathy Rating Scale scores $\geqslant -21$) and 15 non-apathetic ($-36 \leqslant$ Lille Apathy Rating Scale scores $\leqslant -22$) drug-naïve *de novo* parkinsonian patients were enrolled in the present study and underwent detailed clinical assessment and positron emission tomography imaging, using both dopaminergic [^{11}C-*N*-(3-iodoprop-2E-enyl)-2-beta-carbomethoxy-3-beta-(4-methylphenyl)-nortropane (PE2I)] ($n = 29$) and serotonergic [^{11}C-*N*,*N*-dimethyl-2-(-2-amino-4-cyanophenylthio)-benzylamine (DASB)] ($n = 27$) presynaptic transporter radioligands. Apathetic parkinsonian patients presented higher depression ($P = 0.0004$) and anxiety ($P = 0.004$) scores – as assessed using the Beck Depression Inventory and the part B of the State-Trait Anxiety Inventory, respectively – compared to the non-apathetic ones – who were not different from the age-matched healthy subjects ($n = 15$). Relative to the controls, the non-apathetic parkinsonian patients mainly showed dopaminergic denervation ($n = 14$) within the right caudate nucleus, bilateral putamen, thalamus and pallidum, while serotonergic innervation ($n = 15$) was fairly preserved. Apathetic parkinsonian patients exhibited, compared to controls, combined and widespread dopaminergic ($n = 15$) and serotonergic ($n = 12$) degeneration within the bilateral caudate nuclei, putamen, ventral striatum, pallidum and thalamus, but also a specific bilateral dopaminergic disruption within the substantia nigra–ventral tegmental area complex, as well as a specific serotonergic alteration within the insula, the orbitofrontal and the subgenual anterior cingulate cortices. When comparing the two parkinsonian groups, the apathetic patients mainly displayed greater serotonergic alteration in the ventral striatum, the dorsal and the subgenual parts of the anterior cingulate cortices, bilaterally, as well as in the right-sided caudate nucleus and the right-sided orbitofrontal cortex. Regression analyses also revealed that the severity of apathy was moreover mainly related to specific serotonergic lesions within the right-sided anterior caudate nucleus and the orbitofrontal cortex, while the degree of both depression and anxiety was primarily linked to serotonergic disruption within the bilateral subgenual parts and/or the right dorsal part of the anterior cingulate cortex, without prominent role of the dopaminergic degeneration in the pathogenesis of these three non-motor signs. Altogether, these findings highlight a prominent role of the serotonergic degeneration in the expression of the neuropsychiatric symptoms occurring at the onset of Parkinson's disease.

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Abbreviations: ACC = anterior cingulate cortex; BDI = Beck Depression Inventory; BP_{ND} = non-displaceable binding potential; DASB = *N*,*N*-dimethyl-2-(-2-amino-4-cyanophenylthio)-benzylamine; DAT = dopaminergic transporter; GPe = globus pallidus pars externa or external pallidum; LARS = Lille Apathy Rating Scale; MDS-UPDRS = Movement Disorders Society Unified Parkinson's Disease Rating Scale; OFC = orbitofrontal cortex; PE2I = *N*-(3-iodoprop-2E-enyl)-2-beta-carbomethoxy-3-beta-(4-methylphenyl)-nortropane; SERT = serotonergic transporter; SN-VTA = substantia nigra–ventral tegmental area; STAI-YB = State-Trait Anxiety Inventory part B

Introduction

Parkinson's disease is traditionally characterized by levodopa-responsive motor symptoms that are mainly due to nigrostriatal dopamine depletion. In addition, a wide range of disabling non-motor signs is also present throughout the course of the disease. These non-motor features include more particularly fatigue, pain, but also autonomic and neuropsychiatric manifestations, such as apathy, anxiety and depression. These neuropsychiatric signs, which are inherent to the disease, and not a consequence or a side-effect to long-term dopaminergic medication, indeed constitute a triad of symptoms traditionally grouped under the umbrella of 'hypodopaminergic' symptoms (Ardouin *et al.*, 2009). Such comorbidities are frequent and can be encountered at every stage of Parkinson's disease, from the premotor and the early untreated phases of the disease, to the advanced stages of Parkinson's disease, and can notably appear after the reduction of antiparkinsonian drugs (Aarsland *et al.*, 2009; Barone *et al.*, 2009; Chaudhuri *et al.*, 2009; Thobois *et al.*, 2010; de la Riva *et al.*, 2014; Dujardin *et al.*, 2014; Pagonabarraga *et al.*, 2015; Schrag *et al.*, 2015). Recent clinical reports have more specifically revealed that apathy is encountered in 16–36% of recently diagnosed patients with Parkinson's disease (Barone *et al.*, 2009; de la Riva *et al.*, 2014; Dujardin *et al.*, 2014; Pagonabarraga *et al.*, 2015), whereas depression and anxiety are detected in 9 to 61% of cases (Barone *et al.*, 2009; de la Riva *et al.*, 2014; Dujardin *et al.*, 2014; Schrag *et al.*, 2015). Although apathy can occur separately, this manifestation is frequently associated with anxiety and/or depression, as previously reported in patients with advanced Parkinson's disease after dopaminergic medication withdrawal (Thobois *et al.*, 2010; Pagonabarraga *et al.*, 2015). Apathy is twice as frequent in this latter case, and the patients suffering from depressive symptoms always also display apathy. Thus, apathy, which is less likely to be reactive to the handicap and stigma of the disease than anxiety or depression, seems to be the core neuropsychiatric symptom of Parkinson's disease. These three neuropsychiatric features most often respond to dopaminergic treatments (Remy *et al.*, 2005; Barone *et al.*, 2010; Thobois *et al.*, 2013).

Beyond the well-known role of nigrostriatal dopaminergic dysfunction in the pathophysiology of Parkinson's disease motor symptoms (Brooks *et al.*, 1990; Boileau *et al.*, 2009), dopaminergic disruption of the mesolimbic and mesostriatal pathways is involved in the occurrence of several non-motor manifestations, such as apathy, depression, anxiety, fatigue, or impulse control

disorders (Remy *et al.*, 2005; Weintraub *et al.*, 2005, 2015*a*; Aarsland *et al.*, 2009; Boileau *et al.*, 2009; Chaudhuri *et al.*, 2009; Pavese *et al.*, 2010; Thobois *et al.*, 2010; Pagonabarraga *et al.*, 2015; Castrioto *et al.*, 2016). In addition, increasing lines of evidence support a specific causal role of serotonergic dysfunction in the pathogenesis of several parkinsonian signs, such as tremor and dyskinesia, but also depression, fatigue, cognitive decline and hallucinations, at moderate-to-advanced stages of the disease (Doder *et al.*, 2003; Boileau *et al.*, 2008; Pavese *et al.*, 2010; Politis *et al.*, 2010*b*; Ballanger *et al.*, 2012).

The neurochemical mechanisms underpinning Parkinson's disease-related disorders therefore appear multifactorial and remain incompletely elucidated to date. This may be because most of the neuroimaging studies performed so far have included patients with Parkinson's disease who have been treated chronically with dopaminergic medication or have been exposed to serotonergic drugs, which may consequently hinder the pathophysiological interpretation of the associated results (Brooks *et al.*, 1990; Doder *et al.*, 2003; Kerenyi *et al.*, 2003; Remy *et al.*, 2005; Weintraub *et al.*, 2005; Albin *et al.*, 2008; Boileau *et al.*, 2008; Pavese *et al.*, 2010; Politis *et al.*, 2010*a*, *b*; Thobois *et al.*, 2010; Strecker *et al.*, 2011; Ballanger *et al.*, 2012; Joutsa *et al.*, 2015). In addition, the delineation of non-motor signs is not always detailed, which limits the clinical interpretation of these investigations (Qamhawi *et al.*, 2015). Furthermore, post-mortem and neuroimaging studies performed in early-stage patients have sometimes reported conflicting results, either showing serotonergic disruption at Parkinson's disease onset (Albin *et al.*, 2008; Politis *et al.*, 2010*a*; Joutsa *et al.*, 2015; Qamhawi *et al.*, 2015) or not (Beucke *et al.*, 2011; Strecker *et al.*, 2011). Finally, most of these previous works have explored the dopaminergic or the serotonergic systems separately, but not simultaneously in the same patients suffering from Parkinson's disease.

The present work therefore aims to fill this gap, by providing a further understanding of the respective contribution of dopaminergic and serotonergic degeneration in the pathogenesis of the most prominent neuropsychiatric signs that can be encountered in early Parkinson's disease. For all the reasons previously mentioned, we have chosen to focus our study on patients with *de novo* Parkinson's disease, before any exposure to antiparkinsonian drugs and to their related potential psychotropic effects, to dispose of a relatively pure model of the disease, but also to avoid confounding effects related to the dopaminergic and/or serotonergic ongoing medications on the exploration of the underlying pathophysiology.

We have thus investigated apathetic and non-apathetic patients with *de novo* Parkinson's disease, whether or not accompanied by depression and/or anxiety, through both detailed clinical evaluation and PET imaging, using specific presynaptic dopaminergic [*N*-(3-iodoprop-2E-enyl)-2-beta-carbomethoxy-3-beta-(4-methylphenyl)-nortropane (^{11}C-PE2I)] and serotonergic [*N*,*N*-dimethyl-2-(-2-amino-4-cyanophenylthio)-benzylamine (^{11}C-DASB)] transporter radioligands.

Subjects and methods

Participants

Fifteen apathetic and 15 non-apathetic recently diagnosed, untreated patients with Parkinson's disease, and 15 age-matched healthy controls were enrolled at the Lyon and Grenoble University Hospitals (Table 1). Apathetic behaviour was defined by the score obtained on the Lille Apathy Rating Scale (LARS) (Sockeel *et al.*, 2006). Apathetic patients had a score on the LARS scale of $\geqslant -21$, while the LARS score of the non-apathetic patients ranged between -36 and -22. The other inclusion criteria were as follows: (i) patients under 70 years of age; (ii) diagnosis of a parkinsonian syndrome with onset less than 2 years prior to the start of the investigation; and (iii) absence of any atypical symptoms incompatible with a diagnosis of Parkinson's disease according to the United Kingdom Parkinson Disease Society Brain Bank Diagnostic Criteria for idiopathic Parkinson's disease (Gibb and Lees, 1988). Exclusion criteria included: (i) cognitive impairment [i.e. a Mattis Dementia Rating Scale (MDRS) score of $<130/144$ (Schmidt *et al.*, 1994), and a Frontal Assessment Battery (FAB) score of $<15/18$ (Dubois *et al.*, 2000)]; (ii) present or past therapy with (pro-) dopaminergic agents; (iii) a marked resting tremor (to avoid movement artefacts during neuroimaging acquisitions); (iv) severe concomitant illnesses and/or psychiatric disturbances other than apathetic, depressive, and anxious disorders; and (v) current use of serotonergic drugs altering ^{11}C-DASB binding. The healthy subjects had to be free of psychiatric or neurological disorders and did not receive any serotonergic drugs. The present study (project #2012.722) was approved by the local Ethics Committee (Grenoble University Hospital) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Clinical and neuropsychological assessment

Part III of the Movement Disorders Society Unified Parkinson Disease Rating Scale (MDS-UPDRS; Goetz *et al.*, 2008) was used to rate motor signs. Akinesia–rigidity and rest tremor (excluding consequently the action tremor item) subscores were also computed for Parkinson's disease group comparisons (by adding the scores related to the items 3.3 to 3.8, and the item 3.14, for the first one, and the scores related to the items 3.15, 3.17 and 3.18 for the second one) (Table 1).

Detailed neuropsychological assessment was performed to measure: (i) apathy, using the LARS scale (Sockeel *et al.*, 2006); (ii) trait-anxiety, using the part B of the State-Trait Anxiety Inventory (STAI-YB; Spielberger *et al.*, 1980); (iii) depression, using the Beck Depression Inventory (BDI-2; Beck *et al.*, 1988); (iv) fatigue, using the Parkinson's Disease Fatigue Scale (PFS-16; Brown *et al.*, 2005); (v) pain, using the Neuropathic Pain Symptoms Inventory (NPSI; Bouhassira

Table 1 Main demographic, clinical and neuropsychological features of the subjects included in the study

Main scales	Parameter assessed	Normative values	Non-apathetic patients ($n = 15$)	Apathetic patients ($n = 15$)	Controls ($n = 15$)	Between-group comparisons	
						Main group effect	*Post hoc P*-value
–	Age	–	53.5 ± 12.6 [32; 70]	60.7 ± 7.2 [45; 69]	55.3 ± 8.6 [43; 69]	*ns*. $F(2,42) = 2.20$; $P = 0.123$	–
–	Gender	–	7 F, 8 M	4 F, 11 M	6 F - 9 M	–	–
MDRS (/144)	Global cognition	⩾130	141.3 ± 2.9 [134; 144]	140.6 ± 2.4 [137; 144]	138.6 ± 4.1 [131; 144]	*ns*. $F(2,40) = 2.98$; $P = 0.062$	–
MDS-UPDRS III (/132)	Global motor disability	–	27.5 ± 10.9 [9; 45]	32.3 ± 5.3 [25; 47]	–	*ns*. $F(1,26) = 0.89$; $P = 0.35$	–
MDS-UPDRS III (/64)	Akinesia-rigidity	–	21.2 ± 8.8 [2; 34]	24.5 ± 5.1 [17.5; 38]	–	*ns*. $F(1,26) = 0.76$; $P = 0.39$	–
MDS-UPDRS III (/32)	Tremor[a]	–	3.6 ± 4.1 [0; 14]	3.1 ± 3.2 [0; 8]	–	*ns*. $F(1,26) = 0.29$; $P = 0.60$	–
LARS (−36; 36)	Apathy	−36; −22	−29.1 ± 3.4 [−34; −24]	−13.7 ± 8.0 [−21; 2]	−29.3 ± 5 [−34; 14]	$F(2,40) = 28.68$; $P < 0.0001$	C versus NAP: *ns* (1.0) **C versus AP: $P < 0.0001$** **NAP versus AP: $P < 0.0001$**
BDI-2 (/63)	Depression	0–11	6.3 ± 5.1 [2; 23]	15.1 ± 6.3 [5; 31]	6.1 ± 5.5 [0; 17]	$F(2,40) = 10.48$; $P = 0.0002$	C versus NAP: *ns* (1.0) **C versus AP: $P = 0.0003$** **NAP versus AP: $P = 0.0004$**
STAI-YB (/80)	Trait anxiety	–	38.4 ± 8.5 [29; 55]	50.5 ± 9.0 [33; 63]	34.2 ± 10.8 [20; 57]	$F(2,40) = 10.84$; $P = 0.0001$	C versus NAP: *ns* (0.72) **C versus AP: $P = 0.0001$** **NAP versus AP: $P = 0.004$**
PFS-16 (/80)	Fatigue	0–40	38.4 ± 19 [16; 75]	55.7 ± 14.0 [17; 75]	–	$F(1,26) = 9.19$; $P = 0.005$	**$P = 0.005$**
NPSI (/100)	Pain	–	4.7 ± 7.8 [0; 24]	14.1 ± 14.1 [0; 42]	–	$F(1,26) = 7.17$; $P = 0.013$	*ns*.

The quantitative variables are shown as the mean and standard deviation (SD). All the significant statistics surviving after correction for multiple comparisons ($n = 10$) are highlighted in bold. Values in square brackets are [min; max]. Regarding the STAI-YB scale, the normative values take into account age and gender. Akinesia-Rigidity subscores were computed by adding the scores related to the items 3.3 to 3.8, and the item 3.14, and the tremor subscores were calculated from the items 3.15, 3.17 and 3.18, of the motor part of the MDS-UPDRS scale.

[a]The item associated with the action tremor has not been considered when calculating the mean tremor subscore.

AP = apathetic patients; C = controls; F = female; M = male; NAP = non-apathetic patients; ns = non-significant; PFS = Parkinson's Disease Fatigue Scale.

et al., 2004); and (vi) global cognition, using the MDRS scale (Schmidt *et al.*, 1994), and executive functions, using the FAB scale (Dubois *et al.*, 2000) (Table 1).

Statistical analysis of the clinical manifestations

Suitable statistical tests were used for between-group comparisons, after assessing for normal distribution (using Shapiro-Wilk tests) and variance homogeneity (using the Fisher-Snedecor F-test) within the data. For continuous data, one-way ANOVAs (with three levels of groups, for the main neuropsychological scales, and two levels of groups, for the fatigue, pain, and motor scales) were performed using appropriate co-variates (i.e. age and gender, except for the analysis related to age). Within each analysis showing a significant main effect, a *post hoc* examination was conducted, using the Bonferroni test. Pearson correlations were also conducted to explore the covariations between the severity of the main Parkinson's disease non-motor and motor signs, using the dedicated ordinal scores. Corrections for multiple comparisons ($n = 10$ items) were also applied, and only α-values $\leqslant 0.005$ were considered statistically significant. Statistical analyses were performed using Statistica® (Statistica 8, Statsoft, Tulsa, USA).

Neuroimaging and data processing

Scanning procedure and data collection

A maximum of 8 weeks separated the two (behavioural and imaging) sessions. Forty-one subjects underwent one anatomical MRI and two PET scans to specifically study dopamine transporter (DAT) binding using ^{11}C-PE2I, and serotonin transporter (SERT) binding using ^{11}C-DASB. Three apathetic patients did not undergo the SERT PET scan as they were on serotonergic antidepressant medication. One non-apathetic patient did not undergo the DAT PET scan due to an episode of claustrophobia.

Neuroimaging was performed at the CERMEP Cyclotron Center (Lyon, France). The subjects were positioned supine on the scanner beds, with their head held in place. A camera monitored the head's position during scanning. Anatomical MRI acquisition consisted of two 8-min 3D sagittal T_1-weighted sequences obtained on a 1.5 T Magnetom scanner (Siemens) equipped with an emitting/receiving head coil. The anatomical volume covered the entire brain using 176 adjacent slices of 1-mm thickness (repetition time = 1970 ms; echo time = 3.93 ms; flip angle = 15°; field of view = 256 mm; voxel size = $1 \times 1 \times 1$ mm^3). PET scans were performed using a PET/CT tomograph (Siemens Biograph mCT/S 64, with a spatial transverse resolution of 4.4 mm; Jakoby *et al.*, 2011) in 3D mode over a 90-min period. Dynamic acquisition began with the bolus injection of the radiotracers, i.e. ^{11}C-DASB for SERT binding and ^{11}C-PE2I for DAT binding, through an intravenous forearm catheter. Mean ^{11}C-DASB injected activity [± standard error of the mean (SEM)] ranged between 274 and 292 MBq (for controls: 274.9 ± 10.3 MBq; for non-apathetic patients: 280.2 ± 14.9 MBq; and for apathetic patients: 291.8 ± 18.8 MBq) whereas mean ^{11}C-PE2I injected activity (±SEM) was between 156 and 196 MBq (for controls: 195.3 ± 11.7 MBq; for non-apathetic patients: 156.6 ± 8.3 MBq; for apathetic patients: 188.7 ± 7.7 MBq). A 1-min low-dose CT scan (<0.2 mSv) acquired prior to emission was used to correct tissue attenuation during PET data reconstruction. Biograph mCT/S64 emission images were reconstructed using the Siemens ultra-HD PET algorithm with 12 iterations, 21 subsets and a zoom factor of 3, then sampled into 30 sequential-frame series for the ^{11}C-DASB tracer, and 28 sequential-frame series for the ^{11}C-PE2I tracer. The reconstructed images displayed a transaxial resolution of 4 mm full-width at half-maximum in a 128×128 matrix-size, resulting in 109 slices of 2.03-mm thickness generating a voxel size of approximately 2.12×2.12 mm^2. The order of the PET-scans (using ^{11}C-DASB or ^{11}C-PE2I tracers) was counterbalanced between subjects and groups. The interval between the two PET-scan sessions was <2 months.

Kinetic modelling

PET images were analysed using suitable tracer kinetic modelling at the voxel-based level. A representative value of the non-displaceable binding potential (BP_{-ND}) of ^{11}C-DASB and ^{11}C-PE2I tracers was computed for each voxel of the images by applying the Simplified Tissue Reference Model (SRTM) (Lammertsma and Hume, 1996; Gunn *et al.*, 1997). The white matter of the cerebellum, as defined in the Hammers atlas (Hammers *et al.*, 2003; Gousias *et al.*, 2008), was transformed into PET native space and used as the reference area, as this region is assumed to be devoid of SERT (Kish *et al.*, 2005) and DAT (Hall *et al.*, 1999) transporters.

Voxel-based analyses for between-group comparisons and symptom correlations

Binding potential images were spatially normalized onto the common Montreal Neurological Institute (MNI) template space using the following procedure: (i) the binding potential images were first co-registered on the corresponding individual structural MRI; (ii) the parameters for transforming individual structural MRI native space to the standard grey matter stereotactic template (MNI/ICBM152) (Ashburner and Friston, 1997) were calculated using the 'new segment' algorithm of the SPM8 software (Statistical Parametric Mapping, Wellcome Department for Cognitive Neuroscience, London, UK) implemented in Matlab® 2012b 8.0 (MathWorks Inc., Natick, Massachusetts, USA); and (iii) the binding potential images were normalized using these transformation parameters. The normalized binding potential images were then smoothed using an isotropic Gaussian kernel filter (8 mm full-width at half-maximum) to reduce variance due to interindividual anatomical variability and to improve the signal-to-noise ratio.

Voxel-based analysis was conducted using SPM8 within an appropriate mask of interest composed of the following areas, delineated from those of the Hammers atlas (Hammers *et al.*, 2003; Gousias *et al.*, 2008): the inferior frontal cortices (IFC), the orbitofrontal cortices (OFC, including the anterior, medial, lateral, posterior orbital and straight gyri), the dorsal anterior cingulate cortices (dorsal ACC), the subgenual anterior cingulate cortices [subgenual ACC, including the (pre-) subgenual frontal cortices and subcallosal areas], the posterior cingulate cortices, the insula, the caudate nuclei, the putamen, the pallidum, the substantia nigra-ventral tegmental area (SN-VTA), the amygdala, the hippocampus, the parahippocampal/ambient

gyri and the brainstem. Only the ligand-dependent voxels presenting mean binding potential values of $\geqslant 0.1$ in controls were used in the mask of interest.

Between-group comparisons (i.e. non-apathetic versus apathetic patients; controls versus non-apathetic patients; and controls versus apathetic patients) were performed using two-sample Student's *t*-tests. In addition, multiple regression analyses were conducted to explore the covariations between the binding of each tracer and the severity of both non-motor and motor Parkinson's disease symptoms, from the dedicated ordinal scores using appropriately weighted categorical contrasts to generate statistical parametric maps representing both increase and decrease in ^{11}C-DASB and ^{11}C-PE2I BP_{-ND} in each considered voxel. In all these analyses, age and gender are implemented as covariates of non-interest. For the main analyses related to apathy (that is, the Parkinson's disease group comparison, and the associated regression analysis), the effect of depression was also controlled by adding the patients' scores as covariate of non-interest.

Regional analyses

Because the voxel-based analyses are very conservative, a regional approach was also performed. Regional analysis focused on the following eight bilateral subcortical regions of interest: the anterior and posterior caudate nuclei, the anterior and posterior putamen, the ventral striatum (VS), the pallidum, the thalamus, and the SN-VTA complex. These areas were delineated using the Hammers atlas (Hammers *et al.*, 2003; Gousias *et al.*, 2008). Because this atlas does not allow the anterior and posterior parts of the caudate nuclei and of the putamen to be differentiated, manual parcellation of these territories was conducted on individual normalized MRIs, after extracting the homologous regions of the atlas. The anterior commissure was used as a benchmark to separate these boundaries along a postero-anterior axis, on 28 consecutive axial sections. In addition to this segmentation, the ventral (i.e. limbic) part of the striatum (corresponding anatomically to the anterior parts of the caudate nuclei and of the putamen) was manually drawn on 11 consecutive individual coronal MRI sections (Levitt *et al.*, 2013). Regional ^{11}C-DASB and ^{11}C-PE2I BP_{-ND} values were calculated by averaging the values from all of the voxels constituting each of these regions of interest. For each group, these values are represented as the mean and the SEM (Table 2). The regions whose BP_{-ND} values were below 0.1 were not considered for the analyses. As the mean ^{11}C-DASB BP_{-ND} value for the posterior caudate nuclei, and the mean ^{11}C-PE2I BP-ND value for the thalamus, were <0.1, we have only studied the ^{11}C-PE2I BP_{-ND} value within the posterior caudate nuclei, and the ^{11}C-DASB BP_{-ND} value within the thalamus. In total, seven bilateral subcortical regions of interest were defined for each tracer (i.e. the anterior and posterior caudate nuclei, the anterior and posterior putamen, the ventral striatum, the pallidum, and the SN-VTA complex, for the dopaminergic tracer; and the anterior caudate nuclei, the anterior and posterior putamen, the ventral striatum, the pallidum, the thalamus and the SN-VTA complex, for the serotonergic tracer).

Between-group comparisons (i.e. non-apathetic versus apathetic patients; controls versus non-apathetic patients; and controls versus apathetic patients) were performed by means of appropriate statistical tests, that is, one-way ANOVAs with three levels of groups, using age and gender as covariates, followed by *post hoc* analyses (Bonferroni tests) when applicable. Pearson correlations were also conducted on the complete *de novo* cohort in order to explore the covariations between the binding of each tracer in each bilateral region of interest and the severity of both Parkinson's disease non-motor and motor signs, using the dedicated ordinal scores. Also, Pearson correlations between the ^{11}C-DASB and ^{11}C-PE2I binding values collected within each of the subcortical regions of interest were also carried out, for all the patients with Parkinson's disease having undergone the two PET scans ($n = 26$). These statistical analyses were performed under Statistica® (Statistica 8, Statsoft, Tulsa, USA).

Reported statistics

For all of the voxel-based analyses, overall changes in PET signal were covaried out for all voxels and comparisons across conditions were performed using t-statistics and then converted into *Z*-scores. For the voxel-based between-group comparison analyses, multiple comparisons were applied at the cluster-level with a family wise error (FWE) corrected significance threshold ($P_{FWE\text{-}corrected} < 0.05$). Because the associated results were subsequently employed as *a priori* hypotheses regarding the cortical and subcortical dysfunction in the complete Parkinson's disease *de novo* cohort, a non-corrected statistical threshold of $P\text{-value}_{\text{-uncorrected}} \leqslant 0.001$ was then applied at the voxel-level (Z-scores > 3.10) for the main regression analyses. For all these analyses, only clusters of a minimum extent k of 10 contiguous voxels were considered. Reported *x*, *y*, and *z* coordinates were consistent with the MNI space. For the regional analyses, *P*-value significance ($\leqslant 0.05$) was corrected for multiple comparisons ($n = 7$ for each tracer used), and α-values $\leqslant 0.007$ were considered statistically significant.

Results

Demographic, clinical and neuropsychological characteristics of the subjects

There were no significant statistical differences between groups regarding age [$F(2,42) = 2.20$; $P = 0.123$] and cognition (MDRS scores) [$F(2,40) = 2.98$; $P = 0.062$]. The apathetic patients did not differ from the non-apathetic patients with regard to the severity of motor signs [total MDS-UPDRS score: $F(1,26) = 0.89$; $P = 0.35$; akinesia-rigidity subscore: $F(1,26) = 0.76$; $P = 0.39$; and tremor subscore: $F(1,26) = 0.29$; $P = 0.60$] (Table 1).

As concerns the non-motor features, group effects were found regarding the depression [$F(2,40) = 10.48$; $P = 0.0002$] and trait-anxiety [$F(2,40) = 10.84$; $P = 0.0001$] scores, respectively. *Post hoc* analyses revealed that these scores were significantly higher in the apathetic group, relative both to the control group (depression scores: $P = 0.0003$; trait-anxiety scores: $P = 0.0001$), and to the non-apathetic group (depression scores: $P = 0.0004$; trait-anxiety scores: $P = 0.004$). The scores obtained in apathetic patients more particularly indicated mild depression (mean

Table 2 Subcortical ^{11}C-PE2I and ^{11}C-DASB binding values obtained using the regional analysis

	^{11}C-PE2I tracer					^{11}C-DASB tracer				
	Controls (n = 15)	Non-apathetic patients (n = 14)	Apathetic patients (n = 15)	Between-group comparisons		Controls (n = 15)	Non-apathetic patients (n = 15)	Apathetic patients (n = 12)	Between-group comparisons	
				Main group effect	*Post-hoc P*-value				Main group effect	*Post hoc P*-value
Bilateral ant. caudate	2.90 ± 0.15	2.63 ± 0.16 (9%)	2.28 ± 0.14 (21%)	$F(2,39) = 3.13$; $P = 0.054$	C versus NAP: $P = 0.53$ C versus AP: *$P = 0.009$* NAP versus AP: $P = 0.28$	0.60 ± 0.04	0.57 ± 0.05 (5%)	0.36 ± 0.07 (40%)	$F(2,37) = 2.95$; $P = 0.064$	C versus NAP: $P = 1.0$ C versus AP: $P = 0.008$ NAP versus AP: $P = 0.02$
Bilateral post. caudate	1.35 ± 0.13	1.26 ± 0.12 (7%)	0.99 ± 0.09 (27%)	$F(2,39) = 1.48$; $P = 0.24$	*ns.*	–	–	–	–	–
Bilateral ant. putamen	3.72 ± 0.10	2.54 ± 0.18 (32%)	2.26 ± 0.14 (39%)	$F(2,39) = 30.41$; $P < 0.0001$	**C versus NAP: $P < 0.0001$** **C versus AP: $P < 0.0001$** NAP versus AP: $P = ns.$ (0.45)	1.08 ± 0.06	1.04 ± 0.04 (4%)	0.92 ± 0.06 (15%)	$F(2,37) = 1.19$; $P = 0.32$	*ns.*
Bilateral post. putamen	3.39 ± 0.14	1.61 ± 0.25 (53%)	1.18 ± 0.20 (65%)	$F(2,39) = 35.99$; $P < 0.0001$	**C versus NAP: $P < 0.0001$** **C versus AP: $P < 0.0001$** NAP versus AP: $P = ns.$ (0.35)	0.86 ± 0.03	0.92 ± 0.05	0.76 ± 0.06 (12%)	$F(2,37) = 1.52$; $P = 0.23$	*ns.*
Bilateral vent. striatum	3.02 ± 0.08	3.02 ± 0.10	2.59 ± 0.11 (14%)	$F(2,39) = 4.58$; $P = 0.016$	C versus NAP: $P = ns.$ (1.0) C versus AP: *$P = 0.006$* NAP versus AP: *$P = 0.008$*	1.03 ± 0.05	1.00 ± 0.03 (3%)	0.86 ± 0.05 (17%)	$F(2,37) = 2.74$; $P = 0.08$	C versus NAP: $P = 1.0$ C versus AP: $P = 0.014$ NAP versus AP: $P = 0.046$
Bilateral pallidum (GPe + GPi)	1.11 ± 0.05	0.77 ± 0.08 (31%)	0.67 ± 0.05 (40%)	$F(2,39) = 16.17$; $P < 0.0001$	**C versus NAP: $P = 0.0002$** **C versus AP: $P < 0.0001$** NAP versus AP: $P = ns.$ (0.66)	0.71 ± 0.04	0.72 ± 0.04	0.64 ± 0.04 (10%)	$F(2,37) = 0.20$; $P = 0.81$	*ns*
Bilateral thalamus	–	–	–	–		0.93 ± 0.03	0.93 ± 0.05	0.84 ± 0.05 (10%)	$F(2,37) = 0.14$; $P = 0.87$	*ns*
Bilateral SN-VTA complex	0.69 ± 0.04	0.52 ± 0.06 (25%)	0.39 ± 0.04 (43%)	$F(2,39) = 9.81$; $P = 0.0003$	**C versus NAP: $P = 0.027$** **C versus AP: $P < 0.0001$** NAP versus AP: *$P = 0.097$*	0.76 ± 0.05	0.74 ± 0.09 (3%)	0.60 ± 0.06 (21%)	$F(2,37) = 0.77$; $P = 0.47$	*ns*

The regional ^{11}C-PE2I and ^{11}C-DASB BP_{ND} values obtained for each bilateral region of interest and group are shown as mean and SEM. Significant between-group differences (after correction for multiple comparisons) are highlighted in bold. Trends toward significance are indicated in italics.

ant = anterior; AP = apathetic patients; inf = inferior; NAP = non-apathetic patients; post = posterior; vent = ventral.

(%) = Per cent of BP_{ND} decrease in ^{11}C-PE2I or ^{11}C-DASB tracers in patients, compared to controls.

BDI-2 score: 15.1 ± 6.3) and a trend towards pathological anxiety (mean STAI-YB score: 50.5 ± 9.0). No significant differences appeared between the healthy subjects and the non-apathetic group. If a significant difference was also found between the two Parkinson's disease groups regarding the fatigue scores [$F(1,26) = 9.19$; $P = 0.005$], the effect observed for the pain scores [$F(1,26) = 7.17$; $P = 0.013$] did not survive to correction for multiple comparisons (Table 1).

Correlation between the clinical and neuropsychological scales

Correlation analyses between the scores related to the main neuropsychological scales demonstrated a positive covariation between the severity of apathy scores with those of depression ($r = 0.66$; $P = 0.00007$) and anxiety ($r = 0.64$; $P = 0.0001$). A positive covariation was also found between the degree of depressive and anxious symptoms ($r = 0.81$; $P < 0.00001$). However, no significant covariations were found between the severity of non-motor signs and the motor impairment in the complete *de novo* Parkinson's disease cohort.

PET imaging data

Differences in dopaminergic and serotonergic transporter binding between groups

The voxel-based analysis revealed that non-apathetic patients, compared to controls, expressed a significant and bilateral reduction in ^{11}C-PE2I binding within the putamen, the external part of the pallidum (GPe), the thalamus, as well as a significant decrease of ^{11}C-PE2I binding within the right anterior caudate nucleus [Table 3 (controls versus non-apathetic patients) and Fig. 1A]. Serotonergic innervation moreover appeared to be well preserved in these patients (Table 4). The regional approach overall confirmed these findings, with a significant decrease in ^{11}C-PE2I binding within the anterior putamen (*post hoc* $P < 0.0001$), the posterior putamen (*post hoc* $P < 0.0001$), the pallidum [including both its internal (Gpi) and external (Gpe) segments] (*post hoc* $P = 0.0002$), and the SN-VTA complex (*post hoc* $P = 0.027$), bilaterally, in apathetic patients, relative to controls. No significant effect was found for the anterior caudate nuclei. Again, no significant alteration appeared regarding the ^{11}C-DASB binding (Table 2).

Relative to the controls, the apathetic patients showed, when considering first the voxel-based level, a significant combined, widespread and bilateral decrease in ^{11}C-PE2I and ^{11}C-DASB binding within the caudate nuclei, putamen, ventral striatum, GPe, and thalamus. Significant voxel-wise reductions in ^{11}C-PE2I binding were specifically highlighted within the bilateral SN-VTA complex, whereas a significant decrease in ^{11}C-DASB binding was also specifically noticed within the medial part of the OFC, the subgenual ACC and the insula, bilaterally, as well as in the right-sided hippocampus [Tables 3, 4 controls versus apathetic patients and Fig. 1B and C]. The analyses performed at the regional level also attested, after applying *post hoc* analyses, a significant reduction in ^{11}C-PE2I binding within the anterior putamen ($P < 0.0001$), the posterior putamen ($P < 0.0001$), the pallidum ($P < 0.0001$), and the SN-VTA complex ($P < 0.0001$), bilaterally, in the apathetic group relative to the control group. No significant disruption in ^{11}C-PE2I and ^{11}C-DASB binding was found within the bilateral anterior caudate nuclei, although trends toward a group effect were observed for both the dopaminergic tracer [$F(2,39) = 3.13$; $P = 0.054$], and the serotonergic tracer [$F(2,37) = 2.95$; $P = 0.064$], with a decrease in binding in apathetic patients compared to controls, as revealed by *post hoc* examination ($P = 0.009$ and $P = 0.008$, respectively). A trend toward a group effect was also found in ^{11}C-DASB binding within the bilateral ventral striatum [$F(2,37) = 2.74$; $P = 0.08$], with a reduction in binding in apathetic patients compared to controls (*post hoc* $P = 0.014$). An effect of group regarding the ^{11}C-PE2I binding also appeared within the bilateral ventral striatum [$F(2,39) = 4.58$; $P = 0.016$], suggesting a decrease of this binding in apathetic patients (*post hoc* analysis: $P = 0.006$), but such effect did not survive to correction for multiple comparisons. No alteration in ^{11}C-DASB binding was observed within the bilateral putamen, the pallidum, the thalamus, and the SN-VTA complex, in apathetic patients relative to controls, with the regional approach (Table 2).

When comparing apathetic and non-apathetic patients, at the voxel-wise level no significant reduction in ^{11}C-PE2I binding was found, except a trend toward significance regarding the bilateral SN-VTA complex ($P_{FWE\text{-}corrected}$ at the cluster level = 0.07) [Table 3 (non-apathetic versus apathetic patients)]. Greater and significant reductions in ^{11}C-DASB binding were however observed within the ventral striatum, the subgenual ACC and the dorsal ACC, bilaterally, as well as in the right anterior and posterior parts of caudate nucleus, and the right OFC in the apathetic group, compared to the non-apathetic group, using the voxel-based approach [Table 4 (non-apathetic versus apathetic patients) and Fig. 1D]. After having controlled for the specific effects of the depression, Parkinson's disease group differences in ^{11}C-DASB binding were found within the bilateral OFC (left part: $x = -6$; $y = 33$; $z = -9$; $k = 1486$; Z-score = 4.869; right part: $x = 3$; $y = 33$; $z = -9$; $k = 1486$; Z-score = 5.042), the bilateral subgenual ACC (left part: $x = -8$; $y = 27$; $z = -9$; $k = 1486$; Z-score = 4.583; right part: $x = 11$; $y = 24$; $z = -8$; $k = 1486$; Z-score = 3.456) and the bilateral dorsal ACC (left part: $x = -5$; $y = 41$; $z = 1$; $k = 1486$; Z-score = 4.352; right part: $x = 8$; $y = 38$; $z = -9$; $k = 1486$; Z-score = 3.315) ($P_{FWE\text{-}corrected\text{-}cluster} < 0.001$ for all these areas), while the effects previously observed within the bilateral ventral striatum and the right caudate nucleus disappeared. The region of interest analysis also indicated trends toward a decrease in ^{11}C-DASB binding within the bilateral anterior caudate nuclei [$F(2,37) = 2.95$; $P = 0.064$] and the bilateral ventral striatum [$F(2,37) = 2.74$; $P = 0.08$], confirmed by *post hoc* examinations ($P = 0.02$, and $P = 0.046$, respectively), in the

Table 3 Between-group differences in dopaminergic (^{11}C-PE2I) binding revealed by the voxel-based analysis

Between-group comparisons ^{11}C-PE2I			Controls versus non-apathetic patients				Controls versus apathetic patients				Non-apathetic versus apathetic patients			
Location of the main brain areas		Lat.	Coordinates	*Z*-score	P-FWE-corr cluster	*k*	Coordinates	*Z*-score	P-FWE-corr cluster	*k*	Coordinates	*Z*-score	*P-FWE-corr* cluster	*k*
Anatomical labels	BA		*x*, *y*, *z*				*x*, *y*, *z*				*x*, *y*, *z*			
Anterior caudate nuclei	*ND*	L	–	–	–	–	−17, 20, 5	4.865	<0.001	5611	–	–	–	–
		R	18, 17, 3	3.967	<0.001	3647	18, 18, 1	6.303	<0.001	4663	–	–	–	–
Middle caudate nuclei	*ND*	L	–	–	–	–	−17, 14, 3	4.796	<0.001	5611	–	–	–	–
		R	–	–	–	–	20, 14, 7	5.141	<0.001	4663	–	–	–	–
Posterior caudate nuclei	*ND*	L	–	–	–	–	−18, −6, 18	3.833	<0.001	5611	–	–	–	–
		R	–	–	–	–	18, 5, 14	3.833	<0.001	4663	–	–	–	–
Ventral striatum	*ND*	L	–	–	–	–	−15, 15, −5	4.947	<0.001	5611	–	–	–	–
		R	–	–	–	–	15, 15, 1	4.764	<0.001	4663	–	–	–	–
Anterior putamen	*ND*	L	−17, 14, 3	4.051	<0.001	3416	−18, 14, −2	4.634	<0.001	5611	–	–	–	–
		R	20, 14, 7	3.794	<0.001	3647	21, 18, −2	6.199	<0.001	4663	–	–	–	–
Middle putamen	*ND*	L	−27, 0, −2	5.227	<0.001	3416	−21, 2, 11	5.039	<0.001	5611	–	–	–	–
		R	27, 2, −3	7.393	<0.001	3647	23, −3, 11	5.018	<0.001	5611	–	–	–	–
Posterior putamen	*ND*	L	−23, −4, 9	4.905	<0.001	3416	−30, −7, −4	7.542	<0.001	5611	–	–	–	–
		R	27, −13, 7	4.639	<0.001	3647	29, −1, −3	65535	<0.001	4663	–	–	–	–
External pallidum	ND	L	−20, 6, 4	5.197	<0.001	3416	−20, −6, 7	4.992	<0.001	5611	–	–	–	–
		R	21, −7, 6	5.180	<0.001	3647	20, −7, 7	5.122	<0.001	4663	–	–	–	–
Thalamus	*ND*	L	−20, −18, 9	3.577	<0.001	3416	−17, −11, 9	3.849	<0.001	5611	–	–	–	–
		R	18, −12, 7	5.208	<0.001	3647	18, −14, 3	4.920	<0.001	4663	–	–	–	–
Midbrain / SN–VTA complex	*ND*	L	–	–	–	–	−9, −21, −15	5.481	<0.001	5611	–	–	–	–
		R	–	–	–	–	9, −20, −17	5.595	<0.001	5611	3, −17, −14	4.075	0.07	175

BA = Brodmann area; *k* = cluster size (number of voxels); Lat = laterality; L = left; ND = not defined as Brodmann areas; R = right; *x* = medio–lateral, *y* = rostro–caudal, *z* = dorso–ventral coordinates according to the MNI space. Statistical maps were thresholded at a *P*-value < 0.001 uncorrected and $k > 10$ contiguous voxels. *Z*-scores indicate the statistical value of the most significant voxel in the associated cluster which survived at a FWE-corrected *P*-value $\leqslant 0.05$ (P-FWE-corr) at the cluster-level in the voxel-based analysis.

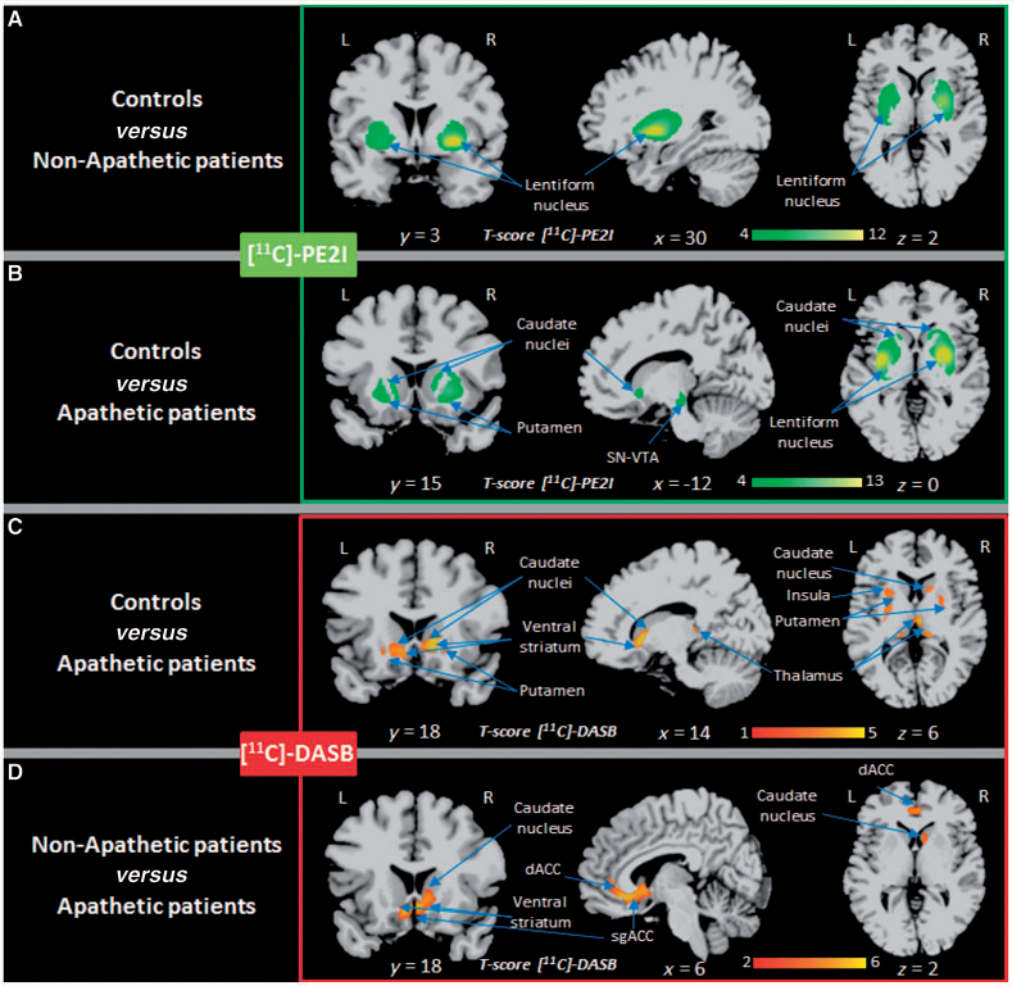

Figure 1 Between-group differences in dopaminergic and serotonergic innervation. The maps illustrate the brain areas with a higher dopaminergic binding (upper panels, in green) in the control group, relative to the non-apathetic Parkinson's disease group (**A**), and to the apathetic Parkinson's disease group (**B**), and with a higher serotonergic binding (lower panels, in red) in the control group, compared with the apathetic Parkinson's disease group (**C**), as well as in the non-apathetic Parkinson's disease group compared to the apathetic Parkinson's disease group (**D**). L = left; R = right; *x* = medio-lateral; *y* = rostro-caudal; *z* = dorso-ventral coordinates according to the MNI space. The lentiform nucleus was defined as the association of the putamen and of the pallidum.

apathetic group, relative to the non-apathetic one. An effect of group regarding the ^{11}C-PE2I binding was finally observed within the bilateral ventral striatum [$F(2,39) = 4.58$; $P = 0.016$], suggesting a reduction of such binding in apathetic patients (*post hoc* analysis: $P = 0.008$), but this effect did not survive to correction for multiple comparisons (Table 2).

Correlations between the severity of motor manifestations and dopaminergic and serotonergic degeneration

The regional correlation analyses highlighted covariations between the severity of motor signs (reflected both by the MDS-UPDRS III total scores, as well as the akinesia-rigidity and rest tremor subscores, computed from the MDS-UPDRS III scores) and the reduction in ^{11}C-PE2I binding within several subcortical areas, especially within the bilateral posterior putamen ($r = -0.47$; $P = 0.009$), the bilateral pallidum ($r = -0.45$; $P = 0.013$), and the bilateral SN-VTA ($r = -0.48$; $P = 0.008$), regarding the MDS-UPDRS III total scores, and within the bilateral anterior putamen ($r = -0.41$, $P = 0.025$), the bilateral posterior putamen ($r = -0.52$; $P = 0.003$), the bilateral pallidum ($r = -0.49$; $P = 0.007$) and the bilateral SN-VTA ($r = -0.44$; $P = 0.017$), for the akinesia-rigidity subscores, at uncorrected thresholds. No link was found between the alteration of ^{11}C-PE2I binding and the degree of tremor (not shown).

Moreover, no relationship between the alteration in ^{11}C-DASB binding and the severity of motor signs was noticed (not shown).

Correlations between the severity of non-motor manifestations and dopaminergic and serotonergic degeneration

The voxel-based analysis revealed that the severity of apathy was correlated with a reduction in ^{11}C-DASB

Table 4 Between-group differences in serotonergic (^{11}C-DASB) binding revealed by the voxel-based analysis

Between-group comparisons ^{11}C-DASB			Controls versus non-apathetic patients				Controls versus apathetic patients				Non-apathetic versus apathetic patients			
Location of the main brain areas		Lat.	Coordinates	*Z*-score	P-FWE-corr cluster	*k*	Coordinates	*Z*-score	P-FWE-corr cluster	*k*	Coordinates	*Z*-score	*P-FWE-corr* cluster	*k*
Anatomical labels	**BA**		*x*, *y*, *z*				*x*, *y*, *z*				*x*, *y*, *z*			
Median orbitofrontal cortex	11	L					−3, 30, −11	3.703	<0.001	1209	–	–	–	–
		R					2, 29, −9	3.923	<0.001	1209	3, 33, −9	5.504	<0.001	2266
Subgenual anterior cingulate cortex	25	L					−8, 23, −9	4.015	<0.001	1209	−5, 18, −9	4.787	<0.001	2266
		R					2, 18, −8	3.807	<0.001	1209	3, 20, −9	4.644	<0.001	2266
Dorsal anterior cingulate gyrus	24	L					–	–	–	–	−3, 42, 0	4.200	<0.001	2266
		R					–	–	–	–	5, 41, 1	4.236	<0.001	2266
Insula	ND	L					−30, 11, 4	3.574	0.002	627	–	–	–	–
		R					39, −12, −2	3.656	0.011	417	–	–	–	–
Anterior caudate nuclei	ND	L					−8, 18, −6	3.876	<0.001	1209	–	–	–	–
		R					15, 18, −2	4.550	<0.001	1209	9, 11, 0	4.132	<0.001	2266
Middle caudate nuclei	ND	L					−14, 18, −1	3.700	0.002	627	–	–	–	–
		R					13, 14, 2	3.900	0.011	417	9, 18, −3	4.154	<0.001	2266
Posterior caudate nuclei	ND	L					−15, −4, 20	3.524	0.002	627	–	–	–	–
		R					18, −10, 22	3.436	0.011	417	18, 0, 17	3.569	0.012	70
Ventral striatum	ND	L		*No suprathreshold clusters*			−14, 15, −2	3.728	0.002	627	−3, 9, −8	5.024	<0.001	2266
		R					14, 16, −3	3.856	0.011	417	2, 12, −6	4.573	<0.001	2266
Anterior putamen	ND	L					−24, 12, 6	3.369	0.002	627	–	–	–	–
		R					20, 17, −2	3.699	<0.001	1209	–	–	–	–
Middle putamen	ND	L					−27, 8, 6	3.570	0.002	627	–	–	–	–
		R					26, 3, 7	3.403	0.011	417	–	–	–	–
Posterior putamen	ND	L					−26, −7, 3	3.696	0.002	627	–	–	–	–
		R					26, −1, 4	3.395	0.011	417	–	–	–	–
External pallidum	ND	L					−21, 3, 0	3.306	0.002	627	–	–	–	–
		R					26, −7, 0	3.346	0.011	417	–	–	–	–
Thalamus	ND	L					−2, −16, 9	3.794	0.010	428	–	–	–	–
		R					5, −15, 9	4.605	0.010	428	–	–	–	–
Hippocampus	ND	L					–	–	–	–	–	–	–	–
		R					12, −32, 9	3.986	0.010	428	–	–	–	–

BA = Brodmann area; *k* = cluster size (number of voxels); Lat. = Laterality; L = left; ND = not defined as Brodmann areas; R = right; *x* = medio–lateral, *y* = rostro–caudal, *z* = dorso–ventral coordinates according to the MNI space. Statistical maps were thresholded at a *P*-value < 0.001 uncorrected and $k > 10$ contiguous voxels. Z-scores indicate the statistical value of the most significant voxel in the associated cluster which survived at a FWE-corrected *P*-value $\leqslant 0.05$ (P-FWE-corr) at the cluster-level in the voxel-based analysis.

Table 5 Correlations between apathy, depression, and anxiety scores and serotonergic lesions revealed by the voxel-based analysis

Location of the main brain areas		Lat.	^{11}C-PE2I tracer				^{11}C-DASB tracer			
Anatomical labels	**BA**		**Coordinates *x, y, z***	**Z-score**	**P-FWE-corr cluster**	***k***	**Coordinates *x, y, z***	**Z-score**	**P-FWE-corr cluster**	***k***
Apathy LARS scale										
Median orbitofrontal cortex	11	R	*No suprathreshold clusters*				32, 59, −8	3.686	0.734	12
Anterior caudate nucleus	*ND*	R					12, 21, −6	3.872	0.112	275
Depression BDI-2 scale										
Subgenual anterior cingulate gyrus	25	L	*No suprathreshold clusters*				−2, 8, −11	3.542	0.606	35
		R					2, 8, −9	3.355	0.736	12
Trait-Anxiety STAI-YB scale										
Subgenual anterior cingulate gyrus	25	L	*No suprathreshold clusters*				−2, 12, −14	3.400	0.633	30
		R					3, 12, −14	3.570	0.423	81
Dorsal anterior cingulate gyrus	24	R					6, 29, 12	3.542	0.565	44

BA = Brodmann area; *k* = cluster size (number of voxels); Lat. = laterality; L = left; ND = not defined as Brodmann areas; R = right; *x* = medio-lateral, *y* = rostro-caudal, *z* = dorso-ventral coordinates according to the MNI space. The ordinal scores used correspond to the total scores respectively obtained on the LARS, STAI-YB, and BDI-2 scales for each patient in the study. Statistical maps were thresholded at a *P*-value < 0.001 uncorrected and *k* > 10 contiguous voxels. Z-scores indicate the statistical value of the most significant voxel in the associated cluster which survived at a *P*-value ⩽ 0.001 uncorrected at the voxel-level in the voxel-based analysis.

binding within the right-sided anterior caudate nucleus and OFC (Table 5 and Fig. 2). These results were preserved after controlling for the effects of depression (for the right anterior caudate nucleus: $x = 12$; $y = 23$; $z = -5$; $k = 39$; Z-score = 3.311; $P_{\text{uncorrected-voxel}} < 0.001$; for the right OFC: $x = 32$; $y = 59$; $z = -8$; $k = 20$; Z-score = 3.894; $P_{\text{uncorrected-voxel}} < 0.001$). The regression analysis performed at the regional level also highlighted a link between the severity of apathy and a significant decrease in ^{11}C-DASB binding within the bilateral anterior caudate nuclei (r = −0.56; $P = 0.002$), the bilateral posterior caudate nuclei (r = −0.54; $P = 0.004$), the bilateral ventral striatum (r = −0.64; $P = 0.0003$), the bilateral anterior putamen (r = −0.51; $P = 0.007$), and the bilateral posterior putamen (r = −0.56; $P = 0.002$).

The voxel-wise analysis has, moreover, reported that the degree of depression was exclusively related to the reduction in ^{11}C-DASB binding within the bilateral subgenual ACC (Table 5 and Fig. 3A), whereas the severity of trait-anxiety was linked to the decrease in ^{11}C-DASB binding within the bilateral subgenual ACC and the right dorsal ACC (Table 5 and Fig. 3B). The correlation analysis performed from the manually-drawn regions of interest has not revealed significant link between the severity of both depression and anxiety and the reduction in ^{11}C-DASB binding.

No significant alteration in ^{11}C-PE2I binding was highlighted for each of these three signs. As well, no significant link was found between ^{11}C-PE2I and ^{11}C-DASB binding and the degrees of fatigue and pain.

Correlation between dopaminergic and serotonergic degeneration

Region of interest analysis demonstrated significant positive covariations between ^{11}C-PE2I and ^{11}C-DASB binding values within the bilateral anterior (r = 0.60; $P = 0.001$) and posterior (r = 0.64; $P = 0.0004$) caudate nuclei, the thalamus (r = 0.58; $P = 0.002$) and the SN-VTA complex (r = 0.68; $P = 0.0001$), in the studied *de novo* Parkinson's disease cohort.

Discussion

To our knowledge, the present PET study is the first to simultaneously explore the presynaptic dopaminergic and serotonergic changes occurring in *de novo* drug-naïve patients with Parkinson's disease, using combined DAT (^{11}C-PE2I) and SERT (^{11}C-DASB) tracers. This work confirms the well-known, exclusive role of the dopaminergic degeneration in the pathophysiology of motor symptoms in non-tremulous patients with Parkinson's disease, which, consequently, will not be discussed here. More interestingly, this study highlights that, at Parkinson's disease onset, the pathogenesis of apathy, anxiety and depressive manifestations is clearly related to widespread serotonergic alteration, and to a much more limited dopaminergic disruption.

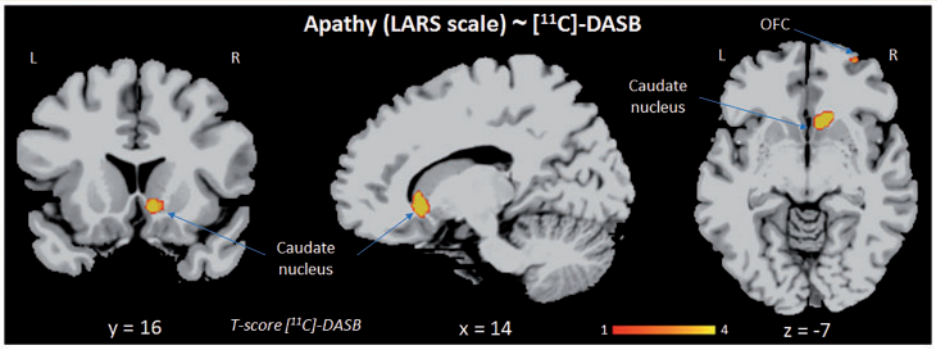

Figure 2 Correlation between the reduction in serotonergic innervation and the severity of apathy. The map illustrates the relationships between the severity of apathy (based on the LARS scores) and the reduced binding of serotonin (SERT) tracer within cortico-subcortical areas (namely, the right-sided orbitofrontal cortex and the ventral part of the right-sided anterior caudate nucleus), in the complete *de novo* Parkinson's disease cohort. L = left; R = right; *x* = medio-lateral; *y* = rostro-caudal; *z* = dorso-ventral coordinates according to the MNI space.

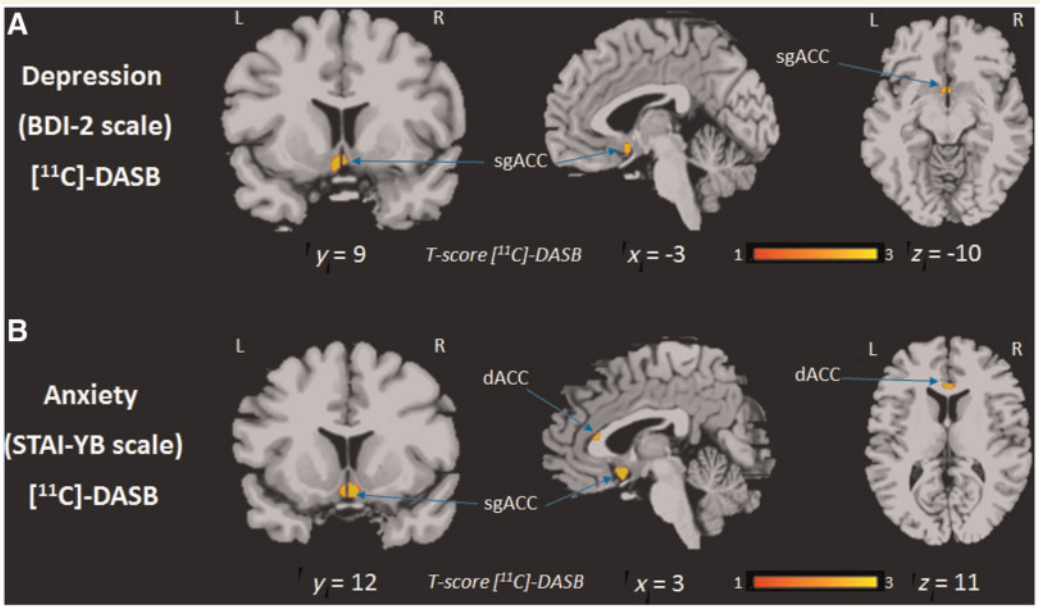

Figure 3 Correlation between the reduction in serotonergic innervation and the severity of depression and anxiety. The maps illustrate the relationships between both the severity of depression (based on the BDI-2 scores, **A**) and the severity of anxiety (based on the STAI-YB scores, **B**), and the reduced binding of serotonin (SERT) tracer within cortical limbic areas (that is, the subgenual and/or the dorsal parts of the anterior cingulate cortex), in the complete *de novo* Parkinson's disease cohort. L = left; R = right; *x* = medio-lateral; *y* = rostro-caudal; *z* = dorso-ventral coordinates according to the MNI space.

Before discussing these pathophysiological results, it has to be acknowledged that only a long term follow-up of the *de novo* population included in the present study, and presenting with good responsiveness to levodopa therapy, will definitively confirm the diagnosis of Parkinson's disease.

The serotonergic system is altered from the early stages of Parkinson's disease

A growing body of evidence, provided by neuroimaging studies, points towards global serotonergic disruption in Parkinson's disease (Doder *et al.*, 2003; Boileau *et al.*, 2008; Pavese *et al.*, 2010; Politis *et al.*, 2010*b*; Ballanger *et al.*, 2012; Qamhawi *et al.*, 2015).

It has been shown that such serotonergic alteration progresses slowly during the course of the disease (Kerenyi *et al.*, 2003; Politis *et al.*, 2010*a*). Some studies have reported serotonergic degeneration at Parkinson's disease onset (Albin *et al.*, 2008; Politis *et al.*, 2010*a*; Joutsa *et al.*, 2015; Qamhawi *et al.*, 2015), while others have not (Beucke *et al.*, 2011; Strecker *et al.*, 2011). Here, we demonstrate not only that the serotonergic innervation is altered from the inaugural stages of Parkinson's disease,

compared to controls, but also that this serotonergic disruption is clearly more pronounced in patients suffering from a combination of apathetic, anxious, and depressive manifestations.

Respective topographical differences in dopaminergic versus serotonergic degeneration within the striatum

Previous studies have demonstrated that dopaminergic lesions predominated in the putamen, whereas serotonergic lesions were more marked in the caudate nuclei (Kerenyi *et al.*, 2003; Kish *et al.*, 2008). Such predominance of dopaminergic degeneration in the putamen compared to the caudate nuclei is confirmed in our non-apathetic patients, whereas such a distinction is less clear in apathetic patients, as serotonergic and dopaminergic innervation appear affected throughout the entire striatum.

Dopamine, serotonin and non-motor manifestations in early Parkinson's disease

Among the wide range of non-motor features, apathy, anxiety and depression, which can also occur separately, are frequently combined at the early, or the premotor stages, of Parkinson's disease (Aarsland *et al.*, 2009; Barone *et al.*, 2009; Boileau *et al.*, 2009; Chaudhuri *et al.*, 2009; de la Riva *et al.*, 2014; Dujardin *et al.*, 2014; Pagonabarraga *et al.*, 2015; Santangelo *et al.*, 2015; Schrag *et al.*, 2015; Weintraub *et al.*, 2015*b*). Our findings corroborate such observation. Indeed, we had separated our two populations of patients with Parkinson's disease based on the presence or absence of apathy, assuming a simultaneous expression of apathy with both anxiety and depression. Our clinical results have indeed confirmed the large overlap between these three manifestations, showing more particularly a positive covariation between the severity of apathy with those of anxiety and depression. This result is thus in favour of the clinical notion of a behavioural 'non-motor' triad in Parkinson's disease, with apathy as the core feature (Ardouin *et al.*, 2009; Thobois *et al.*, 2010; Pagonabarraga *et al.*, 2015).

In addition, our present PET findings demonstrate the dysfunction of the limbic cortico-basal ganglia circuit—including namely the OFC, ACC, and limbic part of basal ganglia—in the pathophysiology of apathy, depression and anxiety, independently of the underlying neurotransmitter dysfunction. This is consistent with the results of previous studies supporting functional, structural and metabolic abnormalities within this network in apathetic (Thobois *et al.*, 2010; Skidmore *et al.*, 2013) and depressed (Weintraub *et al.*, 2005; Skidmore *et al.*, 2013) patients with Parkinson's disease.

Although the present study did not reveal any correlation between the serotonergic and dopaminergic alteration and either fatigue or pain severity, the absence of such results does not rule out a possible role of serotonergic and dopamine disruption in their pathogenesis. Indeed, these two symptoms were more pronounced in the apathetic patients, who exhibited clearly more severe serotonergic degeneration, and a more limited dopaminergic disruption. Such assertion is consistent with previous works, having suggested a role of these alterations in the pathophysiology of these two manifestations (Pavese *et al.*, 2010; Attal *et al.*, 2015).

More importantly, one of the most striking results of this work is that, despite a trend for greater dopaminergic degeneration within both the bilateral ventral striatum and the right SN-VTA complex in apathetic versus non-apathetic patients with Parkinson's disease, the present study did not highlight, in this specific Parkinson's disease population, any significant role of dopaminergic alteration in the pathogenesis of the non-motor triad (thus comprising apathy, depression and anxiety). However, it is likely that increasing the number of subjects included in this study would have permitted us to reach statistical significance for the dopaminergic denervation within the ventral striatum, which is of interest regarding the importance of this brain area in emotional processes. Some methodological issues could also partly explain such results. Indeed, the dopaminergic tracer used here (^{11}C-PE2I) has a low and mainly non-specific binding outside the basal ganglia, which could have contributed to difficulties in assessing for differences in dopaminergic innervation in mesolimbic cortical areas, in comparison to what had been observed in our previous work using, for example, ^{11}C-raclopride (a dopaminergic D2 receptor ligand) (Thobois *et al.*, 2010). Nevertheless, the present results clearly differ from those obtained in previous studies, having shown a pronounced role of dopamine in the pathogenesis of apathy, in accordance with its well-known implication in mood regulation and reward, goal-directed and motivational behaviours (Weintraub *et al.*, 2005; Boileau *et al.*, 2009; Thobois *et al.*, 2010; Santangelo *et al.*, 2015). Such dopaminergic involvement in the underlying mechanisms of apathy was further supported by the improvement of this symptom after administrating dopaminergic drugs (Chaudhuri *et al.*, 2009; Thobois *et al.*, 2013; de la Riva *et al.*, 2014; Pagonabarraga *et al.*, 2015).

Thus, the present results clearly challenge the dopaminergic nature of apathy, anxiety and depression at Parkinson's disease onset, and rather underline the crucial involvement of the serotonergic degeneration. This constitutes the major and most important finding of the present study. Indeed, the more apathetic, but also more anxious and more depressed patients display greater serotonergic lesions within the meso-cortico-limbic and meso-striatal pathways, as well as in the pallidum and thalamus. When controlling for the specific effect of depression, it appears that the serotonergic alteration observed within the bilateral ventral striatum and the right caudate nucleus could in fact contribute, altogether, to apathetic and depressive

manifestations while the serotonergic degeneration observed within both the OFC and the ACC could be more specifically associated with apathy. Interestingly, the severity of apathy seems also to be especially related to serotonergic denervation within the limbic cortical and subcortical circuits, more particularly within the right-sided OFC and the anterior part of caudate nucleus, while the degree of both depression and anxiety was mainly associated with serotonergic lesions in the cortical limbic areas, namely the subgenual ACC.

These observations are consistent with the well-documented role of the serotonergic alteration in the pathogenesis of depression and anxiety in non-Parkinson's disease subjects (Spies *et al.*, 2015), as well as with previous findings showing that brain stimulation of the subgenual cingulate cortex improves pharmaco-resistant depression (Holtzheimer *et al.*, 2012). In more advanced Parkinson's disease, the link between the serotonergic disruption and the expression of both anxiety and depression has already been established (Boileau *et al.*, 2008; Politis *et al.*, 2010*b*; Ballanger *et al.*, 2012) and is supported by pharmacological studies (Ohno *et al.*, 2015). Indeed, previous works have reported at least a partial improvement of the Parkinson's disease depression and anxiety following the administration of serotonergic antidepressant drugs (Richard *et al.*, 2012). However, the major role of the serotonergic dysfunction in early Parkinson's disease, and its particular involvement in apathy, had never been addressed to date. In the present study, it is striking to note that the severity of both apathy, depression and anxiety seems almost exclusively linked to the serotonergic degeneration, although such disruption is obviously not isolated, as the dopaminergic denervation is, by definition, inherent to Parkinson's disease.

The discrepancy between the present work, which clearly highlights the role of the serotonergic denervation in the pathophysiology of both apathy, depression and anxiety, and those underlying the involvement of the dopaminergic degeneration for the same non-motor manifestations, argues for the complexity of the pathogenesis of these Parkinson's disease non-motor signs. Indeed, for the same neuropsychiatric clinical presentation (i.e. apathy, depression and anxiety), the underlying mechanisms may vary according to the Parkinson's disease stage, with, for instance, a greater serotonergic involvement at disease onset, and a greater dopaminergic disruption with the disease progression (Remy *et al.*, 2005; Thobois *et al.*, 2010). More particularly, the worsening of the dopaminergic mesolimbic degeneration with the disease evolution, while these territories are relatively spared at Parkinson's disease onset, might explain why apathy could become more levodopa-responsive when disease progresses.

Overall, the present findings suggest that, beyond the stage of Parkinson's disease, there are in fact not one, but several 'types' of apathy—such as emotional-affective, auto-activation and cognitive apathy—which could be underpinned by different functional, metabolic and neurotransmission abnormalities, as described by Pagonabarraga *et al.* (2015). Thus, one might assume that the extent of both serotonergic and dopaminergic damages is probably a key factor explaining different types of apathy. Neuropsychological and neuroimaging evidence collected in our patients rather suggest an emotional-affective or auto-activation apathy, which is more closely related to depression and anxiety, instead of the cognitive one (Pagonabarraga *et al.*, 2015). This fits well with the underlying widespread serotonergic disruption observed here. A longitudinal follow-up of our *de novo* Parkinson's disease cohort, using both clinical, and dopaminergic and serotonergic PET imaging, will allow validation of our dual serotonin/dopamine hypothesis by drawing correlations, at different stages of the disease, between the neurotransmission abnormalities and the severity of the non-motor presentation.

Finally, it must be kept in mind that the situation may be even more complex, considering other neurotransmission abnormalities, beyond the serotonergic and the dopaminergic ones. Indeed, we did not analyse here the contribution of other neurotransmission systems, such as the noradrenergic or the cholinergic ones that are also involved in the pathogenesis of the Parkinson's disease-related neuropsychiatric symptoms. Some neuroimaging studies have, for instance, reported the involvement of norepinephrine in the pathogenesis of several non-motor signs, such as depression or anxiety (Remy *et al.*, 2005). Similarly, the role of the cholinergic system dysfunction as a possible pathophysiological mechanism in apathy has been suggested, notably by some pharmacological studies having demonstrated an improvement of apathy following anticholinesterasic drug intake (Devos *et al.*, 2014). Moreover, and not surprisingly, several anatomical, biochemical and electrophysiological studies also point toward interactions between different neurotransmitters in the behavioural control (Flik *et al.*, 2015; De Deurwaerdère and Giovanni, 2016). Further exploration of the respective roles of these other neurotransmission systems in the emergence of Parkinson's disease neuropsychiatric signs, and their potential interactions with the serotonergic and dopaminergic alterations, will be of major interest.

Conclusion

Beyond the dopaminergic depletion, the present findings stress the importance of the serotonergic degeneration in Parkinson's disease, and show that this serotonergic alteration is primarily associated with the expression of apathy, anxiety and depression, at the beginning of the disease. These three neuropsychiatric manifestations, which clearly constitute a 'non-motor' triad, and which were traditionally grouped until now under the aegis of 'hypodopaminergic' Parkinson's disease features, can no longer be considered as hypodopaminergic signs, despite their association with both dopaminergic disruption and good response to dopatherapy, in particular for apathy, in more advanced stages of Parkinson's disease. Thus, the present results rather suggest

that the diversity of phenotypes at Parkinson's disease onset appears closely related to the heterogeneity of the underlying dopaminergic and serotonergic degeneration, without excluding the role of other neurotransmitters. Moreover, it remains to be seen whether the PET neuroimaging might be an adapted tool to use in routine to guide the choice of the pharmacological arsenal in order to alleviate for the Parkinson's disease-related neuropsychiatric symptoms.

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