Published Ahead of Print on February 26, 2016 as 10.1212/WNL.0000000000002494

Serotonin-to-dopamine transporter ratios in Parkinson disease

Relevance for dyskinesias

Andreas-Antonios Roussakis, MD
Marios Politis, PhD
David Towey, PhD
Paola Piccini, PhD, FRCP

Correspondence to Dr. Piccini: paola.piccini@imperial.ac.uk

ABSTRACT

Objective: To investigate whether a serotonin-to-dopamine terminal ratio is related to the appearance of dyskinesias in patients with Parkinson disease (PD).

Methods: Twenty-eight patients with idiopathic PD (17 with levodopa-induced dyskinesias [LIDs], 11 without dyskinesias) and 12 age-matched healthy controls were studied with PET and 5[^{11}C]-3-amino-4-(2-dimethylaminomethylphenyl-sulfanyl)-benzonitrile (^{11}C-DASB) and with SPECT and [^{123}I]N-w-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane (^{123}I-ioflupane), which are in vivo specific markers of the serotonin and dopamine transporters' availability, respectively. We have employed a simplified reference tissue model for the quantification of ^{11}C-DASB, whereas a semiquantification approach was used for ^{123}I-ioflupane data. We calculated ^{11}C-DASB binding to ^{123}I-ioflupane uptake ratios for the caudate and the putamen.

Results: Patients with PD showed striatal decreases in ^{11}C-DASB binding potential ($p < 0.01$) and in ^{123}I-ioflupane mean uptake ($p < 0.001$) compared to controls. The mean ^{11}C-DASB binding to ^{123}I-ioflupane uptake ratio in the putamen was 0.779 (increased by 75.8% of the controls' mean) for the nondyskinetic group and 0.901 (increased by 103.4% of the controls' mean) for the patients with dyskinesias. There was a statistically significant difference ($p < 0.001$) in ^{11}C-DASB binding to ^{123}I-ioflupane uptake ratio in the putamen between the group of patients with and without dyskinesias. Higher ^{11}C-DASB to ^{123}I-ioflupane binding ratios correlated with longer disease duration for the 28 patients with PD ($r = 0.52$; $p < 0.01$).

Conclusions: Serotonin-to-dopamine transporter binding ratio increases as PD progresses and patients experience LIDs. Our findings suggest that, when the dopaminergic innervation in the striatum is critically low, the serotonergic system plays an important role in development of LIDs. ***Neurology*® 2016;86:1–7**

GLOSSARY

AIMS = Abnormal Involuntary Movement Scale; **BP** = binding potential; **^{11}C-DASB** = 5[^{11}C]-3-amino-4-(2-dimethylaminomethylphenyl-sulfanyl)-benzonitrile; **DAT** = dopamine transporter; **HAM-D** = Hamilton Depression Rating Scale; **^{123}I-ioflupane** = [^{123}I]N-w-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane; **LID** = L-dopa-induced dyskinesia; **MMSE** = Mini-Mental State Examination; **PD** = Parkinson disease; **ROI** = region of interest; **SERT** = serotonin transporter; **UPDRS** = Unified Parkinson's Disease Rating Scale; **VDR** = volume of distribution ratio.

Studies in the animal model of Parkinson disease (PD)[1] as well as in humans[2–4] have indicated that degeneration of dopaminergic presynaptic terminals in the striatum is critical in the development of L-dopa-induced dyskinesias (LIDs). Due to the progressive degeneration, striatal dopaminergic terminals lose their dopamine storage capacity and the ability to maintain a stable dopamine release rate in the synapse.[5]

Serotonergic terminals have been found capable of converting exogenous levodopa into dopamine, store it in synaptic vesicles, and release it in an activity-dependent manner.[6–9] The above studies propose that serotonergic terminals in the degenerating striatum are responsible for mishandling exogenous levodopa and exacerbating dyskinesia in the animal model[10–12] and PD.[13] Accordingly, the presence of dyskinesia could be a reflection of serotonergic over

Supplemental data at Neurology.org

From the Neurology Imaging Unit (A.-A.R., P.P.), Centre of Neuroinflammation and Neurodegeneration, Division of Brain Sciences, Hammersmith Campus, Imperial College London; Neurodegeneration Imaging Group (M.P.), Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London; and Radiological Sciences Unit (D.T.), Imperial College Healthcare NHS Trust, London, UK.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

dopaminergic terminals' activity. Nonetheless, the above mechanisms have not been fully understood in humans.

Dopamine transporter (DAT) and serotonin transporter (SERT) are densely located in the presynaptic terminals in the striatum.[14,15] The SPECT tracer [^{123}I]N-w-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane (^{123}I-ioflupane) and the PET ligand 5[^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (^{11}C-DASB) are in vivo specific markers of DAT[16] and SERT[17] availabilities. This study intended to assess the interaction of dopaminergic and serotonergic presynaptic mechanisms in the development of LIDs. We hypothesized that a SERT-to-DAT ratio in the striatum is related to the occurrence of LIDs and employed the above imaging techniques with ^{11}C-DASB and ^{123}I-ioflupane to assess this ratio.

METHODS

Standard protocol approvals, registrations, and patient consents. All participants provided their written consent in accordance with the Declaration of Helsinki. This study was reviewed and approved by the West London Research Ethics Committee, the Imperial College Joint Research Compliance Office, and the Administration of Radioactive Substances Advisory Committee, UK.

Participants. Patients with PD were recruited from our Movement Disorders Clinic at the Imperial College Healthcare NHS Trust, London, UK. Patients with PD fulfilled the UK Brain Bank Criteria for idiopathic PD.[18] At screening, patients with PD were on levodopa treatment for at least 2 years. Patients with a history of dementia or depression were excluded from this study. Clinical data were acquired by detailed medical history including medication history cross-checked with patients' medical notes and clinical letters to their general practitioners. Patients with PD were assessed in an outpatient clinical setting for their motor and nonmotor symptoms including the Unified Parkinson's Disease Rating Scale (UPDRS) and the modified Hoehn & Yahr staging scale.

All participants were assessed for depression using the Hamilton Depression Rating Scale (HAM-D) and for cognitive impairment using the Mini-Mental State Examination (MMSE). None of the patients with PD had a history of depression and HAM-D scores above 7 were an exclusion criterion. Patients with PD with cognitive impairment were excluded from this study; an MMSE score below 26 was an exclusion criterion.

Thirty-six patients with idiopathic PD were screened for enrollment in the study, 6 of whom failed one of the exclusion criteria, and 2 declined participation in the study. Twenty-eight patients with idiopathic PD were included in the study. Seventeen patients experienced LIDs and 11 did not experience LIDs.

Clinical evaluation. Presence of LIDs was then assessed on separate days within 1 hour after the patients had taken their usual levodopa dose (range of single dose: 100–200 mg). LIDs were scored using the Abnormal Involuntary Movement Scale (AIMS) every 15 minutes for the next 120 minutes.

The LED_{Total}, LED_{Ldopa}, and LED_{Dag} doses were calculated in milligrams for each individual following the formulas[13] described in table e-1 on the *Neurology*® Web site at Neurology.org. DD_{diagn} was defined as the time from the date of the PD diagnosis at the movement disorders clinic and DD_{onset} as the time since each individual first experienced a PD motor symptom. At the time of diagnosis, all patients with PD were classified as stage 1 on the modified Hoehn & Yahr staging scale. We also calculated the time from diagnosis to initiation of dopaminergic medication per individual. None of the patients with PD was treated with any drugs that directly act on the serotonergic system.

We also included 12 healthy controls who undertook the same imaging procedures as described below (table 1).

Scanning procedures. All participants had brain SPECT imaging with ^{123}I-ioflupane and brain PET imaging with ^{11}C-DASB. All participants also had a 1.5 T1-weighted MRI scan for coregistration to the PET imaging data.

Patients with PD were asked to withdraw from medication 18 hours prior to any procedure involving ionizing radiation and this was defined as the "off" dopaminergic medication state. All patients with PD were assessed for motor symptoms with the UPDRS part III scale in "off" dopaminergic medication state.

All imaging were performed at the Hammersmith and Charing Cross Hospitals of Imperial College in London.

^{11}C-DASB PET scan images were obtained with an EXAT EXACT HR+ scanner (Siemens; Munich, Germany) and details on the PET and MRI scanners have been described previously.[17] Briefly, patients with PD underwent a low-dose CT brain scan prior to the administration of the ^{11}C-DASB PET tracer to measure tissue attenuation of radiation. A mean activity dose of 450 MBq was administered to each individual undertaking a ^{11}C-DASB PET scan. PET images were obtained for 90 minutes starting after IV bolus injection of the PET tracer.

For the administration of ^{123}I-ioflupane, thyroid gland blockade was performed by administering potassium iodide tablets 60 mg twice daily for 3 consecutive days, starting 24 hours prior to the SPECT scan day, in accordance with the clinical protocol of our nuclear medicine department. All ^{123}I-ioflupane SPECT scan images were obtained with a Symbia T SPECT-CT scanner (Siemens). A mean activity dose of 185 MBq was administered to each individual undergoing a ^{123}I-ioflupane SPECT scan. SPECT images were obtained 180 minutes after IV bolus injection of ^{123}I-ioflupane for approximately 45 minutes.

^{11}C-DASB PET imaging data analysis. We have employed a simplified reference tissue model using the cerebellum as the reference tissue[19] for the quantification of ^{11}C-DASB. Following reconstruction of the dynamic PET image volume, a summed image volume was created from the entire dynamic dataset using an in-house software package. A template of high-contrast regions of interest (ROIs) was defined directly on the summed image and these ROIs were then applied to the dynamic dataset. Any movement detected was corrected using a frame-by-frame realignment procedure. Individual participant MRIs were coregistered to the summed PET volume using the SPM2 software package (Wellcome Department of Cognitive Neuroscience, Institute of Neurology) implemented in Matlab 6.5 (MathWorks; Natick, MA). Following coregistration, the definition of ROIs was performed on the coregistered MRI using Analyze medical imaging software (version 8.1; Mayo Foundation; Rochester, NY). ROIs were standardized for volume throughout and were manually defined for caudate and putamen. Volume of distribution ratios (VDRs) were computed

Table 1 Demographics and clinical characteristics of patients with PD and healthy controls

	Healthy controls	PD all	Non-LID	LID
No. of participants	12	28	11	17
Sex, M:F	7:5	19:9	10:1	9:8
Age at the time of the scan, y	61.41 ± 8.64	64.87 ± 8.21	69.32 ± 4.67	61.69 ± 8.89[a]
MMSE	29.7 ± 0.67	28.28 ± 1.22	28 ± 1.26	28.44 ± 1.20
HAM-D	1.8 ± 1.62	4.28 ± 1.25	4.27 ± 0.65	4.28 ± 1.53
Disease duration from diagnosis, y	—	7.89 ± 5.01	5.82 ± 4.88	9.56 ± 5.48[a]
Disease duration from onset, y	—	9.79 ± 4.99	7.82 ± 3.66	11.07 ± 5.41[a]
H&Y "off"	—	2.34 ± 0.57	2.27 ± 0.47	2.39 ± 0.63
UPDRS-III "off"	—	27.59 ± 8.32	26.7 ± 7.25	28.11 ± 9.07
UPDRS total "off"	—	45.03 ± 10.89	40.55 ± 10.30	47.78 ± 10.59
Tremor dominant/akinetic-rigid/mixed	—	2:15:11	2:5:4	0:10:7
AIMS	—	—	0	8.06 ± 4.26
Duration on dopaminergic medication, y	—	6.69 ± 4.68	4.41 ± 2.07	8.40 ± 5.07[a]
Time from diagnosis to initiation of DA medication, y	—	—	1.45 ± 1.05	1.03 ± 1.27
Time from PD onset to initiation of DA medication, y	—	—	3.42 ± 2.66	2.55 ± 1.60
Daily LED_{Total}, mg	—	—	537.59 ± 199.87	826.59 ± 350.70[a]
Daily LED_{Ldopa}, mg	—	—	376.68 ± 167.68	650.82 ± 369.69
Daily LED_{Dag}, mg	—	—	160.91 ± 193.87	175.76 ± 207.02

Abbreviations: AIMS = Abnormal Involuntary Movement Scale; DA = dopamine; H&Y = modified Hoehn & Yahr staging scale in "off" medication state; HAM-D = Hamilton Depression Rating Scale; LID = L-dopa-induced dyskinesia; MMSE = Mini-Mental State Examination; PD = Parkinson disease; UPDRS = Unified Parkinson's Disease Rating Scale.
Data represent mean ± 1 SD.
[a] $p < 0.05$ between the non-LID and the LID groups.

for ROIs with the graphical analysis method of Logan et al.[20] and the binding potential (BP) of the specifically bound PET tracer relative to the nondisplaceable tracer in tissue was calculated as VDR-1.[21] The specific SERT binding as reflected by ^{11}C-DASB BP was calculated for each caudate and putamen for both hemispheres. The average caudate and average putamen binding was calculated per individual as the mean uptake value for both hemispheres.

^{123}I-ioflupane SPECT imaging data analysis. A semiquantification analysis approach for each individual was used to quantify reconstructed tomographic imaging data. Acquired data were transferred to a HERMES workstation (HERMES Medical Solutions; Stockholm, Sweden) and reconstructed with attenuation, scatter, and resolution corrections. The reconstructed data were analyzed using BRASS software (HERMES Medical Solutions). The software uses automatic image registration to align the patient's image to a template. This contains a series of predefined volumes of interest that can be then applied to the image being analyzed. Following automatic alignment, all scans were inspected visually and manually realigned to fit to the predefined template where necessary. Uptake ratios were measured for each caudate and putamen relative to the nonspecific uptake measured from the occipital cortex. The uptake is defined as the specific binding ratio ([striatal counts − background counts]/background counts). The specific DAT binding as reflected by the ^{123}I-ioflupane uptake values was calculated for each caudate and putamen for both hemispheres. The average caudate and average putamen binding was calculated per individual as the mean uptake value for both hemispheres.

SERT-to-DAT ratios. We estimated SERT-to-DAT ratios as reflected by ^{11}C-DASB BP to ^{123}I-ioflupane uptake for the caudate and the putamen comprising the average caudate and average putamen uptake values for each individual. SERT-to-DAT ratios were calculated as described previously in patients with PD who received neural transplantation.[22]

Statistical analysis. Statistical analysis and graph illustration was performed using IBM (Armonk, NY) SPSS (version 22) and GraphPad (La Jolla, CA) Prism (version 5) software for Windows. For all variables, we performed normality tests for homogeneity and Gaussianity with Bartlett and Kolmogorov-Smirnov tests and then proceeded into parametric tests for all our values that were normally distributed and nonparametric tests where applicable. For correlations of the clinical values to the imaging values, we performed one-tailed Spearman correlations. For specific clinical characteristics between the groups of PD with and without LIDs, we calculated 2-tailed p values using unpaired t tests.

RESULTS

Clinical findings. The demographics and clinical characteristics of patients with PD and healthy controls are summarized in table 1.

Imaging data. The mean uptake of ^{123}I-ioflupane, ^{11}C-DASB BP, and the SERT-to-DAT ratios are listed in table 2. Representative ^{11}C-DASB PET

Table 2 Mean uptake values of ^{123}I-ioflupane, ^{11}C-DASB, and the SERT-to-DAT binding

	Healthy controls	PD all	Non-LID	LID
No. of participants	12	28	11	17
Region of interest				
^{11}C-DASB specific binding (SERT)				
Caudate	1.31 ± 0.06	0.58 ± 0.20[a]	0.62 ± 0.22	0.56 ± 0.18
Putamen	1.36 ± 0.11	0.90 ± 0.25[b]	0.86 ± 0.24	0.94 ± 0.22
^{123}I-ioflupane specific to nonspecific binding (DAT)				
Caudate	3.42 ± 0.44	1.98 ± 0.52[a]	2.10 ± 0.49	1.90 ± 0.53
Putamen	3.07 ± 0.28	1.26 ± 0.42[a]	1.51 ± 0.40	1.15 ± 0.33
SERT-to-DAT binding ratios				
Caudate	0.383	0.304	0.301	0.306
Putamen	0.443	0.851	0.779	0.901[c]

Abbreviations: ^{11}C-DASB = 5[^{11}C]-3-amino-4-(2-dimethylaminomethylphenyl-sulfanyl)-benzonitrile; DAT = dopamine transporter; ^{123}I-ioflupane = [^{123}I]N-w-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane; LID = L-dopa-induced dyskinesia; PD = Parkinson disease; SERT = serotonin transporter.
Data represent mean ± 1 SD. Mean values are calculated as an average for both hemispheres.
[a] $p < 0.01$ between the PD and the healthy controls group.
[b] $p < 0.05$ between the PD and the healthy controls group.
[c] $p < 0.001$ between the non-LID and LID groups.

and ^{123}I-ioflupane SPECT images at the level of dorsal basal ganglia in 2 patients with PD with and without LIDs are shown in figure e-1.

Patients with PD showed reduced ^{11}C-DASB BP ($p < 0.01$) in the putamen compared to healthy controls (34%). Patients with PD without LIDs showed 37% loss, while the patients with PD with LIDs showed 31% loss relative to the mean value of the healthy controls (between-group comparison for the putamen; $p = 0.19$) (figure 1A). No differences were found for the caudate (between-group comparison for the caudate; $p = 0.23$) (figure e-2).

Patients with PD showed reduced ^{123}I-ioflupane uptake values ($p < 0.001$) compared to healthy controls in the caudate and the putamen. Patients with PD without LIDs showed 51% loss, while patients with LIDs showed 62% loss relative to healthy controls (between-group difference for putamen; $p = 0.13$) (figure 1B). No differences were found for the caudate (between-group comparison for the caudate; $p = 0.16$) (figure e-3).

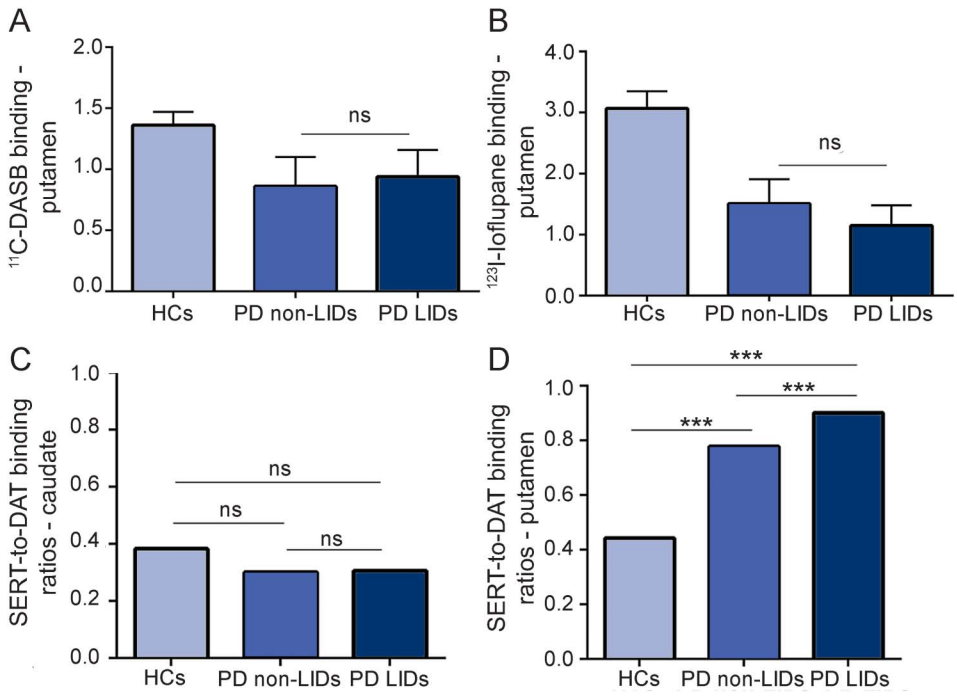
Healthy controls had a mean ^{11}C-DASB BP to ^{123}I-ioflupane uptake ratio in the putamen of 0.443. All patients with PD had increased ^{11}C-DASB BP to ^{123}I-ioflupane uptake ratio in the putamen compared to healthy controls ($p < 0.001$). In particular, patients with PD with LIDs had a mean ^{11}C-DASB BP to ^{123}I-ioflupane uptake ratio in the putamen of 0.901 (increased by 103.4% compared to healthy controls), while in the group of patients without LIDs, the mean ratio was 0.779 (increased by 75.8%, relative to healthy controls) with a significant between-group difference ($p < 0.001$) (figure 1D). No significant differences were found in the caudate for the ^{11}C-DASB BP to ^{123}I-ioflupane uptake ratios (figure 1C).

There was a statistically significant correlation between putaminal ^{11}C-DASB BP to ^{123}I-ioflupane uptake ratios and disease duration from diagnosis for all patients with PD ($r = 0.52$; $p < 0.01$) (figure 2).

No correlation was found between putaminal ^{11}C-DASB BP and either age, UPDRS, AIMS scores, the mean LED_{Total}, the mean LED_{Ldopa}, or the times from diagnosis to initiation of dopaminergic medication. No correlation was found between putaminal ^{123}I-ioflupane uptake values and either age, UPDRS, AIMS scores, the mean LED_{Total}, the mean LED_{Ldopa}, or the times from diagnosis to initiation of dopaminergic medication. No correlation was found between putaminal ^{11}C-DASB BP to ^{123}I-ioflupane uptake ratios and either age, UPDRS, AIMS scores, the mean LED_{Total}, the mean LED_{Ldopa}, or the times from diagnosis to initiation of dopaminergic medication.

DISCUSSION We found a statistically significant correlation between the putaminal SERT-to-DAT ratio and the disease duration in patients with PD. Our findings indicate that as PD progresses, the putaminal SERT-to-DAT ratio, as reflected by the ^{11}C-DASB BP to ^{123}I-ioflupane uptake ratio, becomes higher, thus potentially implicating an important role for the development of LIDs. We have shown that the density of DAT in the putamen of patients with PD with LIDs declines more than the SERT density compared to patients without LIDs. These data indicate that as disease

Figure 1 Histograms of mean uptake values of ^{123}I-ioflupane, ^{11}C-DASB binding, and serotonin transporter (SERT)-to-dopamine transporter (DAT) binding



(A) Histogram of mean ^{11}C-DASB binding for the putamen shown in 12 healthy controls (HCs), the Parkinson disease PD non–L-dopa-induced dyskinesia (LID) group (n = 11), and the PD LID group (n = 17). (B) Histogram of mean ^{123}I-ioflupane binding for the putamen shown in 12 HCs, the PD non-LID group (n = 11), and the PD LID group (n = 17). (C) Histogram of mean SERT-to-DAT binding ratios calculated for the caudate in 12 HCs, non-LID (n = 11), and LID (n = 17). (D) Histogram of mean SERT-to-DAT binding ratios calculated for the putamen in 12 HCs, non-LID (n = 11), and LID (n = 17). Data represent mean ± 1 SD. Mean values are calculated as an average for both hemispheres. ***Statistical significance ($p < 0.001$). NS = not significant.

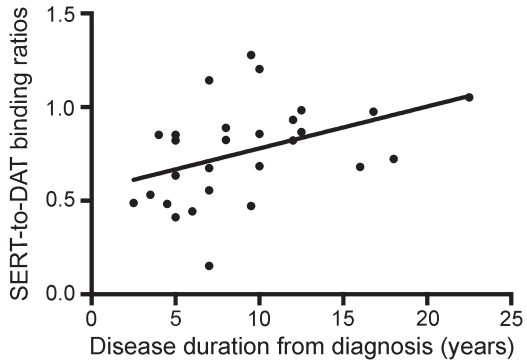
progresses, SERT over DAT availabilities increase in the striatum and that it is the ratio rather than the DAT/SERT components of it that may be important for the appearance of dyskinesias in advanced disease.

As PD progresses, reductions in striatal DAT density have been proposed to support presynaptic mechanisms as responsible for the development of LIDs due to the subsequent loss of dopamine storage capacity.[5] Patients with advanced PD lose their ability to maintain a stable rate of dopamine release in the striatum. Thus, the dopamine release is fairly dependent on the levodopa delivery rate into the synapse.

PET studies with ^{11}C-raclopride, which reflects postsynaptic dopamine D2 receptor distribution, are able to estimate in vivo the dopamine release in the striatum.[23] Tedroff et al.[2] have shown that the same dose of exogenous levodopa can induce higher levels of synaptic dopamine in advanced PD in comparison to early disease. Furthermore, standard levodopa doses can cause high swings in synaptic dopamine increases in PD with LIDs,[3] the magnitude of which correlated with higher dyskinesia scores.[4]

Wide fluctuations in the synaptic concentration of dopamine in patients with motor fluctuations have been also shown to precede clinically apparent LIDs[24] and were linked with longer disease duration and a younger age at onset.[25]

Figure 2 Correlation of serotonin transporter (SERT)–to-dopamine transporter (DAT) binding ratios and disease duration in 28 patients with Parkinson disease (PD)



Statistical analysis was performed with one-tailed Spearman correlation between disease duration from diagnosis (in years) and SERT-to-DAT binding ratios in the putamen of 28 patients with PD ($r = 0.521$, $p < 0.01$). Each point in the graph represents one patient with PD.

Alongside these dopaminergic mechanisms, serotonergic terminals in the striatum have been shown to be also involved in the development of LIDs.

In experimental animals, the chemical blockade of serotonin neurons as well as selective lesions in the serotonin terminals led to a dramatic reduction of the induced involuntary movements without counteracting levodopa's main effects.[10,12,26,27] In addition, serotonin receptor agonists have been shown to have antidyskinetic effects in both rodent and nonhuman primate models of dyskinesias.[12,28]

In humans, a recent clinical and PET imaging study from our group showed that buspirone, a 5-HT1a partial agonist, when administered acutely prior to levodopa in patients with PD with LIDs, is able to normalize levodopa-derived levels of synaptic dopamine and to alleviate dyskinesias.[12] Similarly, a recent phase I/IIa clinical trial in patients with PD[29] confirmed antidyskinetic effects of the 5-HT1a/5-HT1b partial agonist eltoprazine following the promising results of the same drug in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–treated macaques.[12]

Taken together, these studies suggest that the development of LIDs is dependent on compromised dopaminergic function and on aberrant serotonergic function in the striatum.

A recent imaging study estimated midbrain-SERT to striatal-DAT binding ratio in early-stage, drug-naive patients with PD. The authors showed that imbalanced ratios do not predict the development of LIDs in early stages of the disease, but hypothesized that imbalance ratios may occur at later stages of PD.[30]

Our study suggests that the putaminal SERT-to-DAT terminals ratio is a good index to quantify in vivo the serotonergic over dopaminergic terminals' activity. Using PET and SPECT imaging, we have shown that the presence of LIDs is related to an imbalanced serotonergic-over-dopaminergic terminals density ratio in the putamen. Our results indicate that while the striatum becomes critically depleted of the dopaminergic terminals with the progression of the disease, the serotonergic terminals remain reasonably preserved to uptake levodopa and release dopamine. Hence, the serotonergic terminals in the striatum may start to contribute to the development of dyskinesias once the dopaminergic innervation becomes critically low.

Previous studies looking for clinical risk factors in LIDs have shown that the occurrence of LIDs is linked with younger age at onset of PD,[31–33] as well as with longer disease duration.[32,34] The LIDs and non-LIDs groups of our PD cohort were clinically different (so that reached statistical significance) for sex, age, disease duration, the duration on dopaminergic medication, and LED_{Total}. We performed separate analysis corrected for sex and found similar but less significant results compared to our whole cohort (male and female). Our dyskinetic patients with PD had longer disease duration, had a younger age at onset, and were treated with higher daily levodopa doses. This may reflect that the 2 groups represent 2 different factions within our studied population, which may have had an influence on the SERT-to-DAT terminals ratio. Nonetheless, we did not find any correlation between the SERT-to-DAT binding ratios and the age at onset or the duration of dopaminergic medication. Hence, we propose that sex, age, and duration of dopaminergic medication may have not affected the outcome of our findings in this cohort and that disease duration may indeed be a good index to reflect the serotonergic over dopaminergic terminals' changes that occur in the striatum during PD progression.

Serotonin-to-dopamine transporter binding ratio increases as PD progresses and patients experience dyskinesias. Our findings support the hypothesis that the serotonergic terminals are involved in the occurrence of LIDs, once the dopaminergic innervation in the striatum is critically low. There may be a threshold during the progression of the disease that dyskinesias occur; nonetheless, this study makes it difficult to address this point. Future longitudinal studies with SERT and DAT imaging in a larger number of patients may be able to address this point by studying the time from initiation of dopaminergic medication to the first time dyskinesias are observed in relation to serotonergic terminals' activity. Future clinical trials with highly selective 5-HT1A/B drugs as antidyskinetic agents could also provide robust support to the above findings.

AUTHOR CONTRIBUTIONS

P.P. and M.P. conceptualized the experimental design of the study. A.A.R., M.P., D.T., and P.P. organized the study. A.A.R. acquired the data. A.A.R. analyzed the clinical data. A.A.R. and D.T. analyzed the imaging data. A.A.R. and M.P. conducted the statistical analysis. A.A.R. wrote the first draft of the manuscript and interpreted the findings. A.A.R. and P.P. were responsible for the clinical supervision of the patients. All authors gave input and revised the manuscript.

ACKNOWLEDGMENT

The authors thank the participants and their families; the medical physicists and the nuclear medicine radiographers from the Imperial College Healthcare NHS Trust; and the UK Medical Research Council for providing infrastructure and funding part of the imaging studies.

STUDY FUNDING

Supported by a grant awarded by the Michael J. Fox Foundation for Parkinson's Research. Part of the imaging studies was funded by the UK Medical Research Council.

DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received July 23, 2015. Accepted in final form December 1, 2015.

REFERENCES

1. Winkler C, Kirik D, Björklund A, Cenci MA. L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of Parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. Neurobiol Dis 2002; 10:165–186.
2. Tedroff J, Pedersen M, Aquilonius SM, Hartvig P, Jacobsson G, Långström B. Levodopa-induced changes in synaptic dopamine in patients with Parkinson's disease as measured by [11C]raclopride displacement and PET. Neurology 1996;46:1430–1436.
3. de la Fuente-Fernández R, Sossi V, Huang Z, et al. Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias. Brain 2004;127:2747–2754.
4. Pavese N, Evans AH, Tai YF, et al. Clinical correlates of levodopa-induced dopamine release in Parkinson disease: a PET study. Neurology 2006;67:1612–1617.
5. Leenders KL, Palmer AJ, Quinn N, et al. Brain dopamine metabolism in patients with Parkinson's disease measured with positron emission tomography. J Neurol Neurosurg Psychiatry 1986;49:853–860.
6. Ng KY, Chase TN, Colburn RW, Kopin IJ. Dopa-induced release of cerebral monoamines. Science 1970; 170:76–77.
7. Tanaka H, Kannari K, Maeda T, Tomiyama M, Suda T, Matsunaga M. Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. Neuroreport 1999;10:631–634.
8. Maeda T, Nagata K, Yoshida Y, Kannari K. Serotonergic hyperinnervation into the dopaminergic denervated striatum compensates for dopamine conversion from exogenously administered l-DOPA. Brain Res 2005;1046: 230–233.
9. Kannari K, Shen H, Arai A, Tomiyama M, Baba M. Reuptake of L-DOPA-derived extracellular dopamine in the striatum with dopaminergic denervation via serotonin transporters. Neurosci Lett 2006;402:62–65.
10. Carta M, Carlsson T, Kirik D, Björklund A. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. Brain 2007;130: 1819–1833.
11. Rylander D, Parent M, O'Sullivan SS, et al. Maladaptive plasticity of serotonin axon terminals in levodopa-induced dyskinesia. Ann Neurol 2010;68:619–628.
12. Bézard E, Tronci E, Pioli EY, et al. Study of the antidyskinetic effect of eltoprazine in animal models of levodopa-induced dyskinesia. Mov Disord 2013;28:1088–1096.
13. Politis M, Wu K, Loane C, et al. Serotonergic mechanisms responsible for levodopa-induced dyskinesias in Parkinson's disease patients. J Clin Invest 2014;124:1340–1349.
14. Ciliax BJ, Heilman C, Demchyshyn LL, et al. The dopamine transporter: immunochemical characterization and localization in brain. J Neurosci 1995;15:1714–1723.
15. Kish SJ, Furukawa Y, Chang LJ, et al. Regional distribution of serotonin transporter protein in postmortem human brain: is the cerebellum a SERT-free brain region? Nucl Med Biol 2005;32:123–128.
16. Piccini P. Dopamine transporter: basic aspects and neuroimaging. Mov Disord 2003;18(suppl 7):S3–S8.
17. Politis M, Wu K, Loane C, et al. Staging of serotonergic dysfunction in Parkinson's disease: an in vivo 11C-DASB PET study. Neurobiol Dis 2010;40:216–221.
18. Hughes AJ, Ben-Shlomo Y, Daniel SE, Lees AJ. What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study. Neurology 1992;42:1142–1146.
19. Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. Neuroimage 1997;6:279–287.
20. Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL. Distribution volume ratios without blood sampling from graphical analysis of PET data. J Cereb Blood Flow Metab 1996;16:834–840.
21. Ginovart N, Wilson AA, Meyer JH, Hussey D, Houle S. Positron emission tomography quantification of [(11)C]-DASB binding to the human serotonin transporter: modeling strategies. J Cereb Blood Flow Metab 2001;21: 1342–1353.
22. Politis M, Oertel WH, Wu K, et al. Graft-induced dyskinesias in Parkinson's disease: high striatal serotonin/dopamine transporter ratio. Mov Disord 2011;26:1997–2003.
23. Piccini P, Brooks DJ, Björklund A, et al. Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient. Nat Neurosci 1999;2:1137–1140.
24. de la Fuente-Fernández R, Lu JQ, Sossi V, et al. Biochemical variations in the synaptic level of dopamine precede motor fluctuations in Parkinson's disease: PET evidence of increased dopamine turnover. Ann Neurol 2001;49:298–303.
25. Sossi V, de la Fuente-Fernández R, Schulzer M, Adams J, Stoessl J. Age-related differences in levodopa dynamics in Parkinson's: implications for motor complications. Brain 2006;129:1050–1058.
26. Nevalainen N, Af Bjerkén S, Gerhardt GA, Strömberg I. Serotonergic nerve fibers in L-DOPA-derived dopamine release and dyskinesia. Neuroscience 2014;260:73–86.
27. Conti MM, Ostock CY, Lindenbach D, et al. Effects of prolonged selective serotonin reuptake inhibition on the development and expression of L-DOPA-induced dyskinesia in hemi-parkinsonian rats. Neuropharmacology 2014;77:1–8.
28. Muñoz A, Li Q, Gardoni F, et al. Combined 5-HT1A and 5-HT1B receptor agonists for the treatment of L-DOPA-induced dyskinesia. Brain 2008;131:3380–3394.
29. Svenningsson P, Rosenblad C, Af Edholm Arvidsson K, et al. Eltoprazine counteracts l-DOPA-induced dyskinesias in Parkinson's disease: a dose-finding study. Brain 2015; 138:963–973.
30. Suwijn SR, Berendse HW, Verschuur CV, Winogrodzka A, de Bie RM, Booij J. SERT-to-DAT ratios in early Parkinson's disease do not correlate with the development of dyskinesias. EJNMMI Res 2013;3:44.
31. Grandas F, Galiano ML, Tabernero C. Risk factors for levodopa-induced dyskinesias in Parkinson's disease. J Neurol 1999;246:1127–1133.
32. Wickremaratchi MM, Knipe MD, Sastry BS, et al. The motor phenotype of Parkinson's disease in relation to age at onset. Mov Disord 2011;26:457–463.
33. Olanow WC, Kieburtz K, Rascol O, et al. Factors predictive of the development of Levodopa-induced dyskinesia and wearing-off in Parkinson's disease. Mov Disord 2013; 28:1064–1071.
34. Schrag A, Quinn N. Dyskinesias and motor fluctuations in Parkinson's disease: a community-based study. Brain 2000;123:2297–2305.