Behavioral/Systems/Cognitive

Lack of Self-Administration of Cocaine in Dopamine D_1 Receptor Knock-Out Mice

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Evidence suggests a critical role for dopamine in the reinforcing effects of cocaine in rats and primates. However, self-administration has been less often studied in the mouse species, and, to date, "knock-out" of individual dopamine-related genes in mice has not been reported to reduce the reinforcing effects of cocaine. We studied the dopamine D_1 receptor and cocaine self-administration in mice using a combination of gene-targeted mutation and pharmacological tools. Two cohorts with varied breeding and experimental histories were tested, and, in both cohorts, there was a significant decrease in the number of D_1 receptor knock-out mice that met criteria for acquisition of cocaine self-administration (2 of 23) relative to wild-type mice (27 of 32). After extinction of responding with saline self-administration, dose–response studies showed that cocaine reliably and dose dependently maintained responding greater than saline in all wild-type mice but in none of the D_1 receptor knock-out mice. The D_1-like agonist SKF 82958 (2,3,4,5,-tetrahydro-6-chloro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine hydrobromide) and the D_2-like agonist quinelorane both functioned as positive reinforcers in wild-type mice but not in D_1 receptor mutant mice, whereas food and intravenous injections of the opioid agonist remifentanil functioned as positive reinforcers in both genotypes. Finally, pretreatment with the D_1-like antagonist SCH 23390 [*R*-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-01] produced surmountable antagonism of the reinforcing effects of cocaine in the commonly used strain C57BL/6J. We conclude that D_1 receptor knock-out mice do not reliably self-administer cocaine and that the D_1 receptor is critical for the reinforcing effects of cocaine and other dopamine agonists, but not food or opioids, in mice.

Key words: **dopamine; cocaine; D_1; self-administration; knock-out; mouse**

Introduction

Cocaine dependence is a serious public health problem for which there are no effective treatment medications (Mendelson and Mello, 1996; National Institute on Drug Abuse, 2003). Evidence suggests that the reinforcing effects of cocaine are related to its action as an indirect dopamine agonist (Wise and Bozarth, 1987; Koob, 1992). Accordingly, understanding the roles of the various dopamine receptors may suggest avenues for the development of medications (Mello and Negus, 1996). Gene targeting may offer one strategy toward this end, but in previous studies, "knock-out" of individual dopamine-related genes, including those for the dopamine transporter and the D_2 receptor, failed to reduce the reinforcing effects of cocaine (Rocha et al., 1998; Caine et al., 2002).

Genes for five dopamine receptors have been cloned, and there are several reasons that the D_1 receptor is of particular interest regarding the abuse-related effects of cocaine. First, there has been growing interest in the D_1 receptor as a target for pharmacotherapies for stimulant dependence in humans (Haney et al., 1999, 2001; Romach et al., 1999). Second, although D_1-like and D_2-like receptors have both been implicated in the discriminative and reinforcing effects of cocaine in rats and primates (Woolverton et al., 1984; Bergman et al., 1990; Kleven et al., 1990; Spealman et al., 1991; Witkin et al., 1991; Self and Stein, 1992; Caine and Koob, 1993, 1994; Weed and Woolverton, 1995), important differences in the effects produced by stimulating D_1 and D_2 receptors have been observed (Grech et al., 1996; Self et al., 1996; Caine et al., 1999a, 2000a,b; Khroyan et al., 2000). Third, D_2 receptor knock-out mice acquired cocaine self-administration (Caine et al., 2002), suggesting that receptors other than D_2 were sufficient to mediate the reinforcing effects of cocaine. Moreover, in contrast to rats, psychomotor stimulant effects in several strains of mice were more prominently reproduced by direct stimulation of D_1-like receptors than D_2-like receptors, and those

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effects were absent in D_1 receptor mutant mice but remained intact in D_2 receptor mutant mice (Ralph-Williams et al., 2002, 2003; Ralph and Caine, 2005). Thus, D_1-selective compounds may have important and qualitatively distinct effects from other dopaminergic drugs in both humans and laboratory animals, and evidence from recent studies suggests that D_1-like receptors may play an especially critical role in psychomotor effects in mice.

The goal of the present study was to test the hypothesis that the D_1 receptor is critical for the reinforcing effects of cocaine in mice. First, acquisition of self-administration was evaluated in two cohorts of D_1 receptor mutant and wild-type mice with varying experimental and drug histories. Second, various cocaine doses were systematically evaluated, and food and cocaine were made available alternately over consecutive sessions to enhance the probability of acquisition of cocaine self-administration in the mutant mice. Third, reinforcing effects of the D_1 agonist SKF 82958 (2,3,4,5,-tetrahydro-6-chloro-7,8-dihydroxy-1-phenyl-1*H*-3-benz-azepine hydrobromide), the D_2 agonist quinelorane, and the opioid agonist reminfentanil were evaluated. Finally, the effects of pharmacological blockade with the D_1-selective antagonist SCH 23390 [*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride] on cocaine self-administration were assessed in the commonly used C57BL/6J strain for comparison with effects previously reported in other species.

Materials and Methods

Animals and housing conditions. The dopamine D_1 receptor-deficient mice were generated using homologous recombination as described previously, using the 129/SvJ strain for genetic and embryonic material and the C57BL/6J strain for breeding (Xu et al., 1994a). Mice homozygous for the D_1 receptor gene were identified by Southern blot analysis. Mutant mice were ~30% lower in body weight than their wild-type littermates but exhibited no other gross physiological abnormalities. It should be noted that experimenters could not easily be "blinded" to the genotype of the animals because the D_1 receptor mutant mice were sometimes overtly smaller than wild-type mice. Self-administration data are shown for a total of 27 mutant and 36 wild-type mice. The numbers of male and female mice for the data shown in each figure were as follows: 21 male and 11 female wild type, 14 male and 9 female mutant, Figure 1; 14 male and 6 female wild type, 10 male and 6 female mutant, Figures 2–4; all male mice, Figures 5–7.

Male C57BL/6J mice were obtained from Charles River Laboratories (Wilmington, MA) (data from five mice shown in Fig. 8).

Mice were shipped at age 10–16 weeks and studied for up to 4 months thereafter.

Mice were group housed up to four per cage (8.8 × 12.1 × 6.4 inches). Water was available *ad libitum* in the home cage. Standard rodent chow was available *ad libitum* except during the initial several days of operant training for food reinforcement in the second cohort of mice (see below). The temperature was maintained at ~68°F, and illumination was provided for 12 h/d.

Overview of breeding and experimental history of mice. There were two cohorts of mice with different breeding and experimental histories in the present study, as follows.

The first cohort of mice (n = 19) was of the F2 generation from heterozygous breeding pairs and was transferred from Massachusetts Institute of Technology to The Scripps Research Institute, where they were maintained on a reverse 12 h light/dark cycle (lights on at 7:00 P.M.) and tested during the dark phase. Before any self-administration tests, preliminary studies of drug-induced locomotor activity were conducted that were later extended and reported previously (Xu et al., 2000). Specifically, intraperitoneal injections of D-amphetamine sulfate (1.0, 3.0, 5.6, and 3.0 mg/kg, in that order) and cocaine (20.0 mg/kg) were administered to all mice with 2 d separating injections, and the animals were monitored for their locomotor activity for several hours in a photocell apparatus. After locomotor studies were completed, this first cohort of mice was tested in acquisition of self-administration with no previous operant experience (see Fig. 1, left bars).

For all other studies, a few years after the F2 generation were generated and studied (see above), a second cohort of mice (n = 44) were used that were initially backcrossed for three generations to C57BL/6J mice (obtained from The Jackson Laboratory, Bar Harbor, ME), and then the various genotypes were generated using heterozygous breeding pairs. These mice were transferred from the University of Cincinnati to McLean Hospital, where they were maintained on a normal 12 h light/dark cycle (lights on at 7:00 A.M.) and tested during the light phase. These mice were drug naive and were trained and evaluated with food reinforcement (see below) before tests of intravenous drug self-administration (see Figs. 1, right bars, 2–8).

Animal health and welfare. Vivarium conditions were maintained in accordance with the guidelines provided by the *NIH Committee on Laboratory Animal Resources*. All experimental protocols were approved by the Institutional Animal Care and Use Committee. Animal experimentation adhered to the guidelines described in the *Policy on the Use of Animals in Neuroscience Research* for the Society for Neuroscience. The health of the mice was evaluated by research technicians on a daily basis and was also occasionally monitored by consulting veterinarians. In addition, sentinel animals were periodically evaluated by Charles River Laboratories Comprehensive Health Monitoring Program.

Behavioral test apparatus. Experimental chambers (6.3 × 5.5 × 5.0 inches) inside sound-attenuating cubicles were equipped with a house light, ventilator fan, drug infusion pump, liquid swivel with counterbalance arm, and two manipulanda with cue lights that were located on either side of an apparatus for delivering liquid food. The manipulanda were holes (~1.2 cm diameter) equipped with photocells (for nose-poke activation). All equipment was either manufactured in-house or obtained from Med Associates (Georgia, VT) except for the liquid swivel and counterbalance assembly (Instech, King of Prussia, PA). Scheduling of experimental events and data collection were accomplished using a DOS-based microcomputer system equipped with programs written in C++ or Med Associates MedState Notation.

Surgical procedures. Mice were anesthetized with halothane or isofluorane vapor mixed with oxygen and prepared with chronic indwelling intravenous catheters as described previously (Caine et al., 1993, 1999b; Thomsen and Caine, 2005, 2007). Briefly, a 6 cm length of SILASTIC tubing (0.25 mm inner diameter, 0.76 mm outer diameter) was fitted to a 26 gauge stainless steel cannula that was bent at a right angle and then embedded in a cement disk with an underlying nylon mesh. The catheter tubing was inserted 1.2 cm into the right external jugular vein and anchored with suture (Barr et al., 1979). In some cases, implantation in the right jugular vein was unsuccessful because of vein constriction during the procedure, in which case the catheter was implanted in the left jugular vein. The remaining tubing ran subcutaneously to the cannula, which exited at the midscapular region. All incisions were sutured and coated with triple antibiotic ointment. Ticarcillin disodium or Cefazolin (20 μl of 67 mg/ml saline) was administered through the catheter immediately after surgery to forestall infection. For the next 4–7 d, mice were allowed to recover from surgery, and antibiotic was administered as before but with 30 U/ml heparin in the solution. Thereafter, catheters were flushed with saline containing heparin only (30 U/ml).

The patency of intravenous catheters was evaluated periodically (usually once per week, on Fridays after behavioral testing, and/or at the completion of each experimental phase, and whenever drug self-administration behavior appeared to deviate dramatically from that observed previously). Catheter evaluations were always performed at least 2 h or more before or after a drug self-administration test session. Approximately 20 μl of 1% methohexital sodium (Brevital; Eli Lilly & Co., Indianapolis, IN) or a mixture containing 15% Ketaset (ketamine, 100 mg/ml), 15% Versed (midazolam, 5 mg/ml), and 70% saline was infused through the catheter. If prominent signs of anesthesia were not apparent within 3 s of infusion and the left jugular vein had not been implanted previously (see above), then the catheter was surgically removed from the right jugular vein and a new catheter was implanted in the left jugular vein using the surgical procedures described above. Most of the data were

collected from mice with their first catheter in the right jugular vein. In ~25% of the mice, data were also collected after a second catheter was implanted in the left jugular vein because of failure of the first catheter (for details, see Thomsen and Caine, 2005, 2007).

Food-maintained responding in mice. In the second cohort of mice, operant responding with food reinforcement was trained and evaluated before self-administration studies. Mice were first acclimated to the food that was subsequently used to reinforce operant responding. Mice were deprived of food for 20 h, which resulted in a mean reduction in body weight of ~5%. Mice were then placed in test chambers for 2 h with the fan and house light activated, and with a small cup containing 5 ml of vanilla-flavored Ensure (a nutritional supplement, hereafter referred to as "liquid food"). After the acclimation session, mice were given 3 g each of mouse chow in their home cage. This procedure was repeated until a minimum of 1.5 ml of liquid food was consumed during a 2 h acclimation session (typically within one or two sessions, although sometimes longer for D_1 receptor mutant mice). Thereafter, mouse chow was available *ad libitum* in the home cage, and the small cup was removed from the test chamber.

During subsequent 2 h sessions, liquid food was available under a fixed ratio (FR) 1 timeout (TO) 30 s schedule of reinforcement. When one manipulandum was activated (the "active" nose-poke hole), the adjacent cue light was illuminated, the house light was extinguished, and 17 μl of liquid food was delivered into the chamber. Nose pokes in the other ("inactive") hole and nose pokes in the active hole while the dipper was raised (timeout responses) were recorded but were without scheduled consequences. Each session was preceded by presentation of one reinforcer, together with the cue light, for 60 s. The session was terminated after 100 reinforcers were delivered or after 2 h, whichever occurred first.

To fully evaluate the relationship between behavior and the magnitude of a nondrug reinforcer in D_1 receptor mutant and wild-type mice, nose pokes in the active hole were reinforced with various concentrations of food or water in a series of three training phases that have been described previously (Caine et al., 1999b). Briefly, nose pokes in the active hole were reinforced under the FR 1 TO 30 s schedule with 100% liquid food for a minimum of five 2-h sessions and until responding met criteria for baseline ("acquisition"). The criteria for baseline were (1) stable daily responding (within 20% across two consecutive sessions), (2) a minimum of 20 responses in the active hole, and (3) at least 70% of responses in the active hole. These criteria were selected because they are identical or nearly identical to criteria commonly used in previous studies designed to evaluate acquisition of operant responding in mutant mice (Rocha et al., 1997, 1998; Caine et al., 1999b, 2002; Thomsen and Caine, 2006).

After these baseline criteria were met, responses on the active manipulandum resulted in the presentation of water rather than liquid food for three subsequent sessions ("extinction"). Finally, responding produced liquid food (100%) or water presentations on alternate days over the next six sessions ("alternation"). These three training phases were designed to establish nose-poke behavior that was related to presentations of a positive reinforcer (i.e., appetitive liquid food, but not water, in mice that had standard mouse chow and water available *ad libitum* in the home cage).

After the training phases of acquisition, extinction, and alternation were completed, responding maintained by various concentrations of liquid food (water or 3, 10, 32, and 100% Ensure in water) was evaluated to assess general operant performance across a range of reinforcer magnitudes. Concentrations of liquid food were presented for single sessions according to a Latin square design, and then this was repeated. Thus, over the course of several weeks, one concentration–effect function was fully determined and then a second concentration–effect function was determined.

Drug self-administration in mice. In operant naive mice, or after training and evaluation of food-maintained behavior was completed, animals were implanted with chronic indwelling jugular catheters. Drug self-administration sessions were conducted using the schedule parameters described above for food-maintained responding, with the following exceptions. Responding was maintained by intravenous infusions of cocaine delivered in 18 μl over 2 s [~1.0 mg/kg per injection, assuming a body weight of 33 g per mouse (Fig. 1, leftmost bars); or precisely 1.0 mg/kg per injection, adjusted daily according to each mouse's body weight (rightmost pair of bars in Fig. 1 and all subsequent figures)]. One deviation to this protocol was that data shown for Figure 7 were from animals with previous operant training with food and then drug self-administration sessions with remifentanil (precisely 0.01 mg/kg per injection, adjusted daily according to each mouse's body weight) as the reinforcer rather than cocaine. A 28 s post-reinforcer timeout period was selected to match the parameters used for food-maintained responding (FR 1 TO 30 s). Sessions were initiated with an infusion that filled the catheter volume and then delivered one unit dose of cocaine that was available for self-administration. To prevent adverse long-term health consequences of high-dose stimulant self-administration (e.g., chronic hypothermia and hypophagia were observed after termination of higher-dose self-administration sessions), a maximum of 10 infusions was allowed per session for the highest dose of each self-administered drug, and a maximum of 30 infusions was allowed per session for the next lower dose (no limit was set for remifentanil studies). For all other doses of self-administered drugs, a maximum of 100 infusions was allowed per session because of volume limitations. All self-administration sessions were 3 h in duration or until the maximum number of reinforcers was earned, whichever occurred first.

Criteria for stable cocaine-maintained responding were the same as for acquisition of food-maintained responding: (1) stable daily responding (within 20% across two consecutive sessions), (2) a minimum of 20 responses in the active hole, and (3) at least 70% of responses in the active hole. These criteria were selected because they are identical or nearly identical to criteria commonly used in previous studies designed to evaluate acquisition of cocaine self-administration in mutant mice (Rocha et al., 1997, 1998; Caine et al., 1999b, 2002; Thomsen and Caine, 2006). After these criteria were met, either immediately or after one additional drug self-administration session (depending on the robustness and stability of responding during that session and previous sessions), saline injections were substituted for drug injections until responding was <50% of the animal's cocaine baseline, and then responding maintained by various doses of cocaine (0.03–3.2 mg/kg per injection) or saline was evaluated, according to a Latin square design. For remifentanil studies, the same basic design was used but only a single dose of remifentanil was studied (i.e., 0.01 mg/kg per injection until baseline criteria were met, then extinction with saline, then 0.01 mg/kg per injection remifentanil again). For mice that did not acquire cocaine self-administration within seven sessions, approximately half the mice continued testing with cocaine (1.0 mg/kg per injection) and the other half were tested in sessions in which food and cocaine were made available alternately over at least six additional consecutive sessions.

In subsequent studies after evaluation of cocaine self-administration, self-administration of the D_1-like agonist SKF 82958 (0.1–32.0 μg/kg per injection) and the D_2-like agonist quinelorane (0.003–1.0 mg/kg per injection) were evaluated. The criteria for acquisition of self-administration were the same as those for acquisition of cocaine self-administration (see above), except that criteria could be met in a single session (rather than two consecutive sessions with <20% variability in the number of injections, a single session with a minimum of 17 injections and greater than or equal to 70% of nose pokes in the active manipulandum was sufficient). This change in criteria was instituted for two reasons. First, higher variability in self-administration of SKF 82958 and quinelorane was noted in previous (Caine et al., 2002) and preliminary (data not shown) studies. Second, by this point in the series of studies, after cocaine self-administration tests were completed, catheter failure was considered a greater risk and so extinction with saline self-administration and dose–effect studies with SKF 82958 and quinelorane were conducted as expeditiously as possible. If mice did not meet criteria for self-administration of SKF 82958 (10.0 μg/kg per injection) or quinelorane (0.32 mg/kg per injection) within seven consecutive sessions, liquid food (100%) or drug was made available alternately in consecutive sessions, and drug unit doses were tested across a log unit range, as described above for cocaine self-administration acquisition studies.

In a group of C57BL/6J mice ($n = 5$), the effects of pretreatment with the D_1-like antagonist SCH 23390 (0.032–0.32 mg/kg, i.p.) on cocaine self-administration were evaluated. Doses of SCH 23390 were selected

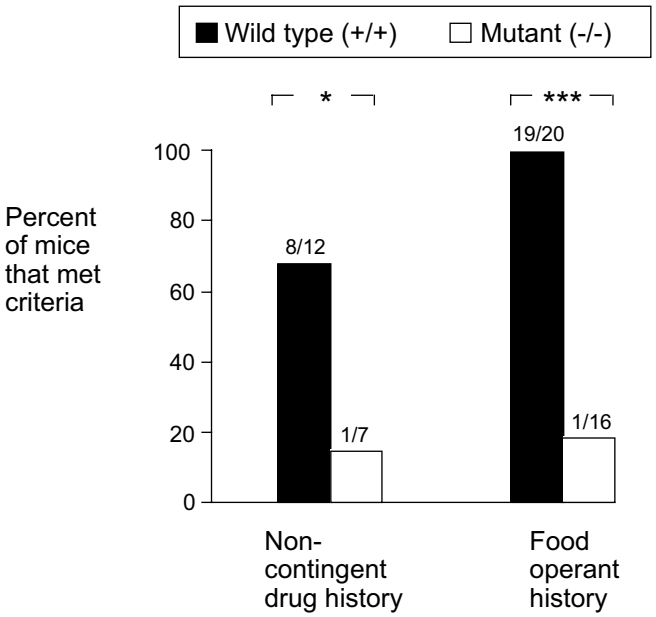

Figure 1. Summary of data for acquisition of cocaine self-administration (0.5 or 1.0 mg/kg per injection). Abscissa, Experimental history including operant naive mice (left pair of bars) and mice that had previous operant training with food reinforcement (right pair of bars). Ordinate, Percentage of mice that met criteria for acquisition of cocaine self-administration under the FR 1 schedule. Criteria for acquisition were stable levels of responding (within 20% across 2 consecutive sessions), a minimum of 20 active nose pokes, and at least 70% of nose pokes in the active hole. Numbers at top of each bar indicate number of mice that met criteria/group size. Asterisks indicate statistical significance (*$p < 0.05$, ***$p < 0.001$; all data analyzed by χ^2 test for genotype). The cocaine dose for all data shown was 1.0 mg/kg per injection.

from previous studies in mice (Ralph-Williams et al., 2002). SCH 23390 was administered intraperitoneally ~10 min before cocaine self-administration sessions.

Data analysis. For acquisition data, the numbers of mice that met criteria were analyzed by an information statistic (χ^2 test) by genotype (D_1 receptor mutant and wild type) for cocaine self-administration or by reinforcer type (food and cocaine) in groups of D_1 receptor mutant mice only. For all other measures, data were analyzed with ANOVA using genotype and sex as between-subjects factors and using repeated measures for food concentration, self-administered drug dose, or pretreatment drug dose as within-subjects factors. After acquisition tests for self-administration in the second cohort of mice, 2 of the 20 wild-type mice did not complete dose–effect determinations because of catheter failure or illness and were excluded from the data analyses and presentation. There were no missing cells in ANOVA analyses. Significant main effects or interactions were followed by pairwise comparisons using Newman–Keuls *post hoc* tests. The criterion for significance was $p < 0.05$ for all analyses, and, in the figures, asterisks were used to indicate significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Drugs. Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (National Institutes of Health). D-Amphetamine sulfate, SKF 82958, quinelorane, and SCH 23390 were obtained from Research Biochemicals (Natick, MA). Remifentanil hydrochloride (Ultiva) was obtained from Cardinal Health (Peabody, MA). All doses refer to the weights of the respective salts. Pretreatment doses labeled "zero" indicate administration of the vehicle. The vehicle was saline for all drugs and was administered in a volume of 10 ml/kg for intraperitoneal injections.

Results

Acquisition of cocaine self-administration in two cohorts of mice

Figure 1 shows the percentage of mice in each experimental group that met criteria for acquisition of cocaine self-administration (i.e., at least 20 injections earned and at least 70% of nose pokes in the active hole during at least two 3-h test sessions over a minimum of seven consecutive test sessions). There were no significant sex effects; therefore, female and male mice were combined for the group data shown.

Taking into account all mice that were studied in cocaine self-administration regardless of previous drug and operant training history ($n = 55$), 27 of 32 wild-type mice met criteria for acquisition of cocaine self-administration, and 2 of 23 mutant mice met the criteria, and the effect of genotype was statistically significant (Fig. 1, all bars) ($\chi^2 = 24.05$; $p < 0.001$).

The "noncontingent drug history" mice were of the F2 generation and had been injected with D-amphetamine (1.0, 3.0, 5.6, and 3.0 mg/kg) and cocaine (20 mg/kg) (with 2 d between injections) for preliminary screening of their responses to the psychomotor stimulants and had no previous operant training. Of 12 wild-type mice, eight met criteria for acquisition of cocaine self-administration (1.0 mg/kg per injection), and only one of seven homozygous mutant mice met the criteria, and the effect of genotype was statistically significant (Fig. 1, left pair of bars) ($\chi^2 = 5.27$; $p < 0.05$).

The "food operant history" mice were backcrossed for three generations to C57BL/6J and then generated exclusively from heterozygous breeding pairs, and all mice were drug naive and trained with food reinforcement (see below) before cocaine self-administration. Under these conditions, 19 of 20 wild-type mice met criteria for acquisition of cocaine self-administration (1.0 mg/kg per injection), whereas only 1 of 16 homozygous mutant mice met the criteria, and the effect of genotype was statistically significant (Fig. 1, right pair of bars) ($\chi^2 = 31.65$; $p < 0.001$).

Cocaine self-administration in mice initially trained with food reinforcement

Figure 2 shows cocaine self-administration data in wild-type mice and D_1 receptor mutant mice after training and evaluation of food-maintained responding was completed (see below). Top row shows tests of acquisition of cocaine self-administration (1.0 mg/kg per injection) for up to seven consecutive sessions (until a mouse reached criteria for acquisition and was moved to another phase of the study), and bottom row shows dose–effect tests for cocaine self-administration. There were no significant sex effects; therefore, female and male mice were combined for the group data shown.

Of 20 wild-type mice, 19 met criteria for acquisition of cocaine self-administration (1.0 mg/kg per injection) within seven sessions (Fig. 2, top left). In the first eight mutant mice examined, none of the mutant mice acquired cocaine self-administration during the first few sessions. Thereafter, all of those eight mutant mice, as well as eight additional mutant mice, were subjected to a slightly modified protocol, as follows. With the self-administration tether attached to the catheter port but with saline in the drug syringe and the syringe pump turned off, liquid food-maintained responding was reestablished to baseline criteria. Thereafter, acquisition of cocaine self-administration (1.0 mg/kg per injection) was tested for a minimum of seven consecutive sessions. Under these conditions, 1 of 16 D_1 receptor mutant mice met criteria for acquisition of cocaine self-administration (on the 12th consecutive test session). After seven consecutive sessions of testing with cocaine (1.0 mg/kg per injection), all of the wild-type mice and half of the D_1 receptor mutant mice were transferred to cocaine dose–effect studies (Fig. 2, bottom row). In the group of 18 wild-type mice (2 of 20 were removed from the dose–effect study because of catheter malfunction or illness),

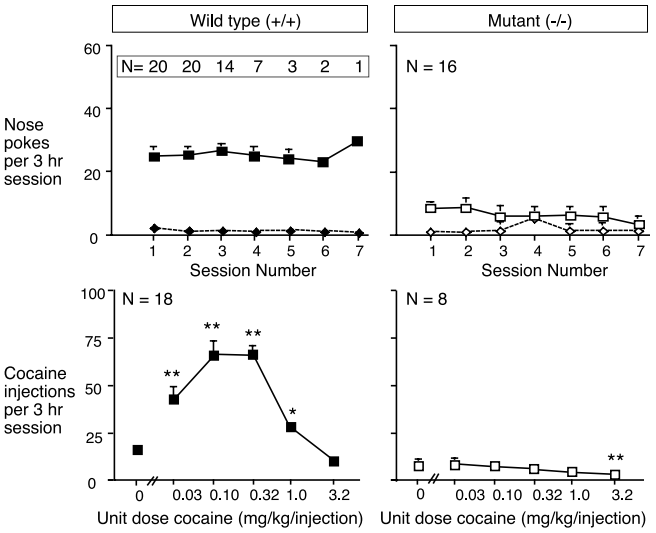

Figure 2. Cocaine self-administration in mice previously trained with food reinforcement: acquisition and effect of cocaine dose. Abscissas, Session number (top row) or cocaine dose in milligrams per kilogram per injection (bottom row); points above zero depict data from saline self-administration. Ordinates, Nose pokes (top row) and cocaine injections self-administered (bottom row) in 3 h sessions under the FR 1 schedule. Cocaine dose was 1.0 mg/kg per injection during acquisition (top row). Filled symbols show data for wild-type (+/+) mice, and open symbols show data for mutant (−/−) mice. Squares show data for reinforced nose pokes (active hole), and diamonds show data for nonreinforced nose pokes (inactive hole). In top left, group size decreases with increasing session number as acquisition tests ceased for each mouse as soon as they met criteria for self-administration. After acquisition tests, 2 of the 20 wild-type mice did not complete dose–effect determinations because of catheter failure or illness (bottom left). After acquisition tests, half of the mutant mice were tested with various cocaine doses in consecutive sessions (bottom right), and the other half were tested with food and cocaine reinforcement alternately (see Fig. 4). All symbols depict group means and SEM. Asterisks indicate significantly different from saline self-administration by pairwise comparison (*$p < 0.05$, **$p < 0.01$) following significant main effect of cocaine dose by ANOVA.

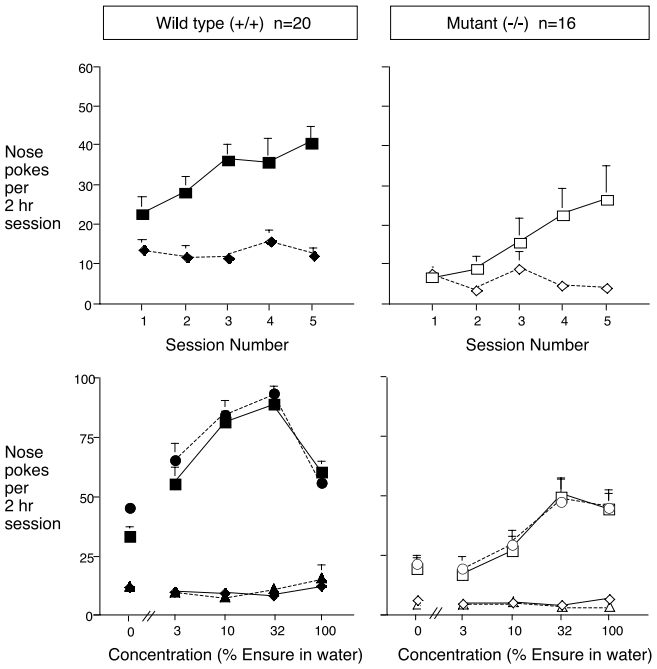

Figure 3. Nose-poke behavior reinforced with liquid food: acquisition and effect of food concentration. Abscissas, Session number (top row) or liquid food concentration (bottom row). Ordinates, Nose pokes in 2 h sessions under the FR 1 schedule. Liquid food concentration was 100% during acquisition (top row). Filled symbols show data for wild-type (+/+) mice, and open symbols show data for mutant (−/−) mice. Squares show data for reinforced nose pokes (active hole), and diamonds show data for nonreinforced nose pokes (inactive hole). Circles and triangles show data for a second determination of the concentration–effect curve for reinforced and nonreinforced nose pokes, respectively. All symbols depict group means and SEM. Significant main effects included genotype, session number, hole selection, food concentration, and multiple main interactions (for details, see Results).

there was a highly significant effect of cocaine dose ($F_{(5,85)} = 37.1$; $p < 0.0001$). Moreover, the dose–effect function for cocaine self-administration in wild-type mice was an inverted U-shaped function, as is typical for drug self-administration under these conditions (Fig. 2, bottom left). In contrast to wild-type mice, none of the D_1 receptor mutant mice exhibited an inverted U-shaped function relating self-administration behavior to cocaine dose (Fig. 2, bottom right). Even the one mutant mouse that met criteria for acquisition of cocaine self-administration (1.0 mg/kg per injection) exhibited extinction of responding during dose–effect tests. There was a significant effect of cocaine dose in the D_1 receptor mutant mice ($F_{(5,35)} = 5.44$; $p < 0.001$), but, relative to the wild-type mice, the dose–effect function was flat, and the only cocaine dose that differed significantly from saline self-administration by pairwise comparison was the highest cocaine dose (3.2 mg/kg per injection; $p < 0.01$). Thus, the group of eight D_1 receptor mutant mice did not self-administer any cocaine dose at higher rates than saline, and the highest dose of cocaine engendered significantly lower rates than saline, suggesting some behavioral effect of this cocaine dose in the D_1 receptor mutant mice but no positive reinforcing effects.

Training and evaluation of food-maintained behavior

Figure 3 shows behavior maintained by 100% liquid food under a FR 1 TO 30 s schedule of reinforcement in wild-type mice and D_1 receptor mutant mice during five sessions of acquisition (top row) and when the concentration of liquid food was varied (bottom row). There were no significant sex effects; therefore, female and male mice were combined for the group data shown.

Nose-poke behavior reinforced by liquid food was acquired by all of the mice (Fig. 3, top row). However, the number of food-reinforced nose pokes per hour was significantly lower in mutant mice than in wild-type mice across the five acquisition sessions (genotype, $F_{(1,34)} = 10.9$; $p < 0.01$). There were significant main effects of session number and nose-poke selection (active and inactive) and session number × nose-poke selection interactions, but there was not a significant session number × nose-poke selection × genotype interaction ($F_{(4,136)} = 1.73$; $p > 0.1$). Thus, both wild-type and D_1 receptor mutant mice acquired food-maintained responding, although nose-poke behavior was significantly lower for D_1 receptor mutant mice throughout acquisition and for both active and inactive nose-poke responses.

When responding was reinforced with water or with various concentrations of food (3, 10, and 32% liquid food in water), rates of nose-poking behavior were related to the concentration of the liquid food in both wild-type and mutant mice (Fig. 3, bottom row). There were significant main effects of genotype ($F_{(1,34)} = 50.5$) and also response selection, food concentration, and genotype × response selection × food concentration interactions ($p < 0.0001$ for all of these main effects and interactions). Overall, rates of nose-poke behavior maintained by all concentrations of food were significantly lower in mutant mice compared with wild-type mice ($p < 0.05$ by pairwise comparison for each food concentration between genotypes). There was no difference between the first and second determinations of the food concentration–effect curve for either genotype (Fig. 3, bottom

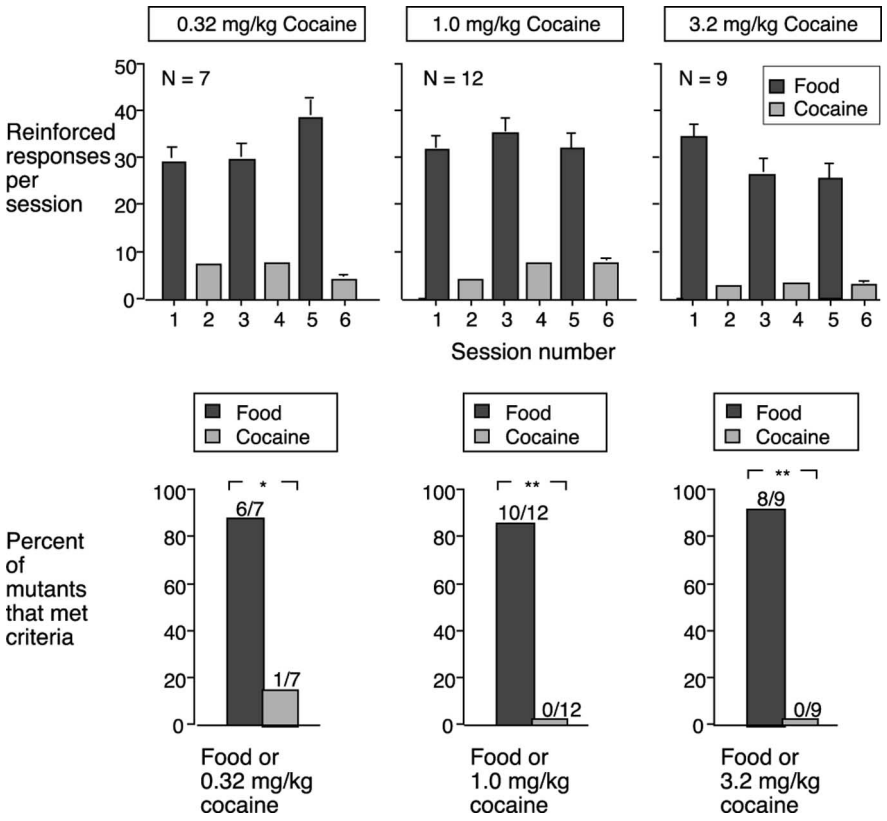

Figure 4. Alternating daily test sessions with either cocaine or food as the reinforcer in groups of D_1 receptor mutant (−/−) mice. Abscissas, Session number (top row) or reinforcers available (bottom row). Ordinates, Reinforced nose-poke responses per session under the FR 1 schedule (top row) or percentage of mutant mice that met criteria for acquisition of responding (bottom row). Food concentration was 100% in all panels and is depicted in dark gray bars, and cocaine was 0.32, 1.0, or 3.2 mg/kg per injection and is depicted in lighter gray bars. Numbers at top of each bar indicate number of mice that met criteria/group size. Asterisks indicate statistical significance (*$p < 0.05$, **$p < 0.01$; all data analyzed by χ^2 test for reinforcer type, i.e., food or cocaine).

row, circles and squares). Thus, behavior was related to the magnitude of the food reinforcer in both mutant and wild-type mice, and, once the behavior was acquired, the effect of varying the food concentration did not change over time.

Cocaine self-administration and food reinforcement tested on alternate days in D_1 receptor mutant mice

In D_1 receptor mutant mice that failed to acquire cocaine self-administration during consecutive daily tests, as well as in the one D_1 receptor mutant mouse that met criteria for acquisition but then extinguished during dose–effect tests, nose-poke behavior was reinforced with a single dose of cocaine (0.32, 1.0, or 3.2 mg/kg per injection) for a 3 h session for 1 d and then with liquid food (100%) for a 2 h session on another day, alternately, over at least six consecutive sessions (Fig. 4). In these studies, only one of the D_1 receptor mutant mice met the criteria for cocaine self-administration (0.32 mg/kg per injection), and this was the same mouse that had acquired cocaine self-administration (1.0 mg/kg per injection) before alternating daily test sessions with either cocaine or food as the reinforcer. In contrast to cocaine, for liquid food, criteria for stable responding were met by most of the D_1 receptor mutant mice (24 of 28 observations; $n = 7$, 12, and 9 for cocaine doses of 0.32, 1.0, and 3.2 mg/kg, respectively). The reinforcing effects of 100% liquid food and 0.32, 1.0 mg/kg per injection or 3.2 mg/kg per injection of cocaine were significantly different in D_1 receptor mutant mice ($p < 0.05$ or $p < 0.01$; $n = 7$, 12, and 9, respectively) (Fig. 4).

Response patterns in individual mice for cocaine self-administration and food reinforcement

Cumulative response records for individual mice suggested stable nose-poke behavior maintained by liquid food (32%) during a 2 h session (Fig. 5, top) and by cocaine (1.0 mg/kg per injection) during a 3 h session (Fig. 5, middle) for a wild-type mouse (#30) and a mutant mouse (#40). With regard to rates and patterns of food-maintained responding, these mice were representative of the group of mice for each genotype. Specifically, a drop off in rates of food-maintained responding over the course of the sessions was more frequently observed for mutant mice relative to wild type. With regard to rates and patterns of cocaine-maintained responding, mouse #40 was not representative of the group of mutant mice but rather was one of the two mutant mice that reached criteria for acquisition of cocaine self-administration in this report. In those two mutant mice that reached the criteria, behavior maintained by 1.0 mg/kg per injection of cocaine appeared relatively stable for the duration of the sessions and nose pokes were emitted almost exclusively in the active manipulandum (e.g., only one inactive nose poke was emitted by mouse #40 for the session shown in the middle). However, even in those two mutant mice that met criteria for acquisition of cocaine self-administration, relative to wild-type mice, pauses were more frequently observed during the course of the sessions (middle), responding was less reliable across sessions, and dose–effect functions were flat (bottom). Thus, cocaine did not maintain rates of responding higher than saline in any D_1 receptor mutant mouse in any of the experiments.

Self-administration of dopamine D_1- and D_2-selective agonists

Figure 6 shows data from wild-type and D_1 receptor mutant mice that completed cocaine self-administration tests (described above) and were then tested with the D_1 agonist SKF 82958 and/or the D_2 agonist quinelorane as an intravenous reinforcer. There were no significant sex effects; therefore, female and male mice were combined for the group data shown.

Given a minimum of seven consecutive sessions, four of five wild-type mice met criteria for acquisition of self-administration of the D_1 agonist SKF 82958 (10.0 μg/kg per injection) (Fig. 6, top left). In contrast, none of the five D_1 receptor mutant mice met criteria for acquisition of SKF 82958 self-administration (10.0 μg/kg per injection). Several D_1 receptor mutant mice were tested with various other doses of SKF 82958 as a reinforcer (3.2 and 32.0 μg/kg per injection), and several D_1 receptor mutant mice were also tested in sessions in which 100% liquid food or SKF 82958 (3.2, 10.0, or 32.0 μg/kg per injection) were available

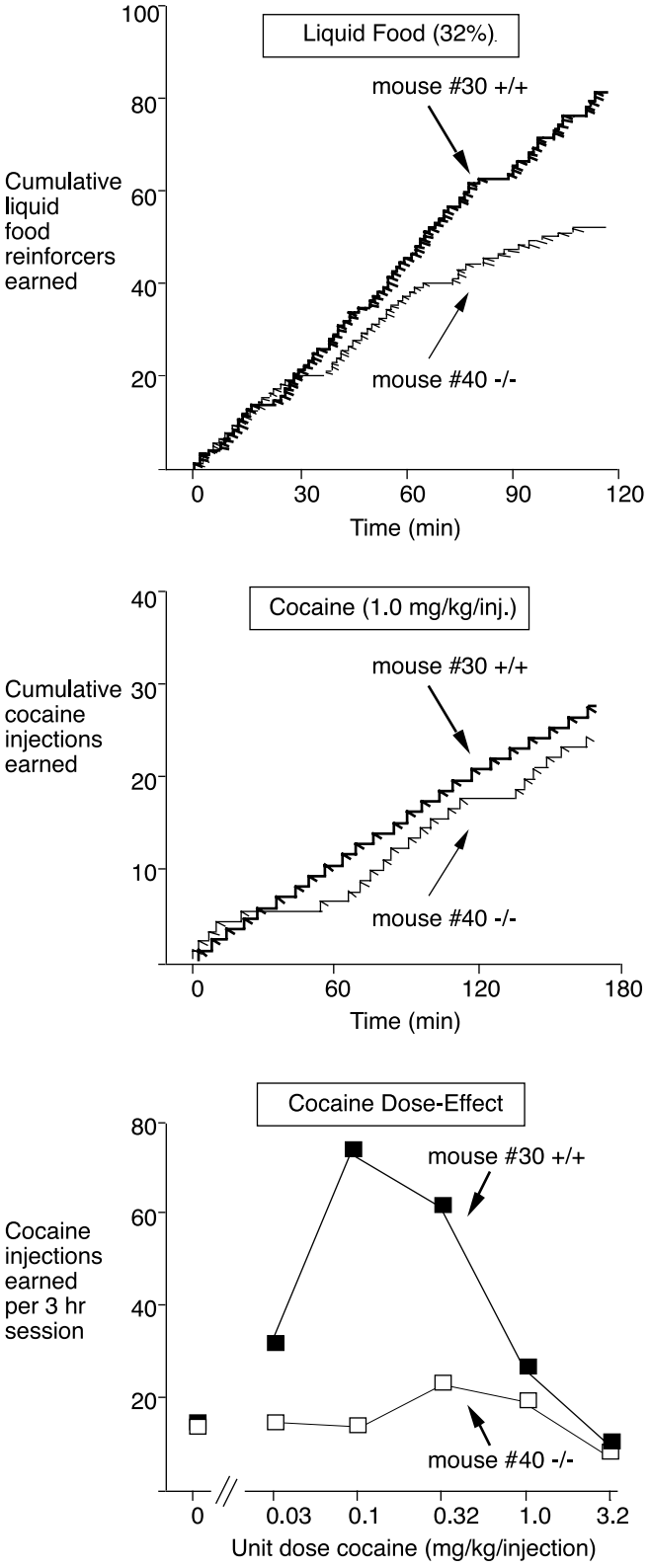

Figure 5. Operant behavior in representative individual mice. Abscissas, Time within a session (top and middle) or cocaine dose in milligrams per kilogram per injection (bottom). Ordinates, Cumulative number of liquid food deliveries (top) or cocaine injections (middle) within a session, or total cocaine injections earned in a 3 h session under the FR 1 schedule (bottom). Each slanted tick mark indicates a reinforced nose-poke response (top and middle). Bold cumulative records and filled squares show data from a wild-type mouse, and thin cumulative records and open squares show data from a mutant mouse. Top and middle show data from single sessions, and bottom shows data averaged from two determinations for each dose of cocaine for each mouse. Mouse #30 was representative of the group of wild-type mice, whereas mouse #40 was representative of those two mutant mice that initially met criteria for cocaine self-administration but did not self-administer any dose of cocaine at rates profoundly higher than saline. Nonreinforced (inactive) nose pokes were extremely low throughout the tests (e.g., 3 inactive nose pokes for mouse #30 and 1 inactive nose poke for mouse #40 during the tests shown in the middle).

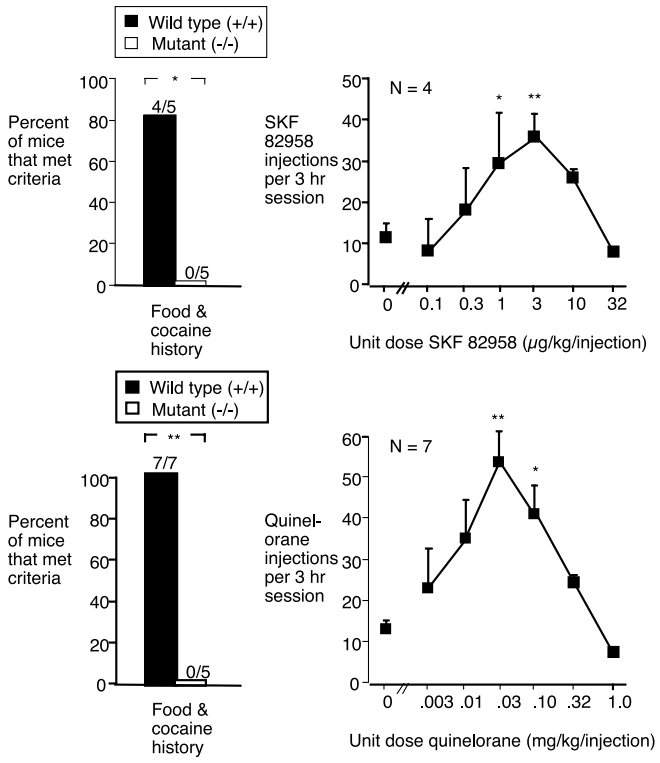

Figure 6. Self-administration of the D_1-selective agonist SKF 82958 and the D_2-selective agonist quinelorane in wild-type (+/+) or D_1 receptor mutant (−/−) mice. Abscissas, Training history (left column) or unit dose in micrograms per kilogram per injection of SKF 82958 (top right) or in milligrams per kilogram per injection of quinelorane (bottom right); points above zero depict data from saline self-administration. Ordinates, Percentage of mice that met criteria for self-administration of direct dopamine agonists (left top, 10 μg/kg per injection of SKF 82958; bottom left, 0.32 mg/kg per injection of quinelorane) or number of injections per 3 h session under the FR 1 schedule (right column). Numbers at top of each bar in left column indicate number of mice that met criteria/group size. All symbols in right column depict group means and SEM. Asterisks in left column indicate statistical significance ($*p < 0.05$, $**p < 0.01$) analyzed by Mann–Whitney *U* test for genotype. Asterisks in right column indicate significantly different from saline self-administration by pairwise comparison ($*p < 0.05$, $**p < 0.01$) following significant main effect of unit dose by ANOVA. Some D_1 receptor mutant (−/−) mice were also given access to several doses of each dopamine agonist but none of them met criteria for self-administration (data not shown).

alternately over consecutive sessions, with no evidence of reinforcing effects of SKF 82958 in D_1 receptor mutant mice (data not shown). In contrast, varying the dose of SKF 82958 in wild-type mice produced an inverted U-shaped function characteristic of drug self-administration under these conditions (effect of drug dose, $F_{(6,18)} = 7.03$; $p < 0.001$) (Fig. 6, top right). Moreover, SKF 82958 maintained rates of nose poking significantly higher than saline in wild-type mice at doses of SKF 82958 of 1.0 μg/kg per injection ($p < 0.05$) and 3.2 μg/kg per injection ($p < 0.01$).

Within seven consecutive test sessions, all seven wild-type mice met criteria for acquisition of self-administration of the D_2 agonist quinelorane (0.32 mg/kg per injection) (Fig. 6, bottom left). In contrast, none of the five D_1 receptor mutant mice met criteria for acquisition of quinelorane self-administration (0.32

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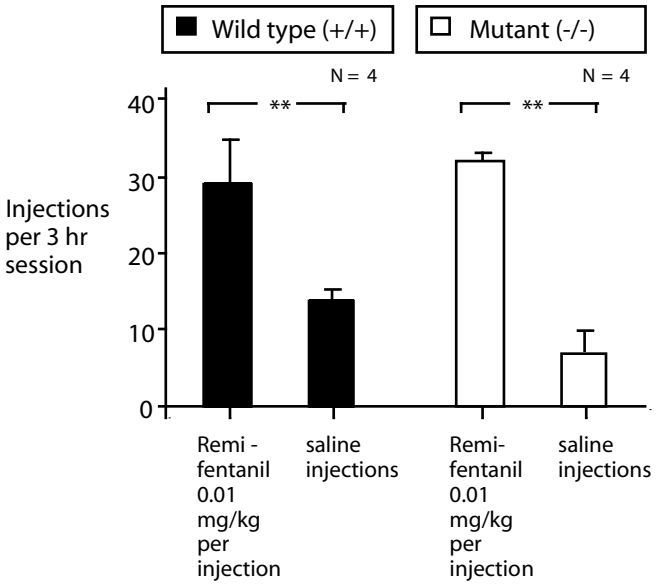

Figure 7. Self-administration of the opioid agonist remifentanil in wild-type (+/+) or D_1 receptor mutant (−/−) mice. Abscissas, Dose of remifentanil self-administered (0.01 mg/kg per injection or zero, i.e., saline). Ordinates, Injections per 3 h session under the FR 1 schedule. All bars indicate group means and SEM. Asterisks indicate significant main effect of dose by ANOVA (**$p < 0.01$).

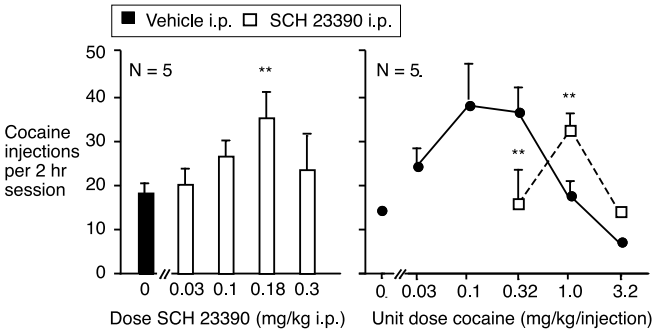

Figure 8. Self-administration of cocaine after pretreatment with vehicle or the D_1-selective antagonist SCH 23390 in C57BL/6J mice. Abscissas, Dose of SCH 23390 administered before self-administration of 1.0 mg/kg per injection of cocaine (left) or dose of cocaine (milligram per kilogram per injection) self-administered after pretreatment with vehicle or 0.18 mg/kg of the D_1-selective antagonist SCH 23390 (right). Symbols above zero depict data from vehicle pretreatment (left) or saline self-administration (right). Ordinates, Cocaine injections per 2 h session. All symbols depict group means and SEM. Asterisks indicate significantly different from vehicle pretreatment by pairwise comparison (**$p < 0.01$) following significant main effect of SCH 23390 dose (left) or a SCH 23390 × cocaine dose interaction (right) by ANOVA.

mg/kg per injection). Several D_1 receptor mutant mice were tested with various other doses of quinelorane as a reinforcer, and several D_1 receptor mutant mice were also tested in sessions in which 100% liquid food or quinelorane (0.10, 0.32, or 1.0 mg/kg per injection) were available alternately over consecutive sessions, with no evidence of reinforcing effects of quinelorane in D_1 receptor mutant mice (data not shown). In contrast, varying the dose of quinelorane in wild-type mice produced an inverted U-shaped function characteristic of drug self-administration under these conditions (effect of drug dose, $F_{(6,36)} = 5.45$; $p < 0.001$) (Fig. 6, bottom right). Moreover, quinelorane maintained rates of nose poking significantly higher than saline in wild-type mice at doses of quinelorane of 0.03 mg/kg per injection ($p < 0.01$) and 0.10 mg/kg per injection ($p < 0.05$).

Self-administration of the opioid agonist remifentanil

Figure 7 shows data from wild-type and D_1 receptor mutant mice that were initially trained with food-maintained responding (100% liquid food and then extinction with water, described above) and were then tested with the opioid agonist remifentanil (0.01 mg/kg per injection) as an intravenous reinforcer. There were four male mice per group. All mice regardless of genotype met criteria for remifentanil self-administration (i.e., a minimum of 20 injections per session, 70% of responses in the active manipulandum, and a deviation of responses <20% over two consecutive sessions). There was a significant overall main effect of dose (saline vs remifentanil 0.01 mg/kg per injection, $F_{(1,1)} = 19.21$; $p < 0.01$) (Fig. 7) but no significant effect of genotype ($F_{(1,6)} = 0.31$; $p > 0.1$) or dose × genotype interaction ($F_{(1,6)} = 0.94$; $p > 0.1$).

Effects of the D_1-like antagonist SCH 23390 on cocaine self-administration behavior in C57BL/6J mice

To determine the effects of pharmacological blockade of D_1-like receptors on cocaine self-administration in this species, the effects of pretreatment with the D_1-like antagonist SCH 23390 were examined in C57BL/6J mice. Pretreatment with SCH 23390 dose dependently and significantly increased rates of self-administration of 1.0 mg/kg per injection cocaine in C57BL/6J mice ($F_{(4,16)} = 8.02$; $p = 0.001$) (Fig. 8, left). Rates of cocaine self-administration were significantly increased by pretreatment with 0.18 mg/kg SCH 23390 compared with vehicle pretreatment ($p < 0.01$). A higher dose of SCH 23390 (0.3 mg/kg) disrupted patterns of cocaine-maintained responding and produced long pauses in responding at various times throughout the test sessions, as well as high rates of cocaine-maintained responding at other times during the test sessions. In some instances, C57BL/6J mice were immobile after pretreatment with the highest dose of SCH 23390 (0.3 mg/kg), particularly during early portions of the test sessions.

When the self-administration of a range of unit doses of cocaine was examined, pretreatment with 0.18 mg/kg SCH 23390 produced a rightward shift in the dose–effect function for cocaine self-administration in C57BL/6J mice (Fig. 8, right). There was a main effect of cocaine dose ($F_{(2,8)} = 13.5$; $p < 0.01$) and a cocaine dose × pretreatment interaction ($F_{(2,8)} = 31.2$; $p < 0.001$). Pretreatment with SCH 23390 (0.18 mg/kg) significantly decreased rates of self-administration of 0.32 mg/kg per injection of cocaine ($p < 0.01$) and increased rates of self-administration of 1.0 mg/kg per injection of cocaine ($p < 0.01$).

Discussion

There were three major findings in the present study. First, cocaine did not function as a positive reinforcer in D_1 receptor knock-out mice, whereas both food and the opioid agonist remifentanil (intravenous) reliably maintained responding in the mutants. Second, neither a D_1- nor a D_2-selective agonist functioned as a positive reinforcer in D_1 receptor knock-out mice, whereas these drugs were self-administered by wild-type littermates. Third, pharmacological blockade of D_1 receptors in C57BL/6J mice produced surmountable antagonism of the reinforcing effects of cocaine, as shown previously in rats and nonhuman primates. These results suggest that mutation of a single dopamine-related gene, the D_1 receptor gene, is sufficient to eliminate the reinforcing effects of cocaine and D_1 and D_2 dopamine agonists in mice.

Decreased acquisition of cocaine self-administration in D_1 receptor mutant mice

Decreased acquisition in D_1 receptor mutant mice relative to wild-type littermates was observed across a range of conditions. First, the breeding of the mice was varied because of practical considerations, and this can introduce confounds associated with genetic background (Kelly et al., 1998; Phillips et al., 1999). Indeed, the background strains used to generate D_1 receptor mutant mice in the present study (129/SvJ and C57BL/6J) differed in cocaine conditioned place preference, a tail-vein procedure for cocaine self-administration, and rates of acquisition of food-maintained behavior (Miner, 1997; Kuzmin and Johansson, 2000; Zhang et al., 2002; Thomsen and Caine, 2006). Nevertheless, significantly decreased acquisition was observed in mutants relative to wild-type mice in both the F2 generation and after several generations of backcrossing to C57BL/6J. Moreover, rates of acquisition of food-maintained behavior were comparable in mutant and wild-type mice in the present study. Finally, cocaine clearly served as a positive reinforcer in both background strains used for our D_1 knock-out mice (Thomsen and Caine, 2006). Accordingly, it is unlikely that genetic background contributed significantly to decreased acquisition of cocaine self-administration in D_1 receptor mutants.

A second factor that was varied in the present study was experimental history. One cohort of mice had no experience with operant conditioning before evaluation of cocaine self-administration, although noncontingent amphetamine and cocaine injections were administered during a preliminary study of motor activity (Xu et al., 2000), and this approach has been shown to increase acquisition of cocaine self-administration (Suto et al., 2004). Another cohort of mice was trained and evaluated with food reinforcement, including acquisition and extinction phases, before cocaine self-administration studies. Importantly, acquisition of cocaine self-administration was significantly decreased in D_1 receptor mutants in both cohorts of mice. In fact, only two D_1 receptor mutants (of a total of 23 studied) met the established criteria for acquisition of cocaine self-administration, and, even in those two mice, no dose of cocaine maintained rates of responding higher than saline self-administration.

We also alternated cocaine and food as the reinforcer to recover stable levels of responding with food reinforcement after extinction occurred during cocaine self-administration tests in the D_1 receptor mutants. This allowed D_1 receptor mutant mice to be tested for acquisition of self-administration of a single dose of cocaine repeatedly, as well as tests with lower and higher cocaine doses, after repeated reinstatement of responding with food as a reinforcer. As such, the decreased acquisition of cocaine self-administration in D_1 receptor mutant mice is not easily attributable to a one-time, rapid and sustained extinction of operant behavior, or to selection of an inappropriate cocaine dose.

Food-maintained responding and remifentanil self-administration in D_1 receptor mutant mice

Rates of food-maintained responding were significantly lower in D_1 receptor mutants relative to wild-type mice. However, rates of acquisition (i.e., number of sessions to meet criteria) were comparable in mutant and wild-type mice, and, relative to their rates of water-reinforced responding, liquid food-maintained responding was robust in both genotypes. There did not appear to be delayed learning with respect to the operant response in D_1 receptor mutant mice, because two determinations of food concentration–effect curves yielded identical data. Accordingly, it appears that response rates were uniformly lower for food-maintained behavior in D_1 receptor mutants compared with wild-type mice. These findings are consistent with findings of decreased exploratory behavior, movement generation and sequencing, spatial navigation, and motor dexterity in D_1 receptor mutants relative to wild-type mice (Cromwell et al., 1998; Smith et al., 1998; El-Ghundi et al., 1999; Karasinska et al., 2000). In contrast, D_1 receptor mutants were not impaired in tests of visual acuity, muscle strength, coordination, and equilibrium (El-Ghundi et al., 1999). Additionally, decreased rates of food-maintained responding are not necessarily predictive of impaired sensitivity to reinforcers generally or to cocaine specifically, because D_2 receptor mutant mice also exhibited low rates of food-maintained responding, yet D_2 mutants acquired cocaine self-administration and exhibited high rates of responding maintained by cocaine (Caine et al., 2002).

Importantly, the opioid agonist remifentanil served as a positive reinforcer and maintained comparable rates of responding in the two genotypes. These findings are consistent with findings in rats suggesting that the reinforcing effects of opioids and alcohol do not always depend on intact dopaminergic systems (Pettit et al., 1984; Rassnick et al., 1993). Overall, we conclude that D_1 knock-out mice are not insensitive to positive reinforcers including drug reinforcers generally, but rather are impaired in self-administration of cocaine and dopamine direct agonists specifically.

A permissive role for D_1 receptors in the effects of dopamine agonists

The present findings are consistent with a permissive role for D_1 receptors in the full expression of effects of dopaminergic stimulation in rodents. For example, both pharmacological blockade and gene knock-out of the dopamine D_1 receptor attenuated the physiological effects of dopamine and D_1- and D_2-selective agonists (Carlson et al., 1987; Clark and White, 1987; Walters et al., 1987; White et al., 1988; Wachtel et al., 1989; Johansen et al., 1991; Xu et al., 1994a,b; Cooper et al., 1998; Waszczak et al., 2002). Conversely, D_1 stimulation enabled the full expression of behavioral effects and molecular and cellular adaptations induced by dopamine indirect and direct D_1 and D_2 agonists (Walters et al., 1987; White et al., 1988; Xu et al., 1994a,b; Gerfen et al., 1995; Keefe and Gerfen, 1995; Xu et al., 2000; Ralph-Williams et al., 2002; Waszczak et al., 2002; Fetsko et al., 2003; Zhang et al., 2004; Ralph and Caine, 2005; Zhang et al., 2005; Zhang et al., 2006).

With regard to reinforcing effects, pharmacological blockade of D_1 receptors produced rightward shifts in dose–effect functions for cocaine self-administration in both rats and monkeys (Bergman et al., 1990; Caine and Koob, 1994, 1995; Barrett et al., 2004). The present findings with SCH 23390 in C57BL/6J mice extend those observations to include this species. Moreover, the positive reinforcing effects of SKF 82958 in the present study are consistent with findings that the drug potentiated brain stimulation reward in mice (Gilliss et al., 2002). The lack of reinforcing effects of cocaine, a D_1-selective agonist and a D_2-selective agonist in D_1 receptor knock-out mice, and the aforementioned findings from previous studies are all consistent with a critical, permissive role for D_1 receptors in the reinforcing effects of dopaminergic drugs in mice.

Comparison of self-administration with place conditioning and motor activity

Our findings with self-administration contrast with previous findings of a cocaine conditioned place preference in D_1 receptor

mutant mice (Miner et al., 1995). One explanation for this discrepancy is that the two procedures measure qualitatively different aspects of the abuse-related effects of cocaine. An alternative explanation is that the present findings are attributable, at least in part, to a decrement in rate-increasing effects of cocaine in D_1 receptor mutant mice and that these psychomotor stimulant effects contribute to cocaine self-administration behavior to a greater extent than to cocaine conditioned place preference. There are several reports of a diminution in the psychomotor stimulant effects of cocaine in D_1 receptor mutants (Xu et al., 1994b, 2000; Miner et al., 1995).

Comparison with other knock-out mice and future studies

In the present study, the reinforcing effects of cocaine were diminished by targeted mutation of the D_1 receptor much more so than by other dopamine-related mutations, including the dopamine transporter (Rocha et al., 1998), the D_2 receptor (Caine et al., 2002), or the D_3 receptor (Caine et al., 2003). Indeed, such profound effects of a gene mutation on cocaine self-administration have only rarely been observed (Chiamulera et al., 2001). Future studies with inducible and conditional knock-outs that allow temporal and anatomical control should provide additional information in the absence of chronic, organism-wide developmental and regulatory alterations, and allow identification of critical D_1 receptors within specific brain regions and cell populations. Such information may clarify the potential of D_1 receptors as targets for the development of pharmacotherapies for cocaine abuse and dependence.

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