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Increases in Dopamine D_3 Receptor Binding in Rats Receiving a Cocaine Challenge at Various Time Points after Cocaine Self-Administration: Implications for Cocaine-Seeking Behavior

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Previous research suggests that cocaine dysregulates dopamine D_3 receptors. The present study examined the time course of changes in dopamine D_3 receptor binding after terminating a cocaine self-administration regimen. [^{125}I]-7-hydroxy-2-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)-amino]-tetralin was used to label dopamine D_3 receptors in rats that had undergone testing for cocaine-seeking behavior reinstated by a cocaine priming injection (15 mg/kg, i.p.; the behavior results have been previously published), and were killed 24 h after the test at time points that were either 2, 8, or 31–32 days after their last cocaine self-administration session. The results indicated a time-dependent increase in D_3 receptor binding relative to controls that received saline yoked to the delivery of cocaine in an experimental animal. Specifically, there was no significant change in D_3 receptor binding in cocaine-experienced rats killed at the 2- or 8-day time points relative to controls, but there was an increase in D_3 receptor binding in the nucleus accumbens core and ventral caudate-putamen in rats killed at the 31- to 32-day time point. In a subsequent experiment, we replicated the increase in D_3 receptor binding in rats that underwent a less extensive self-administration regimen, then were tested for cocaine-primed reinstatement of cocaine-seeking behavior, and then were killed 24 h later at a time point of 22 days after their last self-administration session. Furthermore, the increase in binding was attenuated by repeated 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin administration (1 mg/kg/day, s.c. for 14 days), a regimen that also reduces cocaine-seeking behavior in animals when tested in a nondrug state. Collectively, the findings suggest that regulatory responses of D_3 receptors may be functionally related to changes in propensity for cocaine-seeking behavior.
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INTRODUCTION

A growing body of literature suggests that dopamine D_3 receptors play a role in drug abuse-related behavior. An early indicator was the unique anatomical distribution of D_3 receptors with the highest concentrations found in mesolimbic systems that have been implicated in drug abuse (Lévesque *et al*, 1992). D_3-preferring agonists were later found to modulate cocaine self-administration (Caine and Koob, 1993, 1995; Nader and Mach, 1996; Parsons *et al*, 1996), and their relative potency to shift the cocaine self-administration dose-effect function to the left corresponds to their relative potency to stimulate mitogenesis in cell lines expressing D_3, but not D_2, dopamine receptors (Caine *et al*, 1997). In drug-naïve animals, however, the D_3-preferring agonist 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) fails to support self-administration (Nader and Mach, 1996). Recent findings with more selective partial agonists and antagonists suggest these compounds exert little, if any, effect on cocaine self-administration on a continuous reinforcement schedule (Campiani *et al*, 2003; Di Ciano *et al*, 2003; Gál and Gyertyán, 2003). However, the D_3-selective antagonist SB-277011 does inhibit cocaine-primed reinstatement of cocaine-seeking behavior (Vorel *et al*, 2002), suggesting that D_3 receptors mediate the incentive motivational effects of cocaine.

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D_3 receptors may be particularly important for modulating behaviors elicited by cocaine-associated stimuli, although the mechanism for these effects is unclear. For instance, a low dose (0.1 mg/kg) of the D_3-preferring agonist 7-OH-DPAT and the partial D_3-preferring agonist BP897 attenuates the ability of cocaine or amphetamine to establish conditioned place preference for a drug-paired environment (Duarte *et al*, 2003; Khroyan *et al*, 1998; Khroyan *et al*, 1999), and the partial agonist BP897 also attenuates conditioned activity elicited by exposure to a cocaine-paired environment (Aujla *et al*, 2002; Le Foll *et al*, 2002). Moreover, a low dose of 7-OH-DPAT attenuates cocaine-seeking behavior following exposure to a cocaine self-administration environment (Fuchs *et al*, 2002), and the partial agonist BP897 attenuates cocaine-seeking behavior elicited by a cocaine discriminative stimulus (Cervo *et al*, 2003) or a cocaine-conditioned reinforcer on a second order schedule of cocaine reinforcement (Pilla *et al*, 1999). Surprisingly, however, the D_3-selective antagonist SB-277011 also decreases cocaine-seeking behavior elicited by cocaine-associated stimuli (Di Ciano *et al*, 2003; Vorel *et al*, 2002), as well as cocaine-conditioned place preference (Vorel *et al*, 2002). It is unclear whether the effects of these drugs involve different mechanisms due to differences in D_3 receptor selectivity and/or efficacy or indicate that either increases or decreases in stimulation of D_3 receptors reduce the motivational effects of cocaine-paired stimuli as reported previously for D_1 receptors (Alleweireldt *et al*, 2002).

D_3 receptors are dysregulated following cocaine exposure. Mash and colleagues reported an increase in D_3 receptor binding and mRNA in the nucleus accumbens (NAc) of cocaine overdose fatalities (Segal *et al*, 1997; Staley and Mash, 1996); however, others have failed to find an increase in D_3 receptor mRNA in cocaine abusers (Meador-Woodruff *et al*, 1995). In rats, repeated intravenous administration of cocaine increases D_3 receptor binding in the caudate-putamen (CPu), but decreases D_3 receptor binding in the NAc (Wallace *et al*, 1996), whereas continuous subcutaneous administration of cocaine fails to alter D_3 receptor binding (Stanwood *et al*, 2000b). Converging lines of evidence suggest that D_3 receptors exhibit paradoxical regulation in response to changes in dopamine levels. Dopamine depletion by the dopamine-selective neurotoxins 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), or the vesicular storage-depleting drug reserpine down regulates D_3 receptor binding (Guillin *et al*, 2001; Lévesque *et al*, 1995; Morissette *et al*, 1998; Stanwood *et al*, 2000b). In contrast, increased dopamine associated with knockout of the dopamine transporter gene upregulates D_3 receptor mRNA in the CPu and NAc (Fauchey *et al*, 2000). Furthermore, exposure to D_3-preferring agonists upregulates D_3 receptors in C6 glioma cells *in vitro* (Cox *et al*, 1995) and upregulates D_3 receptors in the ventral pallidum and substantia nigra when administered *in vivo* (Cox *et al*, 1995; Stanwood *et al*, 2000b).

Dysregulation of monoamine systems may contribute to the neuropathology underlying cocaine dependence (Koob and Le Moal, 2001). For instance, dynamic changes occur in brain dopamine levels after varying lengths of abstinence from cocaine self-administration in rats, with basal and cocaine-primed dopamine levels decreased after relatively short periods of abstinence ($\leqslant$7 days), and increased after longer periods of abstinence (30 days) (Neisewander *et al*, 1996; Parsons *et al*, 1995; Tran-Nguyen *et al*, 1998). The changes in dopamine levels during abstinence from cocaine are accompanied by changes in the incentive motivational effects of cocaine priming and cocaine-associated stimuli, evident as enhanced cocaine-seeking behavior elicited by these stimuli later during abstinence relative to early during abstinence (Grimm *et al*, 2003; Neisewander *et al*, 2000; Tran-Nguyen *et al*, 1998).

The purpose of the present study was to examine whether dopamine D_3 receptor binding is altered in a time-dependent manner after terminating a cocaine self-administration regimen. Twenty-four h before killing, all animals in this study received a cocaine challenge (15 mg/kg, i.p.), which was given as a prime for tests of reinstatement of cocaine-seeking behavior (behavioral results have been published previously; Fuchs *et al*, 2002; Tran-Nguyen *et al*, 1998). [^{125}I]-7-hydroxy-2-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)-amino]-tetralin ([^{125}I]-7-OH-PIPAT) was used to label dopamine D_3 receptors in animals killed either 2, 8, or 31–32 days after their last cocaine self-administration session. The results indicated an increase in D_3 receptor binding in the ventral striatum in animals killed 31–32 days after terminating self-administration relative to a saline-yoked control group. In a subsequent experiment, we examined whether repeated 7-OH-DPAT administration would alter the increase in D_3 receptor binding.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats, weighing 250 $\pm$ 25 g at the start of the experiment, were housed individually in a climate-controlled colony room with a reversed 12-h light–dark cycle (lights on at 1800). Housing conditions were in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996). Animals were acclimated to handling for at least 5 days prior to the start of the experiment.

Surgery

Animals were deeply anesthetized with pentobarbital sodium (50 mg/kg, i.p.; Sigma Chemical Co., St Louis, MO), which was administered 5 min after pretreatment with atropine sulfate (10 mg/kg, i.p.; Sigma Chemical Co.) to reduce bronchial secretions. Incisions were made in clean, shaven areas on the neck to expose the jugular vein and on the head to expose the skull. A burrow was then made subcutaneously from the incision on the neck to the incision on the head, and a catheter was pulled through the burrow. The catheters were constructed from silastic tubing (10 cm; ID 0.012′ $\times$ OD 0.025′; Dow Corning, Midland, MI) connected to a bent 22-gauge metal cannula encased within a plastic screw connector (Plastics One, Roanoke, VA) at one end and affixed with a small ball of aquarium sealant approximately 4 cm from the other end. A small incision was made in the jugular vein, and the catheter was inserted into the vein until flush with the ball of

aquarium sealant. The catheter was then secured to the vein with sutures on both sides of the ball. The metal end of the catheter was secured to the skull using dental acrylic cement and small anchor screws drilled into the skull. The incisions were then sutured and treated with a topical antibiotic. To maintain patency, catheters were flushed daily throughout the experiment with 0.1 ml of a solution of bacteriostatic saline, heparin sodium (10 U/ml; Elkins-Sinn Inc., Cherry Hill, NJ), streptokinase (0.67 mg/ml; Astra Pharmaceutical Products, Westborough, MA), and ticarcillin (66.67 mg/ml; SmithKline Beecham Pharmaceuticals, West Chester, PA). Catheter tips were sealed with a plastic cap when not in use. Catheter patency was verified periodically by administering 0.3 ml of methohexital sodium (i.v., 16.6 mg/ml, Eli Lilly and Co., Indianapolis, IN), which produces a rapid loss of muscle tone only when administered intravenously. Animals were given at least 5 days to recover from surgery prior to self-administration training.

Experiment 1: Changes in D_3 Receptor Binding after Terminating a High-Dose Cocaine Self-Administration Regimen

Design. Animals were randomly assigned to groups that were either trained to self-administer cocaine or were yoked ($N=11$) to a self-administering animal such that they received a saline infusion each time their self-administering counterpart received cocaine. The cocaine self-administering animals were later assigned to one of three groups, counterbalanced for previous cocaine intake, that were killed either 2 ($N=5$), 8 ($N=9$), or 31–32 ($N=9$) days after the last self-administration session. The animals in this experiment had also undergone testing for concomitant changes in reinstatement of cocaine-seeking behavior by a cocaine priming injection (15 mg/kg, i.p.) and extracellular dopamine levels in the amygdala. This testing took place 24 h prior to killing the animals and the results have been published previously (Tran-Nguyen *et al*, 1998).

Self-administration training. Animals were given daily 3-h sessions of self-administration training for 14 consecutive days during their light cycle. Sessions took place in operant conditioning chambers ($20 \times 28 \times 20$ cm high; Med Associates Inc., St Albans, VT) equipped with an active lever, an inactive lever, a stimulus light, a house light, and a tone generator. Initially, animals were placed on a fixed ratio (FR) 1 schedule of cocaine (0.75 mg/kg/0.1 ml, i.v.) reinforcement and then progressed to a variable ratio (VR) 2 schedule, and finally to a VR5 schedule. Schedule completions by cocaine-trained animals resulted in inactivation of the house light and activation of the cue light, tone (2.9 kHz) and, 1 s later, the infusion pump, which delivered a 6-s infusion of cocaine. All stimuli were inactivated simultaneously when the infusion was completed, and after a 20-s timeout, the house light was re-activated. Animals in the control group received the same stimulus complex and a 0.1 ml infusion of saline contingent upon schedule completions by their yoked counterpart. To facilitate acquisition of cocaine self-administration (Carroll *et al*, 1981), animals were restricted to a 1 h/day feeding schedule until they began responding on the VR5 schedule of reinforcement. In addition, animals that failed to respond within 10 min were given priming infusions (0.75 mg/kg/0.1 ml i.v.) every 2–3 min for a maximum of three primes/session.

Experiment 2: Changes in D_3 Receptor Binding after Terminating a Low-Dose Self-Administration Regimen and Repeated 7-OH-DPAT Administration

Design. Animals were randomly assigned to cocaine self-administration or saline-yoked control groups. After the last self-administration session, animals were given a 7-day drug-free period. Then all saline-yoked controls received daily repeated saline administration for 14 days (Saline/Saline group, $N=7$) and cocaine self-administering animals received either repeated saline (Cocaine/Saline group, $N=6$) or 7-OH-DPAT (Cocaine/7-OH-DPAT group, $N=7$) administration with group assignment counterbalanced for cocaine intake during self-administration. Animals were tested for the effects of 7-OH-DPAT on extinction of cocaine-seeking behavior immediately following their first and 13th 7-OH-DPAT/saline administration. All animals also received a cocaine priming injection (15 mg/kg, i.p.) co-administered with their last 7-OH-DPAT/saline treatment and were then tested for cocaine-seeking behavior. They were then killed 24 h later (ie 22 days after terminating cocaine self-administration).

Self-administration training. To expedite cocaine self-administration training, animals were trained to press a lever for food reinforcement (45-mg food pellets; Noyes, Lancaster, NH) across five daily sessions prior to surgery for catheter implantation. This training was conducted in operant conditioning chambers ($25 \times 30 \times 27$ cm high; BRS/LVE Inc., Laurel, MD) that were different from the drug self-administration chambers and were equipped with a response lever, a food dispenser, and a house light. An autoshaping procedure was used, and after animals met a minimum criterion of responding ($\geqslant 10$ schedule completions/30-min session) on an FR1 schedule, the schedule demand was increased to a VR2 and then finally to a VR5 schedule of food reinforcement. Two days prior to and throughout autoshaping, animals were restricted to 15–18 g of food per day.

After recovering from surgery, the animals were given daily 2-h sessions of self-administration training for 21 consecutive days during their dark cycle in the operant conditioning chambers described in Experiment 1. Initially, animals were placed on an FR1 schedule of cocaine (0.25 mg/kg/0.1 ml, i.v.) reinforcement and then progressed to a VR2, and finally to a VR5 schedule of reinforcement. Schedule completions resulted in the same stimulus complex as described in Experiment 1 except that the tone frequency was changed to 500 Hz. To facilitate acquisition of self-administration, animals were restricted to 15-18 g of food per day until they met the minimum criterion of $\geqslant 10$ schedule completions/any given h/2-h session for 3 consecutive days. Food was available *ad libitum* thereafter. No priming injections of cocaine were given during training.

[I^{125}]-7-OH-PIPAT Binding

At the time points specified in the design sections above, animals were decapitated and their brains were rapidly

harvested, frozen in 2-methylbutane at −20°C, and stored at −70°C. The brains were later sectioned in the coronal plane at 20 μm in a cryostat at −12°C. D_3 receptor binding was assayed using the procedure of Gurevich and Joyce (1999). Prior to the binding assay, the sections were placed in a −20°C freezer for 30 min. The sections were then pre-incubated in 50 mM Tris buffer (pH 7.4) containing 100 mM NaCl for 30 min at 30°C in order to dissociate dopamine from the receptors. To estimate total binding, sections were incubated for 60 min in Tris buffer containing 0.4 nM [^{125}I]-7-OH-PIPAT (New England Nuclear, Boston, MA), 40 mmol NaCl, 100 μM 5′-guanylylimidodiphosphate (Sigma-Aldrich Co., St Louis, MO), and 0.5 μM 1,3-di(2-tolyl)guanidine (Research Biochemicals International, Natick, MA) to protect sigma receptors. To estimate nonspecific binding, adjacent sections were incubated in the same buffer in the presence of 10 μM 7-OH-DPAT. Incubation was followed by three washes of 1 h each in ice-cold buffer. The slides were then dipped in ice-cold deionized water and dried. Labeled sections were apposed to Hyperfilm-^{3}H (Amersham, Piscataway, NJ) in light-proof X-ray cassettes for 18 h. Total and nonspecific [^{125}I]-7-OH-PIPAT binding was estimated based on an average of at least four measures from each brain region. Optical density values were derived from the autoradiograms of [^{125}I]-7-OH-PIPAT binding using Brain image analysis software version 3.0 (Drexel University, Philadelphia, PA). Standards calibrated against ^{125}I-tissue mash standards for the appropriate exposure time were used according to the method described by Artymyshyn *et al* (1990). This procedure allowed for the transformation of gray values to the quantity of radioligand bound per milligram of protein. Measurements were taken in portions of the CPu and in the core and shell regions of the NAc at levels 1.7–2.2 mm anterior to Bregma as shown in Figure 1.

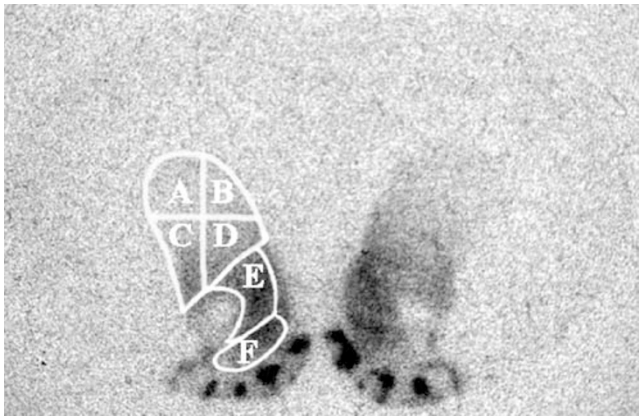

Figure 1 Representative autoradiogram of total [^{125}I]-7-OH-PIPAT binding from an animal in the Cocaine/Saline group in Experiment 2 (see caption for Figure 4 for more details regarding drug history). The regions analyzed in this study are depicted as follows: A = dorsolateral CPu; B = dorsomedial CPu; C = ventrolateral CPu; D = ventromedial CPu; E = NAc core; F = NAc shell. Nonspecific binding was close to background and, therefore, is not shown. The labeled section shown is approximately 2.2 mm anterior to Bregma. Other sections extended back to approximately 1.7 mm anterior to Bregma, and measurements of the NAc subregions in these sections were adjusted since the shell extends along the medial regions of the core at more caudal levels as diagrammed in the Paxinos and Watson (1997) atlas.

Data Analysis

[^{125}I]-7-OH-PIPAT binding was analyzed by separate ANOVAs for each brain region, and significant main effects were further analyzed using Newman–Keuls tests. In addition, we anticipated variability in binding across time points due to dynamical changes that occur in dopamine systems after terminating chronic cocaine regimens (eg Neisewander *et al*, 1994). Therefore, we conducted two-tailed, planned *t*-test comparisons between the control group and the 2- and 32-day withdrawal groups to test the hypotheses that cocaine self-administration or withdrawal from a self-administration regimen, respectively, may dysregulate dopamine D_3 receptors. This approach resulted in an acceptable number of planned comparisons that was less than the degrees of freedom associated with the independent variable (Keppel, 1982).

RESULTS

Experiment 1: Changes in D_3 Receptor Binding after Terminating a High-Dose Self-Administration Regimen

A time-dependent and region-specific increase in D_3 receptor binding was observed in the ventral striatum after terminating the self-administration regimen (see Figures 2 and 3). Although separate ANOVAs of [^{125}I]-7-OH-PIPAT binding in each region failed to reveal significant effects, planned comparisons between saline-yoked controls and animals killed 31–32 days after terminating self-administration revealed region-specific, significant differences. Relative to saline-yoked controls, animals killed 31–32 days after termination of self-administration exhibited a 66.8% increase in binding in the ventrolateral (VL) CPu ($t(18) = 2.42$, $p < 0.05$), a 42.9% increase in the ventromedial (VM) CPu ($t(18) = 2.15$, $p < 0.05$), and a 37.9% increase in the NAc core ($t(18) = 2.40$, $p < 0.05$). No differences were found in the dorsolateral (DL) CPu, dorsomedial (DM) CPu, or the NAc shell (see Figure 3). As reported previously (Tran-Nguyen *et al*, 1998), the average daily cocaine intake of rats in this experiment was 7.6 ± 1.0 mg. The mean total number of cocaine infusions ($\pm$SEM) obtained across the 14 self-administration sessions by animals killed at 2, 8, or 31–32 days after terminating self-administration, respectively, was 422.1 ± 35, 391 ± 25, and 327 ± 35. There was no significant difference in number of cocaine infusions across groups.

Experiment 2: Changes in D_3 Receptor Binding after Terminating a Low Dose Self-Administration Regimen and Repeated 7-OH-DPAT Administration

Animals killed 22 days after terminating the cocaine self-administration regimen exhibited increases in D_3 receptor binding, and the effects were attenuated by repeated 7-OH-DPAT administration (see Figure 4). Separate ANOVAs of [^{125}I]-7-OH-PIPAT binding in each region revealed significant group effects in the NAc core and the VM, VL, and DM subregions of the CPu ($F_{(2,17)} = 3.88$–5.21, $p < 0.05$). In these regions, [^{125}I]-7-OH-PIPAT binding was significantly

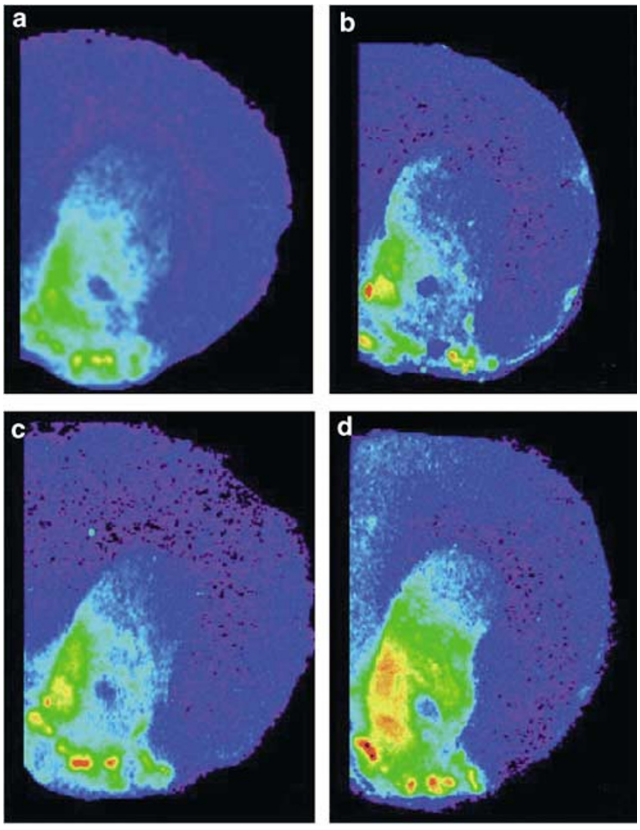

Figure 2 Color-encoded images of total [^{125}I]-7-OH-PIPAT binding in a control animal (a), and in animals that were killed either 2 days (b), 8 days (c), or 31–32 days (d) after terminating the cocaine self-administration regimen (0.75 mg/kg/0.1 infusion, i.v., 3 h/day for 14 days) in Experiment 1. At 24 h prior to killing, all animals received a challenge injection of cocaine–HCl (15 mg/kg, i.p.). The concentration of [^{125}I]-7-OH-PIPAT binding from lowest to highest is represented by blue, green, yellow, orange, and red, respectively.

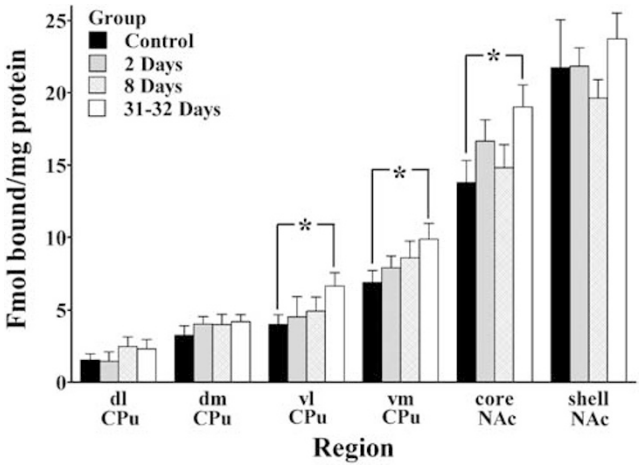

Figure 3 Quantified dopamine D_3 receptor binding (mean fmol [^{125}I]-7-OH-PIPAT bound/mg protein + SEM) in animals from Experiment 1 that were killed either 2, 8, or 31–32 days after terminating the cocaine self-administration regimen (0.75 mg/kg/0.1 ml infusion, i.v., 3 h/day for 14 days) or yoked saline administration (controls). Animals remained drug-free after terminating cocaine self-administration, except for a challenge injection of cocaine–HCl (15 mg/kg, i.p.) given 24 h prior to killing. Asterisks (*) represent a significant difference from controls, planned *t*-test, $p<0.05$, two-tailed. Abreviations: CPu, caudate-putamen; DL, dorsolateral; DM, dorsomedial; VL, ventrolateral; VM, ventromedial; NAc, nucleus accumbens.

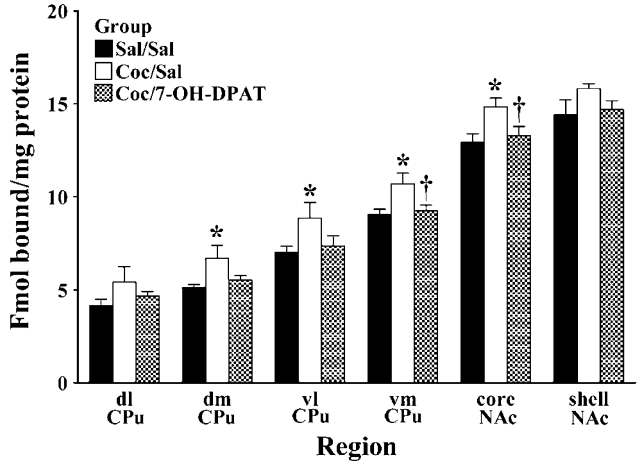

Figure 4 Quantified dopamine D_3 receptor binding (mean fmol [^{125}I]-7-OH-PIPAT bound/mg protein + SEM) in animals from Experiment 2. Group designations represent self-administration history/repeated drug treatment following termination of self-administration. Animals either self-administered cocaine–HCl (0.25 mg/kg/0.1 ml/infusion, i.v., 2 h/day for 21 days) or received saline infusions yoked to delivery of cocaine in an animal self-administering cocaine. Beginning 7 days after the last self-administration session, animals received daily injections of either saline or 7-OH-DPAT (1 mg/kg, i.p.) for 14 days, and all received a challenge injection of cocaine–HCl (15 mg/kg, i.p.) co-administered with their last saline/7-OH-DPAT treatment. The animals were killed 24 h later, corresponding to 22 days after their last self-administration session. Asterisks (*) represent a significant increase in binding relative to the Saline/Saline control group, $p<0.05$, Newman–Keuls test. Dagger represents a significant decrease in binding relative to the Cocaine/Saline group, $p<0.05$, Newman-Keuls test.

increased by 14.8, 18.2, 25.9, and 30.6%, respectively, in the Cocaine/Saline group relative to the Saline/Saline group (Newman–Keuls tests, $p<0.05$). Furthermore, [^{125}I]-7-OH-PIPAT binding was attenuated in the NAc core and VM CPu in the Cocaine/7-OH-DPAT group relative to the Cocaine/Saline group (Newman–Keuls tests, $p<0.05$) and there was no difference between the Saline/Saline and Cocaine/7-OH-DPAT groups in these regions. There were also no significant differences in the NAc shell or the DL CPu. As reported previously (Fuchs *et al*, 2002), the average daily cocaine intake of rats in this experiment was 2.16 ± 0.39 mg. The mean total number of cocaine infusions ($\pm$SEM) obtained across the 21 self-administration sessions by animals in the Cocaine/Saline and Cocaine/7-OH-DPAT groups, respectively, was 596.8 ± 167 and 519 ± 101. There was no significant difference in number of cocaine infusions across these two groups.

DISCUSSION

The findings indicate a region-specific, time-dependent increase in dopamine D_3 receptor binding across time after terminating a cocaine self-administration regimen that is attenuated by repeated administration of 7-OH-DPAT. In Experiment 1, animals that were killed 31–32 days after terminating a cocaine self-administration regimen exhibited an increase in D_3 receptor binding relative to saline-yoked controls in the NAc core and ventral CPu, but not in the

NAc shell or dorsal CPu. Animals killed either 2 or 8 days after terminating cocaine self-administration did not differ from controls. In Experiment 2, an increase in dopamine D_3 receptor binding was also found in animals that were killed 22 days after terminating self-administration. It is important to note that all animals in both experiments received a cocaine challenge approximately 24 h prior to killing, and therefore it is possible that the changes observed involve an interaction between length of time since terminating the self-administration regimen and the cocaine challenge. It is also interesting to note that the increase in binding in Experiment 2 appeared to be more expansive, although smaller in magnitude, relative to Experiment 1. Factors that may contribute to the differences across experiments are that relative to Experiment 1, Experiment 2 employed a lower cocaine self-administration dose, a longer self-administration phase with shorter access/session, and less time between self-administration and killing. There was also more variability in binding measures in Experiment 1 relative to Experiment 2, and consequently there may have been greater sensitivity for detecting changes in the dorsomedial CPu in Experiment 2. Finally, the results from Experiment 2 also indicated that repeated administration of 1 mg/kg 7-OH-DPAT on days 8–21 after terminating self-administration attenuated the increases in D_3 receptor binding. The effects of 7-OH-DPAT alone on dopamine D_3 receptor binding were not examined in the present study; however, previous research suggests that the 7-OH-DPAT regimen used does not alter D_3 receptor binding in the ventral striatum of drug-naïve animals (Stanwood *et al*, 2000b). Overall, the findings suggest that there are dynamic changes in dopamine D_3 receptor binding in the ventral striatum that occur after terminating a cocaine self-administration regimen that may be counteracted by repeated administration of a high, nonselective dose of 7-OH-DPAT.

The distribution of D_3 receptor binding across brain regions in the present study is consistent with the pattern reported previously (Lévesque *et al*, 1992; Stanwood *et al*, 2000a), with higher concentrations of binding in regions of the ventral striatum relative to the dorsal striatum. Receptor binding measures were taken in the rostral portion of the NAc because the density of D_3 receptors is greater in the rostral NAc relative to the caudal NAc (Diaz *et al*, 1995). It is important to note, however, that the core and shell subregions of the NAc are less anatomically distinct in the rostral NAc relative to the caudal NAc (Brog *et al*, 1993; Zahm and Brog, 1992; Zahm and Heimer, 1993). The finding that termination of the cocaine self-administration regimens reliably increased binding in the ventral, but not the dorsal, striatum is interesting because the ventral striatum, which includes the NAc, is thought to play an important role in the rewarding and incentive motivational effects of cocaine (Robinson and Berridge, 1993; Wise, 1996). Furthermore, it is unlikely that the lack of an effect in the dorsolateral striatum was simply due to its low concentration of D_3 receptors because regulatory responses of D_3 receptors in this region have been reported previously under similar assay conditions as used in the present study (Joyce *et al*, 2000). Thus, the region-specific effects observed in the present study suggest that the changes in D_3 receptor binding may be functionally related to plasticity in the neural systems thought to underlie the rewarding and incentive motivational effects of cocaine.

Dopamine D_3 receptors typically exhibit a paradoxical downregulation in response to dopamine depletion (Guillin *et al*, 2001; Lévesque *et al*, 1995; Morissette *et al*, 1998; Stanwood *et al*, 2000b) and upregulation in response to dopamine and dopamine agonist administration (Cox *et al*, 1995; Fauchey *et al*, 2000; Stanwood *et al*, 2000b). Therefore, we suggest that the increase in D_3 receptor binding during the course of abstinence from cocaine self-administration may involve enhancement in synaptic concentrations of dopamine. Indeed, dialysate measures in the amygdala from animals in Experiment 1 that have been reported previously (Tran-Nguyen *et al*, 1998) demonstrate a time-dependent increase in baseline dopamine levels and dopamine levels after a cocaine priming injection in animals abstinent for 1 month relative to saline-yoked controls. These increases in dopamine may be due to enhanced phasic release in response to either environmental stimuli (baseline) or to the cocaine challenge that emerges during the course of abstinence from a cocaine self-administration regimen, and which may in turn underlie the increase in dopamine D_3 receptor binding in the ventral striatum observed in the present study. Alternatively, D_3 receptors are also upregulated by brain-derived neurotrophic factor (BDNF, Guillin *et al*, 2001), and BDNF also exhibits a time-dependent increase during the course of abstinence from a cocaine self-administration regimen (Grimm *et al*, 2003). Thus, an abstinence-induced increase in BDNF may also underlie the increase in D_3 receptor binding observed in the present study. These are not mutually exclusive hypotheses since increased DA may also regulate the release of BDNF (Guillin *et al*, 2001).

There have been some discrepancies among previous studies examining cocaine-induced changes in D_3 receptor binding and mRNA levels that may be reconciled, at least in part, by considering the influence of the cocaine administration regimen on tonic *vs* phasic dopamine concentrations. For instance, increases in dopamine D_3 receptor mRNA and/or binding are observed in cocaine overdose fatalities and in rats challenged with cocaine after experiencing protracted abstinence from a cocaine self-administration regimen (present findings; Segal *et al*, 1997; Staley and Mash, 1996). In these studies, phasic dopamine levels were likely abnormally high, and we speculate that the increase in phasic dopamine may invoke D_3 receptor regulatory responses. Furthermore, it is reasonable to suggest that an episode leading to cocaine overdose would be sufficient to upregulate D_3 receptors given that agonist-induced upregulation has been shown to occur rapidly in cell lines, reaching a maximal level within 6–8 h during acute exposure (Cox *et al*, 1995). In contrast, another post-mortem analysis of brains from drug abusers failed to observe a change in D_3 receptor mRNA (Meador-Woodruff *et al*, 1995). The lack of effect in the latter study may be due to a lack of an increase in phasic dopamine in close proximity to harvesting brain tissue given that there was a fairly long post-mortem interval (mean of 72 h) and an undetermined amount of cocaine consumed prior to death. Finally, with a 24-h abstinence period, intermittent intravenous administration of cocaine increases D_3 receptor binding in the CPu and decreases D_3 receptor binding in the

NAc (Wallace *et al*, 1996), whereas continuous subcutaneous administration of cocaine fails to alter D_3 receptor binding (Stanwood *et al*, 2000b). The lack of effect in the latter case may be due to a lack of phasic increase in dopamine levels since continuous administration produces an increase in tonic, rather than phasic, levels of dopamine that may not be sufficient to regulate D_3 receptors. In contrast, intermittent intravenous administration of cocaine results in phasic increases in dopamine, which may have induced D_3 receptor regulatory responses. Specific regulatory changes in dopamine D_3 receptor binding observed after cocaine regimens may vary across time and brain regions in a manner dependent on the specific regimen employed given the differences in region-specific effects observed across studies.

It was somewhat surprising that repeated 7-OH-DPAT administration attenuated the time-dependent increase in D_3 receptor binding given that previous research suggests D_3 receptors are upregulated by dopamine and dopamine agonists (Cox *et al*, 1995; Fauchey *et al*, 2000; Stanwood *et al*, 2000b). However, repeated administration of dopamine agonists does not always follow this general rule. For instance, although Fauchey *et al* (2000) observed increases in dopamine D_3 receptor mRNA in the NAc of dopamine transporter knockout mice with enhanced extracellular dopamine levels, Stanwood *et al* (2000b) failed to observe increases in D_3 receptor density in the NAc of cocaine-naïve animals following a similar repeated 7-OH-DPAT regimen as that used in the present study. These findings suggest that dopamine and dopamine agonists may differentially regulate dopamine D_3 receptors in the NAc, which may result from differences in relative amounts of stimulation of dopamine receptor subtypes. Indeed, some regulatory responses of dopamine D_3 receptors may involve interactions with other dopamine receptor subtypes. For instance, downregulation of D_3 receptors resulting from lesions of dopamine neurons is reversed by the D_1-like receptor agonist SKF 82958, but not by the D_2-like receptor agonist cabergoline (Morissette *et al*, 1998). In fact, cabergoline further decreased D_3 receptor binding in the CPu (Morissette *et al*, 1998), similar to the decrease in D_3 binding observed in the ventral striatum with 7-OH-DPAT in the present study. Collectively, the findings suggest that dysregulated striatal D_3 receptors are susceptible to downregulation by chronic D_2-like agonist administration.

The functional significance of the increase in dopamine D_3 receptor binding in the ventral striatum observed in the present study remains to be determined; however, we suggest that it may be related to enhanced motivation for cocaine. This suggestion is based on the observation that animals abstinent from cocaine self-administration for 31–32 days exhibit concomitant enhancement of dopamine D_3 receptor binding and cocaine-seeking behavior in extinction and following a cocaine prime relative to animals abstinent for a shorter period of time (Tran-Nguyen *et al*, 1998). Furthermore, dopamine D_3 receptors are elevated in cocaine overdose fatalities, in which the subjects presumably died while, or shortly after, experiencing motivation for cocaine. Moreover, repeated administration of the D_3-preferring agonist 7-OH-DPAT attenuated the increase in D_3 receptor binding and *decreased* cocaine-seeking behavior in these animals when they were placed back into the cocaine self-administration environment 17–23 h after their last 7-OH-DPAT administration without cocaine available (Fuchs *et al*, 2002). Collectively, these findings suggest that increased dopamine D_3 receptor binding is associated with an increase in incentive motivation for cocaine.

The mechanism underlying the relationship between the increase in D_3 receptor binding and increased motivation is likely complex and may involve interactions with other dopamine receptor subtypes. In the Fuchs *et al* (2002) study for instance, cocaine-experienced animals that received repeated 7-OH-DPAT administration and were tested in a drug-free state 17–23 h later exhibited decreased cocaine-seeking behavior, whereas another group tested after a high-dose 7-OH-DPAT challenge (1 mg/kg, s.c.) exhibited *enhanced* cocaine-primed reinstatement of cocaine-seeking behavior. The opposite changes in cocaine-seeking behavior in these animals when tested in a drug *vs* drug-free state may involve differences in relative stimulation of dopamine D_2 and D_3 receptors in these states. In a cocaine-free state 17–23 h after 7-OH-DPAT administration, the relatively low synaptic concentration of dopamine would preferentially bind to dopamine D_3 receptors because dopamine has a higher affinity for D_3 receptors relative to other dopamine receptor subtypes (Seeman, 1999; Sokoloff *et al*, 1992). Furthermore, we suggest that an action of dopamine at D_3 receptors may have an inhibitory effect on cocaine-seeking behavior based on our previous finding that acute administration of low, presumably D_3-preferring, doses of 7-OH-DPAT decreases cocaine-seeking behavior (Fuchs *et al*, 2002). Moreover, it seems likely that a higher proportion of available D_3 receptors would be occupied by endogenous dopamine in animals receiving repeated 7-OH-DPAT administration relative to saline-treated controls because the former group exhibited a decrease in D_3 receptor binding relative to the latter group. By contrast, following cocaine and 7-OH-DPAT administration, both 7-OH-DPAT and the relatively high cocaine-induced synaptic concentration of dopamine would likely bind to a large proportion of D_2 receptors. The increased stimulation of D_2 receptors relative to D_3 receptors would likely facilitate cocaine-seeking behavior as suggested previously (De Vries *et al*, 2002; Self *et al*, 1996). Furthermore, it has been suggested that tolerance to the inhibitory effects of D_3 receptor stimulation may contribute to sensitization of D_2 receptor-mediated behaviors following repeated administration of dopamine agonists (Richtand *et al*, 2001). Consistent with this idea, we observed cross tolerance to 7-OH-DPAT-induced hypolocomotor activity in cocaine-experienced animals (Fuchs *et al*, 2002), and this behavior is thought to be mediated by preferential binding to dopamine D_3 receptors (Daly and Waddington, 1993). This finding suggests that even though dopamine D_3 receptor binding is increased, there is tolerance to the functional inhibitory effects of 7-OH-DPAT. This putative functional tolerance to inhibitory effects of 7-OH-DPAT following cocaine self-administration experience may contribute to the enhanced cocaine-primed reinstatement of cocaine-seeking behavior observed after prolonged abstinence.

In summary, termination of a cocaine self-administration regimen increases dopamine D_3 binding over time, which

we speculate may occur through regulatory responses to an increase in phasic dopamine levels and/or BDNF. Further research is needed to test these hypotheses and to determine whether the increases in D_3 receptor binding observed in this study are due to a change in receptor density and/or affinity. In any case, the increase in D_3 receptor binding is accompanied by an increase in cocaine-seeking behavior (Tran-Nguyen *et al*, 1998), and both of these measures are attenuated by repeated 7-OH-DPAT administration (Fuchs *et al*, 2002; present findings). These findings suggest that D_3 receptors may play an important role in mediating cocaine-seeking behavior and may provide a useful target for developing medications for cocaine dependence.

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