Imaging of Dopamine Transporters with Iodine-123-β-CIT and SPECT in Parkinson's Disease

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The aim of the present study was to demonstrate the degeneration of the dopaminergic nigrostriatal pathway in Parkinson's disease by using the cocaine derivative [^{123}I]β-CIT (2-β-carbomethoxy-3-β(4-iodophenyl)-tropane or RTI-55) and SPECT and to relate the findings to the severity of the disease (Hoehn and Yahr scale, H/Y) and to clinical data such as motor score and activities of daily living. **Methods:** Thirteen volunteers and 47 patients with idiopathic Parkinson's disease (PD) of H/Y Stage I-V (I:n = 16, II:n = 6, III:n = 14, IV:n = 9, V:n = 2) were investigated. Acquisitions were performed 2, 4, 16, 20 and 24 hr postinjection. ROIs were drawn over the striatum and the cerebellum. Specific β-CIT binding was defined as striatal minus cerebellar binding. The ratio of specific over nondisplaceable binding (striatum/cerebellum-1) was determined as well as the percent deviation of this ratio from age-expected control values. **Results:** The time-activity curve of striatal [^{123}I]β-CIT binding demonstrated a maximum around 20 hr postinjection in controls and a peak 4 hr postinjection in PD patients. Ratios differed significantly between the two groups. A significant correlation existed between this ratio and clinical measures. Hemiparkinsonian patients revealed significantly diminished [^{123}I]β-CIT binding not only contralateral to the clinically affected but also contralateral to the clinically unaffected side. [^{123}I]β-CIT binding showed a significant decrease in comparison to age-expected values ranging from 36% in H/Y stage I to 71% in H/Y stage V. **Conclusion:** The present study demonstrates that it is possible to visualize and quantify the degeneration of dopaminergic nigrostriatal neurons in PD using [^{123}I]β-CIT and SPECT with good correlation to clinical parameters.

Key Words: iodine-123-β-CIT; SPECT; Parkinson's disease

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Until recently, imaging of the striatal dopaminergic system with SPECT was restricted to the postsynaptic side. By using [^{123}I]IBZM alterations of D2 dopamine (DA), receptor binding could be detected for example in Huntington's disease or in patients under neuroleptic treatment (*1*), whereas in Parkinson's disease (PD) only minor changes were found with this method. Recently, a new radiotracer for the presynaptic side has been developed: 2β-carboxymethoxy-3β(4-iodophenyl)tropane (β-CIT (*2,3*), also designated as RTI-55 (*4*)), a cocaine derivative with high affinity for monoamine transporters. β-CIT can be labeled with ^{123}I for SPECT or ^{11}C for PET. It has been shown to bind with high affinity to DA and serotonin (5-HT) transporters (*2,4–8*), with the highest binding in striatal and hypothalamus/midbrain regions. Displacement studies in nonhuman primates demonstrated that β-CIT binding in the striatum is almost exclusively to DA transporters, whereas binding in the hypothalamus/midbrain is mainly to 5-HT transporters (*7*). This finding was confirmed by our group, in which we found no reduction of striatal [^{123}I]β-CIT binding in depressive patients treated with the selective serotonin reuptake inhibitor citalopram (*9*).

In PD, a degeneration of dopaminergic neurons in the substantia nigra leads to a reduction of neuronal projections to the striatum. Consequently, the presynaptically located DA transporters in the striatum are lost. A reduction of DA reuptake sites in the striatum of patients with PD had already been demonstrated by Kaufmann and Madras (*10*) and Niznik et al. (*11*) in postmortem studies.

Few in vivo studies using [^{123}I]β-CIT and SPECT have shown a loss of DA transporters in PD (*12–17*). The aim of the present study was to investigate patients with PD of different severity to evaluate alterations of DA transporters in the striatum in comparison with healthy volunteers and to correlate these results with clinical data.

MATERIALS AND METHODS

Patients

Thirteen healthy volunteers (4 women, 9 men; age range 24–75 yr; median age 57 yr) and 47 patients (16 women, 31 men; age range 42–82 yr; median age 66 yr) with idiopathic PD were investigated. Volunteers were free of medication and had no history of neuropsychiatric disorders.

Patients were examined neurologically by five experienced physicians. The severity of the disease was classified according to Hoehn and Yahr (H/Y) (*18*). In addition, motor disabilities and activities of daily living were rated using the Unified Parkinson's Disease Rating Scale (UPDRS) (*19*). Patients showed no major depression, dementia or other neurological disorders besides PD. Sixteen patients ranged in H/Y Stage I, 6 in Stage II, 14 in Stage III, 9 in Stage IV and 2 in Stage V. UPDRS motor scores and activities of daily living scores are given in Figures 5 and 6. Eighteen patients were untreated or stopped therapy 24 hr before tracer administration; the remaining 29 patients were allowed to take antiparkinsonian medication on the day of tracer administration with L-DOPA/decarboxylase inhibitors of various doses (n = 28), in several cases in combination with dopamine agonists (n = 9) or amantadine (n = 6) and anticholinergic drugs (n = 5) except benztropine. Five patients were treated with a low-dose neuroleptic medication and two were treated with a tetracyclic antidepressant. Therapy with l-(-)-deprenyl (n = 18) was stopped at least 18 hr before [^{123}I]β-CIT application.

The study was approved by the local ethics committee and informed consent was obtained from each subject.

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SPECT Imaging

After blockade of thyroid uptake with 600 mg sodium perchlorate taken orally 30 min before tracer application, subjects received a mean dose of 3.76 mCi (139 MBq) (range 2.57–5.38 mCi) of [^{123}I]β-CIT intravenously as a bolus.

The normal subjects and 25 of the 47 patients were investigated 2, 4, 16, 20 and 24 hr after tracer administration and the other 22 patients only 20 hr postinjection. SPECT studies were performed with a triple-headed rotating scintillation camera equipped with medium-energy collimators and a dedicated computer system. Two- and 4-hr acquisitions lasted for 20 min (20 sec per frame) and 16-, 20- and 24-hr acquisitions for 40 min (40 sec per frame), so that 180 frames in a step-and-shoot mode were achieved. The subject's head was positioned in a headholder using a crossed-laser beam system for repositioning. Parallel to the cantomeatal plane 3.5-mm thick cross-sections were reconstructed by filtered back-projection in 128 × 128 matrices using a Butterworth filter (cutoff frequency 0.7, order 7). Attenuation correction was then performed with a uniform attenuation coefficient of 0.12/cm after manually drawing an ellipse around the head contour.

ROIs were drawn manually on single-slice views by one investigator over the right and left striatum (size: 40–45 pixels each), respectively, and cerebellum (size: 50–55 pixels each) using a brain atlas for help. Striatal ROIs were drawn on several consecutive (3.5-mm thick) axial slices and the highest value for each striatum was taken to avoid tilting errors. Only for patients in H/Y Stage I (unilateral PD) right and left striatal values were calculated separately. Cerebellar ROIs were drawn on the slice of best visualization, usually 10 slices below the striatum, and on the two adjacent slices. These three consecutive sections and left and right cerebellar hemispheres were pooled together and average cerebellar counts were calculated. Cerebellar values were taken as reference (nondisplaceable activity) because postmortem studies (*20–22*) had shown a very low density of DA (and 5-HT) transporters and receptors in this region. For semiquantification, three different methods of describing brain activity were applied: (a) average regional radioactivity normalized to injected dose and body mass

(cpm/pixel/mCi × body weight, decay-corrected for the time of the SPECT investigation after tracer injection)

was calculated for each ROI and specific binding was determined as target minus cerebellar activity; (b) a binding ratio of

striatum over cerebellum − 1,

which represents specific/nondisplaceable binding based on average counts/pixel as another measure of specific binding was calculated

[total − nondisplaceable activity/nondisplaceable activity
= total/nondisplaceable activity − 1
= specific/nondisplaceable binding],

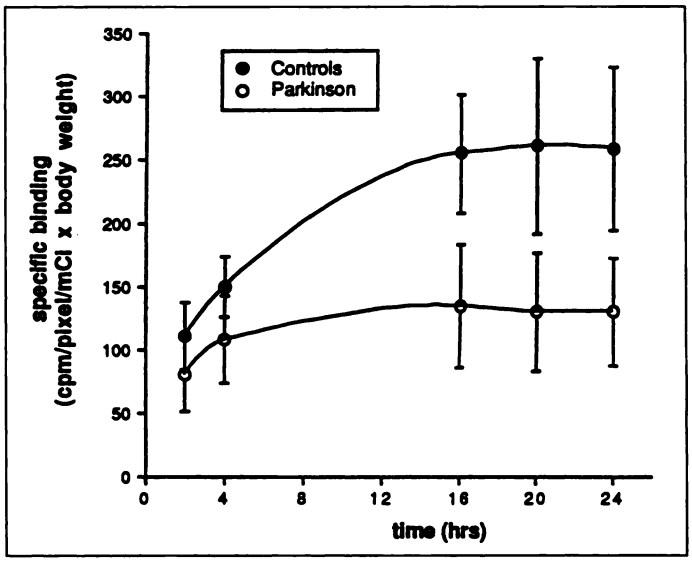

FIGURE 1. Time-activity curve of specific [^{123}I]β-CIT binding in the striatum (difference between striatum and cerebellum in cpm/pxl/mCi × body weight, decay corrected) in healthy volunteers and patients with Parkinson's disease. (For details of control and patient populations see Table 1 and Methods section.)

according to (*23*); and (c), in addition, age-expected control values of the binding ratio were obtained from regression analysis of the data of the healthy volunteers. The percent deviation of each patient's ratio from this age-expected control value was calculated for the investigation 20 hr after tracer application.

Statistical Analysis

The calculated specific binding as well as binding ratios were compared between controls and patients for each SPECT acquisition using a one-way ANOVA. In patients of H/Y Stage I striatal ratios ipsi- and contralateral to clinical symptoms were compared with Student's t-test for paired data as well as with values of the volunteers applying one-way ANOVA. Correlations between striatal ratios and H/Y stages were evaluated with one-way ANOVA and regression analysis, and correlations to UPDRS values (motor score, activities of daily living) were evaluated with regression analysis. A $p < 0.05$ was regarded as significant.

RESULTS

Kinetic Study

Striatal specific binding and binding ratios differed significantly between patients and controls at each acquisition time. (Fig. 1 and Table 1). The time-activity curve in the control subjects showed a maximum around 20 hr after tracer administration with a plateau between 16 and 24 hr postinjection. Therefore, further calculations were based on the data obtained 20 hr postinjection. In PD group, the highest striatal activity was reached earlier.

Visual evaluation demonstrated relatively high activity in the caudate nucleus in PD patients, whereas the putamen could

TABLE 1
Iodine-123-β-CIT Binding Ratio (Striatum/Cerebellum − 1 = Specific/Nondisplaceable Binding) in Healthy Volunteers and Patients with Parkinson's Disease (± s.d.)

	Time postinjection (hr)				
	2	4	16	20	24
Control subjects	1.8 ± 0.46	2.76 ± 0.57	6.82 ± 1.09	7.37 ± 1.26	8.71 ± 1.54
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
PD patients	1.14 ± 0.33	1.75 ± 0.48	3.36 ± 1.09	3.44 ± 1.11	4.49 ± 1.86

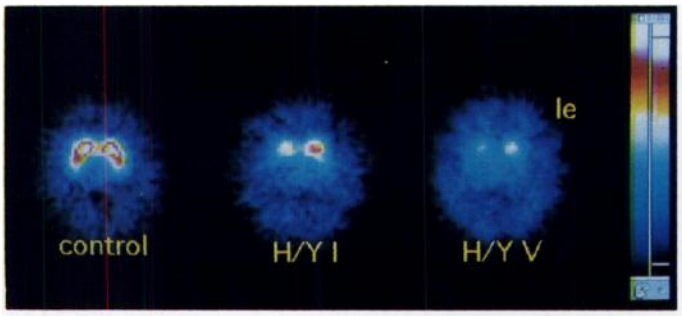

FIGURE 2. Iodine-123-β-CIT SPECT scan obtained 20 hr postinjection in a healthy volunteer (control), a patient with PD, H/Y Stage I and a patient with PD, Stage V. All three studies are scaled to the maximum of the control.

hardly be differentiated even in patients with H/Y Stage I (Fig. 2). A separate calculation of the caudate nucleus and putamen values was not performed in the present study.

Regression analysis of the binding ratio of controls revealed a highly significant age dependency of [^{123}I]β-CIT binding in the striatum 20 hr postinjection, with an average loss of [^{123}I]β-CIT binding of about 5% per decade ($r = -0.71$, $p < 0.007$) (Fig. 3).

Correlation between Iodine-123-β-CIT Binding and Clinical Data

A correlation was found between disease severity according to H/Y and the binding ratio 20 hr postinjection ($F = 14.5$, $p < 0.0001$) (Fig. 4). In addition, this ratio correlated significantly with UPDRS values: ratio versus motor score: $F = 14.2$, $p < 0.0005$; ratio versus activities of daily living: $F = 6.6$, $p < 0.013$. (Figs. 5 and 6).

The percent deviation from age-expected control values in PD patients revealed a significant correlation to H/Y stages ($F = 10.6$, $p < 0.0001$), ranging from −36% in H/Y Stage I to −71% in H/Y Stage V (H/Y Stage II: −50%, H/Y Stage III: −52%, H/Y Stage IV: −64%).

Patients with H/Y Stage I ($n = 16$) had significantly lower striatal-to-cerebellar radioactivity ratios contralateral to clinical symptoms compared to ipsilateral values (contralateral ratio = 4.05 (±0.82); ipsilateral ratio = 4.91 (±1.03); $t = 8.2$, $p < 0.0001$). Both ratios were significantly lower than corresponding values of

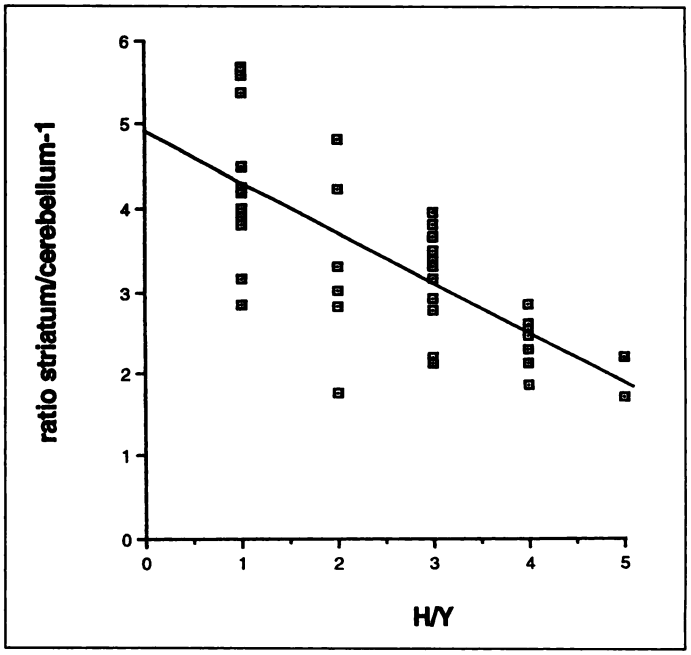

FIGURE 4. Correlation between the [^{123}I]β-CIT binding ratio (striatum/cerebellum − 1 = specific/nondisplaceable binding) 20 hr postinjection and the severity of PD ($n = 47$), classified according to H/Y ($r = -0.75$, $p < 0.0001$).

13 controls (ipsilateral: $F = 64.1$, $p < 0.0001$, contralateral: $F = 27.4$, $p < 0.0001$). The decrease to age-expected control values was 42% (±13%) in the contralateral striatum and 29% (±16%) in the ipsilateral striatum.

DISCUSSION

In vivo studies of the presynaptic dopaminergic function in the striatum of PD patients are mostly performed with 6-L-[^{18}F]fluoro-DOPA ([^{18}F]-FDOPA), which reflects the activity of the aromatic amino acid decarboxylase and therefore the ability of nigrostriatal dopaminergic terminals to take up and metabolize exogenous DOPA (*23–26*). Recently it has become possible to visualize dopamine reuptake sites as a measure of the regional density of dopaminergic nerve endings. For that

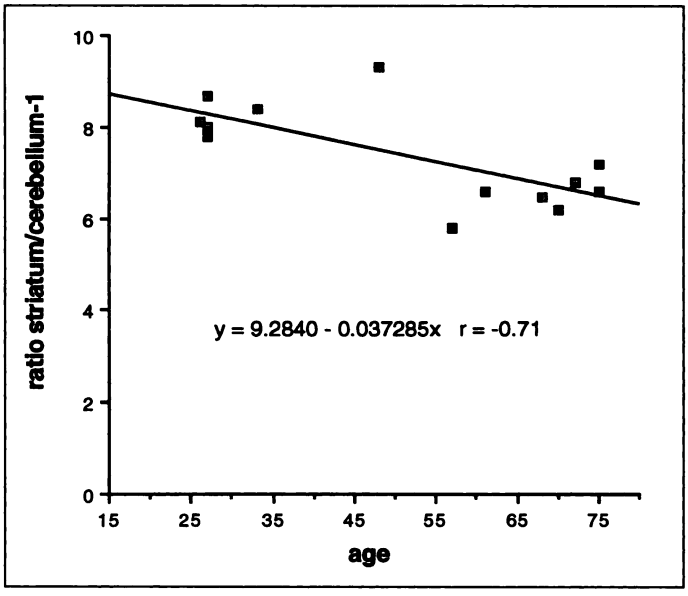

FIGURE 3. Regression analysis of the [^{123}I]β-CIT binding ratio (striatum/cerebellum − 1 = specific/nondisplaceable binding) 20 hr postinjection and age of the healthy volunteers ($n = 13$) ($r = -0.71$, $p < 0.007$).

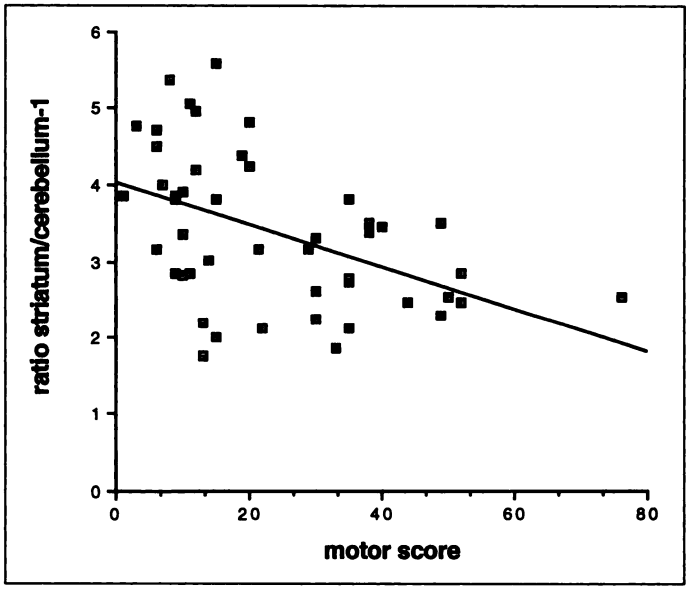

FIGURE 5. Correlation between the [^{123}I]β-CIT binding ratio (striatum/cerebellum − 1 = specific/nondisplaceable binding) 20 hr postinjection and UPDRS motor score in 47 patients with PD ($r = -0.49$, $p < 0.0005$).

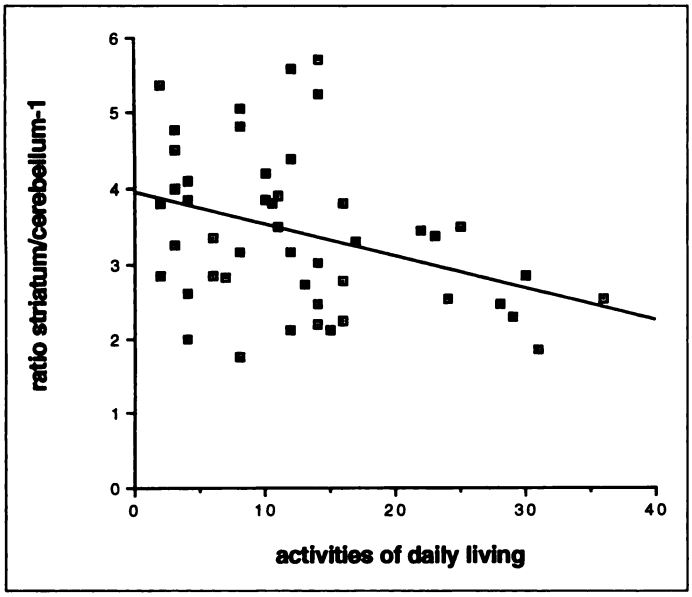

FIGURE 6. Correlation between the [^{123}I]β-CIT binding ratio (striatum/cerebellum − 1 = specific/nondisplaceable binding) 20 hr postinjection and UPDRS activities of daily living scores in 47 patients with PD ($r = -0.36$, $p < 0.013$).

purpose, various cocaine analogs with high affinity for DA reuptake sites have been developed, labeled with ^{3}H (*27*) respectively, or positron single-photon emitting isotopes ([^{11}C] WIN 35,428 [^{11}C]CFT (*28*), [^{123}I]β-CIT ([^{123}I]RTI-55) (*2–5*), [^{11}C]β-CIT (*8*)).

In this study, we used [^{123}I]β-CIT to visualize DA transporters and demonstrated significantly reduced binding in the striatum of patients with PD, which correlates with disease severity. Moreover, an age-related decline of striatal [^{123}I]β-CIT binding in the control due to a loss of dopamine reuptake sites with age was found. This subject's finding is in line with previous reports (*29–32*).

Diminished DA transporters in PD have already been found with [^{11}C]nomifensine and PET (*23,25*) and in postmortem brain tissue using [^{3}H]GBR 12935 (*33,34*) or [^{3}H]cocaine (*35*). The finding of a reduced binding of [^{123}I]β-CIT in the striatum of patients with PD in this investigation is also in accordance with earlier studies applying [^{123}I]β-CIT (*12–17*) or other cocaine derivatives such as [^{11}C]WIN 35,428 (*36*).

Iodine-123-β-CIT binding in the striatum of PD patients was reduced by 36% to 71% in normal controls, depending on the severity of the disease. In patients with PD of H/Y Stage V, the ratio was reduced by 71% in comparison with age-expected control values. In a similar study with [^{123}I]β-CIT and SPECT, Innis et al. (*14*) described an uptake reduction of 55%–75% in PD of H/Y Stage II in comparison control subjects.

These data obtained with [^{123}I]β-CIT indicate a similar degree of degeneration of nigrostriatal dopaminergic neurons as found in studies with [^{11}C]nomifensine (*23,25*) or [^{18}F]FDOPA (*23–25*). In these studies, uptake reduction of both tracers of about 60% in the putamen has been reported, which is in accordance with postmortem studies describing a 60% loss of neurons in the lateral part of the substantia nigra pars compacta (*37–39*). In contrast, it has been described that a dopamine loss of 70%–80% is necessary for the decompensation of dopamine metabolism (*40,41*) and the appearance of parkinsonian symptoms. Kish et al. (*42*) reported a dopamine loss from 89% to near-total depletion in the putamen of patients with clinically severe illness. Therefore, the indicated decreases of dopamine transporters measured in the cited and present study are lower than the expected reduction of endogenous dopamine. One explanation for this finding would be that a subpopulation of dopaminergic neurons loses its ability to synthesize DA in the course of the degenerating process but is still morphologically intact and could thus be visualized with [^{123}I]β-CIT. Terminal sprouting of dopaminergic nerve endings for compensation of the degeneration of neighboring axons and/or an upregulation of DA transporters might be another explanation. Thus, changes in the amount of DA transporters theoretically might be influenced not only by the loss of dopaminergic nerve endings but also by regulatory processes. Unfortunately, there is no way to objectively measure the degree of degeneration of dopaminergic nerve endings in the striatum in postmortem brain samples of PD patients.

Specific binding of [^{123}I]β-CIT, as well as the binding ratio, was significantly lower in patients with PD in comparison to normal subjects at any time of the investigation. As discussed elsewhere (*13*), the time-activity curve of specific binding in the volunteers showed a maximum about 20-hr postinjection. Apparently, patients with PD showed only a minor increase of [^{123}I]β-CIT binding after 4-hr postinjection. Similar observations were reported by Innis et al. (*14*) and recently confirmed by Laruelle et al. (*43*). This earlier peak uptake in PD patients can be explained by a diminished number of binding sites due to nigrostriatal degeneration and, concomitantly, a decreased number of DA transporters. Due to the lower number of binding sites, equilibrium of [^{123}I]β-CIT across the blood-brain barrier is reached more rapidly (*14*).

In all patients, a clear difference of [^{123}I]β-CIT binding between the caudate and putamen was evident, indicating a more severe loss of DA fibers in the putamen. A different effect on the caudate and putamen, with relative sparing of caudate function in PD, has been demonstrated in vivo elsewhere (*23–26,36,44*) and was recently shown by Seibyl et al. (*16*) in a [^{123}I]β-CIT study. This is in accordance with postmortem studies in PD, where a more pronounced loss of cells in the ventrolateral substantia nigra, primarily projecting to the posterior putamen, is described and with the finding of a more pronounced loss of DA in the putamen (*37–42*).

Iodine-123-β-CIT binding in the striatum showed a significant inverse correlation to the degree of disability as measured by the H/Y stage and UPDRS scores. Similar results have recently been reported by Seibyl et al. (*16*), Rinne et al. (*17*) and in preliminary form by our own group (*45*). These significant correlations with simple measures of disease severity such as the H/Y scale and also with the more detailed UPDRS motor and activities of daily living scores suggest that the loss of [^{123}I]β-CIT binding in the striatum 20-hr postinjection actually is a robust index for dopaminergic nerve cell loss in PD.

Laruelle et al. (*43*) compared kinetic and graphical analyses with the simple ratio method on the second day after injection of [^{123}I]β-CIT in five healthy volunteers and concluded that the calculation of the specific/nonspecific-bound ratio at the time when a binding equilibrium is reached (about 16–24-hr postinjection) gives the closest estimate of the binding potential B_{max}/K_d. Furthermore, the coefficient of variance of repeated measures was only 6% in their study. These findings underline the validity of the ratio method of [^{123}I]β-CIT binding at a time of binding equilibrium.

Patients with unilateral parkinsonian symptoms (H/Y Stage I) demonstrated diminished [^{123}I]β-CIT uptake in the striatum not only contralateral to the clinical symptoms but also ipsilateral. Asymmetric [^{18}F]FDOPA uptake, [^{11}C]nomifensine or [^{123}I]β-CIT binding in the putamen with a more marked decrease contralateral to the clinically more affected side has been described previously (*15–17,23,46–48*). Kempster et al. (*49*)

reported an asymmetric cell loss in the substantia nigra postmortem in correlation to asymmetrical disease onset. In this study using [^{123}I]β-CIT and SPECT, it was not only possible to demonstrate different side-to-side affection corresponding to clinical symptoms but also to show a subclinical lesion of the nigrostriatal dopaminergic system contralaterally to the clinically still intact side. With disease progression, a continuous decline of striatal [^{123}I]β-CIT binding was evident. Similar correlations between [^{18}F]FDOPA uptake in the putamen and the H/Y stage have been described in PET studies (*24,25,36*). Iodine-123-β-CIT and SPECT now seem to offer the possibility of early identification of subclinically affected or high-risk persons and the opportunity of investigating or monitoring the effect of neuroprotective agents in long-term studies.

The effect of medication should be discussed, as patients in the recent study were under long-term antiparkinsonian therapy. Laruelle et al. (*7*) have shown that acute intravenous infusions of L-DOPA did not influence [^{123}I]β-CIT binding to the DA transporter in the striatum. There is no evidence that dopamine agonists or amantadine, regarded as a noncompetitive NMDA (N-methyl-D-aspartate) antagonist, have any effect on DA reuptake sites. L-(-)-deprenyl is metabolized to l-amphetamine and l-metamphetamine with a half-life of several hours (*50*). Patients who had received l-(-)-deprenyl within 18 hr of the first investigation were not included in this study because these metabolites might compete with β-CIT binding and may displace it from the DA transporter as demonstrated by Laruelle et al. (*7*) for d-amphetamine. None of the study subjects received benztropine, which acts as a DA uptake inhibitor and would influence striatal β-CIT binding (*51*).

CONCLUSION

Iodine-123-β-CIT fulfilled the expectations of demonstrating nigrostriatal dopaminergic neuronal loss in PD. A high signal-to-noise ratio makes it possible to detect even discrete alterations. Because SPECT is easily available and comparatively inexpensive, [^{123}I]β-CIT seems to be a promising new ligand for early diagnosis of PD, for differential diagnosis in clinically unclear cases such as vascular lesions or essential tremor resembling PD, and for monitoring the disease progression and evaluating possible neuroprotective agents.

REFERENCES

1. Brücke T, Podreka I, Angelberger P, et al. Dopamine D2 receptor imaging with SPECT: studies in different neuropsychiatric disorders. *J Cereb Blood Flow Metab* 1991;11:220–228.
2. Neumeyer JL, Wang S, Milius RA, et al. Iodine-123-2β-carboxymethoxy-3β-(4-iodophenyl)tropane (β-CIT): high affinity SPECT radiotracer of monoamine reuptake sites in brain. *J Med Chem* 1991;34:3144–3146.
3. Innis R, Baldwin R, Sybirska E, et al. Single photon emission computed tomography imaging of monoamine reuptake sites in primate brain with [^{123}I]β-CIT. *Eur J Pharmacol* 1991;200:369–370.
4. Shaya EK, Scheffel U, Dannals RF, et al. In vivo imaging of dopamine reuptake sites in the primate brain using SPECT and iodine-123-labeled RTI-55. *Synapse* 1992;10: 169–172.
5. Boja JW, Patel A, Carroll FI, et al. Iodine-123-RTI-55: a potent ligand for dopamine transporters. *Eur J Pharmacol* 1991;194:133–134.
6. Scheffel U, Dannals RF, Cline EJ, et al. Iodine-123-RTI-55, an in vivo label for serotonin transporters. *Synapse* 1992;11:134–139.
7. 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 non-human primates. *Synapse* 1993;13:295–309.
8. Müller L, Halldin C, Farde L, et al. Carbon-11-β-CIT, a cocaine analog. Preparation, autoradiography and preliminary PET investigations. *Nucl Med Biol* 1993;20:249–255.
9. Pirker W, Asenbaum S, Kasper S, et al. β-CIT SPECT demonstrates blockade of 5HT-uptake sites by citalopram in the human brain in vivo. *J Neural Transm* 1995;100:247–256.
10. Kaufmann MJ, Madras BK. Severe depletion of cocaine recognition sites associated with the dopamine transporter in Parkinson's diseased striatum. *Synapse* 1991;49:43–49.
11. Niznik HB, Fogel EF, Fassos FF, Seeman P. The dopamine transporter is absent in parkinsonian putamen and reduced in the caudate nucleus. *J Neurochem* 1991;56:192–198.
12. Kuikka JT, Bergström KA, Vanninen E, Laulumaa V, Hartikainen P, Länsimies E. Initial experience with single-photon emission tomography using iodine-123-labeled 2β-carbomethoxy-3β-(4-iodophenyl)tropane in human brain. *Eur J Nucl Med* 1993; 20:783–786.
13. 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* 1993;94:137–146.
14. Innis RB, Seibyl JP, Scanley BE, et al. Single-photon emission computed tomographic imaging demonstrates loss of striatal dopamine transporters in Parkinson's disease. *Proc Natl Acad Sci USA* 1993;90:11965–11969.
15. Brücke T, Asenbaum S, Angelberger P, et al. Iodine-123-β-CIT binding in the human brain: SPECT studies in parkinsonian patients and normal controls. *Soc Neurosci Abstr* 1994;20(part 2):1464.
16. Seibyl JP, Marek KL, Quinlan D, et al. Decreased single-photon emission computed tomography [^{123}I]β-CIT striatal uptake correlates with symptom severity in Parkinson's disease. *Ann Neurol* 1995;38:589–598.
17. Rinne JO, Kuikka JT, Bergström KA, Rinne UK. Striatal dopamine transporters in different disability stages of Parkinson's disease studied with [^{123}I]β-CIT SPECT. *Parkinsonism and Related Disorders* 1995;1:47–51.
18. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology* 1967;6:253–259.
19. Fahn S, Elton RL, Members of the Unified Parkinson's Disease Rating Scale Development Commitee Unified Parkinson's disease rating scale. In: Fahn S, Marsden CD, Calne DB, Goldstein M, eds. *Recent developments in Parkinson's disease*, vol. 2. Florham Park, NY: Macmillan Healthcare Information; 1987:153–164.
20. Laruelle M, Vanisberg M-A, Maloteaux J-M. Regional and subcellular localization in human brain of [^{3}H]paroxetine binding, a marker of serotonin uptake sites. *Biol Psychiatry* 1988;24:299–309.
21. Palacios JM, Camps M, Cortes R, Probst A. Mapping dopamine receptors in the human brain. *J Neural Transm* 1988;27(suppl 27):227–235.
22. De Keyser J, DeBacker J-P, Ebinger G, Vauquelin G. Hydrogen-3-GBR-12935 binding to dopamine uptake sites in the human brain. *J Neurochem* 1989;53:1400–1404.
23. 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.
24. Brooks DJ, Ibanez V, Sawle GV, et al. Differing patterns of striatal ^{18}F-dopa uptake in Parkinson's disease, multiple system atrophy and progressive supranuclear palsy. *Ann Neurol* 1990;28:547–555.
25. Brooks DJ, Salmon EP, Mathias CJ, et al. The relationship between locomotor disability, autonomic dysfunction and the integrity of the striatal dopaminergic system in patients with multiple system atrophy, pure autonomic failure and Parkinson's disease, studied with PET. *Brain* 1990;113:1539–1552.
26. Otsuka M, Ichiya Y, Hosokawa S, et al. Striatal blood flow, glucose metabolism and ^{18}F-Dopa uptake: difference in Parkinson's disease and atypical parkinsonism. *J Neurol Neurosurg Psychiatry* 1991;54:898–904.
27. Madras BK, Spealman RD, Fahey MA, Neumeyer JL, Saha JK, Milius RA. Cocaine receptors labeled by [^{3}H]-2β-carbomethoxy-3β-(4-fluorophenyl)tropane. *Mol Pharmacol* 1989;36:518–524.
28. Dannals RF, Neumeyer JL, Milius RA, et al. 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.
29. Zednik N, Angel I, Paul SM, Kleinmann JE. Decreased density of human striatal dopamine uptake sites with age. *Eur J Pharmacol* 1986;126:175–176.
30. De Keyser J, Ebinger G, Vauquelin G. Age-related changes in the human nigrostriatal dopaminergic system. *Ann Neurol* 1990;27:157–161.
31. Volkow ND, Fowler JS, Wang GJ, et al. Decreased dopamine transporters with age in healthy human subjects. *Ann Neurol* 1994;36:237–239.
32. 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.
33. Janowsky A, Vocci F, Berger P, et al. Hydrogen-3-GBR 12935 binding to the dopamine transporter is decreased in the caudate nucleus in Parkinson's disease. *J Neurochem* 1987;49:617–621.
34. Maloteaux J-M, Vanisberg M-A, Laterre C, et al. Hydrogen-3-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.
35. Pimoule C, Schoemaker H, Javoy-Agid F, Scatton B, Agid Y, Langer SZ. Decrease in [^{3}H]-cocaine binding to the dopamine transporter in Parkinson's disease. *Eur J Pharmacol* 1983;95:145–146.
36. Frost JJ, Rosier AJ, Reich SG, et al. Positron emission tomography imaging of the dopamine transporter with ^{11}C-WIN 35,428 reveals marked declines in mild Parkinson's disease. *Ann Neurol* 1993;34:423–431.
37. German DC, Manaye K, Smith WK, Woodward DJ, Saper CB. Midbrain dopaminergic cell loss in Parkinson's disease: computer visualization. *Ann Neurol* 1989;26:507–514.
38. Goto S, Hirano A, Matsumoto S. Subdivisional involvement of nigrostriatal loop in idiopathic Parkinson's disease and striatonigral degeneration. *Ann Neurol* 1989;26: 766–770.
39. Rinne JO, Rummukainen J, Paljärvi L, Rinne UK. Dementia in Parkinson's disease is related to neuronal loss in the medial substantia nigra. *Ann Neurol* 1989;26:47–50.
40. 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.
41. Hornykiewicz O, Kish SJ. Biochemical pathophysiology of Parkinson's disease. *Adv Neurol* 1986;45:19–34.
42. Kish SJ, Shannar K, Hornykiewicz O. Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiological and clinical implications. *N Engl J Med* 1988;318:876–880.
43. 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.
44. Salmon E, Brooks DJ, Leenders KL, et al. A two-compartment description and kinetic procedure for measuring regional cerebral [^{11}C]nomifensine uptake using positron emission tomography. *J Cereb Blood Flow Metab* 1990;10:307–316.
45. Brücke T, Asenbaum S, Pozzera A, et al. Dopaminergic nerve cell loss in Parkinson's disease quantified with [I-123]-β-CIT and SPECT correlates with clinical findings. *Mov Disord* 1994;9(suppl 1):120.
46. Garnett ES, Nahmias C, Firnau G. Central dopaminergic pathways in hemiparkinsonism examined by positron emission tomography. *Can J Neurol Sci* 1984;11:174–179.
47. Karbe H, Holthoff V, Huber M, et al. Positron emission tomography in degenerative disorders of the dopaminergic system. *J Neural Transm* 1992;4:121–130.
48. Marek KL, Seibyl JP, Zoghbi S, et al. Iodine-123-β-CIT/SPECT imaging demonstrates bilateral loss of dopamine transporters in hemi-Parkinson's disease. *Neurology* 1996;46:231–237.
49. Kempster PA, Gibb WRG, Stern GM, Lees AJ. Asymmetry of substantia nigra neuronal loss in Parkinson's disease and its relevance to the mechanism of levodopa related motor fluctuations. *J Neurol Neurosurg Psychiatry* 1989;52:72–76.
50. Heinonen EH, Anttila MI, Lammintausta RAS. Pharmacokinetics and clinical pharmacology of selegilin. In: Szelenyi, ed. *Inhibitors of monoamine oxidase B. Pharmacology and clinical use in neurodegenerative disorders.* Basel: Birkhäuser; 1993:201–213.
51. Xu C, Coffey LL, Reith ME. Translocation of dopamine and binding of 2-beta-carbomethoxy-3-beta-(4-fluorophenyl) tropane (WIN 35,428) measured under identical conditions in rat striatal synaptosomal preparations. Inhibition by various blockers. *J Biochem Pharmacol* 1995;49:339–350.

Regional Stability of Cerebral Blood Flow Measured by Repeated Technetium-99m-HMPAO SPECT: Implications for the Study of State-Dependent Change

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The replicability of resting state rCBF has implications for the analysis of cerebral activation protocols and the interpretation of rCBF in disease states. This study examined the stability of rCBF as measured by two resting state ^{99m}Tc-HMPAO brain SPECT scans with an emphasis on examining the contribution of specific cerebral regions to within and between subjects variance. **Methods:** Nine normal, medically healthy subjects underwent two ^{99m}Tc-HMPAO brain SPECT scans under identical conditions separated by 48 hr. A reference system and semiautomated computer ROI method was used to enable accurate alignment and cortical analysis of the two scans. **Results:** Mean within-subject difference between Scans 1 and 2 was 2.8% (range 0%-7.8%) for the 36 cortical ROIs. The mean between-subject coefficient of variation was 10% (range 7%-15%) for these ROIs. Correlation analysis of rCBF pattern replication for all slice levels yielded a highly significant overall consistency of pattern within subjects (Pearson r = 0.698, p = 0.0001). Variance component analysis revealed regional heterogeneity in between-subjects variance, with significantly greater variability found in frontal regions. The within-subject repeated measures variability was not significantly different across regions. **Conclusion:** Good within-subject 48-hr replicability indicates that individual resting state rCBF reflects fairly stable, subject-specific factors. This also justifies comparing state-dependent studies separated by a modest length of time. Although individual patterns of rCBF replicate well, the larger contribution of frontal regions to normal between-subjects variance makes evaluating the frontal effects of disease or activation more difficult.

Key Words: normal cerebral blood flow; activation states; technetium-99m-HMPAO; SPECT; rCBF

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Activation studies of regional cerebral blood flow (rCBF) during stimulation, motor activity, psychological tasks and other state manipulations have become a prominent part of functional neuroimaging research. Such investigations were originally conducted using ^{133}Xe cortical blood flow measures and later using ^{15}O-water rCBF PET, since these methods allow relatively rapid sequential repeat measures on the same subject. The ability to study the rCBF effects of several different conditions in the same session is clearly convenient and possibly reduces the errors associated with repositioning subjects in protocols involving testing sessions separated in time. Sequential studies are also thought to be best suited for activation research because it is generally believed that proximity in time reduces variability associated with uncontrolled-for changes in a subject's physiology, mental state and mood, i.e., state dependent factors not being specifically evaluated by the experimental protocol.

SPECT tracers such as ^{99m}Tc-HMPAO are not readily suited for immediately repeated studies because clearance of the tracer from cerebral tissue is essentially dependent on radioactive decay (based on a 6-hr physical half-life in the case of ^{99m}Tc). The lack of redistribution and clearance is at the same time an extremely useful property, allowing investigators to capture a state present at the time of ^{99m}Tc-HMPAO injection and scan the subject later. This offers advantages for experimental situations in which the state to be studied is difficult to capture, achieve or control within a scanner, such as an ictal seizure state (*1*), psychiatric research paradigms involving complex state changes (*2*) and for situations where anesthesia may be required to properly scan an uncooperative patient but where the anesthetic may mask the effects of the disease state under study, such as in the case of autistic patients (*3*). Brain SPECT scans using ^{99m}Tc-HMPAO can be used for activation protocols either by a split dose technique, in which one condition is evaluated using a low ^{99m}Tc-HMPAO dose, and another condition is scanned using a much higher dose, or by separating each study condition by two days, allowing the initially injected tracer to decay. The former method unfortunately results in the

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