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Dopamine Depletion and *In Vivo* Binding of PET D_1 Receptor Radioligands: Implications for Imaging Studies in Schizophrenia

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Recent positron emission tomography (PET) studies have assessed the level of dopamine (DA) D_1 receptors in the prefrontal cortex (PFC) in patients with schizophrenia and have generated contradictory findings. In the PFC of patients with schizophrenia, the binding potential (BP) of [^{11}C]NNC 112 has been reported as increased, while the BP of [^{11}C]SCH 23390 was reported as decreased or unchanged. In this study, the effect of acute and subchronic DA depletion on the *in vivo* binding of [^{11}C]NNC 112 and [^{3}H]SCH 23390 was evaluated in rats. Acute DA depletion did not affect [^{11}C]NNC 112 *in vivo* binding, but paradoxically decreased [^{3}H]SCH 23390 *in vivo* binding. Subchronic DA depletion was associated with increased [^{11}C]NNC 112 *in vivo* binding and decreased [^{3}H]SCH 23390 *in vivo* binding. Together, these data demonstrate that the *in vivo* binding of these radiotracers is differentially affected by changes in endogenous DA tone, and suggest that alterations in the binding of these tracers in the PFC of patients with schizophrenia might reflect changes in D_1 receptors secondary to sustained deficit in prefrontal DA function.
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INTRODUCTION

Multiple lines of evidence suggest that schizophrenia is associated with alterations of prefrontal functions, and that these alterations are implicated in the cognitive deficits presented by these patients (Weinberger, 1987; Goldman-Rakic and Selemon, 1997; Lewis and Akil, 1997). Among the neuronal systems in the prefrontal cortex (PFC), the mesocortical dopamine (DA) system is essential for normal cognitive functions (Weinberger *et al*, 1988; Goldman-Rakic *et al*, 2000; Jentsch *et al*, 2000; Robbins, 2000). Indirect evidence supports the hypothesis that a deficit in prefrontal DA function might contribute to prefrontal impairment in schizophrenia. Studies in nonhuman primates have shown that working memory, a function reliably shown to be altered in schizophrenia, is critically dependent on prefrontal DA function and appropriate stimulation of D_1 receptors, the most abundant DA receptors in the PFC (Brozoski *et al*, 1979; Sawaguchi and Goldman-Rakic, 1991, 1994; Arnsten *et al*, 1994; Arnsten and Goldman-Rakic, 1998). Clinical studies have suggested a relationship between low cerebrospinal fluid homovanillic acid and poor performance at tasks involving working memory in schizophrenia (Weinberger *et al*, 1988; Kahn *et al*, 1994). The administration of DA agonists might have beneficial effects on the pattern of prefrontal activation measured with PET during these tasks (Daniel *et al*, 1989, 1991; Dolan *et al*, 1995). Atypical antipsychotic drugs preferentially activate cortical DA neurotransmission in the PFC, suggesting that increasing DA function in the PFC mediates the therapeutic effects of these drugs (Moghaddam, 1994; Yamamoto and Cooperman, 1994; Youngren *et al*, 1999). More direct evidence for such a deficit was recently provided by one post-mortem study suggesting a decrease in DA innervation in the dorsolateral PFC (DPFC) (Akil *et al*, 1999).

Over the last decade, the development of radiotracers suitable to image DA D_1 receptors with positron emission tomography (PET) allowed a direct assessment of this critical component of the prefrontal DA system. The first PET radiotracer for the D_1 receptor to be introduced was the benzazepine [^{11}C]SCH 23390 (Halldin *et al*, 1986; Farde *et al*, 1987; Chipkin *et al*, 1988; Andersen *et al*, 1992). *In vitro*, SCH 23390 displayed only moderate D_1 to 5-HT_{2A} receptor selectivity (Laruelle *et al*, 1991). Yet, *in vivo* studies in mice suggested that the binding of [^{11}C]SCH 23390 is selective for D_1 receptors, even in the PFC (Suhara *et al*, 1992). Despite the lower density of D_1 receptors in the PFC compared to the striatum (STR) (Hall *et al*, 1994), a test/retest study recently demonstrated appropriate reproducibility of the measurement of [^{11}C]SCH 23390 binding

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potential (BP) in the human PFC (Hirvonen *et al*, 2001). More recently, [^{11}C]NNC 112 has been developed as a superior PET D_1 receptor radiotracer (Andersen *et al*, 1992; Halldin *et al*, 1998). In humans, [^{11}C]NNC 112 provides higher specific to nonspecific ratios compared to [^{11}C]SCH 23390 (Halldin *et al*, 1998; Abi-Dargham *et al*, 1999). The *in vivo* selectivity of [^{11}C]NNC 112 toward D_1 receptors has been demonstrated in monkeys (Halldin *et al*, 1998), and the reproducibility of measurement of [^{11}C]NNC 112 BP in the human PFC has been established (Abi-Dargham *et al*, 2000).

Three PET studies of prefrontal D_1 receptor availability in patients with schizophrenia have recently been published, and generated conflicting results. Two studies were performed with [^{11}C]SCH 23390. The first reported a decrease in [^{11}C]SCH 23390 BP in the PFC of patients with schizophrenia (Okubo *et al*, 1997), and the other reported no changes (Karlsson *et al*, 2002). One study was performed with [^{11}C]NNC 112 (Abi-Dargham *et al*, 2002), and reported an increase in [^{11}C]NNC 112 BP in the DLPFC of the patients. Many potential factors, including patient heterogeneity, might account for these discrepancies. However, the severity of deficits at tasks involving working memory was reported to be associated with both decreased PFC [^{11}C]SCH 23390 BP (Okubo *et al*, 1997) and increased PFC [^{11}C]NNC 112 BP (Abi-Dargham *et al*, 2002), suggesting that both alterations might reflect a common underlying deficit. Owing to the prevalent view that schizophrenia is associated with a deficit in prefrontal DA activity, the present investigation was undertaken to examine the impact of acute (24 h) and subchronic (14 days) DA depletion on the *in vivo* binding of both radioligands in rats.

MATERIALS AND METHODS

DA Depletion Regimen

Male Sprague–Dawley rats (250–300 g) were kept under a 12 h light/12 h dark cycle, with free access to food and water. For acute DA depletion, rats were pretreated with reserpine (5 mg/kg, i.p.) 24 h before the experiment. On the day of the experiment, the pretreated rats received two injections of α-methyl-para-tyrosine (AMPT), 400 mg/kg, i.p. The first injection was given 4 h before the experiment, and the second was given 1 h before the experiment. Since the drug treatment slightly lowered the body temperature to 36°C compared to control animals (38°C), the treated rats were placed in a 38°C warm chamber (ThermoCare, Incline Village, NV) 1 h before the experiments to bring the body temperature back to normal. Control animals were also placed in the warm chamber for 1 h prior to the experiments.

For subchronic DA depletion, rats received an injection of reserpine (1 mg/kg, i.p.) once daily for 14 days. Control animals were treated with vehicle (100 μl of 40% acetic acid in 1 ml H_2O) once daily for 14 days. The reserpine-treated rats were fed with 5–8 ml of a liquid diet twice daily by gavage for the first 10 days of the study to minimize dehydration and weight loss. The diet consisted of 200 ml of water, a package of chocolate-flavored instant breakfast mix, 100 ml of sweetened condensed milk, and 22.5 ml of Kaopectate (Neisewander *et al*, 1991a, b). The rats were also given access to the liquid diet mixed with ground Purina rat chow No. 5012 and tap water throughout the treatment period. Control rats were restricted to 30 gm of rat chow daily for the first 10 days of the study to maintain similar body weights as reserpine-treated rats (Neisewander *et al*, 1991a, b).

Assessment of DA Depletion

Following the drug pretreatment, the rats were anesthetized with carbon dioxide and killed by decapitation. The brains were quickly removed and the STR and PFC were dissected. The tissue samples were weighed and the tissue homogenates were processed for neurochemical analysis following procedures described previously (Guo *et al*, 1995). Briefly, STR and PFC samples were placed in a cold homogenizing solution (0.22 N perchloric acid, 0.05% EDTA-Na_2, and 0.15% sodium bisulfite). The tissues were sonicated for 2×30 s on ice and centrifuged at 10 000*g* for 3×20 min at 4°C, and the supernatants were stored at −40°C until use. Regional analysis of brain DA concentrations was performed by an ion-paring reverse phase high-pressure liquid chromatography (HPLC) with an electrochemical detection system (Guo *et al*, 1995).

Measurement of *In Vivo* D_1 Radioligand Binding

In vivo radioligand-binding studies were performed with [^{11}C]NNC 112 and [^{3}H]SCH 23390. Initial experiments were performed to characterize the time course of [^{11}C]NNC 112 and [^{11}C]SCH 23390 accumulation in the rat brain (three animals were studied per time point, three and four time points were collected for [^{11}C]NNC 112 and [^{3}H]SCH 23390, respectively, for a total of 21 rats). The goal of these experiments was to identify the time postinjection at which binding equilibrium occurs. For this purpose, the binding equilibrium was defined as peak-specific binding, measured as the difference between activity in the regions of interest (ROIs) and region of reference. The average specific binding was calculated for each time point, and the point with highest specific binding was then chosen for subsequent experiments.

For DA depletion studies, a total of 100 rats were studied. For both acute and subchronic DA depletion studies with [^{11}C]NNC 112, three cohorts of rats including five control and five DA-depleted rats were studied, for a total of 15 rats per experimental group. For both acute and chronic DA depletion studies with [^{3}H]SCH 23390, two groups of rats, including five controls and five DA-depleted rats were studied, for a total of 10 rats per experimental group. Thus, each cohort included the same number of control and depleted rats.

Within each cohort, rats were randomly assigned to control or depletion conditions, and both treatment groups were studied on the same experimental days. More animals were studied with [^{11}C]NNC 112 ($n = 15$ per group) than with [^{3}H]SCH 23390 ($n = 10$ per groups) because we observed, in preliminary experiments, a larger between-animal variability in measured [^{11}C]NNC 112 binding parameters compared to [^{3}H]SCH 23390.

[^{11}C]NNC 112 was radiolabeled as previously described (Abi-Dargham *et al*, 2000). [^{3}H]SCH 23390 was obtained from New England Nuclear, Boston, MA (specific activity of 70 μCi/nmol). Rats were injected through a lateral tail vein. The injected doses and injected masses were 122.5 ± 82.6 μCi and 60 ± 29 ng for [^{11}C]NNC 112, and 8 μCi and 32.8 ng for [^{3}H]SCH 23390. These injected masses are comparable to injected masses used in clinical studies (range from 1 to 10 μg in a 70 kg adult).

Rats were restrained for the tail vein injection, then returned to the cages for the duration of the tracer uptake. At the appropriate time post-tracer injection, carbon dioxide anesthesia was used and rats were killed by decapitation. The brain regions were dissected on ice. ROIs included STR, PFC, and hippocampus (HIP). The cerebellum (CER) was used as a region of reference to assess nonspecific binding.

In [^{11}C]NNC 112 experiments, blood and brain tissue samples were weighed and counted with a gamma-counter (Cobra II) (Packard, Meriden, CT). For rats who received [^{3}H]SCH 23390, blood and brain tissue samples were weighed and incubated with 1.5 ml of Solvable (Packard, Meriden, CT) at 60°C overnight. Blood samples were decolorized with 100 μl of 30% H_2O_2 for 30 min. Digested brain tissue samples and decolorized blood samples were added to 18 ml of scintillation solution (Packard, Meriden, CT). The samples were mixed thoroughly and kept at room temperature for at least 2 h before being counted with a liquid scintillation counter (1500 Tri-Carb) (Packard, Meriden, CT). Tissue activities were normalized to the injected dose and expressed as percent of injected dose per gm of tissue (%ID/g).

The outcome measure was the specific binding index (SBI), calculated as the ROI/CER activity ratio minus one. Under binding equilibrium conditions, the SBI is equal to V_3'', the equilibrium specific to nonspecific partition coefficient (Abi-Dargham *et al*, 2000). V_3'' is related to receptor parameters by $V_3'' = B_{max}/K_D V_2$, where B_{max} is the density of sites, K_D is the affinity of the tracer, and V_2 is the nonspecific distribution volume. Under the assumption that treatment conditions do not affect V_2 and that SBI provides a close approximation of V_3'', between-group differences in SBI reflect differences in radiotracers' BP (B_{max}/K_D ratio).

Statistical Analysis

Prior to pooling results obtained from cohorts studied on different experimental days, the potential impact of this factor was assessed. Thus, SBI variability was initially assessed with repeated measures ANOVA (RM ANOVA), with experimental day and treatment condition as factors and regional SBI as dependent measures. A p-value less than 0.05 was selected as a significance threshold, and p-values for interactions were provided when significant.

This test was followed by examination of the effect of treatment on individual regions, using one-way ANOVA (in case of no significant experimental day factor) or two-way ANOVA (with experimental day and treatment conditions in case of significant experimental day factor). All tests were two-tailed. Given a coefficient of variability of 17 and 12% for [^{11}C]NNC 112 and [^{3}H]SCH 23390 regional SBI measurements, respectively, this study was designed to have adequate power (>80%) to detect treatment effects on SBI of 18% or higher for [^{11}C]NNC 112 ($n = 15$ animals) and 16% or higher for [^{11}C]SCH 23390 ($n = 10$).

RESULTS

DA Tissue Concentration

Acute drug (reserpine plus AMPT) treatment caused severe impairment in the mobility of the animals. The acute drug treatment produced significant reduction in DA tissue concentrations, with a loss of 94.8% in STR. DA levels were not detectable in PFC (Table 1).

Subchronic reserpine treatment impaired the mobility of the animals in the first week of the drug treatment. The animals started to move normally during the second week of the treatment period. The reserpine-induced diarrhea ceased 2–3 days after the rats were fed with the diet, which contained Kaopectate. The weight loss of the drug-treated animals was in a range of 0–15% of the controls 2 weeks after the repeated treatment. Repeated reserpine treatment for 2 weeks produced extensive reduction in DA tissue concentrations, with a loss of 98.9% in STR and 98.1% in PFC (Table 1).

Time Course of [^{11}C]NNC 112 and [^{3}H]SCH 23390 Uptake

[^{11}C]NNC 112 uptake in rat brain is shown in Figure 1, left panel. The highest uptake was found in STR, with 1.11 ± 0.19 percent of injected dose per gram of tissue (%ID/g) at 10 min and stable levels from 30 min (1.62 ± 0.11%ID/g) to 50 min (1.57 ± 0.10%ID/g). Specific binding, calculated as the difference between STR and CER activities, showed a protracted and stable peak during that period. A comparable period of stable specific binding was observed in the PFC. Therefore, 50 min was chosen as the time point in DA depletion studies.

[^{3}H]SCH 23390 uptake in rat brain is shown in Figure 1, right panel. The highest uptake was found in STR, with 1.44 ± 0.41 percent of injected dose per gram of tissue (%ID/g) at 15 min and relatively stable levels from 30 min (1.81 ± 0.10%ID/g) to 60 min (2.04 ± 0.31%ID/g). STR and PFC specific binding reached stable levels at 45 min. Therefore, 45 min was chosen as the time point in

Table 1 Effects of Acute and Subchronic DA Depletion Treatment on DA Levels in the Forebrain

		DA (pmol/mg)	
Experiment	**Condition**	**STR**	**PFC**
Acute DA depletion ($n = 5$)	Control	40.91 ± 1.88	0.67 ± 0.16
	Depleted	2.10 ± 0.96*	ND
Subchronic DA depletion ($n = 6$)	Control	45.02 ± 3.64	3.57 ± 1.66
	Depleted	0.48 ± 0.15*	0.07 ± 0.02*

Mean (± SEM) DA tissue concentrations.*Significant ($p < 0.001$) difference from corresponding control.
n = number of animals per group.
ND = nondetectable.

DA depletion studies, a time similar to that used in previous rodent [^{3}H]SCH 23390 *in vivo* studies (Inoue *et al*, 1991).

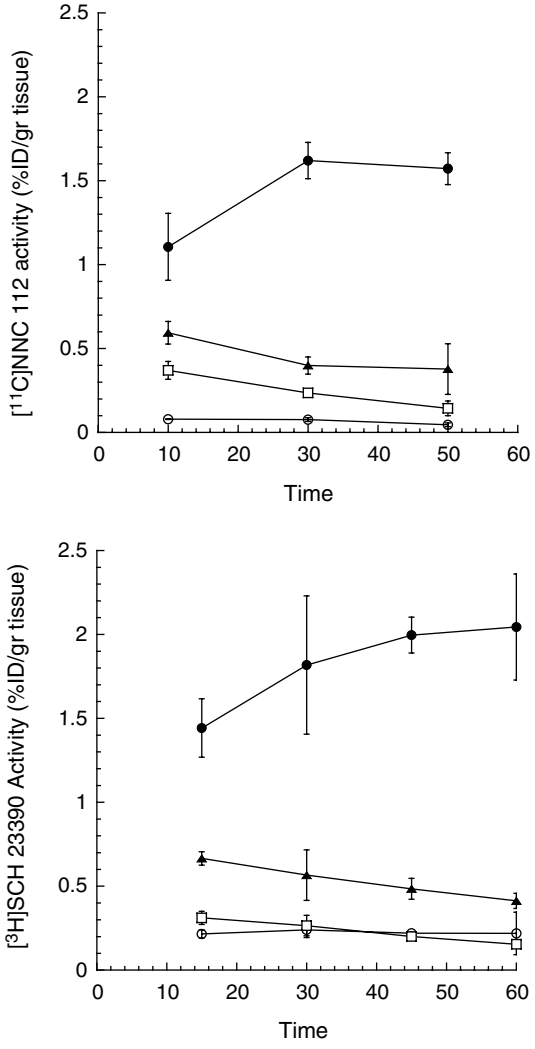

Figure 1 *In vivo* uptake of [^{11}C]NNC 112 (left panel) and [^{3}H]SCH 23390 (right panel) in rat brain ($n=3$ animals per time point) in STR (closed circles), PFC (closed triangles), CER (open squares), and blood (open circles).

Effects of DA Depletion on *In Vivo* D_1 Radioligand Binding

Regional values of brain uptake and SBI are provided in Table 2.

Acute DA Depletion

[^{11}C]NNC 112. One acutely DA-depleted animal died before the *in vivo* binding experiment, so data were obtained in 29 animals. No significant effect of experimental day ($n=3$) or treatment condition ($n=2$) were detected in [^{11}C]NNC 112 regional SBIs (RM ANOVA, experimental day, $p=0.46$; treatment condition, $p=0.96$; region, $p<0.001$). Compared to controls ($n=15$), no changes were detected in [^{11}C]NNC 112 SBIs in acutely DA-depleted animals ($n=14$) in STR (control rats, 9.63 ± 1.56; acutely DA-depleted rats: 9.33 ± 2.40; $p=0.69$), PFC (control rats, 1.68 ± 0.29; acutely DA-depleted rats: 1.84 ± 0.41; $p=0.24$), and HIP (control rats, 1.12 ± 0.20; acutely DA-depleted rats: 1.20 ± 0.17; $p=0.27$). Thus, acute DA depletion failed to affect *in vivo* [^{11}C]NNC 112 binding (Figure 2).

[^{3}H]SCH 23390. No significant effect of experimental day ($n=2$) was detected, but a significant treatment condition effect ($n=2$) was detected on [^{3}H]SCH 23390 regional SBIs (RM ANOVA, experimental day, $p=0.46$; treatment condition, $p<0.001$, region, $p<0.0001$; region by condition interaction, $p<0.001$). Compared to controls ($n=10$), [^{3}H]SCH 23390 SBI in STR was significantly decreased in acutely DA-depleted animals ($n=10$): control: 9.80 ± 0.65; acutely DA-depleted rats: 6.79 ± 1.48; $p<0.0001$. No significant differences were observed in the PFC (control rats: 1.97 ± 0.34; acutely DA-depleted rats: 1.83 ± 0.31; $p=0.36$) or HIP (control: 1.18 ± 0.17; acutely DA-depleted rats: 1.32 ± 0.28; $p=0.21$). Thus, acute DA depletion was associated with a marked (−31%) decrease in [^{11}C]SCH 23390 *in vivo* specific binding in the STR, and no detectable changes in PFC and HIP (Figure 3).

Subchronic DA Depletion

[^{11}C]NNC 112. Significant effects of experimental day ($n=3$) and treatment condition ($n=2$) were detected on [^{11}C]NNC 112 regional SBIs (RM ANOVA, experimental day, $p<0.0001$; treatment condition, $p=0.003$, region,

Table 2 Effects of Acute and Subchronic DA Depletion Treatment on [^{11}C]NNC 112 and [^{3}H]SCH 23390 Parameters

				Uptake (%ID/g)				SBI		
Studies	**Ligand**	**Group**	***n***	**CER**	**STR**	**PFC**	**HIP**	**STR**	**PFC**	**HIP**
Acute DA studies	[^{11}C]NNC 112	Controls	15	0.19 ± 0.04	2.05 ± 0.43	0.52 ± 0.10	0.41 ± 0.09	9.63 ± 1.56	1.68 ± 0.29	1.12 ± 0.20
		Treated	14	0.20 ± 0.07	2.02 ± 0.45	0.57 ± 0.16	0.44 ± 0.13	9.33 ± 2.40	1.84 ± 0.41	1.20 ± 0.17
	[^{3}H]SCH 23390	Controls	10	0.21 ± 0.08	2.24 ± 0.71	0.61 ± 0.17	0.44 ± 0.15	9.80 ± 0.65	1.97 ± 0.34	1.18 ± 0.17
		Treated	10	0.21 ± 0.03	1.66 ± 0.38*	0.61 ± 0.11	0.49 ± 0.06	6.79 ± 1.48*	1.83 ± 0.31	1.32 ± 0.28
Chronic DA studies	[^{11}C]NNC 112	Controls	15	0.28 ± 0.10	1.93 ± 0.61	0.55 ± 0.20	0.48 ± 0.15	6.51 ± 2.24	1.13 ± 0.65	0.80 ± 0.32
		Treated	15	0.22 ± 0.07	1.82 ± 0.41	0.53 ± 0.12	0.45 ± 0.10	7.85 ± 2.28*	1.59 ± 0.68*	1.12 ± 0.35*
	[^{3}H]SCH 23390	Controls	10	0.15 ± 0.02	1.82 ± 0.29	0.45 ± 0.07	0.36 ± 0.06	11.36 ± 2.36	2.01 ± 0.37	1.46 ± 0.36
		Treated	10	0.17 ± 0.04	1.77 ± 0.56	0.49 ± 0.13	0.39 ± 0.11	9.20 ± 1.64*	1.89 ± 0.24	1.26 ± 0.23

Values are mean ($\pm$ SD).*Significant ($p<0.05$) difference from corresponding control.
n = number of animals per group.
CER = cerebellum, STR = striatum, PFC = Prefrontal Cortex, HIP = hippocampus.

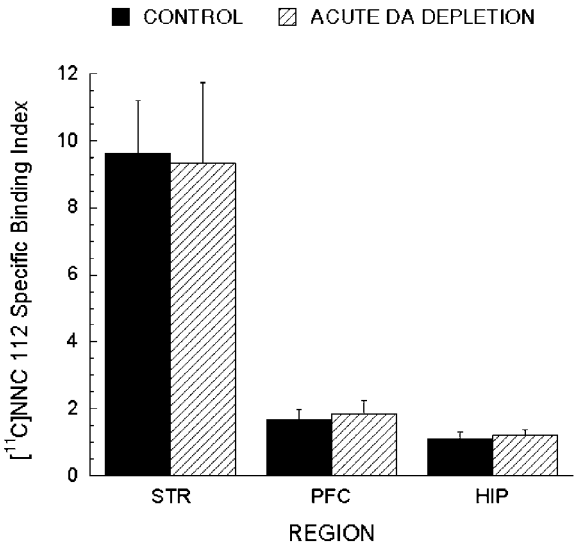

Figure 2 Effects of acute DA depletion on *in vivo* binding of [^{11}C]NNC 112 in the rat brain. Bars represent mean ± SD SBI, calculated as (ROI/CER)−1, in STR, PFC, and HIP. Black bars indicate control animals ($n=15$), shaded bars indicate acutely DA-depleted animals ($n=14$). Acute DA depletion had no detectable effect on the *in vivo* binding of [^{11}C]NNC 112 in any of these regions.

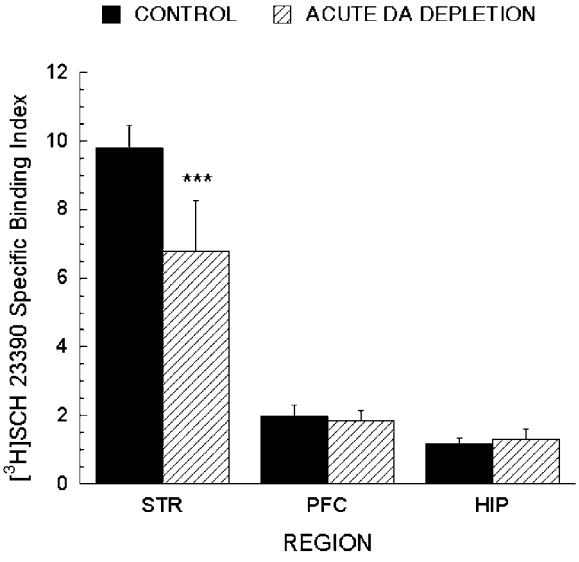

Figure 3 Effects of acute DA depletion on *in vivo* binding of [^{3}H]SCH 23390 in the rat brain. Bars represent mean ± SD SBI, calculated as (ROI/CER)−1, in STR, PFC, and HIP. Black bars indicate control animals ($n=10$), shaded bars indicate acutely DA-depleted animals ($n=10$). Acute DA depletion was associated with a significant decrease in [^{11}C]SCH 23390 *in vivo* binding in STR ($p<0.0001$).

$p<0.001$). When regions were examined individually, a significant increase in [^{11}C]NNC 112 SBIs was detected in STR (control rats, 6.52 ± 2.24, $n=15$; subchronically DA-depleted rats, 7.85 ± 2.28, $n=15$, $p=0.01$), PFC (control rats: 1.13 ± 0.65; subchronically DA-depleted rats, 1.59 ± 0.68, $p=0.002$), and HIP (control rats: 0.80 ± 0.32; subchronically DA-depleted rats, 1.12 ± 0.35, $p<0.001$). Thus, subchronic DA depletion was associated with

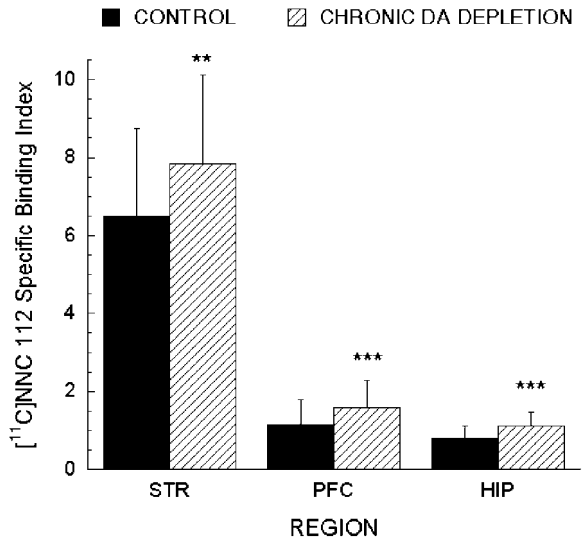

Figure 4 Effects of subchronic DA depletion on *in vivo* binding of [^{11}C]NNC 112 in the rat brain. Bars represent mean ± SD SBI, calculated as (ROI/CER)−1, in STR, PFC, and HIP. Black bars indicate control animals ($n=15$), shaded bars indicate subchronically DA-depleted animals ($n=15$). Subchronic DA depletion was associated with a significant increase in [^{11}C]NNC 112 *in vivo* binding in STR ($p=0.01$), PFC ($p=0.002$), and HIP ($p<0.001$).

increases in [^{11}C]NNC 112 *in vivo* binding in the STR (21%), PFC (41%), and HIP (40%) (Figure 4).

[^{3}H]SCH 23390. No significant effect of experimental days ($n=2$) was detected, but a significant treatment condition ($n=2$) effect was detected on [^{3}H]SCH 23390 regional SBIs (RM ANOVA, experimental day, $p=0.54$; treatment condition, $p=0.045$; region, $p<0.001$; region by condition interaction: $p=0.007$). When regions were examined individually, a significant decrease in [^{3}H]SCH 23390 SBI was detected in STR (control rats: 11.36 ± 2.36, $n=10$; subchronically DA-depleted rats, 9.20 ± 1.64, $n=10$; $p=0.03$), but not in PFC (control rats, 2.01 ± 0.37; subchronically DA-depleted rats, 1.89 ± 0.24, $p=0.41$) or in HIP (control rats, 1.46 ± 0.36; subchronically DA-depleted rats, 1.26 ± 0.23, $p=0.16$). Thus, subchronic DA depletion was associated with a decrease in [^{3}H]SCH 23390 *in vivo* binding in the STR (−19%), and no detectable changes in PFC and HIP (Figure 5).

DISCUSSION

The results of the present study suggest that the *in vivo* binding of the two commonly used PET D_1 receptor radiotracers, [^{11}C]NNC 112 and [^{3}H]SCH 23390, is differentially affected by DA depletion. Acute DA depletion does not affect *in vivo* [^{11}C]NNC 112 binding, but is associated with a paradoxical decrease in [^{11}C]SCH 23390 *in vivo* binding in the STR, and no change in PFC and HIP. Subchronic DA depletion is associated with an increase in [^{11}C]NNC 112 binding in all regions examined, and either a decrease (STR) or no change (PFC and HIP) in [^{3}H]SCH 23390 binding. These results might be relevant to the interpretation of the PET studies performed with these

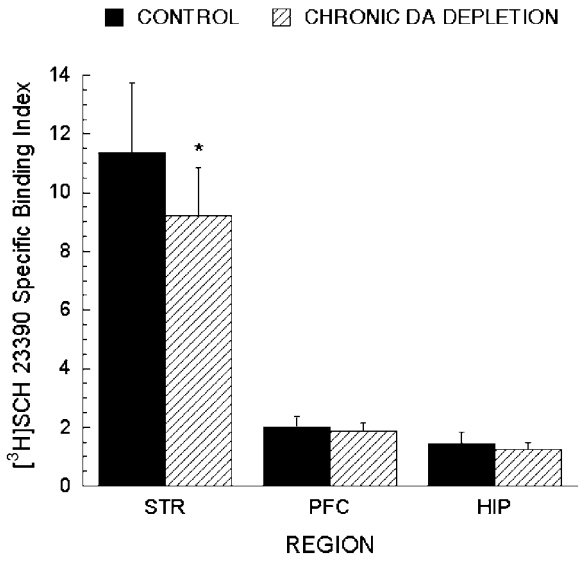

Figure 5 Effects of subchronic DA depletion on *in vivo* binding of [^{3}H]SCH 23390 in the rat brain. Bars represent mean ± SD SBI, calculated as (ROI/CER)−1, in STR, PFC, and HIP. Black bars indicate control animals ($n=10$), shaded bars indicate chronically DA-depleted animals ($n=10$). DA depletion was associated with a significant decrease in [^{11}C]SCH 23390 *in vivo* binding in STR ($p=0.03$).

tracers in schizophrenia (Okubo *et al*, 1997; Abi-Dargham *et al*, 2002; Karlsson *et al*, 2002).

The main limitation of the present study is that the *in vivo* binding was measured only at one time point, and that a simple tissue activity ratio was used as outcome measure. The vulnerability of simple ratio methods to condition-induced changes in tracer peripheral clearance and cerebral regional blood flow has been well described (Carson *et al*, 1993; Slifstein and Laruelle, 2001). Therefore, the difference in SBI change observed following DA depletion between the two tracers might be due to differences in basic pharmacokinetic profiles of the tracers, although these differences do not appear to be major (Figure 1). Multiple time points and kinetic modeling are required to generate a more robust outcome measure. Thus, the results from this study warrant replication with different methods, such as imaging the binding of the tracers with small animal-dedicated PET cameras. However, even with such a microPET camera, the ability to properly disentangle the PFC signals from the much stronger STR signals has yet to be established. As the impact of DA depletion in the PFC was the main focus of this study, the *ex vivo* method was selected for the initial evaluation of this question.

Another potential limitation of this study is that different isotopes were used for the radiotracers, C-11 for [^{11}C]NNC 112 and H-3 for [^{3}H]SCH 23390. Each isotope has advantages and limitations. C-11 requires a dedicated radiolabeling for each experiment, while H-3 does not. Thus, experiments performed with C-11 are more expensive. The specific activity of a C-11-labeled compound is much higher than that of 3-H compounds (which is a plus), but the activity of C-11 decays much faster than that of 3-H (which is a minus). As far as the injected mass is concerned, both factors almost cancelled each other. Owing to the faster decay of C-11, the injected dose activity of [^{11}C]NNC 112 was 15 times higher than that of [^{3}H]SCH 23390. However, due to the higher specific activity of C-11, the injected masses of [^{11}C]NNC 112 were only 1.8 times higher than that of [^{3}H]SCH 23390. Another difference between using the different isotopes is that C-11 activity in the tissue samples can be counted directly, while measuring the 3-H activity requires tissue processing. Yet, since the same isotope was used to compare the tracer uptake in both DA-depleted and control rats, differences in isotope do not bias the results of this study.

Results of these studies are relatively consistent with previously published reports using different pharmacological challenges and different species. The lack of impact of acute DA depletion on the *in vivo* binding of [^{11}C]NNC 112 is consistent with results of PET experiments in nonhuman primates that demonstrated that the BP of [^{11}C]NNC 112 is not affected by acute changes in endogenous DA (Laruelle *et al*, 1998; Chou *et al*, 1999). A similar observation was made with [^{11}C]NNC 756, a close analog of [^{11}C]NNC 112 (Abi-Dargham *et al*, 1999).

The decrease in [^{3}H]SCH 23390 *in vivo* binding following acute DA depletion was a paradoxical change in the context of a simple occupancy model. If anything, removal of the endogenous competitor should increase the binding of the radiotracer. However, such a paradoxical change in [^{3}H]SCH 23390 binding has been previously reported. In mice, inhibition of DA release with reserpine (Inoue *et al*, 1991; Yonezawa *et al*, 1991) or flunitrazepam (Inoue *et al*, 1992) induced decreased striatal [^{3}H]SCH 23390 accumulation, while stimulation of DA release with MK-801 induced increased striatal [^{3}H]SCH 23390 binding (Kobayashi and Inoue, 1993). Interestingly, the decreased striatal accumulation of [^{3}H]SCH 23390 following reserpine was reversed by amphetamine, confirming that this effect was mediated by monoamine depletion (Inoue *et al*, 1991). Furthermore, the amphetamine-induced reversal of the reserpine effect was blocked by haloperidol, suggesting that D_2 receptors are involved in the paradoxical regulation of [^{3}H]SCH 23390 binding following changes in endogenous DA (Inoue *et al*, 1991). Some studies failed to observe this effect. In mice, the inhibition of DA neurons firing with gammabutyrolactone decreased striatal [^{3}H]SCH 23390 uptake, but this effect did not reach significance (Thibaut *et al*, 1996). In humans, acute and partial DA depletion induced by AMPT did not significantly affect [^{3}H]SCH 23390 BP (Verhoeff *et al*, 2002).

The mechanism(s) underlying this paradoxical decrease in [^{11}C]SCH 23390 binding observed upon DA depletion is unknown. Such a paradoxical change has been previously described with [^{3}H]spiperone, and attributed to the effect of D_2 receptor internalization on ligand binding (Chugani *et al*, 1988). These data suggest that receptor trafficking might play a role in the change in *in vivo* binding of radiotracers observed following changes in endogenous transmitter tone (see Laruelle, 2000a for discussion). Trafficking of D_1 receptors between the cell surface and internalized compartment is well characterized. Elevations in synaptic DA levels promote internalization (Dumartin *et al*, 1998; Vickery and von Zastrow, 1999), whereas decreases in synaptic DA levels promote externalization (Dumartin *et al*, 2000). Externalization of D_1 receptors upon acute DA depletion might result in decreased *in vivo* affinity of [^{3}H]SCH 23390, which shows a much faster dissociation

rate in homogenates' preparation compared to intact slides (Gifford *et al*, 1998). However, there is no direct evidence supporting this hypothesis, and studies directly measuring the affinity of [^{3}H]SCH 23390 for internalized and externalized receptors are required to test this hypothesis. Irrespective of the precise mechanism responsible for the marked decrease in the accumulation of [^{11}C]SCH 23390 observed here, this mechanism does not appear to affect the *in vivo* binding of [^{11}C]NNC 112. This ligand, which is more lipophilic than [^{3}H]SCH 23390, might be less vulnerable to receptor trafficking between surface and intracellular compartments (NNC 112 log *P* is 3.2 and SCH 23390 log *P* is 2.5).

The increase in [^{11}C]NNC 112-specific binding following sustained DA depletion is a critical finding of this study, as this observation supports the hypothesis that the increased binding of [^{11}C]NNC 112 observed in the DLPFC in patients with schizophrenia might be secondary to chronic deficit in prefrontal DA (Abi-Dargham *et al*, 2002). Under this hypothesis, increased [^{11}C]NNC 112 BP in the DLPFC and poor performance at working memory tasks are associated as both phenomena derive from an underlying deficit in prefrontal endogenous DA. The increased [^{11}C]NNC 112 binding in subchronically reserpine-treated animals observed here presumably reflects a compensatory increase in the expression of D_1 receptors in response to the sustained deficit in DA transmission (Butkerait and Friedman, 1993).

Interestingly, this putative upregulation of D_1 receptors detected by [^{11}C]NNC 112 was not detected by [^{3}H]SCH 23390. The striatal accumulation of [^{3}H]SCH 23390 was in fact still decreased following sustained DA depletion (albeit to a lesser extent than in the acute studies).

The divergence in the effect of sustained DA depletion on the *in vivo* binding of [^{11}C]NNC 112 and [^{11}C]SCH 23390 might be relevant to the apparently contradictory results of prefrontal D_1 receptor PET studies in patients with schizophrenia. As in subchronically depleted rats, in the PFC of patients with schizophrenia, PET studies observed decreased (or no change) *in vivo* [^{3}H]SCH 23390 binding and increased *in vivo* [^{11}C]NNC 112 binding (Okubo *et al*, 1997; Abi-Dargham *et al*, 2002; Karlsson *et al*, 2002). Direct comparison of [^{11}C]NNC 112- and [^{11}C]SCH 23390-binding alterations in the same patients with schizophrenia is critical to test the hypothesis that both alterations might be related to the same underlying process.

The results of the present study might be integrated into a hypothetical model. This model predicts that changes in the *in vivo* binding of [^{11}C]NNC 112 and [^{3}H]SCH 23390 upon DA depletion are affected by two factors, externalization and upregulation. Based on the results of the acute depletion study, it is postulated that externalization decreases the *in vivo* [^{11}C]SCH 23390 binding, but does not affect *in vivo* [^{11}C]NNC 112 binding. On the other hand, it is postulated that upregulation increases the binding of both radiotracers. Under these assumptions, acute DA depletion (which induces externalization but no upregulation) is expected to decrease [^{3}H]SCH 23390 *in vivo* binding, but not [^{11}C]NNC 112 binding. The situation following sustained DA depletion is more complex, since both externalization and upregulation comes into play. Regarding *in vivo* [^{11}C]SCH 23390 binding, both effects affect the binding in opposite directions. [^{11}C]NNC 112 *in vivo* binding, which is not influenced by externalization, is increased due to upregulation. This hypothesis is summarized in Table 3. Additional research, including *in vitro* measurement of the expression and cellular localization of D1 receptors following DA depletion, is required to test this hypothesis. Other factors, such as differences in ligand binding due to receptor conformation changes and oligomerization state under different pharmacological conditions, might also play roles in the observed differences.

CONCLUSIONS

The results of this study demonstrate that (1) subchronic reserpine treatment increases *in vivo* [^{11}C]NNC 112 binding in the PFC and other brain regions, suggesting that this radiotracer detects an upregulation of D_1 receptors in response to subchronic DA depletion; (2) this observation supports the hypothesis that increased [^{11}C]NNC 112 observed in the PFC of patients with schizophrenia (Abi-Dargham *et al*, 2002) might be secondary to sustained deficit in prefrontal DA function (3) the *in vivo* binding of different D_1 radioligands is affected differently following changes in DA transmission, since the same drug treatment produced opposite changes in the *in vivo* binding of [^{11}C]NNC 112 and [^{3}H]SCH 23390; (4) these data provide possible avenues to reconcile the contradictory clinical findings from the [^{11}C]NNC 112 and [^{11}C]SCH 23390 PET studies and illustrate the complexity of factors affecting the *in vivo* binding of PET radiotracers. Additional studies, including investigations of the impact of receptor traffick-

Table 3 Hypothetical Model of Effects of Acute and Chronic DA Depletion on [^{11}C]NNC 112 and [^{3}H]SCH 23390 *In Vivo* Binding

		Cellular events affecting D1 receptor		
Condition	**Ligand**	**Externalization**	**Upregulation**	**Net effect on *in vivo* binding**
Acute DA depletion		Yes	No	
Effect on *in vivo* binding	[^{11}C]NNC 112	↔	—	↔
	[^{3}H]SCH 23390	↓↓	—	↓↓
Chronic DA depletion		Yes	Yes	
Effect on *in vivo* binding	[^{11}C]NNC 112	↔	↑	↑
	[^{3}H]SCH 23390	↓↓	↑	↓ ↔

Hypothetical interpretation of data from this study.
↑, *in vivo* binding; ↓, decreased *in vivo* binding; ↔, unchanged *in vivo* binding.

ing on the binding affinity of PET radiotracers, are warranted to further explore these issues.

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