Test–Retest Reproducibility of Extrastriatal Dopamine D₂ Receptor Imaging with [¹²³I]Epidepride SPECT in Humans

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This study evaluated the test–retest reproducibility of D₂ receptor quantification in the thalamic and temporal cortex using [¹²³I]epidepride SPECT. Methods: Ten healthy volunteers (4 men, 6 women; age range, 19–46 y) underwent 2 SPECT studies (interval, 2–26 d) using a bolus-plus-constant-infusion paradigm (bolus-to-infusion ratio = 6 h; infusion time = 9 h). Plasma clearance (in liters per hour) and free fraction (f₁) of the parent tracer were measured. Radioactivity (in becquerels per gram) in the thalamus, temporal cortex, and cerebellum were normalized to the infusion rate (in becquerels per hour). Normalized striatal radioactivity was also measured to assess reproducibility in regions with a high density of receptors and better counting statistics. The outcome measures obtained were V₃ (receptor density (Bₘax)/equilibrium dissociation constant [Kₐ]), V₃’ (f₁ × Bₘax/Kₐ), and R₇ (specific-to-nondisplaceable tissue ratio). Results: Test–retest variability and reliability (intraclass correlation coefficient) were 10.8% and 0.88, respectively, for plasma clearance and 15.3% and 0.77, respectively, for f₁. The test–retest variability of brain-specific (target minus nondisplaceable) radioactivity was higher in the thalamus and temporal cortex than in the striatum, although reliability was comparable. Among the outcome measures, V₃’ showed better test–retest variability and reliability in the thalamus (13.3% and 0.75, respectively) and temporal cortex (13.4% and 0.86, respectively). Conclusion: Brain radioactivity was the main source of variability for quantification of extrastriatal D₂ receptors with [¹²³I]epidepride. The reproducibility of outcome measures in extrastriatal regions was good. However, because receptor density was lower in extrastriatal regions than in the striatum, the counting statistics in these regions were low and reproducibility was affected by the higher test–retest variability of brain-specific radioactivity. Compared with V₃ and V₃’, R₇ showed less test–retest variability in the thalamus and temporal cortex but lower reliability. Moreover, measurement of R₇ may be affected by the presence of potential lipophilic metabolites entering the brain.

Key Words: [¹²³I]epidepride; dopaminergic system; reproducibility; D₂ receptor quantification


The extrastriatal dopaminergic system is implicated in the pathophysiology of schizophrenia (2). In vitro and in vivo rodent studies as well as human postmortem studies using [¹²³I]epidepride have shown the presence of dopamine D₂ receptors in extrastriatal brain regions such as the thalamic nuclei, medial and lateral temporal cortex, hypothalamus, and pituitary gland (2–4). Moreover, [¹²³I]epidepride and its fluorine analog 5-[¹⁸F]fluoropropylidepride have been used for imaging of striatal and extrastriatal D₂ receptors with either SPECT (5–7) or PET (8). Epidepride is a suitable tracer for imaging extrastriatal D₂ receptors with SPECT because of its low nonspecific binding and high affinity for D₂ receptors (dissociation constant [Kₐ] = 24 pmol/L) (2). Because of the high affinity for D₂ receptors, washout of the tracer from the region with a high receptor density (i.e., the striatum) is slow, and quantification of D₂ receptors in the striatum and extrastriatal regions requires a lengthy study for either bolus or constant-infusion experiments (9). Multiple arterial blood samples are required in bolus studies to measure the binding potential, or the specific distribution volume relative to free tracer concentration in plasma. Because binding potential equals Bₘax/Kₐ, i.e., the product of the density (Bₘax) and the affinity (1/Kₐ) of the receptors, this value reflects receptor density provided that Kₐ is the same across subjects and across different brain regions. A simpler quantification of receptor density is also possible with methods based on analysis of time–activity curves in receptor-rich and receptor-free regions (10,11). The outcome measure obtained with such methods is V₃”, or the specific-to-nondisplaceable ratio that is proportional to receptor density if the between-subjects variation of nondisplaceable distribution volume is negligible. However, in situations in which the metabolic pathway of a tracer generates lipophilic metabolites that cross the blood–brain barrier and contribute to nondisplaceable radioactivity, V₃” is underestimated and the outcome measure can be referred to as the R₇, the specific-to-nondisplaceable equilibrium tissue ratio (12). The presence of labeled lipophilic metabolites of [¹²³I]epidepride has been shown (13). These metabolites can enter the...
brain and complicate quantification of D2 receptors. Because the radioactivity levels of parent tracers and lipophilic metabolites cannot be measured separately in the brain, bloodless analyses do not allow correction for the presence of lipophilic metabolites. However, a method to correct for the presence of such metabolites in the brain is a constant-infusion paradigm in which the tracer is infused at a constant rate until equilibrium is reached between the specific, nondisplaceable compartment and the plasma. This method obtains as outcome measures the specific distribution volume relative to free (V1) or total (free and protein-bound [V1']) tracer in the plasma. A lipophilic metabolite with negligible receptor affinity would be expected to have a uniform distribution in the brain. In fact, D2 receptor-blocking studies in monkeys have shown that the residual (i.e., nondisplaceable) activity of [123I]epidepride is uniformly distributed (14). Thus, under equilibrium conditions of stable levels of parent tracer and metabolite, the simple subtraction of target (e.g., thalamus or temporal cortex) and background (e.g., cerebellum) activities yields specific receptor binding corrected for the presence of lipophilic metabolites. However, a limitation of the constant-infusion paradigm with [123I]epidepride is the time required to reach equilibrium receptor-binding conditions in the brain. Even with an optimized bolus-to-infusion ratio (B/I) of 10 h, constant infusion for approximately 24 h is required to achieve equilibrium in both the striatal and the extrastriatal regions with [123I]epidepride (9). Nevertheless, if the primary interest is quantification of extrastriatal D2 receptors, a shorter infusion protocol may be applied with the advantage of improving counting statistics in regions that have a lower density of D2 receptors than does the striatum.

The aim of this study was to evaluate the test–retest reproducibility of quantification of D2 receptors in the thalamus and temporal cortex using [123I]epidepride SPECT and a constant-infusion paradigm with a 9-h infusion protocol and a B/I of 6 h. Such a paradigm is particularly designed to quantify D2 receptor density only in extrastriatal regions and may be applied to evaluate the effects of pharmacologic changes of endogenous dopamine concentration in regions that have a lower receptor density than does the striatum.

MATERIALS AND METHODS

Radiolabeling

[123I]epidepride was prepared by iododestannylation from N-[(1-ethyl-2-pyrridinyl)-methyl]-2,3-dimethoxy-5-(tributylstannyli)-benzamide with no carrier added, as previously described (15). The average radiochemical yield of the preparation was 73.6% ± 14.2% (n = 20, with these and subsequent values expressed as mean ± SD). Radiochemical purity was 98.3% ± 1.3%.

Volunteers

Ten healthy volunteers (4 men, 6 women; age range, 19–46 y; mean age, 27 y) underwent 2 [123I]epidepride SPECT studies with an interval ranging from 2 to 26 d (mean, 9 d). The volunteers were found to be free of physical or mental illness on the basis of history, physical examination, blood chemistries, complete blood counts, thyroid function studies, urinalysis, and electrocardiography. Female volunteers of child-bearing potential had negative results from urine pregnancy testing on the day of the study. The volunteers received 0.6 g potassium iodide in saturated solution before tracer injection. All gave written informed consent for the study, which was approved by the local institutional review board.

Data Acquisition

The volunteers were asked to consume a low-fat breakfast in the morning and to fast during the scanning study. Two indwelling 20-gauge catheters were inserted into each forearm vein. One intravenous line was used to infuse 0.45% normal saline at a rate of 167 mL/h through a computer-controlled pump (LifeCare, model 4; Abbott Laboratories, North Chicago, IL) and to sample blood. The other intravenous line was used for constant infusion of the tracer. A total dose of 314 ± 14 MBq epidepride was administered as a bolus (142 ± 7 MBq) followed by constant infusion (172 ± 8 MBq) using a B/I of 6 h and a total infusion duration of 9 h. The tracer was infused at a rate of 23,600 ± 1,190 Bq/h through a computer-controlled pump (IMED Gemini; Alaris Medical Systems, San Diego, CA). Five fiducial markers, 3 on the left and 2 on the right, each containing 111–185 kBq (3–5 μCi) 99mTc-pertechnetate, were placed on each volunteer’s head along the canthomeatal line before imaging to facilitate reorientation of transaxial images afterward.

SPECT was performed with a triple-head camera (PRISM 3000 XP; Marconi, Cleveland, OH) equipped with low-energy, high-resolution fanbeam collimators with an axial resolution of 12.2 mm, measured using a 123I line source in a 20-cm cylindrical phantom filled with water.

Multiple images were acquired: 15-min acquisitions at 0, 15, 30, and 45 min after injection; 24-min acquisitions at 75 min and 2, 3, 4, 5, 6, and 7 h after injection; and a 48-min acquisition at 8 h after injection. One transmission image using a 57Co line source was also acquired at approximately 7 h after injection to obtain a map for nonuniform attenuation correction, as previously described (16). The images were acquired in continuous mode with a 128 × 128 matrix and a 16-cm radius.

All volunteers underwent MRI of the brain within 1–2 wk of the test or retest. Three-millimeter contiguous slices were obtained with a 1.5-T scanner (Signa; General Electric Medical Systems, Milwaukee, WI) using spoiled gradient-recalled acquisition in the steady state with a 25-ms repetition time, a 5-ms echo time, 1 excitation, a 256 × 256 matrix, and a 24-cm field of view. MR images were used to identify brain regions on the SPECT images.

Multiple blood samples were drawn at the midpoint of the acquisitions (3, 4, 5, 6, 7, and 8 h after injection). The last blood sample was obtained in triplicate to check the reproducibility of metabolite analysis. Two blood samples were drawn before tracer injection and used for in vitro measurement of tracer stability and protein binding.

Image Analysis

SPECT projection data were processed with a 2-dimensional Butterworth filter (cutoff = 0.24 cycles/pixel; order = 10) and reconstructed with a ramp backprojection filter on a 128 × 128 matrix to obtain 60 slices with a pixel size of 2.06 × 2.06 × 3.56 mm in the x-, y-, and z-axes, respectively. Multiple reconstructed images of the test and retest studies were coregistered using either the automated image registration (17) module available in MEDX 2.1 (Sensor Systems, Inc., Sterling, VA) or the coregister function
in Statistical Parametric Mapping, version 96 (SPM96; Wellcome Department of Cognitive Neurology, London, UK) (18). The attenuation map was reconstructed from the projection data obtained during transmission scanning with a Bayesian algorithm using locally developed software to obtain a map of pixel-by-pixel attenuation coefficients for $^{123}$I, as previously described (16). The attenuation map was reconstructed using the same matrix as was used for the emission images and coregistered to the emission images using the coregister function in SPM96. Nonuniform attenuation correction was performed for all emission scans of both the test studies and the retest studies. A mean image of the first 5 acquisitions of the test and retest studies was generated using the image calculator in MEDx 2.1. This image was used for posthoc reorientation along the canthomeatal line and coregistration of the retest studies to the test studies. The transformation matrix generated for the mean image of the test and retest studies was applied to all images. Afterward, the mean image of the retest study was coregistered to the mean image of the test study using the coregister function in SPM96, and the same transformation matrix was applied to all images of the retest study. MR images were coregistered to the mean image of the test study using either the coregister function in SPM96 or the automated image registration module in MEDx 2.1 (19).

Volumes of interest (VOIs) were drawn on coregistered MR images (Figure 1) to overlie the putamen, caudate nucleus, and thalamus. Two VOIs were drawn on the lateral temporal cortex (1 VOI on the anterior half, including the superior and middle temporal gyri; another VOI on the posterior half, including the middle and inferior temporal gyri), and 2 VOIs were drawn on the cerebellar hemispheres. The VOI for the thalamus was elliptic and placed on the radioactivity approximately corresponding to bound $^{123}$Iepidepride in the anterior and dorsomedial thalamic nuclei (3,4) and to the hypothalamus around the third ventricle. VOIs were drawn on 4 slices of 3.56-mm thickness. The sizes of the VOIs were 2326 ± 60 mm$^3$ for the caudate nucleus, 3763 ± 7 mm$^3$ for the putamen, 5373 ± 17 mm$^3$ for the thalamus, 5696 ± 527 mm$^3$ for the anterior lateral temporal cortex, 7649 ± 639 mm$^3$ for the posterior lateral temporal cortex, and 11,393 ± 1,252 mm$^3$ for the cerebellum.

Data from VOIs for the caudate nucleus and putamen were averaged and reported as a single value for the striatum, and data from VOIs for the anterior and posterior halves of the temporal cortex were averaged and reported as a single value for the temporal cortex. VOI data were decay corrected to the time of injection, and counts per minute per voxel were converted to becquerels per gram of tissue using a calibration factor of 34 Bq/cpm measured as the average of 3 different experiments performed with an anthropomorphic brain phantom (Radiologic Support Devices, Long Beach, CA) filled with a uniform solution of $^{123}$I.

**Plasma Analysis**

Blood samples were extracted 3 times with ethyl acetate. The extractable fraction was then analyzed with high-performance liquid chromatography using a reversed-phase 3.9 × 300 mm C$_{18}$ column (Novapak; Waters, Milford, MA) and a mobile phase of a 75:25:0.1 mixture of methanol:water:triethylamine to separate parent tracer from lipophilic metabolites, as previously described (15).

Clearance of $^{123}$Iepidepride, in liters per hour, was calculated as $R_0/C_w$, where $R_0$ is the infusion rate of the tracer in becquerels per hour and $C_w$ is the total (free and protein-bound) tracer concentration in becquerels per milliliter in the plasma at equilibrium (steady-state concentration) (20).

The free fraction ($f_1$) of the tracer was measured as previously described (21). An aliquot of stored plasma pool was also used to measure protein binding under control conditions at the time of each test and retest study. The purpose of this measurement was to

**FIGURE 1.** Transaxial SPECT images of $^{123}$Iepidepride, acquired for 48 min between 8 and 9 h after injection in test (A) and retest (B) studies, and MR images (C) at level (from left to right) of cerebellum and temporal lobes, lateral temporal cortex, striatum, and thalamus of volunteer 4. SPECT images of retest study and MR image were coregistered to SPECT images of test study. Relative distribution of tracer in striatum and extrastriatal regions (cerebellum, temporal cortex, and thalamus) is represented by color bar showing relative percentage uptake. Regions of interest for cerebellum, lateral temporal cortex, striatum (caudate nucleus and putamen), and thalamus are shown on single slices of MR image (C).

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correct for possible variations in the measurement of tracer protein binding between test and retest studies.

**Imaging Outcome Measures**

The total radioactivity, in becquerels per gram, in the cerebellum was subtracted from the total radioactivity in the thalamus and temporal cortex at equilibrium and in the striatum at the last time point to obtain the specific (target minus nondisplaceable) radioactivity in receptor-rich regions.

Cerebellar radioactivity and specific radioactivity in the striatum, thalamus, and temporal cortex were then divided by the infusion rate of the radiotracer, in becquerels per hour, to normalize differences in brain radioactivity caused by infusion of different amounts of tracer. This measure was used to assess the reproducibility of brain radioactivity in receptor-rich and receptor-free regions. Specific radioactivity in the striatum was measured even if not at equilibrium to estimate differences in the reproducibility of radioactivity in a region with a high counting statistic (striatum) compared with regions with a lower counting statistic (thalamus and temporal cortex). Because the cerebellum has been shown to have negligible levels of D₂ receptors, it was used as the region of nondisplaceable radioactivity (4).

Outcome measures obtained for the thalamus and temporal cortex were \( V_3 \) (\( B_{max}/K_D \)), \( V_1' \) (\( f_I \times B_{max}/K_D \)), and \( R_2 \). These outcome measures were obtained by averaging time points at which the percentage change per hour of total regional radioactivity was less than 2% in the thalamus and less than 1% in the temporal cortex. For studies in which the percentage change per hour was higher than 2%, only the last point was used for deriving outcome measures.

**Statistical Analysis**

Test–retest variability was measured as the absolute difference between the test and the retest divided by the average of the 2 studies and expressed as a percentage.

The reliability of measurements was assessed by measuring the intraclass correlation coefficient, \( r_I \), calculated as \( (s_x^2 - s^n)^2) / (s_x^2 + (n - 1)s^n)^2 \), where \( s_x^2 \) is the mean sum of the square between subjects, \( s^n \) is the mean sum of the square within subjects, and \( n \) is the number of within-subject measurements (in this study, \( n = 2 \)).

The intraclass correlation is the proportion of the total variance of an observation that is associated with the class (in this study, subject) to which it belongs. The value of \( r_I \) may range from 0 (no reliability, when \( s_x = s^n \)) to 1 (maximal reliability, when \( s = 0 \) and there is identity between the test and the retest). The 95% CIs for \( r_I \) were also calculated.

Differences in the variability of normalized radioactivity in the striatum, thalamus, temporal cortex, and cerebellum were evaluated with 1-way ANOVA. Differences in variability among outcome measures were evaluated with repeated-measures ANOVA. Differences in \( r_I \) were evaluated on the basis of the value of \( r_I \) and the lower and upper bounds of its 95% CI.

Changes in plasma and brain radioactivity were evaluated by measuring the percentage change per hour and its 95% CI.

All tests were performed on a personal computer with SPSS 8.0 software (SPSS Inc., Chicago, IL). All tests were 2-tailed, and the significance level corresponded to \( \alpha = 0.05 \).

**RESULTS**

**Clearance, \( f_I \), and Plasma Metabolites**

Figure 2 shows plasma time–activity curves for the test and retest studies of 2 volunteers. The plasma parent tracer achieved a steady-state concentration at approximately 5 h after injection in all volunteers (percentage change per hour = 0.1% ± 2.1%; 95% CI = −0.9%–1.1%). Clearance of the plasma parent tracer showed good variability (11%) and reliability (\( r_I = 0.88; 95\%\) CI = 0.61–0.97; Table 1).

The \( f_I \) of the plasma parent tracer showed lower reproducibility (variability = 15%; \( r_I = 0.77; 95\%\) CI = 0.34–0.93; Table 1) than did plasma clearance. The \( f_I \) of an aliquot of a stored pool of human plasma was also measured on the same day as each test and retest study. The reproducibility of this control \( f_I \) was lower than that of the experimental \( f_I \) (variability = 15.4% ± 13.5%; \( r_I = 0.44; 95\%\) CI = −0.19–0.82). Therefore, the experimental \( f_I \) was used for calculating \( V_3 \) because correcting the experimental \( f_I \) using the average and the individual values of the control \( f_I \) as previously reported for [123I]iomazenil (23) decreased the reproducibility of the \( f_I \) value (variability = 21.1% ± 13.4%; \( r_I = 0.61; 95\%\) CI = 0.04–0.88).

The composition of plasma at 8.4 ± 0.2 h after injection was 57.5% ± 9.2% polar compound, 28.4% ± 3.2% parent tracer, and 14.1% ± 7.5% lipophilic metabolite. The ratio of parent tracer to lipophilic metabolite at the same time after injection was 2.8 ± 1.9.
**TABLE 1**

Plasma Clearance and Free Fraction

<table>
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<th>Cₗ (L/h)</th>
<th>Variability (%)</th>
<th>fᵢ</th>
<th>Variability (%)</th>
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Mean

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<th>11.5</th>
<th>8.2</th>
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rᵢ = 0.88

95% CI of rᵢ = 0.61–0.97

**Brain Radioactivity and Outcome Measures**

The values for normalized brain radioactivity were 1.26 ± 0.39 for the striatum, 0.38 ± 0.13 for the thalamus, 0.14 ± 0.06 for the temporal cortex, and 0.11 ± 0.03 for the cerebellum (Table 2). The variability of normalized specific radioactivity was better in the striatum (12%) than in the thalamus (14%) and temporal cortex (18%), but reliability was similar (rᵢ = 0.86, 0.88, and 0.88, respectively). The variability of nondisplaceable radioactivity (cerebellum) was comparable with other regions (14%), although reliability was lower (rᵢ = 0.78). However, differences of variability among these brain regions were not found to be significantly different by 1-way ANOVA.

Representative brain time–activity curves for the striatum and extra striatal regions are shown in Figure 3. For the duration (9 h) and the B/I (6 h) used in this study, the striatum did not achieve equilibrium. The thalamus, temporal cortex, and cerebellum achieved equilibrium within 5–9 h from the beginning of infusion. The slight undershooting observed for the thalamus might have been related to scattered radioactivity from the striatum, in which the radioactivity was progressively increasing during infusion. Because of scatter from the striatum, equilibrium in the thalamus and temporal cortex was evaluated using an average percentage change per hour of 2% and 1%, respectively, as the threshold. Therefore, from approximately 5 h

**TABLE 2**

Specific and Nondisplaceable Radioactivity Normalized to Infusion Rate

<table>
<thead>
<tr>
<th>Index</th>
<th>Striatum Specific radioactivity (mean)</th>
<th>Variability (%)</th>
<th>Thalamus Specific radioactivity (mean)</th>
<th>Variability (%)</th>
<th>Temporal cortex Specific radioactivity (mean)</th>
<th>Variability (%)</th>
<th>Cerebellum Nondisplaceable radioactivity (mean)</th>
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</tbody>
</table>

Radioactivity is expressed as becquerels per gram divided by becquerels per hour. Specific radioactivity is target minus cerebellum. Values for striatum were obtained with last time point (not at equilibrium).
after injection, the change per hour in radioactivity in the thalamus, temporal cortex, and cerebellum was 2.2% ± 1.4% (95% CI = 1.5%–2.8%), 0.7% ± 1.5% (95% CI = 0.1%–1.4%), and 0.6% ± 1.7% (95% CI = –0.2%–1.4%), respectively. However, the change in radioactivity over time was above the threshold for the thalamus in 50% of the studies and above the threshold for the temporal cortex in 35% of the studies. In these cases, the percentage change per hour was 3.3 ± 0.9 in the thalamus and 1.9 ± 1.9 in the temporal cortex, and either later time points below the threshold of the thalamus (2%) were selected or only the last time point was used for calculating outcome measures.

To test equilibrium conditions, the change in $V_3'$ and $R_T$ over time was evaluated for the thalamus and temporal cortex. After approximately 5 h from the beginning of the infusion, the change per hour in $V_3'$ was 2.7% ± 2.1% for the thalamus (95% CI = 1.6%–3.6%) and 0.8% ± 2.9% for the temporal cortex (95% CI = –0.6%–2.1%). The change per hour in $R_T$ was 2.1% ± 2.2% for the thalamus (95% CI = 1.1%–3.1%) and 0.3% ± 3.3% for the temporal cortex (95% CI = –1.3%–1.8%). As shown in Figures 3B and D, the thalamus achieved equilibrium later after the beginning of the infusion than did the temporal cortex. Therefore, in 60% of the volunteers, outcome measures were obtained using only the last point, to minimize the degree of underestimation of receptor density.

The values of outcome measures for the thalamus and temporal cortex are reported in Table 3. The average $V_3$ was 196.1 ± 43.1 mL/g for the thalamus and 70.5 ± 20.6 mL/g for the temporal cortex; the average $V_3'$ was 14.9 ± 3.4 mL/g for the thalamus and 5.5 ± 1.7 mL/g for the temporal cortex; and the average $R_T$ was 3.5 ± 0.5 for the thalamus and 1.3 ± 0.4 for the temporal cortex. Repeated-measures ANOVA did not show any significant differences in variability among the 3 outcome measures for the thalamus and temporal cortex, although the average variability in $R_T$ for these 2 regions (11% and 13%, respectively) was lower than the average variability in $V_3$ and $V_3'$, as shown in Table 3. However, for the thalamus, $R_T$ showed lower reliability than did either $V_3$ or $V_3'$ ($r_T = 0.61$).

**DISCUSSION**

This study was designed to quantify $D_2$ receptor density in extrastriatal regions (i.e., the thalamus and lateral temporal
cortex) and to evaluate the reproducibility of such measurements with $[^{123}\text{I}]$epidepride SPECT. The constant-infusion paradigm was chosen because it can correct for the effect of potential lipophilic metabolites entering the brain. This method allows one to obtain $V_3$ or $V_3'$. The reproducibility of measurements of binding potential depends on the reproducibility of brain radioactivity and metabolite analysis of plasma. In this study, to improve the reproducibility of measurements of tracer binding in regions that have a lower receptor density than does the striatum, a short infusion protocol was designed to achieve equilibrium in extrastriatal regions, reducing the effect of radioactivity decay and improving counting statistics. Moreover, to reduce possible interference from metabolic changes throughout the 9-h infusion, the volunteers fasted and half-normal saline was constantly infused. This method minimized changes in tracer clearance caused by changes in metabolic rate or hydration status.

**Reproducibility of Clearance and $f_1$**

Plasma clearance of $[^{123}\text{I}]$epidepride showed the best reproducibility among the different plasma and brain parameters analyzed. Because the B/Is in the test and retest studies were almost identical (variability = 0.4% ± 0.3%), only slight differences were found in the infusion rates (variability = 3.5% ± 7.1%) and in the radioactivity levels of total plasma normalized by the infusion rate (variability = 4.7% ± 5.8%). Therefore, most of the variability in clearance was likely related to biologic differences between test and retest conditions.

Measurements of the $f_1$ of tracer in plasma were poorly reproducible, as were measurements of the $f_1$ in control plasma. Therefore, to obtain $V_3$, we did not apply a correction to the experimental $f_1$ using the value of the control plasma. Protein binding of $[^{123}\text{I}]$epidepride is high (in this study, 92%), and measurement of $f_1$ may be expected to have large within- and between-subject variability, related mainly to the method of measurement.

**Reproducibility of Brain Radioactivity and Outcome Measures**

Brain radioactivity was the main source of variability. The reproducibility of brain radioactivity was lower in extrastriatal regions than in the striatum, although $[^{123}\text{I}]$epidepride binding in the striatum was not measured at equilibrium. The average variability of brain radioactivity was less in the striatum than in the thalamus and less in the thalamus than in the temporal cortex. This order corresponds to the order of $D_2$ receptor density, which is greater in the striatum than in the thalamus and greater in the thalamus than in the temporal

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**TABLE 3**

Outcome Measures in Thalamus and Temporal Cortex

<table>
<thead>
<tr>
<th>Volunteer no.</th>
<th>Study</th>
<th>$V_3$ (mL/g)</th>
<th>Variability (%)</th>
<th>Value (mL/g)</th>
<th>Variability (%)</th>
<th>Value</th>
<th>Variability (%)</th>
<th>Value (mL/g)</th>
<th>Variability (%)</th>
<th>Value</th>
<th>Variability (%)</th>
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<tr>
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<tr>
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<td>90.3</td>
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<tr>
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<td>14.8</td>
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<td>4.7</td>
<td></td>
<td>59.7</td>
<td></td>
<td>4.1</td>
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</table>

Mean | SD  | $\eta_1$ | 95% CI
---|-----|---------|---
196.1 | 43.1 | 0.70 | 0.20–0.91 | 0.30–0.93 | 0.04–0.88 | 0.49–0.95 | 0.57–0.96 | 0.46–0.95

Variability is absolute value (test – retest)/(mean test and retest) × 100.
cortex. The variability of nondisplaceable radioactivity (i.e., in the cerebellum) was similar to variability in the thalamus and lower than variability in the temporal cortex, although the reliability in the cerebellum was lower than in the other 2 regions.

Under this constant-infusion paradigm, the average activity 8–9 h after injection was much higher in the striatum (612 ± 191 cpm/g) than in the thalamus (218 ± 73 cpm/g) or temporal cortex (113 ± 42 cpm/g). The variability of brain radioactivity in the striatum and extrastriatal regions directly correlated with the counting statistics. Therefore, although the protocol was designed to improve counting statistics in extrastriatal regions, the reproducibility of \[^{123}\text{I}^\text{epidepride} Binding in these regions was still affected by low counts measured with the constant-infusion paradigm.

The test–retest variability of striatal \[^{123}\text{I}^\text{epidepride} binding reported by Volkow et al. (24), using \[^{11}\text{C}^\text{raclopride} and a kinetic approach with graphic analysis, and by Laruelle et al. (25,26), using \[^{123}\text{I}^\text{(S)-(\text{\textdaggerleft})-3-iodo-2-hydroxy-6-methoxy-N(\text{\textdaggerleft})-ethyl-2-pyrrolidinyl) methyl}benzamide and a bolus-plus-constant-infusion paradigm, was approximately 5%–6%, with \( r_1 \) values of 0.85 and 0.96, respectively. Our bolus-plus-constant-infusion paradigm has an intrinsic limitation in that it includes counting statistics for regions, such as the thalamus and temporal cortex, that have much lower \( \text{D}_2 \) receptor densities than does the striatum. In other words, the differences in counting statistics between the striatum (high counts) and the extrastriatal regions (low counts) are expected to reduce reproducibility in regions with low counting statistics. Although the reproducibility of \( \text{D}_2 \) receptor imaging can be improved by increasing counting statistics with a bolus injection of the tracer, this approach would require arterial plasma sampling to measure the input function of the parent tracer and metabolites and to correct for the effects of lipophilic metabolites (9). The bolus-plus-constant-infusion paradigm to measure \( \text{D}_2 \) receptor density in extrastriatal regions was selected for several reasons. First, this method corrects for the presence of lipophilic metabolites by simply subtracting the radioactivity of the nondisplaceable compartment (cerebellum) from the total radioactivity in each target brain region (thalamus and temporal cortex) at equilibrium, without the need for laborious compartmental modeling. Second, the method uses venous sampling, which subjects tolerate more easily than arterial sampling. Third, after achieving equilibrium at 8–9 h, receptor density can be measured with only a single SPECT acquisition and a single or a few venous samples. This simplification of the constant-infusion approach also reduces the difficulty of performing a 9-h study in patients with mental illness.

Another aspect to consider is scattering of activity from the striatum to the extrastriatal regions. To estimate the effect of scatter, we applied an anthropomorphic phantom used to estimate a calibration factor for converting SPECT activity (counts per minute) to absolute units of radioactivity (bequerels). The phantom included an actual human skull and contained 3 fillable regions (right striatum, left striatum, and remainder of brain). The striatal chambers and the entire brain had realistic shapes and total volumes. If activity was added only to the striatal regions, the measured activity in the extrastriatal regions reflected either limited camera resolution or scattered activity. Using this phantom, we estimated that scatter from the striatum contributed significantly to the radioactivity detected in extrastriatal regions. In the thalamus, scatter from the striatum accounted for 11%–17% of the counts detected at 2 h after injection and 17%–30% of the counts detected at 8 h after injection. In the temporal cortex, these values were 2%–4% and 4%–7%, respectively. Therefore, we believe that the time–activity curves of this study have been influenced by scatter from the striatum, with more significant effects in regions closer to the striatum.

For the outcome measures that used plasma \[^{123}\text{I}^\text{epidepride} concentrations, \( V_{3}^{\prime} \) was more reproducible than \( V_{3} \), the latter being influenced by the higher variability of \( f_{i} \) measurements. Thus, if plasma measurements are included, we recommend using the total plasma concentration of \[^{123}\text{I}^\text{epidepride}. This practice is probably the most common for both PET and SPECT radiotracer studies. The bloodless measurement \( R_{T} \) showed lower reliability than did \( V_{3}^{\prime} \), probably because of the limited accuracy of measuring the low concentration of radioactivity in the nondisplaceable compartment. Moreover, measurement of \( R_{T} \) is likely to be affected by differences between the partition coefficients of parent tracers and lipophilic metabolites in the nondisplaceable compartment. Thus, this outcome measure is influenced by within- and between-subject variations in the rate of tracer metabolism. In the presence of a lipophilic metabolite, \( R_{T} = V_{3}^{\prime} / (V_{2}^{P^\prime} + \delta V_{2}^{M^\prime}) \), where \( V_{2}^{P^\prime} \) and \( V_{2}^{M^\prime} \) are the distribution volume of the parent tracer (\( P \)) and lipophilic metabolite (\( M \)) in nondisplaceable compartments, and \( \delta \) is the ratio of \( M \) to \( P \) in the plasma at equilibrium (12). Therefore, \( R_{T} \) underestimates receptor density by a factor of \( 1 + \delta V_{2}^{M^\prime}/V_{2}^{P^\prime} \); the degree of such underestimation depends on the variability of \( V_{2}^{P^\prime} \), \( V_{2}^{M^\prime} \), and \( \delta \). In this study, \( \delta \) ranged from 0.2 to 0.9 (coefficient of variation = 51%), with a test–retest variability of 17% ± 17%. Therefore, although this outcome measure has the practical advantage of not requiring careful metabolite analysis, within- and between-subject variations in tracer metabolism do not seem to be negligible. Thus, \( R_{T} \) may not be a reliable outcome measure in the presence of lipophilic metabolites. In contrast, \( V_{3}^{\prime} \) seems to be a more reliable outcome measure when metabolite analysis is reliably performed.

CONCLUSION

\( \text{D}_2 \) receptor quantification in extrastriatal regions with \[^{123}\text{I}^\text{epidepride} SPECT showed good reproducibility and reliability with a bolus-plus-constant-infusion paradigm that relatively quickly achieved equilibrium receptor-binding conditions in the low-density extrastriatal regions. Among the 3 imaging outcome measures examined, \( V_{3}^{\prime} \) is probably
best. In contrast to V3, V3′ is not confounded by errors from measuring the relatively small f1 values in plasma (~8%). In contrast to R2, V3′ incorporates measurement of the parent tracer in plasma and, therefore, corrects for between-subject variations in the production of lipophilic metabolites that may enter the brain. However, all 3 of these measurements are vulnerable to errors from partial-volume effects or scatter from the much higher concentration of activity in the striatum. In future clinical studies, such contamination from striatal activity needs to be either controlled (e.g., by matching groups on this variable) or removed from the images through scatter-corrected image reconstruction.

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