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Concentration and Occupancy of Dopamine Transporters in Cocaine Abusers with [^{11}C]Cocaine and PET

JEAN LOGAN,[1]* NORA D. VOLKOW,[2,3] JOANNA S. FOWLER,[1] GENE-JACK WANG,[2] MARIAN W. FISCHMAN,[5] RICHARD W. FOLTIN,[5] NAJI N. ABUMRAD,[6] STEPHEN VITKUN,[4] S. JOHN GATLEY,[2] NAOMI PAPPAS,[2] ROBERT HITZEMANN, AND COLLEEN E. SHEA[1]

[1]*Chemistry Department, Brookhaven National Laboratory, Upton, New York*
[2]*Medical Department, Brookhaven National Laboratory, Upton, New York*
[3]*Department of Psychiatry, SUNY-Stony Brook, Stony Brook, New York*
[4]*Department of Anesthesiology, SUNY-Stony Brook, Stony Brook, New York*
[5]*Department of Psychiatry, Columbia University, New York, New York*
[6]*Department of Surgery, Northshore University Hospital, Manhasset, New York*

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ABSTRACT The concentration (Bmax) of the dopamine transporter (DAT) and the maximum and effective occupancies by cocaine doses of 0.1 mg/kg or 0.05 mg/kg were measured in the striatum of cocaine abusers ($n = 12$) by using [^{11}C]cocaine as a radiotracer for the DAT and positron emission tomography (PET). Two methods based on a three-compartment model with one binding site (the nonlinear least squares (NLSQ) and the Farde pseudoequilibrium method) were used to estimate Bmax. Effective occupancies and maximum occupancies were calculated from the distribution volume ratios (DVR) and a three-compartment model, respectively. The NLSQ and Farde methods gave similar values of Bmax (average, 650 ± 350 pmol/ml and 776 ± 400 pmol/ml, respectively), but the individual estimates of Bmax were found to be very sensitive to small variations in other model parameters and were not correlated with the parameter Bmax/Kd ($r = .07$). The average maximum (and effective) occupancies were found to be 67% (50%) and 52% (39%) for the 0.1-mg/kg and the 0.05-mg/kg studies, respectively. The ED_{50} based on the effective occupancy corresponds to 0.1 mg/kg, which is significantly smaller than the ED_{50} of 3 mg/kg calculated from studies in which [^{123}I]β-CIT is displaced by cocaine. The effect on the Bmax estimate of two binding sites with different Kd's is also considered by simulation.

We conclude (1) that the lack of robustness in the Bmax estimate limits the usefulness of any one subject's Bmax and suggests that the combination parameter Bmax/Kd (or the DVR), which has been used extensively, is a more stable measure of free receptor/transporter concentration. The average Bmax may, however, provide an estimate of the expected concentration in humans. (2) The DVR can be used as a measure of DAT occupancy without applying an explicit model. **Synapse 27:347–356, 1997.**

INTRODUCTION

The dopamine transporter, which is localized on the presynaptic terminal of the dopamine neuron, is currently an important subject of research not only because it is linked to the addictive properties of cocaine but also because it is a marker for the integrity of the dopamine neuron. Cocaine, which binds to the dopamine transporter (DAT), blocks dopamine reuptake leading to increases in synaptic dopamine, a process that has been linked to cocaine's reinforcing effects (Ritz et al., 1987). Estimates of the concentration of the DAT in humans have been made from postmortem tissue. Some of these results are presented in Table I along with some examples of estimates in nonhuman primates. The variability in the human values may be due to factors such as the postmortem interval and storage time of the specimens used. Recent results from PET studies in nonhuman primates indicate a wide range of values. Volkow et al. (1995) estimated a concentration (Bmax) of 2,300 nM from a single baboon PET study

*Correspondence to: Jean Logan, Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973. email: logan@modbrain.chm.bnl.gov

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TABLE I. Bmax values for the dopamine transporter from in vitro studies

Bmax (nM)			
High	Low	Compound	Species
13[1]	47	RTI—55	Human (caudate)[2]
60[3]		GBR 12935	Human (putamen)[4]
147[3]	4220	Cocaine	Human (putamen)[5]
50	290	WIN 35428	Monkey (caudate-putamen)[6]
28	430	Cocaine	Monkey (caudate-putamen)[7]
22	423	[125I]β-CIT	Baboon (striatum)[8]

[1]High and low refer to high- and low-affinity sites for the transporter.
[2]Little et al. (1993).
[3]Assuming 100 mg protein/g tissue.
[4]DeKeyser et al. (1989).
[5]Shoemaker et al. (1985).
[6]Madras et al. (1989a).
[7]Madras et al. (1989b).
[8]Laruelle et al. (1994b).

with a cocaine dose of 2 mg/kg by using the pseudoequilibrium analysis. A recent PET study in rhesus monkeys reports an average of 113 nM from three studies, although the values range from 36 to 450 nM (Morris et al., 1996b). The use of PET allows an in vivo estimate of the concentration of transporters, although there are certain limitations to performing these experiments in humans. To estimate a transporter concentration, it is necessary to administer a sufficient quantity of drug to block a substantial number of receptors, on the order of 50%, thereby potentially causing physiologic effects. Also, it is desirable to have the drug and tracer be the same chemical entity, the only difference being the radiolabel, so that when they are coinjected, the mass of drug taken up by tissue can be measured from the PET image. The use different drugs for blocking and as a radiotracer introduces another level of assumptions about the actual mass of the unlabeled drug in tissue and leads to additional uncertainty in the Bmax estimates. In the protocol reported here, a baseline [11C]cocaine scan at high specific activity followed by a scan in which unlabeled cocaine is coadministered intravenously with [11C]cocaine in 12 cocaine abusers allowed the measurement of the concentration of cocaine in the brain and permitted the estimate of Bmax. In two of the subjects, stability of the Bmax measure was made by repeating the experiment 1 week later.

We have used two methods for estimating Bmax. One method uses the traditional three-compartment model with plasma input function and relies on estimating model parameters by a fitting procedure. Results from these calculations are also used to compare the effective occupancy derived from the distribution volume ratio (DVR) to the maximum occupancy calculated from the three-compartment model. A second method for the determination of Bmax, which is easier to carry out and also has the advantage of not requiring blood samples, has been described by Farde et al. (1989). Originally, this method was used to estimate dopamine D2 density by using [11C]-raclopride. We also consider the impact of the differences in radiotracer kinetics of β-CIT vs [11C]-cocaine on the estimates of DAT occupancy by (unlabeled) cocaine.

THEORY AND METHODS

Background

The differential equations that govern the uptake and loss of ligand from a receptor containing region with a single receptor type are

$$\frac{dF}{dt} = K_1 Cp(t) - k_2 F(t) - k_{on}(\mathrm{Bmax} - B/\sigma)F + k_{off} B \quad (1)$$

$$\frac{dB}{dt} = k_{on}(\mathrm{Bmax} - B/\sigma)F - k_{off} B. \quad (2)$$

Although these equations represent a simplification of the processes involved, they have generally been effective in describing PET data from receptor-ligand studies (e.g., Farde et al., 1989; Huang et al., 1986; Mintun et al., 1984; Wong et al., 1986), although some problems have appeared with the use of slowly dissociating ligands (Votaw et al., 1993). F and B in Equations 1 and 2 designate the "free" and "bound" ligand, respectively, $Cp(t)$ refers to the plasma radioactivity due to [11C]cocaine at time t, k_{on} and k_{off} are the receptor ligand binding constants (the dissociation equilibrium constant is $Kd = k_{off}/k_{on}$), and σ is the specific activity (SA). For the dopamine system, the cerebellum (CB) is frequently used as a reference (nonreceptor) region, and the time course of ligand radioactivity can be described by the single equation

$$\frac{d\mathrm{CB}(t)}{dt} = K_1 Cp(t) - k_2 \mathrm{CB}(t). \quad (3)$$

The distribution volume (DV) for the cerebellum is $\lambda = K_1/k_2$. In the above equations, nonspecific binding is not explicitly included but, following Mintun et al. (1984), is considered to be instantaneous and can therefore be included in the parameters k_2 and k_{on} as the product with the free fraction f_{NS} (for example $k_2 = k'_2 f_{NS}$) (see Logan et al., 1994, for more details concerning the model). In the case of high SA (low mass) of the drug, the term B/σ can be neglected, and the equations for the receptor region become

$$\frac{dF}{dt} = K_1 Cp(t) - k_2 F(t) - k_{on}\,\mathrm{Bmax}\,F + k_{off} B \quad (4)$$

$$\frac{dB}{dt} = k_{on}\,\mathrm{Bmax}\,F - k_{off} B. \quad (5)$$

Because of the large number of parameters to be estimated (five) (K_1, k_2 k_{on}, k_{off}, and Bmax from Equa-

tions 1 and 2), information from both high and low specific activity studies as well as from a nonreceptor region must be used to reduce the number of parameters to be determined from a single study. As described previously (Logan et al., 1990), the ratio K_1/k_2 is fixed at the value found for the cerebellum (the nonreceptor/transporter region in this case), reducing by one the number of parameters to be determined from the high SA study. From the high SA study, values for $k_3 = k_{on} \cdot$ Bmax and $k_4 = k_{off}$ (k_3/k_4 = Bmax/Kd) can be found. The high and low SA studies have been separated in time so that residual radioactivity from the first study is negligible. More complex strategies have been proposed for estimating receptor parameters; for example, Delforge et al. (1989) displaced the hot specifically bound ligand with a mass of unlabeled ligand. Morris et al. (1996a) have considered the implications of these multiple injection studies when cold ligand and/or an injection of low SA is given to displace the "hot" bound tracer.

The method proposed by Farde involves using a reference region (the cerebellum) that has no receptor binding as a measure of nonspecific binding and subtracting the radioactivity at each time point from radioactivity in the receptor region. The difference curve is then an approximation to the receptor bound ligand. When $dB/dt = 0$, we recover the equilibrium equation, and B/F and B can be used in a Scatchard-type analysis. The high and low SA values for B/F and B provide a two-point Scatchard, allowing the calculation of Bmax and Kd.

Validity of the "equilibrium" method

To consider the validity of the pseudoequilibrium method of Farde for [^{11}C]cocaine studies, we can apply simulations using typical cocaine parameters and a cocaine input function to generate data from Equations 1, 2, and 3 above. These data can then be used to test the method. Using parameters given in Figure 1, the simulated caudate-putamen (ST) and cerebellum curves illustrated in Figure 1 were generated. What is clear is that the bound curve from the model does not correspond to the difference curve between ST and CB except at one point. However, we can verify that application of the method does lead to the correct value for Bmax. To understand why this method works, consider the plots illustrated in Figure 2. Each curve is a plot of B (pmol/ml) vs B/F for data simulated with different specific activities ranging from 0.35 nCi/umol (upper curve), 0.5 (second curve), 0.75 (third curve), and 1.0 (lowest curve). The points on each curve (●) correspond to the values of bound and B/F generated by application of the pseudoequilibrium method. The point on the x axis corresponds to the high specific activity point in which the bound is negligible (this point slightly overestimates the true value). After the maximum in each plot of B vs B/F, the simulated curves fall on one line, which corresponds to a "Scatchard" (with x and y reversed) for this system. This is illustrated by the straight line in Figure 2, which recovers the correct value of Bmax on the y intercept. The reason for this can be seen by rearranging Equation 2 above to give

$$\frac{B}{F} = \frac{\text{Bmax} - B}{\text{Kd}} + \frac{1}{Fk_{off}}\frac{dB}{dt}. \qquad (6)$$

When the term involving dB/dt is small compared with (Bmax − B)/Kd, the equation becomes essentially the

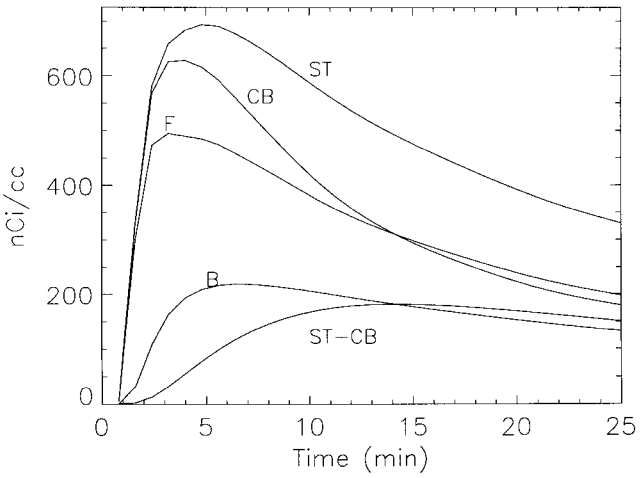

Fig. 1. Comparison of "true" B generated from Equation 2 compared with the difference between the region with specific binding (ST = F + B) generated from Equations 1 and 2 and the reference region (CB) Equation 3. These are simulations using a measured cocaine input function with model parameters $K_1 = 0.5$, $\lambda = 3.0$, $k_3 = 0.34$, and $k_4 = 0.4$.

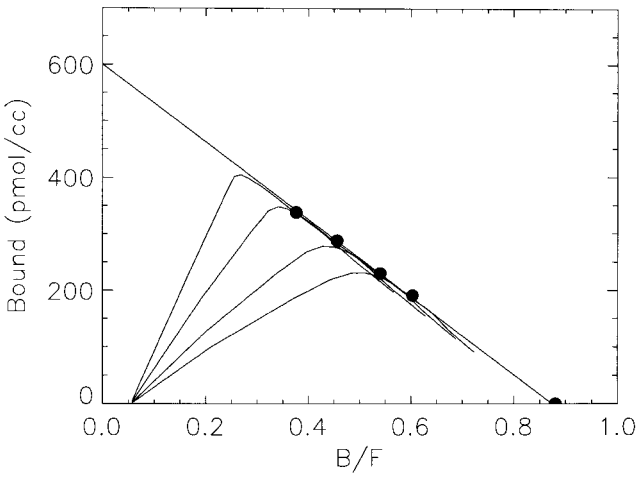

Fig. 2. The curves B vs B/F were generated from Equations 1 and 2 with varying specific activities 0.35 mCi/μmol (upper curve), 0.50 (second curve), 0.75 (third curve), and 1.0 (lowest curve). The points on each curve (●) correspond to the values of the bound and B/F generated by application of the pseudoequilibrium method using simulated curves for ST and CB, $K_1 = .55$/min and $\lambda = 3.0$, Bmax = 600 pmol/g, Bmax/Kd = 0.81. The point on the x axis is the high SA value for B/F, which is 0.87, slightly higher than the "true" value of 0.81.

equilibrium equation. The success of the method depends on the fact that the pseudoequilibrium values for B and B/F (obtained from the difference between ST and CB) also fall on this line and will therefore give the correct Bmax. The condition for which F = CB is the pseudoequilibrium condition, which can be seen by combining Equations 1, 2, and 3 to give

$$\frac{d(\mathrm{ST} - \mathrm{CB})}{dt} = k_2(F - \mathrm{CB}) \tag{7}$$

where we have assumed that K_1 and k_2 are the same for both regions. A difference in blood flow between the two regions is a potential source of error in the method, although because the CB uptake curves resulting from the same distribution volume but different values of transport constants (blood flow) will tend to converge after a few minutes (Fig. 7), this error may not be very important. This point is discussed later.

Experimental

Cocaine abusers (11 male, two female; average age, 35 ± 5 years) were used in these studies. All met the following criteria: (1) DSM IV diagnostic criteria for active cocaine dependence, (2) continuous use of cocaine for at least the prior 6 months with claimed cocaine use of at least "3 g" a week (estimated cost $120–150/week), (3) smoked or intravenous use of cocaine, (4) no current or past psychiatric disease other than cocaine dependence, (5) no neurologic signs and/or history of neurologic disease, (6) no history of head trauma with loss of consciousness, (7) no history of cardiovascular or endocrinologic disease, and (8) no current medical illness. All participants were oriented and signed consent forms approved by the institutional review board at both Brookhaven National Laboratory and Columbia Presbyterian Medical Center. PET studies were performed with a Siemens CTI 931 tomograph (6 × 6 × 6.5 full width half max, 15 slices) by using [^{11}C]cocaine (Fowler et al., 1989). Emission scans were started at the time of injection of 4–8 mCi of [^{11}C]cocaine (specific activity >0.2 Ci/μmol at the time of injection). Twenty emission scans were obtained up to 54 min after injection. Arterial sampling was used to quantitate total [^{11}C] and unchanged [^{11}C]cocaine in plasma (Alexoff et al., 1995). A baseline scan was performed on each subject, and 2 h later a second scan was performed in which a pharmacologically active dose of unlabeled cocaine was coadministered with [^{11}C]cocaine. In nine subjects, the dose of unlabeled cocaine given was 0.1 mg/kg. In three subjects, 0.05 mg/kg was given. For two of the subjects, both baseline and coinjection studies were repeated to assess reproducibility for a total of five studies. Two regions of interest were used in this study; the cerebellum, the reference region, and the caudate putamen (ST). See Volkow et al. (1993) for details concerning ROIs.

Determination of Bmax

Nonlinear least squares (NLSQ) method

Using K_1/k_2 from the cerebellum and setting k_{off} = 0.4/min, K_1 and the product $k_3 = k_{on}$ Bmax were determined from the high SA (baseline) study. Using this value for k_3, K_1 and Bmax were obtained from the low SA study. Optimum values for the parameters were determined by a least squares fit (see Logan et al., 1994, for details of the calculations). The value for the receptor-ligand dissociation constant was fixed in this calculation because it was previously found that the tissue clearance was apparently slower than the dissociation, thus limiting the ability to accurately determine this parameter (Logan et al., 1990).

Farde method

The Farde method was implemented as described by Farde et al. (1989). The CB and ST were fit with three exponentials and the difference curve corresponding to B formed from the difference of the exponential fits. This is slightly different from the approach of Farde in which the difference curve itself was fit to three exponentials. The maximum in the difference curve was taken as the pseudoequilibrium point determining B and F. The high and low SA studies provided two points for the Scatchard analysis. Because the values of B/F could change considerably with small changes in the time point determined to correspond to the maximum in B, Bmax was also calculated by using DVR − 1 because this corresponds to an effective B/F, that is,

$$\begin{aligned}\mathrm{DVR} - 1 &= \mathrm{DV(ST)/DV(CB))} - 1 \\ &= (\mathrm{Bmax} - B)/Kd = B/F\end{aligned} \tag{8}$$

where

$$\mathrm{DV(ST)} = \lambda(1 + \mathrm{Bmax}'/\mathrm{Kd}) \quad \text{and}$$
$$\mathrm{Bmax}' = \mathrm{Bmax} - \mathrm{B}.$$

Determination of effective occupany

We have previously reported effective occupancies derived from distribution volume ratios of caudate-putamen to cerebellum using DVs calculated graphically (Volkow et al., 1996). Because DVR − 1 = Bmax/Kd or Bmax′/Kd, an effective occupancy (Occ) (i.e., the fraction of receptors occupied with cocaine) can be defined as

$$\begin{aligned}\mathrm{Occ} &= 1 - (\mathrm{DVR}_2 - 1)/(\mathrm{DVR}_1 - 1) \\ &= 1 - (\mathrm{Bmax} - B)/\mathrm{Bmax}\end{aligned} \tag{9}$$

where DVR_1 is the ratio DV (ST/DV/CB) for the baseline (high SA) study and DVR_2 is that ratio calculated for the low SA study with the coinjection of unlabeled cocaine.

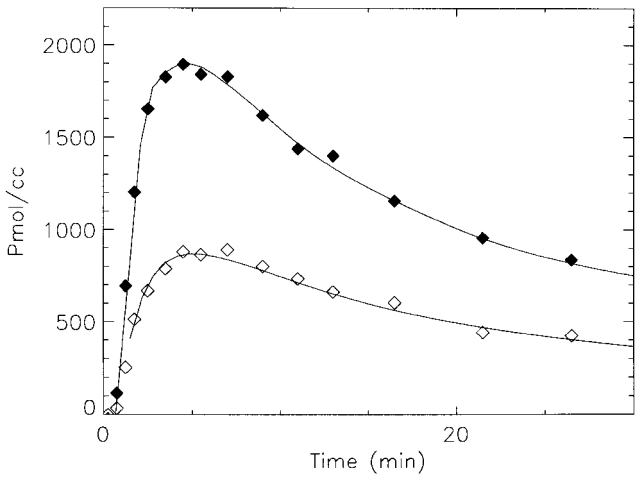

Fig. 3. Comparison of uptake in pmol/ml cocaine for 0.1 mg/kg (solid symbol) and for 0.05 mg/kg cocaine (open symbol). K_1 values were within 4% of each other. The solid lines are the model fits from Equations 1 and 2.

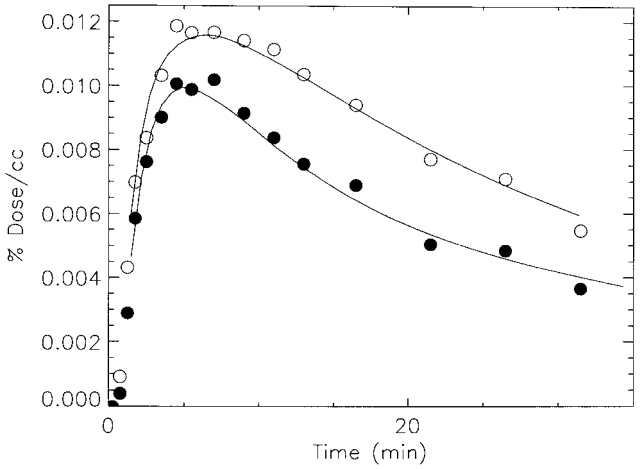

Fig. 4. Uptake of [^{11}C]cocaine in ST for baseline (○) and with injection of cocaine 0.05 mg/kg (●). Solid lines are the model fits from Equations 4 and 5 (high SA) and Equations 1 and 2 for the cocaine mass study.

TABLE II. Model parameters Bmax/Kd and λ from ST and CB for baseline and cocaine mass studies[1]

Study	Bmax Kd (1)	Bmax Kd (2)	λ_a	λ_b
(0.1 mg/kg)				
ccs001	0.87	0.45	2.28	2.74
ccs002	0.67	0.34	3.23	3.09
ccs003	0.70	0.34	3.40	3.10
ccs004	0.90	0.31	2.88	2.85
ccs006	0.97	0.32	2.47	2.61
ccs008	0.60	0.29	2.07	1.90
ccs010	0.92	0.44	4.11	4.00
ccs011	0.77	0.54	3.18	3.17
ccs012	0.87	0.42	2.22	2.12
Avg		0.38 ± 0.08		
(0.05 mg/kg)				
ccs018	0.85	0.38	3.48	3.50
ccs218	0.99	0.65	3.55	3.59
ccs019	0.90	0.47	3.54	3.51
ccs219	0.87	0.44	3.60	3.82
ccs220	0.80	0.55	3.20	3.18
Avg	0.83 ± 0.12	0.50 ± 0.09	3.09 ± 0.59	3.08 ± 0.58

[1]Bmax/Kd calculated by using the three-compartment model (1) for the baseline study (high SA) and graphically for the cocaine mass study (low SA) (2). The distribution volumes for the cerebellum λ are given for baseline and low SA study (a) and (b), respectively.

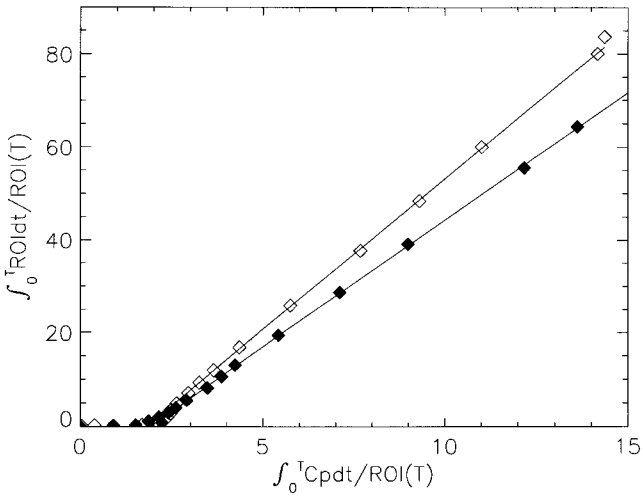

Fig. 5. Graphical analysis of data in Figure 4, high SA (open symbol), low SA (solid symbol) for a 0.05-mg/kg study

RESULTS AND DISCUSSION

The concentration of cocaine in pmol/ml in the caudate-putamen is obtained from PET time-radioactivity curves and is illustrated in Figure 3 for the 0.1-mg/kg cocaine (upper curve) and the 0.05-mg/kg (lower curve) studies. Figure 4 illustrates the three-compartment model fit to data from a baseline study (upper curve) using Equations 4 and 5 and with the injection of a cocaine mass of .05 mg/kg using Equations 1 and 2 (lower curve). For the high SA studies, the average Bmax/Kd for the three-compartment model was 0.83 ± 0.12 (Table II) and for the graphical method, 0.81 ± 0.12. Even though the low SA study should be described by Equations 1 and 2, it can also be well described by Equations 4 and 5 (i.e., the linear form with an effective Bmax). The graphical analysis of both a baseline and a low SA study are shown in Figures 5 and 6 for the 0.05- and the 0.1-mg/kg studies, respectively. The average Bmax/Kd was 0.38 ± 0.08 and 0.50 ± 0.09 for the 0.1- and 0.05-mg/kg studies, respectively (Table II). There was no difference in the average values for the distribution volumes of CB for the baseline and cocaine mass studies (Table II). Also no difference was found between K_1 in the baseline and cocaine studies (average, 0.54 ± 0.07 and 0.52 ± 0.10, respectively). The high values indicate that K_1 is dominated by blood flow rather than permeability. K_1 was found to be smaller in CB (average, 0.43 ± 0.06, baseline, and 0.44 ± 0.05, low SA). The transporter densities Bmax are given (Table III) for both the 0.1-mg/kg and 0.05-mg/kg cocaine studies.

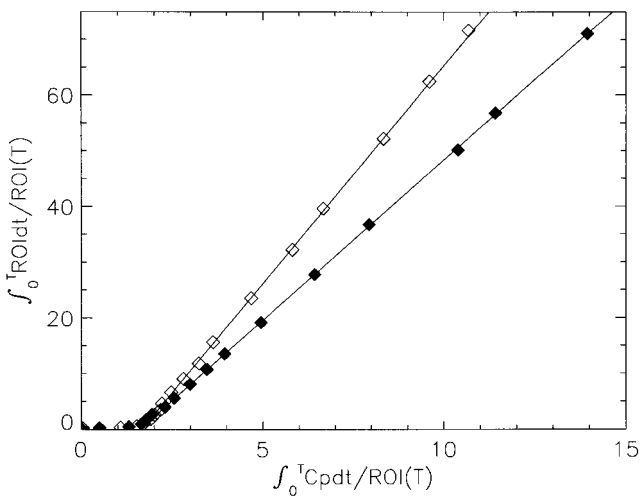

Fig. 6. Graphical analysis of [^{11}C]cocaine in ST for baseline (open symbol) and with injection of cocaine 0.10 mg/kg (closed symbol).

TABLE III. Transporter densities (Bmax) calculated by using the three-compartment model (1), the method of Farde (2), and with B/F *estimated from the DVR (3)*[1]

Study	Bmax (1)	Bmax (2)	Bmax (3)
(0.1 mg/kg)			
ccs001	825	900	773
ccs002	642	1015	930
ccs003	680	811	639
ccs004	530	980	830
ccs006	330	401	424
ccs008	350	516	525
ccs010	765	825	960
ccs011	1700	2000	1580
ccs012	620	674	860
Avg (a)	761 ± 380	902 ± 433	836 ± 314
Avg (b)	627 ± 150	765 ± 205	742 ± 182
(0.05 mg/kg)			
ccs018	370	350	325
ccs218	830	710	900
ccs019	430	576	521
ccs219	350	404	440
ccs020	680	712	750
Avg	523 ± 200	550 ± 150	587 ± 210
Avg for both (a)	650 ± 340	776 ± 400	747 ± 305
Avg for both (b)	569 ± 179	683 ± 215	683 ± 210

[1]The averages were calculated with (a) and without (b) ccs011.

With the exception of ccs011, most values of Bmax are 1,000 nM or less. The Farde method tends to give higher estimates for the 0.1-mg/kg studies. Some indication of the difficulties in estimating transporter density can be seen in the test/retest studies, in which the Bmax value for study ccs218 is two times the value of ccs018 and the effective occupancies for both studies are 0.48 and 0.33, respectively.

Table IV compares the maximum occupancy (at a single time point) calculated from the three-compartment model solution to the effective occupancy defined in Equation 9 by using the graphical analysis. The effective occupancy is about 75% of the maximum occupancy achieved in both the 0.1-mg/kg and 0.05-mg/kg studies. The half-time of cocaine occupancy,

TABLE IV. Comparison of maximum and average effective occupancy[1]

Study	Max occupancy	Eff occupancy	Eff/Max × 100	$t_{1/2}$
(0.1 mg/kg)				
ccs001	0.64	0.49	77	35
ccs002	0.71	0.47	67	32
ccs003	0.61	0.47	78	33
ccs004	0.80	0.65	82	40
ccs006	0.79	0.65	83	50
ccs008	0.62	0.49	79	31
ccs010	0.65	0.51	79	45
ccs011	0.50	0.34	67	38
ccs012	0.71	0.52	73	29
Avg	0.67 ± 0.09	0.51 ± 0.09	76 ± 6	37 ± 7
(0.05 mg/kg)				
ccs018	0.63	0.48	76	42
ccs218	0.44	0.33	75	32
ccs019	0.57	0.46	81	32
ccs219	0.61	0.45	74	35
ccs020	0.34	0.24	73	35
Avg	0.52 ± 0.11	0.39 ± 0.09	76 ± 3	35 ± 4

[1]Maximum occupancy was calculated at a single time point as the maximum in *B*/Bmax from the NLSQ model fit. The average effective occupancy was calculated from the DVR and represents an average over the time of the experiment. $t_{1/2}$ is the time from maximum occupancy to one-half maximum. Maximum occupancy occurs at 6 min.

defined as the time for the occupancy to decline to one-half of the maximum value, is also estimated and found to be 35 min. Foltin and Fischman (1991) found that the "high" from intravenous (i.v.) cocaine returned to baseline in about 30 min.

Malison et al. (1995) have estimated an effective cocaine occupancy by using SPECT with the DAT ligand [^{123}I]β-CIT. Cocaine doses of 0.28 mg/kg and 0.56 mg/kg were found to decrease the equilibrium binding of β-CIT by 6–17%. By using PET, Farde et al. (1994) report that an i.v. dose of 7 mg/kg cocaine was required to displace 50% of [^{11}C]-β-CIT 60 min after the cocaine injection. Similar results were obtained with cocaine pretreatment. Based on our results, even the smaller doses of 0.28 and 0.56 mg/kg should produce a much greater maximum cocaine occupancy than is evident with β-CIT. Malison points out that the small observed displacement is due to the slow dissociation of β-CIT from the DAT and that the measured occupancy is most likely equivalent to the cocaine occupancy at times greater than 1 h postinjection. To estimate the maximum occupancy compared with the effective occupancy for cocaine with β-CIT, we used the kinetic constants for a three-compartment model of β-CIT binding taken from Laruelle et al. (1994b) ($K_1 = 0.49$, $k_2 = 0.0173$, $k_3 = 0.0265$, and $k_4 = 0.0039$ (min^{-1}). Assuming equilibrium conditions for β-CIT and introducing a cocaine dose by using a measured cocaine input function with cocaine kinetic parameters $K_1 = 0.55$, $k_3 = 0.325$, and $k_4 = 0.4$ with Bmax = 600 pmol/ml, we calculated the maximum occupancy of cocaine compared with the decrease in β-CIT binding for doses of cocaine equivalent to 0.1 mg/kg, 0.28 mg/kg, and 0.56 mg/kg. For purposes of this calculation, the cocaine input function was extrapolated to 2 h, although the measured values

TABLE V. Analysis of sensitivity of Bmax to variations in parameters used in its calculation with the NLSQ method[1]

	+5%	−5%
λ	−23	33
K_1	−6	8
k_3	−10	14
σ	−5	5

[1]The resulting percentage change in Bmax values are given for a ±5% variation in the parameters used in its calculation.

were actually to 60 min. For the 0.1-mg/kg dose, the simulated maximum cocaine occupancy was found to be 71% whereas the decrease in "specific" β-CIT binding at 2 h was 5%. At 0.28 mg/kg, the maximum was 87%, with a 10% decrease in β-CIT, and at 0.56 mg/kg the maximum was 93%, with 15% decrease. The decrease in specific binding was calculated by using the difference between the reference region at equilibrium and the total binding; however, the nonspecific compartment was larger in the simulated receptor region due to displacement of receptor bound ligand so the decrease was actually slightly greater. These simulations agree with Malison's conclusion that decreases in β-CIT binding do not reflect the in vivo potency of cocaine for the DAT. This also holds if cocaine is coinjected with β-CIT. From simulations using a bolus input for both ligands, there is little difference in β-CIT binding at early times with a cocaine input of 0.28 mg/kg. At the maximum cocaine occupancy, β-CIT binding is within 1% of the simulated baseline. At 60 min, total β-CIT binding is 17% lower than the baseline value when the cocaine occupancy is 50%. Both cocaine and β-CIT have similar K_1 so that the difference in kinetics is due to the difference in tissue efflux (k_2) and the ligand binding constants. Interestingly, both Bmax/Kd (k_3/k_4) and λ are on the order of four times greater for β-CIT than for cocaine.

The inability of β-CIT to compete with cocaine is indicated by the difference in ED_{50}. Laruelle et al. (1994a) and Malison et al. (1995) calculated an ED_{50} of 3 mg/kg for the displacement of β-CIT by cocaine. Farde et al. (1994) reported that 7 mg/kg was required to displace 50% of the striatal specific binding of [^{11}C]-β-CIT as measured by PET at 60 min after the injection of cocaine. Similar results were obtained with cocaine pretreatment (4 min p.i.) (Farde et al., 1994). From our data, the ED_{50} for the effective occupancy of cocaine with cocaine is 0.1 mg/kg. (The average logit transformed occupancy × 100 for the 0.1-mg/kg studies is 9×10^{-3}, indicating that this is very close to the x intercept.) The conclusion is that β-CIT cannot be used to accurately estimate cocaine occupancy.

An analysis of the sensitivity of the estimated value of Bmax to variations in other model parameters is given in Table V for the NLSQ method. Simulated data were generated by using the following model values: $K_1 = 0.50$, $\lambda = 2.8$, $k_3 = 0.32$, and Bmax = 600 pmol/ml with $\sigma = 0.45$ nCi/μmol. The optimum value of Bmax was then calculated with variations of ±5% in the indicated parameter. From Table V, the value of Bmax was found to be very sensitive to small variations in λ and also to small variations in k_{on} Bmax (related to the baseline value of Bmax/Kd). In general, the difficulty with cocaine is due to the low ratio of specific to nonspecific binding, which at high SA is reflected in a Bmax/Kd value of less than 1.0. This can also be seen in the calculation of percentage occupancy, which is very sensitive to small variations in λ. The sensitivity of the cocaine signal taken as λ(1 + Bmax/Kd) (the DV from the striatum) can be expressed in terms of the normalized derivative with respect to λ and with respect to Bmax/Kd, giving 1 and (DVR − 1)/DVR, respectively (see Kim et al., 1990). Because (DVR − 1)/DVR ≈ 0.44, this indicates that the signal is much more sensitive to changes in λ than to changes in Bmax/Kd. Drugs with larger values of Bmax/Kd will have an increased sensitivity, although with different kinetics.

Because of the low sensitivity of the signal with respect to the receptor parameter, the individual values calculated for Bmax have a rather large uncertainty considering that variations of ±5% are within the range of what is observed in previous baseline test/retest studies (Logan et al., 1990). The large variation between study ccs018 and ccs218 is evidence of this, with a ±45% deviation from the average, which is greater than the difference in baseline Bmax/Kd of 20%. The variability of ccs019/219 is somewhat less, on the order of 10–20%. As a result of the large uncertainty associated with the each of the Bmax values, the average value of approximately 650 ± 400 pmol/ml is presumably more meaningful due to the cancellation of random errors from the individual studies.

Bmax estimates from the Farde method generally appear larger than values calculated by using the NLSQ method for the lower SA (0.1-mg) studies. The 0.05-mg studies agree quite well with the NLSQ method. In the 0.1-mg studies, the difference in blood flow between caudate-putamen and cerebellum can lead to a maximum in the bound curve (ST-CB) that reflects the difference in blood flow rather than specific binding. Figure 7 illustrates for simulated data the difference in the bound curves determined from cerebellum with $K_1 = 0.55$ and $K_1 = 0.47$ ($\lambda = 2.8$ for both) with upper curve (ST) $K_1 = 0.55$. Differences due to blood flow (K_1) are minimized at later times (12 min). This plot also illustrates another problem with the technique, which is that the bound curve is rather constant over a period of time while the ratio B/F is changing rapidly due to the washout from the cerebellum. Small variations in the time of the maximum can lead to large variations in B/F. This is analogous to the large errors encountered with variations in λ when Bmax is calculated by using the NLSQ method. From model simulations, the use of a CB curve with a 15% lower value of K_1 than that of ST leads to a 4–5% lower value of B (for specific activities

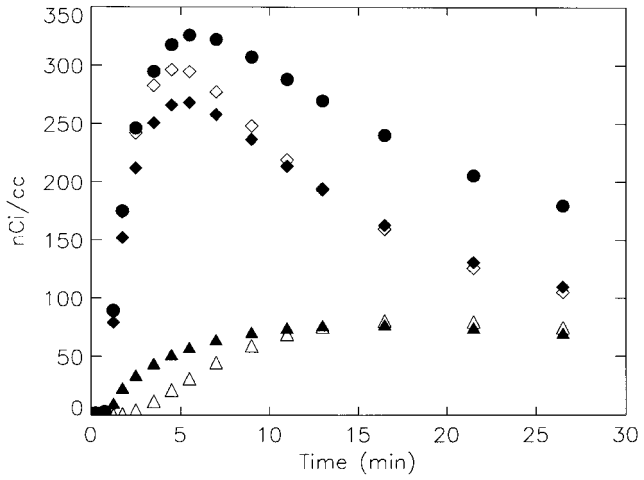

Fig. 7. ST − CB curves generated from simulated data in which the CB had the same K_1 as the ST and in which K_1 was 15% less. The upper curve (●) corresponds to the ST. CB curves for K_1 the same as ST (open diamond) and with K_1 lower (solid diamond). Solid triangles correspond to the difference using CB with lower K_1, open triangles CB with the same K_1.

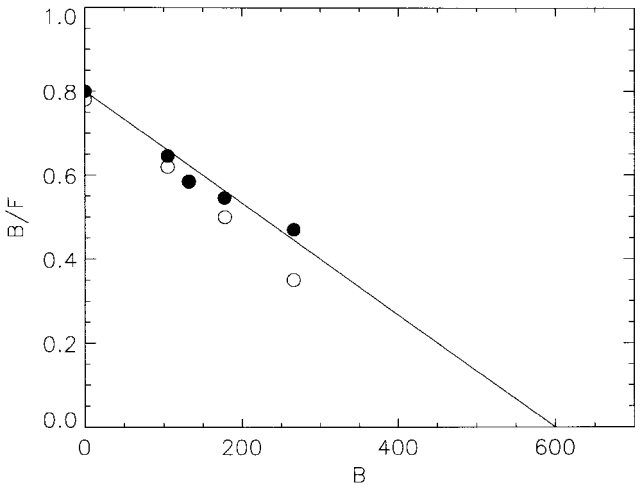

Fig. 8. "Scatchard" analysis with simulated data generated from ST-CB in which the CB has K_1 15% less than K_1 of ST. The solid line corresponds to the "true" Scatchard. The bound was estimated from the difference between the ST and CB with B/F estimated by the Farde method (○) and from the DVR (●).

ranging from 0.75 to 0.3 mCi/μmol) and a lower value for *B*/*F*. This leads to an underestimate of Bmax. This is illustrated in Figure 8 in which the pseudoequilibrium values indicated by (○) fall below the Scatchard line. This is in contrast to the apparent overestimate observed (compared with the NLSQ estimate) in experimental data at the lower SA. This may be related to the fact that the true model may have two sites with different kinetics, so that the one site simulation does not really produce the same details of the uptake curve as the experimental data.

Another approach to the Farde method is to approximate B/F with DVR − 1 (Equation 8). In simulations using model parameters given previously, the use of the DVR gives a close estimate to the correct Bmax even with a 15% difference in blood flow between ST and CB. This is illustrated in Figure 8, in which the DVR-derived values for B/F are given by ●. The DVRs were generated directly by using our recent method, which does not require blood sampling but uses data from the cerebellum (Logan et al., 1996).

Further indication of the ill-conditioned nature of the Bmax determination is the fact that the correlation coefficient between Bmax and Bmax/Kd is 0.07, whereas one would expect that a high Bmax would lead to a high Bmax/Kd, with Kd remaining relatively unchanged. This is also evident in the work of Morris et al. (1996b), in which a Bmax of 450 pmol/ml was associated with a Bmax/Kd of 2.6 and Bmax of 36 pmol/ml with Bmax/Kd of 3.9. This variability could also be associated with the fact that there may be high- and low-affinity binding sites.

Other sources of error are associated with the resolution of the PET camera, leading to partial volume effects in which the true radioactivity in a structure smaller than the camera resolution is underestimated. Differences in positioning between the low and high SA studies could lead to a different partial volume effect between the two studies due to the large interslice distance. Also, even though every effort was made to ensure that the subjects in the studies did not take cocaine during the times before the scans (drug testing was performed at the time of the study), it is not possible to completely rule this out as a possible source of error.

The previously mntioned potential difficulty encountered in estimating the DAT Bmax is that, by necessity, the model used is a one-site model whereas much of the previous in vitro work suggests that there are two binding sites for cocaine as well as for most other ligands that bind to the DAT. It is not possible with our experimental data to uniquely determine the model parameters of a two-site model. In nonhuman primates, Madras et al. (1989b) find both a high-affinity site (Bmax = 28 pmol/g) and a low-affinity site (Bmax = 430 pmol/g). Bmax/Kd is 1.47 and 0.38 for the high and low sites, respectively, for a total of 1.86. This is somewhat higher than the effective Bmax/Kd observed from PET studies, which is on the order of 0.8. The high SA Bmax/Kd should reflect the total of all binding sites because it is derived from the distribution volume (Logan et al., 1990). The effective Kd calculated from the average Bmax/Kd of 0.8 and Bmax = 550 pmol/g is 625 pmol. This Kd differs from the in vitro values in that it contains the effect of nonspecific binding. That is,

$$\mathrm{Kd(PET)} = \frac{k_{\mathrm{off}}}{f_{NS} k_{\mathrm{on}}} = \frac{\mathrm{Kd(in\ vitro)}}{f_{NS}} \qquad (10)$$

Because f_{NS} is a fraction representing the fraction of radioactivity available to bind to the receptor, the PET Kd should be larger than the in vitro Kd. This is

consistent with the Bmax/Kd for PET being smaller than the in vitro value. Although the total Bmax/Kd can be estimated for multiple binding sites, the estimate of Bmax from a one-site model when the true model contains two sites with different Kd will be in error (i.e., it will not in general reflect the total Bmax for both sites). The presence of a high-affinity site, even one with a small Bmax, will lead to an underestimate of the total Bmax by using the techniques and doses reported here. The problem in this case is that the largest contribution to the DV is made by the high-affinity low-concentration site (for cocaine this is 1.47; Madras et al., 1989b). In a simulation study using a measured experimental plasma, input function data were generated from a two-site model with $Bmax_1$ = 25 pmol/g and $Bmax_2$ = 400 pmol/g ($Bmax_1/Kd_1$ = 0.833 and $Bmax_2/Kd_2$ = 0.388) with Kd of 30 and 1,052 nM. The dissociation was assumed to be very rapid for both sites because cocaine exhibits a rapid washout. Setting k_{off} to 0.2 and 0.4 (min^{-1}) for the high- and low-affinity sites, respectively, the largest difference in Kd occurs in the association constant k_{on} rather than k_{off}. Using the one-site model of Equations 1 and 2, we find Bmax values of 170 and 225 pmol/g for cocaine masses corresponding to the 0.05 mg/kg and 0.1 mg/kg studies, respectively. In the high specific activity case, the true DV was recovered with the one-site model. If the Kd was not very different for both sites, then the one with the greater Bmax would dominate and the error should be less. This may be the case if the in vivo Kd for the two sites is more similar to each other than the in vitro values. In any case, it is not possible to uniquely determine Bmax values from a two-binding site model from these PET studies.

A natural extension of this work is to perform these experiments in normal subjects with [^{11}C]*d*-threo-methylphenidate, which has a greater sensitivity to changes in transporter occupancy than cocaine and could be coadministered with *d*-threo-methylphenidate. Bmax/Kd is between 1 and 2 for methylphenidate compared with 0.8 for cocaine. Also, experiments performed at a wider range of specific activities would determine whether two binding sites can be observed on the transporter.

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