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# A Genetic Approach to Study Mechanisms of Cocaine Action[a]

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## INTRODUCTION

Dopamine is the principal neurotransmitter for several major neural systems in the brain.[1-4] The nigrostriatal pathway originates from dopamine-synthesizing neurons of the midbrain substantia nigra complex and innervates the dorsal striatum (caudoputamen). This system is involved in modulating sensorimotor coordination and initiation of movement.[5] Degeneration of this pathway leads to Parkinson's disease.[6] The mesolimbic pathway arises in the midbrain ventral tegmental area and innervates the ventral striatum (nucleus accumbens and olfactory tubercle) and parts of the limbic system. This system is thought to influence motivated behaviors including activity related to reward.[7-9] The mesocortical pathway also arises in the ventral tegmental area and innervates part of the frontal cortex. It may be involved in certain aspects of learning and memory.[10] Together, these dopaminergic systems are thought to be the major targets for the neuropharmacological actions of psychomotor stimulants such as cocaine and amphetamine.[7,11,12]

Dopamine receptors are strongly expressed in the targets of these pathways and on many dopamine-containing neurons. The dopamine receptor genes so far cloned fall into two classes, the D1-like ($D1_A$ and D5 or $D1_B$) and the D2-like (D2, D3, and D4) receptors.[13,14] These classes were originally differentiated pharmacologically and in terms of their positive (D1-like) or negative (D2-like) coupling to cAMP.[15]

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Previous studies suggest that cocaine primarily binds to the dopamine transporter and interferes with the uptake of dopamine by the transporter.[16,17] Such binding would result in excessive dopamine in the synapse and therefore, it is reasonable to assume that multiple dopamine receptor subtypes would be involved in the effects of cocaine. Indeed, both D1 and D2 receptor subfamilies have been suggested to mediate the effects of cocaine on behavior. For example, antagonists of both D1 and D2 receptor classes have been shown to block several well-characterized behavioral effects of cocaine, including locomotor hyperactivity,[18,19] subjective effects measured by drug discrimination techniques,[20–22] and rewarding effects assessed by self-administration paradigms.[23–27] 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.[28–31] Nevertheless, although there has been general agreement regarding the essential role played particularly by the mesolimbic dopamine system in mediating the psychomotor stimulant activities of cocaine, disagreement exists with respect to the relative contributions of different dopamine receptor subtypes. This is because of the lack of ligands with selectivity within the D1 and D2 subfamilies. For example, available ligands do not distinguish between D1 and D5 receptors, or 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. To date, which dopamine receptor subtypes within these two subfamilies are particularly important for the behavioral effects of cocaine remains unknown.

To gain further knowledge of how the brain dopamine system functions in the presence of cocaine, we have used a genetic approach, the gene-targeting technique, and generated D1 dopamine receptor mutant mice. These mutant mice provide an unprecedented opportunity to evaluate, in a very selective and precise manner, the roles of this receptor in cocaine- and dopamine-mediated neuronal function.[32,33] 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. These mice should be very useful for further investigation of mechanisms of cocaine action.

## MATERIALS AND METHODS

For details of the generation of D1 dopamine receptor mutant mice and subsequent behavioral and electrophysiological analyses, see Xu *et al.* and references therein.[32,33]

## RESULTS

### *Generation of D1 Dopamine Receptor Mutant Mice*

To generate D1 receptor mutant mice, we first cloned the mouse D1 receptor gene. The restriction map of the mouse D1 gene locus and the D1 gene targeting construct are shown in FIGURE 1A. The D1 receptor gene is about 2 kilobase pairs (kb) long and is intronless. The targeting construct contains a 4.5 kb DNA fragment from the 5′ of the D1 gene and a 4.2 kb DNA fragment from the 3′ of the D1 gene. It also contains a Neo gene coding for G418 resistance for selection. Upon

successful homologous recombination, 95% of the D1 gene would be deleted. The predicted sizes of the DNA fragment before and after the desired homologous recombination and the D1 specific DNA probes used are indicated (see FIG. 1A). After stable transfection, we were able to identify five cell clones that carry the desired D1 mutation. We introduced three of the clones into recipient mice and bred the resulting mice extensively to obtain mice homozygous for D1 receptor gene deletion.

To identify the D1 receptor mutant mice, we performed genomic Southern analysis with a D1 specific DNA probe, the 5′ probe. As shown in FIGURE 1B, in this family of mice, two are homozygous for D1 gene mutation. The sizes of the DNA fragment matched the predicted sizes indicating homologous recombination occurred correctly. The others are either heterozygous or do not carry such mutation. Another D1 specific DNA probe, the 3′ probe, confirms the results from the first D1 specific probe. Therefore, in this family of 10 pups, the distribution of the D1 gene alleles reflects typical Mendelian segregation.

The D1 mutant mice appeared healthy and had no gross abnormalities. However, they were smaller than their wildtype littermates. For instance, ten-week-old adult mice ($n$ = 46) weighed, on the average, 30% less than the wildtype controls ($n$ = 46). Because of the reduced body weight, the mutant mice were weaned three to five days later than the wildtype littermates. The litter sizes of the mutants seemed normal and there was no obvious sex bias in the offspring.

As reported by Xu *et al.*,[32] ligand binding and locomotor studies confirmed the absence of expression and function of the D1 receptor gene in the mutant mice. Furthermore, extensive histological analysis indicates that the general anatomy of the dopamine system in the D1 mutant brains are similar to that of the controls.[32] We used the D1 receptor mutant mice as valuable tools to study the role of this receptor in mediating the actions of cocaine and dopamine.

### *Cocaine Fails to Induce Locomotor Hyperactivity in D1 Dopamine Receptor Mutant Mice*

To investigate whether D1 receptor is involved in locomotor-stimulating effect of cocaine, we performed locomotor activity analysis in response to cocaine. To do this, different groups of mutant and control mice were allowed to habituate in photocell cages for 30 min. Then, they were given ip injections of different doses of cocaine or its vehicle and placed back into photocell cages for 1 h. As shown in FIGURE 2, while there is a dose-dependent increase in locomotor activity in the control mice, cocaine-induced locomotor hyperactivity was not observed in the mutant mice. In fact, with increasing doses of cocaine, there is a decrease in locomotor activity.

To make sure the lack of locomotor hyperactivity is not due to increased stereotyped behavior, at the same time of recording cocaine-induced locomotor activity, we also scored each mouse for cocaine-induced stereotyped behaviors. As shown in FIGURE 3, only the wildtype mice engaged in typical cocaine-induced stereotyped behaviors such as rearing and sniffing. 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 wildtype 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

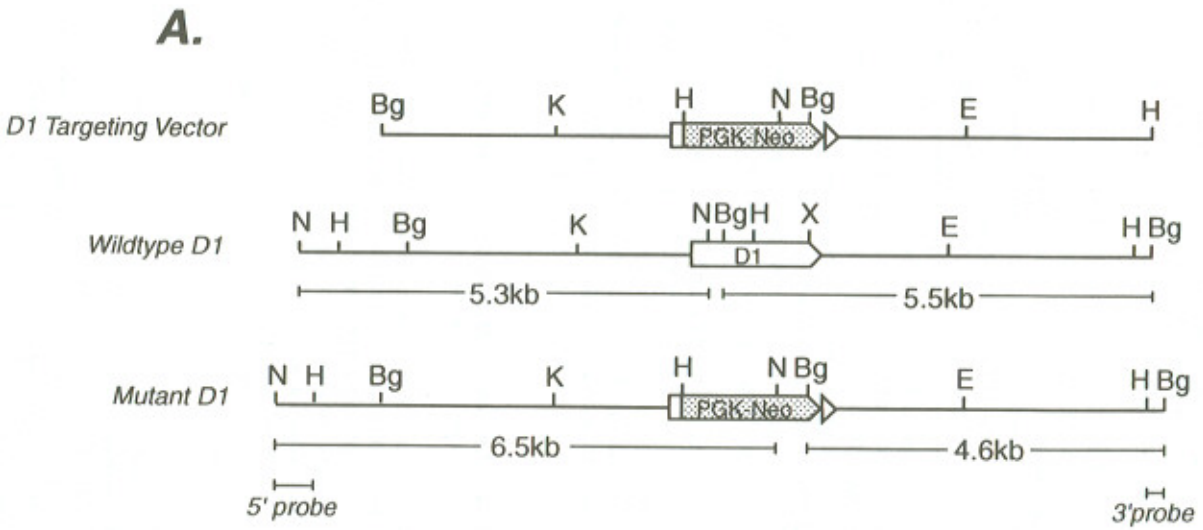
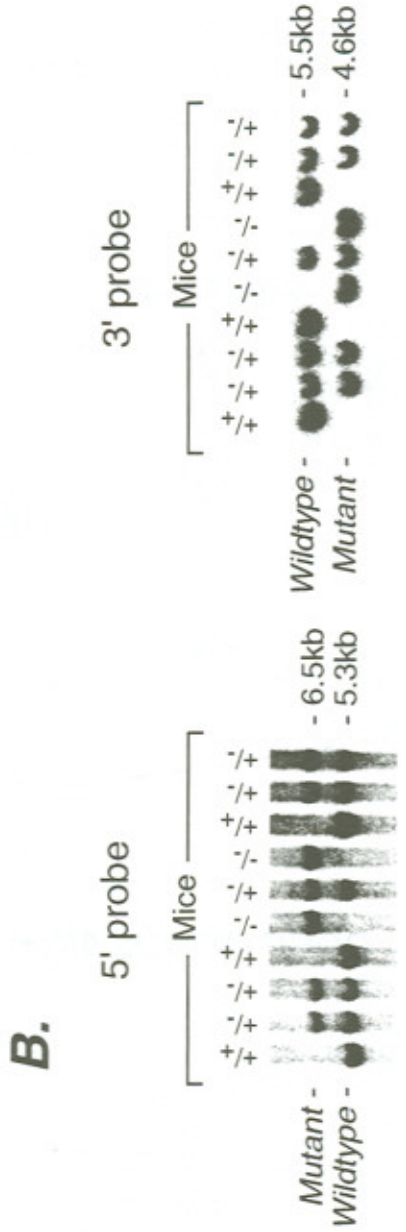



**FIGURE 1.** Generation of D1 dopamine receptor mutant mice. **(A)** The D1 gene targeting construct, wildtype and mutant loci of the mouse D1 dopamine receptor gene. The *open box* represents the entire D1 gene. The *shaded box* depicts the neo gene driven by a PGK promoter. The *solid line* represents extragenic sequences of the D1 gene. The expected sizes of the hybridizing restriction fragments for both the wildtype and the mutant alleles are indicated under the corresponding wildtype and the mutant loci sequences. Abbreviations for restriction enzyme sites are: Bg, BglII; E, EcoRI; H, HindIII; K, KpnI; N, NcoI; X, XbaI. **(B)** Genomic Southern analyses of tail biopsies. Genomic DNA was isolated from a litter of 10 pups from one heterozygous breeding pair. DNA was digested either with NcoI and hybridized with a 5′ probe (*left*) or with BglII and hybridized with a 3′ probe (*right*). The genotype of each pup is indicated. (From Xu *et al.*[32] Reprinted by permission from *Cell*.)


B.
5' probe
Mice
+/+
-/+
-/+
+/+
-/-
-/+
-/-
+/+
-/+
-/+
Mutant -
Wildtype -
- 6.5kb
- 5.3kb
3' probe
Mice
-/+
-/+
+/+
-/-
-/+
-/-
+/+
-/+
-/+
+/+
Wildtype -
Mutant -
- 5.5kb
- 4.6kb

hindlimbs. Therefore, cocaine-induced locomotor hyperactivity is eliminated in the D1 mutants, implying the importance of D1 receptor in mediating such effect.

### *Nucleus Accumbens Neurons in D1 Dopamine Receptor Mutant Mice Are Less Sensitive to Cocaine*

Since nucleus accumbens has been implicated in mediating the effect of cocaine, we studied the electrical properties of the nucleus accumbens neurons in the

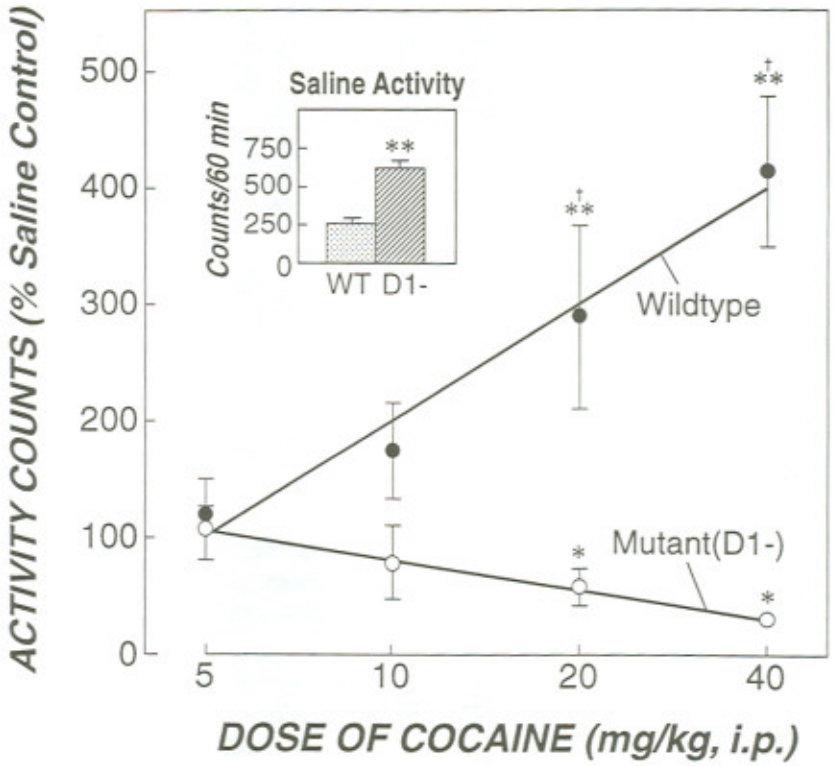



**FIGURE 2.** Dose-response curves for cocaine-induced locomotor activity. Because the D1 mutant mice (D1-) were significantly more active than wildtype (WT) mice during habituation to the test chamber as well as during saline injections (bar graph, *inset*; $^{**}t_{54} = 5.95$, $p < 0.0001$), the data 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,[45] 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 wildtype mice, but a significant ($p < 0.001$, Kruskal-Wallis ANOVA) dose-related decrease in activity counts in the mutant mice ($^{*}p < 0.05$, $^{**}p < 0.01$ as compared to saline test for that group; $\#\#p < 0.01$ as compared to D1 mutant mice at the same dose, Mann-Whitney test). (From Xu *et al.*[33] Reprinted by permission of *Cell*.)

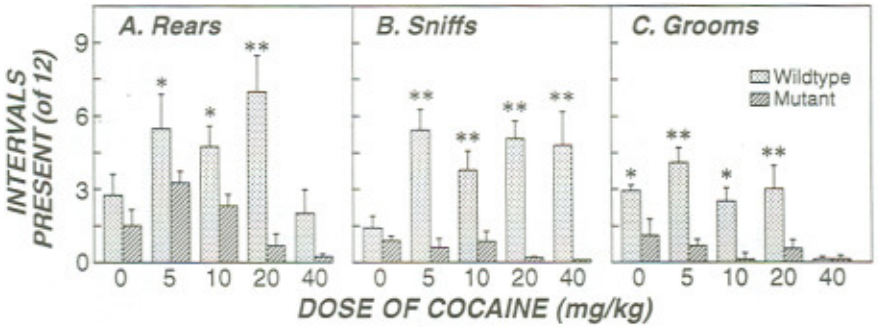



**FIGURE 3.** Effects of cocaine on different aspects of behavior generally referred to as stereotypy. Cocaine caused significant increases in rearing, sniffing, and grooming in wildtype, but not in D1 mutant mice (*$p$ <0.05, **$p$ <0.01, between groups comparison for each dose, Mann-Whitney test). (From Xu *et al.*[33] Reprinted by permission of *Cell*.)

presence of cocaine. To accomplish this, we used extracellular single unit recording and microiontophoresis *in vivo*, and recorded the effects of cocaine on the firing of the nucleus accumbens neurons. We inserted multibarrel electrodes into mice that have been anesthetized and manipulated the electrodes in such a way that only the electrical activity of a single neuron would be recorded. Most neurons in the nucleus accumbens are quiescent or fire at very slow and irregular rates. Therefore, the excitatory amino acid transmitter glutamate, which is the endogenous neurotransmitter of cortical and limbic inputs to the nucleus accumbens,[34] was used to induce neuronal activity. We detected no significant difference between the wildtype 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/30 s generated by glutamate during basal conditions (wildtype: 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 wildtype mice (FIG. 4A). This effect was significantly reduced in the D1 mutant mice (FIG. 4C). 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 (FIG. 5A).

### *Elimination of Dopamine-Mediated Neurophysiological Responses in D1 Dopamine Receptor 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.[35] This dual transmitter mediation of cocaine's effects results from the potent inhibition by cocaine of both dopamine and serotonin transporters.[16] Therefore, we conducted additional experiments to determine the inhibitory efficacies of dopamine and serotonin in the wildtype and

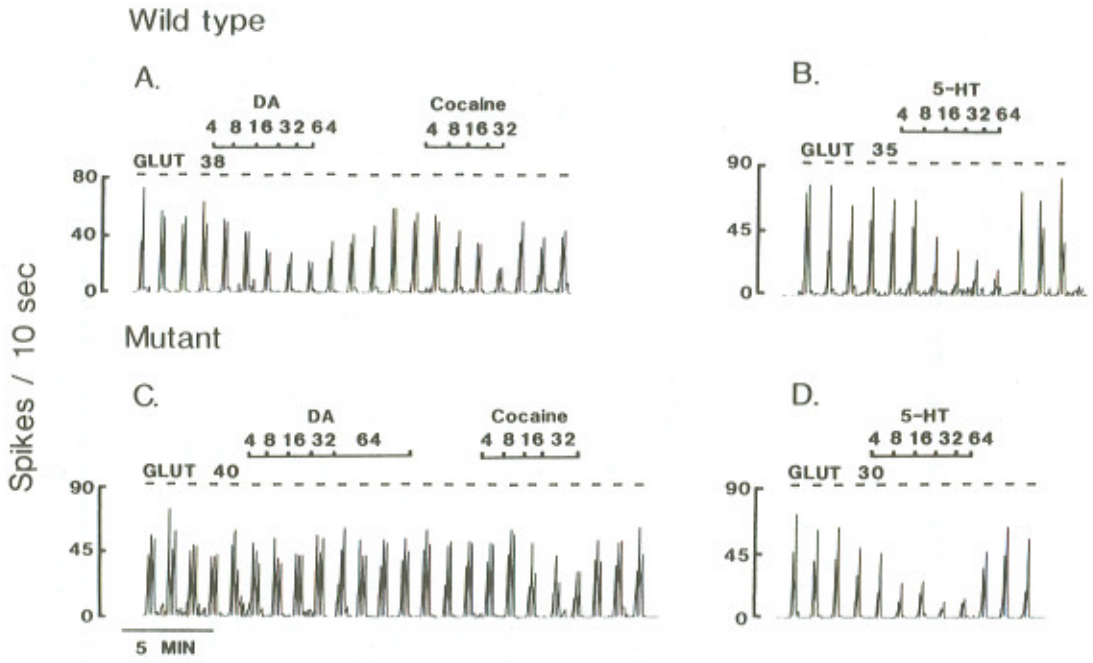



**FIGURE 4.** Cumulative rate histograms illustrating the reduced efficacy of dopamine and cocaine at inhibiting glutamate (GLUT) activated nucleus accumens neurons in the mutant mice. **(A)** and **(B)**: Dopamine (DA), cocaine, and serotonin (5-HT) inhibited glutamate-induced firing of nucleus accumbens neurons in wildtype 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 two minutes 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. (From Xu *et al.*[33] Reprinted by permission from *Cell*.)

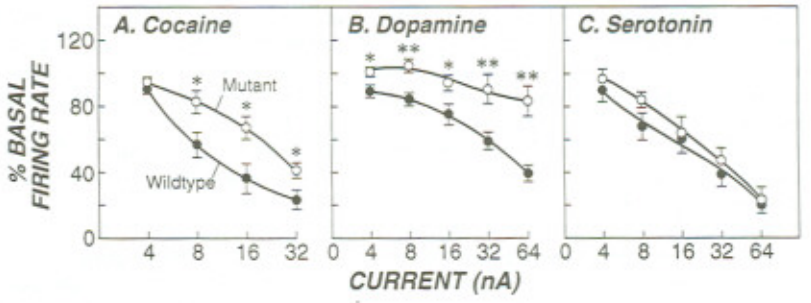



**FIGURE 5.** Current-response curves showing the reduced inhibitory efficacy of **(A)** cocaine and **(B)** dopamine, but not **(C)** serotonin in D1 mutant mice as compared to wildtype controls. Each point represents the mean ± SEM of 10–12 neurons obtained from 4–5 mice. Statistical comparison revealed 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 to wildtype mean at the same dose). (From Xu *et al.*[33] Reprinted by permission from *Cell*.)

mutant mice. Whereas both dopamine and serotonin inhibited glutamate-induced firing of nucleus accumbens neurons in wildtype mice (FIG. 4A and 4B), only serotonin was effective in the D1 mutant mice (FIG. 4C and 4D). 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 to the wildtype controls (FIG. 5B). In contrast, there was no significant difference between the two groups of mice with respect to serotonin-induced inhibition (FIG. 5C). Therefore, D1 receptor plays a critical role in normal dopamine-mediated neurophysiological effects in the nucleus accumbens.

## DISCUSSION

We have used a genetic approach and generated mice that do not carry the D1 receptor gene in their genome. We used these mice and started to study the mechanisms of cocaine action. Our behavioral and electrophysiological analyses demonstrated the essential roles of the D1 dopamine receptor in cocaine-induced locomotor activity and in cocaine- and dopamine-mediated inhibitory neuronal effects within the nucleus accumbens. This ventral forebrain structure is closely allied to both limbic and motor systems,[36–38] and is considered as the primary site of the locomotor stimulating, subjective, and positive reinforcing effects of cocaine and other drugs of abuse.[7,39,40] Previous work has implicated both D1-like and D2-like dopamine receptors in these behavioral effects of cocaine. The present findings explicitly demonstrate that without the D1 dopamine receptor, and in the absence of detectable changes in the brain dopamine system, 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. Thus, our studies have provided definitive evidence that the D1 dopamine receptor is essential for the psychomotor stimulant effects of cocaine. In addition, we have demonstrated that the D1 receptor is required for dopamine-mediated inhibitory effects 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.[7,40,41] Certainly with respect to animal studies of drug-seeking 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 mesolimbic dopamine system.[7,39,40] The availability of D1 mutant mice should provide the possibility to test whether D1 dopamine receptor is a primary target for the reinforcing effects of cocaine and other drugs of abuse. In addition, psychomotor stimulants like cocaine are known to be able to induce the expression of immediate early genes through dopamine receptors.[11,42] The generation of D1 receptor mutant mice should be very valuable to address which dopamine receptor subtypes mediate these inductions *in vivo* and help to elucidate the molecular targets of these immediate early gene products.

Deletion of D1 dopamine receptor gene in the mouse genome is only the beginning of using genetic approaches to study brain dopamine receptor function in the presence of cocaine. Recent advances in gene targeting techniques[43,44] make it possible to generate region-specific, developmental stage-specific knockout, and subtle changes (such as point mutations) of dopamine receptors in the mouse genome to study when, where and how they function in the presence of cocaine. Furthermore, mice with simultaneous deletion of different dopamine receptor gene subtypes can be generated and thus provide the opportunity to study synergistic or antagonistic effects among these dopamine receptor subtypes. The use of this powerful genetic approach will be essential for dissecting the components of the signal transduction pathway leading to anatomical, cellular, and behavioral changes in mice upon cocaine administration and dopamine neurotransmission. These studies will undoubtedly bring insights to, in addition to mechanisms of cocaine action, many aspects of the molecular mechanisms of behavior and help to understand the manifestation of neurological diseases as well as provide new strategies in cure and prevention of these diseases.

## SUMMARY

The brain dopamine system is thought to be the major target for the neuropharmacological actions of psychomotor stimulants such as cocaine. To investigate the mechanisms of cocaine action, we used a genetic approach, the gene-targeting technique, and generated D1 dopamine receptor mutant mice. Locomotor activity analysis in response to cocaine indicates that, in contrast to control mice which showed a dose-dependent increase in locomotion, D1 receptor mutant mice exhibited a dose-dependent decrease, suggesting that D1 receptors play an essential role in mediating such effects. Extracellular single unit recording of dopamine sensitive nucleus accumbens neurons in the D1 receptor mutant mice and control mice revealed a marked reduction in the inhibitory effects of cocaine and dopamine on the generation of action potentials, suggesting that D1 receptors play a fundamental role in cocaine- and dopamine-mediated neurophysiological effects within the nucleus accumbens. From these analyses, we conclude that the D1 dopamine receptor plays essential roles in mediating these effects of cocaine. In the future, the use of this powerful genetic approach will be essential for elucidating the molecular components of the signal transduction pathway leading to anatomical,

cellular and behavioral changes upon cocaine administration and dopamine neurotransmission.

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## REFERENCES

1. Dahlström, A. & K. Fuxe. 1964. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol. Scand. (Suppl.) **232:** 1–55.
2. Fallon, J. H. & R. Y. Moore. 1978. Catecholamine innervation of the basal forebrain III. Olfactory bulb, anterior olfactory nuclei, olfactory tubercle and piriform cortex. J. Comp. Neurol. **180:** 533–544.
3. Graybiel, A. M. & C. W. Ragsdale. 1983. Biochemical anatomy of the striatum. *In* Chemical Neuroanatomy. P. C. Emson, Ed.: 427–504. Raven Press. New York, NY.
4. Lindvall, O. & A. Björklund. 1983. Dopamine and norepinephrine-containing neuron systems: their anatomy in the rat brain. *In* Chemical Neuroanatomy. P. C. Emson, Ed.: 229–255. Raven Press, New York, NY.
5. Robbins, T. W. & B. J. Everitt. 1992. Functions of dopamine in the dorsal and ventral striatum. Semin. Neurosci. **4:** 119–127.
6. Hornykiewicz, O. 1966. Dopamine and brain function. Pharmacol. Rev. **18:** 925–964.
7. Koob, G. F. 1992. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol. Sci. **13:** 177–184.
8. Koob, G. F. 1992. Dopamine, addiction and reward. Semin. Neurosci. **4:** 139–148.
9. Koob, G. F. & F. E. Bloom. 1988. Cellular and molecular mechanisms of drug dependence. Science **242:** 715–723.
10. Le Moal, M. & H. Simon. 1991. Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol. Rev. **71:** 155–234.
11. Graybiel, A. M., R. Moratalla & H. A. Robertson. 1990. Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc. Natl. Acad. Sci. USA **87:** 6912–6916.
12. Nestler, E. J., B. T. Hope & K. L. Widnell. 1993. Drug addiction: a model for the molecular basis of neural plasticity. Neuron **11:** 995–1006.
13. Civelli, O., J. R. Bunzow & D. K. Grandy. 1993. Molecular diversity of the dopamine receptors. Annu. Rev. Pharmacol. Toxicol. **32:** 281–307.
14. Gingrich, J. A. & M. G. Caron. 1993. Recent advances in the molecular biology of dopamine receptors. Annu. Rev. Neurosci. **16:** 299–321.
15. Kebabian, J. W. & D. B. Calne. 1979. Multiple receptors for dopamine. Nature **277:** 93–96.
16. Ritz, M. C., R. J. Lamb, S. R. Goldberg & M. J. Kuhar. 1987. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science **237:** 1219–1223.
17. Kuhar, M. J., M. C. Ritz & J. W. Boja. 1991. The dopamine hypothesis of the reinforcing properties of cocaine. Trends Neurosci. **14:** 299–302.
18. Cabib, S., C. Castellano, V. Cestari, V. Filibeck & S. Puglisi-Allegra. 1991. D1 and D2 receptor antagonists differently affect cocaine-induced locomotor hyperactivity in the mouse. Psychopharmacol. **105:** 335–339.
19. Tella, S. R. 1994. Differential blockade of chronic versus acute effects of intravenous

cocaine by dopamine receptor antagonists. Pharmacol. Biochem. Behav. **48:** 151–159.
20. BARRETT, R. L. & J. B. APPEL. 1989. Effects of stimulation and blockade of dopamine receptor subtypes on the discriminative stimulus properties of cocaine. Psychopharmacology **99:** 13–16.
21. CALLAHAN, P. M., J. B. APPEL & K. A. CUNNINGHAM. 1991. Dopamine D1 and D2 mediation of the discriminative stimulus properties of *d*-amphetamine and cocaine. Psychopharmacology **103:** 50–55.
22. SPEALMAN, R. D., J. BERGMAN, B. K. MADRAS & K. F. MELIA. 1991. Discriminative stimulus effects of cocaine in squirrel monkeys: involvement of dopamine receptor subtypes. J. Pharmacol. Exp. Ther. **258:** 945–953.
23. KOOB, G. F., H. T. LE & I. CREESE. 1987. The D1 dopamine receptor antagonist SCH23390 increases cocaine self-administration in the rat. Neurosci. Lett. **79:** 315–320.
24. BERGMAN, J., J. B. KAMIEN & R. D. SPEALMAN. 1990. Antagonism of cocaine self-administration by selective dopamine D1 and D2 antagonists. Behav. Pharmacol. **1:** 355–363.
25. BRITTON, D. R., P. CURZON, R. G. MACKENZIE, J. W. KEBABIAN, J. E. G. WILLIAMS & D. KERKMAN. 1991. Evidence for involvement of both D1 and D2 receptors in maintaining cocaine self-administration. Pharmacol. Biochem. Behav. **39:** 911–915.
26. HUBNER, C. B. & J. E. MORETON. 1991. Effects of selective D1 and D2 dopamine antagonists on cocaine self-administration in the rat. Psychopharmacology **105:** 151–156.
27. CAINE, S. B. & G. F. KOOB. 1994. Effects of dopamine D-1 and D-2 antagonists on cocaine self-administration under different schedules of reinforcement in the rat. J. Pharmacol. Exp. Ther. **270:** 209–218.
28. PHILLIPS, A. G., C. L. BROEKKAMP & H. C. FIBIGER. 1983. Strategies for studying the neurochemical substrates of drug reinforcement in rodents. Prog. Neuropsychopharmacol. Biol. Psychiatry **7:** 585–590.
29. WOOD, D. M. & M. W. EMMETT-OGLESBY. 1989. Mediation in the nucleus accumbens of the discriminative stimulus produced by cocaine. Pharmacol. Biochem. Behav. **33:** 453–457.
30. MALDONADO, R., P. ROBLEDO, A. J. CHOVER, S. B. CAINE & G. F. KOOB. 1993. D1 dopamine receptors in the nucleus accumbens modulate cocaine self-administration in the rat. Pharmacol. Biochem. Behav. **45:** 239–242.
31. CALLAHAN, P. M., R. DE LA GARZA II & K. A. CUNNINGHAM. 1994. Discriminative stimulus properties of cocaine: modulation by dopamine D1 receptors in the nucleus accumbens. Psychopharmacology **115:** 110–114.
32. XU, M., R. MORATALLA, L. H. GOLD, N. HIROI, G. F. KOOB, A. M. GRAYBIEL & S. TONEGAWA. 1994. Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. Cell **79:** 729–742.
33. XU, M., X. T. HU, D. C. COOPER, R. MORATALLA, A. M. GRAYBIEL, F. J. WHITE & S. TONEGAWA. 1994. Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice. Cell **79:** 945–955.
34. WALAAS, I. 1981. Biochemical evidence for overlapping neocortical and allocortical glutamate projections to the nucleus accumbens and rostral caudatoputamen in the rat brain. Neuroscience **6:** 399–405.
35. WHITE, F. J., X.-T. HU & D. J. HENRY. 1993. Electrophysiological effects of cocaine in the rat nucleus accumbens: microiontophoretic studies. J. Pharmacol. Exp. Ther. **266:** 1075–1084.
36. MOGENSON, G. J., D. L. JONES & C. Y. YIM. 1980. From motivation to action: functional interface between the limbic system and the motor system. Prog. Neurobiol. **14:** 69–97.
37. MOGENSON, G. J. 1987. Limbic-motor integration. Prog. Psychobiol. Physiol. Psych. **12:** 117–170.

38. ROBBINS, T. W., M. CADOR, J. R. TAYLOR & B. J. EVERITT. 1989. Limbic-striatal interactions in reward-related processes. Neurosci. Biobehav. Rev. **13:** 155–162.
39. WHITE, F. J. 1990. Electrophysiological basis of the reinforcing effects of cocaine. Behav. Pharmacol. **1:** 303–315.
40. WISE, R. A. & M. A. BOZARTH. 1987. A psychomotor stimulant theory of addiction. Psychol. Rev. **94:** 469–492.
41. ROBINSON, T. E. & K. C. BERRIDGE. 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res. Rev. **18:** 247–291.
42. MORATALLA, R., H. A. ROBERTSON & A. M. GRAYBIEL. 1992. Dynamic regulation of *NGFI*-A (*zif*268, *egr*1) gene expression in the striatum. J. Neurosci. **12:** 2609–2622.
43. FURTH, P. A., P. GRUSS, M. GOSSEN, A. KISTNER, H. BUJARD & L. HENNIGHAUSEN. 1994. Temporal control of gene expression in transgenic mice by a tetracycline-responsive promoter. Proc. Natl. Acad. Sci. USA **91:** 9302–9306.
44. GU, H., J. D. MARTH, P. C. ORGAN, H. MOSSMANN & K. RAJEWSKY. 1994. Deletion of a DNA polymerase b gene segment in T cells using cell type–specific gene targeting. Science **265:** 103–106.
45. WHITE, F. J., X.-T. HU, D. J. HENRY & X.-F. ZHANG. 1995. Neurophysiological alterations in the mesocorticolimbic dopamine system during repeated cocaine administration. *In* The Neurobiology of Cocaine: Cellular and Molecular Mechanisms. R. P. Hammer, Jr., Ed. 99–119. CRC Press. Boca Raton, FL.