PET Studies on Brain Monoamine Transporters with Carbon-11-β-CIT in Parkinson's Disease

Arto O. Laihinen, Juha O. Rinne, Kjell Å. Någren, Pertti K. Lehikoinen, Vesa J. Oikonen, Ulla H. Ruotsalainen, Hanna M. Ruottinen and Urpo K. Rinne

Department of Neurology, University of Turku; Turku University Cyclotron/PET Center; and Radiopharmaceutical Chemistry Laboratory, Turku, Finland

The cocaine analog 2β-carbomethoxy-3β-[4-iodophenyl]tropane (β-CIT) labeled with ^{11}C was used to study dopamine reuptake sites with PET. **Methods:** Three normal subjects and nine patients with Parkinson's disease were investigated. Each of them underwent a dynamic PET scan (25 timeframes over 80 min) with [^{11}C]β-CIT. A dose of 102.5–211.3 MBq (2.77–5.71 mCi) of this ligand was administered intravenously and a PET examination with an ECAT 931/08 PET camera was carried out. Ratios between the striatal/cortical/thalamic/midbrain and cerebellar uptake of this radioligand were calculated. **Results:** The highest accumulation of [^{11}C]β-CIT was observed in the caudate and putamen, though there was some uptake in the thalamus and the midbrain. Cortical uptake was negligible. Carbon-11-β-CIT accumulated significantly less in the putamen of the Parkinson's patients than in the normal subjects. The putamen-to-cerebellum ratio in the Parkinson's patients was 1.59 ± 0.04 and 1.80 ± 0.13 ($p = 0.028$) in the normal subjects. In the caudate, there was no significant difference between the Parkinson's patients and the normal subjects. **Conclusion:** These results imply that [^{11}C]β-CIT is a useful compound for carrying out a PET examination of the function of the presynaptic monoaminergic neurons both in normal and pathological brains.

Key Words: carbon-11-β-CIT; dopamine reuptake sites; positron emission tomography; Parkinson's disease

J Nucl Med 1995; 36:1263–1267

The monoamine reuptake sites transport dopamine, noradrenaline and serotonin from the synaptic cleft back into the presynaptic monoaminergic nerve terminals. Therefore, they reflect the functional integrity of monoaminergic innervation. This aspect plays an important role in the pathogenesis of several neurological diseases, e.g., the presynaptic degeneration of dopaminergic neurons in Parkinson's disease. Moreover, parkinsonism-inducing neurotoxin MPP^+ is taken up into the presynaptic dopaminergic neurons via the dopamine transporter (*1,2*).

Previously, the presynaptic dopamine reuptake site has been studied in vitro using various ligands, e.g., [^3H]mazindol (*3*), [^3H]nomifensine (*4*), [^3H]BTCP (*5,6*) and [^3H]cocaine (*7*) as well as [^3H]GBR-12935 (*8*).

PET technology has created new possibilities to examine these presynaptic reuptake sites in vivo, e.g., using [^{11}C]nomifensine (*9,10*), [^{18}F]GBR 13119 (*11*) and 3-[^{18}F]fluoromethyl-BTCP (*12*). These ligands are not, however, specific for dopaminergic reuptake sites because they also have affinity for other sites, such as piperazine, noradrenergic and serotonergic reuptake sites. Recently, [^{11}C]cocaine (*13*) and its phenyltropane analogs such as [^{11}C]CFT (*14*) and [^{11}C]β-CIT (*15,16*) have been introduced as the affinity of these analogs for dopamine reuptake sites is higher than that of cocaine itself.

The aim of this study was to carry out a PET examination of the usefulness of presynaptic reuptake function of dopamine in the striatum and that of serotonin in the thalamus, midbrain and neocortex using [^{11}C]β-CIT both in normal subjects and in parkinsonian patients.

METHODS

Subjects and Patients

Three normal age-matched volunteers were studied (1 woman, 2 men, age 36–69 yr, mean ± s.e.m., 57.3 ± 10.7 yr) and nine parkinsonian patients (Table 1). Of these patients, three were untreated de novo patients, three were levodopa-treated compensated patients (individual daily levodopa doses: 400 mg/600 mg/200 mg, the last one also had selegiline 10 mg per day) and three were advanced cases (individual daily drug regimens: levodopa 1500 mg, selegiline 10 mg, pergolide 1.5 mg/levodopa 450 mg/levodopa 200 mg, bromocriptine 40 mg, amantadine 200 mg). The severity of Parkinson's disease was assessed according to the Unified Parkinson's Disease Rating Scale (UPDRS) (*17*) and disability along the modified (*17*) classification of Hoehn and Yahr (*18*). All participants gave informed consent. The study was approved by the Ethical Committee of Turku University Central Hospital.

Radioligand

Carbon-11-β-CIT was prepared as described previously (*15*). Carbon-11-methyl iodide, prepared by a one-pot reaction set up according to the standard procedure at the radiochemistry laboratory (*19*) from [^{11}C]carbon dioxide produced with an Efremov 103-cm isochronous cyclotron, was used in an alkylation reaction of the *N*-desmethyl precursor nor-β-CIT (Research Biochemicals International, Natick, MA). The volume of the formulated (physiological 0.1 *M* phosphate buffer) β-CIT solution was ascertained

Received Sept. 28, 1994; revision accepted Feb. 14, 1995.
For correspondence or reprints contact: Arto O. Laihinen, MD, Department of Neurology, University of Turku, FIN-20520 Turku, Finland.

TABLE 1
Patient Characteristics

Patient group	Sex F/M	Age (yr) Mean ± s.e.m.	PD duration (yr) Mean ± s.e.m.	UPDRS*/motor score Mean ± s.e.m.	H & Y† 1–1.5	2	3	4	Lateralization‡ R	L	Bilat
De novo, no levodopa	2/1	52.7 ± 2.9	0.8 ± 0.5	31.3 ± 3.7	2	1	—	—	—	3	—
Levodopa-treated, compensated	—/3	47.3 ± 3.8	3.3 ± 0.8	27.7 ± 3.0	—	3	—	—	1	2	—
Levodopa-treated, advanced	1/2	59.3 ± 2.2	13.0 ± 3.2	43.0 ± 1.3	—		1	2	1	1	1

*Unified Parkinson's disease rating scale of parkinsonian severity (*17*).
†Modified (*17*) classification of disability according to Hoehn and Yahr (*18*).
‡Side of predominant clinical symptoms.

by weight determination before and after sterile filtration. The concentration of β-CIT solutions was determined by reversed-phase HPLC (28% acetonitrile, 72% 0.05 *M* sodium dihydrogen phosphate, 2 ml/min) with ultraviolet detection at 235 nm. Samples of β-CIT were analyzed in triplicate and the concentration determined from calibration curves made by injection of three known concentrations of β-CIT tartrate (obtained from the same sources as the precursor for radiolabeling) on the same day as the PET study, the standard deviation being less than 2%. The radiochemical purity of [^{11}C]β-CIT was more than 97%.

PET Imaging

The PET scans were carried out with an eight-ring, whole-body PET scanner (ECAT 931/08-12) with an inplane spatial resolution of 6.5 mm FWHM and with an axial resolution of 6.75 mm measured according to Spinks et al. (*20*). The subject's head was fixed in the tomograph with an individually prepared Styrox frame. The PET examinations with [^{11}C]CIT were carried out as dynamic studies between 0 and 80 min (time frames 15 × 60 sec, 7 × 300 sec, 3 × 600 sec). The injected doses of [^{11}C]CIT were 102.5–211.3 MBq (2.77–5.71 mCi). The duration of the intravenous injection was 10 sec. The specific radioactivity at the time of injection was 10.2–29.2 GBq/μmole (276.3–789.6 mCi/μmole). The amount injected varied between 2.33 and 7.35 μg. No untoward effects of this compound were observed during the PET studies.

The region of interest (ROI) analysis was carried out by taking the head of the caudate and the putamen, as well as the total striatum, as separate ROIs. Moreover, frontal, temporal and parietal cortical ROIs were made, as well as thalamic and midbrain ROIs. All the ROIs were drawn freely except the cerebellar ROIs, which were elliptical. In this context, high-field (1.5 T) MRI images of the brain were available for reference. Ratios between these regions and the cerebellum were determined by calculating the mean ratio using the time frames from 60 to 80 min from the injection reflecting the maximum region-to-cerebellum ratio. Samples of arterialized venous blood (23 samples; 10 during the first 3 min and then at 5, 7, 8, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 min after the injection) were drawn from an antecubital vein during the PET scan for assessment of radioactivity.

Statistics

After the dynamic time-activity curves had been generated, the differences between the region-to-cerebellum ratios in the normal subjects and in the parkinsonian patients were compared using the nonparametric Kruskal-Wallis test. Correlation analyses concerning the effects of age and the degree of disability on the region-to-cerebellum ratios were calculated with Pearson's method.

RESULTS

A peak of radioactivity was observed in the venous blood within 2 min of the injection (Fig. 1A). After that, there was a decline of radioactivity in the blood. The transfer of radioactivity into the brain was rapid (Fig. 1). As indicated in Fig. 2A, [^{11}C]β-CIT was taken up into the putamen and caudate, the thalamus and a midbrain region under the thalamus. The cortical uptake of this compound was negligible as the ratios of frontal, temporal and parietal cortical regions to cerebellum were around 1. With reference to total radioactivity in the tissue, [^{11}C]β-CIT accumulated slowly during the PET scan period of 80 min towards a plateau (Fig. 1A).

As shown in Figure 3, the putamen-to-cerebellum ratios in normal subjects were 1.57–2.02 (range 1.80 ± 0.13, mean ± s.e.m.), whereas the corresponding values in parkinsonian patients were 1.41–1.76 (range 1.59 ± 0.04; mean ± s.e.m.). This difference is significant ($p = 0.028$). In those parkinsonian patients whose symptoms were more pronounced on one side than on the other side, there was less accumulation of [^{11}C]β-CIT in the putamen contralateral to the clinical symptoms than in the relatively normal ipsilateral putamen (Fig. 2B). In the caudate, there was no significant difference between the normal subjects and the parkinsonian patients.

The disability of the patients according to the Hoehn and Yahr scale had a significant negative correlation with the accumulation of [^{11}C]β-CIT in the putamen expressed as a putamen-to-cerebellum ratio ($r = -0.677$, $p < 0.05$) and in the caudate ($r = -0.737$, $p <= 0.05$). On the other hand, age did not correlate significantly with the uptake of [^{11}C]β-CIT.

Concerning the uptake of [^{11}C]β-CIT into the thalamus and into the midbrain area (Fig. 4), no significant differences were detected between the normal subjects and the parkinsonian patients. The thalamo-to-cerebellar ratios were 1.33–1.72 (range, 1.51 ± 0.12, mean ± s.e.m.) in the

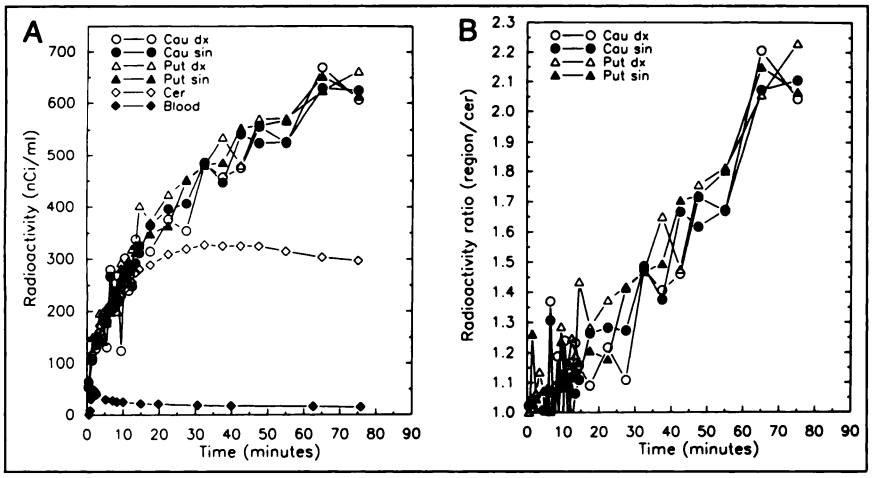

FIGURE 1. Radioactivity in brain regions and venous blood of a normal subject after intravenous injection of [^{11}C]β-CIT (216.5 MBq = 5.85 mCi). (A) Total radioactivity and (B) region-to-cerebellum ratios as a function of time. Cau dx = right caudate; Cau sin = left caudate; Put dx = right putamen; Put sin = left putamen; Cer = cerebellum.

normal subjects and 1.37–1.63 (range, 1.51 ± 0.03, mean ± s.e.m.) in the parkinsonian patients. The midbrain-to-cerebellar ratios were 1.38–1.77 (range, 1.60 ± 0.11 mean ± s.e.m.) in the controls and 1.32–1.76 (range, 1.52 ± 0.06, mean ± s.e.m.) in the patient group.

DISCUSSION

Our results show that [^{11}C]β-CIT accumulates in the striatum and, though to a lesser extent, in the thalamus and midbrain, whereas cortical uptake is negligible. In this sense, [^{11}C]β-CIT is a marker of the presynaptic dopaminergic system, although the thalamic and midbrain binding clearly indicate that there is also affinity for other neurotransmitter systems. The pattern of distribution including midbrain binding has been described previously by using [^{11}C]CFT PET (*21*) and with [^{123}I]β-CIT SPECT (*22,23*).

Our results differ, however, from those of Farde et al. (*16*) in the sense that they did not describe any midbrain binding with [^{11}C]β-CIT. The exact nature of the midbrain binding has been discussed previously. Frost et al. (*21*) did not interpret it in exact anatomical terms, whereas Laruelle et al. (*24*) suggested, on the basis of autoradiography, that the midbrain binding refers to the uptake of the radioligand into the hypothalamic area.

According to our results, there is a decreased accumulation of [^{11}C]β-CIT in the putamen of parkinsonian patients contralateral to the clinical symptoms, indicating a decreased dopamine reuptake function. This finding agrees with the conclusions obtained in vivo with 6-[^{18}F]fluoro-L-dopa (*25,26*) and [^{11}C]nomifensine (*10*).

The fact that there is a significant negative correlation between the disability of the patients and [^{11}C]β-CIT uptake means that the accumulation of this radioligand in the striatum sensitively monitors the basic pathogenetic processes in Parkinson's disease. The lack of a significant age correlation in vivo may be due either to the small number of patients or the limited age range of the subjects in this study. On the other hand, Wong et al. (*27*) found no positive age correlation in their studies using [^{11}C]CFT. By contrast, an analysis of postmortem samples (*28,29*) has shown that there is a decrease of about 10% per decade for dopamine transporters.

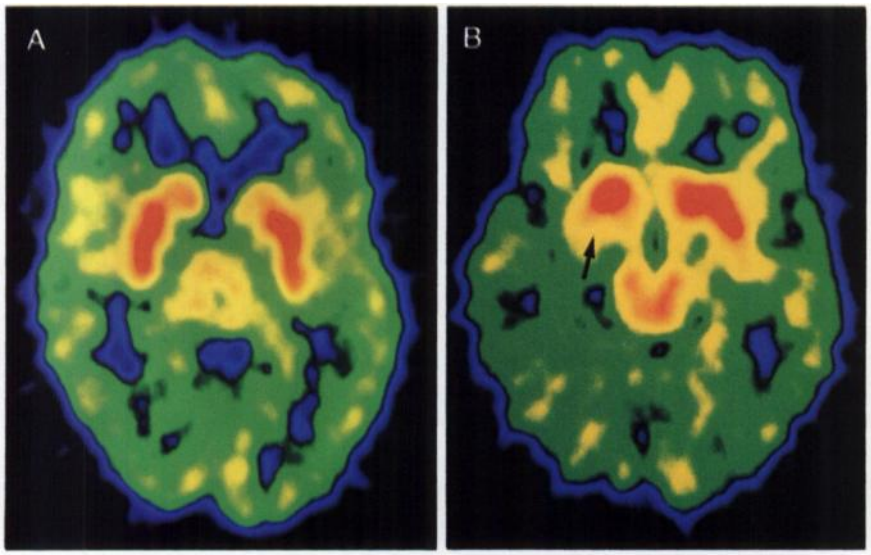

FIGURE 2. PET images through the caudate/putamen level of (A) a normal subject and (B) a de novo parkinsonian patient with left-sided symptoms. Note the decreased accumulation of radioactivity in the right putamen contralateral to the clinical symptoms (arrow).

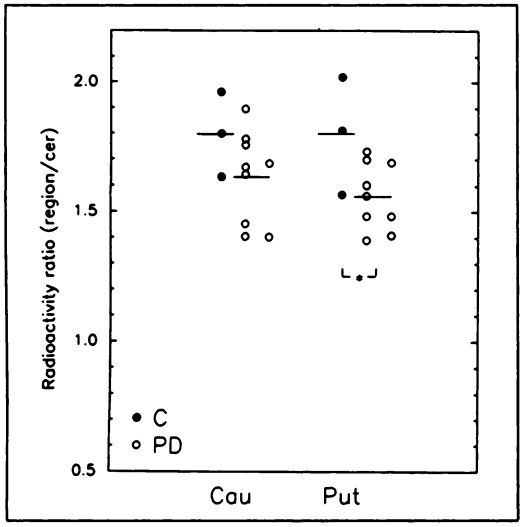

FIGURE 3. Caudate-to-cerebellum and putamen-to-cerebellum ratios of [^{11}C]β-CIT. Controls: both the hemispheres have been averaged; parkinsonian patients: only the hemispheres contralateral to the side of predominant clinical symptoms have been taken into account. In the only patient with symmetrical clinical symptoms, averaged values have been used. In the putamen, parkinsonian patients have significantly less accumulation of radioactivity than controls * = $p < 0.05$. C = control; PD = Parkinson's disease; and ——— = mean.

Furthermore, postmortem studies with [^{3}H]GBR-12935 (*30–33*) have shown that the presynaptic dopaminergic reuptake sites in Parkinson's disease are significantly diminished. Uhl et al. (*34*) showed that those neurons which survived in the pars compacta of the substantia nigra in brains of Parkinson's disease patients displayed only 57% of the dopamine transporter mRNA hybridization intensity displayed by nigral neurons in normal control brains.

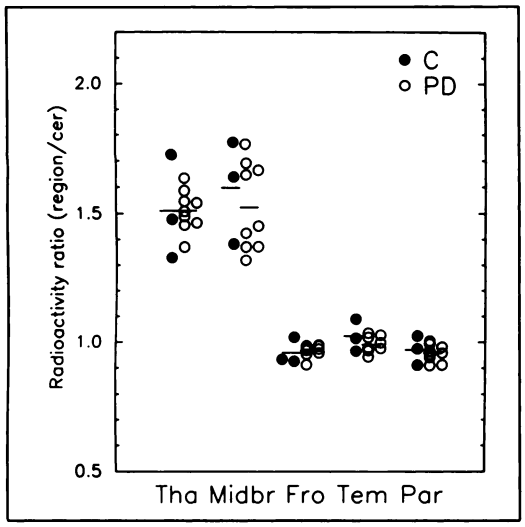

FIGURE 4. Thalamus/midbrain/frontal cortex/temporal cortex/parietal cortex-to-cerebellum ratios of [^{11}C]β-CIT. All the differences between controls and parkinsonian patients are nonsignificant. Tha = thalamus; midbr = midbrain; fro = frontal cortex; tem = temporal cortex; and par = parietal cortex.

Displacement studies using [^{123}I]β-CIT in nonhuman primates (*24*) have shown that the striatal uptake is associated with dopamine transporters, as it is displaced by GBR 12909, a selective dopamine uptake inhibitor, but not by citalopram, a selective serotonin (5-HT) uptake inhibitor. The inverse is true for the hypothalamo-midbrain area, suggesting that the uptake in this area is associated primarily with 5-HT transporters (*24*). The same result has been obtained with autoradiography in mice using [^{125}I]β-CIT (*35*).

Preliminary SPECT results obtained with [^{123}I]β-CIT (*22,23*) have been promising. There are certain quantitative differences between these findings and PET results: SPECT shows remarkably high striatum-to-cerebellum ratios 10–20 hr after the injection of the tracer. This discrepancy is due to the fact that [^{11}C]β-CIT, because of the short half-life of [^{11}C], only shows the first part of a prolonged accumulation process, whereas [^{123}I] displays these processes more extensively for a longer duration. Therefore, β-CIT may be better suited for SPECT studies than for PET. Comparison between [^{11}C]β-CIT and [^{18}F]6-fluorodopa is difficult because no direct comparative studies in identical patients have been published. In any case, it is clear that these compounds measure different aspects of presynaptic dopaminergic function, which makes them complementary to each other.

CONCLUSION

Carbon-11-β-CIT is a ligand with which monoaminergic neurotransmission can be studied in a way which reflects the pathogenetic mechanisms of various dopaminergic and serotonergic CNS disorders.

ACKNOWLEDGMENTS

The authors thank the staff of the Cyclotron/PET Center for their assistance and the Medical Research Council/Academy of Finland for their financial support.

REFERENCES

1. Kitayama S, Shimada S, Uhl GR. Parkinsonism-inducing neurotoxin MPP$^+$: uptake and toxicity in nonneuronal COS cells expressing dopamine transporter cDNA. *Ann Neurol* 1992;32:109–111.
2. Uhl GR. Parkinson's disease: neurotransmitter and neurotoxin receptors and their genes. *Eur J Neurol* 1990;30:21–30.
3. Javitch AJ, Blaustein RO, Snyder SH. [^{3}H]mazindol binding associated with neuronal dopamine and norepinephrine uptake sites. *Mol Pharmacol* 1984;26:35–44.
4. Dubocovich ML, Zahniser NR. Binding characteristics of the dopamine uptake inhibitor [^{3}H]nomifensine to striatal membranes. *Biochem Pharmacol* 1985;34:1137–1144.
5. Vignon J, Pinet V, Cerruti C, Kamenka J-M, Chicheportiche R. [^{3}H]N-[1-(2-benzo(b)thiophenyl)cyclohexyl]piperidine ([^{3}H]BTCP): a new phencyclidine analog selective for the dopamine uptake complex. *Eur J Pharmacol* 1988;148:427–436.
6. Maurice T, Barbanel G, Kamenka J-M, Vignon J. Modulation by dopamine of [^{3}H]*N*-[1-(2-benzo(b)thiophenyl)cyclohexyl]piperidine ([^{3}H]BTCP, a phencyclidine derivative) binding to the dopamine uptake complex. *Neuropharmacology* 1991;30:591–598.
7. Reith MEA, Sershen H, Lajtha A. Saturable (^{3}H)cocaine binding in central nervous system of mouse. *Life Sci* 1980;27:1055–1062.
8. Janowsky A, Berger P, Vocci F, Labarca R, Skolnik P, Paul SM. Characterization of sodium-dependent [^{3}H]GBR-12935 binding in brain: a radio-

ligand for selective labeling of the dopamine transport complex. *J Neurochem* 1986;46:1272–1276.
9. Aquilonius S-M, Bergström K, Eckernäs S-Å, et al. In vivo evaluation of striatal dopamine reuptake sites using ^{11}C-nomifensine and positron emission tomography. *Acta Neurol Scand* 1987;76:283–287.
10. Tedroff J, Aquilonius S-M, Laihinen A, et al. Striatal kinetics of [^{11}C]-(+)-nomifensine and 6-[^{18}F]fluoro-L-dopa in Parkinson's disease measured with positron emission tomography. *Acta Neurol Scand* 1990;81:24–30.
11. Kilbourn MR, Carey JE, Koeppe RA, et al. Biodistribution, dosimetry, metabolism and monkey PET studies of [^{18}F]GBR 13119. Imaging the dopamine uptake system in vivo. *Nucl Med Biol* 1989;16:569–576.
12. Ponchant M, Varastet M, Hantraye P, Chicheportiche R, Kamenka J-M, Crouzel C. Synthesis of 3-[^{18}F]fluoromethyl-BTCP and evaluation as a potential PET radioligand for the dopamine transporter in baboons. *Nucl Med Biol* 1993;20:727–733.
13. Fowler JS, Volkow ND, Wolf AP et al. Mapping cocaine binding sites in human and baboon brain in vivo. *Synapse* 1989;4:371–377.
14. Dannals RF, Neumeyer JL, Milius RA, Ravert HT, Wilson AA, Wagner HN, Jr. Synthesis of a radiotracer for studying dopamine uptake sites *in vivo* using PET: 2β-carbomethoxy-3β-(4-fluorophenyl)-[N-^{11}C-methyl]tropane ([^{11}C]CFT or [^{11}C]WIN-35,428). *J Lab Compd Radiopharm* 1993;33:147–152.
15. Müller L, Halldin C, Farde L, et al. [^{11}C]β-CIT, a cocaine analogue. Preparation, autoradiography and preliminary PET investigations. *Nucl Med Biol* 1993;20:249–255.
16. Farde L, Halldin C, Müller L, Suhara T, Karlsson P, Hall H. PET study of [^{11}C]β-CIT binding to monoamine transporters in the monkey and human brain. *Synapse* 1994;16:93–103.
17. Fahn S, Elton RL, members of the UPDRS Development Committee. Unified Parkinson's disease rating scale. In: Fahn S, Marsden CD, Calne DB, Goldstein M, eds. *Recent developments in Parkinson's disease*. Florham Park, NJ: MacMillan Healthcare Information; 1987:153–163.
18. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology (Minneap)* 1967;17:427–442.
19. Någren K, Takahashi T, Lehikoinen P, Bergman J. Preparation of the antioestrogenic compound *N*-[methyl-^{11}C]-toremifene for the study of oestrogen-receptor positive tumors in vivo. *J Labelled Compd Radiopharm* 1991;29:1085–1089.
20. Spinks TJ, Jones T, Gilardi MC, Heather JD. Physical performance of the latest generation of commercial positron scanner. *IEEE Trans Nucl Sci* 1988;35:721–725.
21. Frost JJ, Rosier AJ, Reich SG, et al. Positron emission tomographic imaging of the dopamine transporter with ^{11}C-WIN35,428 reveals marked declines in mild Parkinson's disease. *Ann Neurol* 1993;34:423–431.
22. Innis RB, Seibyl JP, Scanley BE, et al. Single photon emission computed tomographic imaging demonstrates loss of striatal dopamine transporters in Parkinson disease. *Proc Natl Acad Sci* 1993;90:11965–11969.
23. 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 [GenSect]* 1993;94:137–146.
24. 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.
25. Nahmias C, Garnett ES, Firnau G, Lang A. Striatal dopamine distribution in Parkinsonian patients during life. *J Neurol Sci* 1985;69:223–230.
26. Leenders KL, Salmon EP, Tyrrel P, et al. The nigrostriatal dopaminergic system assessed in vivo by PET in healthy volunteer subjects and patients with Parkinson's disease. *Arch Neurol* 1990;47:1290–1298.
27. Wong DF, Yung B, Dannals RF, et al. In vivo imaging of baboon and human dopamine transporters by positron emission tomography using [^{11}C]WIN 35,428. *Synapse* 1993;15:130–142.
28. Zelnik N, Angel I, Paul SM, Kleinman JE. Decreased density of human striatal dopamine uptake sites with age. *Eur J Pharmacol* 1986;126:175–176.
29. De Keyser JD, Ebinger G, Vauquelin G. Age-related changes in the human nigrostriatal dopaminergic system. *Ann Neurol* 1990;27:157–161.
30. Janowsky AA, Vocci F, Berger P, et al. [^{3}H]GBR-12935 binding to the dopamine transporter is decreased in the caudate nucleus in Parkinson's disease. *J Neurochem* 1987;49:617–621.
31. Hirai M, Kitamura N, Hashimoto T, et al. [^{3}H]GBR-12935 binding sites in human striatal membranes: binding characteristics and changes in parkinsonians and schizophrenics. *Jpn J Pharmacol* 1988;47:237–243.
32. Maloteaux JM, Vanisberg M-A, Laterre C, Javoy-Agid F, Agid Y, Laduron PM. [^{3}H]GBR-12935 binding to dopamine uptake sites: subcellular localization and reduction in Parkinson's disease and progressive supranuclear palsy. *Eur J Pharmacol* 1988;156:331–340.
33. Allard PO, Rinne J, Marcusson JO. Dopamine uptake sites in Parkinson's disease and in dementia of the Alzheimer type. *Brain Res* 1994;637:262–266.
34. Uhl GR, Walther D, Mash D, Faucheux B, Javoy-Agid F. Dopamine transporter messenger RNA in Parkinson's disease and control substantia nigra neurons. *Ann Neurol* 1994;35:494–498.
35. Cline EJ, Scheffel U, Boja JW, et al. In vivo binding of [^{125}I]RTI-55 to dopamine transporters: pharmacology and regional distribution with autoradiography. *Synapse* 1992;12:37–46.