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Reproducibility of Iodine-123-β-CIT SPECT Brain Measurement of Dopamine Transporters

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Iodine-123-β-CIT has been used as a probe of monoamine transporters in human and nonhuman primates utilizing SPECT. To assess the utility of this tracer for measurement of striatal dopamine (DA) transporters in human disease, we studied the test/retest variability and reliability of SPECT measures obtained after bolus injection of [^{123}I]β-CIT 0–7 hr (Day 1) and 18–24 hr (Day 2) after administration. **Methods:** For the Day 2 study, seven healthy humans (4 men, 3 women; aged 19–74 yr) participated in two [^{123}I]β-CIT SPECT scans separated by 7–14 days. Subjects were imaged at 18, 21 and 24 hr postinjection of 370 MBq (10 mCi) [^{123}I]β-CIT. Two outcome measures were evaluated: (a) the ratio of specific striatal (activity associated with DA transporter binding) to nondisplaceable uptake, also designated V_3'' and (b) the total specific striatal uptake (%SSU) expressed as a percentage of injected radiotracer dose. Test/retest variability associated with V_3'' and total specific striatal uptakes were compared for scans acquired at 18, 21 and 24 hr with 24 hr only postinjection scans. For the Day 1 study, three of the subjects participated in two kinetic studies of [^{123}I]β-CIT uptake. A three-compartment model was used for determination of konBmax and binding potential (BP = B_{max}/K_d) and the reproducibility of the measures assessed. **Results:** In the Day 2 study, both outcome measures demonstrated excellent test/retest reproducibility with variability of $V_3'' = 6.8 \pm 6.8\%$ and percent striatal uptake = $6.6 \pm 4.3\%$ using data acquired from all time points. There were no significant differences in variability for the two outcome measures obtained. The intraclass correlation coefficient ρ was 0.96 and 0.98 for V_3'' and %SSU, respectively. Considering the 24 hr postinjection scans only, there was a nonsignificant trend toward lower test/retest variability for %SSU compared to V_3'' ($6.6 \pm 4.2\%$ and $12.8 \pm 9.0\%$, respectively). The test/retest variability for the Day 1 kinetic modeling data showed marked differences depending on the fitting strategy and assumptions about the reversibility of [^{123}I]β-CIT in striatum. Using a model that assumed a low, fixed value for reversible striatal binding (k_4) produced low variability ($12 \pm 9\%$). **Conclusion:** These data suggest that SPECT imaging performed at either 0–7 hr or 18–24 hr after [^{123}I]β-CIT injection permits calculation of reliable and reproducible measures of dopamine transporters and supports the feasibility of using [^{123}I]β-CIT in serial evaluation of human neuropsychiatric disease.

Key Words: iodine-123-β-CIT; SPECT; dopamine transporter

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Iodine-123-β-CIT ([^{123}I]2β-carbomethoxy-3β-(4-iodophenyl-tropane) binds with high affinity to dopamine (IC_{50} = 1.6 n*M*) and serotonin (IC_{50} = 3.78 n*M*) transporters and has been used as a SPECT probe in human and nonhuman primates (*1–5*). In baboons, striatal activity was largely associated with dopamine transporters based on dynamic SPECT studies demonstrating displacement of this activity following administration of dopamine transporter-selective, but not serotonin transporter-selective agents (*3*). Following bolus administration of [^{123}I]β-CIT in humans, decay-corrected striatal time-activity data showed a prolonged time to highest uptake occurring by 18 hr posttracer injection and very slow striatal washout. Occipital and free parent plasma time-activity data achieved a plateau earlier than striatum and also demonstrated extremely slow rates of washout.

The ratio of striatal activity specifically bound to receptors divided by nondisplaceable activity is equal to the binding potential (BP) divided by the nonspecifically-bound compartment distribution volume (V_2) under conditions of equilibrium binding; i.e., when the concentration of parent compound is unchanging in plasma, receptor-bound, and nonspecifically-bound brain compartments. For a tracer like [^{123}I]β-CIT, the protracted steady levels of parent activity in plasma and activity within brain compartments closely approximates the equilibrium condition (*5*). Thus, the simple ratio of specific striatal to nondisplaceable activity calculated during the plateau phase of uptake provides an outcome measure that may be directly proportional to dopamine transporter density. Another consequence of the unchanging striatal time-activity data is the stability of other SPECT outcome measures, including specific striatal uptake expressed as a percent of injected radiopharmaceutical dose which provides a measure related to total receptor number.

The demonstration of reproducible SPECT outcome measures is critical and preliminary to the extension of [^{123}I]β-CIT to clinical populations, including the serial monitoring of progressive disorders like idiopathic Parkinson's disease. To extend our previous evaluation of quantitative [^{123}I]β-CIT SPECT outcome measures in humans, we undertook an evaluation of the test/retest reproducibility of two outcome measures

obtained 18–24 hr after [^{123}I]β-CIT injection: the ratio of specific:nondisplaceable striatal uptake, (also designated V_3''), and the total specific striatal uptake expressed as a percent of the administered dose (%SSU). In addition, we assessed the reproducibility of a kinetic analysis of data acquired during seven hours following the bolus injection (Day 1). For this purpose, scanning on Day 1 and arterial sampling were performed during the test and retest experiments in three subjects.

METHODS

Subjects

Subjects were evaluated by a research physician and determined to be free of medical or psychiatric illness on the basis of history, normal physical examination, blood chemistries, complete blood counts, thyroid function studies, urinalysis and EKG. Female subjects of child-bearing potential had negative serum pregnancy tests performed 24 hr prior to tracer injection. Following the provision of informed consent and the oral administration of supersaturated potassium iodide (SSKI, 800 mg), seven healthy subjects (4 men, 3 women; aged 19–74) participated in two [^{123}I]β-CIT SPECT studies separated by 7–21 days.

Radiopharmaceutical Preparation

High specific activity [^{123}I]β-CIT was prepared from the corresponding trimethylstannyl precursor (*1*) (Research Biochemicals International, Natick, MA) and high radionuclidic purity [^{123}I]NaI (Nordion International, Ltd., Vancouver, BC, Canada) as described previously (*6*). Radiochemical purity was 97.6 ± 1% (with this and subsequent measures expressed as mean ± s.d.) as measured with high-performance liquid chromatography (HPLC) and the specific activity was >5000 Ci/mmole.

Data Acquisition and Day 2 Data Analysis

Four fiducial markers each filled with 4–5 μCi [^{99m}Tc]$NaTcO_4$ were attached to both sides of the subject's head at the level of the canthomeatal line prior to imaging to facilitate post hoc reorientation of transaxial images. At 18, 21 and 24 hr following the intravenous bolus injection of 358 ± 19 MBq (9.7 ± 0.5 mCi) [^{123}I]β-CIT, SPECT brain scans were acquired in a 64 × 64 × 32 matrix (n = 4 subjects test/retest) or 128 × 128 × 64 matrix (n = 3 subjects test/retest). Three 15-min acquisitions were obtained at each of the three time points postinjection.

Raw data were reconstructed from photopeak counts within a 20% symmetric energy window centered around 159 keV using a Butterworth filter (power factor = 10, cutoff = 1 cm). Transaxial images were reoriented parallel to the canthomeatal plane and attenuation-corrected using Chang zero-order correction (*7*) based on an ellipse fit to brain using a linear attenuation factor (μ = 0.15 cm^{-1}) determined empirically from an ^{123}I containing distributed source phantom.

Two outcome measures were evaluated: the ratio of specific striatal uptake to nonspecific uptake (V_3'') and the total left and right specific striatal uptake expressed as a percent of injected radioactivity. For V_3'', four (64 × 64 × 32 matrix) or eight (128 × 128 × 64 matrix) contiguous transaxial slices representing the most intense striatal uptake were summed. A standard region of interest (ROI) template was constructed based on coregistered MRI scans obtained from previous [^{123}I]β-CIT studies in four healthy controls. This template included regions for the left and right striatum, frontal cortex, occipital cortex, midbrain and cerebellum. Small variations in individuals' brain required movement of the ROIs within the template without changing the individual ROI shape or pixel size. Data were expressed as counts/pixel/minute for each brain region. Estimates of specific striatal uptake were made by subtracting occipital counts/pixel/min from total striatal counts/pixel/min based on the low density of monoamine transporters in the occipital cortex. This method assumes equivalence of nondisplaceable uptake in the striatum and occipital cortex. V_3'' was derived by dividing the operationally-defined specific striatal uptake by occipital uptake, this measure is equal to total specific activity/occipital activity −1. The final ratio was calculated as the mean of all V_3'' measurements over 18, 21 and 24 hr (9 scans total) or the mean of V_3'' measurements made at 24 hr (3 scans).

The percent uptake in striatal and occipital ROIs used a larger number of transaxial slices (n = 14 for the 64 × 64 × 32 matrix size and n = 28 for the 128 × 128 × 64 matrix) than the ratio of specific to nondisplaceable uptake (n = 4 or n = 8 for 64 × 64 × 32 and 128 × 128 × 64 matrices, respectively) to recover all specific activity associated with the striatum. In all subjects, the summed slices included one or two slices extending beyond visually-identified striatum. A striatal ROI slightly larger than that used in the other ROI analysis above was placed over the summed slices over regions corresponding to left and right striatum and occipital cortices bilaterally. The size of these ROIs (left striatum, right striatum, left occipital, right occipital) were identical and therefore produced identical volumes for measurement of total counts in the striatal regions and occipital cortex. As in the previous analysis, nondisplaceable striatal uptake was estimated from occipital counts. Total counts within the occipital volume of interest were subtracted from the total counts within the striatal volumes to generate a measure of counts associated with specific striatal uptake. In this instance, the measure is more akin to total receptor number rather than receptor concentration provided by V_3''. Counts were corrected for physical decay and converted to microcuries of activity based on ^{123}I distributed source phantoms containing ~500 μCi of activity. The mean of nine measurements made at 18, 21 and 24 hr and three measurements made at the 24 hr time point are reported.

Day 1 Kinetic Analyses

Three subjects (1 woman, 2 men; 27 ± 11 yr) were scanned on Day 1 as well as on Day 2 at 18 ± 10-day interval. The first experiment (test) of these three subjects was previously published (*5*). Immediately after injection, scans were acquired according to the following protocol: four 3-min scans, followed by four 6-min scans and four 10-min scans. After this initial session (91 min total duration), the subjects were allowed to rest out of the camera gantry for 45 min. Two 10-min scans were then acquired every 45 min up to 420 min postinjection.

Arterial samples were obtained every 20 sec for the first 2 min with a peristaltic pump. Subsequent samples were obtained manually at 3, 4, 6, 8, 10, 12, 16, 20 and 30 min, and every 30 min until 420 min. Arterial samples were analyzed as previously described (*6*). Extraction in ethyl acetate was followed by reverse phase HPLC to measure the metabolite corrected total plasma activity ($C_a(t)$, μCi/ml). Plasma protein binding was measured in vitro by ultrafiltration through Centrifree membrane filters (Amicon, Beverly, MA) in quadruplicate at 37°C as previously described (*8*). SPECT images were analyzed as described for Day 2 data.

Kinetic analysis of Day I data were performed as previously described (*9*). Briefly, a three-compartment model was used. The model included the arterial plasma compartment (C_a), the intracerebral rapidly reversible compartment (C_2, also designated as the nondisplaceable compartment) and the slowly reversible compartment (C_3, also designated as the specific compartment). C_2 included the free, the nondisplaceable binding and binding to the 5-HT transporters (shown to equilibrate much faster than the binding to the DA transporters) (*3*). Nonlinear regression was performed to fit the convolution of the arterial input function by the impulse response function to the measured values, using a Levenberg-Marquart minimization procedure (*10*) implemented in MAT-

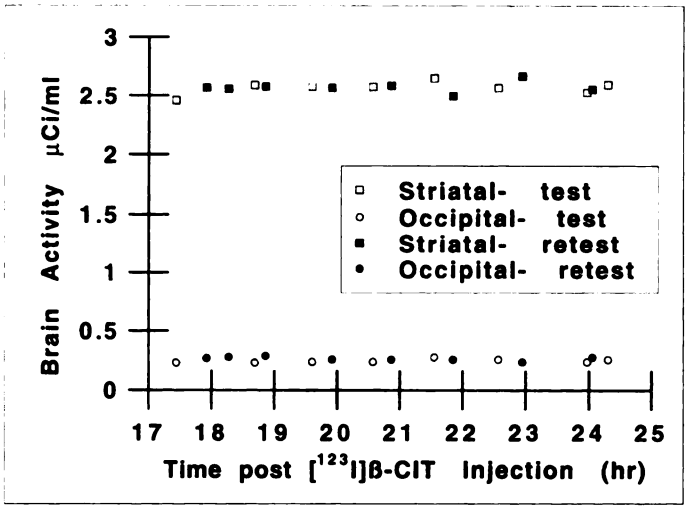

FIGURE 1. Test/retest decay-corrected time-activity data for striatum and occipital cortex in a representative healthy subject with [^{123}I]β-CIT SPECT imaging at 18–24 hr following the bolus administration of 370 MBq (10.0 mCi) test and 374 MBq (10.11 mCi) retest. Tests were separated by 7 days. Data are expressed as µCi/ml based on a calibration factor determined with a ^{123}I-containing distributed-source phantom and corrected for physical decay. Regional activity is essentially unchanging over the course of the study due to extremely slow biological washout of tracer.

LAB (The Math Works Inc., South Natick, MA) as previously described.

Two models and four fitting strategies were used. In the first model, the binding of [^{123}I]β-CIT was assumed to be irreversible, i.e., k_4, the tracer-receptor complex dissociation constant, was set to zero. This assumption appeared justified by the fact that the tracer activity increased in the striatum during Day 1 and by the linearity of the striatal Patlak slope on Day 1 (*2,5,11*). Two fitting strategies were used with this model. No constraints were used for the first fit (Fit A), while in the second fit (Fit B), the volume of distribution of the nondisplaceable compartment (V_2) in the striatum was assumed to be equal to the occipital volume of distribution.

In the second model, we assumed that the binding was reversible, i.e., that $k_4 > 0$. A first fit was performed (Fit C) with the same constraint as in fit B (striatal V_2 = occipital V_2). In fit C, k_4 was free to float. A second fit (Fit D) was performed with this model, using a fixed value of k_4 (0.00386 min^{-1}), a value derived from previous kinetic analysis of [^{123}I]β-CIT uptake combining Days 1 and 2 data (*5*). The assumption and constraining strategies of each fit are summarized in Table 3. Because the irreversible model assumed no dissociation, the outcome measure was the $k_{on}B_{max}$ product (ml · g^{-1} · min^{-1}), where k_{on} (ml · $pmole^{-1}$ · min^{-1}) is the association rate constant and B_{max} (pmole · gr^{-1}) is the density of receptors. In the reversible model, the outcome measure is the binding potential (BP, ml · g^{-1}), the ratio of B_{max} to the K_D (pmole · ml^{-1}), the equilibrium dissociation constant.

Statistical Analyses

For all outcome measures determined on Days 1 and 2, within subject variability between test and retest conditions, were calculated as the absolute value of the difference of the test and retest measure divided by the mean of test and retest and expressed as a percentage. Repeated measures ANOVA was utilized for evaluating within and between subject differences for V''_3, %SSU, and percent occipital uptake determined from the mean of nine scans from 18 to 24 hr post-tracer injection and from the mean of three scans at the 24-hr time point. Post hoc analyses utilized Dunnet's t-test. ANOVA was performed on the calculated variability for each of the two SPECT outcome measures determined on the basis of scans obtained at 18, 21, 24 hr and at 24 hr postinjection of radiotracer. A measure of the reliability of the two Day 2 outcome measures was determined relative to between subject variance by calculation of the intraclass correlation coefficient, ρ (*12*). This coefficient is an estimate of the reliability of the measurement and varies from 0 (no reliability) to 1 (total reliability, when test = retest measure) and is expressed by:

TABLE 1
Reproducibility of Day 2 Outcome Measures

					Uptake as % ID											
					Striatal V3"				Specific striatal uptake				Occipital uptake			
					Mean 9		Mean 3		Mean 9		Mean 3		Mean 9		Mean 3	
Subject no.	Condition	Age	Sex	Matrix	scans	Variability	scans	Variability	scans	Variability	scans	Variability	scans	Variability	scans	Variability
1	Test	24	F	64 × 64	8.87		8.47		1.03		1.06		0.15		0.16	
	Retest			64 × 64	8.65	2.5	9.07	6.8	1.06	2.8	1.17	10.1	0.08	54.4	0.07	80.3
2	Test	39	M	64 × 64	5.11		5.72		0.55		0.53		0.06		0.09	
	Retest			64 × 64	4.12	21.5	4.30	28.3	0.56	1.3	0.56	4.7	0.10	43.7	0.10	9.1
3	Test	19	F	64 × 64	5.83		5.84		0.86		0.84		0.13		0.12	
	Retest			64 × 64	5.61	3.8	5.26	10.4	0.79	8.8	0.76	10.1	0.12	5.3	0.11	10.6
4	Test	37	M	64 × 64	7.92		8.32		0.55		0.54		0.09		0.09	
	Retest			64 × 64	8.00	1.0	8.05	3.4	0.51	6.8	0.55	0.5	0.09	0.4	0.10	12.0
5	Test	34	M	128 × 128	9.50		9.87		0.94		0.89		0.11		0.12	
	Retest			128 × 128	8.82	7.4	8.08	20.0	0.86	9.1	0.85	5.4	0.11	2.7	0.12	0.5
6	Test	62	M	128 × 128	6.04		6.81		0.44		0.45		0.08		0.08	
	Retest			128 × 128	5.73	5.3	5.86	15.1	0.42	3.6	0.43	2.9	0.09	5.7	0.08	1.0
7	Test	74	F	128 × 128	6.09		6.29		0.37		0.37		0.09		0.09	
	Retest			128 × 128	5.74	5.9	5.95	5.6	0.42	14.0	0.42	12.4	0.09	2.2	0.09	1.7
		Mean variability				6.8		12.8		6.6		6.6		16.4		16.5
		COV(%)				100.0		70.0		67.0		66.0		138.0		173.0
		Reliability				0.96		0.82		0.98		0.97		0.35		*

*MSWS > MSBS; Variability = abs (test − retest)/(mean test and retest) × 100; Reliability = (MSBS − MSWS)/(MSBS + (n − 1) MSWS).
MSBS = mean sum of squares between subjects; MSWS = mean sum of squares within subjects.

TABLE 2
Mean Day 2 Outcome Measures

	V_3''				Uptake as %ID							
					Striatum		Occipital		Striatum		Occipital	
	Mean 9 scans		Mean 3 scans		Mean 9 scans				Mean 3 scans			
Measure	Test	Retest	Test	Retest	Test	Retest	Test	Retest	Test	Retest	Test	Retest
Mean	7.05	6.67	7.33	6.65	0.68	0.66	0.20	0.20	0.67	0.68	0.22	0.18
% COV	24	27	21	26	39	37	39	40	30	14	26	18

$$\rho = \frac{\text{MSBS} - \text{MSWS}}{\text{MSBS} + (n-1)\text{MSWS}},$$

where MSBS and MSWS are the mean sum of squares between and within subjects, respectively, and n is the number of within subject measurements.

RESULTS

Test/Retest Variability in V_3'' and Percent-Specific Striatal Uptake

Subjects achieved a plateau of both occipital and striatal activity during imaging at 18 through 24 hr after [^{123}I]β-CIT injection (Fig. 1). Table 1 summarizes results of the two Day 2 outcome measures: V_3'' and %SSU as the percent injected dose. V_3'' had a within-subject test/retest variability of 12.8 ± 9.0% for the mean of three scans at 24 hr and 6.8 ± 6.8% for the mean of the nine scans acquired at 18–24 hr, with these and subsequent measures expressed as mean ± s.d. The test/retest variability of %SSU was 6.6% ± 4.4% and 6.6% ± 4.3% for the mean of three and nine scans, respectively. The test/retest variability in occipital uptake was higher than striatum with variability of 16.5% ± 22.6% and 16.4% ± 28.5% for the mean of three scans at 24 hr and 9 scans at 18–24 hr, respectively.

There were no statistically significant differences in test/retest variability for either of the Day 2 outcome measures calculated as the mean of nine scans from 18–24 hr or three scans at 24 hr. Specifically, ANOVA comparing the variability of the three with nine scans showed no significant differences for V_3'' ($F = 2.008$ $p = 0.18$), percent striatal uptake ($F = 0.0001$ $p = 0.99$) or percent occipital uptake ($F = 0.0005$ $p = .994$). There were also no significant differences on ANOVA comparing V_3'' and percent striatal uptake as the mean of nine ($F = 0.003$ $p = 0.96$) or three scans ($F = 2.72$ $p = 0.125$).

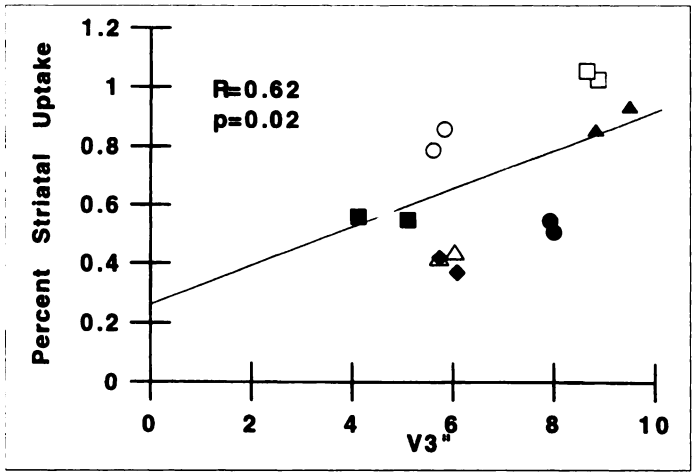

FIGURE 2. Correlation of V_3'' and percent striatal uptake for test and retest scans in seven healthy subjects. Individual subjects' test and retest data are indicated by the same markers. Data are based on means of nine scans per test condition obtained at 18–24 hr following [^{123}I]β-CIT injection.

The mean striatal V_3'' and percent uptake measures for test and retest conditions using the 18–24 hr time points (nine scans) and the 24 hr postinjection time point (three scans) are summarized in Table 2. Repeated measures ANOVA of V_3'' obtained at 18–24 hr showed significant between subject ($F = 48.4$ $p = 0.0001$) and within-subject ($F = 8.49$ $p = 0.027$) differences, although post hoc analysis was not significant for the within-subject effect. When V_3'' was calculated from only the 24 hr scans, there were again significant between-subject differences ($F = 10.47$ $p = 0.0034$), while within-subject comparison showed only a trend toward significance ($F = 5.11$ $p = 0.64$). The %SSU for data taken from the mean of either nine or three scans demonstrated statistically significant differences between subjects ($F = 106.55$ $p = 0.0001$, $F = 80.62$ $p = 0.0001$, for nine and three scans respectively), but not within subjects for the test/retest conditions ($F = 0.84$ $p = 0.39$, $F = 0.13$ $p = 0.73$, for nine and three scans, respectively). Occipital percent uptake showed no significant within subject or between subject differences for either the mean of nine scans or three scans.

Figure 2 graphically summarizes the variability of V_3'' and percent striatal uptake for the seven subjects. A regression fit of the data for the measures obtained at 18–24 hr postinjection showed a moderate correlation ($r = 0.62$ $p = 0.02$) of these measures.

Reliability of 18–24-Hour Postinjection Outcome Measures

Intraclass correlation coefficients provide a measure of test reliability and are summarized in Table 1. V_3'' calculated as the mean of all data points (18, 21 and 24 hr) demonstrated better reliability ($\rho = 0.96$) compared to V_3'' based on three scans acquired at 24 hr ($\rho = 0.82$). Striatal percent uptake based on three scans obtained at 24 hr ($\rho = 0.97$) had similar reliability to uptakes calculated over 18–24 hr ($\rho = 0.98$). Occipital percent uptake obtained from nine scans had much poorer reliability than the striatal measures ($\rho = 0.35$). Reliability of occipital uptake obtained from three scans at 24 hr could not be calculated because the mean sum of squares within subjects was greater than the mean sum squares between subjects.

Day 1 Kinetic Analyses

There were marked differences in the test/retest variability of the outcome measure between the fitting strategies (Table 3). The unconstrained irreversible model (Fit A) had the worse test/retest variation (70% ± 83%). This variability was dramatically improved when the striatal nondisplaceable compartment was assumed to be equal to the occipital compartment (Fit B, 8% ± 5%). The addition of a non-zero, unconstrained k_4 to this configuration (reversible model, Fit C) induced a large increase in the test/retest variability of the outcome measure (37% ± 36%). Constraining the value of k_4 to a fixed value restored the good reproducibility of the fitting strategy (12% ± 9%).

DISCUSSION

This study demonstrated low test/retest variability for three different [^{123}I]β-CIT SPECT outcome measures in healthy humans: two measures obtained at 18–24 hr after tracer administration and one outcome measure obtained on day 1 which used a kinetic analysis that constrained k_4 and set striatal V_2 equal to occipital V_2. Of the three outcome measures, one is clearly limited to research studies (kinetic measurement of BP) and two are potentially useful for clinical applications (specific striatal uptake and V_3''). Table 4 summarizes the underlying assumptions, sources of intersubject variation, and experimental requirements of each of these measures.

Day 1 Outcome Measures

Test/retest variability of Day 1 data were assessed to define which model provided the most reproducible outcome measure. The poor reproducibility of the unconstrained irreversible model demonstrated the inability of the fitting procedure to adequately separate the specific from nondisplaceable compartments. Introducing a constraint on the value of the nondisplaceable compartment considerably improved the reproducibility of the $k_{on}B_{max}$ derivation. The poor reproducibility of the reversible model with free k_4 revealed the difficulty of estimating k_4 in the absence of any noticeable washout. k_4 could be estimated by combining the Day 1 and Day 2 data (*5*). Fixing k_4 to a small but nonzero value provided reproducibility comparable to fit B.

Several criteria should be considered for the choice of an appropriate model and fitting strategy. The identifiability of the parameters, the goodness-of-fit, the sensitivity of the outcome measure to the duration of the experiment, and the test/retest reproducibility should be integrated with the physiological meaning of the parameters to guide the choice of the strategy. We previously showed that Fit D was superior to fits A to C for the first three criteria. We now observed that, as far as reproducibility is concerned, fit D and B are equivalent and acceptable, while fit A and C are much more sensitive to the noise in the data collection and do not provide reliable outcome measures. Taken together, fit D appear to be the best fitting strategy for kinetic analysis of [^{123}I]β-CIT experiments in human.

Although the number of kinetic Day 1 experiments was small compared to equilibrium Day 2 experiments, the variability of BP derivation by kinetic analysis with fit D appeared of the same magnitude as the variability of V_3'' measured on day 2 by the equilibrium method. However, BP measurement is not comparable to V_3'' measurement. The BP is a ratio of the receptor binding to the free plasma concentration, and this ratio is only dependent on receptor parameters (B_{max}/K_D). Measuring BP requires relating the plasma activity to the brain activity and is thus potentially sensitive to variation of cross calibration of both counting devices and to the noise associated with each of these measurements. In contrast, V_3'' is the ratio of the receptor binding to the nondisplaceable binding ($=BP/V_2$, where V_2 is the equilibrium distribution volume of the nonspecific striatal tracer uptake), and this value depends on both the BP and the nondisplaceable binding. Because V_3'' does not require plasma measurement, it is expected to be a more robust and reproducible outcome measure. The price, however, associated with the use of V_3'' is the assumption that the nondisplaceable binding does not vary between subjects, an assumption so far not validated.

In summary, measurement of BP with compartmental analyses may appear to be the most accurate from a theoretical perspective, since it uses multiple data points of brain and plasma activities and provides a value which is independent of

TABLE 3
Reproducibility of Day 1 Outcome Measures

Model	Fit	Constraints	Outcome measure	Free parameters	Subject			Mean ± s.d.
					Pair 1	Pair 2	Pair 3	
Irreversible (k4 = 0)	A	None	konBmax	K1, k2, k3	42%	163%	4%	70% ± 83%
	B	Striatal V2 = Occipital V2	konBmax	K1, k3	10%	11%	2%	8% ± 5%
Reversible (k4 > 0)	C	Striatal V2 = Occipital V2	Bmax/Kd	K1, k3, k4	31%	75%	4%	37% ± 36%
	D	Striatal V2 = Occipital V2 (k4 = 0.00386)	Bmax/Kd	K1, k3	15%	18%	2%	12% ± 9%

TABLE 4
Inherent Assumptions for Days 1 and 2 SPECT Measures

	Day BP	Day 2	
		V_3'' (Striatal/Occ) − 1	Specific striatal uptake (%ID)
Assumptions			
• Occipital activity equals nondisplaceable striatal activity	No—Fit A Yes—Fits B, C, D	Yes	Yes
• State of sustained equilibrium receptor binding	No	Yes	No
Influenced by intersubject variations in			
• Free fraction of tracer in plasma (F1)	No	No	Yes
• Nonspecific binding	No	Yes	No
• Kd	Yes	Yes	Yes
• Peripheral clearance of tracer	No	No	Yes
• Striatal volume/Precision of ROI placement	Yes	Yes	No
Experimental requirements			
• Blood sampling and metabolite analysis	Yes	No	No
• Accurate SPECT calibration (cpm to μCi)	Yes	No	Yes

between-subject variations in peripheral clearance of the radiotracer. Iodine-123-β-CIT, however, shows very slow kinetics in human subjects and striatum does not reach a peak specific (or equilibrium) value on Day 1. These slow kinetics significantly impair the ability of a three compartment/four parameter analysis of day 1 results to provide estimates of rate constants which show either good identifiability or reasonable test/retest reproducibility. Nonetheless, the use of two constraints (setting the striatal nondisplaceable activity equal to the occipital activity; and assuming a specific non-zero value for k_4) significantly improves both identifiability and reproducibility. One major use of the more complicated and more invasive modeling procedures has been to validate the utility of simpler outcome measures which may be more easily applied in clinical settings.

Day 2 Outcome Measures

With regard to the two "clinically applicable" outcome measures, we had predicted on theoretical grounds that V_3'' would be superior to %SSU, since V_3'' would be less vulnerable than striatal uptake to variations in peripheral clearance (Table 4). That is, if equilibrium or near-equilibrium receptor binding conditions exist on Day 2, then V_3'' will be immune to between-subject variations in clearance of the tracer on Day 1. The %SSU on Day 2 represents a cumulative value, and would therefore be sensitive to variations in uptake achieved on Day 1. Prior to this study, we were aware that V_3'' has an intrinsic theoretical flaw of vulnerability to between-subject variation in nonspecific binding. That is, since V_3'' is calculated from the striatal to occipital ratio, variations between subjects could be due to changes in either the numerator or denominator of the ratio. Studies, however, in nonhuman primates have shown that occipital activity is a good measure of nondisplaceable (and, thus, nonspecific) uptake. Although it has not yet been demonstrated for [^{123}I]β-CIT, we believe that between-subject variations of nonspecific uptake will not be significant. That is, nonspecific binding is not likely to be different between individuals—and significant alterations of basic constituents of the brain involved with nonspecific binding would likely be associated with widespread neuropathology.

The percent specific striatal uptake showed a nonsignificant trend toward lower within-subject variability and better reliability than the ratio measurement, V_3''. This is due to the error associated with calculating V_3'' which is a quotient of two measurements (striatal and occipital), each with an attendant error. Considering the occipital measure, the unsuspected vulnerability of V_3'' is largely the result of the low accuracy of measuring occipital activity on Day 2, because of washout of the tracer and decay of the radionuclide. In fact, the error was so great that the reliability for the occipital value (expressed as a percent of injected dose) using the mean of three scans at 24 hr could not be determined (Table 1). An additional source of the occipital error derives from a truncation artifact of events from septal penetration of the high energy emissions of ^{123}I (approximately 2% of the gamma emissions are >500 keV) (*13*). The septal penetration results in a ring artifact which surrounds the brain and can be easily seen on the May 1994 cover of this journal. The ring artifact actually extends into the image of the brain, and variations in placement of the head within the gantry will lead to differing amounts of data corruption. We attempted to remove the ring artifact with software from the manufacturer in our initial human studies with [^{123}I]β-CIT (*2*), but not in this or an earlier study (*5*) so that results between the early and later studies cannot be directly compared. Studies performed after those reported in this paper use a new collimator which has markedly less septal penetration. In addition, more accurate measurements of occipital activity may be obtained with a tracer which equilibrates rapidly and which could be imaged on day 1. The N-fluoroalkyl analogs of β-CIT seem promising in this regard, based upon monkey studies (*14*) and preliminary results in human subjects (unpublished observations). Additional studies are needed to examine between subject variations in nondisplaceable uptake and methods to improve quantitation of occipital activity.

As to the error associated with measurement of striatal count concentrations, i.e., the numerator of V_3'', the fact that only four slices were summed for this measurement compared with fourteen slices summed for determination of %SSU could also contribute to the higher test/retest variability of the V_3'' (Table 1). It is possible to sum additional slices for the determination of V_3'', although at the risk of losing the ability to accurately discriminate caudate and putamen.

Another important difference between V_3'' and percent specific striatal uptake lies in the stability of the outcome measure with small changes in ROI placement and susceptibility to differences in striatal volume (Table 4). For %SSU the volume of total counts was obtained in striatum based on generous regions of interest that purposely extended beyond the visually-identified striatum. Hence, this measure is less subject to the effects of ROI placement than V_3''. In particular, ROI size and placement affects V_3'' because the concentration of counts in striatum and occipital cortex vary with both the positioning and the size of the ROI (increasing ROI size produce lower count concentrations). Thus, for two subjects with different size striata, but similar number of DA transporters/gm striatal tissue, the identical ROI will result in a lower V_3'' estimate in the smaller striatum. %SSU is not affected in this fashion if the ROIs are large and care is taken to sum all slices with identified striatal activity. In this instance, as the ROI size increases there is no effect on the measure of total specific striatal counts as long as an identical occipital volume of interest is utilized to estimate the nondisplaceable uptake. A major disadvantage of use of large ROIs in the percent specific striatal uptake calculation is the inability to accurately distinguish caudate and putamen, which may be differentially affected by disease processes.

Finally, the significant between-subject variability in both Day 2 SPECT outcome measures is due to the expected reduction in the density of DA transporters with age. The number of subjects studied in the present investigation was not sufficiently large to assess aging effects. Loss of dopamine transporters reported on the basis of postmortem data is 8–10% per decade (*15,16*). In our other healthy human studies using [^{123}I]β-CIT SPECT in a larger cohort we have demonstrated a significant reduction in V_3'' and percent striatal uptake of 6–7% per decade (*17*).

Application of Iodine-123-β-CIT SPECT to Neuropsychiatric Illness

Is the test/retest variability associated with [^{123}I]β-CIT measures acceptable for serial studies in patients? For diseases characterized by reduced numbers of dopamine transporters like idiopathic Parkinson's disease where postmortem data suggest reductions of DA transporters on the order of 10–30% of baseline, a test/retest variability of 12% would theoretically detected changes of 1.2–3.6% of the baseline value. This estimate, however, is optimistic in that it assumes similar reproducibility of the SPECT measures in patients and healthy subjects. Less striatal uptake in the patient groups would be expected to increase the test/retest variability. Test/retest studies are currently underway in Parkinson's patients.

CONCLUSION

Iodine-123-β-CIT SPECT imaging in healthy humans demonstrates low test/retest variability and good reliability across several outcome measures. This supports the feasibility of utilizing [^{123}I]β-CIT for SPECT measurement of dopamine transporters in the evaluation of neuropsychiatric illness affecting dopamine neuronal function. Further studies of the variability of these measures in patient populations would be useful for better delineating the utility of [^{123}I]β-CIT SPECT for serial evaluations in patients groups.

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Iodine-131 Treatment of Hyperthyroidism: Significance of Effective Half-life Measurements

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Our goals were to evaluate the effect of half-life determination and differences in the half-life of ^{131}I between patients with Graves' disease and toxic nodular goiter, and the influence of antithyroid drugs on iodine uptake. **Methods:** We reviewed the records of 555 patients who had received radioiodine treatment for Graves' disease and toxic nodular goiter to analyze iodine uptake, half-life values and pretreatment with antithyroid drugs. Two different methods of dose calculation were compared: one using repeated uptake measurements at 24 and 48 hr and 4 or 6 days to define the effective half-life. The other method assumed a half-life of 5 days and uptake at 24 hr only. All patients were treated according to the first method. A follow-up questionnaire was sent to 327 patients (238 responders) to assess the treatment outcome. **Results:** After comparing the results of the two methods, we found that repeat uptake measurements and determination of effective half-life results in administered activities that differ considerably from those calculated when an assumed, fixed half-life and a single uptake measurement are used. The simpler method would lead to over- as well as undertreatment of the patient. There was a functional difference between patients with Graves' disease and toxic nodular goiter, as reflected by the shorter ^{131}I half-life in Graves' disease (mean 5.0 days) than toxic nodular goiter (mean 6.0 days) and a skewed distribution in toxic nodular goiter. Patients pretreated with antithyroid drugs had shorter ^{131}I half-lives in both categories. Ten percent of the patients required more than one treatment; 94% of the patients with Graves' disease and 45% with toxic nodular goiter had thyroxine substitution 1–5 yr after treatment. **Conclusion:** A dose calculation method that uses three uptake measurements provides sufficient data about the effective half-life of ^{131}I in the thyroid. There is considerable difference in the half-life based on the disease being treated (Graves' disease or toxic nodular goiter). The ^{131}I half-life also is shorter after pretreatment with anti-thyroid drugs. Thus, the simpler method leads to significant uncertainty, leading to over- as well undertreatment of the patient.

Key Words: hyperthyroidism; iodine-131; effective half-life; Graves' disease; toxic nodular goiter

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Hyperthyroidism may be treated in three ways: medical therapy with antithyroid drugs, radioiodine therapy or surgery. Therapeutic strategies vary within and between different countries. In many clinics, radioiodine treatment is the most commonly used method for treating adult patients with hyperthy-

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