Brief Communications

Dramatically Decreased Cocaine Self-Administration in Dopamine But Not Serotonin Transporter Knock-Out Mice

Morgane Thomsen,[1] F. Scott Hall,[2] George R. Uhl,[2] and S. Barak Caine[1]

[1]Alcohol and Drug Abuse Research Center, McLean Hospital–Harvard Medical School, Belmont, Massachusetts 02478, and [2]Molecular Neurobiology Branch, National Institute on Drug Abuse–Intramural Research Program, National Institutes of Health, Baltimore, Maryland 21224

There has been much interest in the relative importance of dopamine and serotonin transporters in the abuse-related-effects of cocaine. We tested the hypotheses that mice lacking the dopamine transporter (DAT$^{-/-}$), the serotonin transporter (SERT$^{-/-}$), or both (DAT$^{-/-}$SERT$^{-/-}$) exhibit decreased reinforcing effects of cocaine. We also assessed whether observed effects on self-administration are specific to cocaine or if operant behavior maintained by food or a direct dopamine agonist are similarly affected. We used a broad range of experimental conditions that included acquisition without previous training, behavior established with food training and subsequent testing with food, cocaine or a direct dopamine agonist as reinforcers, fixed ratio and progressive ratio schedules of reinforcement, and a reversal procedure. Wild-type mice readily acquired cocaine self-administration and showed dose–response curves characteristic of the schedule of reinforcement that was used. While some DAT$^{-/-}$ mice appeared to acquire cocaine self-administration transiently, almost all DAT$^{-/-}$ mice failed to self-administer cocaine reliably. Food-maintained behaviors were not decreased by the DAT mutation, and IV self-administration of a direct dopamine agonist was robust in the DAT$^{-/-}$ mice. In contrast to those mice, cocaine's reinforcing effects were not diminished in SERT$^{-/-}$ mice under any of the conditions tested, except for impaired initial acquisition of both food- and cocaine-maintained behavior. These findings support the notion that the DAT, but not the SERT, is critical in mediating the reinforcing effects of cocaine.

Key words: **cocaine; drug abuse; DAT; SERT; knock-out mouse; progressive ratio**

Introduction

Much evidence suggests that cocaine's reinforcing effects are directly related to its ability to rapidly block the dopamine transporter (DAT). For instance, other dopamine reuptake inhibitors are also self-administered, with relative potencies that generally correlate positively with their potencies in inhibiting the DAT, but not the serotonin or norepinephrine transporters (SERT, NET) (Ritz et al., 1987; Bergman et al., 1989; Howell and Byrd, 1995). Direct dopamine agonists are self-administered in animals trained to self-administer cocaine (Woolverton et al., 1984; Wise et al., 1990; Caine and Koob, 1993). Destruction of dopamine nerve terminals can lead to extinction of cocaine self-administration behavior (Roberts et al., 1977). In view of all those previous findings, it was surprising that DAT$^{-/-}$ mice were reported to self-administer cocaine, and to show a cocaine-conditioned place preference (CPP) (Rocha et al., 1998; Sora et al., 1998). However, DAT$^{-/-}$ mice show profound compensatory changes in monoamine systems, which complicate the interpretation of those results (Giros et al., 1996; Jones et al., 1998). Importantly, cocaine increased extracellular nucleus accumbens dopamine levels in the line of DAT$^{-/-}$ mice used in that previous self-administration study (Carboni et al., 2001).

To further test the hypothesis that the DAT is essential to cocaine's reinforcing effects, we tested a different line of DAT$^{-/-}$ mice, which have displayed no increases in accumbens dopamine after cocaine administration (Shen et al., 2004). We also evaluated the potential role of the SERT in the reinforcing effects of cocaine by testing SERT$^{-/-}$ mice, which previously showed increased cocaine CPP (Sora et al., 1998), and the double knock-out (DAT$^{-/-}$SERT$^{-/-}$) mice, which previously showed no cocaine CPP (Sora et al., 2001). We tested the mice in chronic cocaine self-administration under a wide range of experimental conditions. Acquisition of IV cocaine self-administration was evaluated in experimentally naive mice. In a second group of mice, operant responding was established with liquid food and evaluated over a range of food reinforcer magnitudes, followed by substitution of cocaine as the reinforcer. Cocaine self-administration dose-effect functions were determined under a fixed ratio (FR) 1 and a progressive ratio (PR) schedule of reinforcement. IV self-administration of the direct dopamine agonist SKF 82958 was also assessed in wild-type and DAT$^{-/-}$ mice to further verify that operant behavior generally was not compromised by the mutation, and that relevant dopamine systems were functional in the DAT$^{-/-}$ mice.

Materials and Methods

See supplemental material (available at www.jneurosci.org) for additional details. Mutant mice lacking DAT or SERT were generated as

Received Aug. 24, 2008; revised Nov. 4, 2008; accepted Dec. 22, 2008.

This work was supported by grants from National Institute on Drug Abuse (NIDA)–National Institutes of Health (NIH) (DA12142, DA14644, DA17323, DA14528, DA07252) and in part from intramural funding from the NIDA–Intramural Research–NIH–Department of Health and Human Services (G.R.U., F.S.H.). We thank Joon Y. Boon, Jennifer Dohrmann, Jill Berkowitz, and Dana Angood for expert technical assistance.

Correspondence should be addressed to Dr. Morgane Thomsen, Alcohol and Drug Abuse Research Center, McLean Hospital–Harvard Medical School, 115 Mill Street, Belmont, MA 02478. E-mail: mthomsen@mclean.harvard.edu.

DOI:10.1523/JNEUROSCI.4037-08.2009

 0270-6474/09/291087-06$15.00/0

previously described (Bengel et al., 1998; Sora et al., 1998). DAT/SERT double knock-out mice were obtained by intercrossing the single knock-out lines (Sora et al., 2001). All subjects were littermates derived from the DAT/SERT double knock-out line. Animals were kept group housed under standard laboratory conditions, with some enrichment. All procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee. Operant conditioning chambers, training and evaluation of food-maintained behavior under a fixed-ratio schedule have been described in detail (Caine et al., 1999; Thomsen et al., 2005). Briefly, each chamber contained two nose-poke holes and a plate into which liquid food could be delivered. In all except the reversal procedure, responding in the right hole resulted in delivery of a reinforcer and illumination of the cue light for 20 s during which no reinforcer could be earned. Responses in the left hole were counted but had no scheduled consequences. The house light was on during the session. Training and evaluation of food-maintained behavior under the FR schedule, implantation and maintenance of indwelling jugular catheters, and cocaine self-administration behavior under the FR 1 schedule, including the reversal procedure were performed as described by Thomsen et al. (2005). Acquisition of cocaine self-administration was performed as described by Thomsen et al. (2005) except that a dose of 0.32 mg/kg/infusion was used in all mice.

In one group of mice, with 1.0 mg/kg/infusion cocaine as the reinforcer, schedules of reinforcement of increasing response requirements were presented in daily sessions, as follows: FR 1, FR 3, PR 1 (a PR schedule in which the initial ratio was 1, and the ratio incremented by 1 after each reinforcer delivery), and PRlgt (a logit function-based PR schedule in which the initial ratio was 3, and ratio incremented according to the function: ratio = $19 \cdot [1+\log(\text{step}/(7-0.3 \cdot \text{step}))]$, i.e., 3, 9, 13, 16, 18…) (Thomsen et al., 2005). The breaking point was defined as the step value associated with the last completed ratio (i.e., number of reinforcers earned) after a 60 min limited hold (i.e., period with no reinforcer earned). If a breaking point had not been reached within 6 h, the session was terminated to prevent health hazard and the last reached ratio was used. Under the PRlgt schedule, mice self-administered 1.0 mg/kg/infusion cocaine until a stable baseline was achieved (two consecutive sessions with breaking points >10 and <20% variation), then saline was substituted until responding extinguished to <10 reinforcers and ≤50% of the baseline breaking point. Then a dose-effect curve (0, 0.032, 0.32, and 1.0 mg/kg/infusion cocaine) was determined. Subsequently liquid food replaced cocaine as the reinforcer, and baseline (100% Ensure), extinction (water), and a concentration-effect curve (0, 3, 10, 32, 100% food in water) were determined using the same methods and criteria as for cocaine. Generally, drug doses and food concentrations were each presented for 2 or 3 consecutive sessions according to a Latin-square design, with some exceptions. The latter included additional baseline sessions, as few as one test with a drug dose or food magnitude in some cases (e.g., subsequent catheter or health complications).

In a different group of mice, pretreatment with a dopamine D_1-like receptor antagonist was tested after the determination of cocaine dose-effect functions under the FR 1 schedule as described by Caine et al. (2007), with pretreatment doses of 0.01–0.32 mg/kg SCH 39166. These mice were not tested under PR schedules. Substitution of the dopamine D_1-like receptor agonist SKF 82958 (0.32–32 μg/kg/infusion) in DAT$^{-/-}$ mice after exposure to cocaine was conducted as described previously (Caine et al., 2007).

For acquisition of cocaine self-administration, the proportions of mice meeting criteria (including extinction and re-baseline) were compared using a two-sided χ^2 test. For food, the latencies to acquisition criteria were analyzed using the Logrank test with genotypes as groups. For drug dose-effect functions and food concentration-effect functions, numbers of reinforcers earned were compared by mixed model ANOVA with DAT genotype and SERT genotype as between-subjects variables and drug dose or food concentration as within-subjects (repeated measures) variables. For the reversal procedure, reinforced and non-reinforced nose-pokes per session were compared using SERT genotype as a between-subjects variable and hole and session as within-subjects variables. Significant effects were followed where appropriate by two-sided paired-sample (within subjects) or unpaired (between subjects) *t* test. Pretreatment data in the SERT$^{-/-}$ mice were analyzed by one-way repeated measures ANOVA followed by Dunnett's multiple comparisons test. Significance level was set at $p < 0.05$.

Results

Experimentally naive mice were allowed to acquire self-administration of 0.32 mg/kg/infusion cocaine. Most wild-type mice acquired self-administration within 10 sessions (8 of 11 mice, 82%), all of which extinguished their behavior when saline was substituted for cocaine and reestablished baseline-level responding when cocaine was again made available. The DAT$^{-/-}$ mice showed initial behavior suggestive of self-administration (6 of 8 mice, 75%) but only 2 of those mice (25%) met extinction and re-establishment criteria ($p = 0.04$ vs wild-type, two-sided χ^2 test). Importantly, using a palatable food instead of drug in the same procedure, there was no significant effect of DAT genotype (see supplemental Fig. 1, available at www.jneurosci.org as supplemental material). In contrast, the SERT$^{-/-}$ mice showed impaired initial acquisition of both cocaine self-administration and food-maintained behavior, but mice that acquired the behavior maintained robust self-administration after extinction (cocaine: 2 of 9, 22%; $p = 0.02$ vs wild-type, two-sided χ^2 test; food: median number of sessions to criteria: 12.0 vs 6.0, $p = 0.02$, Logrank test; see supplemental Fig. 1, available at www.jneurosci.org as supplemental material). DAT$^{-/-}$SERT$^{-/-}$ mice showed a combination of the DAT$^{-/-}$ and SERT$^{-/-}$ phenotypes, as they generally failed to initiate (2 of 6 mice, 33%) or maintain cocaine self-administration (1 of 6 mice, 17%; $p = 0.03$ vs wild-type).

Once nose-poking behavior was established as described above, using food-training if necessary, cocaine dose-effect functions were determined under the FR 1 schedule of reinforcement. Results from this procedure further showed that most DAT$^{-/-}$ and DAT$^{-/-}$SERT$^{-/-}$ mice did not self-administer cocaine, across a wide range of doses, even if previously trained with food (Fig. 1*a*). Just one DAT$^{-/-}$ mouse self-administered cocaine exhibiting the inverted-U shaped function that is characteristic of cocaine self-administration under those conditions. In contrast, in all of the wild-type and SERT$^{-/-}$ mice cocaine engendered typical dose-effect curves. ANOVA showed a significant effect of cocaine dose [$F_{(5,170)} = 7.6$, $p = 2 \times 10^{-6}$] and DAT genotype [$F_{(1,34)} = 8.0$, $p = 0.008$], and a cocaine by DAT interaction [$F_{(5,170)} = 4.3$, $p = 0.001$]. In contrast, there was no effect of SERT genotype, no cocaine by SERT, DAT by SERT genotype or three-way interaction.

To further explore the possibility that cocaine retained seemingly full reinforcing efficacy in a minority DAT$^{-/-}$ mice, we trained an additional cohort of DAT$^{-/-}$ mice to identify additional "responders." We used an abbreviated training procedure of food followed by cocaine baseline (100% Ensure, then 1.0 mg/kg/infusion cocaine, then saline extinction). Only 3 of the 20 DAT$^{-/-}$ mice that we tested self-administered cocaine under those conditions. We then tested those three DAT$^{-/-}$ mice along with wild-type and SERT$^{-/-}$ mice with 1.0 mg/kg/infusion cocaine under increasing response requirements. Figure 1*b* shows that the DAT$^{-/-}$ mice earned progressively fewer reinforcers as response requirement increased, whereas wild-type and SERT$^{-/-}$ mice maintained their cocaine intake. There was a significant effect of schedule of reinforcement [$F_{(3,69)} = 14.0$, $p < 1 \times 10^{-7}$], DAT genotype [$F_{(1,23)} = 7.0$, $p = 0.01$] and DAT by schedule interaction [$F_{(3,69)} = 65.4$, $p < 1 \times 10^{-7}$], but not of SERT genotype or SERT by schedule interaction. Preplanned analysis of the effect of schedule in each genotype revealed that

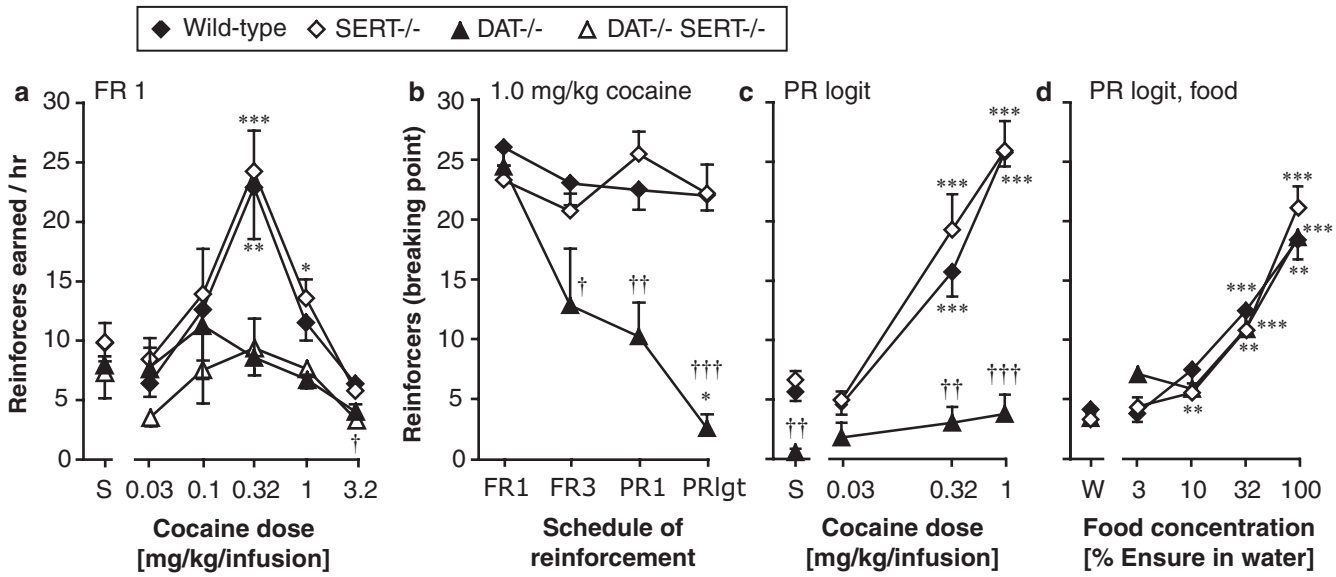

Figure 1. Cocaine self-administration and food-maintained behavior in wild-type, SERT$^{-/-}$, and DAT$^{-/-}$ mice. ***a***, Under an FR 1 schedule of reinforcement, most DAT$^{-/-}$ mice failed to self-administer cocaine, while SERT mutation had no effect. Group sizes: wild-type, $N = 13$; DAT$^{-/-}$, $N = 5$; SERT$^{-/-}$, $N = 11$, DAT$^{-/-}$SERT$^{-/-}$, $N = 7$. ***b***, A minority of DAT$^{-/-}$ mice that responded under the FR 1 schedule stopped self-administering when the response requirement was increased, while wild-type and SERT$^{-/-}$ mice maintained their cocaine intake. Group sizes: wild-type, $N = 11$; DAT$^{-/-}$, $N = 3$; SERT$^{-/-}$, $N = 12$. ***c***, Wild-type mice and SERT$^{-/-}$ mice, but not DAT$^{-/-}$ mice, self-administered cocaine under a PR schedule of reinforcement. Group sizes: wild-type, $N = 8$; DAT$^{-/-}$, $N = 3$; SERT$^{-/-}$, $N = 10$. ***d***, Food maintained comparable responding in all three genotypes under the PR schedule. Group sizes: wild-type, $N = 10$; DAT$^{-/-}$, $N = 4$; SERT$^{-/-}$, $N = 14$. † < 0.05, †† < 0.01, ††† < 0.001 versus wild-type; independent samples *t* test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, versus saline (S) (***a***, ***c***), versus FR 1 (***b***), or versus water (W) (***d***); paired-sample *t* test. Data are group means ± SEM.

only DAT$^{-/-}$ mice showed an effect of schedule [$F_{(3,6)} = 21.4$, $p = 0.001$], with the PRlgt schedule reaching significance relative to FR 1 *post hoc* ($p < 0.05$). The DAT$^{-/-}$ mice earned significantly fewer cocaine infusions than wild-type mice under all but the FR 1 schedule ($p < 0.05$ to $p < 0.001$ *post hoc*) (Fig. 1*b*).

Dose-effect functions were then determined under the PRlgt function, and are shown in Figure 1*c*; the self-administering DAT$^{-/-}$ mice reached only low breaking points at all doses, whereas wild-type and SERT$^{-/-}$ mice reached progressively higher breaking points in a dose-dependent manner. There was a significant effect of cocaine dose [$F_{(3,54)} = 28.5$, $p < 1 \times 10^{-7}$], DAT genotype [$F_{(1,18)} = 17.3$, $p = 0.0006$] and a DAT by dose interaction [$F_{(3,54)} = 6.7$, $p = 0.0006$], but not of SERT genotype or SERT by dose interaction. Preplanned analysis of the cocaine dose effect in each genotype confirmed that only wild-type [$F_{(3,21)} = 33.3$, $p < 1 \times 10^{-7}$] and SERT$^{-/-}$ mice [$F_{(3,27)} = 73.9$, $p < 1 \times 10^{-7}$] showed a significant cocaine effect. In contrast, food concentration-effect curves determined using the same PR procedure revealed no difference between wild-type, DAT$^{-/-}$, and SERT$^{-/-}$ mice (main effect of food concentration [$F_{(4,100)} = 65.4$, $p < 1 \times 10^{-7}$]) (Fig. 1*d*). Food concentration-effect curves determined under a FR 1 schedule were also comparable across wild-type, DAT$^{-/-}$ and SERT$^{-/-}$ mice (data not shown).

In the DAT$^{-/-}$ mice that failed to self-administer cocaine, the dopamine D_1-like agonist SKF 82958 (10 μg/kg/infusion) was substituted for cocaine as the reinforcer. For cocaine, ANOVA showed a significant effect of cocaine availability [$F_{(1,19)} = 14.2$, $p = 0.001$], and a cocaine by DAT genotype interaction [$F_{(1,19)} = 8.2$, $p = 0.01$] (Fig. 2*a*). Cocaine only maintained higher response rates than saline in the wild-type mice ($p = 0.0002$). In contrast, wild-type and DAT$^{-/-}$ self-administered SKF 82958 at comparable rates (Fig. 2*a*). ANOVA showed a significant effect of SKF 82958 availability [$F_{(1,19)} = 39.2$, $p = 5 \times 10^{-6}$], but no effect of DAT genotype nor interaction. SKF 82958 maintained significantly higher response rates than saline in both wild-type mice

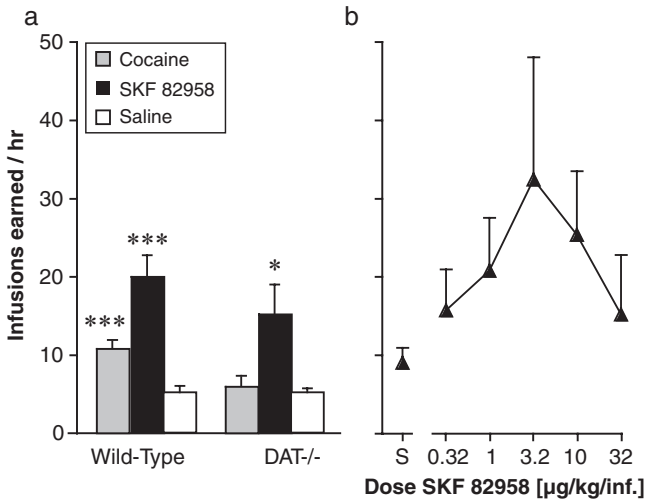

Figure 2. Self-administration of the dopamine D_1-like receptor agonist SKF 82958 in wild-type and DAT$^{-/-}$ mice. ***a***, Levels of self-administration in wild-type and DAT$^{-/-}$ mice of cocaine 1.0 mg/kg/infusion, compared with stable responding maintained by 10 μg/kg/infusion SKF 82958, and saline substitution. Group sizes: wild-type, $N = 13$; DAT$^{-/-}$, $N = 8$. ***b***, SKF 82958 dose-effect function in DAT$^{-/-}$ mice ($N = 7$). *$p < 0.05$, ***$p < 0.001$ versus saline, paired-sample *t* test. Data are group means ± SEM.

($p = 3 \times 10^{-5}$) and DAT$^{-/-}$ mice ($p = 0.024$). After extinction, SKF 82958 maintained dose-dependent self-administration in the DAT$^{-/-}$ mice (Fig. 2*b*), although potency and peak rate of self-administration varied considerably between subjects.

To further investigate potential differences in cocaine self-administration between wild-type and SERT$^{-/-}$ mice, a subset of mice of these two genotypes were tested in a reversal procedure (after the dose-effect determination under the FR 1 schedule). The mean numbers of responses in the right and left nose-poke holes before, during, and after reversal of the active and inactive

nose-poke holes are shown in Figure 3. Most mice in both genotypes rapidly increased their nose-poking behavior in the newly reinforced hole upon reversal. After an initial increase in poking, compatible with an "extinction burst," non-reinforced poking decreased over sessions. An ANOVA on reinforced and non-reinforced nose-pokes during the reversal period showed no significant effect of SERT genotype or any significant interaction of the genotype. There was a significant effect of session [$F_{(4,52)} = 11.9$, $p < 0.001$] and hole by session interaction [$F_{(4,52)} = 16.0$, $p < 0.001$], while the main effect of hole did not reach significance, consistent with the initial high level of non-reinforced behavior. When the holes were again reversed back to their original configuration, nose-poking behavior was again reallocated to maintain cocaine intake (no effect of SERT genotype). Another subset of SERT$^{-/-}$ mice was tested with the dopamine D_1-like receptor antagonist SCH 39166, under the FR 1 schedule. Figure 4 shows that SCH 39166 dose-dependently increased responding maintained by 1.0 mg/kg/infusion cocaine (effect of SCH 39166 dose: [$F_{(5,20)} = 3.1$, $p = 0.03$]). The SCH 39166 doses of 0.03 and 0.10 mg/kg significantly increased rates of cocaine self-administration relative to baseline ($p < 0.01$ and $p < 0.05$).

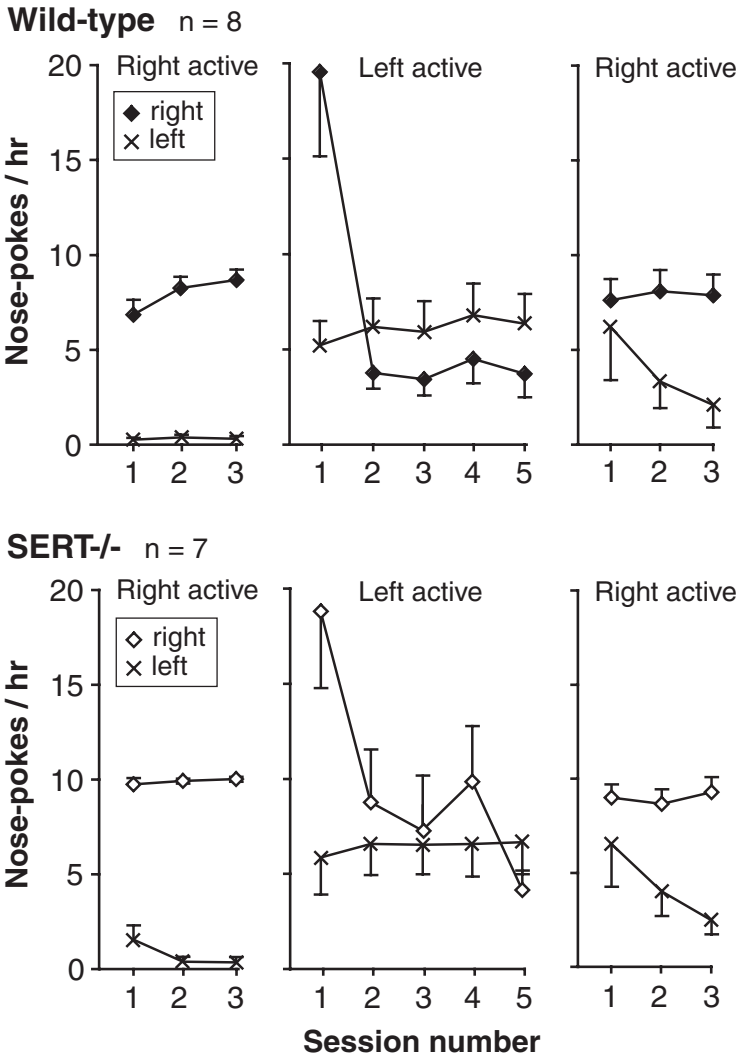

Figure 3. Active and inactive responses during a hole reversal procedure in wild-type and SERT$^{-/-}$ mice. Diamonds represent responses in the right nose-poke hole (reinforced or not), crosses represent responses in the left hole (reinforced or not). Panels show: responding before reversal (left), during reversal (center), and after the second reversal (right). Group sizes: wild-type, $N = 8$; SERT$^{-/-}$, $N = 7$. Data are group means ± SEM.

Discussion

We found that mice lacking the DAT generally failed to acquire and maintain cocaine self-administration, despite high levels of initial activity in the operant chambers and despite normal acquisition and maintenance of the procedure using a food reinforcer. Under both food-trained and naive conditions, both wild-type and DAT$^{-/-}$ mice emitted relatively high numbers of nose-pokes in both holes on the first day, excluding the possibility that reduced spontaneous activity (including nose-poking) in the DAT$^{-/-}$ mice accounted for their lack of acquisition. In addition, due to this initial activity, wild-type and DAT$^{-/-}$ mice received cocaine reinforcers in comparable amounts early on, and thus should have had comparable chances of acquiring self-administration. Despite the initial high levels of nose-pokes, responding was not resumed after saline extinction in most DAT$^{-/-}$ mice, and subsequent dose-effect determinations showed low response levels regardless of cocaine dose. A parsimonious explanation for these data is that the apparent acquisition of cocaine self-administration in some DAT$^{-/-}$ mice was likely an artifact. It is possible that hyperactivity and/or high response to novelty resulted in higher levels of nose-poking in the reinforced hole, which was accompanied by illumination of a cue light and the sound of the drug pump, relative to the inactive hole (in which poking had no scheduled consequences). Indeed, the hyperdopaminergic DAT$^{-/-}$ mice and DAT knock-down mice showed hyperactivity and high response to novelty and/or lack of habituation to novelty in previous studies (Giros et al., 1996; Gainetdinov et al., 1999; Zhuang et al., 2001; Mead et al., 2002). In summary, the acquisition data suggested two things. First, mice lacking the DAT failed to acquire reliable self-administration of cocaine (see below). Second, arbitrary criteria for acquisition such as those used in the present and previous investigations (Rocha et al., 1998) (Caine et al., 2007) may be too lenient and allow for "false positives," particularly when testing mice with high spontaneous or novelty-induced activity levels.

A minority of mice (3 of 20) did maintain cocaine self-administration under a low-requirement schedule of reinforcement (FR 1). However, when the response requirement was increased, self-administration behavior was rapidly extinguished in those DAT$^{-/-}$ mice, to the point that cocaine no longer maintained responding at levels above saline under a PR schedule of reinforcement. Importantly, in a parallel experiment with food reinforcers, DAT$^{-/-}$ mice were identical to wild-type mice under high response requirements. Moreover, in contrast to the DAT$^{-/-}$ mice, wild-type mice maintained their intake of the relatively high "baseline dose" of cocaine, 1.0 mg/kg/infusion, at comparable levels across the four tested schedules of reinforce-

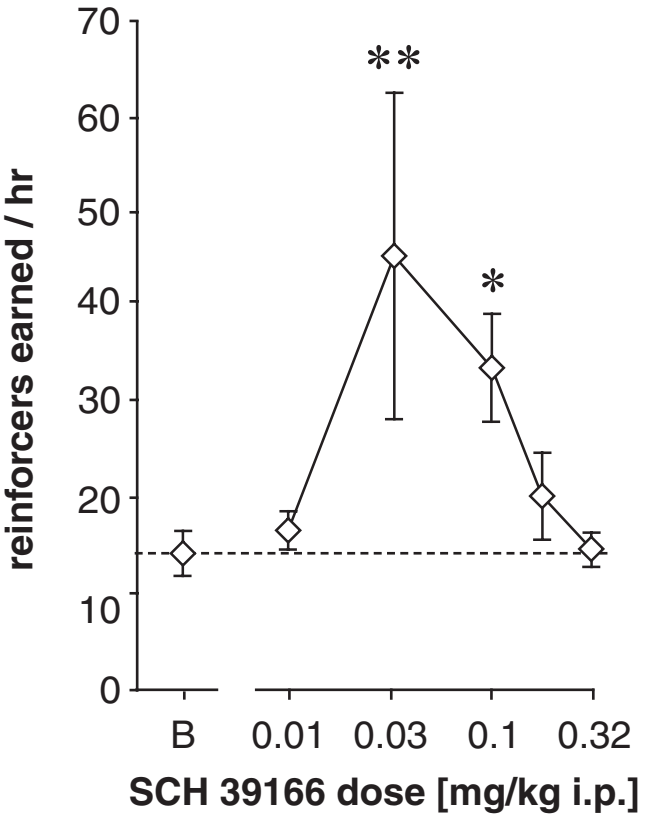

Figure 4. Pretreatment with the dopamine D_1-like receptor antagonist SCH 39166 increased rates of cocaine self-administration in the SERT$^{-/-}$ mice. $N = 5$. *$p < 0.05$, **$p < 0.01$ versus baseline (B), Dunnett's multiple comparisons test. Data are group means ± SEM.

ment, and showed dose-dependent breaking points under the PR schedule. Thus self-administration of a moderately high cocaine dose, but not food, was a very elastic behavior in the few DAT$^{-/-}$ mice that did self-administer, whereas it was an essentially inelastic behavior in the wild-type mice, up to conditions where the final ratio for a single cocaine infusion was between 50 and 100 for most mice. This finding suggests that even in the few DAT$^{-/-}$ mice in which cocaine did serve as a positive reinforcer, its reinforcing strength was significantly diminished relative to wild-type mice.

In contrast to cocaine, the DAT$^{-/-}$ mice self-administered a direct dopamine agonist in a manner comparable with wild-type mice in the present and previous investigations (Caine et al., 2007), showing that constitutive deletion of the DAT did not disrupt the ability of the mice to acquire and maintain self-administration of IV drugs generally. This finding also suggests that the general failure of the DAT$^{-/-}$ mice to self-administer cocaine was not likely attributable to compensatory changes in downstream dopamine systems (e.g., desensitization of D_1 receptor mechanisms).

Our findings are in apparent contrast to the previous report by Rocha et al. (1998) that a line of DAT$^{-/-}$ mice self-administered cocaine. That earlier study used a different line of DAT knock-out mice than the mice used in the present investigation. Importantly, cocaine was also reported to increase accumbens dopamine in those mice, a finding that differs from observations made in the present line of DAT$^{-/-}$ mice (Carboni et al., 2001; Shen et al., 2004). Collectively, the present and previous findings support the notion that cocaine-induced increases in extracellular dopamine in the nucleus accumbens are critical for cocaine self-administration. It remains unknown why the two lines of DAT$^{-/-}$ mice show divergent phenotypes. Both lines were maintained on mixed 129/SvJ/C57BL/6 backgrounds (Rocha et al., 1998), although they certainly would differ in particular 129 and C57 alleles. Different lines of embryonic stem cells were used to generate the knock-outs: the 129/Sv-derived J1 line versus the 129P2-derived E14TG2a (Giros et al., 1996; Sora et al., 1998). Both lines showed similar decreases in striatal D2 receptor binding (Giros et al., 1996; Sora et al., 2001). While we have shown that two 129 substrains and C57BL/6J mice all self-administer cocaine, genetic background has been shown to influence the phenotype of the DAT knock-out (Morice et al., 2004; Thomsen and Caine, 2006).

Conversely, cocaine produced an increase in dorsal striatal dopamine in the present line of DAT$^{-/-}$ mice, but not in the previously tested line (Rocha et al., 1998; Shen et al., 2004). Because the present line of DAT$^{-/-}$ mice was reported to develop cocaine CPP, one might speculate that DAT-mediated increases in dopamine in the dorsal striatum are involved in the development of cocaine CPP (Sora et al., 1998). Alternatively, the prefrontal cortex has been implicated in cocaine's place-conditioning effects, and the present line of DAT$^{-/-}$ mice showed cocaine-induced increases in extracellular dopamine comparable with wild-type mice in this region (Tzschentke and Schmidt, 1998; Shen et al., 2004). Further studies (e.g., using local infusion techniques or tissue selective knock-out mice) are needed to clarify the relative neuroanatomical and neurochemical substrates of the self-administration and CPP procedures.

In contrast to the DAT, we found no evidence that deletion of the SERT reduced the reinforcing strength of cocaine or its mechanism of action. First, SERT$^{-/-}$ mice maintained self-administration comparable with wild-type mice even under high response requirements. Second, SERT$^{-/-}$ mice reallocated their behavior to a previously inactive nose-poke hole to maintain cocaine self-administration in a reversal procedure, further suggesting that cocaine served as a positive reinforcer in the SERT$^{-/-}$ mice. Third, pretreatment with a dopamine D_1-like antagonist dose-dependently increased cocaine self-administration in the SERT$^{-/-}$ mice, consistent with competitive antagonism through dopamine receptors. This behavior was comparable with that observed in wild-type C57BL/6J mice in our laboratory (Caine et al., 2007), suggesting the mechanism of action of cocaine as a reinforcer was essentially the same in the SERT$^{-/-}$ mice as in wild-type mice. This hypothesis is also consistent with the lack of alteration in cocaine-induced increase in extracellular accumbens dopamine in the same line of SERT$^{-/-}$ mice (Shen et al., 2004). The only effect we detected in the SERT$^{-/-}$ mice was a moderate delay in acquisition of operant behaviors, with both cocaine and food. A similar delay in the acquisition of food- and water-maintained behavior in SERT$^{-/-}$ mice was recently reported, and was tentatively attributed to neophobia in the novel operant conditioning environment (Trigo et al., 2007). Last, it is worth noting that in contrast to the previous observation made using cocaine CPP (Sora et al., 2001), there was no significant interaction between DAT and SERT genotype. DAT$^{-/-}$SERT$^{-/-}$ double knock-out mice showed cocaine self-administration and food-maintained behaviors comparable with the DAT$^{-/-}$ mice in all procedures.

In conclusion, there were two major findings of the present study. First, cocaine generally failed to serve as a positive reinforcer in DAT$^{-/-}$ mice, while both food and a direct dopamine agonist reliably maintained operant behavior in these mice at levels comparable with wild-type mice. Second, cocaine's rein-

forcing effects were intact in SERT $^{-/-}$ mice under a wide range of experimental conditions, despite a protracted acquisition of operant procedures generally, including food-maintained behavior. While a different line of DAT knock-out mice were previously reported to self-administer cocaine, cocaine was also reported to increase accumbens dopamine in those mice, a finding which differs from observations in the present line of DAT $^{-/-}$ mice (Carboni et al., 2001; Shen et al., 2004). Collectively, the present and previous findings support the notion that cocaine-induced increases in extracellular dopamine in the nucleus accumbens are critical for cocaine self-administration.

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