Cocaine Sensitization: Modulation by Dopamine D2 Receptors

Repeated administration of cocaine progressively increases drug-induced locomotor activity. This study examined the role of dopamine D1- and D2-like receptors in the medial prefrontal cortex (mPFC) in mediating these sensitized behaviors. For initiation experiments, animals received bilateral intramPFC injections of either saline, the D2-like agonist SKF 81297 (3 nmol/side) or the D2-like agonist quinpirole (5 nmol/side) 5 min before each of four daily peripheral injections of saline or cocaine (15 mg/kg i.p.). Following 1 week of withdrawal, the animals were challenged with a systemic injection of cocaine. For expression studies, the animals received four daily systemic injections of either saline or cocaine and 1 week later were pre-treated with an intramPFC injection of saline, SKF 81297 or quinpirole 5 min before receiving a systemic challenge injection of cocaine. IntramPFC injection of quinpirole blocked the initiation and attenuated the expression of cocaine-induced behavioral sensitization. In contrast, intramPFC SKF 81297 did not alter the induction or expression of behavioral sensitization to cocaine at the dose tested. In addition, in vivo microdialysis studies demonstrated that intracortical quinpirole administration blocked the initiation and blunted the expression of cocaine-induced neurochemical sensitization, as defined by augmented dopamine concentrations in the nucleus accumbens. Collectively, the results show that activation of D2-like receptors in the mPFC may alter the enduring changes responsible for the development of cocaine sensitization.

Introduction

Repeated intermittent administration of cocaine, amphetamine or related psychostimulants elicits a progressive enhancement of locomotor activity and stereotypic behaviors (Kalivas and Duffy, 1990; Kalivas et al., 1993; Robinson and Berridge, 1993). This phenomenon is termed behavioral sensitization or reverse tolerance. It has been conjectured that the neuroadaptive changes governing the development of behavioral sensitization also underlie various facets of human drug dependence and accompanying psychopathologies (Wise and Bozarth, 1987; Robinson and Berridge, 1993; Kalivas, 1995). In this light, sensitization is argued to be a useful animal model of drug addiction. It is widely accepted that the initiation and expression of sensitization result from drug-induced alterations in dopamine (DA) transmission in the ventral tegmental area (VTA) and the nucleus accumbens (NAc) respectively (Kalivas et al., 1993; Robinson and Berridge, 1993). However, accumulating evidence indicates that additional brain regions may contribute to or be responsible for the development of behavioral sensitization.

The medial prefrontal cortex (mPFC), a terminal region of the mesocorticolimbic DA system and a region that innervates the VTA and the NAc, has been implicated in sensitization processes. For instance, behavioral sensitization to cocaine is paralleled by an attenuation of drug-induced elevations in cortical DA concentrations (Sorg and Kalivas, 1993; Sorg et al., 1997) and dopaminergic tone (White et al., 1995), while DA-depleting lesions of the mPFC produce ‘sensitized-like’ responses to subsequent psychostimulant administration (Carter and Pycock, 1980; Sokolowski and Salamone, 1994; Banks and Gratton, 1995; Beyer and Steketee, 1999). Furthermore, repeated electrical stimulation of the mPFC is reported to induce sensitization (Schenk and Snow, 1994), while excitotoxic lesions of the mPFC (Pierce et al., 1998; Tschenkte and Schmidt, 1998; Cador et al., 1999; Li et al., 1999; Tschenkte and Schmidt, 2000) and amphetamine administration into this region (Ben-Shahar and Ettenberg, 1998; Prasad et al., 1999) disrupt the development of behavioral sensitization.

Previously, we have shown that intramPFC microinjection of quinpirole, a DA D2-like agonist, dose dependently blocked acute cocaine-induced locomotor activity and increases in DA overflow in the NAc (Beyer and Steketee, 2000). In contrast to these findings, intramPFC injection of either a selective D1 agonist (Beyer and Steketee, 2001) or a partial (Beyer and Steketee, 2000) or full (Beyer and Steketee, 2001) D1 agonist did not alter the acute motor-stimulant response to cocaine. Collectively, these data suggest that D2 receptor activation in the mPFC can block the acute locomotor effects of cocaine. However, the potential involvement of cortical DA receptors in mediating the development of sensitization has yet to be determined. In the present studies we therefore examined the effects of intramPFC microinjections of DA D1- and D2-like receptor agonists, SKF 81297 and quinpirole respectively, on the initiation and expression phases of behavioral sensitization to cocaine. Based on the results of these experiments, in vivo microdialysis techniques were employed for determining the effects of intracortical quinpirole infusions on cocaine-induced DA concentrations in the NAc (which is defined as neurochemical sensitization).

Materials and Methods

Animals

One hundred and seventy-five male Sprague–Dawley rats (Harlan, Indianapolis, IN), weighing 250–300 g at the time of surgery, were individually housed in a facility approved by the American Association for the Accreditation of Laboratory Animal Care. The animals were maintained on a 12 h light:dark cycle (lights on at 7:00 a.m.) and had continuous access to food and water. All studies were performed according to the National Institutes of Health (NIH) Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1987) and were approved by the Louisiana State University Health Sciences Center Animal Resources Advisory Committee.

Surgery

The animals were anesthetized with 3.3 ml/kg (i.p.) of Equithesin (9.72 mg/ml sodium pentobarbital and 42.50 mg/ml chloral hydrate) immediately before placement in a Kopf stereotaxic instrument (Tujunga, CA). For behavioral experiments the animals received bilateral implants of stainless-steel, 26-gauge guide cannulae (14 mm) secured 1 mm above the mPFC. The coordinates for these surgeries were anteroposterior (A/P)
Intra-mPFC / i.p.; S, saline; D, drug (quinpirole = 5 nmol/side or SKF 81297 = 3.0 nmol/side); C, cocaine (15 mg/kg, i.p.). Uppercase letters represent days when activity was monitored, and lowercase letters represent injections given in the animal’s home cage. Microdialysis experiments were conducted on days that are underlined. All intracortical injections were administered ~5 min prior to peripheral injections.

### Table 1

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Once during these experiments, with each NAc (e.g. left or right) being probed in a random fashion within each treatment group.

### In vivo microdialysis

The effects of intra-mPFC quinpirole (5 nmol/side) administration on cocaine-induced DA concentrations were studied in a separate group of animals using *in vivo* microdialysis techniques. On day 1 of the dialysis studies the animals underwent ‘sham dialysis’ procedures in which they were given injections in the experimental chambers but were not dialyzed (see Table 1). For the next 3 days, the animals received the same treatments in their home cages. Six days following the last daily injection (day 10) and ~16 h before the beginning of the dialysis experiments, concentric-style dialysis probes (5 mm active membrane) were introduced into the guide cannulae. The next morning (day 11), dialysis buffer (KCl = 2.7 mM, NaCl = 1.40 mM, CaCl2 = 1.2 mM, MgCl2 = 1.2 mM and phosphate-buffered saline (PBS) = 0.2 mM, pH = 7.4) was advanced through the dialysis probe at a rate of 2 µl/min. The dialysis probes were perfused with dialysis buffer for at least 1 h before the start of the dialysis experiments, after which four 20 min dialysis samples were collected. Intracortical injections of quinpirole or saline preceded peripheral administration of saline or cocaine by ~5 min and were given in the same manner as described above. Following injections, dialysis samples were collected in 20 min time intervals. Each animal was dialyzed only once during these experiments, with each NAc (e.g. left or right) being probed in a random fashion within each treatment group.

**Histology**

After all experiments the animals were overdosed with sodium pentobarbital and were perfused with PBS (0.2 mM) and 4% formaldehyde. Their brains were removed and stored in a solution of 30% sucrose for at least 5 days. Fixed brains were sliced on a vibratome (TPI Inc., St Louis, MO) and coronal sections (100 µm) were mounted on gelatin-coated slides and stained with cresyl violet. The placement of mPFC injections and NAc dialysis probes was verified according to *The Rat Brain in Stereotaxic Coordinates* (Paxinos and Watson, 1997). It is noteworthy to mention that severe scarring (i.e. gliosis) was not visualized in the histologies of animals receiving multiple cortical microinjections.
Results

Histology
Figure 1 shows the microinjector cannula and dialysis probe placements in the mPFC and NAc respectively. Representative photomicrographs of cresyl violet-stained coronal sections are shown on the left side of each panel. The right side of each panel shows line drawings of the injector and probe placements for all animals included in the study. The drawings were adapted from the CD-ROM version of *The Rat Brain in Stereotaxic Coordinates* (Paxinos and Watson, 1997).

Intra-mPFC Quinpirole and Behavioral Sensitization to Cocaine
Figure 2A illustrates the effect of intracortical quinpirole (5 nmol/side) pre-treatment on the acute motor-stimulant response to cocaine (15 mg/kg i.p.). Acute cocaine administration (saline/cocaine) significantly increased horizontal motor activity, an effect that was blocked by intra-mPFC pre-treatment cannula or dialysis probe placements.
with the D2-like agonist quinpirole (quinpirole/cocaine). Intracortical injection of quinpirole did not alter the locomotor response to a peripheral saline (1 ml/kg i.p.) injection (quinpirole/saline), indicating that the inhibitory effects of quinpirole were not the result of non-specific behavioral effects of the drug.

Following day 1 the animals received the same treatment for three consecutive days in their home cages (no locomotor activity was measured). Six days later (day 10) all animals were challenged with a peripheral injection of saline. On this day no differences in locomotor activity were found regardless of previous treatment (data not shown) \(F(21,168) = 1.374\) and \(P = 0.137\). The next day (day 11) the initiation of cocaine-induced behavioral sensitization was determined (Fig. 2B). A cocaine challenge produced a sensitized locomotor response during the first 45 min post-injection in animals previously exposed to cocaine [cocaine (saline/saline)] as compared to animals receiving cocaine for the first time [cocaine (saline/saline)]. However, the animals that were pre-treated with quinpirole before each of their daily cocaine injections [cocaine (quinpirole/cocaine)] did not show an enhanced behavioral response to a challenge injection of cocaine. Interestingly, the behavioral
responses of these animals were not shown to be significantly different from the animals receiving cocaine for the first time [cocaine (saline/saline) or cocaine (quinpirole/saline)].

Figure 3 illustrates the effects of quinpirole on the expression of cocaine-induced behavioral sensitization. On day 1 (Fig. 3A) systemic cocaine produced an increase in horizontal motor activity for at least the initial 60 min of the test session as compared to saline-treated animals. The animals received the same treatment (i.e. saline or cocaine) for three consecutive days in their home cages. Six days later (day 10) all animals were challenged with a peripheral injection of saline preceded by an intra-mPFC injection of saline or quinpirole. On this day locomotor activity did not differ between treatment groups (data not shown) [F(21,161) = 0.980 and P = 0.491]. The next day (day 11) the animals received an intra-mPFC injection of saline or quinpirole before a peripheral challenge injection of cocaine (Fig. 3B). A cocaine challenge [saline/cocaine (saline)] in animals previously exposed to cocaine evoked a sensitized locomotor response during the first 45 min post-injection when compared to control animals [saline/cocaine (saline)]. Conversely, intra-mPFC quinpirole administration given before a cocaine challenge injection [quinpirole/cocaine (cocaine)] attenuated the sensitized response in animals previously exposed to cocaine. This latter effect was not observed to be...
significantly different from those animals receiving their first injection of cocaine [saline/cocaine (saline)]. It was also demonstrated that intra-mPFC quinpirole administration blocked the locomotor response of animals receiving cocaine for the first time [quinpirole/cocaine (saline)].

**Intra-mPFC SKF 81297 and Behavioral Sensitization to Cocaine**

Figure 4 illustrates the effect of intracortical SKF 81297 (3 nmol/side) pre-treatment on the acute motor-stimulant response to cocaine (15 mg/kg i.p.). Acute cocaine administration (saline/cocaine) significantly increased horizontal motor activity for at least 75 min post-injection, an effect that was unaltered by intracortical injection of the selective D1 agonist SKF 81297 (SKF 81297/cocaine). In addition, the dose of SKF 81297 was not demonstrated to alter the locomotor response to a peripheral saline injection (SKF 81297/saline).

Following day 1 the animals received the same treatment for three consecutive days in their home cages (no locomotor activity was measured). Six days later (day 10) all animals received a systemic injection of saline regardless of previous treatment. On this day no differences in locomotor activity were observed (data not shown). Next day (day 11) the effects of intracortical SKF 81297 treatment on the initiation of cocaine-induced behavioral sensitization were determined (Fig. 4B). A cocaine challenge produced a sensitized locomotor response during the first 45 min post-injection in animals previously exposed to cocaine [cocaine (saline/cocaine)] as compared to animals receiving cocaine for the first time [cocaine (saline/saline)]. This effect was unaltered in animals pre-treated with SKF 81297 before each of their daily cocaine injections [cocaine (SKF 81297/cocaine)]. In addition, repeated injections of SKF 81297 into the mPFC did not alter the response to the first cocaine exposure [cocaine (SKF 81297/saline)].

Figure 5 illustrates the effects of SKF 81297 on the expression of cocaine-induced behavioral sensitization. On day 1 (Fig. 5A) systemic cocaine produced an increase in horizontal motor activity for at least the initial 60 min of the test session as compared to saline-treated animals. The animals received the same treatments in their home cages for the following 3 days. Six days after their last daily injection (day 10) the animals were given an intra-mPFC injection of either saline or SKF 81297 before a peripheral injection of saline. On this day locomotor activity was not found to be different between treatment groups (data not shown). A systemic cocaine challenge injection [saline/cocaine (cocaine)] produced a sensitized locomotor response during the first 45 min post-injection in animals previously exposed to cocaine as compared to animals receiving cocaine for the first time [saline/cocaine (saline)]. This effect was unchanged in animals receiving intra-mPFC SKF 81297 pre-treatment before the cocaine challenge [SKF 81297/cocaine (cocaine)].

**Intra-mPFC Quinpirole and Microdialysis**

Based on the findings from the behavioral studies, the effects of intra-mPFC quinpirole (5 nmol/side) administration on cocaine (15 mg/kg i.p.)-induced increases in extracellular DA concentrations in the NAc were examined (neurochemical sensitization). In vitro microdialysis techniques were employed only on cocaine challenge day 11 (see Table 1). The effects of intracortical

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**Figure 6.** Effects of intra-mPFC quinpirole (5 nmol/side) microinjection on the initiation of neurochemical sensitization as defined by cocaine-induced (15 mg/kg i.p.) DA concentrations in the NAc. The measurements of DA were made following the cocaine challenge and are expressed as mean percent of baseline ± SEM. The basal DA concentrations did not statistically differ between treatment groups (see the Results section for values). The arrow represents the time that injections were administered. Significant differences between groups were determined by repeated-measures ANOVA and multiple comparisons were made according to methods described elsewhere (Milliken and Johnson, 1984). The F-scores were as follows. Treatment effect, $F(3,24) = 1.750$ and $P = 0.184$; time effect, $F(11,264) = 7.903$ and $P < 0.01$; interaction effect, $F(33,264) = 1.677$ and $P = 0.022$.

**Figure 7.** The effects of intra-mPFC quinpirole (5 nmol/side) injection on the expression of neurochemical sensitization to cocaine (15 mg/kg i.p.). The measurements of DA in the NAc were made following the cocaine challenge and the neurochemical data are expressed as mean percent of baseline ± SEM. The basal DA concentrations did not statistically differ and the values are reported in the Results section. The arrow represents the time that injections were administered. Significant differences between groups were determined by repeated-measures ANOVA and multiple comparisons were made according to methods described elsewhere (Milliken and Johnson, 1984). The F-scores were as follows. Treatment effect, $F(3,25) = 1.280$ and $P = 0.303$; time effect, $F(11,275) = 18.196$ and $P < 0.01$; interaction effect, $F(33,275) = 3.720$ and $P < 0.01$. 

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quinpirole on the initiation of neurochemical sensitization are illustrated in Figure 6. The basal (i.e. pre-injection) concentrations of DA [43.3 ± 1.5 fmol/20 min cocaine (saline/saline), 39.3 ± 1.2 fmol/20 min cocaine (saline/cocaine), 41.6 ± 1.7 fmol/20 min cocaine (quinpirole/saline), and 46.3 ± 1.0 fmol/20 min cocaine (quinpirole/cocaine)] did not significantly differ in rats from each treatment group (F(3,24) = 0.378 and P = 0.794). A peripheral challenge injection of cocaine significantly increased DA concentrations in the NAc of animals previously exposed to cocaine [cocaine (saline/cocaine)] as compared to animals receiving cocaine for the first time [cocaine (saline/saline)]. However, the animals that received intra-mPFC quinpirole injections before each of their daily injections of cocaine [cocaine (quinpirole/cocaine)] exhibited a significantly lower dopaminergic response to a cocaine challenge. This response was not demonstrated to be significantly different from animals receiving cocaine for the first time.

The expression of neurochemical sensitization is shown in Figure 7. The basal concentrations of DA [114.3 ± 4.0 fmol/20 min saline/cocaine (saline), 84.6 ± 1.9 fmol/20 min saline/cocaine (cocaine), 119.3 ± 4.6 fmol/20 min quinpirole/cocaine (saline) and 71.6 ± 1.9 fmol/20 min quinpirole/cocaine (cocaine)] did not significantly differ in rats from each treatment group (F(3,25) = 0.43 and P = 0.731). A cocaine challenge produced a sensitized DA response in the NAc [saline/cocaine (cocaine)] in animals previously exposed to cocaine. This effect was attenuated in rats that received an intra-mPFC quinpirole injection before their peripheral challenge injection of cocaine [quinpirole/cocaine (cocaine)]. This response was not found to be significantly different from animals that were injected with cocaine for the first time [saline/cocaine (saline)]. Similar to previous findings (Beyer and Steketee, 2000), intra-mPFC quinpirole administration produced a strong trend, albeit not statistically significant, towards blunting acute cocaine-induced DA concentrations in the NAc [quinpirole/cocaine (saline)]. Locomotor activity was simultaneously measured throughout both components of the microdialysis studies and was found to parallel the earlier behavioral findings in this study (data not shown).

Discussion
The present study demonstrated that intra-mPFC microinjection of quinpirole blocked the acute motor-stimulant response as well as the induction of behavioral and neurochemical sensitization to cocaine. Once sensitization was induced, intracortical administration of quinpirole attenuated the cocaine-induced behavioral and neurochemical responses, producing an effect indistinguishable from that of animals receiving cocaine for the first time. In contrast, intra-mPFC injections of SKF 81297 were not found to alter the development of behavioral sensitization at the dose tested. The main conclusion from these experiments is that DA D2 receptor activation in the mPFC, in particular the cingulate and infralimbic cortices, can alter the development of sensitization to cocaine.

Cortical DA receptors and Locomotor Activity
Dopaminergic innervation of the mPFC functions to inhibit cortical excitatory efferent projections that innervate subcortical areas (Carter and Pycock, 1980; Thierry et al., 1986; Beyer and Steketee, 1999). This hypothesis is congruent with the ability of cortical DA transmission for inhibiting spontaneous (Bubser and Schmidt, 1990), novelty-induced (Radcliffe and Erwin, 1996) and psychostimulant-induced (Vezina et al., 1991; Banks and Gratton, 1995; Beyer and Steketee, 1999; Prasad et al., 1999) locomotor activity. Since the mPFC provides a significant drive to subcortical sites including the mesolimbic and nigrostriatal DA systems (Byne and Davis, 1999) a decrease in cortical DA transmission would theoretically remove the inhibitory influence of DA in this region and produce exaggerated responses in subcortical areas that govern locomotor activity (Deutch, 1992). Recent studies (Sorg et al., 1997) have supported this conjecture by showing that, unlike DA transmission in the mesoaccumbens system, cocaine-induced increases in cortical DA concentrations are attenuated rather than enhanced in sensitized animals. From this study, it may be inferred that behavioral sensitization to cocaine results in part from a decrease in cortical DA levels.

Cortical DA receptors and Acute Locomotor Activity
Despite these findings, little is known about which DA receptor subtypes (D1 like versus D2 like) in the mPFC mediate the purported inhibitory influence of DA in this region. In the present study, it was found that intra-mPFC injection of the D2-like agonist quinpirole blocked the acute motor-stimulant response to cocaine. This finding is consistent with and extends those of previous reports to show that cortical D2 receptor activation blocks the acute locomotor response to psychostimulants (Karler et al., 1998a; Beyer and Steketee, 2000, 2001). While quinpirole has been shown to bind to other types of DA receptor subtypes (Malmberg and Mohell, 1995) the inhibitory behavioral effects of quinpirole in the present study are suggested to occur by D2 receptor mechanisms. First, co-administration of the selective D2 antagonist sulpiride into the mPFC blocked quinpirole-induced decreases in the motor-stimulant response to cocaine (Beyer and Steketee, 2001). Second, intracortical injection of selective D2 (Karler et al., 1998a; Beyer and Steketee, 2001) and D1 (Beyer and Steketee, 2000) agonists were not found to disrupt the acute locomotor response to cocaine.

One concern associated with studies involving intracranial drug injection is the potential effects of diffusion of the drug away from the injection site. Of particular concern in the present study is the possibility that the drug diffused from the mPFC into the NAc, which then produced alterations in cocaine-induced activity. Several points argue against this concern. First, direct infusion of quinpirole into the NAc has been reported to increase motor activity (Gong et al., 1999), an effect not seen when quinpirole was injected into the mPFC. Second, infusion of dopamine antagonists rather than agonists into the NAc has been shown to decrease the stimulant response to cocaine (Neisewander et al., 1995; Baker et al., 1996). Finally, in a previous study we demonstrated that injections of 6-hydroxydopamine into the mPFC depletes dopamine in this region, but did not affect dopamine levels in the NAc (Beyer and Steketee, 1999). Thus, it is unlikely that the inhibitory effects seen following infusion of quinpirole into the mPFC on cocaine-induced motor activity was the result of diffusion of this drug into the NAc.

Cortical DA receptors and Behavioral Sensitization
Consistent with previous reports (Post and Rose, 1976; Kalivas and Duffy, 1990), repeated and intermittent peripheral injections of cocaine produced sensitized locomotor responses to a subsequent cocaine challenge. In the present study, this effect was not observed to be the result of conditioned effects of the treatment regimen because the animals were not found to behave differently when challenged with a systemic saline injection (data not shown). Administration of quinpirole into
the mPFC before each daily peripheral injection of cocaine (initiation) or before the cocaine challenge (expression) blocked and attenuated the sensitized behaviors respectively. These findings are consistent with the ability of intracortical injections of amphetamine, an indirect DA agonist, for decreasing psychostimulant-induced behavioral sensitization (Ben-Shahar and Ettenberg, 1998; Prasad et al., 1999). Furthermore, the present study also showed that intracortical quinpirole injections blocked mesolimbic DA responses to cocaine, an effect consistent with reports showing that alterations in DA transmission in the mPFC mediated DA concentrations in the NAc in response to psychostimulants (Taber and Fibiger, 1995; Beyer and Steketee, 1999) and to stress (Deutch et al., 1990). Taken together, these studies suggest that D2 receptors in the mPFC are important in mediating the development of behavioral sensitization to cocaine and their activation can alter subcortical DA responses to cocaine.

Unlike the effects of quinpirole, intra-mPFC injection of SKF 81297 did not alter the acute or the sensitized behavioral responses to cocaine. This finding is consistent with previous studies showing that intracortical D1 agonist administration did not significantly affect psychostimulant-induced stereotypy or motor activity (Karler et al., 1998a; Beyer and Steketee, 2000, 2001). However, the present findings are inconsistent with recent studies showing that intra-mPFC injection of SKF 81297 blocked the expression of cocaine-induced behavioral sensitization (Sorg et al., 2001) and the mesolimbic DA response to stress (Doherty and Gratton, 1996). The present results also differ from previous reports showing that behavioral sensitization to amphetamine is blocked by systemic injection of a D1 antagonist (Vezina and Stewart, 1989; Vezina 1996). These findings suggest that D1 receptors in the mPFC could be important in mediating the development of sensitization. It is not clear why the differences between these studies exist. It is noteworthy to mention that the region of the mPFC injected varies between studies. An earlier study (Sorg et al., 2001) injected SKF 81297 into more dorsolateral regions of the mPFC, while in the present study SKF 81297 was injected into the more ventral areas. In addition, it should be noted that, in some studies, peripheral injections of the D1 drugs were administered (Vezina and Stewart, 1989; Vezina, 1996). Therefore, DA receptors in other brain regions and in the periphery may also be involved in the development of sensitization. For example, infusion of the D1 antagonist SCH 33390 into the VTA blocked the acute stimulant response to amphetamine and cocaine (Vezina, 1996; Steketee and Braswell, 1997; Steketee, 1998). However, intra-VTA injections of SCH 23390 blocked the development of behavioral sensitization to amphetamine, but not to cocaine. An alternative study demonstrated that infusion of the D1 agonist SKF 82958 into the NAc enhanced the development of sensitization (De Vries et al., 1998). Thus, non-cortical dopamine D1 receptors may be more important than cortical dopamine receptors in the development of sensitization to cocaine. Finally, another difference between this and other studies is the dose of drug administered. For example, a previous study (Sorg et al., 2001) found that a lower dose of SKF 81297 blocked the expression of cocaine sensitization. However, in a previous report we found that doses of SKF 81297 comparable to those used in that study (Sorg et al., 2001) did not alter acute cocaine-induced locomotor activity (Beyer and Steketee, 2001).

Neural Circuitry in the mPFC
Quantitative autoradiographic assays have demonstrated that both D1 and D2 receptors are located in the less superficial layers of the mPFC (Boyson et al., 1986). It is likely that quinpirole blocked the development of sensitization by acting at DA D2 receptors (see earlier comments). However, the neuroanatomical mechanisms by which quinpirole decreases the development of sensitization are unclear. One hypothesis is that D2 receptors residing on excitatory amino acid (EAA) cell bodies in the mPFC (Seguela et al., 1988) may serve to attenuate excitatory output from this region directly, ultimately dampening psychostimulant-induced locomotor activity. Alternatively, D2 receptors are located on γ-aminobutyric acid (GABA)ergic interneurons in the mPFC (Pesack et al., 1995) and have been demonstrated to interact with both DA neurons and EAA cell bodies in the mPFC (Penit-Soria et al., 1987; Linderfors et al., 1989). Support for this latter mechanism comes from studies showing that D2 but not D1 receptor agonists increase cortical GABA concentrations in vivo (Grobin and Deutch, 1998) and in cortical neuronal slices (Retaux and Besson, 1991). In consideration of these findings, it may be hypothesized that intra-mPFC quinpirole administration either directly or indirectly dampened excitatory output from the mPFC and subsequent responses to cocaine.

Interestingly, quinpirole pre-treatment in the mPFC in animals previously exposed to cocaine (expression studies) did not completely block cocaine-induced locomotor activity and DA concentrations in the NAc. Thus, the ability of intra-mPFC quinpirole to block cocaine-induced motor activity completely is attenuated in animals sensitized to cocaine. These data are similar to results reported earlier (Karler et al., 1998b), in which the ability of intracortical injections of the dopamine D2 agonist PDPT in blocking amphetamine-induced motor activity is attenuated in sensitized animals. Such findings may reflect changes in cortical D2 receptors that are a direct result of repeated cocaine-induced changes in the receptor binding site density (Kleven et al., 1990). A role for enhanced activity of protein kinase C, an enzyme that readily phosphorylates D2 receptors (Elazar and Fuchs, 1991), has also been reported in the mPFC of animals sensitized to cocaine (Steketee et al., 1998). Therefore, cocaine-induced changes in the levels of this protein kinase could conceivably result in changes in the phosphorylation state and subsequent function of D2 receptors. Such molecular events may have produced less functional cortical D2 receptors and, thus, contributed to the present results. However, more research is required in order to support such a hypothesis.

Summary
The mPFC is heavily innervated by DA neurons originating in the VTA (Oades and Halliday, 1987). DA receptors are located in a critical position for mediating cocaine-induced DA transmission in the mPFC (Boyson et al., 1986; Fuster 1997) and, as such, may play an important role in mediating the development of behavioral sensitization to cocaine. The present study supports this hypothesis and showed that D2 receptor activation could disrupt the development of behavioral sensitization. However, other studies have suggested an equally important role for D1 receptors in psychostimulant sensitization (Vezina and Stewart, 1989; Vezina 1996; Sorg et al., 2001). When considering the discrepancies between these studies, it is important to note that DA in the mPFC has been recently conjectured to be a modulatory neurotransmitter (Yang et al., 1999). Therefore, depending on the contribution and interactions of DA with other cortical systems, including GABA and glutamate, different responses may occur. These complex interactions between multiple neurotransmitter systems and/or brain regions warrant consideration when interpreting the results of this and other studies.
Notes
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