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Elimination of Cocaine-Induced Hyperactivity and Dopamine-Mediated Neurophysiological Effects in Dopamine D1 Receptor Mutant Mice

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Summary

The brain mesoaccumbens dopamine system is intricately involved in the psychomotor stimulant activities of cocaine. However, the extent to which different dopamine receptors mediate these effects has not yet been firmly established. The present study used dopamine D1 receptor mutant mice produced by gene targeting to investigate the role of this receptor in the effects induced by cocaine. In contrast with wild-type mice, which showed a dose-dependent increase in locomotion, D1 mutant mice exhibited a dose-dependent decrease. Electrophysiological studies of dopamine-sensitive nucleus accumbens neurons demonstrated a marked reduction in the inhibitory effects of cocaine on the generation of action potentials. In addition, the inhibitory effects of dopamine as well as D1 and D2 agonists were almost completely abolished, whereas those of serotonin were unaffected. D2-like dopamine receptor binding was also normal. These results demonstrate the essential role of the D1 receptor in the locomotor stimulant effects of cocaine and in dopamine-mediated neurophysiological effects within the nucleus accumbens.

Introduction

The neurotransmitter dopamine is expressed in brain pathways involved in many aspects of behavior. The nigrostriatal dopamine system, projecting from the substantia nigra to dorsal and lateral striatum (caudoputamen), modulates sensorimotor coordination and initiation of movement (Robbins and Everitt, 1992). This system is the site of progressive neurodegeneration in Parkinson's disease (Marsden, 1992). The mesocorticolimbic dopamine system, through its anatomical connections with the limbic system, is involved in the regulation of goal-directed (motivated) behavior (Mogenson et al., 1980; Mogenson, 1987; Robbins et al., 1989). There are two main branches of the mesocorticolimbic system, both of which originate in the midbrain ventral tegmental area, just medial to the substantia nigra. The larger mesoaccumbens projection terminates in the ventral striatal region known as the nucleus accumbens, whereas the mesocortical projection innervates primarily the medial prefrontal and anterior cingulate cortices (Lindvall and Björklund, 1983). These two projections play essential roles in the locomotor-stimulating and positive-reinforcing (rewarding, hedonic) effects of cocaine and other drugs of abuse (Wise and Bozarth, 1987; White, 1990; Koob, 1992).

Although there has been general agreement regarding the essential role played by the mesocorticolimbic dopamine system in mediating the psychomotor stimulant activities of cocaine, disagreement exists with respect to the relative contributions of different dopamine receptor subtypes. Dopamine receptors identified to date can be divided into two classes, the D1-like (D1 and D5, also referred to as $D1_A$ and $D1_B$) and the D2-like (D2, D3, and D4) receptors (Civelli et al., 1993; Gingrich and Caron, 1993). These receptor classes were originally defined on the basis of their responses to distinct sets of ligands and their linkages to distinct cascades for signal transduction. D1 receptors were shown to mediate stimulation of cAMP formation, whereas D2 receptors were found to be unlinked or negatively coupled to this cascade (Spano et al., 1978; Kebabian and Calne, 1979; Stoof and Kebabian, 1984). The functional expression of the cloned dopamine receptor genes in cell lines has helped in identifying the individual functions of the different dopamine receptors in signal transduction systems (Civelli et al., 1993; Gingrich and Caron, 1993). The roles of the different dopamine receptors in specific aspects of behavioral regulation have been more difficult to determine because of the paucity of ligands with true selectivity within the D1 and D2 subfamilies. For example, available ligands do not distinguish between D1 and D5 receptors, nor between D2 and D3 receptors. Accordingly, studies have been limited, in large part, to broad distinctions between behaviors associated, respectively, with D1 and D2 receptor subfamilies.

Both D1 and D2 receptor subfamilies have been suggested to mediate the effects of cocaine on behavior. Thus, antagonists of both D1 and D2 receptor classes have been shown to block several well-characterized behavioral effects of cocaine, including locomotor hyperactivity (Cabib et al., 1991; Tella, 1994), subjective effects measured by drug discrimination techniques (Barrett and Appel, 1989; Callahan et al., 1991; Spealman et al., 1991), and rewarding effects assessed by self-administration paradigms (Koob et al., 1987; Bergman et al., 1990; Britton et al., 1991; Hubner and Moreton, 1991; Caine and Koob, 1994). Moreover, the relevant D1-like and D2-like receptor–bearing neurons appear to be localized within the nucleus accumbens, which implicates the mesoaccumbens dopamine system in these effects of cocaine (Phillips et al., 1983; Wood and Emmett-Oglesby, 1989; Maldonado et al., 1993; Callahan et al., 1994).

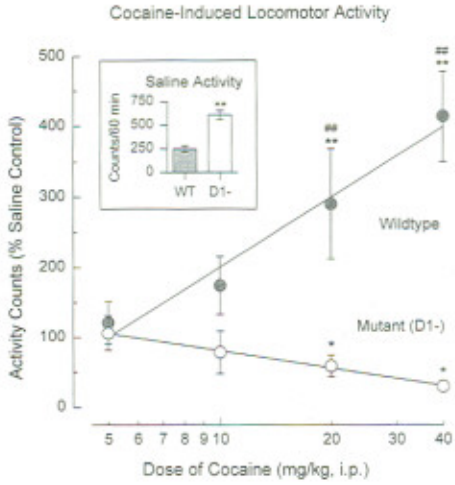

Figure 1. Dose-Response Curves for Cocaine-Induced Locomotor Activity

Because the D1 mutant mice (D1−) were significantly more active than wild-type (WT) mice during habituation to the test chamber (see text) as well as during saline injections (bar graph, inset; $^{**}t_{54} = 5.95$, $p < 0.0001$), the data have been expressed as the percentage of activity observed following saline administration (statistics were performed on raw data). Each point represents the mean of 7–11 mice. To avoid potential confounds resulting from sensitization to cocaine upon repeated administration (White et al., 1994), each mouse was tested with only one dose of cocaine or with saline. Cocaine produced a highly significant ($p < 0.001$, Kruskal–Wallis ANOVA) dose-related increase in activity counts in the wild-type mice, but a signficant ($p < 0.001$, Kruskal–Wallis ANOVA) dose-related decrease in activity counts in the mutant mice ($^{*}p < 0.05$, $^{**}p < 0.01$ as compared with saline test for that group; $\#\#p < 0.01$ as compared with D1 mutant mice at same dose, Mann–Whitney tests).

It is not surprising that multiple dopamine receptor subtypes would be involved in the effects of cocaine, given that a primary molecular target for the acute effects of cocaine is the dopamine transporter (Ritz et al., 1987; Kuhar et al., 1991). By binding to a site on the transporter, cocaine blocks the reuptake of released dopamine, causing a buildup of synaptic concentrations of the transmitter (Church et al., 1987; Di Chiara and Imperato, 1988). Accordingly, any synaptic (as well as nonsynaptic) dopamine receptor should be activated during cocaine administration. Nevertheless, there is reason for presuming that D1-like receptors are particularly important for the behavioral effects of cocaine. A considerable body of evidence indicates that D1-like receptors exert a permissive or "enabling" regulation of D2-like receptors, so that stimulation of D1-like receptors by endogenous dopamine is required for the expression of D2-like receptor–mediated behaviors (Clark and White, 1987; Waddington and Daly, 1993) and gene regulation (Paul et al, 1992). This relationship has also been observed at the single-cell level. The neurophysiological effects of D2 agonists on striatal neurons (and their targets) require D1-like receptor stimulation (Walters et al., 1987; White, 1987; Wachtel et al., 1989; White and Hu, 1993). Coregulation of intracellular signal transduction and membrane ion channel activity by the D1 and D2 receptor families has further been demonstrated in isolated striatal neuron preparations (Bertorello et al., 1990; Surmeier et al., 1992), suggesting that representatives of these receptor families are colocalized in most striatal neurons (for reviews see White and Hu, 1993; Surmeier et al., 1994; for an alternative view see Gerfen, 1992).

The enabling role of D1-like receptors has been demonstrated by using D1 receptor antagonists or manipulations that lead to acute dopamine depletion, such as inhibition of dopamine synthesis, disruption of vesicular storage mechanisms, or both. However, these techniques suffer from a relative lack of pharmacological specificity. For instance, the inhibition of dopamine synthesis also inhibits synthesis of other catecholamine neurotransmitters (e.g., norepinephrine), and drugs that disrupt vesicular stores of dopamine, such as reserpine, produce similar effects in other catecholamine-containing neurons and in indoleamine (serotonin)-containing neurons. D1 receptor antagonists do not discriminate between subtypes of D1-like receptors and also exhibit significant effects at nondopamine receptors (McQuade et al., 1988; Bischoff et al., 1986; Briggs et al., 1991).

Mutant mice lacking the D1 dopamine receptor, produced by gene targeting, provide an unprecedented opportunity to evaluate, in a more selective and precise manner, the roles of this receptor in dopamine-mediated neuronal function (Xu et al., 1994). With these D1 mutant mice, we have demonstrated an essential role for the D1 receptor in the locomotor stimulant effects of cocaine and in dopamine-mediated neurophysiological effects within the nucleus accumbens.

Results

D1 Mutant Mice Are Hyperactive in a Novel Environment

As was suggested in the initial report on the D1 mutants (Xu et al., 1994), the mutant mice exhibited greater locomotor activity than their littermate controls while habituating to a novel testing environment during the light phase of the light/dark cycle. Measurements of locomotion during the 30 min preexposure to the locomotor activity boxes were significantly higher for the D1 mutants than for their wild-type littermates (948.9 ± 92.9 counts versus 351.5 ± 38.1 counts, $t_{54} = 5.95$, $p < 0.0001$, $n = 28$ mice/group). Experiments were conducted to determine whether this hyperactivity persisted despite habituation. The results showed a decrease in the extent of hyperactivity during a 48 hr habituation period. However, during the final 30 min of that 48 hr period, the mutant mice were still more active than their wild-type littermates (362.5 ± 29.9 counts versus 129.4 ± 35.6, $t_{22} = 3.79$, $P < 0.001$, $n = 12$ mice/group). Hyperactivity in the mutant mice was also evident during measurements of locomotor activity after saline injection (Figure 1 inset).

Cocaine Fails to Induce Locomotor Hyperactivity in D1 Mutant Mice

In marked contrast with their wild-type littermates, D1 mutant mice failed to exhibit locomotor hyperactivity following treatment with cocaine over a wide range of doses (Fig-

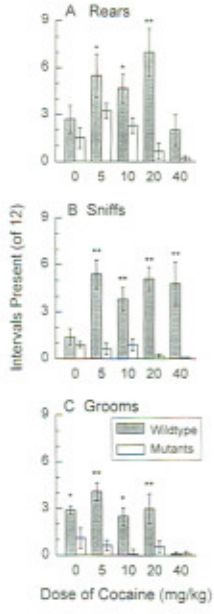

Figure 2. Effects of Cocaine on Different Aspects of Behavior Generally Referred to as Stereotypy

Cocaine caused signficant increases in rearing, sniffing, and grooming in wild-type, but not in D1 mutant mice (*$p < 0.05$, **$p < 0.01$, between groups comparison for each dose, Mann–Whitney test).

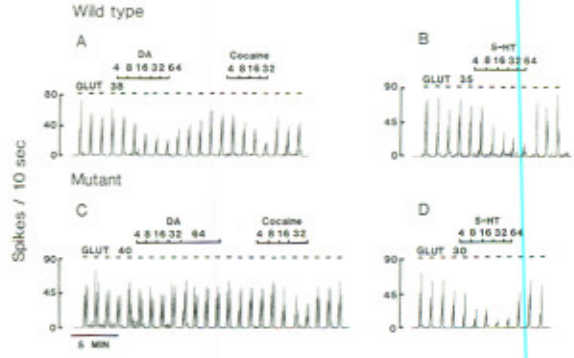

Figure 3. Cumulative Rate Histograms Illustrating the Reduced Efficacy of Dopamine and Cocaine at Inhibiting Glutamate-Activated Nucleus Accumbens Neurons in the Mutant Mice

(A and B) Dopamine (DA), cocaine, and serotonin (5-HT) inhibited glutamate (GLUT)-induced firing of nucleus accumbens neurons in wild-type mice in a current-dependent manner.

(C and D) The inhibitory effects of dopamine were completely absent in the D1 mutant mice, even when the transmitter was administered continuously for 2 min at a high ejection current. The inhibitory efficacy of cocaine was greatly diminished in the D1 mutant mice, whereas that of serotonin was unaffected. Lines represent the onset and offset of drug or transmitter iontophoresis, and numbers indicate the iontophoretic current in nanoamperes.

ure 1). Whereas the wild-type mice exhibited a dose-dependent increase in locomotor activity that was significant at the 20.0 and 40.0 mg/kg doses, the D1 mutant mice were significantly hypoactive at these higher cocaine doses. This hypoactivity could not be attributed to an induction of more focused repetitive "stereotyped" behaviors, as is often observed with increasing doses or increased sensitivity to other psychomotor stimulants such as amphetamine (Segal and Kuczenski, 1987). In fact, only the wild-type mice engaged in typical cocaine-induced stereotyped behaviors such as rearing, sniffing, and grooming (Figure 2). Other stereotyped behaviors known to result from exposure to higher doses of amphetamine (e.g., oral behaviors such as licking and gnawing) were never observed following cocaine treatment in either the wild-type or mutant mice. At the two highest cocaine doses, the mutant mice were often completely immobile during the first 10–15 min of the test period, during which they displayed a flattened body posture and abducted hindlimbs. It was not the purpose of this study to quantitate other behaviors elicited in the mutant mice by cocaine, but we noted that the mutant mice sometimes engaged in a repertoire of behaviors highly reminiscent of the "serotonin syndrome," i.e., forepaw treading or "padding," Straub (raised and rigid) tail, hindlimb abduction, and head-shaking or twitching (Green and Grahame-Smith, 1976; Jacobs, 1976).

Nucleus Accumbens Neurons in D1 Mutant Mice Are Less Sensitive to Cocaine

Most neurons in the nucleus accumbens are quiescent or fire at very slow and irregular rates. Therefore, as described in the Experimental Procedures, the excitatory amino acid transmitter glutamate, which is the endogenous neurotransmitter of cortical and limbic inputs to the nucleus accumbens (Walaas, 1981), was used to induce neuronal activity. We detected no significant difference between the wild-type mice (34.5 ± 2.4 nA, n = 28 neurons) and the mutant mice (36.2 ± 2.1 nA, n = 26 neurons) with respect to the iontophoretic currents required to drive nucleus accumbens neurons to a firing rate of 4–5 spikes/s. Moreover, there was no significant difference between the two groups of mice in the total number of spikes per 30 s generated by glutamate during basal conditions (wild type, 137.3 ± 3.3 spikes/30 s; mutant, 136.2 ± 3.0 spikes/30 s).

Local administration of cocaine onto nucleus accumbens neurons produced a current-dependent inhibition of the generation of action potentials in the wild-type mice (Figure 3A). This effect was significantly reduced in the D1 mutant mice (Figure 3C). When we averaged the results obtained from the 12 neurons tested in each group of mice, we observed a highly significant decrease in the inhibitory efficacy of cocaine in the mutant mice (Figure 4A).

Electrophysiological Effects of Dopamine Are Selectively Abolished in D1 Mutant Mice

Previous experiments conducted on neurons in the rat nucleus accumbens have demonstrated that cocaine-induced inhibition is often dependent upon stimulation of both dopamine and serotonin receptors (White et al., 1993). This dual transmitter mediation of the effects of cocaine results from the potent inhibition by cocaine of both dopamine and serotonin transporters (Ritz et al., 1987). Therefore, we conducted additional experiments to determine the inhibitory efficacies of dopamine and se-

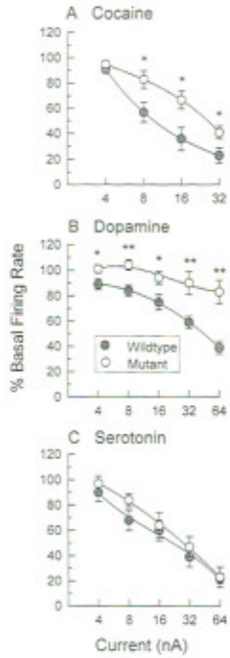

Figure 4. Current–Response Curves Showing the Reduced Inhibitory Efficacy of Cocaine and Dopamine, but Not Serotonin in D1 Mutant Mice as Compared with Wild-Type Controls

Each point represents the mean ± SEM of 10–12 neurons obtained from 4–5 mice. Statistical comparison revealed a significant reduction in the efficacy of cocaine ($F_{1,22}$ = 7.04, p = 0.014) and dopamine ($F_{1,19}$ = 12.36, p = 0.0026), but not serotonin ($F_{1,18}$ = 0.96, n.s.), in the D1 mutant mice (*p < 0.05, **p < 0.01, Dunnett's test as compared with wild-type mean at the same dose).

rotonin in the wild-type and mutant mice. Whereas both dopamine and serotonin inhibited glutamate-induced firing of nucleus accumbens neurons in wild-type mice (Figures 3A and 3B), only serotonin was effective in the D1 mutant mice (Figures 3C and 3D). Analysis of the current–response curves for these two neurotransmitters revealed a near complete loss of dopamine-induced inhibition in the mutant mice as compared with the wild-type controls (Figure 4B). In contrast, there was no significant difference between the two groups of mice with respect to serotonin-induced inhibition (Figure 4C).

Electrophysiological Effects of Both D1 and D2 Agonists Are Absent in D1 Mutant Mice

As has previously been demonstrated for neurons in the nucleus accumbens of the rat (White and Wang, 1986; Wachtel et al., 1989), agonists with selectivity for both D1-like (SKF38393) and D2-like (quinpirole) dopamine receptor subfamilies inhibited glutamate-induced activation of neurons in the wild-type mouse nucleus accumbens (Figures 5A and 5B). As in the rat, the D1-like agonist was considerably more effective than the D2-like agonist at inhibiting nucleus accumbens neurons (compare Figures 6A and 6B). In marked contrast with these results for the wild-type mice, in the D1 mutants, inhibitory effects of SKF38393 and quinpirole were almost completely absent in the nucleus accumbens (Figure 5C). Current–response

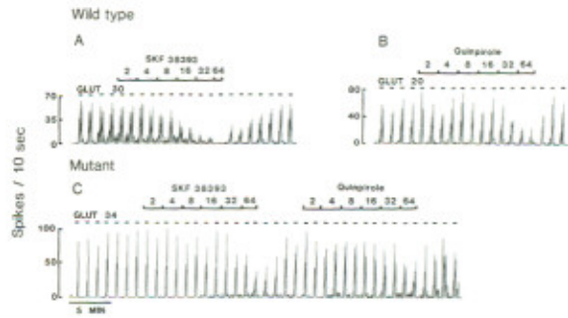

Figure 5. Cumulative Rate Histograms Illustrating the Markedly Reduced Efficacy of D1 and D2 Class Agonists at Inhibiting Nucleus Accumbens Neurons in D1 Mutant Mice

(A and B) Typical inhibitory effects of the D1-like agonist SKF38393 and the D2-like agonist quinpirole at inhibiting glutamate (GLUT)-activated nucleus accumbens neurons.

(C) Marked subsensitivity to the inhibitory effects of SKF38393 and quinpirole in D1 mutant mice. This particular neuron is illustrated because it exhibited the highest degree of sensitivity to these agonists of all the nucleus accumbens cells tested in the D1 mutant mice. Lines represent the onset and offset of drug or transmitter iontophoresis, and numbers indicate the iontophoretic current in nanoamperes.

determinations indicated that only the highest iontophoretic current for delivery of SKF38393 (64 nA) significantly suppressed the firing of nucleus accumbens neurons in the mutant mice (Figure 6A). No inhibitory effect of quinpirole was seen in the mutant mice within the current range tested (Figure 6B).

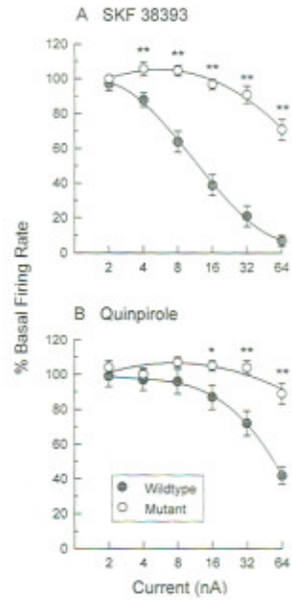

Figure 6. Current–Response Curves Showing the Markedly Reduced Inhibitory Efficacy of the D1-Like Agonist SKF38393 and the D2-Like Agonist Quinpirole in D1 Mutant Mice

Each data point represents the mean ± SEM from 10–12 nucleus accumbens neurons obtained from 4–5 mice in each group. Statistical comparison revealed a highly significant reduction in the efficacy of SKF38393 ($F_{1,21}$ = 101.42, p < 0.000001) and quinpirole ($F_{1,20}$ = 8.77, p = 0.0076) in the D1 mutant mice (*p < 0.05, **p < 0.01, Dunnett's test as compared with wild-type mean at the same dose).

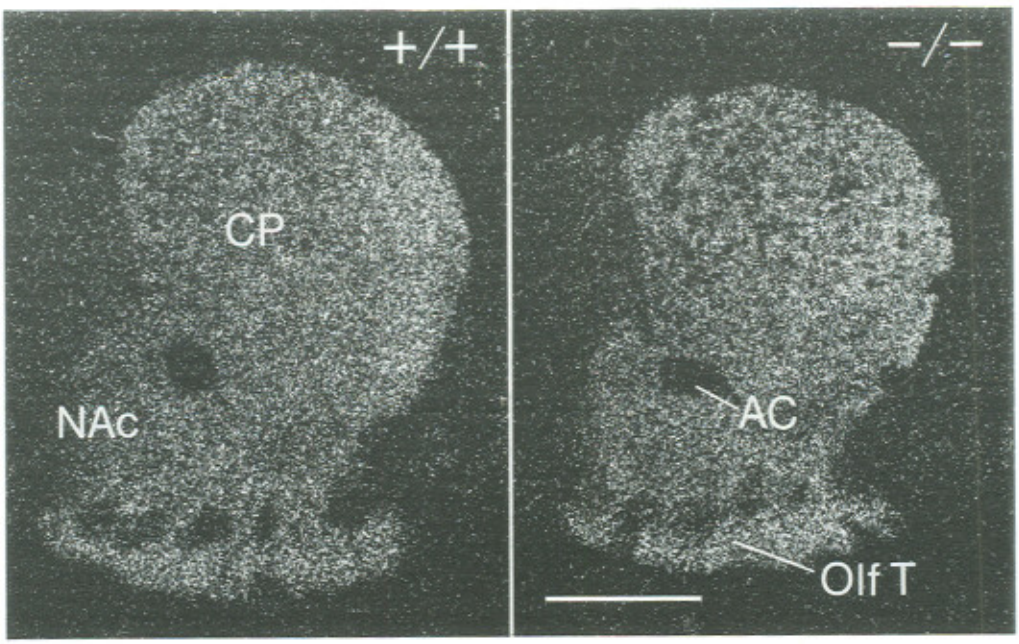

Figure 7. Ligand Binding for D2-Like Dopamine Receptors in the Mutant and Wild-Type Mice

[^{3}H]spiroperidol binding autoradiograms illustrating qualitatively similar distributions of D2-like dopamine receptor–binding sites in the mutant (−/−) (right) and wild-type (+/+) (left) mice. Autoradiographic signal appears white. CP, caudoputamen; NAc, nucleus accumbens; Olf T, olfactory tubercle; AC, anterior commisure. Scale bar indicates 1 mm.

Preserved Ligand Binding for D2-Like Dopamine Receptor Sites in the D1 Mutant Mice

As reported previously, ligand binding for D1-like dopamine receptor–binding sites is nil in the D1 mutants (Xu et al., 1994). Because of the clear-cut electrophysiological evidence for reduced responses to D2-like receptor agonists in the nucleus accumbens of D1 mutants, we carried out ligand binding for D2-like receptor–binding sites with two D2-preferring ligands, [^{3}H]raclopride and [^{3}H]spiroperidol. Dense D2-like ligand binding was present in the nucleus accumbens and other striatal regions both in the wild type and in the D1 mutants, and the distributions of binding sites in the two sets of mice were qualitatively similar (Figure 7). Semiquantitative densitometry of the [^{3}H]spiroperidol binding suggested no significant differences between the density of D2-like binding sites in the mutants and the wild-type mice in the medial (shell), lateral (core), and rostral parts of the nucleus accumbens (Table 1). Nor were differences detected in the caudoputamen (Table 1). Although such autoradiographic assays demonstrate binding sites that may or may not be equivalent to functional receptors, these results favor the view that the functional abnormalities in D2-like receptor responsiveness that we found in the D1 mutants were not due to a major deficiency (or to a massive up-regulation) in D2-like receptor–binding sites in the mutants.

Discussion

The results reported here demonstrate the essential role of the D1 dopamine receptor in cocaine-induced locomotor activity and in dopamine-mediated inhibitory neuronal effects within the nucleus accumbens. This ventral forebrain structure is closely allied to both limbic and motor systems (Mogenson et al., 1980; Mogenson, 1987; Robbins et al., 1989) and is considered as the primary site of the locomotor-stimulating, subjective, and positive-reinforcing effects of cocaine and other drugs of abuse (Wise and Bozarth, 1987; White, 1990; Koob, 1992). Previous work has implicated both D1-like and D2-like dopamine receptors in

Table 1. Dopamine D2-Like Receptor Binding Density in the Striatum of the D1 Mutant and Wild-Type Mice Estimated by [^{3}H]Spiroperidol Bound (nCi/g of tissue ± SEM)

	Nucleus Accumbens			Caudoputamen		
	Rostral	Shell	Core	Rostral	Middle	Caudal
Wild Type	259 ± 22	197 ± 8	304 ± 12	291 ± 12	333 ± 4	324 ± 4
D1 Mutant	250 ± 16	203 ± 9	298 ± 12	300 ± 14	319 ± 12	355 ± 8

Values for optical density calculated by film densitometry for mutant and wild-type mice. Values are expressed as nCi/g of tissue ± SEM.

these behavioral effects of cocaine (see Introduction for references). The present findings explicitly demonstrate that without the D1 receptor, but in the absence of detectable loss of D2-like receptor–binding sites in the nucleus accumbens, mice are incapable of exhibiting psychomotor stimulation in response to cocaine administration. In fact, D1 mutant mice exhibited a significant dose-dependent decrease in locomotion in response to cocaine.

Hyperactivity in D1 Mutant Mice

As was suggested by our previous behavioral studies of the D1 mutant mice (Xu et al., 1994), the lack of D1 receptors resulted in an exaggerated activity response to a novel environment. Thus, the mutant mice were almost three times as active as their wild-type littermates during a 30 min period of habituation to the test chambers. Similar, albeit less pronounced, hyperactivity was observed in our other study, which utilized a longer (2 hr) habituation period (Xu et al., 1994). The shorter habitation period may account for the greater hyperactivity observed in the present study. However, additional behavioral tests indicated that basal hyperactivity in the mutant mice persists even after a 48 hr habituation test. Taken together, these findings suggest that the congenital lack of D1 receptors results in enhanced levels of basal activity. This suggestion is seemingly at odds with the notion that D1 receptor stimulation within the nucleus accumbens is required for locomotor activity (Clark and White, 1987).

One likely reason for D1 mutant mice showing enhanced basal levels of locomotion is that a reduction in D1 receptor–mediated dopamine transmission in other limbic and cortical areas relieved a normal inhibitory influence by such structures on locomotor activity. Considerable evidence supports the notion that D1 dopamine receptor neurotransmission within the prefrontal cortex and amygdala regulates dopamine neurotransmission within the nucleus accumbens (Tassin et al., 1978; Pycock et al., 1980; Simon et al., 1988; Vezina et al., 1991). Thus, when dopamine neurotransmission is reduced in both the nucleus accumbens and other corticolimbic areas, basal levels of locomotor activity are increased (Galey et al., 1977; Tassin et al., 1978; Joyce et al., 1983). As our electrophysiological results indicate a nearly complete loss of dopamine-mediated inhibitory efficacy within the nucleus accumbens of D1 mutant mice, it seems likely that the heightened basal locomotor activity was due to a coordinated lack of D1 receptor–mediated neurotransmission throughout the mesocorticolimbic dopamine system.

It is also possible that the lack of D1 receptors results in heightened basal activity through developmental adjustments (Xu et al., 1994). To date, the clearest neurodevelopmental abnormality that we have found in the D1 mutant mice is in the striatal dynorphin system (Xu et al., 1994). However, we found apparently associated changes in pallidonigral substance P expression and in striosome–matrix differentiation, and there are many other candidate systems that need to be explored.

Lack of Cocaine-Induced Hyperactivity

Although it might be argued that the lack of locomotor hyperactivity in the mutant mice resulted from a "ceiling effect" related to the already heightened activity levels of the mutant mice, we find this possibility unlikely for several reasons. The wild-type mice exhibited cocaine-induced levels of activity that were significantly higher than those of the mutant mice during habituation periods. This suggests that the activity levels of the D1 mutant mice could, in fact, increase further. Indeed, we have observed that the mutant mice are capable of activity levels at least twice as high as those observed either during the 30 min habituation period or during the saline test, as revealed by basal activity levels during the dark phase of the light/dark cycle (Xu et al, 1994; our unpublished data, using the present behavioral procedures). Furthermore, the other characteristic cocaine-induced behaviors (sniffing, rearing, and grooming) were very low in the mutant mice during the habituation period and also failed to increase in response to cocaine.

The complete absence of cocaine-induced locomotor hyperactivity and stereotyped behavior in D1 mutant mice clearly underscores the fundamental role of the D1 receptor in these effects. This finding is consistent with reports of D1 antagonists completely preventing the locomotor stimulant effects of cocaine in normal mice and rats (Cabib et al., 1991; Tella, 1994). However, it would not be accurate to conclude that D1 receptors are the sole mediators of this and other behavioral responses to cocaine. Antagonists of D2-like dopamine receptors also prevent cocaine-induced behaviors. What is made clear by the present electrophysiological and anatomical results is that elimination of D1 receptors abolished all dopamine receptor–mediated effects within the nucleus accumbens, including those produced by the D2-like agonist quinpirole, in the absence of a detectable change in D2-like receptor binding there. Since previous electrophysiological and behavioral experiments have suggested that the enabling role of D1-like receptors is not reciprocated by D2-like receptors, i.e., that D1-like receptor–mediated effects do not require D2-like receptor stimulation (White et al., 1988; Wachtel et al., 1989, 1992), it might be expected that a reduction in D1 transmission would be more effective in preventing the effects of cocaine than would a similar reduction in D2-like receptor transmission. Although a definitive test of this possibility must await the development of D2 receptor mutant mice, some (but not all) investigators have reported that antagonists of D1-like receptors block cocaine-induced behavior at doses far lower than those causing behavioral disruption, whereas antagonists of D2-like receptors are most likely to be effective at doses causing overall response decrements (e.g., Koob et al., 1987; Cabib et al., 1991; Caine and Koob, 1994). Moreover, D1-like receptor antagonists are considerably more effective than D2-like receptor antagonists at reversing the inhibitory effects of cocaine on rat nucleus accumbens neurons (White et al., 1992).

Cocaine-Induced Hypoactivity in D1 Mutant Mice

An unexpected finding of our studies was the significant decrease in locomotor activity produced by higher doses of cocaine in the D1 mutant mice. This hypoactivity was

most pronounced during the first 15 min of the observation, in which the mutant mice displayed abducted hindlimbs and a flattened body posture. There are several possible explanations for the locomotor decreasing effect of cocaine in D1 mutant mice. One is that removal of D1 receptor stimulation "unmasked" D2-like receptor–mediated behavioral inhibition that is normally under oppositional control by D1 receptors (Waddington and Daly, 1993). The removal of D1 receptor stimulation may have enhanced behaviors controlled by postsynaptic D3 receptors, which have recently been suggested to mediate locomotor suppression (Waters et al., 1993; Svensson et al., 1994). Perhaps the most likely explanation is that, with negligible dopamine receptor influence, cocaine-induced enhancement of synaptic serotonin levels resulted in reduced locomotor activity. The limbic serotonergic projections from the raphe nuclei are thought to exert an inhibitory influence on locomotor activity (Geyer et al., 1976), and opposing influences of increased serotonin neurotransmission on both cocaine-induced locomotor activity (Pradham et al., 1979; Taylor and Ho, 1979) and self-administration (Loh and Roberts, 1990) have been described. This explanation is supported by the occasional presence in the D1 mutant mice of behaviors thought to reflect enhanced serotonin receptor activation (e.g., forepaw treading and headshaking).

Loss of Dopamine-Mediated Inhibition of Neuronal Activity in the Nucleus Accumbens

One of the most striking findings of our investigations was the almost complete loss of dopamine-induced inhibition of nucleus accumbens neurons in D1 mutant mice. This effect appeared to be selective for dopamine receptor–mediated inhibition because serotonin-induced inhibitory effects were unaltered. The lack of effect of dopamine in the D1 mutant mice provides definitive support for previous indications that D1-like receptors are essential for the inhibitory effects of dopamine on nucleus accumbens and striatal neurons, whether those effects are initiated by stimulation of D1-like (a direct effect) or D2-like (indirect enabling effect) receptors (for review see White and Hu, 1993). Further support for this conclusion comes from similar experiments indicating that the inhibitory effects of both the D1-like agonist SKF38393 and the D2-like agonist quinpirole were almost completely abolished in the mutant mice. The slight inhibitory effect of SKF38393, when administered with high iontophoretic currents, may be due to the presence of other D1-like receptors (e.g., D5) in the nucleus accumbens (Rappaport et al., 1993) or to nondopaminergic effects of the drug at high concentrations.

Previous studies have suggested that striatal neurons may express D1–like receptors that are not coupled to adenylyl cyclase (Mahan et al., 1990; Undie and Friedman, 1990). In fact, behavioral and electrophysiological studies have demonstrated that both the direct effects of D1-like agonists and their enabling effects on D2-like receptor–mediated function are poorly correlated with their efficacy at stimulating adenylyl cyclase (Arnt et al., 1987, 1992; Murray and Waddington, 1989; Johansen et al., 1991). However, the present results clearly demonstrate that many of these effects are mediated by the D1 receptor. Thus, it appears that the previously noted poor relationship between efficacy at stimulating cAMP formation and other functional measures may have resulted either from the presence of substantial receptor reserve or from additional transduction systems linked to the D1 receptor (Arnt et al., 1992; Johansen et al., 1991).

In contrast with the near complete lack of dopamine effect on nucleus accumbens neurons in the D1 mutant mice, the inhibitory efficacy of cocaine was significantly attenuated, but not abolished. This is not surprising in view of previous work indicating that both the cocaine-induced inhibition of nucleus accumbens neurons and activation of striatal transcription factor genes (c-*fos* and *zif268*) depend on elevated dopamine and serotonin receptor stimulation (Bhat and Baraban, 1993; White et al., 1993). Given that serotonin-mediated inhibition of nucleus accumbens activity was unaltered in the mutant mice, it seems likely that the partial inhibitory effects of cocaine resulted from the ability of cocaine to block serotonin reuptake (Ritz et al., 1987), and thereby increase synaptic levels of serotonin (Bradberry et al., 1993).

Conclusions

Our studies have provided definitive evidence that the D1 receptor is essential for the psychomotor stimulant effects of cocaine. In addition, we have demonstrated that the D1 receptor is required for the dopamine-mediated inhibitory effect within the nucleus accumbens. The relationship between psychomotor stimulant activity and the addictive potential of a variety of drugs of abuse has been the subject of considerable recent interest (Wise and Bozarth, 1987; Koob, 1992; Robinson and Berridge, 1993). Certainly with respect to animal studies of drug-taking behavior, cocaine self-administration seems to rely on anatomical and neurochemical substrates that are intermixed or overlapped with those involved in locomotor hyperactivity, i.e., the mesocorticolimbic dopamine system (Wise and Bozarth, 1987; White, 1990; Koob, 1992). The availability of D1 mutant mice should provide the possibility to test whether D1 receptors are also a primary target for the reinforcing effects of cocaine and other drugs of abuse.

Experimental Procedures

Mice

The D1 mutant mice were generated as described (Xu et al., 1994). Both the wild-type and mutant male mice were bred at the Massachusetts Institute of Technology under standard animal housing conditions. The mutant and wild-type mice were identified by genomic Southern analyses of tail biopsies (Xu et al., 1994). Male mice 10–16 weeks of age were transported to the Chicago Medical School by commercial overnight couriers. Upon receipt, mutant and wild-type mice were housed separately in standard laboratory cages in groups of three to four. They were allowed 4–7 days for acclimation to the new colony. Food and water were available ad libitum in a room maintained at relatively constant temperature and humidity with a 12 hr light/dark cycle.

Behavioral Methods

Locomotor activity experiments were conducted in a separate room within the vivarium. Standard polypropylene cages (30 × 50 cm) were placed inside adjustable frames equipped with three infrared pho-

tobeams (San Diego Instruments). The photoelectric beams were placed at a height of 1.5 cm at equal distances along the length of the cage. For each cage, all beam breaks were relayed by an interface to a 286-based microcomputer with custom software. Both total beam breaks and sequential interruptions of successive beams were quantified as "total counts" and "ambulation counts," respectively. Because the profile of cocaine effects on these two measures were nearly identical, only "total counts" are presented in this report. On test days, mice were weighed and placed in the test cages for a 30 min habituation period. They were then administered various doses of cocaine (5.0–40.0 mg/kg, i.p.) or its vehicle (physiological saline), and the test was begun. Activity was measured continuously for a 1 hr period between 11:00–14:00 during the light phase of the daily cycle. All injections were administered in volumes of 1.0 ml/100 mg body weight.

During drug tests, trained observers scored each mouse for the presence of repetitive stereotyped behaviors, including rearing, sniffing, and grooming. Each mouse was observed for 15 s every 5 min during the 1 hr test session for the presence or absence of each behavior. Data were expressed as the number of intervals, from a total of 12, in which each behavior was observed.

Electrophysiological Methods

All methods for extracellular single-cell recordings and microiontophoresis were similar to those previously reported (Hu et al., 1990; White et al., 1993; Hu and White, 1994) with modifications. Mice were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Body temperature was maintained at 36°C–37.5°C with a thermostatically controlled heating pad. A 28-guage (3/8 inch) hypodermic needle was placed in a lateral tail vein, through which additional anesthetic was administered as required. A burr hole was drilled in the skull, and the dura was retracted from the area overlying the nucleus accumbens.

Five-barrel glass micropipettes were pulled and broken back under a microscope to approximately 8–10 μm at the tip. The center recording barrel of each pipette was filled with a 2 M NaCl solution saturated with 1% fast green dye. The in vitro impedance of the recording barrel was 1–3 MΩ measured at 135 Hz. One side barrel of the micropipette was filled with a 2 M NaCl solution for automatic current balancing, whereas a second side barrel was always filled with L-glutamic acid monosodium salt (100 mM in 50 mM NaCl, pH 8) for activating quiescent nucleus accumbens neurons. The two remaining side barrels contained combinations of the following drugs (all at 10 mM, pH 4): dopamine HCl, serotonin HCl, cocaine HCl, SKF38393 HCl, and quinpirole HCl. Retaining currents (positive for glutamate and negative for others) of 8–10 nA were applied to drug barrels between ejection periods. The impedance of the side barrels was typically between 20–70 MΩ.

Micropipettes were lowered via a hydraulic microdrive into the nucleus accumbens. The coordinates for recording from wild-type mice were the following: 5.6–5.8 mm anterior (A) to λ, 0.5–0.9 mm lateral (L) to the midline suture, and 3.6–4.7 mm ventral (V) to the cortical surface. Because the mutant mice were, on average, 40% smaller than the wild-type mice (18–25 g versus 30–42 g; see Xu et al., 1994), the coordinates were slightly different: 5.3–5.5 A, 0.4–0.7 L, and 4.5–4.5 V. Nucleus accumbens neurons were either quiescent or fired at extremely slow rates (<1 spike/s) and were thus activated to fire by iontophoretic administration of glutamate. The ejection current of glutamate was set to activate quiescent cells to about 4–5 spikes/s (Hu et al., 1990). On most neurons, this required a current of 20–40 nA. Glutamate administration was conducted using a pulsing paradigm in which current through the glutamate barrel was automatically timed to occur for 30 s with a 40 s off period between each pulse.

Electrical signals were passed through a high impedance amplifier, displayed on an oscilloscope, monitored by an audioamplifier, and led into a window discriminator to detect individual action potentials. Integrated rate histograms generated by the analog output of the window discriminator were plotted on a polygraph recorder. Digital counts of action potentials were also obtained for permanent storage.

The responses of nucleus accumbens neurons to microiontophoretic administration of drugs were determined by comparing the total number of spikes occurring during administration of the test compound to the basal firing rate. A total of 5–8 predrug pulses was used to determine the "basal rate," and spikes were counted only during the 30 s glutamate application. Current–response curves were determined by administering increasing currents (2–64 nA) through the drug barrel. Each current doubled the previous current and was administered for a 1–2 min period. Because iontophoretic drug administration restricts the effects of the drugs to the immediate vicinity of the electrode, more than one neuron (1–4) was usually recorded in each mouse.

At the end of the experiment, the final recording site was marked by passing a 25 μA cathodal current through the recording barrel for at least 15 min to deposit a discrete spot of fast green dye. The mice were then perfused with 0.9% NaCl followed by 10% buffered formalin for 15 min. Serial coronal sections were cut at 50 μm intervals and stained with cresyl violet. Because more than one cell was tested in each mouse, the location of the unmarked cells could only be estimated by using the dye spot as a reference point for the location of all other cells. We estimated that most neurons recorded in the present report were located in the core region of the nucleus accumbens, although shell neurons were also included (Zahm and Brog, 1992). Considerable past experience with rat nucleus accumbens neurons has led us to the conclusion that core and shell neurons do not show significant differences in their response to glutamate, dopamine, cocaine, SKF38393, or quinpirole (White et al., 1993; Hu and White, 1994). Therefore, the results of all nucleus accumbens neurons were presented together in this report.

Ligand Binding

For ligand binding assays, four wild-type and five mutant adult male mice were sacrificed by cervical dislocation. Their brains were rapidly removed from the skulls, frozen in powdered dry ice, and stored at −80°C. Frozen coronal sections were cut on a cryostat at 10 μm, thaw-mounted onto slides (Probe On, Fisher Scientific), and stored at −20°C for at least 2 days. Slides were brought to room temperature and washed for 5 min in the working buffer to remove endogenous ligands before labeling.

Autoradiographic labeling of D2-like dopamine receptor–binding sites was carried out as described by Camps et al. (1989). Slide-mounted sections were incubated at room temperature for 45 min with 0.8 nM [^{3}H]spiroperidol, specific activity 19 Ci/mmol (DuPont, New England Nuclear) in 50 mM Tris–HCl buffer (pH 7.5), containing 120 mM NaCl, 150 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mM ascorbic acid. After incubation, sections were rinsed twice for 2 min in fresh ice-cold buffer and dipped briefly in cold distilled water, dried for at least 2 hr, and exposed together with tritium standards ([^{3}H] Microscales, Amersham) to tritium-sensitive film (Hyperfilm, Amersham). Films were developed in Kodak D 19 developer and were analyzed on a Biocom 200 image analyzer (Les Ulis).

For analysis of binding levels in the striatum, optical density measurements were made in the caudoputamen and in the nucleus accumbens. For each mouse, measurements in the caudoputamen were made in five sections (ten hemispheres) per rostrocaudal level (rostral, middle, and caudal; see Table 1). Measurements in the nucleus accumbens were made in two sections rostrally and in four sections more caudally in regions approximately corresponding to the shell (medial) and the core (lateral) subdivisions, with occasional exceptions where tissue was missing (see Table 1). Optical density measurements of the autoradiographic standards on the films were made, and a correlation curve was constructed to allow conversion of optical density measurements to activity values in nCi/g of tissue ± SEM. Values for left and right hemispheres were pooled.

Statistical Analyses

Because the behavioral data obtained in locomotor activity tests were not normally distributed, comparisons of dose–response effects were conducted with Kruskal–Wallis one-way analysis of variance (ANOVA). Comparisons between the two groups of mice at each dose were conducted with Mann–Whitney tests. Habituation results were compared with Student's t test. The results of behavioral observations were compared with Mann–Whitney tests. Comparisons between the two groups of mice with respect to current–response curves generated with iontophoretic administration of drugs were conducted with a two-way ANOVA with repeated measures (current), followed by Dunnett's test.

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