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Validation of the Reference Tissue Model for Estimation of Dopaminergic D₂-Like Receptor Binding With [¹⁸F] (N-Methyl) Benperidol in Humans

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Abstract

PET measurements of dopaminergic D₂-like receptors may provide important insights into disorders such as Parkinson's disease, schizophrenia, dystonia and Tourette's syndrome. The PET radioligand [¹⁸F] (N-Methyl)Benperidol ([¹⁸F]-NMB) has high affinity and selectivity for D₂-like receptors and is not displaced by endogenous dopamine. The goal of this study is to evaluate use of a graphical method utilizing a reference tissue region for ([¹⁸F]-NMB PET analysis by comparisons to an explicit three-compartment tracer kinetic model and graphical method that use arterial blood measurements. We estimated binding potential (BP) in the caudate and putamen using all three methods in 16 humans and found that the three-compartment tracer kinetic method provided the highest BP estimates while the graphical method using a reference region yielded the lowest estimates ($p < 0.0001$ by repeated measures ANOVA). However, the three methods yielded highly correlated BP estimates for the two regions of interest. We conclude that the graphical method using a reference region still provides a useful estimate of BP comparable to methods using arterial blood sampling, especially since the reference region method is less invasive and computationally more straightforward; thereby simplifying these measurements.

Keywords

[¹⁸F]NMB; graphical method

Introduction

Involvement of cerebral dopaminergic D₂-like receptors in Parkinson's disease [1,2,3], schizophrenia [4,5,6], primary focal dystonia [7], and Huntington's disease [8] has generated

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interest in the use of positron emission tomography (PET) for in vivo assessment of human D₂-like receptor activity. For these PET applications in human subjects, [¹¹C]raclopride or radiolabeled analogs of spiperone have been commonly used [9].

[¹¹C]Raclopride has greater selectivity compared to most radiolabeled analogs of spiperone [10,11,12], but is susceptible to displacement by endogenous dopamine [13,14]. [¹²³I]Epidepride and [¹⁸F]fallypride have relatively higher affinity for D₂-like specific binding sites than [¹¹C]raclopride. However, these two ligands, like [¹¹C]raclopride, also are susceptible to displacement by endogenous dopamine [15]. This susceptibility provides a useful strategy for measuring release of endogenous dopamine but complicates modeling and confounds interpretation of PET-based measurements of D₂-like receptors.

Benperidol is a dopamine antagonist of the butyrophenone class with antipsychotic effects [16] and has high affinity and selectivity for binding to central D₂-like specific binding sites [17]. These characteristics have led to the investigation of benperidol analogs as potential PET pharmaceuticals [18,19,20,21,22]. In particular, [¹⁸F](N-Methyl)Benperidol ([¹⁸F]NMB) is a D₂-like receptor binding radioligand with high receptor-binding selectivity and resistance to displacement by synaptic dopamine, making it preferable for measurement of D₂-like specific binding sites.

We have already established that PET measurement of [¹⁸F]NMB specific binding in vivo can be accomplished with a three-compartment non-steady state tracer kinetic model using an arterial plasma input function [23]. But to make these computations possible, patients have to undergo three PET scans (one for [¹⁸F]NMB, one for blood flow and one for blood volume) and have an arterial line. So far, we have not examined the possibility of applying models where blood flow, blood volume, and in some cases blood sampling, may not be needed. Two graphical methods have been developed to quantify reversible receptor binding measured with PET [24,25,26]. The first graphical method does away with the blood flow and blood volume scans but still requires arterial plasma measurements of radioligand as an input function. This method has been shown to yield values for binding potential ($B_{\max}/K_d$; where $B_{\max}$ is the maximum number of specific binding sites and K_d is the equilibrium dissociation constant) close to the binding potential computed using a compartmental model and a nonlinear least-squares optimization algorithm [24]. The second, reference tissue-based, graphical method only requires the [¹⁸F]NMB PET scan to measure specific binding of [¹⁸F]NMB. We now evaluate the binding potential (BP) estimates from this method to the BP estimated obtained from the three-compartment tracer kinetic model and graphical method that require arterial blood measurements.

Materials and Methods

Subjects

Sixteen subjects, four men and twelve women, age 51 ± 12 years (mean $\pm$ SD) were studied. Twelve had adult-onset primary focal dystonia (either cranial or hand dystonia), two had Tourette's Syndrome, and two were normal. A heterogeneous group of subjects were considered for this study to validate use of the reference tissue-based graphical method in measuring specific binding of [¹⁸F]NMB among subjects with presumably varied levels of D₂-like receptors. The study was approved by the Human Studies Committee of Washington University, and informed consent was obtained from all volunteers.

MRI acquisition

Each subject had an MRI of the brain with a Siemens Vision 1.5T Magnetom scanner for identification of regions of interest. The following pulse sequence was used: MPRAGE (TR=9.7 msec, TE=4 msec, flip angle=12, time=6:36, pixel size= 1×1×1.25 mm).

Radiochemistry

[¹⁸F]Fluoride was produced with use of the ¹⁸O(p,n) ¹⁸F nuclear reaction induced on an isotopically enriched [¹⁸O]water target with a JSW BC-16/8 cyclotron. [¹⁸F]NMB was synthesized from [¹⁸F]fluoride using a three-step reaction sequence [27]. The radiochemical purity of the final product exceeded 95%, and the end-of-synthesis specific activity was greater than 2000 Ci/mmol (74 TBq/mmol) as measured by ultraviolet high-pressure liquid chromatography detection validated by receptor-binding assay [27]. The radioligand was dissolved in 1 mmol/L lactic acid in 0.9% saline for intravenous injection. [¹⁵O] water and [¹⁵O] carbon monoxide were produced for measurement of regional cerebral blood flow (rCBF) and regional cerebral blood volume (rCBV), respectively using an automated production system [28].

PET

PET scans were acquired with a Siemens/CTI 953B in three-dimensional mode (septa retracted) [29,30]. Images were reconstructed using measured attenuation and scatter correction [31] to a resolution of 4.3 mm full-width at half-maximum at the slice centers in all three dimensions.

Protocol

A 20-gauge catheter was inserted into an arm vein for injection of radiopharmaceuticals, and a second catheter was inserted into a radial artery for sampling arterial blood. The head was positioned to include imaging from the top of the striatum to the lower portions of the cerebellum and stabilized with a polyform plastic mask molded to the subject's head. Regional blood volume (rCBV) was measured using bolus inhalation of 556-1482 Bq of C¹⁵O [32], and regional blood flow (rCBF) was measured using bolus injection of 1111-1852 Bq of H₂¹⁵O [33,34]. At least 20 minutes was allowed for radioactive decay before injecting 185–259 Bq of [¹⁸F]NMB intravenously over 30 seconds. Dynamic PET images were acquired for 180 minutes beginning with injection (10 1-min frames followed by 34 5-min frames). Subjects were observed throughout the PET procedures.

Input function measurements

Five milliliters of arterial blood were collected before injection to measure the plasma free fraction of [¹⁸F]NMB [23]. During PET scans, approximately 35 arterial blood samples were collected to measure total radioactivity, and 11 of these samples were assayed in duplicate for the fraction of [¹⁸F]NMB activity that represented unmetabolized [¹⁸F]NMB [23].

Image analysis

All image frames were realigned to correct for head movement. Each sequential frame was aligned to its preceding frame using automatic image registration (AIR) [35] with image resampling to remove bias caused by pixelization [36].

The entire caudate and putamen and a portion of the cerebellum on each side of the brain were outlined on the MPRAGE image of each subject by an investigator blinded to identity of the subject. Each subject's MPRAGE image was co-registered to the corresponding [¹⁸F]NMB PET image using AIR [37]. The MR-defined volumes-of-interest (VOIs) were then resliced to the PET image and decay-corrected regional tissue activity PET measurements were

extracted. An average cerebellar tissue activity curve was obtained by averaging the tissue activity curves from the left and right cerebellar regions.

Quantitative analysis

[¹⁸F]NMB regional binding potentials were derived using three methods: the three-compartment tracer kinetic model using the arterial input function (Method 1), the Logan graphical method using an arterial input function (Method 2), and the Logan graphical method using a reference region (Method 3). For the three-compartment tracer kinetic model approach (Method 1), parameters were estimated using a nonlinear least-squares Marquardt minimization algorithm. This tracer kinetic model has been previously described [38,39]. Briefly, this model utilizes three compartments (one vascular and two tissue spaces), not two compartments, as in other PET models. In addition, this model calculates inflow rates to the vascular space as blood flow multiplied by the arterial concentration ($F \cdot C_a(t)$), where flow is measured by PET. Outflow rates to the systemic circulation are then calculated as flow divided by blood volume (F/BV). Therefore the values of blood flow and blood volume are measured in separate PET scans. The rates of tracer transfer between the vascular space and the nonspecific binding space are assumed to be dependent on the permeability-surface product, the volumes of the vascular and nonvascular spaces, the free fraction in blood (measured), and the free fraction in the nonspecific binding space (estimated by fitting the reference region). The input function for this method uses arterial blood radioactivity measurements corrected for radiolabeled metabolites accumulating in the vascular compartment [23]. This method yields an estimate of the BP.

The Logan graphical method using an arterial input function (Method 2) is a method that uses arterial blood measurements, similar to Method 1, to estimate a distribution volume ratio (DVR). This is computed as the ratio of the slope of the straight line fit for the tissue activity curve of the receptor-containing VOI to the slope of the fit for the tissue activity curve of the reference or nonspecific VOI [24,26]. The slopes are computed from the following equation:

$$\frac{\int_0^T C_R(t) dt}{C_R(T)} = \text{Slope} \frac{\int_0^T C_p(t) dt}{C_p(T)} + \text{int},$$

where $C_R(t)$ represents the concentration in a tissue region, either the reference region (*REF*) or the receptor region (*ROI*), and $C_p(t)$ is the plasma concentration. Then $\text{DVR} = \text{Slope}_{\text{ROI}} / \text{Slope}_{\text{REF}}$.

Finally, the Logan graphical method using a reference region (Method 3) uses a of method for estimation of BP without blood samples [25,26] using the formula:

$$\frac{\int_0^T C_{\text{ROI}}(t) dt}{C_{\text{ROI}}(T)} = \text{DVR} \frac{\int_0^T C_{\text{REF}}(t) dt + C_{\text{REF}}(T) / \bar{k}_2^{\text{REF}}}{C_{\text{ROI}}(T)} + \text{int},$$

where DVR is computed directly. This method requires either an estimate of the average efflux $\bar{k}_2^{\text{REF}}$ or conditions such that the efflux term can be assumed to be zero. If the term containing the efflux value is small relative to the integral term, it can be neglected. Also, the efflux term becomes part of the intercept when it is constant. The [¹⁸F]NMB data in this study adhere closely to both conditions.

Figure 1 shows representative plots of the time-activity data from the striatal and cerebellar region of interest. The striatal curve flattens and is nearly constant during most of the interval used for analysis. The average efflux of this tracer is on the order of 0.1/min based on the kinetic analysis performed in Method 1 so that the term $C_{\text{REF}}(T) / (\bar{k}_2^{\text{REF}})$ is not large relative to the integral term of the numerator.

For both graphical methods, data from 30 to 180 minutes after injection was used as the standard interval for the linear regression.

Between methods comparison

The values of BP derived by the three different methods were compared using repeated measures ANOVA and Pearson correlational coefficients. The variances of the BP values computed using the different methods were compared using an F-test. The BP estimates using Method 3 were also calculated using three different temporal windows. The BP estimates using shorter time points were subsequently compared to the original time point using paired t-test.

Results

Tracer Kinetics

As mentioned before, Figure 1 shows representative plots of the time-activity data from the striatal and cerebellar region of interest. The striatal curve demonstrates rapid uptake and equilibration of [^{18}F]NMB in that region of interest. Figure 1 also illustrates that the tracer kinetic model (Method 1) fits the time activity data for striatum and cerebellum. Although the fit to the cerebellum curve is close to the data, there are slight systematic deviations. In our kinetic analysis, the cerebellum is used only to obtain an estimate for the free fraction in tissue; small deviations in the value of the free fraction have little effect on the subsequent calculations of the binding potential. Figure 2 is an example showing the graphical analysis with reference region (Method 3) applied to time activity data from the cerebellum and the striatum with data before 30 minutes not shown. The slight deviation of the straight line from the first few points may be an indication that conditions for linearity are not satisfied until about 50 minutes rather than 30 minutes. However, because there are 30 points used for the regression analysis, this has little effect on the calculated slopes.

Binding potential comparison

Due to lack of differences seen between the binding potentials observed in the left and right regions of interest, we averaged values of left and right estimates for caudate and putamen to reduce variance. One patient's measurable D₂-like receptor binding in the caudate was so low it produced negative BP values using all three methods. Hence, this measurement was considered an outlier and removed from the analysis.

As shown in Table 1, BP estimates obtained using Method 1 were consistently the highest while BP estimates obtained using Method 3 were consistently the lowest. Repeated measures ANOVA show that BP estimates computed using the three different methods are statistically different from each other ($p < 0.0001$). The standard errors of the mean of the BP estimates are not statistically different among the three methods (F-statistic for Method 3 vs. 1 is 1.07; F-statistic for Method 2 vs. 1 is 1.10, F-statistic for a d.f. of 15 and $\alpha = 0.05$ is 2.4), indicating similar precision of the different methods.

Table 2 shows the ratios and correlation coefficients of BP estimates obtained with the two graphical methods compared to the kinetic model BP estimates. BP estimates obtained with the three methods are similar and correlational coefficients that compares paired measures exceeds 0.91 for all methods. Figure 3 shows the correlational scatter plot for the striatal BP

estimates (average of caudate and putamenal values) obtained from Method 2 versus Method 1 and for the BP estimates obtained from Method 3 versus Method 1.

Table 3 shows the BP estimates using Method 3 calculated using three different temporal windows. BP estimates using shorter time points were statistically different from the original BP estimates, but these estimates only deviated from the original value by 4% at most. In addition, the values from the shorter time points remain highly correlated to the original values. Similar results are seen when estimating BP using Method 2 (data not shown). This confirms that even if conditions for linearity may not be satisfied until about 50 minutes rather than 30 minutes, this has minimal effect on our BP estimates.

Discussion

BP estimates for [^{18}F]NMB with the three different methods are highly correlated, but are not the same. The kinetic model (Method 1) provides the highest BP values whereas the graphical method using a reference region for input (Method 3) yielded the lowest BP values. Initial evaluation of the graphical method with an arterial blood curve for analysis of human PET studies using ^{11}C -cocaine [24] revealed that BP estimated from a tracer kinetic model are slightly higher than BP estimated from the graphical method (0.62 vs. 0.66). In a later paper comparing the distribution volume ratios (DVR) obtained using graphical analysis with and without blood sampling [25], the DVR ($1 + \text{BP}$) values for ^{11}C -raclopride computed with the graphical method using blood curves or a reference region were the same. The estimates of DVR for ^{11}C -d-threo-Methylphenidate were slightly less for the graphical analysis using a reference region compared to the graphical method using an arterial input function. A more recent paper [26] also found a trend towards lower values for the graphical methods. In that paper, the DVR values are highest for the kinetic model and lowest for the graphical method using a reference region. We computed the BP from the DVR values to obtain a value of 2.86 for ^{11}C -raclopride and a value of 1.78 for ^{11}C -d-threo-Methylphenidate for the kinetic model; a value of 2.55 and 1.72, respectively, using graphical analysis with blood sampling and 2.52 and 1.59, respectively, using graphical analysis with a reference region.

Other investigators also have found that estimates of BP (or DVR) obtained with the graphical methods are slightly lower than the kinetic model estimates for some tracers. Estimates for BP measured using either [carbonyl- ^{11}C]WAY-100635 or (-)-N- ^{11}C -propyl-norapomorphine and computed by graphical methods were lower than those derived from the application of kinetic models [40,41]. Similarly, estimates of BP for three different D_2 radioligands, ^{18}F -desmethoxyfallypride, ^{11}C -raclopride, and ^{18}F -fallypride [42], revealed that the estimates of BP for the graphical methods were usually lower than estimates of BP obtained with the compartmental model.

Several factors may contribute to the underestimation of BP by graphical methods. Although the underestimation is sometimes attributed to bias introduced by noise, our regions of interest were large, and noise did not appear to be a problem in the receptor-rich regions. In addition,

we used a regression equation that assumed uncorrelated noise in both the x, $\frac{\int_0^T C_{\text{ROI}}(t) dt}{C_{\text{ROI}}(T)}$ and $y, \frac{\int_0^T C_{\text{REF}}(t) dt + C_{\text{REF}}(T) / \bar{k}_2^{\text{REF}}}{C_{\text{ROI}}(T)}$, variables with the further assumption that the variance of the x and y variables was the same for all points. We recognize that this is probably not an accurate assumption. However it is one that has been previously used in these kinds of problems as it

leads to tractable equations and has been shown to substantially reduce the bias usually encountered with the traditional Logan plot procedure [43].

A more likely reason for the 15% higher BP values computed by our implementation of the kinetic model is that our tracer kinetic model (Method 1) tends to yield higher estimates of some variables due to dependence of inflow via blood flow (typically measured for each subject). As discussed earlier, an inadequate kinetic model for the reference region also could lead to errors in the estimates obtained with the compartment model. The possibility of a small number of specific binding sites in the reference regions cannot be excluded, but this was found to be negligible for another D₂-like butyrophenone radioligand, [¹⁸F]spiperone [12]. An additional factor that may influence estimates of BP is the effect of the efflux term. Neglecting the efflux term in the graphical method with reference region as we did may lead to lower values for DVR and BP (see Table 3 in [26]).

Other studies measuring D₂-like receptor binding have reported similar levels of variability in BP estimates using Method 3 [44,45]. A study measuring D₂-like receptor binding using [¹¹C]raclopride and [¹¹C]N-Methylspiperone have reported standard deviations in BP estimates ranging from 0.27 to 0.36 in the caudate of both healthy controls and Parkinson's disease patients [46]. Similar to our findings, higher variances in BP estimates were seen in the putamen, and these values ranged from 0.28 to 0.67 among all study subjects. Another study using [¹⁸F]desmethoxyfallypride also reported variances in BP from 0.38 to 0.70 in the caudate and from 0.47 to 0.86 in the putamen among their participants [47]. Given this range of variability in their BP estimates, they concluded that the BP estimates measured using [¹⁸F]desmethoxyfallypride had enough specificity and sensitivity that they can be utilized for the differential diagnosis of parkinsonian syndromes. Thus, BP estimates from our study have the potential to detect biological changes in D₂-like receptor binding that may be clinically relevant.

We recognize that there are other methods currently used to calculate reversible receptor binding measured with PET. Some of these include the simplified reference tissue model (SRTM) developed by Lammertsma [48] and linear regression analyses approaches proposed by Ichise [49]. Most studies comparing the Logan graphical method (with and without a plasma input function) with SRTM have reported that BP estimates obtained using both methods were nearly identical [40,50]. In at least one study where VOIs were large size regions with low noise, similar to the striatal regions in this study, the reference-tissue based graphical method was found to be more stable [51]. In addition, the reference-tissue based graphical method is computationally simpler and more straightforward.

One limitation of our study was the lack of repeated measures of the patients. It would have been ideal to determine the reproducibility of the BP measures obtained with the three estimation methods in [¹⁸F]NMB studies. But due to radiation dosimetry limits in human subjects [52], reproducibility may be better measured in nonhuman primates.

In conclusion, our study indicates that the graphical analysis using a reference region (Method 3) provides the lowest estimated BP value. But it does so in a consistent and predictable manner that reflects the BP estimates computed using the tracer kinetic model (Method 1), as exemplified by the high correlation between the BP values from Methods 3 and 1. Due to lack of a need for an arterial line, it is less invasive on subjects, encouraging increased patient participation in future [¹⁸F]NMB PET studies. In addition, it is computationally simpler. Thus, we believe that use of graphical analysis using a reference region, is a feasible, and possibly, a more appealing alternative for BP estimation in [¹⁸F]NMB PET studies.

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References

1. Reisine TD, Fields JZ, Stern LZ, Johnson PC, Bird ED, Yamamura HI. Alterations in dopaminergic receptors in Huntington's disease. *Life Sci* 1977;21:1123–1128. [PubMed: 144230]
2. Lee T, Seeman P, Rajput A, Farley IJ, Hornykiewicz O. Receptor basis for dopaminergic supersensitivity in Parkinson's disease. *Nature* 1978;273:59–61. [PubMed: 692671]
3. Strange PG. Dopamine receptors in the basal ganglia: relevance to Parkinson's disease. *Mov Disord* 1993;8:263–270. [PubMed: 8101967]
4. Burt DR, Creese I, Snyder SH. Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. *Science* 1977;196:326–328. [PubMed: 847477]
5. Owen F, Cross AJ, Crow TJ, Longden A, Poulter M, Riley GJ. Increased dopamine-receptor sensitivity in schizophrenia. *Lancet* 1978;2:223–226. [PubMed: 79025]
6. Lee T, Seeman P, Tourtellotte WW, Farley IJ, Hornykiewicz O. Binding of 3H-neuroleptics and 3H-apomorphine in schizophrenic brains. *Nature* 1978;274:897–900. [PubMed: 683328]
7. Perlmuter JS, Stambuk MK, Markham J, Black KJ, McGee-Minnich L, Jankovic J, Moerlein SM. Decreased [18F]spiperone binding in putamen in idiopathic focal dystonia. *J Neurosci* 1997;17:843–850. [PubMed: 8987805]
8. Reisine TD, Fields JZ, Yamamura HI. Neurotransmitter receptor alterations in Parkinson's disease. *Life Sci* 1977;21:335–343. [PubMed: 197361]
9. Baron, JC.; Comar, D.; Farde, L.; Martinot, JL.; Mazoyer, B., editors. *Brain Dopaminergic Systems: Imaging with Positron Emission Tomography*. Boston: Kluwer Academic Publishers, Boston Academic Publishers; 1991.
10. Frost JJ, Smith AC, Kuhar MJ, Dannals RF, Wagner HN Jr. In vivo binding of 3H-N-methylspiperone to dopamine serotonin receptors. *Life Sci* 1987;40:987–995. [PubMed: 3821368]
11. Coenen HH, Wienhard K, Stocklin G, Laufer P, Hebold I, Pawlik G, Heiss WD. PET measurement of D2 and S2 receptor binding of 3-N-[(2'-18F)fluoroethyl]spiperone in baboon brain. *Eur J Nucl Med* 1988;14:80–87. [PubMed: 2968911]
12. Perlmuter JS, Moerlein SM, Huang DR, Todd R. Non-steady state measurement of in vivo radioligand binding with positron emission tomography: comparison with in vitro binding. *J Neurosci* 1991;11:1381–1389. [PubMed: 1827499]
13. Seeman P, Guan HC, Niznik HB. Endogenous dopamine lowers the dopamine D2 receptor density as measured by [3H]raclopride: implications for positron emission tomography of the human brain. *Synapse* 1989;3:96–97. [PubMed: 2521961]
14. Dewey SL, Smith GS, Logan J, Brodie JD, Fowler JS, Wolf AP. Striatal binding of the PET ligand 11C-raclopride is altered by drugs that modify synaptic dopamine levels. *Synapse* 1993;13:350–356. [PubMed: 8480281]
15. Riccardi P, Li R, Ansari MS, Zald D, Park S, Dawant B, Anderson S, Doop M, Woodward N, Schoenberg E, Schmidt D, Baldwin R, Kessler R. Amphetamine-induced displacement of [18F]fallypride in striatum and extrastriatal regions in humans. *Neuropsychopharmacology* 2006;31:1016–1026. [PubMed: 16237395]
16. Reynolds, JEF., editor. *Martindale The Extra Pharmacopeia*. 30th. London: The Pharmaceutical Press; 1993.
17. Peroutka SJ, Snyder SH. Relationship of neuroleptic drug effects at brain dopamine, serotonin, alpha-adrenergic, and histamine receptors to clinical potency. *Am J Psychiatry* 1980;137:1518–1522. [PubMed: 6108081]

18. Arnett CD, Wolf AP, Shiue CY, Fowler JS, MacGregor RR, Christman DR, Smith MR. Improved delineation of human dopamine receptors using [18F]-N-methylspiroperidol and PET. *J Nucl Med* 1986;27:1878–1882. [PubMed: 3491193]
19. Moerlein SM, Laufer P, Stocklin G, Pawlik G, Wienhard K, Heiss WD. Evaluation of 75Br-labelled butyrophenone neuroleptics for imaging cerebral dopaminergic receptor areas using positron emission tomography. *Eur J Nucl Med* 1986;12:211–216. [PubMed: 2876896]
20. Suehiro M, Dannals RF, Scheffel U, Stathis M, Wilson AA, Ravert HT, Villemagne VL, Sanchez-Roa PM, Wagner HN Jr. In vivo labeling of the dopamine D2 receptor with N-11C-methylbenperidol. *J Nucl Med* 1990;31:2015–2021. [PubMed: 2148346]
21. Moerlein SM, Perlmutter JS. Specific binding of 3N-(2'-[18F]fluoroethyl)benperidol to primate cerebral dopaminergic D2 receptors demonstrated in vivo by PET. *Neurosci Lett* 1992;148:97–100. [PubMed: 1300511]
22. Moerlein SM, Perlmutter JS, Welch MJ. Specific, reversible binding of [18F]benperidol to baboon D2 receptors: PET evaluation of an improved 18F-labeled ligand. *Nucl Med Biol* 1995;22:809–815. [PubMed: 8535343]
23. Moerlein SM, Perlmutter JS, Markham J, Welch MJ. In vivo kinetics of [18F](N-methyl)benperidol: a novel PET tracer for assessment of dopaminergic D2-like receptor binding. *J Cereb Blood Flow Metab* 1997;17:833–845. [PubMed: 9290581]
24. Logan J, Fowler JS, Volkow ND, Wolf AP, Dewey SL, Schlyer DJ, MacGregor RR, Hitzemann R, Bendriem B, Gatlley SJ. Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-11C-methyl]-(-)-cocaine PET studies in human subjects. *J Cereb Blood Flow Metab* 1990;10:740–747. [PubMed: 2384545]
25. Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL. Distribution volume ratios without blood sampling from graphical analysis of PET data. *J Cereb Blood Flow Metab* 1996;16:834–840. [PubMed: 8784228]
26. Logan J. Graphical analysis of PET data applied to reversible and irreversible tracers. *Nucl Med Biol* 2000;27:661–670. [PubMed: 11091109]
27. Moerlein SM, Banks WR, Parkinson D. Production of fluorine-18 labeled (3-N-methyl)benperidol for PET investigation of cerebral dopaminergic receptor binding. *Applied Radiation and Isotopes: Including Data, Instrumentation and Methods for Use in Agriculture, Industry and Medicine* 1992;43:913–917.
28. Welch MJ, Kilbourn MR. A remote system for the routine production of oxygen-15 radiopharmaceuticals. *J Lab Comp Radiopharm* 1985;22:1193–1200.
29. Mazoyer B, Trebossen R, Deutch R, Casey M, Blohm K. Physical characteristics of the ECAT 953B/31: a new high resolution brain positron tomograph. *IEEE Trans Med Imaging* 1991;10:499–504. [PubMed: 18222854]
30. Spinks TJ, Jones T, Bailey DL, Townsend DW, Grootenck S, Bloomfield PM, Gilardi MC, Casey ME, Sipe B, Reed J. Physical performance of a positron tomograph for brain imaging with retractable septa. *Phys Med Biol* 1992;37:1637–1655. [PubMed: 1518905]
31. Ollinger JM. Model-based scatter correction for fully 3D PET. *Phys Med Biol* 1996;41:153–176. [PubMed: 8685253]
32. Martin WR, Powers WJ, Raichle ME. Cerebral blood volume measured with inhaled C15O and positron emission tomography. *J Cereb Blood Flow Metab* 1987;7:421–426. [PubMed: 3497162]
33. Raichle M, Martin W, Herscovitch P, Mintun M, Markham J. Brain blood flow measured with intravenous H₂¹⁵O. II. Implementation and validation. *J Nucl Med* 1983;24:790–798. [PubMed: 6604140]
34. Videen TO, Perlmutter JS, Herscovitch P, Raichle ME. Brain blood volume, flow and oxygen utilization measured with O-15 radiotracers and positron emission tomography: revised metabolic computations. *J Cereb Blood Flow Metab* 1987;7:513–516. [PubMed: 3497165]
35. Woods RP, Cherry SR, Mazziota JC. Rapid automated algorithm for aligning and reslicing PET images. *J Comput Assist Tomogr* 1992;16:620–633. [PubMed: 1629424]
36. Andersson JL. How to obtain high-accuracy image registration: application to movement correction of dynamic positron emission tomography data. *Eur J Nucl Med* 1998;25:575–586. [PubMed: 9618571]

37. Woods RP, Mazziotta JC, Cherry SR. MRI-PET registration with automated algorithm. *J Comput Assist Tomogr* 1993;14:536–546. [PubMed: 8331222]
38. Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann Neurol* 1984;15:217–227. [PubMed: 6609679]
39. Perlmutter JS, Larson KB, Raichle ME, Markham J, Mintun MA, Kilbourn MR, Welch MJ. Strategies for in vivo measurement of receptor binding using positron emission tomography. *J Cereb Blood Flow Metab* 1986;6:154–169. [PubMed: 3485640]
40. Parsey RV, Slifstein M, Hwang DR, Abi-Dargham A, Simpson N, Mawlawi O, Guo NN, Van Heertum R, Mann JJ, Laruelle M. Validation and reproducibility of measurement of 5-HT_{1A} receptor parameters with [carbonyl-¹¹C]WAY-100635 in humans: comparison of arterial and reference tissue input functions. *J Cereb Blood Flow Metab* 2000;20:1111–1133. [PubMed: 10908045]
41. Hwang DR, Narendran R, Huang Y, Slifstein M, Talbot PS, Sudo Y, van Berckel BN, Kegeles LS, Martinez D, Laruelle M. Quantitative analysis of (-)-N-(11)¹¹C-propyl-norapomorphine in vivo binding in nonhuman primates. *J Nucl Med* 2004;45:338–346. [PubMed: 14960658]
42. Siessmeier T, Zhou Y, Buchholz HG, Landvogt C, Vernaleken I, Piel M, Schirmacher R, Rosch F, Schreckenberger M, Wong DF, Cumming P, Grunder G, Bartenstein P. Parametric mapping of binding in human brain of D₂ receptor ligands of different affinities. *J Nucl Med* 2005;46:964–972. [PubMed: 15937307]
43. Varga J, Szabo Z. Modified regression model for the Logan plot. *J Cereb Blood Flow Metab* 2002;22:240–244. [PubMed: 11823722]
44. Ghaemi M, Hiker R, Rudolf J, Sobesky J, Heiss WD. Differentiating multiple system atrophy from Parkinson's disease: contribution of striatal and midbrain MRI volumetry and multi-tracer PET imaging. *J Neuro Neurosurg Psych* 2002;73:517–523.
45. Thobois S, Fraix V, Savasta M, Costes N, Pollak P, et al. Chronic subthalamic nucleus stimulation and striatal D₂ dopamine receptors in Parkinson's disease. A [¹¹C]-raclopride PET study. *J Neurol* 2003;250:1219–1223. [PubMed: 14586606]
46. Schreckenberger M, Hagele S, Siessmeier T, Buchholz HG, Armbrust-Henrich H, et al. The dopamine D₂ receptor ligand ¹⁸F-desmethoxyfallypride: an appropriate fluorinated PET tracer for the differential diagnosis of parkinsonism. *Eur J Nucl Med and Mol Imag* 2004;31:1128–1135.
47. Kaasinen V, Ruottinen HM, Nagren K, Lehtikoinen P, Oikonen V, et al. Upregulation of Putaminal Dopamine D₂ Receptors in Early Parkinson's Disease: A Comparative PET Study with [¹¹C] Raclopride and [¹¹C]N-Methylspiperone. *J Nucl Med* 2000;41:65–70. [PubMed: 10647606]
48. Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. *Neuroimage* 1996;4:153–158. [PubMed: 9345505]
49. Ichise M, Toyama H, Innis RB, Carson RE. Strategies to improve neuroreceptor parameter estimation by linear regression analysis. *J Cereb Blood Flow Metab* 2002;22:1271–1281. [PubMed: 12368666]
50. Sossi V, Holden JE, Chan G, Krzywinski M, Stoessl AJ, Ruth TJ. Analysis of four dopaminergic tracers kinetics using two different tissue input function methods. *J Cereb Blood Flow Metab* 2000;20:653–660. [PubMed: 10779009]
51. Suzuki A, Tashiro M, Kimura Y, Mochizuki H, Ishii K, Watabe H, Yanai K, Ishiwata K, Ishii K. Use of reference tissue models for quantification of histamine H₁ receptors in human brain by using positron emission tomography and [¹¹C]doxepin. *Ann Nucl Med* 2005;19:425–433. [PubMed: 16248378]
52. Moerlein SM, Perlmutter JS, Cutler PD, Welch MJ. Radiation dosimetry of [¹⁸F](N-methyl) benperidol as determined by whole-body PET imaging of primates. *Nucl Med Biol* 1997;24:311–318. [PubMed: 9257329]

Figure 1. Representative time-activity data from striatum (average of caudate and putamen data) and cerebellum regions. Gray triangles represent striatal values; white diamonds represent cerebellar values. The solid lines are the two-(gray line) and three-compartment (black line) model fits for the cerebellum and striatum, respectively, using the arterial plasma activity (corrected for the presence of labeled metabolites) as the input function.

Figure 2. DVR graphical analysis of the time activity data from the striatum and cerebellum. $C_{ROI}(t)$ and $C_{REF}(t)$ represent the radioactivity in the receptor region of interest and the reference region at time t .

Figure 3. Correlational scatter plot for striatal BP estimates obtained from Method 2 (black triangles) and Method 3 (white triangles) as function of estimates obtained with Method 1 (solid line is regression line for Method 2, broken line is regression for Method 3).

Table 1**BP estimates computed by the various methods**

		Method1	Method2	Method3
Caudate* (n=15)	Mean	2.201	2.025	1.844
	SD (%)	0.355 (16)	0.390 (19)	0.380 (21)
	SE (%)	0.092 (4)	0.100 (5)	0.098 (5)
Putamen* (n=16)	Mean	3.023	2.832	2.592
	SD (%)	0.530 (18)	0.536 (19)	0.520 (20)
	SE (%)	0.132 (4)	0.134 (5)	0.130 (5)

*
p<0.0001

Table 2**Comparison of BP estimates between various methods and correlational coefficients, r**

Region		Method 2/1	r	Method 3/1	r
caudate (n=15)	Mean	0.926	0.936	0.833	0.913
	SD	0.085		0.089	
putamen (n=16)	Mean	0.936	0.967	0.856	0.941
	SD	0.046		0.058	

Table 3

Method 3		30-180 min	40-180 min	50-180 min
Caudate (n=15)	Mean $\pm$ SD	1.84 $\pm$ 0.38	1.88 $\pm$ 0.38	1.91 $\pm$ 0.39
	SE	0.098	0.099	0.098
	t-test vs. 30-180 min	N/A	p<0.0001	p<0.0001
	Correlation to 30-180 min (p-value)	N/A	0.999 (p<0.0001)	0.996 (p<0.0001)
Putamen (n=16)	Mean $\pm$ SD	2.6 $\pm$ 0.54	2.66 $\pm$ 0.54	2.70 $\pm$ 0.55
	SE	0.14	0.14	0.14
	t-test vs. 30-180 min	N/A	p<0.0001	p<0.0001
	Correlation to 30-180 min (p-value)	N/A	0.999 (p<0.0001)	0.997 (p<0.0001)