One-Day Protocol for Imaging of the Nigrostriatal Dopaminergic Pathway in Parkinson's Disease by [^{123}I]FPCIT SPECT

Jan Booij, Jos T.G.M. Hemelaar, Johannes D. Speelman, Kora de Bruin, Anton G.M. Janssen and Eric A. van Royen

Graduate School of Neurosciences, Departments of Nuclear Medicine and Neurology, Academic Medical Center, University of Amsterdam, Amsterdam; Amersham Cygne and Eindhoven University of Technology, Eindhoven, The Netherlands

Parkinson's disease is characterized by degeneration of dopaminergic neurons, resulting in loss of dopamine transporters in the striatum. Recently, the tracer ^{123}I-*N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane (FPCIT) was developed for imaging dopamine transporters with SPECT. The purpose of this study was to develop an [^{123}I]FPCIT SPECT protocol for routine clinical studies. **Methods:** We examined the time course of [^{123}I]FPCIT binding to dopamine transporters in 10 healthy volunteers and 19 patients with Parkinson's disease. **Results:** We found that the time of peak specific striatal [^{123}I]FPCIT binding was highly varied among subjects, but specific binding peaked in all controls and patients within 3 h postinjection. Between 3 and 6 h, the ratio of specific-to-nonspecific striatal [^{123}I]FPCIT binding was stable in both groups, although, as expected, it was significantly lower in patients. In the patients, [^{123}I]FPCIT binding in the putamen was lower than in the caudate nucleus, and contralateral striatal binding was significantly lower than ipsilateral striatal binding. The subgroup of patients with hemi-Parkinson's disease showed loss of striatal dopamine transporters, even on the ipsilateral side. **Conclusion:** For routine clinical [^{123}I]FPCIT SPECT studies, we recommend imaging at a single time point, between 3 and 6 h postinjection, and using a tissue ratio as the outcome measure. The [^{123}I]FPCIT SPECT technique is sensitive enough to distinguish control subjects from patients with Parkinson's disease, even at an early stage of the disease.

Key Words: dopamine transporter imaging; SPECT; Parkinson's disease; [^{123}I]FPCIT

J Nucl Med 1999; 40:753–761

The main neuropathological feature of Parkinson's disease is a loss of dopaminergic neurons in the substantia nigra pars compacta. The lack of dopaminergic innervation to the striatum is believed to be responsible for parkinsonian symptoms such as resting tremor, rigidity, bradykinesia and postural instability (*1,2*).

Visualization and quantification of dopaminergic neurons in the living human brain by means of imaging techniques such as PET or SPECT facilitate the detection of Parkinson's disease, even at an early stage of the disease (*3–6*). Moreover, these techniques may be used to assess the rate of progression of Parkinson's disease (*7*) and to detect its presymptomatic period. Finally, these techniques may be used to monitor neuroprotective intervention aimed at attenuating the degeneration of dopaminergic neurons.

The dopamine transporter is considered a reliable marker of dopaminergic neurons. The plasma membrane dopamine transporter is located on the presynaptic nerve terminal and is specific to dopamine neurons. It plays an important role in terminating dopaminergic transmission by transporting dopamine from the synaptic cleft into the dopaminergic neuron. The dopamine transporter also acts as a gate for several neurotoxins (notably 6-hydroxydopamine and 1,2,3,6-tetrahydro-1-methyl-4-phenylpyridine) that destroy dopaminergic neurons. Postmortem binding studies have confirmed the loss of striatal dopamine transporters in Parkinson's diseased brain (*8*).

Recently, radiotracers for imaging of the dopamine transporter were introduced successfully. PET, used with ^{11}C-nomifensine or ^{11}C-labeled cocaine analogs, showed a loss of striatal dopamine transporters in patients with Parkinson's disease (*3,6,9*). SPECT with ^{123}I-labeled cocaine analogs also showed a dramatic loss of striatal dopamine transporters in patients with Parkinson's disease with high signal-to-noise ratios (*5,10–13*).

^{123}I-*N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) nortropane (FPCIT) is a new cocaine analog with a high affinity for the dopamine transporter (*14–16*). Studies in mouse, rat and monkey brain showed that the striatal FPCIT activity is the result of binding to the dopamine transporter (*16–18*). These studies also showed a rapid peak of striatal FPCIT binding. Human studies have emphasized that, because of the fast kinetics of FPCIT, the [^{123}I]FPCIT SPECT technique allows a 1-d protocol for imaging of dopamine transporters in humans, which is convenient for routine clinical studies (*5,17,19,20*). Using such a 1-d protocol, several studies reported a significant decrease of striatal [^{123}I]FPCIT binding in patients with Parkinson's disease compared with control subjects.

Received Apr. 1, 1998; revision accepted Sep. 25, 1998.

For correspondence or reprints contact: Jan Booij, MD, Department of Nuclear Medicine, F2N, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

In clinical SPECT studies, short scanning sessions are preferred, as well as a simple quantification procedure, e.g., a tissue ratio determined at a single point of time after bolus injection of the radiotracer. The purpose of this study was to develop an [^{123}I]FPCIT SPECT protocol suitable for routine clinical studies. Therefore, we examined the time course of [^{123}I]FPCIT binding in large groups of healthy volunteers and patients with Parkinson's disease in different disability stages. Moreover, we compared alterations in the ratios of specific-to-nonspecific striatal [^{123}I]FPCIT binding in the striatum of patients with those of healthy volunteers and correlated these alterations with clinical data.

MATERIALS AND METHODS

Subjects

Ten healthy volunteers and 19 patients with clinically established Parkinson's disease according to the UK Parkinson's Disease Society Brain Bank (*21*) participated in this study. The volunteers (9 women, 1 man) were considered free of medical illness on the basis of screening by medical history, physical examination, serum chemical analysis, complete blood cell count, urine analysis and a standard 12-lead electrocardiogram. The volunteers (mean age 53 y, range 41–67 y) were age-matched with the group of patients with Parkinson's disease (1 woman, 18 men; mean age 57 y, range 47–69 y), because ^{123}I-2ß-carbomethoxy-3ß-(4-iodophenyl)tropane ([^{123}I]ß-CIT) SPECT investigations in healthy human subjects showed reductions in tracer uptake with increasing age that are compatible with postmortem findings of dopamine transporter loss (*22*).

A neurological examination of the patients was performed to assess the stage of illness according to the Hoehn and Yahr staging scale (*23*). Medicated patients underwent a 12-h "drug holiday" to assess a standardized off-status (*24*). In patients with severe on/off fluctuations, the motor signs were scored during the worst off period (defined according to Langston et al. [*24*]). Five patients were rated as Hoehn and Yahr stage I, 10 patients as stage II, 3 patients as stage III and 1 as stage IV. Apart from their parkinsonian symptoms, all patients were considered free of medical illness on the basis of the same screening applied to the volunteers.

For safety measurements, all clinical analyses were repeated after injection of the radiotracer and compared with baseline (screening) scores.

Three patients were drug-naive before radiotracer administration; we rated these patients as Hoehn and Yahr stage I. The remaining 16 patients were allowed to take antiparkinsonian medications on the day of tracer administration, with L-3,4-dihydroxyphenylalanine (L-DOPA)/decarboxylase inhibitors of various doses (n = 13), sometimes in combination with selegiline (L-(-)deprenyl) (n = 8), dopamine agonists (n = 8), amantadine (n = 1) or β-blockers (n = 2) and anticholinergic drugs (n = 2). One patient was treated with only L-(-)deprenyl and 1 with only trihexyphenidylchloride. Finally, 1 patient was treated with amantadine, dopamine agonists and a β-blocker at the time of the SPECT experiments.

The medical ethics committee of the Academic Medical Center, University of Amsterdam (Amsterdam, The Netherlands), gave permission for the study. All participants gave written informed consent.

SPECT Camera

For the SPECT studies, a brain-dedicated SPECT system, the Strichman Medical Equipment 810X (Strichman Medical Equipment, Inc., Medfield, MA), was used. The Strichman camera consists of 12 crystals, each equipped with a focusing collimator. The transaxial resolution of this camera is 7.6 mm full width at half maximum of a line source in air (*5*). The energy window was set at 135–190 keV. Acquisition of data, as well as attenuation and reconstruction of images, was performed as earlier described (*5,17*). The measured concentration of radioactivity was expressed as Strichman Medical Units (SMUs, 1 SMU = 100 Bq/mL as specified by Strichman Medical Equipment, Inc.).

SPECT Experiments

To block thyroid uptake of free radioactive iodide, all subjects received potassium iodide orally according to the protocol previously described (*25*). After intravenous injection of 97–121 MBq [^{123}I]FPCIT, tomographic SPECT studies were performed (specific activity >185 MBq/nmol; radiochemical purity >98%; ^{123}I was labeled by Amersham Cygne, University of Technology, Eindhoven, The Netherlands, using the trimethylstannyl precursor of FPCIT obtained from Research Biochemicals International, Natick, MA). On the basis of dosimetry studies performed in humans, the effective dose equivalent was estimated to be 0.024 mSv/MBq (*25*).

Time-Course Study

A time-course study was performed to determine the optimal time postinjection for in vivo dopamine transporter imaging with [^{123}I]FPCIT SPECT in routine clinical studies. Each subject was imaged during six sessions. Session 1 (started 10 min after injection of the radioligand) consisted of multiSPECT acquisition (starting at and parallel to the orbitomeatal line, 150 s/slice; interslice distance 10 mm) to locate the slice with the best visualization of the striatum, as described previously (5). Sessions 2 (which took place 60–80 min after the onset of the experiment), 3 (120–140 min), 4 (180–200 min), 5 (270–290 min) and 6 (360–380 min) consisted of dynamic SPECT scans (eight consecutive acquisitions of 150 s/slice) performed at the level of the reference slice as identified in session 1. The optimum time of acquisition was defined as when the specific radioactivity in the striatum (calculated as total striatal counts minus counts in the occipital cortex, vide infra) had reached its highest value in all subjects.

In 1 patient, dynamic SPECT scanning could not be performed at 2 h postinjection (session 3), and session 4 lasted only 10 min because the patient was in a severe "off-state" (*24*).

Data Processing

For analysis of striatal [^{123}I]FPCIT binding from data obtained in session 1, a standard template with regions of interest (ROIs) for the whole striatum, caudate nucleus, putamen and occipital cortex was positioned on the image with the highest activity, as previously described (*5*). The same template was used to analyze striatal [^{123}I]FPCIT binding from images obtained in sessions 2–6. Minor variations of individual brains required moving the fixed ROIs, without changing the size and shape, within the template for optimal fitting (*5*). Specific-to-nonspecific [^{123}I]FPCIT binding was then calculated as:

$$[^{123}\text{I}]\text{FPCIT binding} = (\text{ROI} - \text{OCC})/\text{OCC},$$

where ROI represents the mean radioactivity (in SMU) in the ROI (striatum, caudate nucleus or putamen). The occipital cortex (OCC, radioactivity in SMU) was selected as the background region,

because the density of dopamine transporters is negligible in this brain area. The binding ratios were also used to calculate putamen-to-caudate nucleus ratios for the ipsilateral and contralateral striatum. In the patients with Parkinson's disease, the contralateral striatum was defined as the side opposite to that of initial presentation of motor signs. For the control subjects, contralateral was arbitrarily assigned to the left striatum.

Curve Fitting

To estimate the time point of peak specific striatal binding, decay-corrected time-activity data for the occipital cortex and striatum were fitted. The following strategy was used to fit data for the occipital cortex. The time course of [^{123}I]FPCIT binding in the occipital cortex, as reported in previous studies (*5,15,26*), suggested that the occipital time course of radioactivity is characterized by a rapid uptake and a biphasic washout; the washout consists of a fast and a slow component. Therefore, the occipital data were fit to equation 1 with the assumptions that at $t = 0$ the radioactivity is zero and that at $t \rightarrow \infty$ the activity in this brain area approximates zero. Thus, occipital activity at time t[Coc(t)] was given by:

$$Coc(t) = C_1(1 - e^{-\lambda_1 t})[(C_2 \times e^{-\lambda_2 t}) + e^{-\lambda_3 t}], \quad \text{Eq. 1}$$

where C_1 and C_2 are constants, and λ_1, λ_2 and λ_3 (h^{-1}) are elimination rate constants associated with each exponential.

To fit the striatal data, the following assumptions were made. The time course of the activity in the occipital brain area is a part of the time course of the striatal activity. Moreover, the slow component of the striatal washout of activity (λ_3) is equal to the slow component of the washout of occipital activity. This assumption was made after the studies, because the measured data for the striatum and occipital cortex showed a similar rate of washout of activity beyond 3 h after injection of the radiotracer in both the groups of controls and patients with Parkinson's disease. Thus, specific striatal activity at time t[Cspecstr(t)] was given by:

$$Cspecstr(t) = C_4(1 - e^{-\lambda_4 t}) \times e^{-\lambda_3 t}, \quad \text{Eq. 2}$$

where C_4 is a constant, and λ_4 (h^{-1}) represents an elimination rate constant.

Striatal activity at time t[Cstr(t)] was given by:

$$Cstr(t) = Coc(t) + C_4(1 - e^{-\lambda_4 t}) \times e^{-\lambda_3 t}. \quad \text{Eq. 3}$$

For calculation of the time point of peak specific striatal binding, the time derivative of equation 2 was set to zero.

Statistical Analysis

For the purpose of statistical analysis, the data per brain area obtained per dynamic SPECT session (see above) were averaged. A possible time trend in the ratios of specific-to-nonspecific [^{123}I]FPCIT binding in the whole striatum, caudate nucleus and putamen was determined by repeated nonparametric analysis of variance. Differences between groups were analyzed with the nonparametric Mann-Whitney U test. For analysis of the difference in uptake ratios between ipsilateral and contralateral sides within groups, the nonparametric Wilcoxon paired test was used. In case of multiple comparisons, the Bonferroni correction method was used. In all statistical analyses, which were all 2-tailed, $P < 0.05$ was considered significant.

RESULTS

Group Characteristics

There were no significant age differences between the control subjects and patients with Parkinson's disease. Among the patients with Parkinson's disease, 7 had initial left-sided onset of motor signs, 11 had right-sided onset and 1 had bilateral onset.

Safety

After injection of approximately 110 MBq [^{123}I]FPCIT, no adverse effects were noticed in any of the subjects. Their vital signs remained stable throughout the experiment. Moreover, no meaningful changes were observed in any of the clinical laboratory assays performed on blood and urine specimens obtained at 6.5 and 24–72 h after administration of the radioligand. Also, no meaningful changes were observed on electrocardiography (at 4.5 h after injection of the radioligand).

Time-Course Study in Healthy Volunteers

The time-activity curves obtained in healthy volunteers showed accumulation of [^{123}I]FPCIT activity in the striatum. Striatal activity peaked rapidly. Striatal activity reached 95% of its highest value at 0.43 ± 0.40 h (mean ± SD) (Fig. 1; Table 1). Radioactivity in the occipital cortex declined rapidly, until approximately 2 h after injection (Fig. 1). After this time point, the occipital washout of activity was 5.6% ± 1.7% h^{-1}. The specific striatal activity reached 95% of its peak value at 1.69 h after injection and showed a large variation in time between subjects (range 0.76–2.55 h; Table 1). After peak specific binding, the rate of washout of striatal and specific striatal activity was 3.6% ± 2.2% and 3.0% ± 2.0% h^{-1}, respectively.

Time-Course Study in Patients with Parkinson's Disease

As in the control group, time-activity curves of brain uptake of [^{123}I]FPCIT in patients with Parkinson's disease showed accumulation of activity in the striatum, rapidly reaching a peak (striatal activity reached 95% of its highest value at 0.21 ± 0.08 h; Fig. 1; Table 1). The time course of uptake of radioactivity in the occipital cortex was similar in the patients with Parkinson's disease and control subjects (Table 1). The specific striatal activity reached 95% of its peak value at 0.91 h, showing a large variation among patients (range 0.24–1.91 h). Hoehn and Yahr stage of the patients was not significantly correlated (Spearman rank correlation) with the time point of peak specific striatal activity, although a tendency for a significant correlation was noticeable ($P = 0.07$; data not shown). Peak specific striatal binding was reached significantly faster in patients than in control subjects (Table 1). After peak specific binding, the rate of washout of striatal and specific striatal activity was 5.1% ± 2.2% and 3.7% ± 2.2% h^{-1}, respectively (Table 1). These washout rates were not significantly faster in patients than in control subjects.

SPECT Measurements

The ratio of specific-to-nonspecific [^{123}I]FPCIT binding in the striatum was significantly lower in patients with Parkinson's disease than in control subjects, as early as 1 h after injection (Table 2). The time-course studies showed that 95% of the peak specific striatal [^{123}I]FPCIT binding was

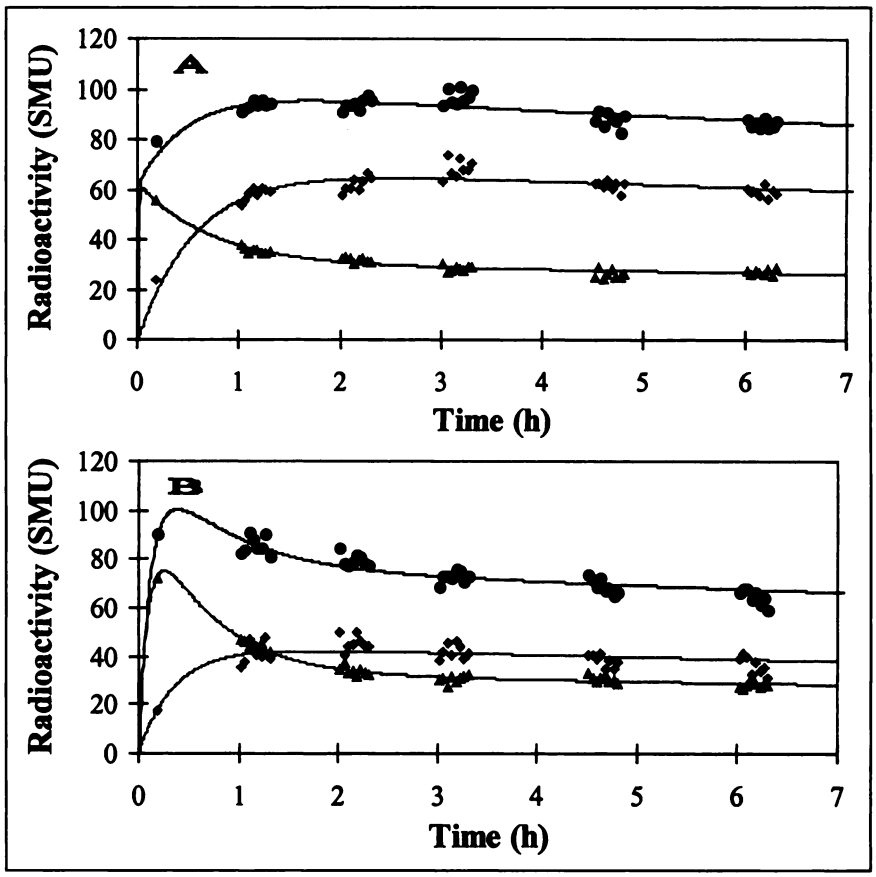

FIGURE 1. Radioactivity in striatum (●), occipital cortex (▲) and specific striatal radioactivity (◆) up to 6 h after injection of [^{123}I] FPCIT. (A) Healthy volunteer. (B) Patient with Parkinson's disease.

reached in all healthy volunteers and patients within 3 h after injection. Moreover, no significant time trend was found for the ratios of specific-to-nonspecific [^{123}I]FPCIT binding in the striatum, caudate and putamen between 3 and 6 h postinjection (Table 2; data for the caudate and putamen are not shown). Therefore, further calculations were based only on data obtained between 3 and 6 h postinjection.

Between 3 and 6 h after injection of the radiotracer, significantly lower ratios of specific-to-nonspecific [^{123}I] FPCIT binding were found in the Parkinson's disease group than in the control group, for all six brain regions measured (both ipsilateral and contralateral for striatum, caudate and putamen; Table 3).

TABLE 1
Time Course of [^{123}I]FPCIT Binding in Healthy Volunteers and Patients with Parkinson's Disease

	Healthy volunteers (n = 10)	Patients (n = 19)
Time (h) of peak striatal binding	0.43 ± 0.40	0.21 ± 0.08
Time (h) of peak specific striatal binding	1.69 ± 0.53	0.91 ± 0.39*
Washout of striatal activity (h^{-1})	3.59 ± 2.23	5.14 ± 2.19
Washout of specific striatal activity (h^{-1})	3.01 ± 2.01	3.71 ± 2.23
Washout of occipital cortex activity (h^{-1})	5.57 ± 1.66	5.27 ± 2.08

*Significantly different from healthy volunteers.
Values are expressed as mean ± SD.

Within the control group, there was no significant difference between the contralateral and ipsilateral ratios of specific-to-nonspecific striatal [^{123}I]FPCIT binding. However, within the Parkinson's disease group, the ratios of contralateral striatal [^{123}I]FPCIT binding were significantly lower than the ratios of ipsilateral binding (Table 3).

The putamen-to-caudate binding ratios obtained in patients with Parkinson's disease indicated that the specific-to-nonspecific [^{123}I]FPCIT binding was less in the putamen than in the caudate nucleus, both for the ipsilateral and the contralateral side. These ratios were also significantly lower in patients than in control subjects. Only ipsilateral putamen-to-caudate binding at 4.5 h postinjection showed no significant difference between control subjects and patients with Parkinson's disease (Table 3).

If only the ratios of specific-to-nonspecific [^{123}I]FPCIT binding in a subgroup of 5 patients with hemi-Parkinson's disease (Hoehn and Yahr stage I) were considered for comparison with those ratios in the control group, still significantly lower ratios were found in the subgroup of patients with hemi-Parkinson's disease (Table 4; Fig. 2). This was true for five out of the six binding measures (both ipsilateral and contralateral for striatum, caudate and putamen) at 3, 4.5 and 6 h postinjection. Only the ipsilateral caudate binding ratio showed no significant difference

TABLE 2
[^{123}I]FPCIT Binding Ratios (Specific Striatal Binding/Nonspecific Binding) in Healthy Volunteers and Patients with Parkinson's Disease

	Time postinjection					
	10 min	1 h	2 h	3 h	4.5 h	6 h
Healty volunteers (n = 10)	0.31 ± 0.14	1.36 ± 0.32	1.93 ± 0.30	2.24 ± 0.32	2.28 ± 0.57	2.20 ± 0.55
	$P > 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
Patients (n = 19)*	0.20 ± 0.16	0.69 ± 0.28	0.94 ± 0.30	0.89 ± 0.34	0.89 ± 0.27	0.88 ± 0.28

*At 2 h postinjection, n = 18. One patient in a severe off-state could not be evaluated.
Values are expressed as mean ± SD.

between the subgroup of patients with hemi-Parkinson's disease and the control group, at 6 h postinjection (Table 4). Moreover, in 4 out of the 5 patients with hemi-Parkinson's disease, the striatal [^{123}I]FPCIT measures were lower on the side contralateral to that on which the motor signs started.

Correlation of Striatal SPECT Signal with Hoehn and Yahr Stage

Hoehn and Yahr stage of the patients with Parkinson's disease was significantly correlated (Spearman rank correlation) with the ratios of specific-to-nonspecific striatal [^{123}I]FPCIT binding at 3, 4.5 and 6 h after injection (Fig. 3).

DISCUSSION

Clinical SPECT studies should be performed at a single time point after injection of the radiotracer during one short imaging session (for example, a scanning time of 40–60 min). Moreover, it is preferable to obtain image acquisitions on the day of injection. Finally, a simple quantification procedure without plasma measurements is recommended. A major finding of this study was that peak striatal [^{123}I]FPCIT binding was reached in all control subjects and patients with Parkinson's disease within 3 h after injection of the radiotracer. In addition, the ratios of specific-to-nonspecific striatal [^{123}I]FPCIT binding were stable between 3 and 6 h after the injection of the radiotracer. Because these ratios were stable over time, conditions for a transient equilibrium (*27*) are fulfilled between 3 and 6 h after injection of [^{123}I]FPCIT. Carson et al. (*27*) showed that the tissue ratio approximates the ratio at true equilibrium, although, depending on the rate of plasma clearance and the local tissue kinetics, the tissue ratio may overestimate the ratio at true equilibrium. Nevertheless, the ratio of specific-to-nonspecific [^{123}I]FPCIT binding reflects the density of dopamine transporters, at least in the period between 3 and 6 h postinjection. For routine clinical studies, we therefore recommend obtaining images within the time period of 3–6

TABLE 3
[^{123}I]FPCIT SPECT Measures in Healthy Volunteers and Patients with Parkinson's Disease

	3 h postinjection		4.5 h postinjection		6 h postinjection	
	Healthy volunteers (n = 10)	Patients (n = 18)*	Healthy volunteers (n = 10)	Patients (n = 18)*	Healthy volunteers (n = 10)	Patients (n = 18)*
Striatum						
Contralateral	2.24 ± 0.32	0.81 ± 0.29†‡	2.25 ± 0.61	0.80 ± 0.22†‡	2.22 ± 0.55	0.77 ± 0.23†‡
Ipsilateral	2.26 ± 0.35	1.01 ± 0.43†	2.31 ± 0.54	0.98 ± 0.37†	2.18 ± 0.57	1.00 ± 0.39†
Caudate						
Contralateral	2.58 ± 0.37	1.21 ± 0.50†	2.55 ± 0.56	1.31 ± 0.39†	2.59 ± 0.59	1.18 ± 0.39†
Ipsilateral	2.59 ± 0.41	1.38 ± 0.52†	2.72 ± 0.56	1.36 ± 0.49†	2.64 ± 0.59	1.44 ± 0.51†
Putamen						
Contralateral	2.24 ± 0.45	0.67 ± 0.23†	2.26 ± 0.77	0.60 ± 0.18†	2.19 ± 0.64	0.62 ± 0.20†
Ipsilateral	2.15 ± 0.41	0.89 ± 0.43†	2.21 ± 0.62	0.84 ± 0.35†	2.05 ± 0.67	0.81 ± 0.35†
Putamen-to-caudate ratio						
Contralateral	0.88 ± 0.19	0.73 ± 0.47†	0.89 ± 0.22	0.49 ± 0.11†	0.86 ± 0.19	0.65 ± 0.37†
Ipsilateral	0.85 ± 0.16	0.65 ± 0.14†	0.83 ± 0.19	0.64 ± 0.14	0.78 ± 0.16	0.61 ± 0.23†

*Because one patient showed bilateral onset of motor signs, n = 18 instead of 19.
†Significantly different from controls.
‡Significantly different from ipsilateral side.
Contralateral is the side opposite that of initial presentation of motor signs. For healthy volunteers contralateral is arbitrarily assigned to the left striatum. All SPECT values are expressed as (region of interest − occipital binding)/occipital binding (mean ± SD).

TABLE 4
[^{123}I]FPCIT SPECT Measures in Healthy Volunteers and Patients with Hemi-Parkinson's Disease (Hoehn and Yahr Stage I)

	3 h postinjection		4.5 h postinjection		6 h postinjection	
	Healthy volunteers (n = 10)	Patients (n = 5)	Healthy volunteers (n = 10)	Patients (n = 5)	Healthy volunteers (n = 10)	Patients (n = 5)
Striatum						
Contralateral	2.24 ± 0.32	0.90 ± 0.29*	2.25 ± 0.61	0.92 ± 0.23*	2.22 ± 0.55	0.91 ± 0.26*
Ipsilateral	2.26 ± 0.35	1.31 ± 0.48*	2.31 ± 0.54	1.25 ± 0.41*	2.18 ± 0.57	1.27 ± 0.49*
Caudate						
Contralateral	2.58 ± 0.37	1.35 ± 0.39*	2.55 ± 0.56	1.47 ± 0.35*	2.59 ± 0.59	1.26 ± 0.44*
Ipsilateral	2.59 ± 0.41	1.63 ± 0.57*	2.72 ± 0.56	1.72 ± 0.54*	2.64 ± 0.59	1.44 ± 0.67
Putamen						
Contralateral	2.24 ± 0.45	0.73 ± 0.30*	2.26 ± 0.77	0.70 ± 0.18*	2.19 ± 0.64	0.77 ± 0.25*
Ipsilateral	2.15 ± 0.41	1.21 ± 0.45*	2.21 ± 0.63	1.01 ± 0.35*	2.05 ± 0.21	1.06 ± 0.39*
Putamen/caudate ratio						
Contralateral	0.88 ± 0.19	0.55 ± 0.09*	0.89 ± 0.22	0.48 ± 0.03*	0.86 ± 0.19	0.76 ± 0.51
Ipsilateral	0.85 ± 0.16	0.75 ± 0.12	0.83 ± 0.19	0.60 ± 0.07	0.78 ± 0.16	0.76 ± 0.37

*Significantly different from controls.
Contralateral is the side opposite that of initial presentation of motor signs. For healthy volunteers, contralateral is arbitrarily assigned to the left striatum. All SPECT values are expressed as (region of interest − occipital binding)/occipital binding (mean ± SD).

h after injection of [^{123}I]FPCIT and quantifying dopamine transporters using the ratio of specific-to-nonspecific striatal binding as the outcome measure. Such a study protocol fulfills the above mentioned recommendations for a clinical protocol and is easy to implement at departments of nuclear medicine. However, future studies must focus on the possible relationship between the empiric ratio of specific-to-nonspecific striatal [^{123}I]FPCIT binding, obtained between 3 and 6 h after injection of the radiotracer, and the density of dopamine transporters. This relationship may be used as a means of assessing disease progression.

Until now, the time course of [^{123}I]FPCIT binding in human brain has been studied only in small groups of control subjects and patients with Parkinson's disease (*5,15,20,26*). In this study, we showed that 95% of peak specific striatal binding had been reached at 1.69 h after injection of the radiotracer in healthy volunteers (n = 10). This is in agreement with other studies performed in control subjects. For instance, Abi-Dargham et al. (*15*) studied the brain kinetics of [^{123}I]FPCIT in four control subjects and showed that the specific striatal binding reached 90% of its peak value at 2.5 h postinjection, whereas Kuikka et al. (*26*) found peak uptake of [^{123}I]FPCIT in the striatum 3–4 h after injection. We now show that specific striatal [^{123}I]FPCIT

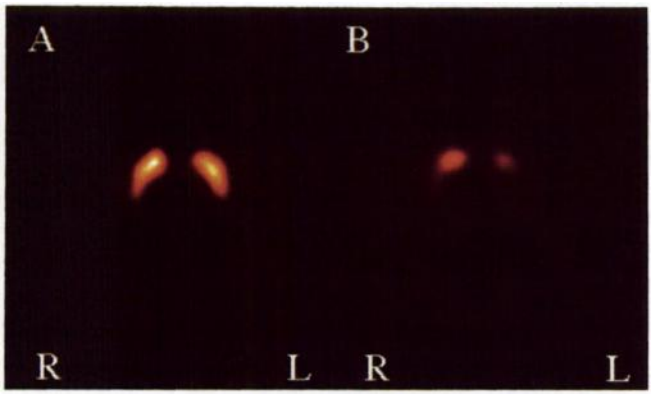

FIGURE 2. [^{123}I]FPCIT SPECT images obtained 3 h after injection of radiotracer of (A) 65-y-old healthy woman and (B) 59-y-old woman with hemi-Parkinson's disease (Hoehn and Yahr stage I). Transverse slices from brain at level of striatum (approximately 3 cm above orbitomeatal line) (L = left side; R = right side). In both images, level of activity is color coded from low (black) to medium (yellow) to high (white) and scaled (corrected for injected dose/kg) to maximum in slice of healthy woman (maximum is 190 SMU).

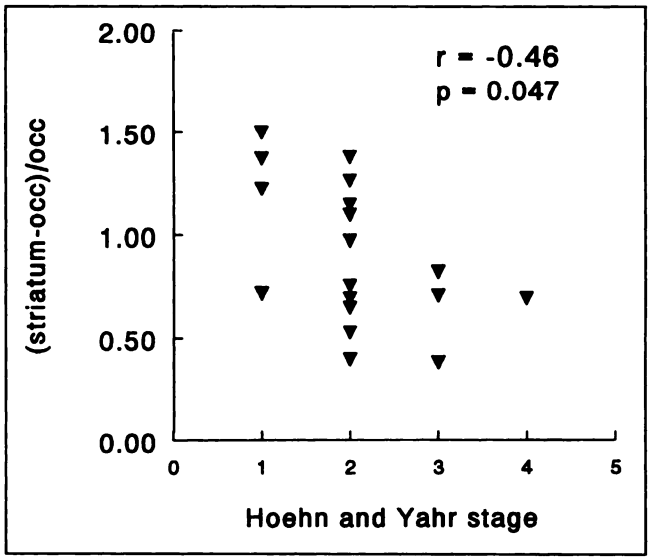

FIGURE 3. Correlation (Spearman rank correlation) of Hoehn and Yahr stage with specific-to-nonspecific binding in striatum (mean left and right side) for patients with Parkinson's disease.

binding equilibrates faster in patients with Parkinson's disease than in control subjects. This finding is in agreement with the results of several human [^{123}I]β-CIT SPECT studies that reported that the peak specific striatal uptake was reached earlier in patients with Parkinson's disease than in control subjects (*10,11,28*). The time point at which equilibrium occurs depends on several factors, including peripheral clearance, blood flow, association and dissociation rate constants and binding sites (*29*). The earlier peak in specific striatal [^{123}I]FPCIT binding in patients with Parkinson's disease can thus be explained by a loss of striatal dopamine transporters caused by the nigrostriatal degeneration. On the basis of this theory, one also may expect that the severity of parkinsonian signs is correlated with the time point of peak specific striatal uptake. Unfortunately, the data of this study showed only a tendency for such a correlation.

Besides the above mentioned "transient equilibrium method," the "peak equilibrium method" (*30*) can be used to quantify dopamine transporters in the striatum by means of PET or SPECT. The peak equilibrium method identifies the point in time of peak specific striatal uptake. At that particular point in time, the association and dissociation to and from the dopamine transporter are equal, and consequently, the specific-to-nonspecific ratio derives an adequate measure of the dopamine transporter density (*15,31*). However, the results of this study show that the time point of peak specific binding is highly variable among subjects. Thus, the peak equilibrium method is not practical for routine clinical [^{123}I]FPCIT applications, because an extended scanning session is needed to define the "peak" specific [^{123}I]FPCIT uptake per subject.

Abi-Dargham et al. (*15*) showed a rapid peak in specific [^{123}I]FPCIT striatal binding with a stable specific striatal [^{123}I]FPCIT binding in time in control subjects. According to these authors, stability of the specific striatal [^{123}I]FPCIT binding, together with stability of the metabolites-corrected plasma concentration, would allow a sustained equilibrium analysis to quantify dopamine transporters. However, the results of this study are not in agreement with the observations of Abi-Dargham et al. (*15*), because we show that peak specific [^{123}I]FPCIT binding was followed by a slow striatal and specific striatal washout of activity, both in control subjects (3.0%–3.6% h^{-1}) and patients with Parkinson's disease (3.7%–5.1% h^{-1}). Interestingly, our observations are in line with the results of two other studies, which showed striatal washout rates of approximately 5%–7% and 3.7% h^{-1} in monkey and human brain, respectively (*26,32*). Moreover, our observations are in agreement with the results of Seibyl et al. (*33*), which showed striatal washout rates of approximately 4.9% and 8.2% h^{-1} in small groups of control subjects and patients with Parkinson's disease, respectively. Our results indicate that basic criteria are not fulfilled to use a sustained equilibrium analysis to quantify dopamine transporters after bolus injection of [^{123}I]FPCIT.

Another important finding of this study was that [^{123}I]FPCIT binding in both the caudate nucleus and putamen (ipsilateral and contralateral) of patients with Parkinson's disease was significantly lower compared with age-matched control subjects. Moreover, the patients with Parkinson's disease showed a preferential loss of [^{123}I]FPCIT binding in the putamen. This finding is in agreement with results from necropsy studies that disclosed a more severe depletion of dopamine in the putamen than in the caudate nucleus in Parkinson's disease (*2*). Moreover, it confirms the findings with PET and SPECT (*3,5,17,19,20*).

In this study, patients with hemi-Parkinson's disease showed a bilateral loss of striatal dopamine transporters. Recent PET and SPECT studies also reported bilateral loss of dopamine transporters in hemi-Parkinson's disease (*4–6,34*). A recent study by Tissingh et al. (*34*) showed that the [^{123}I]FPCIT SPECT technique is completely able to distinguish early, drug-naive patients with Parkinson's disease from age-matched control subjects, as early as 3 h after injection of the radiotracer. On the basis of the results of this and other studies, the [^{123}I]FPCIT SPECT technique may be sensitive enough to detect the preclinical period of Parkinson's disease.

In this study, the ratio of specific-to-nonspecific striatal [^{123}I]FPCIT binding correlated with the severity of Parkinson's disease. This observation is in agreement with results from necropsy studies that showed that the severity of motor signs in patients with Parkinson's disease can be correlated with decreased dopamine concentrations in the striatum (*1*). Moreover, it confirms the recent findings with [^{123}I]FPCIT SPECT in patients with Parkinson's disease (*5,19,20*). Such a correlation supports our statement that the ratio of specific-to-nonspecific [^{123}I]FPCIT binding reflects the density of dopamine transporters.

A source of possible confounding data in this study lies in the fact that most of the patients with Parkinson's disease received antiparkinsonian medication. It is possible that direct or indirect effects of this medication could have interfered with binding measurements of the dopamine transporter. However, Laruelle et al. (*35*) demonstrated in baboons that acute administration of a large dose of L-DOPA (50 mg/kg intravenously) had no influence on specific striatal [^{123}I]β-CIT binding. Moreover, a study showed that dopamine transporter binding in rat striatum is unaltered after chronic changes in dopamine levels (*36*). There is no evidence that β-blockers, dopamine agonists or amantadine (a noncompetitive N-methyl-D-aspartic acid antagonist) have any influence on dopamine transporter binding. L-(-)deprenyl is metabolized to l-amphetamine and l-metamphetamine. It has been suggested that these metabolites might compete with dopamine transporter binding (*28*). However, l-amphetamine and l-metamphetamine were shown to be weak inhibitors of [^{3}H]cocaine binding (IC_{50} = 31,900 nmol/L [*37*]) and GBR12935 binding (IC_{50} > 40,000 nmol/L [*38*]), respectively. The binding of [^{123}I]FPCIT at the dopamine transporter is approximately 1 nmol/L (*22*). Thus, to the extent that in vitro measurement can be extended to the in vivo situation, it is not likely that the metabolites of

L-(-)deprenyl will significantly influence the direct binding of FPCIT to the dopamine transporter. The influence of chronic use of L-(-)deprenyl on dopamine transporter binding sites has been studied in rats, but the results were inconsistent (*39,40*). However, an influence of L-(-)deprenyl on the binding of FPCIT at the dopamine transporter in human brain cannot be excluded. Nonetheless, the effects of medication were not considered in this study but should be in the future.

In a previous study (*25*), we showed that [^{123}I]FPCIT is a pharmacologically safe radioligand in healthy control subjects. As expected, we reproduced this finding in this study and additionally showed that [^{123}I]FPCIT is a pharmacologically safe radioligand in patients with dopaminergic deficits.

CONCLUSION

The results show that peak specific striatal [^{123}I]FPCIT binding was reached faster in patients with Parkinson's disease than in healthy control subjects. This peak was reached in all control subjects and patients within 3 h postinjection, and the ratio of specific-to-nonspecific striatal [^{123}I]FPCIT binding was stable up to 6 h postinjection. Therefore, for routine clinical [^{123}I]FPCIT SPECT studies we recommend obtaining image acquisitions at a single time point between 3 and 6 h after injection of the radiotracer. In that period, the [^{123}I]FPCIT technique is sensitive to distinguish healthy volunteers from patients with Parkinson's disease, even at an early phase of the disease. [^{123}I]FPCIT SPECT allows use of a 1-d protocol, which will be of great advantage in outpatient evaluations.

ACKNOWLEDGMENTS

This study is part of phase II studies and is financially supported by Amersham Cygne and Eindhoven University of Technology, Eindhoven, The Netherlands. We thank cardiologist Jutta M. Schroeder-Tanka, MD, for analysis of the electrocardiograms.

REFERENCES

1. Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F. Brain dopamine and the syndromes of Parkinson and Huntington. *J Neurol Sci.* 1973;20:415–455.
2. Kish SJ, Shannak K, Hornykiewicz O. Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. *N Engl J Med.* 1988;876: 876–880.
3. Frost JJ, Rosier AJ, Reich SG, et al. Positron emission tomographic imaging of dopamine transporter with ^{11}C-WIN 35,428 reveals marked declines in mild Parkinson's disease. *Ann Neurol.* 1993;34:423–431.
4. Marek KL, Seibyl JP, Zoghbi SS, et al. [^{123}I]β-CIT/SPECT imaging demonstrates bilateral loss of dopamine transporters in hemi-Parkinson's disease. *Neurology.* 1996;46:231–237.
5. Booij J, Tissingh G, Boer GJ, et al. [^{123}I]FP-CIT SPECT shows a pronounced decline of striatal labelling in early and advanced Parkinson's disease. *J Neurol Neurosurg Psychiatry.* 1997;62:133–140.
6. Guttman M, Burkholder J, Kish SJ, et al. [^{11}C]RTI-32 PET studies of the dopamine transporter in early dopa-naive Parkinson's disease: implications for the symptomatic threshold. *Neurology.* 1997;48:1578–1583.
7. Vingerhoets FJG, Snow BJ, Lee S, Schulzer M, Mak E, Calne DB. Longitudinal fluorodopa positron emission tomography studies of the evolution of idiopathic parkinsonism. *Ann Neurol.* 1994;36:759–764.
8. Kaufman MJ, Madras BK. Severe depletion of cocaine recognition sites associated with the dopamine transporter in Parkinson's-diseased striatum. *Synapse.* 1991;9:43–49.
9. Leenders KL, Salmon EP, Tyrrell P, et al. The nigrostriatal dopaminergic system assessed in vivo by positron emission tomography in healthy volunteer subjects and patients with Parkinson's disease. *Arch Neurol.* 1990;47:1290–1298.
10. Brücke T, Kornhuber J, Angelberger P, Asenbaum S, Frassine H, Podreka I. SPECT imaging of dopamine and serotonin transporters with [^{123}I]β-CIT. Binding kinetics in the human brain. *J Neural Transm Gen Sect.* 1993;94:137–146.
11. Innis RB, Seibyl JB, Scanley BE, et al. Single photon computed imaging demonstrates loss of striatal dopamine transporters in Parkinson's disease. *Proc Natl Acad Sci USA.* 1993;90:11965–11969.
12. Seibyl JP, Marek KL, Quinlan D, et al. Decreased single-photon emission computed tomographic [^{123}I]β-CIT striatal uptake correlates with symptom severity in Parkinson's disease. *Ann Neurol.* 1995;38:589–598.
13. Booij J, Tissingh G, Winogrodzka A, et al. Practical benefit of [^{123}I]FP-CIT SPET in the demonstration of the dopaminergic deficit in Parkinson's disease. *Eur J Nucl Med.* 1997;24:68–71.
14. Neumeyer JL, Wang S, Gao Y, et al. *N*-ω-fluoroalkyl analogs of (1R)-2β-carbomethoxy-3-β-(4-iodophenyl)-tropane (β-CIT): radiotracers for positron emission tomography and single photon emission computed tomography imaging of dopamine transporters. *J Med Chem.* 1994;37:1558–1561.
15. Abi-Dargham A, Gandelman MS, DeErausquin GA, et al. SPECT imaging of dopamine transporters in human brain with iodine-123-fluoroalkyl analogs of β-CIT. *J Nucl Med.* 1996;37:1129–1133.
16. Scheffel U, Lever JR, Abraham P, et al. N-substituted phenyltropanes as in vivo binding ligands for rapid imaging studies of the dopamine transporter. *Synapse.* 1997;25:345–349.
17. Booij J, Andringa G, Rijks LJM, et al. [^{123}I]FP-CIT binds to the dopamine transporter as assessed by biodistribution studies in rats and SPECT studies in MPTP-lesioned monkeys. *Synapse.* 1997;27:183–190.
18. Lundkvist C, Halldin C, Ginovart N, Swahn C-G, Farde L. [^{18}F]β-CIT-FP is superior to [^{11}C]β-CIT-FP for quantitation of the dopamine transporter. *Nucl Med Biol.* 1997;24:621–627.
19. Ishikawa T, Dhawan V, Kazumata K, et al. Comparative nigrostriatal dopaminergic imaging with iodine-123-βCIT-FP/SPECT and fluorine-18-FDOPA/PET. *J Nucl Med.* 1996;37:1760–1765.
20. Rinne JO, Kuikka JT, Bergström KA, Hiltunen J, Kilpeläinen H. Striatal dopamine transporters in Parkinson's disease: a study with a new radioligand, [^{123}I]β-CIT-FP. *Parkinsonism Related Disord.* 1997;3:77–81.
21. Gibb WRG, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry.* 1988;51:745–752.
22. Van Dyck CH, Seibyl JP, Malison RT, et al. Age-related decline in striatal dopamine transporter binding with iodine-123-β-CIT SPECT. *J Nucl Med.* 1995;36:1175–1181.
23. Hoehn MM, Yahr MD. Parkinsonism: onset, progression, and mortality. *Neurology.* 1967;17:427–442.
24. Langston JW, Widner H, Goetz CG, et al. Core assessment program for intracerebral transplantations (CAPIT). *Mov Disord.* 1992;7:2–13.
25. Booij J, Busemann Sokole E, Stabin MG, Janssen AGM, de Bruin K, van Royen EA. Human biodistribution and dosimetry of [^{123}I]FP-CIT: a potent radioligand for imaging of dopamine transporters. *Eur J Nucl Med.* 1998;25:24–30.
26. Kuikka JT, Bergström KA, Ahonen A, et al. Comparison of iodine-123 labelled 2β-carbomethoxy-3β-(4-iodophenyl)tropane and 2β-carbomethoxy-3β-(4-iodophenyl)-*N*-(3-fluoropropyl)nortropane for imaging of the dopamine transporter in the living human brain. *Eur J Nucl Med.* 1995;22:356–360.
27. Carson RE, Channing MA, Blasberg RG, et al. Comparison of bolus and infusion methods for receptor quantitation: application to [^{18}F]cyclofoxy and positron emission tomography. *J Cereb Blood Flow Metab.* 1993;13:24–42.
28. Asenbaum S, Brücke T, Pirker W, et al. Imaging of dopamine transporters with iodine-123-β-CIT and SPECT in Parkinson's disease. *J Nucl Med.* 1997;38:1–6.
29. Malison RT, Laruelle M, Innis RB. Positron and single photon emission tomography. Principles and application in psychopharmacology. In: Bloom FE, Kupfer DJ, eds. *Psychopharmacology: The Fourth Generation of Progress.* New York, NY: Raven Press; 1995:865–879.
30. Farde L, Hall H, Ehrin E, Sedvall G. Quantitative analysis of D2 dopamine receptor binding in the living human brain by PET. *Science.* 1986;231:258–261.
31. Laruelle M, Wallace E, Seibyl JP, et al. Graphical, kinetic and equilibrium analyses of in vivo [^{123}I]β-CIT binding to dopamine transporters in healthy human subjects. *J Cereb Blood Flow Metab.* 1994;14:982–994.
32. Baldwin RM, Zea-Ponce Y, Al-Tikriti MS, et al. Regional brain uptake and pharmacokinetics of [^{123}I]*N*-ω-fluoroalkyl-2β -carboxy-3β -(4-iodophenyl)nortropane esters in baboons. *Nucl Med Biol.* 1995;22:211–219.

33. Seibyl J, Marek K, Sheff K, et al. Iodine-123-β-CIT and iodine-123-FPCIT SPECT measurement of dopamine transporters in healthy subjects and Parkinson's patients. *J Nucl Med.* 1998;39:1500–1508.
34. Tissingh G, Booij J, Bergmans P, et al. Iodine-123-N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)tropane SPECT in healthy controls and early-stage, drug-naive Parkinson's disease. *J Nucl Med.* 1998;39:1143–1148.
35. Laruelle M, Baldwin RM, Malison RT, et al. SPECT imaging of dopamine and serotonin transporters with [^{123}I]β-CIT: pharmacological characterization of brain uptake in nonhuman primates. *Synapse.* 1993;13:295–309.
36. Moody CA, Granneman JG, Bannon MJ. Dopamine transporter binding in rat striatum and nucleus accumbens is unaltered following chronic changes in dopamine levels. *Neurosci Lett.* 1996;217:55–57.
37. Schoemaker H, Pimoule C, Arbilla S, Scatton B, Javoy-Agid F, Langer SZ. Sodium dependent [^{3}H]cocaine binding associated with dopamine uptake sites in the rat striatum and human putamen decrease after dopaminergic denervation and in Parkinson's disease. *Naunyn Schmiedeberg Arch Pharmacol.* 1985;329:227–235.
38. Fang J, Yu PH. Effect of L-deprenyl, its structural analogues and some monoamine oxidase inhibitors on dopamine uptake. *Neuropharmacology.* 1994;33:763–768.
39. Wiener HL, Hashim A, Lajtha A, Sershen H. Chronic L-deprenyl-induced up-regulation of the dopamine uptake carrier. *Eur J Pharmacol.* 1989;163:191–194.
40. Vander Borght T, Kilbourn M, Desmond T, Kuhl D, Frey K. The vesicular monoamine transporter is not regulated by dopaminergic drug treatments. *Eur J Pharmacol.* 1995;294:577–583.