Carbon-11-Cocaine Binding Compared at Subpharmacological and Pharmacological Doses: A PET Study

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We have characterized cocaine binding in the brain to a high-affinity site on the dopamine transporter using PET and tracer doses of [^{11}C]cocaine in the baboon in vivo. The binding pattern, however, of cocaine at tracer (subpharmacological) doses may differ from that observed when the drug is taken in behaviorally active doses particularly since in vitro studies have shown that cocaine also binds to low affinity binding sites. **Methods:** PET was used to compare and characterize [^{11}C]cocaine binding in the baboon brain at low subpharmacological (18 μg average dose) and at pharmacological (8000 μg) doses. Serial studies on the same day in the same baboon were used to assess the reproducibility of repeated measures and to assess the effects of drugs which inhibit the dopamine, norepinephrine and serotonin transporters. Time-activity curves from brain and the arterial plasma input function were used to calculate the steady-state distribution volume (DV). **Results:** At subpharmacological doses, [^{11}C]cocaine had a higher binding and slower clearance in striatum than in other brain regions. At pharmacological doses, [^{11}C]cocaine had a more homogeneous distribution. Bmax/Kd for sub-pharmacological [^{11}C]cocaine corresponded to 0.5–0.6 and for pharmacological [^{11}C]cocaine it corresponded to 0.1–0.2. Two-point Scatchard analysis gave Bmax = 2300 pmole/g and Kd'= 3600 n*M*. Bmax/Kd for sub-pharmacological doses of [^{11}C]cocaine was decreased by cocaine and drugs that inhibit the dopamine transporter, to 0.1–0.2, but not by drugs that inhibit the serotonin or the norepinephrine transporter. None of these drugs changed Bmax/Kd for a pharmacological dose of [^{11}C]cocaine. **Conclusion:** At subpharmacological doses, [^{11}C]cocaine binds predominantly to a high-affinity site on the dopamine transporter.

Key Words: cocaine; positron emission tomography; pharmacokinetics; low-affinity binding sites; dopamine transporters

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Cocaine is considered one of the most reinforcing and addictive drugs of abuse. The reinforcing properties of cocaine have been associated with its binding to the dopamine transporter and not to the norepinephrine or the serotonin transporters (*1*). In vitro studies have shown that cocaine binds to both high- and low-affinity sites on the dopamine transporter. The range of Kd values is between 16 n*M* and 210 n*M* for high-affinity sites and between 660 n*M* and 26,400 n*M* for low-affinity binding sites (*2–6*). By using PET and [^{11}C]cocaine we demonstrated cocaine binding to dopamine transporters in vivo (*7*). Because the studies with [^{11}C]cocaine were done at subpharmacological levels of cocaine, [^{11}C]cocaine's binding to the dopamine transporter probably represented high-affinity sites. This may, however, not be the only pharmacologically relevant binding site when cocaine is administered in behaviorally active, pharmacological doses. Recent studies using in vitro human brain autoradiography showed significantly different profiles of distribution of tritiated cocaine at 1 μ*M* concentration (*8*), which is similar to that found in the brains of cocaine abusers, than at 10 n*M* concentration which is in the range of the subpharmacological [^{11}C]cocaine studies done with PET (*7*). At the higher concentration, binding is more homogeneous and low-affinity binding sites are observed in the hippocampus and in the temporal cortex. Although these low-affinity sites have yet to be characterized pharmacologically, they may play a significant role in the pharmacological and toxicological spectrum of cocaine.

The ability of PET to measure moment-to-moment changes in the distribution of positron-labeled compounds makes it an ideal technique to investigate binding characteristics of pychoactive drugs in vivo. Since pharmacological responses are observed in living subjects, it is important to validate the binding parameters of drugs under in vivo conditions. Because of the possible relevance of low-affinity sites in the behavioral, addictive and toxic properties of cocaine, we used PET to examine low-affinity binding sites in the living baboon brain. For this purpose, we compared in vivo [^{11}C]cocaine binding in the baboon brain at subpharmacological (18 μg) and at pharmacological (8 mg) doses. Pharmacological doses were used to assess the degree of [^{11}C]cocaine binding to low-affinity sites. Percentage occupancy of high- and low-affinity cocaine bind-

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TABLE 1
Percent Occupancy of High- and Low-Affinity Binding Sites for Cocaine at Subpharmacological (18 μg) and Pharmacological Doses (0.5 mg/kg and 1.0 mg/kg) Using Equation 4 and Striatal Uptake of 0.05% Dose/cc as Measured with PET

Cocaine binding site	Species	Region	Kd (n*M*)	Bmax (pmole/g tissue)	Bmax/Kd	Ref	% occ. (18 μg)	% occ. (7500 μg)	% occ. (15,000 μg)
High affinity	Human	Putamen	210	147*	0.7	2	7	98	99
High affinity	Nonhuman primate	Caudate-putamen	19.2	28.3	1.47	3	41	99	100
Low affinity	Human	Putamen	26,400	4220†	0.16	2	0.06	30	46
Low affinity	Nonhuman primate	Caudate-putamen	1120	431	0.38	3	1	91	96

*Reported as 1.47 pmole/mg protein (*2*).
†Reported as 42.2 pmole/mg protein (*2*) converted to pmole/g tissue by multiplying by 100, considering protein content of the brain to be 10% (*36*).

ing sites by the 18 μg and the 8 mg doses were calculated using literature values of binding parameters determined in vitro. To compare the degree of specific versus nonspecific binding, we assessed the effects of cocaine pretreatment on subpharmacological and pharmacological doses of [^{11}C]cocaine. To compare the degree of binding to serotonin, dopamine and norepinephrine transporters, we assessed the effects of pretreatment with citalopram (*9*) and fluoxetine (*10*), nomifensine (*11*) and methylphenidate (*12*), and desipramine (*13*) and tomoxetine (*14*), respectively, on [^{11}C]cocaine binding at subpharmacological and at pharmacological doses.

METHODS

Synthesis

[N-^{11}C-methyl] cocaine was prepared by the methylation of norcocaine (*7*) using [^{11}C]methyl iodide prepared by the method of Langstrom (*15*). Cocaine and norcocaine were supplied by the National Institute on Drug Abuse.

Scanning Protocol

Seven adult female baboons (*Papio anubis*) were used in this investigation. A total of 16 paired studies were done in these animals 2 hr apart. The first scan was obtained as a baseline and was used to compare changes induced by the pharmacological intervention on the second scan. Paired studies are required because of the large intrasubject variability when scanning baboons on separate days (unpublished data). Baboons were anesthetized, catheterized and prepared for the PET study as previously described (*16*). Imaging was performed on a CTI 931 tomograph (Computer Technologies, Inc., Knoxville, TN) (spatial resolution of 6.5 mm × 5.9 mm FWHM at the center of the field of view). Dynamic scans were started immediately after injection of a fast bolus of [^{11}C]cocaine (5–8 mCi; SA 100 mCi/μmole at time of injection) and were continued for a total of 54 min. Sequence for dynamic scans corresponded to four 30-sec, four 60-sec, four 120-sec and four 600-sec scans. For the subpharmacological dose of [^{11}C]cocaine, an average of 17.8 ± 12.2 μg of cocaine was injected and for the pharmacological dose [^{11}C]cocaine, 8 mg of unlabeled cocaine was coadministered with [^{11}C]cocaine. A calculation of the percent occupancy of high- and low-affinity sites at these doses using binding parameters reported in the literature (*2,3*) is presented in Table 1. Vital signs (heart rate and blood pressure) were monitored during the entire scanning period and during drug administration.

Pharmacological Challenge. To assess binding of [^{11}C]cocaine at subpharmacological and at pharmacological doses to the dopamine, serotonin and norepinephrine transporters, various pharmacological challenge experiments were conducted. The drugs, their doses and the timing of their administration for these studies are described in Table 2. In addition, a paired study was also done to compare subpharmacological and pharmacological dose of [^{11}C]cocaine in the same baboon and two paired studies were done to assess the test-retest reproducibility for subpharmacological and pharmacological dose of [^{11}C]cocaine when repeated studies were done with no intervention. Test and re-test studies were performed to serve as comparisons with which to monitor the magnitude of the changes induced by pharmacological interventions.

Analyses. Procedures regarding blood sampling and quantification of total radioactivity and unchanged labeled tracer in plasma were previously described (*7*).

Regions for the corpus striatum, cerebellum, thalamus, cortex and mesencephalon were obtained in the slice or slices where the regions were identified (*16*). The radioactivity concentration in these regions of interest (ROIs) was used to obtain the time-activity curve for regional tissue concentration. An approximate value for whole brain uptake was obtained by averaging the radioactivity in the five central slices.

Time-activity curves for tissue concentration and for unchanged tracer in plasma were used to calculated the plasma to tissue transport constant (K1) and the distribution volume (DV) for the various regions using graphical analysis for reversible systems (*17*). For the cerebellum, the DV is given by (*18*):

$$DV(CB) = K_1/k_2(1 + NS), \quad \text{Eq. 1}$$

where NS represents the ratio of nonspecific binding constants (*17,19*). For the striatum:

$$DV(ST) = K_1/k_2(1 + NS + \sum (Bmax_i'/Kd_i), \quad \text{Eq. 2}$$

where i designates the type of receptor and Bmax' refers to the free receptor concentration. The ratio of DVs for striatum to cerebellum is given by:

TABLE 2
Tabulation of Paired Baboon Studies with Subpharmacological and Pharmacological Doses of Carbon-11-Cocaine

	Dopamine			Serotonin		Norepinephrine	
[^{11}C]cocaine	Cocaine	Nomifensine	Methylphenidate	Citalopram	Fluoxetine	Desipramine	Tomoxetine
Subpharmacological dose	2*	1‡	1§	2¶	1§§	1¶¶	—
Pharmacological dose	1†	1‡		1**	1§§	—	1***

*0.5 mg/kg and 2 mg/kg i.v., 5 min prior.
†2 mg/kg i.v., 5 min prior.
‡2 mg/kg i.v., 10 min prior.
§0.5 mg/kg i.v., 20 min prior.
¶2 mg/kg at 180 min prior and 2 mg/kg i.v. at 30 min prior.
**2 mg/kg i.v. at 30 min prior.
§§0.5 mg/kg i.v. at 20 min prior.
¶¶0.5 mg/kg i.v. at 30 min prior.
***2 mg/kg i.v. 20 min prior.

$$DV(ST)/DV(CB) = 1 + \sum_i (Bmax_i'/Kd_i'), \quad \text{Eq. 3}$$

where

$$Kd' = Kd(1 + NS). \quad \text{Eq. 4}$$

The derivation of the equations relating DV and Bmax'/Kd' assumed a constant free receptor concentration. Although this does not apply to most of the experiments reported here in which drug pretreatment and low specific activity tracer result in changing free binding site concentrations over the course of the experiment, data analysis with this method produced linear plots. This allows the interpretation of changes in DVs from these experiments in terms of changes in an effective free binding site concentration.

Results were also analyzed using the equilibrium method of Farde et al. (*20*). By subtracting cerebellar radioactivity from that in striatum, the time at which the rate of change of specific binding is zero can be determined, and at this time:

$$\frac{RL}{F} = \frac{(Bmax - RL)}{Kd},$$

where RL is the concentration of bound cocaine and F that of free cocaine. This allows a Scatchard-type analysis, assuming the cerebellum can be used to represent the free concentration.

In addition, time-activity curves for the ratio of the activity in striatum over that in cerebellum were plotted. Only pharmacological interventions which induced changes three times the size of the test-retest variability were considered significant. Student t-tests (unpaired) were used to compare peak uptake and K_1 values for subpharmacological and pharmacological doses of [^{11}C]cocaine.

Calculation of Receptor Occupancy

A maximum expected occupancy of cocaine receptors in baboon striatum can be calculated for the high- and low-affinity binding sites using values reported in the literature for the binding parameters (Bmax and Kd) (*2,3*) for both sites and assuming equilibrium conditions for both sites. Under these conditions:

$$(Bmax_i - RL_i)L = Kd_i\, RL_i, \quad \text{Eq. 5}$$

where $Bmax_i - RLi$ represents the concentration of free binding sites for receptor type i and L represents the concentration of free cocaine in striatum. The total concentration of [cocaine] is given by:

$$[cocaine] = L + RL_1 + RL_2. \quad \text{Eq. 6}$$

Under equilibrium conditions:

$$(Bmax_1\text{-}RL_1)([cocaine] - RL_1 - RL_2) = Kd_1\, RL_1,$$
$$(Bmax_2\text{-}RL_2)([cocaine] - RL_1 - RL_2) = Kd_2\, RL_2, \quad \text{Eq. 7}$$

and % occupancy for each species is given by:

$$\frac{100[RL_i]}{Bmax_i}. \quad \text{Eq. 8}$$

RESULTS

Estimation of Dopamine Transporters Occupancy

Binding parameters from two different in vitro studies, one with human putamen tissue (*2*) and one with monkey caudate-putamen (*3*), report Kds of 210 n*M* and 19 n*M* for the high-affinity sites and 26.4 μM and 1.12 μM for the low-affinity sites for the human and monkey tissues, respectively. Using these values along with the masses of cocaine administered, and the striatal uptake as measured by PET, we calculate that a maximum of 7%–41% of the high-affinity sites are occupied when the subpharmacological dose was given while a maximum of 0.1%–1% of the low-affinity sites would be occupied at this dose. At pharmacological doses (7.5–15.0 mg), a maximum of 99% of the high-affinity sites and a maximum of 30%–96% of the low-affinity sites would be occupied. These occupancy estimates represent a "maximum" since these are not equilibrium conditions. Binding parameters and percent occupancy are tabulated in Table 1. The large range of occupancies reflect a very large difference in in vitro binding parameters reported in these two studies (*2,3*).

TABLE 3
Brain Uptake and Clearance Rates for Subpharmacological and Pharmacological Doses of Carbon-11-Cocaine*

Regions	[^{11}C]Cocaine (18 μg)			[^{11}C]Cocaine (8 mg)		
	Peak activity % dose/cc	Time to peak activity (min)	Half peak clearance (min)	Peak activity % Dose/cc	Time to peak activity (min)	Half peak clearance (min)
Global	0.033 ± 0.007	2–4	15	0.041 ± 0.002‡	1.5–3	15
Striatum	0.046 ± 0.008	3–5	20	0.053 ± 0.004	1.5–3	15
Thalamus	0.040 ± 0.008	1.5–3	15	0.053 ± 0.002§	1.5–3	13
Cerebellum	0.043 ± 0.01	1.5–3	11	0.055 ± 0.003†	1–2	10
Cortex	0.030 ± 0.008	2–4	15	0.053 ± 0.002§	1.5–3	13
Mesencephalon	0.031 ± 0.007	2–4	15	0.041 ± 0.005	1.5–2	15

*Values represent an average from 11 studies at an average injected mass of 18 μg and from 6 studies with an injected mass of 8 mg of [^{11}C]cocaine. Significant differences in peak uptake between subpharmacological and pharmacological [^{11}C]cocaine are denoted with †= $p < 0.05$; ‡$p < 0.01$; §$p < 0.0005$.

Binding Parameters

There were no measureable changes in heart rate or blood pressure when 18 μg of cocaine was administered. Heart rate and blood pressure, however, increased transiently during the administration of 7.5–15 mg of cocaine and during methylphenidate administration. Whole brain and regional brain peak uptake (except for striatum) was significantly higher with pharmacological than with subpharmacological doses of [^{11}C]cocaine (Table 3). The largest differences were in cortex ($t = 6.7$, $p < 0.0003$) and in thalamus ($t = 5$, $p < 0.0005$). The values for K_1 (transport from plasma to tissue) were also higher for pharmacological than subpharmacological doses of [^{11}C]cocaine: 1.07 ± 0.21 versus 0.68 ± 0.26 ($t = 3.0$, $p < 0.01$). The binding pattern also differed markedly between pharmacological and subpharmacological doses [^{11}C]cocaine. Carbon-11-cocaine bound predominantly to the striatum at subpharmacological doses, but bound more homogeneously with equivalent uptake in striatum, thalamus and cortex (Fig. 1) at pharmacological doses.

The uptake and clearance of [^{11}C]cocaine at pharmacological doses was similar for the various brain regions; peak uptake was achieved 1.5–3 min after injection and half peak clearance 10–15 min after injection. In contrast, the uptake and clearance for subpharmacological doses of [^{11}C]cocaine differed among brain regions. Peak uptake occurred at 3–5 min in striatum and at 1–2 min in cerebellum, and clearance was slower in striatum (half peak clearance = 20 min) than in thalamus (half-peak clearance = 15 min) and cerebellum (half peak clearance = 11 min). The time-activity curves for subpharmacological and pharmacological doses of [^{11}C]cocaine are shown in Figure 2 and the uptake and clearance rates for the various brain regions are shown in Table 3. The slower clearance of a subpharmacological dose [^{11}C]cocaine from striatum than that with the pharmacological dose led to higher STR/CB values (Fig. 3). Graphical analysis plots of the ratio of the distribution volume (DV) in striatum (STR) to that in cerebellum (CB) for the subpharmacological and pharmacological doses of [^{11}C]cocaine are also shown in Figure 3.

Repeated measurements on the same day with no intervention were reproducible. Table 4 provides the values for the DV in STR and in CB as well as for the ratio of the DV in STR to CB for these repeated measures, as well as for the results of the pharmacological challenges. Test and retest percent change for the subpharmacological dose of [^{11}C]cocaine corresponded to 2% and to 4% for the pharmacological dose. The time-course of the STR/CB ratios were also very stable (Fig. 4).

We used the technique of Farde et al. (*20*) and found that the maximum amount of specifically bound cocaine was 9

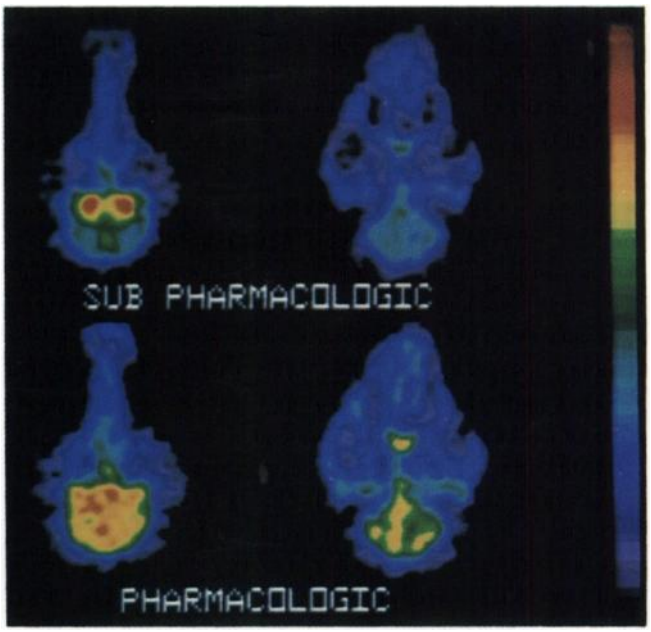

FIGURE 1. Brain images of the baboon obtained with subpharmacological dose of [^{11}C]cocaine (upper images) and with pharmacological dose of [^{11}C]cocaine (lower images) 15 min after injection. Images correspond to planes where striatum and cerebellum are located. Each study was normalized to the maximal activity.

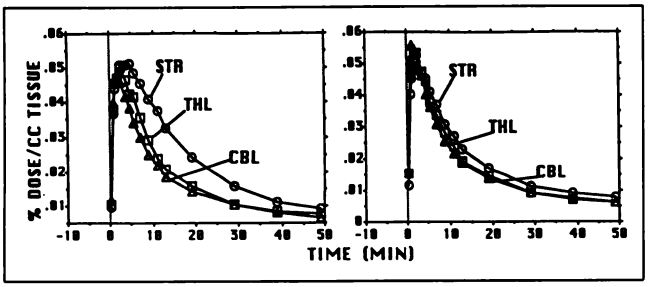

FIGURE 2. Kinetics of subpharmacological (left) and pharmacological (right) doses of [^{11}C]cocaine in striatum (STR), thalamus (THL) and cerebellum (CBL). Notice the relatively slower clearance in striatum for subpharmacological doses but not pharmacological doses of [^{11}C]cocaine. Notice the similar kinetics for pharmacological dose of [^{11}C]cocaine in various brain regions.

pmole/cc in the subpharmacological [^{11}C]cocaine study and 1450 pmole/cc in the pharmacological [^{11}C]cocaine study (Table 5). If the 9 pmole value is bound to the high-affinity site, the occupancy would be 6% (assuming Bmax = 147 pmole/g, Table 1). The bound-to-free fractions were 0.6 and 0.23 for the subpharmacological and the pharmacological [^{11}C]cocaine studies, respectively. A Scatchard analysis with two points gives Bmax = 2300 pmole/g and Kd = 3600 n*M*. For comparison, the calculation of specifically bound cocaine was applied to the test/retest study with pharmacological [^{11}C]cocaine, giving 1900 to 2000 pmole/cc of specifically bound cocaine at the maximum for both studies. The maximum in the specifically bound curve (STR-CB) was found to occur between 7 and 11 min after injection.

Cocaine pretreatment decreased the uptake and distribution volume of the subpharmacological dose of [^{11}C]cocaine in striatum but not in cerebellum. In contrast, the uptake and distribution volume of a pharmacological dose of [^{11}C]cocaine in striatum was not affected by cocaine pretreatment (Table 4). Cocaine pretreatment with a subpharmacological dose of [^{11}C]cocaine decreased the peak in the STR/CB ratio from 2.0 to 1.35 for the 0.5-mg/kg dose and from 1.75 to 1.1 for the 2-mg/kg dose (Fig. 4). It had no effect on a pharmacological dose of [^{11}C]cocaine.

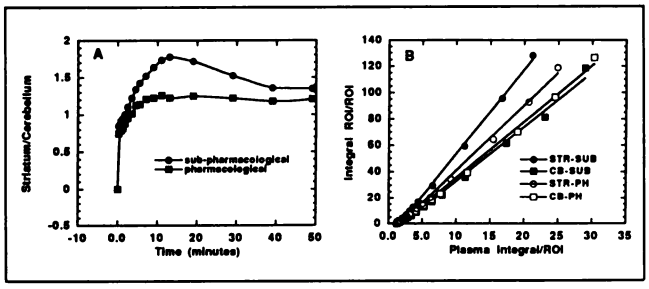

FIGURE 3. (A) Ratios for STR/CB for subpharmacological and pharmacological doses of [^{11}C]cocaine. Closed circles are the subpharmacological dose and open squares are the pharmacological dose. Peak STR/CB ratio for subpharmacological dose was 1.8 and 1.2 for the pharmacological dose. (B) Graphical analysis plots (*17*) for subpharmacological and pharmacological dose [^{11}C]cocaine for striatum and cerebellum.

Effects of Monoamine Transporter Blockers

Pretreatment with nomifensine or with methylphenidate decreased the uptake of a subpharmacological dose of [^{11}C]cocaine in striatum but did not affect other brain regions. Nomifensine did not affect binding of a pharmacological dose of [^{11}C]cocaine (Table 4, Fig. 4).

Citalopram significantly increased the uptake (Fig. 4) of a subpharmacological dose [^{11}C]cocaine and it increased both the STR/CB ratio and the ratio of the DV in STR to that in CB (Table 4). Fluoxetine pretreatment also increased (though to a lesser extent than citalopram) striatal binding of the subpharmacological dose of [^{11}C]cocaine. Neither citalopram nor fluoxetine affected binding of a pharmacological dose of [^{11}C]cocaine (Fig. 4).

Though it was not possible to calculate DVs for the studies with desipramine and tomoxetine, the STR/CB time-activity curves were not changed by either of these drugs (Fig. 4).

DISCUSSION

A number of studies have reported the characterization of high- and low-affinity binding sites for cocaine in vitro (*2–6*) and the range of values is quite large probably reflecting differences in methodology. We selected binding parameters from two different studies: one with human putamen tissue (*2*) and one with nonhuman primate caudate-putamen tissue (*3*) to estimate the percentage occupancy which might be expected with the doses of cocaine used in this study. Binding parameters, as well as Bmax values for these two studies vary dramatically (Table 1) with Kds differing by factors of 10 and 25 for the high- and low-affinity sites respectively. Because of this, the calculated percent occupancy for the high-affinity sites when the subpharmacological dose of 18 μg is administered ranged from 7% to 40%, whereas percentage occupancies for the low-affinity sites ranged from 26% to 95% for the pharmacological doses depending on the binding parameters used (Table 1). The large range of values reported in vitro emphasizes the need for measuring binding parameters in vivo. It is interesting that the ratio of Bmax to Kd for [^{11}C]cocaine determined by graphical analysis (*17*) is 0.62 ± 0.21 for human brain which is in close agreement with the in vitro binding parameters for a Kd of 210 n*M* and a Bmax of 147 pmole/g tissue (0.7) (*2*). It is also important to note that the relationship between the affinity of cocaine and cocaine-related compounds for the dopamine transporter and their reinforcing properties was done using a Ki value for dopamine uptake of 640 n*M* (*1*), which is similar to that reported in the in vitro human study (*2*) and is in accordance with cocaine doses required to observe pharmacological effects (*21*).

Notwithstanding the range of occupancies calculated from in vitro binding parameters and from tissue cocaine concentration as determined with PET for the two doses, we were able to document specific binding of [^{11}C]cocaine only when given at subpharmacological doses. The inabil-

TABLE 4
Values for the Distribution Volume (DV) in Striatum (STR) and Cerebellum (CB) and for the Ratio of the DV in STR to CB

	Subpharmacological dose [^{11}C]Cocaine (avg. dose 18 μg)				Pharmacological dose [^{11}C]Cocaine (8.0 mg)			
Study	DV STR (ml/cc)	DV CB (ml/cc)	DV STR/CB	% Change	DV STR (ml/cc)	DV CB (ml/cc)	DV STR/CB	% Change
Test/Retest								
Baseline[1]	6.07	4.03	1.57	+2%	8.61	6.65	1.29	−4%
Baseline[2]	6.07	3.79	1.60		7.79	6.30	1.24	
Specificity								
Baseline	6.01	3.80	1.58	−25%				
Cocaine[1]	4.11	3.45	1.19					
Baseline	5.70	3.94	1.45	−30%	6.36	5.37	1.18	<1%
Cocaine[2]	3.59	3.57	1.01		6.20	5.22	1.19	
Dopamine								
Baseline	2.72	2.00	1.36	−21%	7.88	6.64	1.19	−2%
Nomifensine	1.98	1.83	1.08		7.73	6.60	1.17	
Baseline	5.52	3.13	1.76	−12%				
Methylphenidate	5.02	3.24	1.55					
Baseline	9.11	5.51	1.65	−35%				
B CIT	6.82	6.35	1.07					
Serotonin								
Baseline	5.95	4.22	1.41	+11%	5.55	4.30	1.29	+2
Citalopram[1]	5.72	3.65	1.57		4.97	3.78	1.31	
Baseline	5.49	3.83	1.43	+11%				
Citalopram[2]	5.38	3.38	1.59					
Baseline	8.49	5.05	1.68	+6%	5.76	5.00	1.15	+2
Fluoxetine	8.70	4.88	1.78		5.86	5.19	1.13	
Norepinephrine								
Baseline*	6.49	3.59	1.81					
Desipramine*								
Baseline†								
Tomoxetine†					5.58	4.97	1.12	

*Blood samples for this second study were lost so no DV values are available.

†The paired baseline scan was lost due to technical error.

Percent change is expressed with respect to the baseline of the paired study. Drug pretreatments prior to the second PET scan (PI) included: cocaine[1]: 0.5 mg/kg i.v., 2 min PI; cocaine[2]: 2.0 mg/kg i.v., 2 min PI; nomifensine: 2.0 mg/kg i.v., 10 min PI; methylphenidate: 0.5 mg/kg i.v., 20 min PI; citalopram[1]: 2.0 mg/kg i.v., 180 min PI; citalopram[2]: 2.0 mg/kg i.v., 30 min PI; fluoxetine: 0.5 mg/kg i.v., 20 min PI; desipramine: 0.5 mg/kg i.v., 30 min PI; tomoxetine: 2.0 mg/kg i.v., 20 min PI.

ity to observe specific binding of a pharmacological dose of [^{11}C]cocaine to low-affinity sites with PET could be interpreted as indicating that in vivo cocaine binding occurs predominantly to high-affinity sites. Alternatively, it may reflect poor sensitivity of PET. Although it has been argued that low-affinity binding sites could be an artifact of in vitro experiments, since their detection requires multiple washings (with a single rinse, only one binding site is evident) (*22*), the proportion of high- to low-affinity sites is highly variable and is dependent on assay and tissue conditions (*23*), most studies have consistently demonstrated the presence of a high- and a low-affinity binding site. It is more likely that the poor sensitivity of PET did not allow us to demonstrate specific binding of [^{11}C]cocaine to low-affinity binding sites.

From Table 4, the values for the total Bmax-to-Kd observed are on the order of 0.5–0.6 which are similar to values determined from in vitro data (Table 1). It is somewhat surprising that the Bmax-to-Kd ratios are as close to the in vitro values as they are, because the Kd value determined by PET contains a contribution from nonspecific binding (Equation 2). The apparent Kd should be greater than the in vitro Kd according to Equation 4. Since the DV in CB can be related to the free fraction of tracer in plasma, fp (*19*):

$$DV(CB) = fp(1 + NS), \qquad \text{Eq. 9}$$

if fp is on the order of 0.1 and DV(CB) is 4, then Kd′ = 40 Kd. Therefore Bmax/Kd should be 0.002 instead of 0.7 or 0.04 instead of 1.5. Since DV(STR)/DV(CB)-1 is on the order of 0.5, either the Kd observed in vivo with PET is smaller than the in vitro value or binding is not restricted to only the free fraction of tracer but can occur directly from the nonspecific compartment so that no Kd correction is required. Assuming that the in vitro values for Bmax/Kd

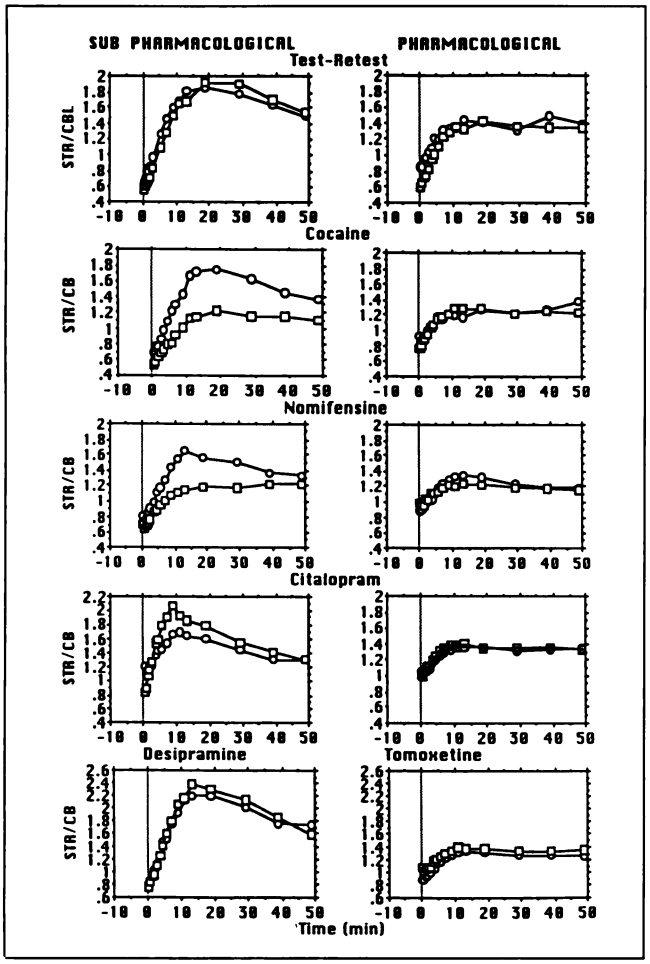

FIGURE 4. Time-activity curves for the striatum-to-cerebellum ratio (STR/CB) for subpharmacological and pharmacological doses of [^{11}C]cocaine for the baseline studies (test-retest), as well as the pharmacological interventions. Data were obtained in the same day with injections performed 2 hr apart, except for tomoxetine study where the time-activity curves for the baseline correspond to the average from the baselines for the other studies (individual baseline was lost). Circles represent baseline scan (first study) and squares represent pharmacological intervention (second study).

are also appropriate for the low-affinity sites, the contribution from the Bmax/Kd term in the DV is small compared to 1 + Bmax/Kd (Eq. 2) for the high-affinity site. If the high-affinity site is effectively saturated, the effect of the low-affinity site is still difficult to detect because it is expected to be small compared to 1 (Eq. 2). A small residual contribution of low-affinity binding sites is consistent with the results reported in Table 4 for which the values of DV(STR)/DV(CB)-1 after pretreatment and for pharmacological [^{11}C]cocaine are on the order of 0.1–0.2. Failure to detect changes in the uptake of pharmacological doses of [^{11}C]cocaine after cocaine pretreatment (15 mg), or to reduce the DV(STR)/DV(CB)-1 below 0.1 to 0.2 may also indicate that pretreatment doses were insufficient. Using the values for binding parameters for the low-affinity site reported for human putamen allows us to estimate that a 200-mg dose of cocaine would be required to occupy 92% of the low-affinity sites (*2*). Since such a high dose would jeopardize the health of the baboon, it could not be examined.

The relatively large amount of specifically bound cocaine (1400 pmole) found in the pharmacological dose [^{11}C]cocaine study reflects the difference between striatum and cerebellum which at the relevant time points is 0.006% dose/cc (Table 5). This is also consistent with a high-capacity, low-affinity site. The validity of this conclusion depends, however, on the appropriateness of the key assumption used for its determination, namely that the cerebellum at these time points represents the appropriate correction for the free and nonspecifically bound cocaine in striatum. From the two-point Scatchard analysis, only one set of parameters can be determined, Bmax = 2300 pmole/cc and Kd 3600 n*M* with Bmax/Kd = 0.63. Whereas Bmax-to-Kd reflects the effects of both types of sites, the large Bmax and Kd appear to be more characteristic of a low-affinity site.

Although these data are not conclusive, they are consistent with other experimental data suggesting the existence of a low-affinity binding site. In general, it is more difficult to determine receptor binding parameters for low-affinity sites with imaging techniques because of the restrictions imposed by Equation 2 (that is that (1 + Bmax/Kd) must be significantly greater than 1.0 to be detected) even though such binding is easily determined in vitro.

The pharmacological challenge experiments confirm that at subpharmacological doses, [^{11}C]cocaine binds predominantly to the dopamine transporter in striatum. Failure to

TABLE 5
Scatchard Analysis of Subpharmacological and Pharmacological Comparison of Carbon-11-Cocaine in the Same Animal Using the Method of Farde et al. (*20*)*

Specific activity (mCi/μmole)	Bound Cocaine (pmole/cc)	Bound/free (pmole/cc)	DV (ST)	DV (CB)	[DV(ST)/DV(CB)]-1
176†	9	0.63	6.40	4.26	0.50
0.44‡	1450	0.23	5.0	4.26	0.15

*The maximum in the specifically bound curve (ST − CB) occurred between 7 and 11 min after injection. The specifically bound and free cocaine were determined as the average of the values in this time range.
†18 μg 10.4 mCi.
‡7500 μg in 10.9 mCi.

corroborate previous in vitro studies documenting cocaine binding to serotonin and norepinephrine transporters probably reflects differences between in vitro and PET studies. The relatively poor counting statistics of dynamic PET studies preclude measurements of sites with relatively low target-to-nontarget ratios. Because there is a relatively low concentration of serotonin transporter sites in the striatum (*24*), particularly when compared with the concentration of dopamine transporters, the signal generated from a relative decrease in binding from striatal serotonin transporters would be undetected. This limitation was recently demonstrated in a SPECT study in which citalopram failed to displace [^{123}I]CIT, a ligand that binds to dopamine and serotonin transporters, from striatum, an area with a dopamine-to-serotonin concentration ratio of at least 4, whereas it displaced it from a mesencephalic region, an area with a dopamine-to-serotonin ratio of 0.3 (*25*). Also, a PET study that compared different cocaine analogs reported almost identical striatal kinetics for [^{11}C]cocaine and 4′[^{18}F]fluorococaine despite a 100-fold higher affinity of 4′-fluorococaine for the serotonin transporter (*26*). In the current study, neither fluoxetine nor citalopram pretreatment changed the uptake of [^{11}C]cocaine in mesencephalon (data not shown). The limitations from the partial volume effect in an area as small as the mesencephalon as well as the short half-life of ^{11}C and consequent poor counting statistics reduce confidence of our measurements in this region.

The assessment of cocaine's binding to serotonin transporters in vivo would require the use of a specific serotonin transporter ligand to evaluate the effects of cocaine pretreatment on its binding. Since we have no specific PET ligand for serotonin transporters, we have been unable to evaluate the degree of cocaine binding to serotonin transporters in vivo in the baboon brain. We have applied, however, the strategy of using specific radiotracer ligands to investigate cocaine's interactions with the norepinephrine transporter in myocardial tissue. Carbon-11-cocaine shows significant binding in the human and baboon heart which is not inhibited by desipramine pretreatment (*27,28*). Cocaine, however, inhibited 6-[^{18}F]fluoronorepinephrine uptake in heart to the same degree as did desipramine (*28*). Since uptake of 6-[^{18}F]fluoronorepinephrine in the heart is a function of its uptake by the norepinephrine transporter (*29*), its inhibition by cocaine corroborates in vivo a significant interaction of cocaine with this transporter. Because of the similarities between peripheral and central monoamine transporters (*30*), it is likely that cocaine would have induced a similar inhibition of norepinephrine transport in brain. Though the different relative regional concentrations of the transporters, as well as the different relative affinities of cocaine for the transporters could account for the discrepancies in the binding studies, it is also possible that they could reflect different binding sites within the transporters as reviewed by Carroll et al. (*6*).

Another variable that needs to be taken into account when comparing in vitro and in vivo studies is that pharmacological challenges in vivo will have secondary effects due to neurotransmitter interactions (*31*). These interactions could account for the relative increases in [^{11}C]cocaine binding after pretreatment with drugs that inhibit the serotonin transporter. Most studies investigating the interactions between serotonin and dopamine have concluded that serotonin inhibits dopamine neurotransmission in the striatum (*32*). PET can be used to evaluate these interactions, and a recent study confirmed decreased striatal dopamine concentration following pretreatment with citalopram, a serotonin transporter inhibitor (*33*). Decreased striatal dopamine concentration after citalopram or fluoxetine would lead to a larger fraction of free dopamine transporters in striatum with a consequent increase in [^{11}C]cocaine binding. Increases in striatal binding after fluoxetine pretreatment have also been observed with the cocaine analogs [^{125}I]RTI-55 (*34*) and 4′-[^{123}I] iodococaine (Gatley SJ, *personal communication*), ligands which bind to serotonin and dopamine transporters. The increase in a subpharmacological dose of [^{11}C]cocaine binding, after citalopram and fluoxetine, could, however, reflect drug-induced changes in ligand bioavailability (*35*). Similarly, the higher brain uptake and K_1 for the pharmacological than for the subpharmacological dose of [^{11}C]cocaine, could result from a larger free fraction of ligand, due to displacement by cold cocaine of binding sites in plasma proteins and cells.

Although the use of [^{11}C]cocaine as a ligand for the dopamine transporter has been criticized on the basis of the nonselectivity of cocaine for monoamine transporters, this study shows that in vivo it is highly selective for the dopamine transporter. Since cocaine has a lower affinity for the dopamine transporter and a lower specific-to-nonspecific binding ratio compared to other dopamine transporter PET ligands, it has rapid kinetics which facilitate its modeling and quantitation (*17*). Also, its relatively low affinity for the dopamine transporter may make it sensitive to synaptic dopamine which may enable its use to monitor synaptic changes in dopamine concentration in an analogous way to the use of [^{11}C]raclopride (*26*).

CONCLUSION

Binding of a subpharmacological dose of [^{11}C]cocaine in brain is predominantly associated with a high-affinity site in the dopamine transporter. Although we were unable to definitively demonstrate low-affinity binding sites for cocaine in vivo, the data is consistent with what has previously been reported for low-affinity binding sites in vitro.

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