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Effects of Binge Pattern Cocaine Administration on Dopamine D_1 and D_2 Receptors in the Rat Brain: An *In Vivo* Study Using Positron Emission Tomography

Hideo Tsukada,[1] Jason Kreuter,[2] Christopher E. Maggos,[2] Ellen M. Unterwald,[2,3] Takeharu Kakiuchi,[1] Shingo Nishiyama,[1] Masami Futatsubashi,[1] and Mary Jeanne Kreek[2]

[1]*Central Research Laboratory, Hamamatsu Photonics K. K., Shizuoka 434, Japan,* [2]*Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, New York 10021, and* [3]*Department of Psychiatry, New York University Medical Center, New York, New York 10003*

The aim of the present study was to determine the effect of "binge" pattern cocaine administration on dopamine D_1 and D_2 receptors in the rat brain. Male Sprague Dawley rats were injected three times at 1 hr intervals with saline or cocaine (15 mg/kg) each day for 2, 7, or 14 d. The *in vivo* binding of [^{11}C]SCH23390 (dopamine D_1 receptor antagonist) and [^{11}C]*N*-methylspiperone (NMSP; dopamine D_2 receptor antagonist) in the striatal region was measured by a high-resolution positron emission tomography at 1 and 3.5 hr, respectively, after the last cocaine or saline injection. Acute (2 d) binge cocaine administration did not change the *in vivo* binding potential of [^{11}C]SCH23390 or the binding of [^{11}C]NMSP in the striatum. After 7 d of binge cocaine administration, a significant decrease in the binding potential of [^{11}C]SCH23390 was observed, whereas no change in the binding of [^{11}C]NMSP was found. After 14 d of binge cocaine administration, the *in vivo* binding was significantly reduced for both [^{11}C]SCH23390 and [^{11}C]NMSP. Separate saturation experiments indicated that the observed alterations of *in vivo* binding were attributable mainly to apparent alterations in the affinity and not the number of binding sites. These results suggest that both dopamine D_1 and D_2 receptors may have altered physiologically available binding sites after binge pattern cocaine administration.

Key words: cocaine; dopamine receptors; [^{11}C]SCH23390; [^{11}C]N-methylspiperone; positron emission tomography; rat brain

Cocaine increases locomotor activity, reduces appetite, increases heart rate and blood pressure, and produces local anesthesia. Behavioral and biochemical studies suggest that cocaine produces its rewarding effects by increasing dopaminergic neurotransmission (Pettit and Justice, 1984; Ritz et al., 1987; Koob and Bloom, 1988). Cocaine binds to the dopamine transporter and blocks dopamine reuptake into presynaptic terminals. Studies using microdialysis have shown that acute cocaine administration leads to an immediate and dose-dependent increase in extracellular dopamine levels (Church et al., 1987; Carboni et al., 1989; Kuczenski et al., 1991; Maisonneuve and Click, 1992; Maisonneuve and Kreek, 1994; Maisonneuve et al., 1995). Cocaine also binds to serotonin and noradrenaline.

Repeated administration of cocaine augments the motor stimulant and stereotypic effects of cocaine (Downs and Eddy, 1932; Post and Rose, 1976). The development of cocaine-induced behavioral sensitization, defined as a greater effect from a given dose of drug after previous exposure to the drug, is dependent on dose, dosing schedule, and environmental context (Hinson and Poulos, 1981; Post et al., 1981; Reith et al., 1987). To approximate the conditions between experimental animals and humans, one administration paradigm was proposed to mimic the human pattern of cocaine abuse both in terms of temporal pattern and in relation to circadian rhythm of rest and activity (Branch et al., 1992; Unterwald et al., 1992). Cocaine abusers often self-administer the drug repeatedly over a short time period (referred to as "bingeing"), most frequently in the early evening hours, followed by a period of abstinence (Gawin, 1991). In our model of "binge" pattern cocaine administration, three doses of cocaine are administered 1 hr apart in the morning, that is, in prerest period for rats, followed by no cocaine administration for 22 hr. It has been reported that different patterns of chronic cocaine administration may alter dopamine receptor densities (Taylor et al., 1979; Goeders and Kuhar, 1987; Kleven et al., 1990; Peris et al., 1990; Unterwald et al., 1994). Recent *in vivo* microdialysis studies have determined that cocaine administered in a binge paradigm caused acute tolerance to the increases in extracellular concentration of dopamine in the ventromedial striatum (nucleus accumbens) and dorsolateral striatum (caudate putamen) (Maisonneuve and Kreek, 1994). Chronic binge cocaine administration abolished the acute tolerance observed during early cocaine binge administration (Maisonneuve et al., 1995). Moreover, chronic binge cocaine administration resulted in alterations in dopamine receptor densities as measured by *in vitro* binding (Unterwald et al., 1994). Several studies, however, have indicated significant differences between *in vitro* and *in vivo* receptor binding (Perry et al., 1980; Leslie and Bennett, 1987; Insel, 1989). For example, *in vivo* dopamine D_2 binding was decreased significantly by reserpine, despite no changes in K_d or B_{max} as determined by an *in vitro* binding assay (Chugani et al., 1988). *In vivo* binding of [^{3}H]*N*-methylspiperone (NMSP) in the mouse brain was altered by swim stress, although there was no change in the binding parameters *in vitro* (Inoue et al., 1991a). It was shown recently that high-

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Correspondence should be addressed to Hideo Tsukada, Central Research Laboratory, Hamamatsu Photonics K. K., 5000 Hirakuchi, Hamakita, Shizuoka 434, Japan.

resolution positron emission tomography (PET) allows *in vivo* quantitative analyses of cerebral blood flow and metabolism, even in the rat brain (Magata et al., 1995; Ouchi et al., 1996a,b). It has also become possible to conduct dynamic neurobiological studies in a rat model using a technique of PET with radiolabeled receptor-selective ligands (Hume et al., 1992; Unterwald et al., 1996).

The aims of the present study were to determine the effects of binge pattern cocaine administration on *in vivo* binding of [^{11}C]SCH23390 (dopamine D_1 receptor antagonist) and [^{11}C]NMSP (dopamine D_2 receptor antagonist) in the rat striatum, measured by high-resolution PET.

MATERIALS AND METHODS

Animals and drug administration. Male Sprague Dawley rats (250–280 gm) obtained from Japan SLC (Hamamatsu, Japan) were caged individually with free access to food and water. Rats were kept on a 12 hr light/dark cycle (lights on at 7:30 A.M.). Rats were randomly assigned to six different experimental groups, with $n = 6$ for groups 1–4 and $n = 15$ for groups 5 and 6, as follows: (1) saline for 2 d, (2) cocaine for 2 d, (3) saline for 7 d, (4) cocaine for 7 d, (5) saline for 14 d, and (6) cocaine for 14 d. After adjustment to the facility for 1 week, each rat received three daily intraperitoneal injections at 8, 9, and 10 A.M. Each rat in groups 1, 3, and 5 received three daily intraperitoneal injections (1 ml/kg of body weight) of saline, and each rat in groups 2, 4, and 6 received three daily intraperitoneal injections (15 mg/kg) of cocaine HCl (Shionogi Pharmaceutical, Osaka, Japan) dissolved in saline, with animals in groups 1 and 2 for 2 d, groups 3 and 4 for 7 d, and groups 5 and 6 for 14 d.

PET scan. On the day of the PET scan, after the second injection of saline or cocaine in the usual binge pattern, animals received initial anesthesia with chloral hydrate (400 mg/kg, i.p.) (Sigma, St. Louis MO). A cannula was implanted into the tail vein of one control and one cocaine-administered animal. Each rat was fixed in a special head holder developed for stereotaxic coordination (Hamamatsu Photonics K. K., Shizuoka, Japan) and maintained under anesthesia during the scan by continuous infusion of chloral hydrate ($100\ mg \cdot kg^{-1} \cdot hr^{-1}$, i.v.). The two rats were secured on a mobile platform, placed in the PET gantry hole, and scanned simultaneously. For the evaluation of the effects of cocaine on D_1 or D_2 receptor binding activities, two different PET studies were conducted in each animal. First, tracer amounts of [^{11}C]SCH23390 were injected simultaneously through the tail vein cannula of each of the two rats at 1 hr after the last of the three administrations of cocaine or saline. After that scan was completed, [^{11}C]NMSP was injected through the tail cannula in each of the same two rats at 3.5 hr after the last administration of cocaine or saline.

It has been shown that the half-life of unmetabolized cocaine in rat striatum is ~30 min; thus, a very small amount of cocaine would be present during the PET scans (Maisonneuve and Kreek, 1994). Because of the very short half-life of ^{11}C (20.4 min), the radioisotope used in these studies, the time lag of 2.5 hr between the two scans provided enough decay time of radioactivity in the rat (~1/180 of injected dose), so that the level of radioactivity associated with the first injection would never interfere with the second scan. Because our preliminary data suggested that the binding of [^{11}C]SCH23390 and [^{11}C]NMSP *in vivo* was not affected by the time interval after the last cocaine injection, at least up to 24 hr after the last cocaine injection (unpublished data), a decision of injection order was made to keep the order of D_1 and D_2 scans constant.

The radioactive purities of [^{11}C]SCH23390 and [^{11}C]NMSP used in this study were >98%, and the specific radioactivity ranged from 37 to 55 GBq/μmol. To allow four PET scans to be conducted in a single day in the final day of binge pattern saline or cocaine administration, one saline- or one cocaine-administered animal received the binge pattern administration deferred by 4 hr. The same procedure was followed for the afternoon studies.

Emission recording began immediately after each radioligand administration. PET scans were performed with an SHR-2000 PET camera (Hamamatsu Photonics K. K.) (Watanabe et al., 1992), which has a transaxial resolution of 3.0 mm full width at half maximum and a center-to-center distance of 3.25 mm with Z-motion of the rat every specific time frame. The PET camera allows seven slices for imaging to be recorded simultaneously. Seven addition slices for imaging were made by moving the platform holding the animals, with a total of 14 slices from each animal. Each individual PET scan was performed for 64 min with 16 time frames at 1 min intervals (total PET scanning time of 16 min), followed by 16 time frames at 3 min intervals (total time of 48 min).

Kinetic analysis of in vivo *binding.* As shown in Figure 1, regions of interest (ROIs), striatum and cerebellum, were identified according to rat brain atlas (Paxinos and Watson, 1986), and time activity curves in ROIs were obtained.

For the quantitation of *in vivo* D_1 dopamine receptor binding, a kinetic three-compartment analysis method was applied as described previously (Huang et al., 1986). The cerebellum was used as a reference region because of the low abundance of dopamine receptors in this region. The total radioactivity in the cerebellum was used as an estimate of the free and nonspecific radioligand and was subtracted from radioligand binding in the striatal region to determine specific binding. A three-compartment model was fitted to the time activity curve of specific binding in the striatal region. The binding potential of [^{11}C]SCH23390 for the dopamine D_1 receptors was calculated by determining the ratio of the estimated k_3 value (association rate) to the estimated k_4 value (dissociation rate).

For the quantitation of *in vivo* D_2 dopamine receptor binding, a graphical analysis method was applied as described previously (Wong et al., 1986). The cerebellum was used as a reference region as performed in [^{11}C]SCH23390 analysis. The estimated k_3 value is equal to the production of the bimolecular association constant (k_{on}) and the number of receptors (B_{max}) and indicates the binding capacity of the ligand with the specific receptors.

Saturation experiments of in vivo *binding.* Saturation experiments were performed to examine the effect of binge cocaine administration on *in vivo* binding parameters (K_d and B_{max}). After 14 d of binge pattern administration with cocaine or saline, [^{11}C]SCH23390 or [^{11}C]NMSP was injected into the rat together with various amounts (from 3 to 300 μg/kg) of corresponding carrier ligands. Emission recording began immediately after tracer injection. For the *in vivo* binding analysis of [^{11}C]SCH23390, the total radioligand concentration in the cerebellum was used as an estimate of the free radioligand concentration (F) in the striatum. Specific binding (B) was defined as radioactivity in the striatum reduced by F. The curve for B was fitted to a set of three exponential functions to determine the time when B reached a peak (Farde et al., 1989). The values for B and F at that time were used in a Scatchard analysis in which the ratio of B/F was plotted against B (Scatchard, 1949). In the case of [^{11}C]NMSP, specific binding in the striatum continued to rise throughout the time of the PET scan. [^{11}C]NMSP concentrations in the striatum and cerebellum 45 min after tracer injection were calculated, and B was defined by the subtraction of radioligand concentration in cerebellum (F) from total radioligand concentration in the striatum. The ratio of B/F was plotted against B. The apparent *in vivo* B_{max} and K_d values were analyzed using LIGAND (Munson and Rodbard, 1980).

Statistical analysis. Results are expressed as mean ± SD. Comparison between saline and cocaine animals at 2, 7, and 14 d was carried out using an unpaired, two-tailed t test, and a probability level <5% ($p < 0.05$) was considered to be statistically significant.

RESULTS

Effects of binge administration of cocaine on *in vivo* binding of [^{11}C]SCH23390 and [^{11}C]NMSP

The time activity curves of [^{11}C]SCH23390 in the saline- and cocaine-treated animals indicate that the maximum accumulation of radioactivity in the striatum occurred 12 min after the tracer injection and decreased gradually thereafter (Fig. 2). The radioactivity of [^{11}C]NMSP accumulated gradually in the striatum of the saline- and cocaine-treated animals during a 64 min period (Fig. 3) and was continuing to rise at the end of the study period. The time for maximum accumulation of radioactivity of [^{11}C]SCH23390 and the continuing rise of [^{11}C]NMSP were similar in the saline- and cocaine-treated animals (Figs. 2, 3).

Administration of cocaine for 2 d did not alter the magnitude of the accumulation of [^{11}C]SCH23390 and [^{11}C]NMSP in the striatum. Administration of cocaine for 7 d resulted in a significant decrease in the accumulation of [^{11}C]SCH23390 in the striatum but no change in the accumulation of [^{11}C]NMSP. After 14 d of binge cocaine administration, the accumulation of both [^{11}C]SCH23390 and [^{11}C]NMSP was decreased (Figs. 2, 3). No

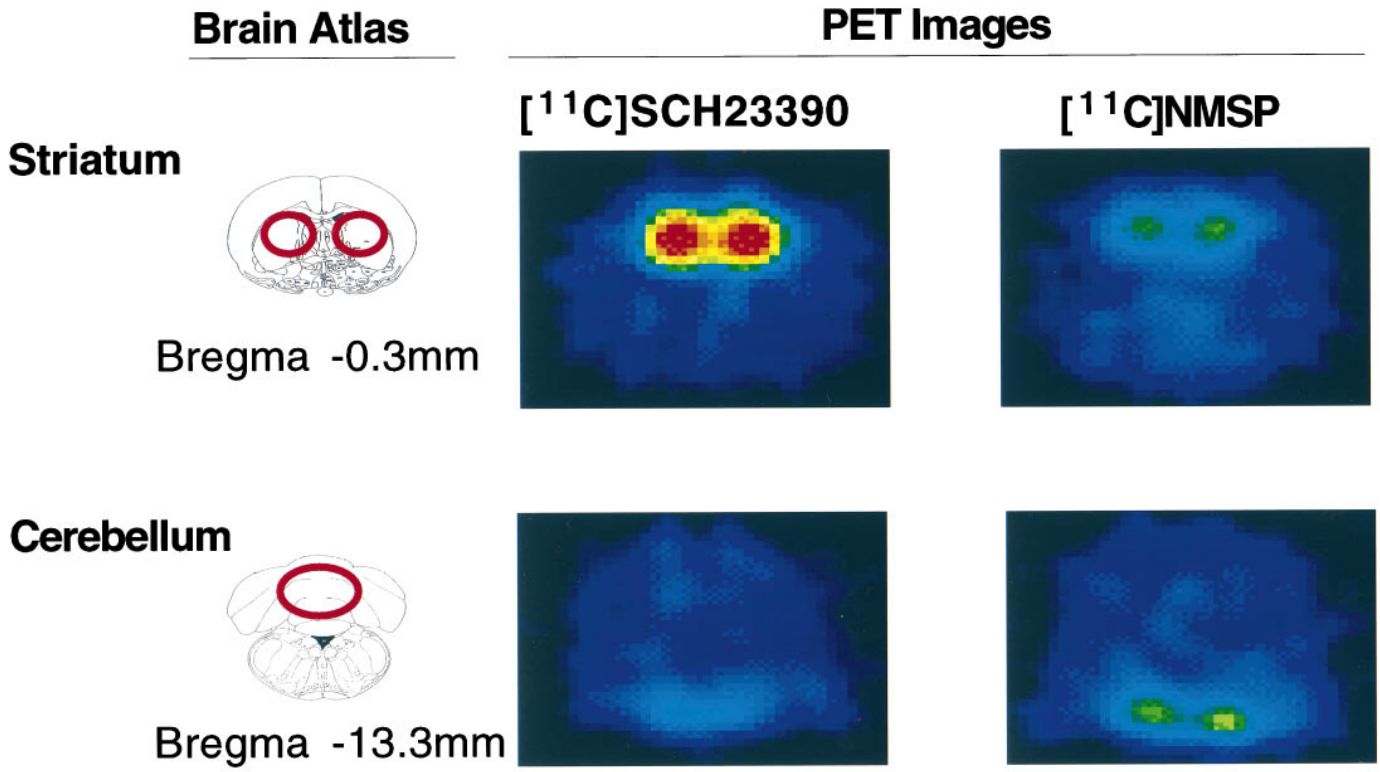

Figure 1. PET Images of [^{11}C]SCH23390 and [^{11}C] *N*-methylspiperone (*NMSP*) in the rat brain. During the scan, the rat was fixed in a stereotaxic frame under anesthesia with a continuous infusion of chloral hydrate (100 mg · kg^{-1} · hr^{-1}, i.v.). ROIs were identified on striatum and cerebellum according to the rat brain atlas (Paxinos and Watson, 1986), as shown by *red circles*. ROIs were placed on corresponding PET images to obtain the time activity curves.

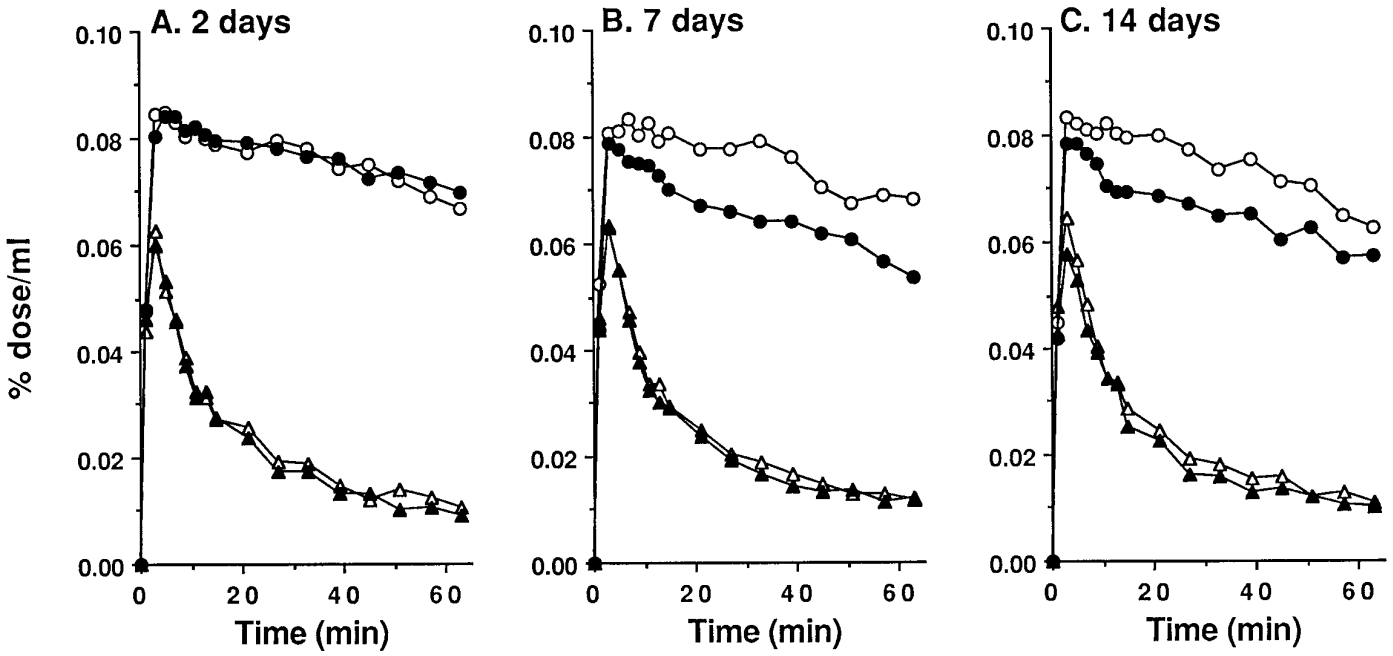

Figure 2. Typical time activity curves in the striatum and cerebellum of the rat brain after intravenous injection of [^{11}C]SCH23390. Rats were administered saline or cocaine (15 mg/kg × 3) in a binge pattern for 2 (*A*), 7 (*B*), and 14 (*C*) d. On the day of the PET scan, the final injection was performed 1 hr before the scan. ROIs were identified according to the rat brain atlas (Paxinos and Watson, 1986). Radioactivity is indicated in the striatum (○) or cerebellum (△) in saline-treated rats, and in the striatum (●) or cerebellum (▲) in cocaine-treated rats.

change was found in the magnitude of the accumulation or the time activity curves of either [^{11}C]SCH23390 or [^{11}C]NMSP in the cerebellum (Figs. 2, 3).

The *in vivo* binding of [^{11}C]SCH23390 and [^{11}C]NMSP in the striatum was not changed after 2 d of binge cocaine administration. After 7 d of binge cocaine administration, a significant

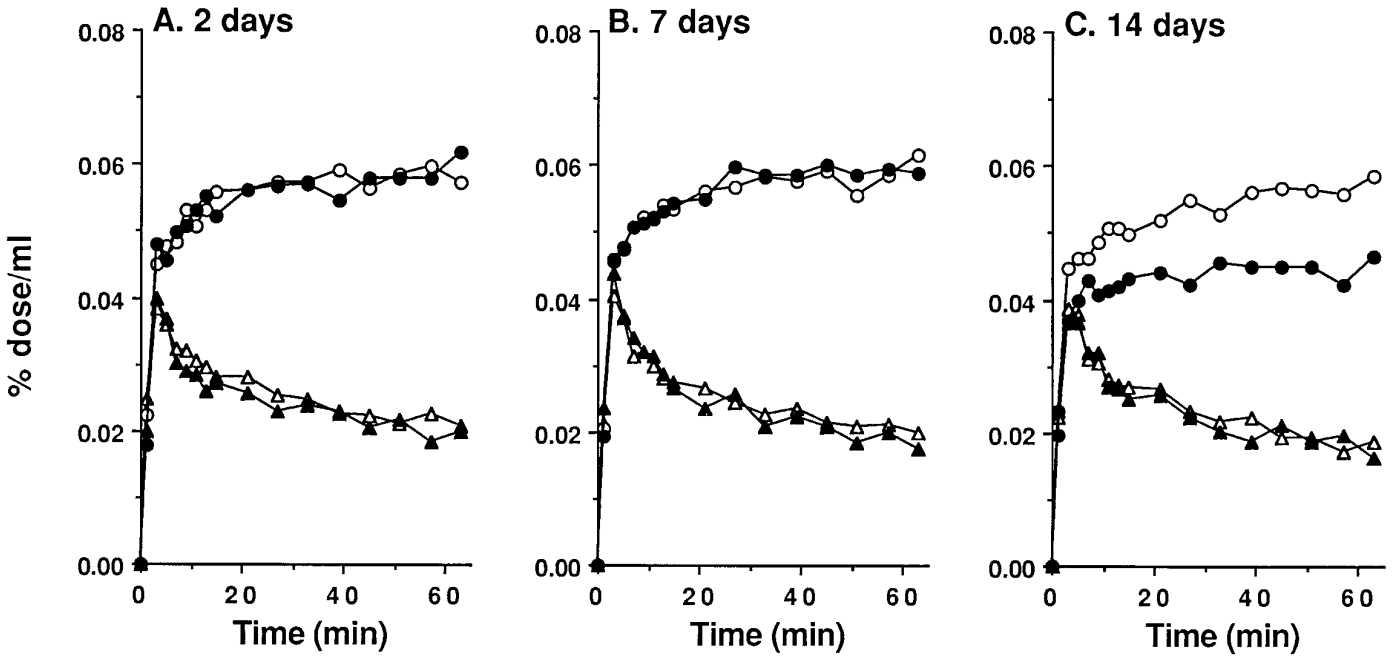

Figure 3. Typical time activity curves in the striatum and cerebellum of the rat brain after intravenous injection of [^{11}C]NMSP. Rats were administered saline or cocaine (15 mg/kg × 3) in a binge pattern for 2 (*A*), 7 (*B*), and 14 (*C*) d. On the day of the PET scan, the final injection was performed 1 hr before the scan. ROIs were identified according to the rat brain atlas (Paxinos and Watson, 1986). Radioactivity is indicated in the striatum (○) or cerebellum (△) in saline-treated rats, and in the striatum (●) or cerebellum (▲) in cocaine-treated rats.

decrease in the binding potential of [^{11}C]SCH23390 was observed (~84% of saline). This was attributable to a decrease in the association rate (k_3) and not to an alteration in the dissociation rate (k_4) (data not shown). No change in the *in vivo* binding of [^{11}C]NMSP was found on day 7 of cocaine administration; however, 14 d of binge cocaine administration decreased the *in vivo* binding of both [^{11}C]SCH23390 (to ~87% of saline) and [^{11}C]NMSP (to ~56% of saline) (Figs. 4, 5). In the saline-treated animals, the levels of the *in vivo* binding of [^{11}C]SCH23390 and [^{11}C]NMSP did not differ at 2, 7, and 14 d (Figs. 4, 5).

Scatchard analysis of *in vivo* binding

After 14 d of binge cocaine or saline administration, [^{11}C]SCH23390 or [^{11}C]NMSP was injected into the rat together with various amounts (from 3 to 300 μg/kg) of corresponding unlabeled carrier ligands. As expected in a saturation study, the addition of increasing amounts of unlabeled carrier ligand reduced in a dose-dependent manner the amounts of bound radiolabeled ligand. In these studies, a significant decrease in radioactivity of bound [^{11}C]SCH23390 and [^{11}C]NMSP in the striatum during the time span of the PET study was found, and the results were similar in both saline- and cocaine-administered rats. In contrast, in the cerebellum no changes in the amount of radioactivity of [^{11}C]SCH23390 and [^{11}C]NMSP were observed over the range of amounts of unlabeled carrier added in both saline- and cocaine-administered animals (Fig. 6*A,B*).

The free radioligand concentration (F) in the striatum was assumed to be comparable to the radioligand concentration in the cerebellum. The specific binding (B) in the striatum was calculated by subtracting the radioligand concentration measured in the cerebellum from total binding in the striatum. For the Scatchard analysis of the binding of [^{11}C]SCH23390 *in vivo*, equilibrium values for B and F were obtained at the time when the B value was maximum. The maximum accumulation occurred at 33 min after the injection of [^{11}C]SCH23390 in both saline- and cocaine-injected rats. The Scatchard plots revealed a linear curve for [^{11}C]SCH23390 in both saline- and cocaine-treated animals.

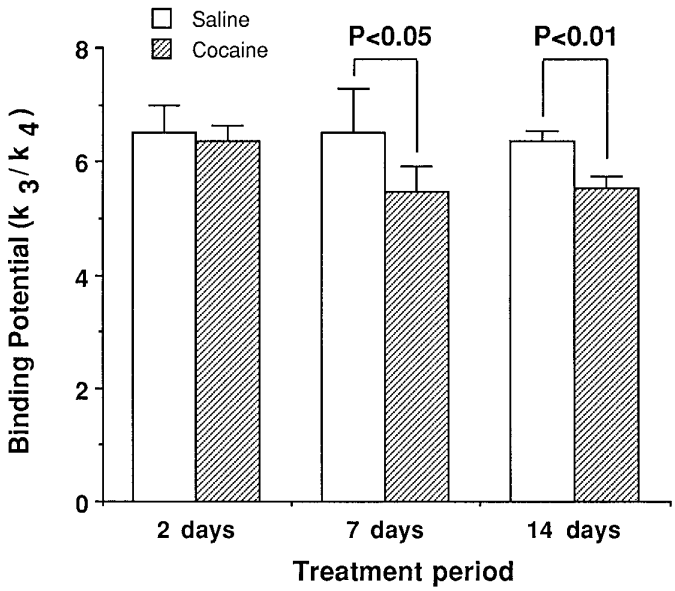

Figure 4. Effect of cocaine on binding potential of [^{11}C]SCH23390 in the striatum of rat brain. Rats were administered saline or cocaine (15 mg/kg × 3) in a binge pattern for 2, 7, and 14 d. On the day of the PET scan, the final injection was performed 1 hr before the scan. The total radioactivity in the cerebellum was used as an estimate of the free and nonspecific binding radioligand concentration in the striatum; the three-compartment model was fitted to the time activity curve of specific binding in the striatum. The binding potential was calculated by the ratio of association rate (k_3) to dissociation rate (k_4). Data are expressed as mean ± SD for six animals per treatment group.

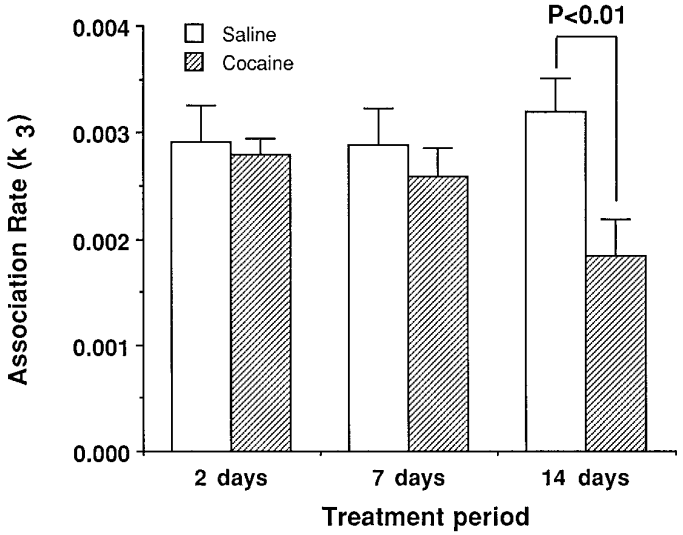

Figure 5. Effect of cocaine on binding of [^{11}C]NMSP in the striatum of rat brain. Rats were administered saline or cocaine (15 mg/kg × 3) in a binge pattern for 2, 7, and 14 d. On the day of the PET scan, the final injection was performed 1 hr before the scan. The total radioactivity in the cerebellum was used as an estimate of the free and nonspecific binding radioligand concentration in the striatum; k_3 value was calculated with a graphical analysis method. Data are expressed as mean ± SD for six animals per treatment group.

Cocaine administration resulted in reduced binding of [^{11}C]SCH23390, which was attributable to an apparent decrease in the affinity rather than a decrease in the maximum number of binding sites (B_{max}) (Fig. 7*A*).

The values for *B* and *F* of [^{11}C]NMSP were determined at 45 min after the injection of various concentrations of carrier ligand. The *B*/*F* ratio was plotted against *B*. The Scatchard plots of [^{11}C]NMSP revealed a linear curve for both saline- and cocaine-treated animals. The administration of cocaine for 14 d reduced the *in vivo* binding of [^{11}C]NMSP, again because of a decrease in the apparent affinity, rather than any change in B_{max} (Fig. 7*B*).

DISCUSSION

The results indicate that cocaine, administered to rats for 14 d in a binge pattern, produced a significant reduction in the *in vivo* binding of [^{11}C]SCH23390 and [^{11}C]NMSP in the striatum. The reduction was primarily attributable to alterations in the affinities of both ligands to their respective dopamine receptors rather than a change in the number of binding sites.

Both [^{11}C]SCH23390 and [^{11}C]NMSP accumulated in the striatum of the rat brain (Figs. 1–3). There were no differences in the time activity curves in the cerebellum between saline and cocaine animals. The cerebellum, because of its low density of dopamine receptors (Creese et al., 1975), was used as the reference region to calculate the specific binding; all ligand present is assumed to be either nonspecifically bound or free. The total radioactivity in the cerebellum is dependent in part on cerebral blood flow. Changes in cerebral blood flow were unlikely to have altered receptor binding in the striatum. It is assumed that specific binding is not affected by blood flow, because the extent of binding is small relative to the extent of transport to the brain tissue.

It has been found using *in vitro* receptor autoradiography that chronic administration of cocaine in a binge pattern produces transient increases in dopamine D_2 receptor number in rostral areas of the nucleus accumbens and caudate putamen after 7 d of treatment (Unterwald et al., 1994). The elevated receptor number returned to baseline levels after 14 d of continued administration. No significant change was found in the number of D_1 receptors in the caudate putamen after 14 d of cocaine administration, although increases in D_1 receptor number were found in the nucleus accumbens. The nucleus accumbens is a very small brain region, which cannot be separated from the caudate putamen in rodent PET study.

The results of the present study are different from the previous study, possibly because of the different technologies. *In vitro* autoradiography studies are performed using thin tissue sections in which selective radiolabeled ligands are applied after the sections are washed to remove both endogenous ligands and any drugs. In addition, binding not only to receptors that are located on the cell membrane but also to receptors that are present in the cytosol might be observed. In PET studies conducted *in vivo*, one would anticipate identifying binding only to cell surface receptors. Also, one does see competition by the presence of endogenous ligands in PET studies, or the presence of any compound that may interfere with access to receptor site by competitive or noncompetitive mechanisms. *In vivo* binding is regulated by all the processes that modulate receptor binding in a living animal. Although it is assumed *in vitro* that some of the components of the signal transduction mechanisms that may be essential in the modulation of binding of a ligand to its receptor are intact, it is not clear whether all such modulatory mechanisms are as intact as they would be in the *in vivo* situation.

Because ligand-receptor binding *in vivo* does not reach an equilibrium state, the analyzed results are affected by kinetic indices (association and dissociation rates). In the *in vitro* autoradiography studies, binding is carried out under equilibrium conditions. In a previous study, D_2 receptors were measured using [^{3}H]raclopride, a more selective D_2 and more sensitive antagonist than NMSP (Young et al., 1991). Each of these factors could contribute to the differences in the findings of the *in vivo* PET versus *in vitro* autoradiography studies.

In the present study, *in vivo* binding of [^{11}C]SCH23390 and [^{11}C]NMSP was measured by PET 1 and 3.5 hr, respectively, after the last injection of cocaine. Previous studies have shown that at these time points extracellular levels of dopamine are still elevated as compared with basal levels (Maisonneuve and Kreek, 1994; Maisonneuve et al., 1995). Simple displacement, or competitive inhibition, of radioligand binding by increased dopamine levels at dopamine receptors is unlikely, because acute binge cocaine administration (2 d) did not alter the *in vivo* binding of [^{11}C]SCH23390 and [^{11}C]NMSP (Figs. 3, 4). It has been reported recently that the occupation of D_1 and D_2 receptors by dopamine was increased during cocaine-induced behavioral sensitization (Burger and Martin-Iverson, 1994). This study indirectly measured dopamine release by assessing the amount of dopamine bound to the receptors *in vivo*. The results suggest that not only the level of released dopamine but also the binding indices were modulated by repeated cocaine administration. Inoue et al. (1991b) reported that *in vivo* binding of [^{3}H]SCH23390 and [^{3}H]NMSP is altered without any changes in the binding parameters *in vitro* (K_d and B_{max}) when dopamine levels are modulated by reserpine and *d*-amphetamine. Taken together, endogenous dopamine might regulate the *in vivo* binding of [^{11}C]SCH23390 and [^{11}C]NMSP at the receptor sites, secondary to the altered receptor affinity attributable to the decreased basal levels of dopamine that have been observed after chronic binge pattern

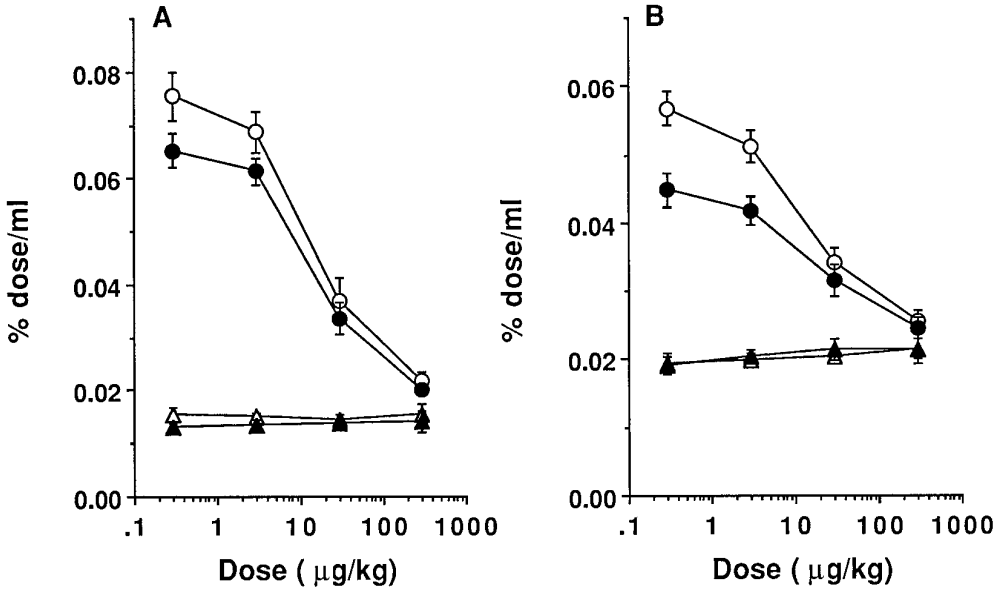

Figure 6. Saturation studies of *in vivo* binding of [^{11}C]SCH23390 (*A*) and [^{11}C]NMSP (*B*) in rat brain. Rats were administered saline or cocaine (15 mg/kg × 3) in a binge pattern for 14 d. On the day of the PET scan, the final injection was performed 1 hr before the scan. Rats were injected with [^{11}C]SCH23390 or [^{11}C]NMSP, with various doses of each carrier ligand ranging from 3 to 300 μg/kg. The radioactivities in striatum and cerebellum at 33 min for [^{11}C]SCH23390 and 45 min for [^{11}C]NMSP after tracer injection were expressed as percent of dose/gm. Data are expressed as mean ± SD for three animals per treatment group. Radioactivity is indicated in the striatum (○) or cerebellum (△) in saline-treated rats, and in the striatum (●) or cerebellum (▲) in cocaine-treated rats.

cocaine administration along with surges in dopamine levels after each cocaine administration.

In our previous studies, it was shown that dopamine levels in the extracellular fluid (ECF) of rat striatum have returned essentially to the baseline levels by 3.5 hr after the last 15 mg/kg of cocaine binge administration (Maisonneuve et al., 1995). Also in the Maisonneuve et al. (1995) study, it was found that the basal levels of dopamine in ECF were lower in rats treated for 14 d with cocaine. It is unlikely that the decreased binding of ligands to dopamine D_1 and D_2 receptors after 14 d of cocaine binge is attributable to the dopamine levels in striatal ECF. The *in vivo* binding is dependent on the kinetics of ligand, that is, the association and dissociation rates have critical roles in the *in vivo* binding. In the present study, cocaine administered in a binge pattern resulted in the different temporal reductions in the *in vivo* binding to dopamine D_1 and D_2 receptors measured by [^{11}C]SCH23390, which has both association and dissociation rates, and [^{11}C]NMSP, which has only an association rate, respectively. It is not known whether the different kinetics of these two ligands may contribute to the different temporal reduction in the *in vivo* binding in rats treated with cocaine.

Binge cocaine administration produced different temporal reductions in the *in vivo* binding to dopamine D_1 and D_2 receptors in the study. Several reports have shown a dissociation of dopamine D_1 and D_2 receptor functions. Behavioral studies have previously shown a significant correlation of locomotor activity with dopamine D_1 receptor binding *in vitro* (Unterwald et al., 1994) and also with increased occupation of dopamine D_1, but not D_2, receptors in the striatum (Burger and Martin-Iverson, 1994). Furthermore, the selective dopamine D_1 receptor antagonist blocks behavioral sensitization to cocaine (McCreary and Marsden, 1993). It is therefore possible that D_1 receptors in the striatum play a role in behavioral sensitization to cocaine. On the contrary, D_2 antagonists blocked the development but not the expression of cocaine-induced behavioral sensitization (Weiss et al., 1989). Moreover, D_1 selective receptor agonist (SKF82958) prevented cocaine-seeking behavior, whereas D_2 agonist (7-OH-DPAT) enhanced the behavior (Self et al., 1996). Although the relation between the temporal difference of changes in dopamine D_1 and D_2 receptor binding and behavioral effects is not clear, these changes in D_1 and D_2 receptors may reflect different roles in response to binge cocaine administration. Several factors might be

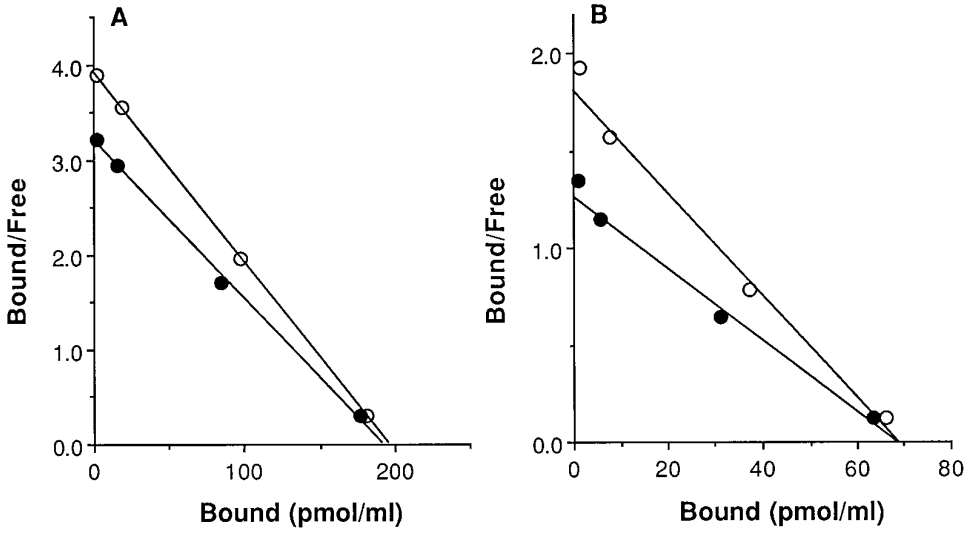

Figure 7. Scatchard plot analysis of saturation studies of the *in vivo* binding of [^{11}C]SCH23390 (*A*) and [^{11}C]NMSP (*B*) in saline- (○) and cocaine-treated (●) rats. The total radioligand concentration in the cerebellum was used as the free radioligand concentration (*F*) in the striatum. Specific binding (*B*) was defined as radioactivity in the striatum reduced with *F*. The values for *B* and *F* at 33 min for [^{11}C]SCH23390 and 45 min for [^{11}C]NMSP after tracer injection were used in a Scatchard analysis in which the ratios *B*/*F* were plotted against *B*.

involved in differential changes, including the interactions with adenylyl cyclase, the interactions with other neurotransmitters, and a permissive role of D_1 receptors in modulating stimulation of D_2 receptors (Walters et al., 1987).

It has also been reported that the administration of desipramine, which acts in part as an uptake inhibitor of serotonin, decreased dopamine receptor binding *in vivo* in mice striatum (Suhara et al., 1990). The binding of cocaine to the serotonin reuptake transporter facilitates serotonergic neural transmission, which might modulate dopamine receptor binding *in vivo* indirectly through the neural network system.

In a previous study from our laboratory, the locomotor activity of rats administered cocaine in a binge pattern was greater than that of the saline-injected rats on all treatment days (Unterwald et al., 1994). In addition, the magnitude of duration of the cocaine-induced hyperactivity was greater in the rats treated with 14 d of binge cocaine administration as contrasted with rats that received saline in the same pattern. In this study, no behavior was measured, and there were no obvious alterations in behavior, as observed informally while the study was being conducted.

In the study reported here, PET studies showed downregulation, as measured by decreased binding of both D_1 and D_2 dopamine receptor ligands after 7 and 14 d of chronic binge cocaine administration. In previous clinical PET studies (Volkow et al., 1990, 1993), decreased binding of [^{18}F]NMSP, a D_2 receptor ligand, was found in early abstinence (2–7 d) chronic cocaine addicts. In one study, other cocaine addicts abstinent for 4–5 weeks had normal binding of D_2 ligand (Volkow et al., 1990). In a second study, decreased D_2 binding was found both during early abstinence and after an interval of 3 months (Volkow et al., 1993). The present study showed the same findings with respect to dopamine D_2 binding after chronic binge pattern cocaine administration in the rat model, but also, and of considerable interest, showed additional findings of depression of D_1 binding in this setting of chronic cocaine administration.

In conclusion, the present results suggest that both D_1 and D_2 receptors may have altered physiologically available binding sites after chronic binge pattern cocaine administration. These findings are important both for understanding the neurobiology of cocaine addiction and potentially for developing effective pharmacotherapy.

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