Positron Emission Tomography of Striatal Serotonin Transporters in Parkinson Disease

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Background: Little is known about serotonin neurons in Parkinson disease (PD).

Objective: To study the serotonin system in PD with positron emission tomography, using the serotonin transporter radioligand [11C](+)McN5652.

Design and Patients: We measured the density of the serotonin transporter and the density of [11C]WIN35428–labeled dopamine transporters in the striatum of 13 adults with PD and 13 age- and sex-matched controls. To assess the effects of possible differences in blood flow or brain atrophy, we also measured regional cerebral blood flow and the size of the regions of interest for the caudate nucleus and putamen.

Results: Patients with PD showed reductions in the specific distribution volumes of [11C](+)McN5652 in the caudate (P<.01) and putamen (P<.01), along with the expected reductions in striatal [11C]WIN35428 binding (P<.01). There were no reductions in regional cerebral blood flow or the sizes of the regions of interest, mitigating against potential confounding effects of blood flow, brain atrophy, or partial volume effects. Reductions in serotonin transporter binding correlated with ratings of disease staging.

Conclusions: These results suggest that the density of serotonin transporters, like that of dopamine transporters, is reduced in the striatum of patients with PD and that these changes are related to disease stage.

Arch Neurol. 2003;60:1223-1229

ARCH NEUROL. 2003;60:1223-1229

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to 75 years (mean ± SD, 65.2 ± 7.6 years), had completed 13.8 ± 4.2 years of education, and included 6 men and 7 women. Patients with PD were recruited from the Johns Hopkins Bayview Medical Center Movement Disorder Clinic (Baltimore, Md), where the diagnosis of idiopathic PD was made based on the clinical criteria described by Hughes et al.17 In addition, 13 NCS, who ranged in age from 38 to 76 years (mean ± SD, 63.0 ± 11.5 years), had completed 13.8 ± 2.4 years of education, and included 7 men and 6 women, participated in the study.

All of the PD patients and NCS were administered the Hamilton Rating Scale for Depression22 and the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.21 None of the patients or control subjects was thought to have major depression, dementia, current alcohol abuse, or any other Axis I mental disorder. Any participant who earned a score of less than 24/30 on the Mini-Mental State Examination20 was excluded. All of the patients underwent neurologic and physical examinations. Disease staging was assessed with the Hoehn-Yahr Scale (HYS).23 On this basis, 5 patients were rated as being in stage 1, 4 in stage 2, 2 in stage 3, and 2 in stage 4. Disease severity, assessed while most patients (12 of 13) were taking antiparkinsonian medication, was determined using the Unified Parkinson’s Disease Rating Scale.21 Finally, each patient completed a self-rating scale of competence for activities of daily living.23 Twelve of the 13 patients with PD were receiving treatment with 1 or more antiparkinsonian medications at study entry; 1 patient was drug naive. Medications included levodopa/carbidopa (n = 7), selegiline (n = 11), and pramipexol (n = 2). None of the patients had taken any medication for at least 12 hours before the PET study. No participants were using medications known to cause persistent alterations in the density of the SERT or DAT. The study was approved by the Johns Hopkins University institutional review board, and all subjects gave written informed consent to participate.

**IMAGING PROCEDURES**

To ensure the reproducibility of positioning for the 3 PET studies, a custom-made face mask was fitted to each participant’s head and attached to the head-holder during magnetic resonance imaging (MRI) and PET. Magnetic resonance imaging was performed to position the subject’s head and delineate the regions of interest (ROIs) using a GE Sigma 1.5-T scanner (GE Medical Systems, Milwaukee, Wis).14

During each PET session, all participants received 3 injections, 1 with [11C](+McN5652, 1 with [11C]WIN 35 428, and 1 with [15O]H2O. The average injected dose and specific activity was 19 mCi (703 MBq) and 6800 mCi/µmol (251600 MBq/µmol) for [11C](+McN5652, and 19 mCi (703 MBq) and 4700 mCi/µmol (173 900 MBq/µmol) for [11C]WIN 35 428. The injected dose for [15O]H2O was 75 mCi (2775 MBq). A GE 4096Plus Whole Body PET Scanner was used for imaging, and 15 simultaneous slices, spaced 6.5 mm apart, were acquired. The transaxial (in-plane) resolution was 5.4 mm (full width at half maximum), with an average axial resolution of 6 mm (full width at half maximum) in the center of the field of view. A transmission scan was obtained with a 10-mCi (370-MBq) 68Ge/68Ga pin. Source for 10 minutes and was used for attenuation correction of the PET images. First, the WIN35428 tracer was injected. [15O]H2O was injected after the first study ended. [11C](+McN5652 was injected 30 minutes after the end of the [15O]H2O study.

Eighteen serial dynamic PET images were acquired after the [11C](+McN5652 injection was given during 95 minutes.14 Seventeen PET scans were obtained during a 75-minute injection with [11C]WIN 35 428. After the [15O]H2O injection, 41 serial PET images were acquired in 300 seconds. After both the [11C](+McN5652 and [11C]WIN 35 428 injections, arterial blood samples were drawn manually to obtain the input function for compartmental analysis. The input function was corrected for metabolized radioligand activity.24

After the [15O]H2O injection, arterial blood was drawn from the radial artery using an automated blood sampler pump and propelled through a coincidence counting device cross-calibrated with the PET scanner. To determine time activity curves from ROIs, PET and MRI images were converted to NetCDF/MINC format (http://www.hic.mni.mcgill.ca/software/minc/minc.html); coregistration was achieved with the Automated Image Registration algorithm software package.25 Regions of interest were drawn on a single slice, which was selected based on the judgment that it contained the best representation of that particular brain area. Regions of interest were drawn for the following brain regions: head of caudate, putamen, cerebellum (Figure 1), and thalamus (not shown). WIN35428 and McN5652 images were coregistered using the same MRI image. The same ROIs were used for both tracers, and fitting was checked visually in each case by codisplay of the PET and MRI images with the ROIs.

Striatal binding of [11C]WIN35428 was expressed by the ROI/cerebellum tissue activity ratio27 between 55 and 75 minutes after injection. [11C](+McN5652 binding was quantified with the (apparent) total distribution volume that was derived from a 1-compartment, 3-parameter model.14 The distribution volume of specific binding (DVsp) was calculated as28 DVsp = (DVtot - DVcer)/DVcer, where DVcer is the radioligand distribution volume in the cerebellum. Using the PET scans of the [15O]H2O study, regional cerebral blood flow was calculated from tissue activity curves derived using the same ROIs as for the radioligand studies. Before fitting these tissue curves, the arterial input function was corrected for bolus delay and dispersion.29

**STATISTICAL ANALYSIS**

The primary hypothesis of the study was that patients with PD would have significant reductions in the DAT and SERT compared with age- and sex-matched NCS. A secondary hypothesis was that reductions in the DAT and SERT would be positively correlated with the stage of illness (HYS). Prior to hypothesis testing, t tests were conducted between the PD and NC groups on the following variables: age; years of education; Mini-Mental State Examination scores; and the mean number of voxels in the various ROIs. To test the primary hypothesis that striatal DAT and SERT binding are reduced in PD, analyses of covariance were conducted, covarying for age. A probability of P < .05 was set as the cutoff for statistical significance, with a correction for multiple test variables to control for a type I error.20 Pearson correlation analyses were used to assess relationships between disease severity and SERT and/or DAT binding. Data are given as mean ± SD unless otherwise indicated.

**RESULTS**

The PD and NC groups did not differ significantly with respect to age (P = .58), years of education (Mini-Mental State Examination score, 28.1 ± 1.6; P = .57), or sex distribution. Figure 1 shows ROIs derived from MRIs to coregister with PET imaging. Comparisons revealed that the patients with PD and the NCS did not differ in the sizes of their respective caudate nuclei (1.19 ± 0.20 vs 1.14 ± 0.19 mL), putamen (2.16 ± 0.34 vs 2.25 ± 0.27 mL), or cerebellum (10.12 ± 1.84 vs 10.34 ± 1.73 mL) (all P values > .10). Thus, the PD patients did not demonstrate...
evidence of atrophic changes in these structures on any of the MRI measurements.

$[^{11}\text{C}]^{(+)}\text{McN5652 BINDING}$

Binding of $[^{11}\text{C}]^{(+)}\text{McN5652}$ was reduced in the caudate of subjects with PD (Table 1 and Figure 2). The distribution volume of the cerebellum, used to assess non-specific binding of $[^{11}\text{C}]^{(+)}\text{McN5652}$, was not statistically different between NCs and PD patients (NC, 21.0±7.0; PD, 18.4±3.9; F=1.77; P=.12), and the covariance effect of age on cerebellar distribution volume was not significant (F=1.77; P=.12). The hypothesized reduction of the SERT in the striatum of patients with PD, as measured by $[^{11}\text{C}]^{(+)}\text{McN5652}$ binding (distribution volume of specific binding), was compared with a P value of 0.4, adjusted for multiple comparisons and correlations.30 The covariance effect of age was not significant (F=0.17; P=.68), but the difference between the NCs (1.39±0.82) and PD patients (0.69±0.30) in the caudate SERT density was significant (F=7.97; P=.01). Similarly, in the putamen, the distribution volume of specific binding of $[^{11}\text{C}]^{(+)}\text{McN5652}$ was reduced in PD patients (NC: 1.50±0.58; PD: 0.98±0.31; F=7.52; P=.01), with no significant covariance effect of age (F=1.52; P=.23) (Table 1). Unlike DAT, SERT binding was not disproportionately reduced in striatal nuclei contralateral to the more affected side of the body among PD patients with asymmetric tremor or rigidity (all P values >.5). There was also a trend for reduced SERT binding in the thalami (NC: 1.15±0.69; PD: 0.86±0.35); however, the difference did not reach the expected level of significance (P=.1).

$[^{11}\text{C}]\text{WIN35428 BINDING}$

The binding of $[^{11}\text{C}]\text{WIN35428}$ to the DAT was tested by the general linear model using diagnosis as a refer-
As expected, the correlation of [11C]WIN35428 binding with HYS was negative (P = .02). No correlation was found between the binding parameters of both ligands in the putamen (part of the striatum) and CPU with the HYS, the Unified Parkinson’s Disease Rating Scale, and activities of daily living (all values > .05), although the correlations between the clinical scores on HYS, the Unified Parkinson’s Disease Rating Scale, and activities of daily living were all significant (r = .84-.92; P < .001).


The hypothesis that reductions in DAT and SERT binding would correlate with a more advanced stage of illness was tested by the Spearman correlation of the binding of these ligands in the putamen (part of the striatum that is more affected in PD than the caudate nucleus) with the HYS. In the 13 PD patients, [11C]-(+)McN5652 demonstrated a negative correlation with HYS (r = -.57; P = .04) and a positive correlation with [11C]WIN35428 binding (r = .57; P = .04). The correlation between [11C]-(+)McNM5652 binding and [11C]WIN35428 binding was even more significant when NCs and PD patients were combined (r = .58; P = .002) (Figure 3). As expected, the correlation of [11C]WIN35428 binding with HYS was negative (r = -.62; P = .02). No correlation was found between the binding parameters of both radioligands with the Unified Parkinson’s Disease Rating Scale and activities of daily living (all P values > .05), although the correlations between the clinical scores on HYS, the Unified Parkinson’s Disease Rating Scale, and activities of daily living were all significant (r = .84-.92; P < .001).

### CEREBRAL BLOOD FLOW AND LIGAND UPTAKE

Striatal cerebral blood flow did not differ significantly between NCs and PD patients (43.1 ± 6.9 vs 49.1 ± 7.6 mL/100 g per minute, respectively; P > .05). Similarly, the group differences in the ligand uptake parameter, K1, were not significant in the caudate (PD: 0.29 ± 0.05 mL/mL of tissue; NC: 0.29 ± 0.06 mL/mL of tissue; P = .78 for main effect; P = .99 for covariance effect of age) and putamen (PD: 0.35 ± 0.05 mL/mL of tissue; NC: 0.35 ± 0.07 mL/mL of tissue; P = .92 for main effect; P = .97 for covariance effect of age).

Our results indicate that patients with PD have reduced specific distribution volumes of [11C]-(+)McN5652 in the caudate and putamen, along with the expected reduction in striatal [11C]WIN35428 binding. Further, no reductions in regional cerebral blood flow or the sizes of the various ROIs were observed in PD patients, mitigating against potential confounding effects of blood flow, brain atrophy, and partial volume averaging. Taken together, these findings suggest that the density of SERTs, similar to the density of DATs, is reduced in the striatum of patients with PD, presumably reflecting the loss of serotonergic and dopaminergic innervation, respectively.

Patients with PD showed the expected decrease in DAT binding in the striatum. These results are in agreement with other studies wherein DAT binding decreases in PD and have been found to range from 20% to 50% in the caudate and from 40% to 70% in the putamen. Notably, patients with asymmetric tremor or rigidity showed a 34% greater decrease in DAT binding in the putamen region contralateral to the most clinically affected side of the body, which is also similar to earlier reports. Also consistent with earlier reports, we found that reductions in striatal DAT binding correlated with more advanced disease, as measured by the HYS. The present study extends these findings by providing preliminary evidence that reductions in striatal SERT binding also correlate with disease stage. Given the small number of subjects included in the analysis (n = 13), additional studies will be necessary to reach definitive conclusions in this regard.

A potential drawback of the neuroimaging methods we employed was the use of [11C]-(+)McN5652, a PET radioligand that demonstrates considerable nonspecific binding even in the striatum. Nonspecific binding could minimize the assessed contrast between the 2 groups of subjects and result in an underestimation of differences. The effect of nonspecific binding was minimized, using the cerebellum as a reference region. Normalization included both subtraction of and division by the cerebellar distribution volume of striatal total distribution volume values, attempting to account for effects of both parameter bias and variance. Although minimal but measurable specific binding in the cerebellum does exist, using the cerebellum to correct for nonspecific binding appeared to be justified since the difference of cerebellar distribution volume between patients and NCs was
not significant. The cerebellar $K_v$ was 14% lower in the PD than in the NC group; if this effect results in a bias, the actual radioligand binding in PD subjects would be 14% lower than calculated, and it could falsely blunt but not augment the difference between the 2 groups.

The potential role of antiparkinsonian medications deserves comment. Although subjects had not taken their antiparkinsonian medications for at least 12 hours at the time of their PET studies, their chronic use prior to these studies could have potentially influenced DAT and/or SERT binding characteristics. Importantly, preclinical studies do not support the view that these medications lead to alterations in monoamine transporter binding or affinity.37-39 The limited available data collected in humans is conflicting regarding this issue.40,41 While it cannot be stated unequivocally that previous chronic medication use did not alter DAT or SERT binding, significant correlations between disease staging and transporter binding suggest that potential medication-related effects on transporter binding were not robust.

Another potential methodologic issue related to medication use is that at the time PD patients underwent clinical evaluation to assess the severity of their disease, they were using antiparkinsonian medications. This could potentially have confounded measures relating to severity of illness. However, despite the use of medications at the time of clinical assessment, significant correlations between striatal $[^{11}C]WIN35428$ and $[^{11}C](+)$

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**Figure 2.** Radioligand binding to the serotonin transporter and dopamine transporter in a normal control (NC) subject and in a patient with Parkinson disease (PD). Dopamine transporter binding was imaged 55 to 75 minutes after injection of $[^{11}C]WIN35428$, and the image was normalized to a minimum of 0 and a maximum activity concentration of 150 nCi/mL per millicurie (injected dose). Serotonin transporter binding was imaged 55 to 95 minutes after injection of $[^{11}C]McN(+)$5652, and the image was normalized to a minimum of 0 and a maximum activity concentration of 100 nCi/mL per millicurie (injected dose).
McN5652 binding and disease stage were noted, suggesting that medication did not mask disease staging. Although data from the present study indicate that both dopaminergic and serotonergic systems are altered in PD, the pattern of changes in the 2 systems differs. In particular, group differences in DAT binding were much greater in the putamen than in the caudate nucleus, and the contralateral-ipsilateral ratio of DAT binding in the putamen (but not the caudate) was strongly related to asymmetry of clinical signs and symptoms. Conversely, group differences in SERT binding were greater in the putamen than in the caudate, and the contralateral-ipsilateral ratio of striatal SERT binding did not relate to asymmetry of clinical presentation. These findings suggest that the disease process affects DA and 5-HT neurons differently. Whether this is due to differences in the topography of the 2 systems or to some other factor remains to be determined.

In conclusion, our findings indicate that the density of striatal SERT, similar to that of DAT, is reduced in PD, and that reductions in SERT correlate with disease stage. Additional studies are needed to identify the functional consequences of loss of serotonergic innervation in PD and to identify potential interactions between DA and 5-HT neurons in the context of the disease process.

Accepted for publication May 9, 2003.

Author contributions: Study concept and design (Drs Ricaurte, McCann, and Szabo); acquisition of data (Drs Kerenyi, Schretlen, McCann, Mathews, Ravert, Dannals, Hilton, Wong, and Szabo); analysis and interpretation of data (Drs Kerenyi, Schretlen, McCann, Varga, Hilton, and Szabo); drafting of the manuscript (Drs Kerenyi, Ricaurte, Schretlen, and Szabo); critical revision of the manuscript for important intellectual content (Drs Ricaurte, Schretlen, McCann, Varga, Mathews, Ravert, Dannals, Hilton, Wong, and Szabo); statistical expertise (Drs Schretlen, Varga, and Hilton); obtained funding (Drs McCann and Szabo); administrative, technical, and material support (Drs Kerenyi, Mathews, Ravert, Dannals, Wong, and Szabo); study supervision (Drs McCann and Szabo).

This research was supported by US Public Health Service awards AG 14400 (Dr Szabo), DA 05938 (Dr Ricaurte), DA 10217 (Dr Ricaurte), DA 09482 (Dr Wong), and NS 38927 (Dr Wong).

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