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Binding of *d-threo*-[^{11}C]methylphenidate to the dopamine transporter in vivo: insensitivity to synaptic dopamine

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Abstract

The regional distribution of [^{11}C]*d-threo*-methylphenidate in mouse brain was very similar to that of [^{3}H]WIN 35,428 ((−)-2β-carbomethoxy-3β-(4-fluorophenyl)tropane), and the two radioligands were displaced from striatum similarly after administration of the potent cocaine analog RTI-55 ((−)-2β-carbomethoxy-3β-(4-iodophenyl)tropane). However, while striatal [^{3}H]WIN 35,428 increased between 5 and 30 min, striatal [^{11}C]*d-threo*-methylphenidate halved. Thus [^{11}C]*d-threo*-methylphenidate binds similarly to but more reversibly than [^{3}H]WIN 35,428. The methyl ester of L-DOPA (L-3,4-dihydroxyphenylalanine; 200 mg/kg) plus benserazide plus clorgyline, which markedly elevates rat striatal extracellular dopamine (Wachtel and Abercrombie, 1994, J. Neurochem. 63, 108), decreased the mouse striatum-to-cerebellum ratio for [^{11}C]*d-threo*-methylphenidate at 30 min by 13% ($P < 0.05$). In positron emission tomographic (PET) baboon studies [^{11}C]*d-threo*-methylphenidate binding was insensitive to drugs expected to lower endogenous dopamine. These experiments suggest that normal synaptic dopamine does not compete for binding with [^{11}C]*d-threo*-methylphenidate, and will not affect PET measures of dopamine transporter availability.

Keywords: Methylphenidate; *d-threo*-Methylphenidate; WIN 35,428; Dopamine; Dopamine transporter; PET (positron emission tomography); (Baboon); (Mouse)

1. Introduction

Inhibition of the dopamine transporter by abused psychostimulant drugs such as cocaine raises extracellular dopamine which in turn has been postulated to lead to euphoria, craving and addiction (Ritz et al., 1987). However, other dopamine transporter inhibitors are therapeutic drugs. For example, pemoline is used to treat attention deficit disorder, and mazindol is prescribed as an anorectic drug. In particular, methylphenidate, although possessing abuse potential (Parran and Jasinski, 1991) is widely prescribed for depression and narcolepsy (Kraus and Burch, 1992) and is commonly used in the treatment of childhood attention deficit disorder (Chiarello and Cole, 1987).

Clinically used methylphenidate (Ritalin) is *dl-threo*-methylphenidate, i.e. a 50/50 mixture of the two *threo* enantiomers. Pharmacological activity and affinity for the dopamine transporter, however, principally reside in *d-threo*-methylphenidate (Patrick et al., 1987; Schweri et al., 1985). We have recently examined the kinetics of methylphenidate in the baboon and human brain using positron emission tomography (PET) and [^{11}C]methylphenidate and demonstrated specific binding of this radiotracer to the dopamine transporter in baboon striatum (Ding et al., 1994). We have also shown that [^{11}C]*d-threo*-methylphenidate exhibits a greater ratio of specific to non-specific binding than *dl-threo*-[^{11}C]methylphenidate, thus making it potentially useful for PET studies of the dopamine transporter, as well as for studies of the regional pharmacokinetics of methylphenidate in humans (Ding et al., 1995; Volkow et al., 1995a,b).

Other radioligands used to image striatal dopamine transporters in PET experiments include [^{11}C]nomi-

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fensine (Salmon et al., 1990), [^{11}C]cocaine (Fowler et al., 1989; Volkow et al., 1992)}, the cocaine analogs [^{11}C]WIN 35,428 ((−)-2β-carbomethoxy-3β-(4-fluorophenyl)tropane) (Frost et al., 1993; Hantraye et al., 1992; and see Madras, 1994)) and [^{11}C]RTI-55 ((−)-2β-carbomethoxy-3β-(4-iodophenyl)tropane) (Muller et al., 1993) and [^{18}F]GBR 13,119 (Kilbourne et al., 1989). SPECT radioligands for the dopamine transporter have also been developed, notably [^{123}I]RTI-55 (e.g. Laruelle et al., 1993). These radioligands differ from one another structurally as well as in several characteristics including affinity for the dopamine transporter, binding site selectivity and pharmacokinetics. Particular interest has been expressed in [^{11}C]WIN 35,428 (Madras, 1994) because of the excellent properties of [^{3}H]WIN 35,428 as a radioligand for homogenate binding and autoradiographic studies (Madras et al., 1989a,b; Shaya et al., 1992; Madras, 1994). However, at present no consensus has been reached on the optimum PET radiotracer or quantification strategy for dopamine transporter, or on the extent to which net binding of high affinity radioligands is controlled by radiotracer delivery rather than dopamine transporter concentration. Another important issue relating to transporter quantification is whether endogenous dopamine competes for binding with exogenous radioligands in vivo. Competition would lower uptake of radioligand and confound estimates of transporter availability made from PET or SPECT measurements. It is also possible that the detailed manner in which a particular psychostimulant drug interacts with the dopamine transporter and competes for binding sites with other drugs and with dopamine in vivo is related to the potential of that drug for therapeutic purposes and/or for abuse. PET experiments could potentially bear on this question, since the ability to measure the concentration and kinetics of carbon-11 labeled compounds using PET provides the opportunity to study interactions of drugs with their binding sites in the living human and non-human primate brain. In the present studies we performed PET measurements of baboon brain uptake and pharmacokinetics of [^{11}C]*d-threo*-methylphenidate using three drugs (sodium 4-hydroxybutyrate, gamma-vinyl-gamma aminobutyric acid (gamma-vinylGABA) and citalopram) which were each expected to decrease striatal dopamine release and thus to increase [^{11}C]*d-threo*-methylphenidate uptake if competition between radiotracer and dopamine occurs (see Dewey et al., 1992, 1993, 1995). Similar experiments with [^{11}C]cocaine have recently suggested that cocaine binding in vivo is sensitive to dopamine depletion (Gatley et al., 1995). L-DOPA (L-3,4-dihydroxyphenylalanine) was also administered in order to evaluate the effects of an increase in the supply of dopamine to the striatum. To complement the PET experiments we compared the binding of *d-threo*-methylphenidate and [^{3}H]WIN 35428 in the mouse brain in vivo and also in vitro, and examined the effects of pharmacological challenges on the uptake of radiotracers in mouse brain.

2. Materials and methods

2.1. Chemicals

RTI-55 and WIN 35,428 were purchased from Research Biochemicals. L-DOPA, L-DOPA methyl ester, benserazide and clorgyline were obtained from the Sigma Chemical Company. *dl-threo*-Methylphenidate was obtained as a gift from Ciba-Geigy, and resolved into its individual enantiomers using the procedure of Patrick et al. (1987).

2.2. Drug dissolution and administration

Benserazide was dissolved in 0.9% saline containing 0.1% ascorbic acid immediately before administration. Sodium 4-hydroxybutyrate was administered as a 20% solution in water. Other drugs were administered to animals as solutions in 0.9% saline.

2.3. Radiotracers

[^{3}H]WIN 35,428 (82.4 Ci/mmol) and [^{3}H]raclopride (81 Ci/mmol) were purchased from Dupont New England Nuclear. [^{11}C]Raclopride was prepared as described previously (Ehrin et al., 1986). [^{11}C]Methylphenidate and [^{11}C]*d-threo*-methylphenidate (300 Ci/mmol) were prepared via reaction of [^{11}C]CH_3I with the *o*-nitrophenylsulfenyl derivative of *dl-threo*- or *d-threo*-ritalinic acid (Ding et al., 1994).

2.4. In vitro binding experiments

Inhibition constants of WIN 35,428, *d-threo*-methylphenidate and *l-threo*-methylphenidate at dopamine transporter sites were determined using 0.5 nM [^{3}H]WIN 35,428 with rat striatal membranes (approximately 1 mg/ml tissue per incubation tube) and a Brandel cell harvester with GF/B filters. Incubations were conducted for 90 min using a final volume of 0.51 ml of buffer containing 320 mM sucrose and 20 mM sodium phosphate, pH 7.4, and terminated by filtration followed by 2 × 5 ml washes with 50 mM Tris chloride buffer, pH 7.6. Binding data were analyzed using the EBDA program on a Macintosh computer.

2.5. Uptake in mouse brain

Outbred Swiss-Webster mice (30–33 g males, $n = 4$–6 per group) were injected intravenously with radio-

tracers dissolved in 0.2 ml of 0.9% saline, and killed at indicated times by cervical dislocation followed by immediate decapitation. The brain was removed and placed on a moist filter paper placed on an inverted glass Petri dish cooled with crushed ice. Brain regions were removed using dissecting forceps. Tissue samples were placed in tared 17×52 mm vials which were immediately reweighed and counted for 1 min using a Packard (Model 5500) autogamma counter for ^{11}C with an efficiency of about 40% and a background of 65 c.p.m. using a window of 430–1100 keV. Tissue solubilizer (1 ml) was added to each vial and the cap tightly replaced. After incubation at 45–50° C for 24–72 h, 4–5 ml of liquid scintillation fluid was added. Brain tissue (300–400 mg) remaining after removal of specific regions was dissolved in 3 ml of solubilizer and the resulting solution transferred to a 28×52 mm vial together with 12 ml of scintillation fluid. Tritium was assayed using a Packard Model 1600 TR liquid scintillation counter after a 6–12 h delay for chemiluminescence decay. A 10 min counting period was used. Counting efficiencies for ^{3}H were 30–45%, and counts were converted to d.p.m. by the instrument using an external standard. Counting standards, in triplicate, contained 20 μl of injectate.

For drug treatments, L-DOPA, L-DOPA methyl ester, clorgyline and benserazide were injected intraperitoneally dissolved in 0.2 ml saline at 25 mg/kg, 200 mg/kg, 2 mg/kg and 5 mg/kg, respectively. RTI-55 was given intravenously at 0.5 mg/kg. Control animals were injected with vehicle alone.

Data were expressed as percent injected activity per gram of brain tissue, and as tissue-to-cerebellum ratios. The cerebellum contains a very low concentration of dopamine transporters (Panagopoulos et al., 1991), and striatum-to-cerebellum ratios are commonly used as an index of specific binding of dopamine receptor and dopamine transporter-binding radioligands.

Simultaneous administration of high specific activity radiotracers labeled with tritium and C-11 allows direct comparison of two radiotracers in the same animals. Differences should thus be detectable with higher precision than when comparing two groups of animals. However, a potential complication of dual isotope studies is competition between two tracers if either of them occupies a significant fraction of tissue binding sites. This should not be a problem for the experiments described here. For [^{3}H]WIN 35,428 striatal uptake of 8%ID/g after injection of 1 μCi corresponds to 80 nCi/g, or 1 pmol/g since the specific activity was 80 Ci/mmol. If the concentration of dopamine transporter in striatum is about 150 pmol/g, then $< 1\%$ of the transporters are occupied by [^{3}H]WIN 35,428. About 10% of the dopamine transporter may be occupied by [^{11}C]*d-threo*-methylphenidate, since although the specific activity was higher (> 300 Ci/mmol) 50 μCi was injected per animal. Under these conditions negligible competition between [^{3}H]WIN 35,428 and [^{11}C]*d-threo*-methylphenidate for dopamine transporter is thus expected.

2.6. PET experiments

PET scans in adult female baboons (*Papio anubis*) were conducted using the test/retest paradigm described previously for [^{11}C]raclopride (Dewey et al., 1992,1993) and [^{11}C]cocaine (Gatley et al., 1995). To assess the effects of sodium 4-hydroxybutyrate (200 mg/kg) or of L-DOPA (50 mg/kg) plus benserazide (5 mg/kg), two intravenous injections of [^{11}C]*d-threo*-methylphenidate were made 2 h apart. Drugs were administered intravenously 30 min before the second scan. The effects of citalopram (2 mg/kg) and of gamma-vinyl GABA (300 mg/kg) were similarly assessed, except that the second scan was performed 3 and 4 h, respectively, after the baseline scan, to allow a 2 or 3 h drug pretreatment time.

2.7. Data analysis

Differences between groups of mice were assessed by *t*-tests. Distribution volumes of [^{11}C]*d-threo*-methylphenidate in striatum and cerebellum in PET experiments were calculated graphically from tissue and plasma kinetic data as described earlier for [^{11}C]cocaine (Logan et al., 1990). The ratio of distribution volumes in striatum and cerebellum, DVR, is related to equilibrium binding parameters by the following relationship (Logan et al., 1990):

$$DVR = 1 + B_{max}/K_d$$

3. Results

3.1. PET experiments

Ratios of striatal to cerebellar distribution volumes for [^{11}C]*d-threo*-methylphenidate in two test-retest experiments were reproducible within 6% (Table 1). After pharmacological treatments between the two scans, differences in the distribution volume ratio of between -4% and 7%, were found. Thus none of the treatments significantly changed the distribution volume ratio.

3.2. Binding of dopamine transporter radioligands in vitro

K_i values for *d-threo*-methylphenidate and WIN 35,428 for binding at the dopamine transporter were 27 ± 3 nM and 12 ± 2 nM, respectively. The less active

Table 1
Test-retest studies with [^{11}C]*d-threo*-methylphenidate in baboons

	Striatum	Cerebellum	DVR	% Change
Baseline	24.8	11.5	2.16	3 *
Baseline	26.3	11.4	2.22	
Baseline	34.3	14.0	2.45	6 *
Baseline	36.1	13.9	2.60	
Baseline	26.0	12.3	2.11	7
GVG	27.1	12.1	2.25	
Baseline	27.1	13.0	2.08	1
4HB	29.8	14.0	2.09	
Baseline	26.8	12.1	2.21	3
4HB	31.2	13.7	2.27	
Baseline	26.3	11.9	2.22	−4
Citalopram	24.8	11.4	2.18	
Baseline	21.6	12.0	1.80	6 *
Citalopram	24.1	12.6	1.91	
Baseline	24.3	11.6	2.16	−3
L-DOPA	22.6	10.8	2.09	

* Data previously reported (Ding et al., 1995). Two sequential injections of [^{11}C]*d-threo*-methylphenidate were made 2–4 h apart in the same scanning session, each followed by a 1 h dynamic PET scan. The indicated drug was administered after the first scan. GVG = gamma-vinylGABA (300 mg/kg); 4HB = sodium 4-hydroxybutyrate (200 mg/kg). The dose of citalopram was 2 mg/kg. DVR = the ratio of the distribution volumes for the striatum to that of the cerebellum. For other details see main text.

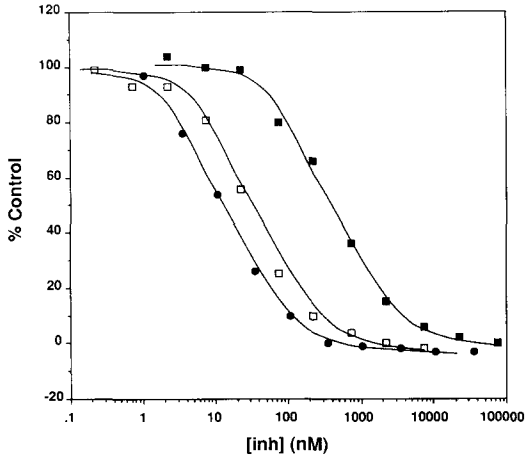

Fig. 1. Concentration dependence of inhibition of binding of [^{3}H]WIN 35,428 to striatal membranes. Circles, WIN 35,428; open squares, *d-threo*-methylphenidate; closed squares, *l-threo*-methylphenidate.

enantiomer of *dl-threo*-methylphenidate, *l-threo*-methylphenidate, exhibited a K_i value of 360 ± 27 nM (Fig. 1). The *d-threo*-methylphenidate-to-*l-threo*-methylphenidate ratio was similar to that observed previously for inhibition of dopamine uptake by striatal synaptosomes (Patrick et al., 1987).

3.3. Kinetics of radiotracer uptake in mouse brain

Table 2 gives tissue concentrations of ^{3}H and ^{11}C (%ID/g) following administration of [^{11}C]*d-threo*-methylphenidate, [^{3}H]WIN 35,428 and [^{11}C]methylphenidate. The striatal concentration of [^{3}H]WIN 35,428 continued to increase up to 30 min. In contrast, while the striatal concentrations of [^{11}C]*d-threo*-methylphenidate were very similar at 5 and 10 min, at 30 min it was only half that at the earlier times. At 5 min, the striatal concentration of [^{11}C]*d-threo*-methylphenidate was about 30% greater than that of [^{3}H]WIN 35,428. Striatal ^{11}C at 5 min after injection of racemic [^{11}C]methylphenidate was similar to that obtained with [^{11}C]*d-threo*-methylphenidate. However, there was less striatal radioactivity at 10 and 30 min as expected because of the presence of the less active enantiomer. Both the dopamine transporter radioligands showed progressive clearance from cerebellum between 5 and 30 min. Striatum-to-cerebellum ratios increased between 5 and 30 min for both [^{11}C]*d-threo*-methylphenidate and [^{3}H]WIN 35,428. At 30 min the ratio for [^{3}H]WIN 35,428 was 50% greater than that for [^{11}C]*d-threo*-methylphenidate.

3.4. Regional distribution of [^{11}C]d-threo-methylphenidate and [^{3}H]WIN 35,428 in mouse brain

The uptakes (%ID/g) of [^{11}C]*d-threo*-methylphenidate in 13 brain regions at 30 min were about half of

Table 2
Time courses of radiotracers in striatum and cerebellum

Radiotracer	Tissue	Uptake		
		5 min	10 min	30 min
d-threo-Methylphenidate	ST (%ID/g)	8.96 ± 0.55	9.52 ± 0.70	4.55 ± 0.27
	CB (%ID/g)	6.03 ± 0.37	5.18 ± 0.52	2.08 ± 0.21
	ST/CB	1.49 ± 0.06	1.85 ± 0.10	2.21 ± 0.23
WIN 35,428	ST (%ID/g)	6.76 ± 0.23	8.96 ± 0.55	12.48 ± 1.30
	CB (%ID/g)	4.86 ± 0.29	5.39 ± 0.45	3.95 ± 0.30
	ST/CB	1.39 ± 0.06	1.78 ± 0.08	3.16 ± 0.08
dl-threo-Methylphenidate	ST (%ID/g)	6.86 ± 0.85	5.61 ± 0.12	2.86 ± 0.33
	CB (%ID/g)	5.07 ± 0.78	3.50 ± 0.28	1.43 ± 0.13
	ST/CB	1.36 ± 0.15	1.61 ± 0.16	2.01 ± 0.17

Mice were injected intravenously with 0.2 ml of saline containing [^{3}H]WIN 35,428 and [^{11}C]*d-threo*-methylphenidate. They were killed at the indicated times. Concentrations of ^{3}H and ^{11}C were determined as described in the main text. ST = striatum; CB = cerebellum.

Table 3
Displacement of striatal radioactivity by RTI-55

Radiotracer	Tissue	Uptake		% Change	$P=$
		Control	RTI-55		
d-threo-Methylphenidate	ST (%ID/g)	3.54 ± 0.65	2.37 ± 0.35	−29	0.01
	CB (%ID/g)	1.56 ± 0.21	1.59 ± 0.15	6	0.53
	ST/CB	2.27 ± 0.27	1.49 ± 0.15	−32	0.001
WIN 35,428	ST (%ID/g)	8.35 ± 0.85	5.38 ± 1.05	−36	0.002
	CB (%ID/g)	2.84 ± 0.15	2.99 ± 0.15	5	0.23
	ST/CB	2.93 ± 0.17	1.82 ± 0.42	−38	0.001

Mice were injected intravenously with 0.2 ml of saline containing [^{3}H]WIN 35,428 and [^{11}C]*d-threo*-methylphenidate. The mice were injected intravenously at 15 min with 0.5 mg/kg RTI-55 or saline (0.2 ml) and killed after a further 15 min. Concentrations of ^{3}H and ^{11}C were determined as describd in the main text. *P* values for the percent change (L-DOPA group minus control group) were calculated using a two-tailed *t*-test. ST = striatum; CB = cerebellum.

those obtained with [^{3}H]WIN 35,428 in the corresponding regions in the same animals (Fig. 2A). Data for the two radiotracers are expressed as tissue-to-cerebellum ratios (T/Cb) in Fig. 2B. The striatum contained the highest concentration of both nuclides. An excellent correlation coefficient (0.96) was obtained. When the striatum was omitted from the calculation, the correlation coefficient was 0.79. In order of increasing concentrations of [^{11}C]*d-threo*-methylphenidate, the regions were: olfactory bulb, cerebellum, superior colliculus, inferior colliculus, brain stem, hypothalamus, thalamus, parietal cortex, rest-of-brain, frontal cortex, hippocampus, olfactory tubercle and striatum.

3.5. Displacement of d-threo-methylphenidate by RTI-55

When the cocaine analog RTI-55 was administered 15 min after radiotracer injection, the concentration of [^{11}C]*d-threo*-methylphenidate in striatum and also ol-

Table 4
Effects of L-DOPA on radioligand uptake

Radiotracer	Uptake		
	5 min	10 min	30 min
d-threo-Methylphenidate			
Baseline, ST/CB	1.49 ± 0.06	1.85 ± 0.10	2.21 ± 0.23
L-DOPA, ST/CB	1.49 ± 0.08	1.88 ± 0.10	2.06 ± 0.15
WIN 35,428			
Baseline, ST/CB	1.39 ± 0.06	1.78 ± 0.08	3.16 ± 0.08
L-DOPA, ST/CB	1.39 ± 0.04	1.85 ± 0.19	2.81 ± 0.15
$^{11}C/^3H$			
Baseline	1.07 ± 0.05	1.04 ± 0.03	0.70 ± 0.08
L-DOPA	1.07 ± 0.05	1.03 ± 0.07	0.73 ± 0.04

Mice were co-injected intravenously with [^{3}H]WIN 35,428 and [^{11}C]*d-threo*-methylphenidate as described in the legend to Table 2. The mice were pretreated intraperitoneally 30 min before radiotracer injection with 25 mg/kg L-DOPA or saline (0.2 ml). Concentrations of ^{3}H and ^{11}C were determined as describd in the main text. ST = striatum; CB = cerebellum.

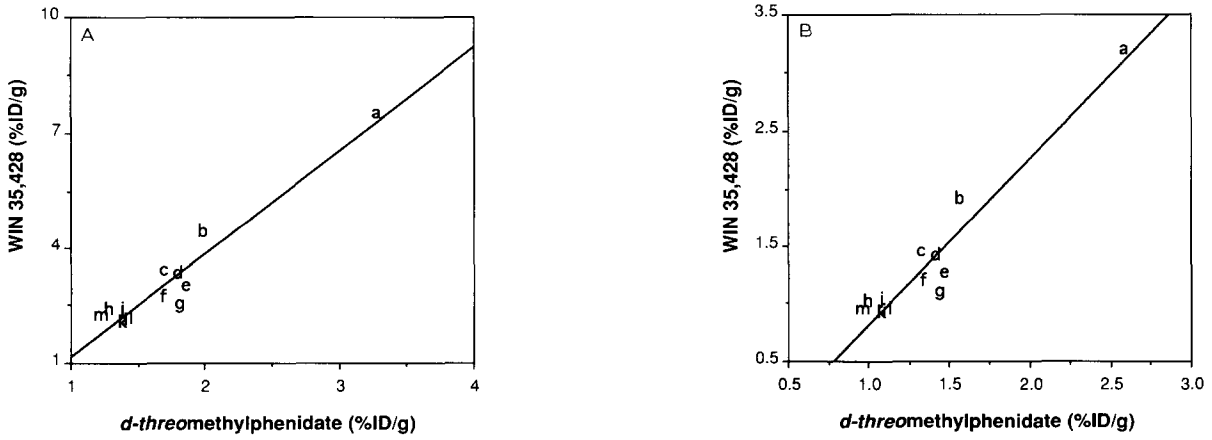

Fig. 2. Left-hand panel: Comparison of binding (percent injected radioactivity per gram of tissue) of [^{3}H]WIN 35,428 (ordinate) versus [^{11}C]*d-threo*-methylphenidate (abscissa) in 13 regions of brain after simultaneous intravenous administration. The brain regions were: a, striatum; b, olfactory tubercle; c, thalamus; d, remainder of brain; e, hippocampus; f, parietal cortex; g, frontal cortex; h, cerebellum; i, hypothalamus; j, brain stem; k, superior colliculus; l, inferior colliculus; m, olfactory bulb. Right-hand panel: Data from the left-hand panel expressed as tissue-to-cerebellum ratios. Correlation coefficients were 0.96 for the whole series of regions, and 0.79 when the striatum was excluded.

Table 5
Effects of L-DOPA methyl ester plus clorgyline

Tissue	Uptake		% Change	$P =$
	Control	L-DOPA		
ST (%ID/g)	5.56 ± 0.75	5.25 ± 0.39	−6	0.4
CB (%ID/g)	2.16 ± 0.25	2.35 ± 0.14	9	0.11
ST/CB	2.58 ± 0.14	2.24 ± 0.20	−13	0.04

Mice ($n = 6$ for the control group and $n = 5$ for the L-DOPA group) were injected intravenously with 0.2 ml of saline containing [^{11}C]*d-threo*-methylphenidate, 30 min after intraperitoneal pretreatment with L-DOPA methyl ester (200 mg/kg) plus benserazide (5 mg/kg) plus clorgyline (2 mg/kg). Control mice were injected with 0.2 ml saline. They were killed at 30 min. Concentrations of ^{3}H and ^{11}C were determined as described in the main text. *P* values for the percent change (L-DOPA group minus control group) were calculated using a two-tailed *t*-test. ST = striatum; CB = cerebellum.

factory tubercle (not shown) was reduced at sacrifice (30 min) compared with control animals injected with saline (Table 3). The proportional displacement of [^{3}H]WIN 35,428 in the same animals was similar.

3.6. Effect of L-DOPA on mouse brain radiotracer binding

L-DOPA did not significantly alter striatum-to-cerebellum ratios for either [^{11}C]*d-threo*-methylphenidate or [^{3}H]WIN 35,428 (Table 4). When striatal radioactivities were expressed as [^{11}C]*d-threo*-methylphenidate-to-[^{3}H]WIN 35,428 ratios, values for control and L-DOPA treated mice were practically superimposable. The ratio declined from 1.07 at 5 min to 0.7 at 30 min.

3.7. Effect of L-DOPA methyl ester plus benserazide plus clorgyline on mouse brain radiotracer binding

A significant ($P < 0.05$) decrease of 13% in striatum-to-cerebellum ratio was seen after administration of L-DOPA methyl ester to animals also treated with clorgyline and benserazide (Table 5). Although no formal quantification of motor activity was made in these studies, the animals were closely observed after the drug treatments and showed no signs of behavioral activation.

4. Discussion

4.1. Comparison of binding of methylphenidate enantiomers and WIN 35,428 in vivo and in vitro

Radiolabeled WIN 35,428 has been extensively used in both in vivo and in vitro experiments because of its high affinity for the dopamine transporter and low level of non-specific binding (Madras et al., 1989b). The extent and time course of striatal accumulation of [^{3}H]WIN 35,428 measured in this study were similar to those observed in other studies (Scheffel et al., 1989). Initial striatal uptake of [^{11}C]*d-threo*-methylphenidate was higher than that of [^{3}H]WIN 35,428 (Table 2), suggesting that *d-threo*-methylphenidate crosses the blood-brain barrier more readily. Loss of striatal radioactivity between 10 and 30 min for [^{11}C]*d-threo*-methylphenidate but not for [^{3}C]WIN 35,428 is consistent with a lower binding affinity to the dopamine transporter for [^{11}C]*d-threo*-methylphenidate, as seen in vitro (Fig. 1). Our comparative data for labeled *d-threo*-methylphenidate and WIN 35,428 in mice (Fig. 2; Table 2 and Table 3) are consistent with previously published baboon PET experiments with [^{11}C]*d-threo*-methylphenidate (Ding et al., 1995) and with [^{11}C]WIN 35,428 (Wong et al., 1993). However, it is not justified at this point to conclude that the 2-fold difference in affinity in vitro is wholly responsible for the differences in the behavior in vivo, since the relative kinetics of association and dissociation as well as brain uptake and clearance are important determinants of the characteristics of in vivo binding. Continued increase in striatum-to-cerebellum ratio up to 30 min was seen for [^{11}C]*d-threo*-methylphenidate as well as [^{3}H]WIN 35,428 (Table 2). The in vitro binding data for *d-threo*- and *l-threo*-methylphenidate (Fig. 1), as well as lower striatum-to-cerebellum ratios for racemic methylphenidate in mice (Table 2), and recently published biodistribution studies in rats (Aoyama et al., 1994), support the use of [^{11}C]*d-threo*-methylphenidate rather than racemic [^{11}C]methylphenidate for in vivo PET studies of the dopamine transporter.

In multiple isotope experiments, the finding that in vivo binding of *d-threo*-methylphenidate for 13 regions of the mouse brain was well correlated with that of WIN 35,428 (Fig. 2) further suggests that the two radiotracers bind predominantly to the same target. Many studies with WIN 35,428 (Madras, 1994; Madras et al., 1989a,b) and methylphenidate (Ding et al., 1994; Schweri, 1990; Schweri et al., 1985) suggest that this target is the dopamine transporter.

The displacement of a high proportion of the striatal uptake of both [^{11}C]*d-threo*-methylphenidate and [^{3}H]WIN 35,428 by subsequent administration of RTI-55 (Table 3) further supports substantial overlap between binding sites on the dopamine transporter for *d-threo*-methylphenidate and cocaine analogs.

4.2. PET experiments with d-threo-methylphenidate

The changes in distribution volume ratio of *d-threo*-methylphenidate after pharmacological challenges are within the test-retest reproducibility of the radiotracer (Table 1). The results with dopamine-depleting drugs contrast with data obtained with

[^{11}C]raclopride where very significant increases in distribution volume ratio were observed (Dewey et al., 1992, 1993, 1995). They also contrast with results with [^{11}C]cocaine where a small but significant average increase was seen for 10 test-retest studies with dopamine-depleting drugs (Gatley et al., 1995). The experiments with dopamine-depleting drugs (Table 1) indicate that [^{11}C]*d-threo*-methylphenidate is insensitive to decreases in synaptic dopamine. This suggests but does not prove that [^{11}C]*d-threo*-methylphenidate binding would also be relatively insensitive to increased synaptic dopamine. Competition by elevated dopamine is harder to demonstrate in vivo for dopamine transporter radioligands than for dopamine receptor radioligands because drugs such as amphetamine which cause large increases in dopamine themselves compete directly at the dopamine transporter. L-DOPA which raises extracellular dopamine to a moderate degree (Buu, 1989) was without significant effect on the DVR of [^{11}C]*d-threo*-methylphenidate (Table 1).

4.3. Effects of L-DOPA in mice

The unchanged striatum-to-cerebellum ratios for both [^{3}H]WIN 35,428 and [^{11}C]*d-threo*-methylphenidate (Table 4) confirm the PET data (Table 1) and suggest that an insufficient increase in the concentration of synaptic dopamine is derived from L-DOPA to compete significantly with either [^{11}C]*d-threo*-methylphenidate or [^{3}H]WIN 35,428 for binding to the dopamine transporter in vivo. This conclusion is supported by the unchanged [^{11}C]*d-threo*-methylphenidate-to-[^{3}H]WIN 35,428 ratios after L-DOPA. If significant competition with dopamine did occur in vivo under these conditions, a smaller effect might be expected for [^{3}H]WIN 35,428 than for [^{11}C]*d-threo*-methylphenidate, since the former ligand binds more tightly. Failure to detect changes in radioligand binding may reflect the moderate increase in synaptic dopamine elicited by L-DOPA (Buu, 1989; Koshimura et al., 1992), as compared with dopamine releasers or dopamine reuptake blockers (Woods and Meyer, 1991).

Combined treatment with L-DOPA methyl ester, benserazide, and the MAO-A inhibitor clorgyline, which has been shown to effect a 20-fold increase in rat striatal extracellular dopamine measured by microdialysis (Wachtel and Abercrombie, 1994), caused a significant decrease of 13% in striatum-to-cerebellum ratio for [^{11}C]*d-threo*-methylphenidate (Table 5). These changes are consistent with sensitivity of [^{11}C]*d-threo*-methylphenidate to a large increase in synaptic dopamine. However, other explanations may be possible including altered radioligand systemic pharmacokinetics induced by this complex drug treatment regimen. An observation which suggests that caution is appropriate in interpreting this result is that no behavioral activation of the animals was apparent, as would be expected from a large increase in synaptic dopamine in the striatum. Furthermore, a recent abstract (Hume et al., 1993) reported *increased* rather than *decreased* uptake of [^{11}C]raclopride in rat striatum following acute administration of L-DOPA. Since in the same rats extracellular dopamine measured by microdialysis was found to be increased, it appears that modulation of radioligand binding in vivo by L-DOPA or derived dopamine may involve other factors in addition to competition for binding sites. Further study of these issues is clearly warranted.

4.4. Competition between dopamine and dopamine transporter radioligands

In vivo binding of dopamine D_2 receptor ligands in humans and non-human primates is known to be sensitive to pharmacological agents which are expected to alter synaptic dopamine concentrations (Ross, 1991; Dewey et al., 1992; Logan et al., 1991; Volkow et al., 1994), but it is not at present clear whether the uptake of dopamine transporter ligands also depends on the concentration of dopamine in the synapse. In vitro competition experiments with dopamine suggest that the binding of dopamine transporter ligands is several-fold less sensitive to dopamine than that of D_2 receptor ligands (see references in Seeman, 1993). This has recently been confirmed by PET studies in baboons using [^{11}C]cocaine (Gatley et al., 1995), which indicate a small but significant degree of competition. Since *d-threo*-methylphenidate and WIN 35,428 have higher affinity for the dopamine transporter than cocaine, they might be expected, by analogy with homogenate binding experiments, to be less sensitive to competition with dopamine than cocaine. However, factors other than competition also need to be considered. Firstly, the degree of competition will depend on the occupancy of the binding site; if occupancy is low, competition should be relatively insensitive to changes in dopamine. Secondly, in a kinetically controlled situation such as exists in vivo following bolus injection of radiotracer, competition will be determined by association and dissociation rate constants of endogenous neurotransmitter and tracer. Finally, it is possible that cocaine and other ligands bind at sites on the dopamine transporter which overlap to varying degrees with the dopamine recognition site, and therefore may compete with dopamine to varying extents.

Thus a radioligand with a higher K_i will not necessarily be more sensitive to competition with endogenous neurotransmitter. To our knowledge, sensitivities of dopamine transporter radioligands other than [^{11}C]cocaine to changes in synaptic dopamine have not been established. Laruelle et al. (1993) found that L-DOPA (ca. 50 mg/kg) and benserazide did not in-

crease the clearance rate of [^{123}I]RTI-55 from baboon striatum, whereas *d*-amphetamine (2 mg/kg) markedly stimulated clearance. However, the relative contributions to competition for binding sites with radioligand of *d*-amphetamine itself on the one hand, and the expected increase in synaptic dopamine on the other, are not yet clear. Scheffel et al. (unpublished observations cited by Wong et al., 1993) found no effect of *d*-amphetamine concentrations up to 0.1 mg/kg on mouse striatum-to-cerebellum ratios obtained with [^{3}H]WIN 35,428.

When viewed in conjunction with our previously reported finding that baboon striatal binding of [^{11}C]cocaine is sensitive to pretreatment with drugs which inhibit dopamine release (Gatley et al., 1995), our present results suggest one or a combination of the following: (a) the dopamine transporter recognition sites for dopamine are normally near to saturation, so that changes in dopamine concentration alter competition to a greater extent with the radioligand of lower affinity (i.e. [^{11}C]cocaine); (b) the recognition sites for cocaine and methylphenidate are not identical and have differential overlap with the dopamine recognition site, or (c) the previous [^{11}C]cocaine findings (Gatley et al., 1995) are due to drug induced effects other than changes in synaptic dopamine.

4.5. Conclusions

The present studies confirm that [^{11}C]*d-threo*-methylphenidate has considerable promise as a PET radiotracer of the dopamine transporter. Its regional distribution in mouse brain is highly correlated with that of [^{3}H]WIN 35,428 which is a well validated in vitro and ex vivo radioligand for the dopamine transporter. Additionally, [^{11}C]*d-threo*-methylphenidate clears from striatum more quickly, and this more reversible in vivo binding minimizes the impact of radiotracer delivery on the quantification of dopamine transporter density using equilibrium binding models (Ding et al., 1995; Logan et al., 1990; Volkow et al., 1994).

The lack of effect of drugs which decrease striatal dopamine release suggests that [^{11}C]*d-threo*-methylphenidate binding in baboon striatum is little affected by competition with endogenous dopamine, a potentially confounding variable in some experimental situations. Although less conclusive, the limited effects of L-DOPA also support the contention that [^{11}C]*d-threo*-methylphenidate binding to the dopamine transporter is fairly insensitive at least to moderate *increases* in dopamine. A practical implication of these findings is that errors in quantification of DVR due to competition with a variable concentration of endogenous dopamine are predicted to be limited in magnitude. This is encouraging for future PET studies of dopamine transporter availability in the living primate and human brain. For example, estimates of dopamine transporter density in untreated parkinsonism using [^{11}C]*d-threo*-methylphenidate should not be compromized by the decreased dopamine associated with this disorder, while interruption of therapy should not be necessary in L-DOPA treated patients. Furthermore, transporter occupancy measurements using PET and [^{11}C]*d-threo*-methylphenidate of other drugs which bind to the dopamine transporter may not be seriously compromised by the increased concentration of dopamine due to blockade of its transport.

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References

Aoyama, T., H. Kotaki, Y. Sawada and T. Iga, 1994, Stereospecific distribution of methylphenidate enantiomers in rat brain: specific binding to dopamine reuptake sites, Pharm. Res. 11, 407.

Buu, N.T., 1989, Vesicular accumulation of dopamine following L-DOPA administration, Biochem. Pharmacol. 38, 1787.

Chiarello, R.J. and J.O. Cole, 1987, The use of psychostimulants in general psychiatry, Arch. Gen. Psychiatry 44, 286.

Dewey, S.L., G.W. Smith, J. Logan, J.D. Brodie, D.-W. Yu, R.A. Ferrieri, P.T. King, R.R. MacGregor, T.P. Martin, A.P. Wolf, N.D. Volkow, J.S. Fowler and E. Meller, 1992, GABAergic inhibition of endogeneous dopamine release measured in vivo with ^{11}C-raclopride and positron emission tomography, J. Neurosci. 12, 3773.

Dewey, S.L., G.S. Smith, J. Logan, J.D. Brodie, J.S. Fowler and A.P. Wolf, 1993, Striatal binding of the PET ligand ^{11}C-raclopride is altered by drugs that modify synaptic dopamine levels, Synapse 13, 350.

Dewey, S.L., G.S. Smith, J. Logan, D.A. Alexoff, Y.-S. Ding, P. King, N. Pappas, J.D. Brodie and C.R. Ashby, 1995, Serotonergic modulation of striatal dopamine measured with positron emission tomography (PET) and in vivo microdialysis, J. Neurosci. 15, 21.

Ding, Y.-S., J.S. Fowler, N.D. Volkow, S.J. Gatley, J. Logan, S.L. Dewey, D. Alexoff and A.P. Wolf, 1994, Pharmacokinetics and in vivo specificity of [^{11}C]*dl-threo*-methylphenidate for the presynaptic dopaminergic neuron, Synapse 18, 152.

Ding, Y.S., J.S. Fowler, N.D. Volkow, J. Logan, S.J. Gatley and Y. Sugano, 1995, [^{11}C]*d-threo*-Methylphenidate: a new PET ligand for the dopamine transporter. I. Characterization of its binding in the baboon brain, J. Nucl. Med. (in press).

Ehrin, E., L. Gawell, T. Hogberg, T. DePaulis and P. Strom, 1986,

Synthesis of [*methoxy*-^{3}H]-and [*methoxy*-^{11}C]-labelled raclopride. Specific dopamine-D_2 receptor ligands, J. Label. Compd. Radiopharm. 24, 931.

Fowler, J.S., N.D. Volkow, A.P. Wolf, S.L. Dewey, D.J. Schlyer and R.R. MacGregor, 1989, Mapping cocaine binding sites in human and baboon brain in vivo, Synapse 4, 371.

Frost, J.J., A.J. Rosier, S.G. Reich, J.S. Smith, M.D. Ehlers, S.H. Snyder, H.T. Ravert and R.F. Dannals, 1993, Positron emission tomographic imaging of the dopamine transporter with ^{11}C-WIN 35,428 reveals marked declines in mild Parkinson's disease, Ann. Neurol. 34, 423.

Gatley, S.J., N.D. Volkow, J.S. Fowler, S.L. Dewey and J. Logan, 1995, Sensitivity of striatal [^{11}C]cocaine binding to decreases in synaptic dopamine, Synapse (in press).

Hantraye, P., A.L. Brownell, D. Elmaleh, R.D. Spealman, U. Wullner, G.L. Brownell, B.K. Madras and O. Isacson, 1992, Dopamine fiber detection by ^{11}C-CFT and PET in a primate model of Parkinsonism, Neuroreport 3, 265.

Hume, S.P., R. Myers, J. Opacka-Juffry, R.G. Ahier, S. Ashworth, D.J. Brooks and A.A. Lammertsma, 1993, Effect of L-DOPA and 6-OHDA lesioning on [^{11}C]raclopride binding in rat striatum, quantified using PET, J. Cereb. Blood Flow Metab. 13, S295.

Kilbourne, M.R., J.E. Carey, R.A. Koeppe, M.S. Haka, G.D. Hutchins, P.S. Sherman and D.E. Kuhl, 1989, Biodistribution, dosimetry, metabolism and monkey PET studies of [^{18}F]GBR 13119 imaging the dopamine uptake system in vivo., Nucl. Med. Biol. 16, 569.

Koshimura, K., T. Ohue, T. Akiyama, A. Itoh and S. Miwa, 1992, L-DOPA administration enhances exocytotic dopamine release in vivo in the rat striatum, Life Sci. 51, 747.

Kraus, M.F. and E.A. Burch, 1992, Methylphenidate hydrochloride as an antidepressant: controversy, case studies, and review, South. Med. J. 85, 985.

Laruelle, M., R.M. Baldwin, R.T. Malison, Y. Zea-Ponce, S.S. Zoghbi, M. Al-Tikriti, E.H. Sybirska, R.C. Zimmermann, G. Wisniewski, J.L. Neumeyer, R.A. Milius, R.A. Wang, E.O. Smith, R.H. Roth, D. Charney, P.B. Hoffer and R.B. Innis, 1993, SPECT imaging of dopamine and serotonin transporters with [I-123] beta-CIT: pharmacological characterization of brain uptake in non-human primates, Synapse 13, 295.

Logan, J., S.L. Dewey, A.P. Wolf, J.S. Fowler, J.D. Brodie, B. Angrist, N.D. Volkow and S.J. Gatley, 1991, Effects of endogeneous dopamine on measures of [^{18}F]*N*-methylspiroperidol binding in the basal ganglia: comparison of simulations and experimental results from PET studies in baboons, Synapse 9, 195.

Logan, J., J.S. Fowler, N.D. Volkow, A.P. Wolf, S.L. Dewey, D.J. Schlyer, R.R. MacGregor, R. Hitzemann, B. Bendriem, S.J. Gatley and D.R. Christman, 1990, Graphical analysis of reversible radioligand binding from time activity measurements applied to [*N*-^{11}C-methyl]-(−)cocaine PET studies in human subjects, J. Cereb. Blood Flow Metab. 10, 740.

Madras, B.K., 1994, ^{11}C-WIN 35,428 for detecting dopamine depletion in mild Parkinson's disease, Ann. Neurol. 35, 376.

Madras, B.K., M.A. Fahey, J. Bergman, D.R. Canfield and R.D. Spealman, 1989a, Effects of cocaine and related drugs in non-human primates. I. [^{3}H]Cocaine binding sites in caudate-putamen, J. Pharmacol. Exp. Ther. 251, 131.

Madras, B.K., R.D. Spealman, M.A. Fahey, J.L. Neumeyer, J.K. Saha and R.A. Milius, 1989b, Cocaine receptors labeled by [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane, Mol. Pharmacol. 36, 518.

Muller, L., C. Halldin, L. Farde, P. Karlsson, H. Hall, C.G. Swahn, J. Neumeyer, Y. Gao and R. Milius, 1993, [^{11}C]β-CIT, a cocaine analog. Preparation, autoradiography and preliminary PET investigations, Nucl. Med. Biol. 20, 249.

Panagopoulos, N.T., G.C. Papadopoulos and N.A. Matsokis, 1991, Dopaminergic innervation and binding in the rat cerebellum, Neurosci. Lett. 130, 208.

Parran, T.V. and D.R. Jasinski, 1991, Intravenous methylphenidate abuse. Prototype for prescription drug abuse, Arch. Intern. Med. 151, 781.

Patrick, K.S., R.W. Caldwell, R.M. Ferris and G.R. Breese, 1987, Pharmacology of the enantiomers of *threo*-methylphenidate, J. Pharmacol. Exp. Ther. 241, 152.

Ritz, M.C., R.J. Lamb, S.R. Goldberg and M.J. Kuhar, 1987, Cocaine receptors on dopamine transporters are related to self administration of cocaine, Science 237, 1219.

Ross, S.B., 1991, Synaptic concentration of dopamine in the mouse striatum in relationship to the kinetic properties of the dopamine receptors and uptake mechanism, J. Neurochem. 56, 22.

Salmon, E., D.J. Brooks, K.L. Leenders, D.R. Turton, S.P. Hume, J.E. Cremer, T. Jones and R.S.J. Frackowiak, 1990, A two compartment description and kinetic procedure for measuring [^{11}C]nomifensine uptake using positron emission tomography, J. Cereb. Blood Flow Metab. 10, 307.

Scheffel, U., J.W. Boja and M.J. Kuhar, 1989, Cocaine receptors: in vivo labeling with ^{3}H-(−)cocaine, ^{3}H-WIN 35,065-2, and ^{3}H-WIN 35,428, Synapse 4, 390.

Schweri, M., 1990, *N*-Ethylmaleimide irreversibly inhibits the binding of [^{3}H]*threo*-(±)-methylphenidate to the stimulant recognition site, Neuropharmacology 29, 901.

Schweri, M.M., P. Skolnick, M.F. Rafferty, K.C. Rice, A.J. Janowsky and S.M. Paul, 1985, [^{3}H]*threo*-(±)-Methylphenidate binding to 3,4-dihydroxyphenylethylamine uptake sites in corpus striatum: correlation with the stimulant properties of ritalinic acid esters, J. Neurochem. 45, 1062.

Seeman, P., 1993, Receptor Tables. Volume 2: Drug Dissociation Constants for Neuroreceptors and Transportes (SZ Research, Toronto).

Shaya, E.K., U. Scheffel, R.F. Dannals, G.A. Ricaurte, F.I. Carroll, H.N. Wagner, M.J. Kuhar and D.F. Wong, 1992, In vivo imaging of dopamine sites in the primate brain using single photon emission computed tomography (SPECT) and iodine 123 labeled RTI-55, Synapse 10, 169.

Volkow, N.D., J.S. Fowler, J. Logan, G.-J. Wang, R. Hitzemann, R.R. MacGregor, S.L. Dewey and A.P. Wolf, 1992, Decreased binding of 11-C-cocaine in the brain of cocaine addicts, J. Nucl. Med. 33, 888.

Volkow, N.D., G.-J. Wang, J.S. Fowler, J. Logan, D.J. Schlyer, R. Hitzemann, J. Lieberman, B. Angrist, N. Pappas, R.R. MacGregor, G. Burr, T. Cooper, S.L. Dewey and A.P. Wolf, 1994, Imaging endogenous dopamine competition with [^{11}C]raclopride in the human brain, Synapse 16, 255.

Volkow, N.D., Y.-S. Ding, J.S. Fowler, G.J. Wang, J. Logan, S.J. Gatley, D.J. Schlyer and N. Pappas, 1995a, [^{11}C]*d-threo*-Methylphenidate: a new PET ligand for the dopamine transporter. II. Studies in the human brain, J. Nucl. Med. (in press).

Volkow, N.D., Y.-S. Ding, J.S. Fowler, G.-J. Wang, J. Logan, S.J. Gatley, S. Dewey, C. Ashby, J. Liebermann, R. Hitzemann and A.P. Wolf, 1995b, Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in human brain, Arch. Gen. Psychiatry (in press).

Wachtel, S. and E.D. Abercrombie, 1994, L-3,4-Dihydroxyphenylalanine-induced dopamine release in the striatum of intact and 6-hydroxydopamine-treated rats, J. Neurochem. 63, 108.

Wong, D.F., B. Yung, R.F. Dannals, E.K. Shaya, H.T. Ravert, C.A. Chen, B. Chan, T. Folio, U. Scheffel, G.A. Ricaurte, J.L. Neumeyer, H.N. Wagner and M.J. Kuhar, 1993, In vivo imaging of baboon and human dopamine transporters by positron emission tomography using [^{11}C]WIN 35,428, Synapse 15, 130.

Woods, S. and J. Meyer, 1991, Exogenous tyrosine potentiates the methylphenidate-induced increase in extracellular dopamine in the nucleus accumbens: a microdialysis study, Brain Res. 560, 97.