Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 2, pp. 327-335, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in the USA. All rights reserved
0091-3057/94 $6.00 + .00

0091-3057(93)E0030-8

Characterization of Dopamine Transporter and Locomotor Effects of Cocaine, GBR 12909, Epidepride, and SCH 23390 in C57BL and DBA Mice

DANIEL E. WOMER,* BYRON C. JONES† AND V. GENE ERWIN*[1]

*School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO 80262
†Health and Human Development, The Pennsylvania State University, University Park, PA 16802

Received 21 June 1993

WOMER, D. E., B. C. JONES AND V. G. ERWIN. *Characterization of dopamine transporter and locomotor effects of cocaine, GBR 12909, epidepride, and SCH 23390 in C57BL and DBA mice.* PHARMACOL BIOCHEM BEHAV 48(2) 327-335, 1994. – C57BL/6 and DBA/2 mice were used to examine genetic differences in locomotor activating effects of acute cocaine administration and to determine whether differences were mediated by dopaminergic systems. C57BL/6 mice were less activated than DBA/2 mice at 5 and 10 min after 10 and 15 mg/kg cocaine. HPLC analysis showed equivalent brain cocaine concentrations in the two strains at 5 and 10 min after 10, 15, or 20 mg/kg doses. The selective dopamine uptake inhibitor, GBR 12909, at 5 and 7.5 mg/kg, produced greater locomotor activation in DBA/2 mice than in C57BL/6 mice. However, binding studies with the selective dopamine uptake ligand [^{3}H]GBR 12935, revealed no between-strain difference in K_d or B_{max} in caudate putamen (CP) or nucleus accumbens (NA) membranes. Competition assays using unlabeled dopamine to compete for [^{3}H]GBR 12935 binding in CP or NA membranes showed no between-strain difference by brain region. The specific D_1 or D_2 antagonists, SCH 23390 or epidepride, respectively, produced dose-dependent decreases in locomotor activity but there were no between-strain differences. However, epidepride, at a dose of 0.003 mg/kg, completely reversed cocaine-induced (15 mg/kg) activation in both strains. These findings show that C57BL/6 and DBA/2 mice differ in dopamine-related behaviors and suggest that dopaminergic processes may mediate genetic differences in cocaine sensitivity.

Cocaine Dopamine transporter Locomotor activity GBR 12909 Epidepride SCH 23390 C57BL mice DBA mice

COCAINE is known to be a potent behavioral reinforcer (18) as well as a psychomotor stimulant in mice acting, in part, through mesocorticolimbic dopamine (DA) pathways (14,21). Many studies have shown that cocaine induces locomotor activation at low to moderate doses (5–30 mg/kg) whereas, at higher doses (>40 mg/kg) stereotyped behaviors predominate (19,23,27). Locomotor activation induced by an acute dose of cocaine has been attributed to its inhibitory action at the dopamine uptake transporter (DAUT) (22) as well as other amine uptake transporters, i.e., serotonin and norepinephrine (4). When selective serotonin or norepinephrine uptake blockers, fluoxetine or desipramine, respectively, are administered to rodents, the resultant behaviors do not match those of an acute dose of cocaine (6,24). However, in recent studies the selective DAUT inhibitors, such as GBR 12909, have been shown to elicit behavioral effects similar to cocaine (26). For example, acute low doses of GBR 12909 cause dose-dependent increases in locomotor activity in rats and at higher doses cause intense stereotypy (10).

Pharmacological (28) and biochemical (3) studies have suggested genetic differences in response to DA drugs and in brain distribution of DA receptors in mice. Recent studies (15) demonstrated genetic differences in cocaine-induced locomotor activation and related motor behaviors in C57BL and DBA

[1] Requests for reprints should be addressed to Dr. V. Gene Erwin, School of Pharmacy, University of Colorado Health Sciences Center, Box C238, 4200 E. 9th Ave., Denver, CO 80262.

mice. In those preliminary studies it was noted that differences in behavioral response could be mediated by pharmacokinetic or tissue sensitivity differences. Pharmacokinetic analysis of cocaine distribution in inbred mice has been studied by several groups (5,13), and Jones et al. (13) reported a significant between strain difference in brain cocaine levels with no significant difference in locomotor activating effects at 15 mg/kg cocaine. However, the latter studies did not compare C57BL and DBA mice. In preliminary experiments, Jones et al. (15) found only minimal differences in brain cocaine levels in C57BL and DBA mice at 5 min after cocaine administration.

Possible genetic differences in tissue sensitivity to cocaine were suggested by Jones et al. (13), and Erwin et al. (8) examined whether differences in cocaine sensitivity in C57BL and DBA mice might be mediated by differences in DA and serotonin transporter or D_1 and D_2 receptors. In this initial report we found no major difference in [^{3}H]CFT, a cocaine analog, or [^{3}H]SCH 23390, ligand binding; but a difference was observed in [^{125}I]epidepride and [^{3}H]sulpiride B_{max} values in caudate putamen (CP) from C57BL and DBA mice. These results were consistent with those of Boehme and Ciaranello (3). Studies to date have not compared locomotor effects for selective DAUT inhibitors or D_1 or D_2 antagonists in C57BL and DBA mice. Therefore, the purpose of this study was to further elucidate the underlying mechanisms mediating genetic differences in cocaine-induced locomotor activation between C57BL and DBA mice by comparing the effects of cocaine, GBR 12909, SCH 23390, and epidepride in these inbred strains. The studies included characterization of the DAUT in NA and CP from these strains using the selective ligand, [^{3}H]GBR 12935.

METHOD

Animals and Materials

Male and female C57BL/6Ibg (C57BL) and DBA/2Ibg (DBA) mice were obtained from the Institute for Behavioral Genetics, University of Colorado, Boulder, CO, and housed in a controlled environment with temperature and humidity maintained at 22°C and 20%, respectively, with light cycle at 0600 L : 1800 D. [C57BL and DBA males and females were compared for locomotor activation at various cocaine doses and there was no significant ($p > 0.05$) gender difference within strain (data not shown); thus, male and female data were combined.] Cocaine and tropacocaine were obtained from Sigma Inc. and GBR 12909 and SCH 23390 were obtained from Research Biochemicals Inc. Epidepride was a generous gift of Dr. Chester Mathis, Pittsburg, PA. All reagents were of the highest purity available from commercial sources. [^{3}H]GBR 12935 was obtained from New England Nuclear.

Locomotor Activity Determinations

C57BL and DBA mice were randomly assigned to either a saline group or one of six cocaine dose groups for activity testing. Following the experimental procedure outlined previously (13,14) with modifications, all mice were activity tested on day 1 with saline and on day 2 with either saline (control) or cocaine. All comparisons were between day 2 control (saline) values and day 2 cocaine values. This method of activity testing is superior to comparing day 1 control values to day 2 cocaine data because it provides some familiarization with the test apparatus rather than testing drug effects in a completely novel environment. Cocaine was dissolved in physiological saline at doses of 2.5, 5, 10, 15, 20, or 30 mg/kg, in an injection volume of 0.01 ml/g given intraperitoneally (IP). Locomotor activity was monitored between 0900 and 1200 h for 15 min, with data collected every 5 min using an automated open field (Omnitech, Inc. Columbus, OH) activity monitor. The activity monitor is a 40 × 40 × 30.5 cm acrylic cage with horizontal infrared sensors.

The effects of GBR 12909, a selective DA uptake inhibitor (10), on locomotor activity were tested in the same manner as outlined for cocaine. Doses of GBR 12909, dissolved in saline (1) were 2.5, 5, 7.5, 10, and 15 mg/kg, IP. Locomotor activity (distance traveled in cm) was monitored for 40 min, with data being summed every 10 min because of slower absorption and elimination rates for GBR 12909.

Epidepride or SCH 23390 were given, IP, in doses of 0.003, 0.01, 0.03, or 0.1 mg/kg, and activity monitored for 30 or 60 min, respectively, with data collected every 5 min. Stock epidepride solution stored in 100% ethanol was diluted greater than 100-fold in physiological saline to make the necessary concentrations for injection; thus, the ethanol concentration was less than 1%. SCH 23390 was dissolved in saline for IP injection.

Brain Cocaine Levels

Brain cocaine levels were determined by the method of Jatlow et al. (12). C57BL and DBA mice were injected with various doses of cocaine, IP, and after 5, 10, or 15 min were sacrificed and the brains removed and weighed and quickly homogenized in 5 ml 0.5 M NaF. Internal standard, tropacocaine (1 mg/20 μl) and 1 ml 1 N H_2SO_4 were added to the homogenate; the homogenates were shaken with 15 ml of anhydrous ethyl ether in 50 ml stoppered tubes for 10 min. After shaking, samples were centrifuged for 10 min at 1000 rpm and the ether layer was removed and discarded. The pH of the remaining samples were adjusted to 9.5 by addition of 200 mg of a dry carbonate buffer (Na_2CO_3 : $NaHCO_3$ in a 17.5 : 20 ratio). Fifteen milliliters of chloroform : absolute ethanol (4 : 1) were added and the mixture shaken for 10 min. The chloroform layer was removed and placed in a clean conical centrifuge tube and evaporated to dryness under a stream of N_2 gas at 30–40°C. Extracted cocaine and tropacocaine were dissolved in 100 ml absolute ethanol by shaking every 15 min for 2 h. After centrifuging samples for 10 min at 2500 rpm to pellet particulates, aliquots were loaded onto a μBondapak C18 HPLC column and separated with an isocratic mobile phase consisting of 50 mM KH_2PO_4 and 25% acetonitrile, pH 2.7 at a flow rate of 2 ml/min. Cocaine and tropacocaine were quantified by absorbance at 235 nm by a ISCO V4 UV detector and Hewlett Packard 3392A integrator.

[^{3}H]GBR 12935 Binding

Mice were sacrificed by cervical dislocation and brains rapidly removed and placed in ice-cold 0.32 M sucrose. Nucleus accumbens and caudate nucleus, punched from brain slices using anatomical guidelines of Slotnick and Leonard (30), were homogenized in 0.32 M sucrose and centrifuged at 1000 rpm for 10 min. The resulting pellet was resuspended in 0.32 M sucrose and centrifuged as described above. The combined supernatant fluids were centrifuged for 30 min at 10,000 rpm. The P_2 pellet containing synaptosomes was then resuspended in ice-cold 50 mM Na_2HPO_4 buffer with a 120 mM final concentration of NaCl and 10 μM ZnCl and centrifuged for 30 min at 3000 rpm. This buffer has been shown to give the

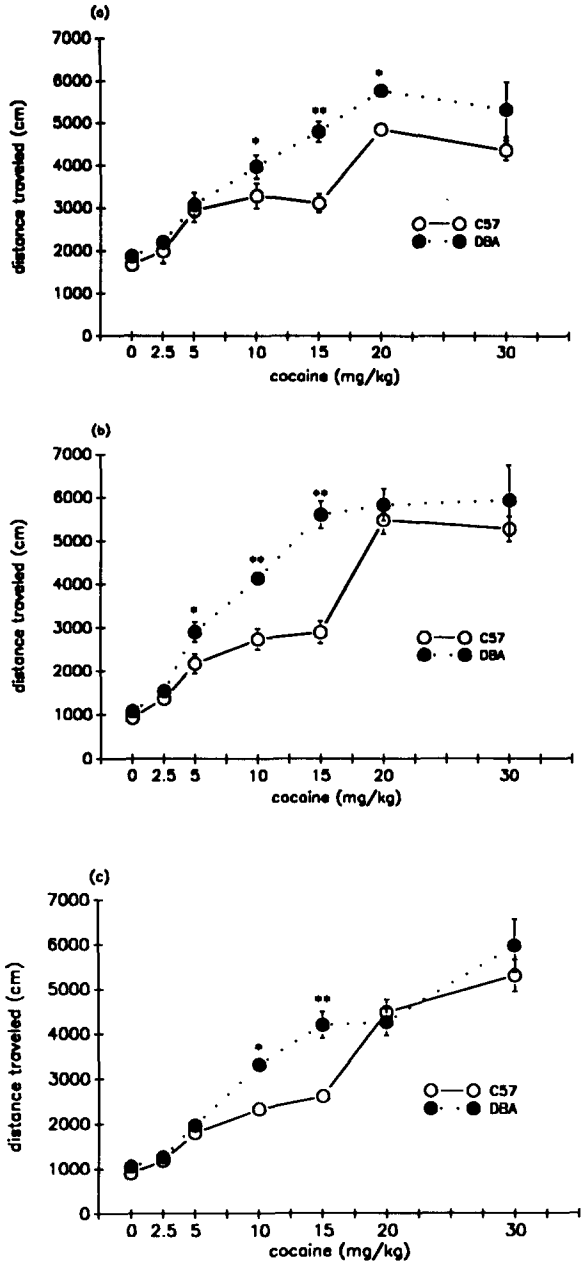

FIG. 1. Effects of cocaine on locomotor activity as a function of dose and time. (a) Shows the effects of cocaine on locomotor activity 5 min after IP administration in C57BL and DBA mice. (b) and (c) show the effects of the same doses of cocaine on locomotor activity at 10 and 15 min postinjection, respectively, in C57BL and DBA mice. Mice were given saline (0.01 ml/g) IP on day 1 and tested for 15 min in an automated open field (Omnitech Inc.). For day 2 testing, mice were given either saline or cocaine IP and activity monitored for 15 min. The results shown here are the means ± SEM for day 2 values with 10 to 24 animals/dose. Two-factor ANOVA, with tests for simple effects, revealed no significant difference between strains for saline, 2.5 or 30 mg/kg cocaine. There was a significant difference between strains (*$p < 0.05$) at the 20 mg/kg dose at 5 min and the 5 mg/kg dose at 10 min. There was a significant difference between strains in locomotor activity at the 10 mg/kg dose across all time (*$p < 0.05$). The 15 mg/kg dose produced a significant (**$p < 0.01$) between-strain difference in locomotor activity.

highest B_{max} for [^{3}H]GBR 12935 binding (25) and, thus, it was used for all binding assays. Brain protein was diluted to a concentration of 1-3 mg/ml for each brain region. Final concentrations of [^{3}H]GBR 12935 ranged from 0.1 to 100 nM, and 10 μM cocaine was used to define nonspecific binding. Final volume for each assay tube was 100 μl. Samples were allowed to incubate for 1 h at room temperature (22-25°C) and filtered through Whatman GF/B filters presoaked in 0.2% PEI using a Brandel Cell Harvester. Analysis of binding parameters was performed by the program Accufit.

In competitive displacement studies with dopamine, tissues were homogenized in 0.32 M sucrose containing 1 μM pargyline and the P_2 fraction was isolated as described above. Binding buffer was 50 mM sodium phosphate plus 120 mM NaCl and 0.1% BSA containing 1 μM ascorbate and 10 μM ZnCl, and nonspecific binding was determined with 10 μM cocaine. IC_{50} values were calculated from pseudo-Hill plots of the competitive displacement data, and these values were entered into Cheng-Prusoff equation to calculate a K_i for DA at the DAUT.

Statistical Analysis

One-factor or two-factor ANOVA with tests for simple main effects were performed where appropriate using the statistical package Crunch 4, with a at 0.05.

RESULTS

Dose-response relationships for the effects of acute doses of cocaine on locomotor activity in C57BL and DBA mice are shown in Fig. 1a, b, and c, representing activity summed at 5 min intervals, as cm traveled at 5, 10, and 15 min after injection. It is important to note that on day 2, locomotor activity following saline administration were similar for C57 and DBA mice, indicating comparable acclimatization to the open field arena. Cocaine produced a dose-dependent increase in activity in both strains. ANOVA with tests for simple main effects showed no significant between-strain difference in locomotor activity for saline or low cocaine doses (2.5 and 5 mg/kg) or for high doses (30 mg/kg). There was a significant difference between strains for locomotor activity at 10 mg/kg, $F(1, 28) = 12.1$, $p < 0.05$ and at 15 mg/kg, $F(1, 54) = 32.7$, $p < 0.01$, across all time points. At 20 mg/kg cocaine there was a significant difference, $F(1, 51) = 5.2$, $p < 0.05$, in activity only at the 5 min time point. It is of interest that the cocaine dose-response function in C57BL, but not in DBA mice was distinctly biphasic.

In order to determine whether the strain differences in co-

TABLE 1

BRAIN COCAINE LEVELS IN C57 AND DBA MICE FOLLOWING 10, 15, OR 20 mg/kg IP INJECTION

	10 mg/kg	15 mg/kg	20 mg/kg
C57	2.92 ± 0.19 (4)	5.33 ± 0.15 (5)	6.12 ± 0.18 (5)
DBA	2.89 ± 0.30 (4)	5.74 ± 0.17 (5)	5.96 ± 0.38 (3)

Time of sacrifice for each animal was 5 min postinjection. Values in each cell are mean ± SEM, with the number of mice used in parentheses. Values are μg cocaine/g wet brain weight. Two factor ANOVA showed no significant between strain differences at any cocaine dose with no strain by dose interaction.

caine-induced locomotor activation might be mediated by differences in cocaine distribution, brain cocaine concentrations were determined for each strain at 5 min (a time when there was a significant strain difference in activation) after 10, 15, or 20 mg/kg cocaine, IP. There was no significant between-strain difference at these doses (Table 1). Two-factor ANOVA of data in Fig. 2 showed no significant between-strain difference in brain cocaine levels over time following a 15 mg/kg dose; however, there was an apparent decrease in brain cocaine concentrations over time which tended (nonsignificant) to be faster in C57BL than in DBA mice.

Locomotor activity induced by acute doses of GBR 12909 in C57BL and DBA mice are shown in Fig. 3a, b, and c. There was a significant within-strain locomotor activation by GBR 12909 over time ($p < 0.05$) for each dose. DBA mice showed significantly greater activation than C57BL mice at 5, $F(1, 18) = 13.2, p < 0.05$, 7.5, $F(1, 16) = 4.4, p < 0.05$, and 10 mg/kg, $F(1, 18) = 5.9, p < 0.05$, doses for time points after the first 10 min. There was no significant difference between strains at the 2.5 mg/kg dose. Locomotor activation peaked at 15 mg/kg and declined at higher doses, perhaps due to marked increases in stereotyped movements (data not shown) (10). The GBR 12909 dose-response functions in both C57BL and DBA mice were biphasic.

Because GBR 12909, a selective inhibitor of DA uptake, produces differential locomotor activation in DBA and C57BL mice, it was of interest to determine whether there are differences in binding characteristics of the DAUT. Data in Fig. 4 show that binding parameters for [^{3}H]GBR 12935 were similar in both strains by region. [^{3}H]GBR 12935 binding was linear, indicating only one affinity site; caudate membranes showed a K_d of 2.86 ± 0.31 nM and 3.34 ± 0.21 nM for C57BL and DBA, respectively, with corresponding B_{max} values of 12.36 ± 0.71 and 13.98 ± 0.56 pmol/mg protein. Nucleus accumbens membranes revealed no between-strain difference in K_d values; $K_d = 2.30 \pm 0.35$ vs. 1.93 ± 0.27 nM for C57BL and DBA mice, respectively. B_{max} values for C57BL and DBA

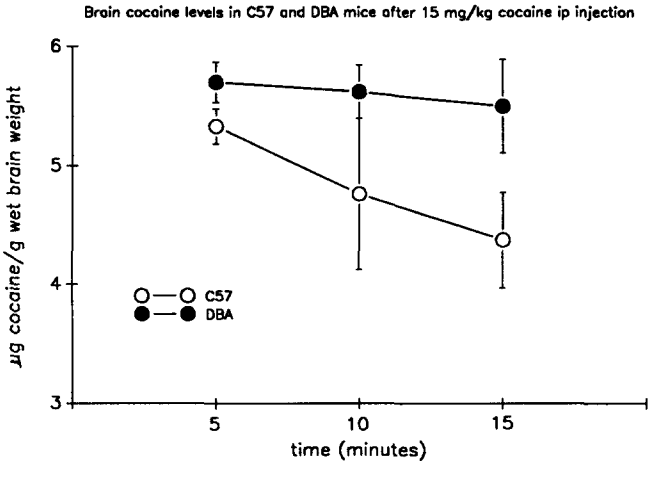

FIG. 2. Brain cocaine concentrations in C57BL and DBA mice after 15 mg/Kg IP injection as a function of time. Brain cocaine levels were determined by the method of Jatlow et al. (12) (see the Method section). Each point is the mean ± SEM for 5–6 animals/time point. One-factor ANOVA revealed a significant difference ($p < 0.05$) between strains over time ($p < 0.05$) with no time or strain × time interaction.

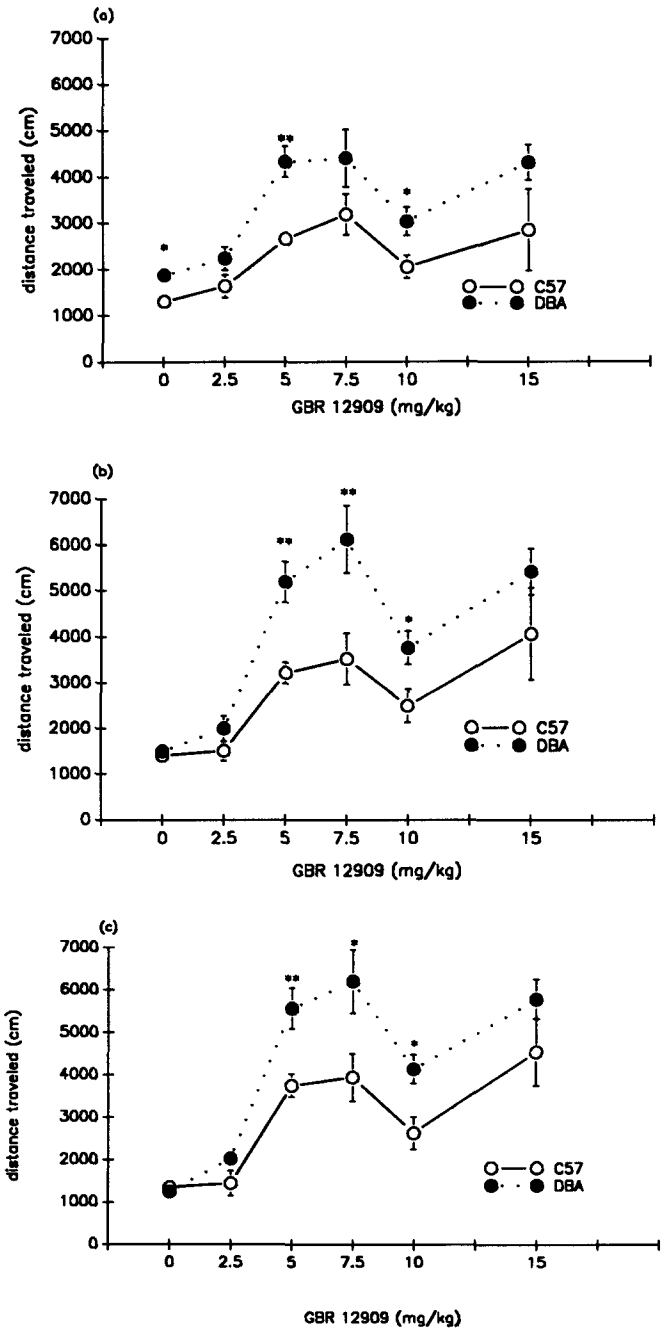

FIG. 3. Effects of GBR 12909 on locomotor activity in C57BL and DBA mice as a function of dose and time. Mice were injected with saline (0.01 ml/g) on day 1 and activity monitored for 40 min in an automated open field (Omnitech Inc.). Values shown are the mean ± SEM (n = 8–12 animals/dose). On day 2, mice were given GBR 12909 and activity monitored as described. ANOVA with tests for simple main effects showed a significant difference for saline treatment at the 10 (data not shown) and 20 min time periods (*$p < 0.05$). At 20 min (a), there was a significant difference at 5 mg/kg (**$p < 0.01$) and at the 10 mg/kg dose (*$p < 0.05$). At 30 min (b), there was a significant difference (**$p < 0.01$) for the 5 and 7.5 mg/kg dose; additionally, there was a significant (*$p < 0.05$) between-strain activity difference at 10 mg/kg. (c) Shows a significant (*$p < 0.05$) difference in activity at the 7.5 and 10 mg/kg doses and (**$p < 0.01$) at 5 mg/kg at 40 min after injection.

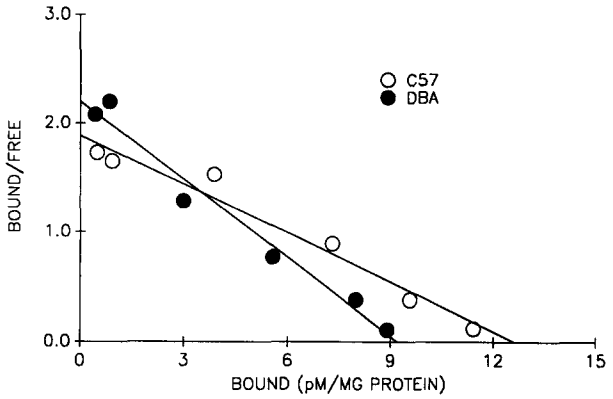

FIG. 4. Representative Scatchard plots of [^{3}H]GBR 12935 binding in C57BL and DBA caudate putamen membranes. Caudate nuclei were dissected, a P_2 fraction prepared, and saturation binding with [^{3}H]GBR 12935 performed as described in the Method section. ANOVA revealed no significant between-strain difference in K_d or B_{max}.

nucleus accumbens membranes were 4.50 ± 0.42 vs. 4.12 ± 0.29 pmol/mg protein, respectively. ANOVA showed a significant difference [C57, $F(1, 8) = 16.3$, $p < 0.01$ and DBA, $F(1, 10) = 14.6$, $p < 0.01$] within-strain by region for B_{max} values.

Competition assays for [^{3}H]GBR 12935 binding revealed no significant difference between strain by region in K_i values for DA (Fig. 5). Competition curves were monophasic in nature, suggesting a single affinity for binding of DA. K_i values for DA in caudate membranes were 105.4 ± 22.6 vs. 108.2 ± 19.8 mM, for C57BL and DBA mice, respectively. Likewise, K_i values for nucleus accumbens membranes showed no between-strain difference with 149.0 ± 24.2 vs. 120.3 ± 12.5 mM, for C57BL and DBA membranes, respectively.

Behavioral studies clearly suggest a role of DA in locomotor activation in C57BL and DBA mice, but biochemical studies suggest similarities in the DAUT. Because cocaine and GBR 12909 may produce differential activation via DA receptors, the effects of the selective D_1 and D_2 antagonist, SCH 23390 and epidepride, respectively, on locomotor activity in C57 and DBA mice were determined. Dose-response relationships for various doses of epidepride are shown in Fig. 6. Two-factor ANOVA with tests for simple main effects show that there was a significant decrease in locomotor activity at 0.1 mg/kg epidepride, $F(1, 6) = 25.3$, $p < 0.05$, and a concomitant time effect within strain at this dose [C57, $F(5, 30) = 58.3$, $p < 0.01$, and DBA, $F(5, 30) = 223.9$, $p < 0.01$]. For all other doses of epidepride there were significant within-strain effects by time with no significant strain difference by

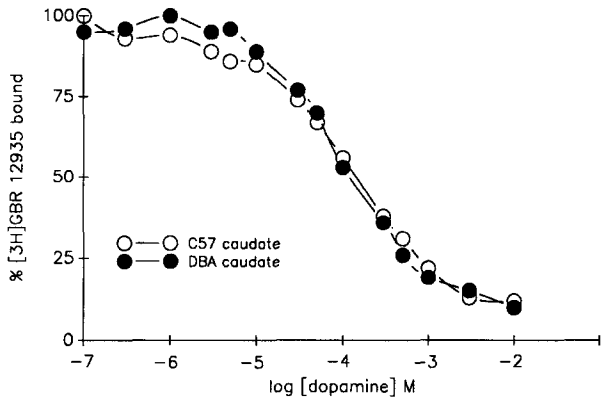

FIG. 5. Dopamine competitive displacement of [^{3}H]GBR 12935 binding in caudate membranes from C57BL and DBA mice. Membranes were prepared as described and competitive binding experiments with [^{3}H]GBR 12935 (~5 nM) and increasing concentrations of DA were conducted as described in the Method section. IC_{50} and K_i values were calculated as described in the Method section. ANOVA showed no significant between-strain difference for K_i values in caudate or nucleus accumbens membranes.

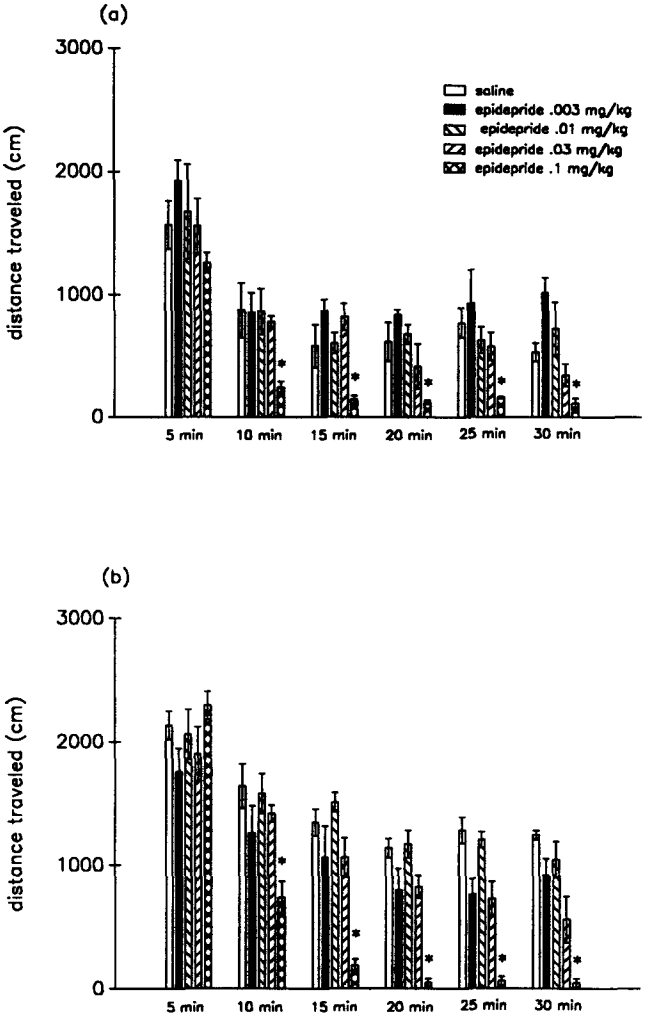

FIG. 6. Effects of epidepride, a selective D_2 antagonist, on locomotor activity are shown in C57BL (a) and DBA (b) mice. Saline was injected IP on day 1 and the mice placed in the automated open field (Omnitech Inc.) for 30 min and data collected every 5 min. On day 2, epidepride was injected IP in the doses indicated, and locomotor activity was monitored for 30 min with data being collected every 5 min. Data shown are the mean ± SEM of 10–15 animals/dose. There was a significant decrease (*$p < 0.05$) in locomotor activity at the 0.1 mg/kg dose and a significant time effect within strain at this dose.

epidepride dose. Similar results were obtained with SCH 23390 (Fig. 8), which shows dose-response relationships for locomotor inhibition in C57BL and DBA mice. There were significant within-strain but no between-strain differences.

Data presented in Fig. 7 show that 0.003 mg/kg epidepride, a dose that had no effect on locomotor activity (Fig. 6), completely blocked cocaine-induced (15 mg/kg) locomotor activation in both C57BL and DBA mice. Higher epidepride doses produced locomotor inhibition even in the presence of cocaine. At the 0.03 mg/kg dose of epidepride followed by cocaine (15 mg/kg) there was a significant, $F(1, 20) = 30.2$, $p < 0.05$, between-strain difference in locomotor inhibition. These data show that DBA were more sensitive than C57BL mice to epidepride inhibition in the presence of cocaine.

The doses of SCH 23390 caused small decreases in locomotor activity in both strains, with no between-strain differences (Fig. 8) when administered alone; however, doses of 0.01 or 0.03 mg/kg given 20 min prior to cocaine injection completely reversed cocaine-induced locomotor activation in DBA mice (Fig. 9b), with little effect on C57BL mice (Fig. 9a). DBA mice were significantly, $F(1, 54) = 32.7$, $p < 0.01$, more activated than C57BL mice following saline/cocaine treatment.

DISCUSSION

The results show a significant difference in locomotor activation between C57BL and DBA mice at 10 and 15 mg/kg cocaine over the 15 min test period, with DBA mice more activated than C57BL. These findings are consistent with

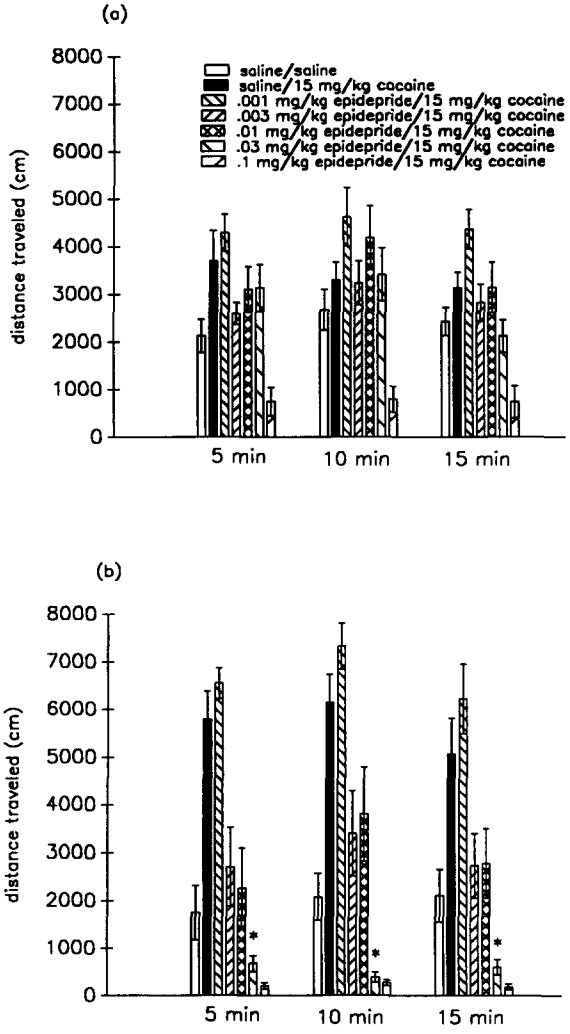

FIG. 7. Effects of epidepride on cocaine-induced locomotor activity. Results are shown for C57BL (a) and DBA (b) mice, respectively. Mice were injected with various doses of epidepride 15 min prior to injection with 15 mg/kg cocaine. The mice were then placed in activity monitors (Omnitech Inc.) and data collected every 5 min for 15 min. The values expressed are the mean ± SEM for 5–15 animals/dose of epidepride. The only significant between-strain difference (*$p <$ 0.05) was noted at the 0.03 mg/kg dose of epidepride where DBA mice were more inhibited than C57BL mice.

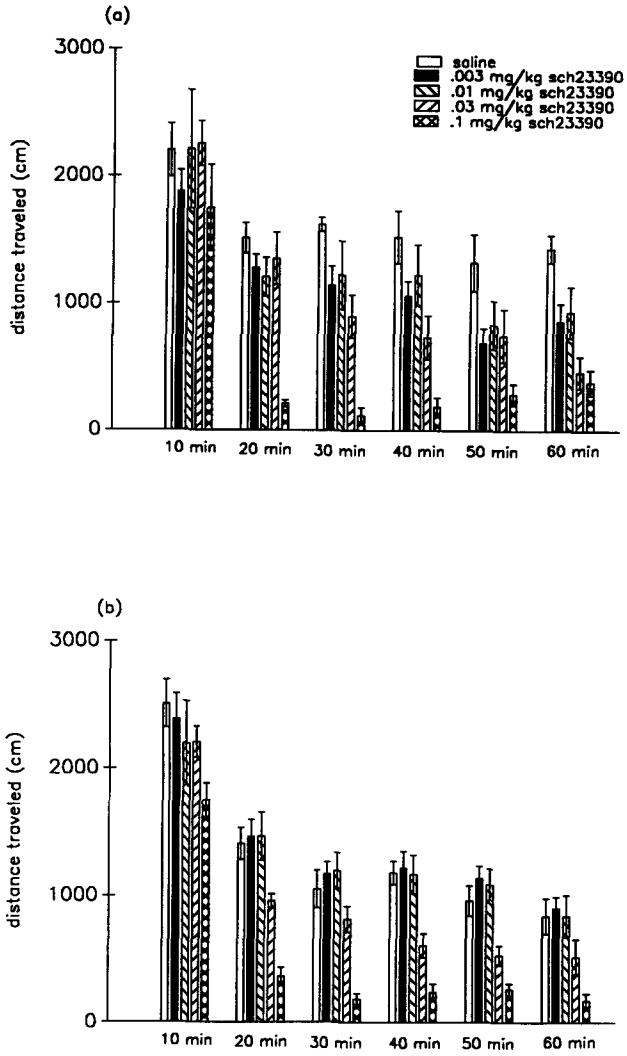

FIG. 8. Effects of SCH 23390, a selective D_1 antagonist on locomotor activity in C57BL (a) and DBA (b) mice. Saline was injected IP (0.01 ml/g) on day 1 and on day 2, SCH 23390 was administered in the doses indicated with activity monitored for 60 min and data collected every 10 min. Values shown are the mean ± SEM of 6–10 animals/dose. Two-factor ANOVA with tests for simple main effects revealed no significant between-strain differences at each dose of SCH 23390. There were significant ($p < 0.05$) within-strain effects of dose across time.

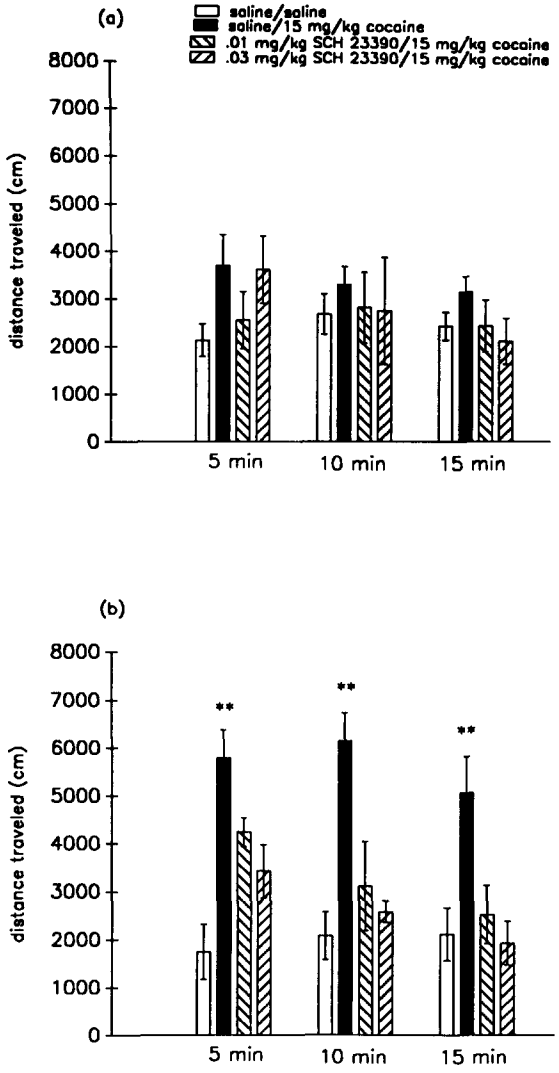

FIG. 9. Effects of SCH 23390 on cocaine-induced locomotor activity in C57BL (a) and DBA (b) mice. Mice were injected with indicated doses of SCH 23390 20 min prior to cocaine (15 mg/kg) administration. Locomotor activity was monitored for 15 min in an automated open field (Omnitech Inc.) and the values reported are the mean ± SEM for 4-10 animals/treatment. There were no between strain differences in activity following SCH 23390 administration, although there was a significant (**$p < 0.01$) difference for saline/cocaine activity between strains.

those previously reported (8). At higher doses (30 mg/kg), both strains are equally activated. The dose-response curve (Fig. 1) for cocaine activation in C57BL mice is clearly biphasic indication more than one mechanism is responsible for activation in this strain. It was hypothesized that differences at the DAUT and not other amine-uptake complexes (4,17) mediates activation by cocaine, and this hypothesis was supported by results with a selective DAUT inhibitor, GBR 12909, which produces greater increases in locomotor activity in DBA mice than in C57BL mice (Figs. 1a-c and 3a-c). Heikkila and Manzino (10) have shown that GBR analogs, which are selective for the DAUT, produce locomotor activation, but this is the first report that the extent of activation is genotype dependent. It is of interest that the dose-response curves for GBR 12909 activation were biphasic in both inbred strains of mice, again suggesting multiple mechanisms associated with DA mediated locomotor activation.

Data showing brain levels of cocaine as a function of dose and time after cocaine administration indicate that differential activation observed between C57BL and DBA mice cannot be attributed in large measure to differences in brain cocaine levels (Fig. 2, Table 1). As noted by Benuck et al. (2) peak brain levels of cocaine are reached within 5 min after IP injection and correlate well with locomotor activation in mice. Our data showed no significant between-strain differences in brain cocaine levels at times (5 and 10 min) and doses (10 and 15 mg/kg) where maximal differences in locomotor activation was observed. Because the differences in locomotor activation between C57BL and DBA mice at 15 mg/kg cocaine are not due to differences in brain cocaine levels, it is likely that C57BL and DBA mice differ in cocaine receptors or in DA receptors that mediate cocaine and GBR 12909 effects through increased synaptic DA levels.

Equilibrium binding studies were conducted to determine whether the DAUT differs in membranes isolated from specific brain regions of C57BL and DBA mice. Binding characteristics, affinity, and binding capacity values for [^{3}H]GBR 12935 to the DAUT from nucleus accumbens and caudate putamen from these inbred strains did not differ (Fig. 4), suggesting no difference in structure or expression of the DAUT in these brain regions. Competition assays showed that there was no strain difference in K_i values for DA for the DAUT (Fig. 5). These results confirm previous observations showing similar K_d and B_{max} values for [^{3}H]CFT, a cocaine analog, binding in brain membranes from C57BL and DBA mice (8). These data are consistent with studies of DAUT function ([^{3}H]DA uptake) using P_2 synaptosomal fractions from brains of C57BL and DBA mice (4). These investigators reported no significant difference in [^{3}H]DA accumulation or IC_{50} values for cocaine inhibition of [^{3}H]DA uptake in synaptosomes isolated from C57BL and DBA brains.

Originally, DA receptors were divided into two distinct classes, D_1 and D_2, shown to differ in ability to stimulate or inhibit adenylate cyclase activity, respectively (16). More recently, DA receptors have been classified as the D_1 family, comprised of D_1 and D_5 receptors and the D_2 family, containing D_2 short, D_2 long, D_3, and D_4 receptors (29). Given the mechanism(s) of cocaine's action, it is reasonable to assume that increased synaptic DA has an effect at all DA receptors. Some evidence for behavioral effects of agents acting on these receptors has been obtained. Locomotor activation and stereotypy can be induced by a selective D_2 agonist, quinpirole, whereas blocking the D_2 receptor with a selective antagonist, raclopride (31), causes dose-dependent decreases in spontaneous locomotor activity. Higher doses of a D_2 antagonist cause catalepsy for extended periods and markedly attenuate amphetamine (31)- and cocaine (9)- induced locomotor activation. A D_1 agonist, SKF 38393, elicits very little effect (32). Darney et al. (7) have shown that with a new selective D_1 agonist, dihydrexidine, locomotor activation can be elicited but only at relatively high doses where the compound has some affinity for D_2 receptors. Hoffman and Beninger (11) have shown that acute doses of the D_1 antagonist, SCH 23390, have little effect on locomotor activity in rats. Others have shown that mixed D_1/D_2 agonist (apomorphine) causes locomotor activation (20).

The present results show no major genetic differences in locomotor effects of relatively selective D_2 and D_1 antagonists

when administered separately and in the absence of cocaine. However, there was a significant difference in the ability of cocaine to reverse epidepride (0.03 mg/kg) locomotor inhibition. This difference is more apparent in DBA mice than in C57 mice (Fig. 7) and might be accounted for by differences in the D_2 receptor or regulation of receptor-coupled processes. For example, DA D_2 receptors from these strains may differ genetically in their ability to regulate DA release from presynaptic terminals or in D_2 coupling to postsynaptic effector systems. Behavioral results obtained with the D_1 antagonist SCH 23390 on cocaine-induced locomotor activation (Fig. 9), were similar to D_2 antagonist pretreatment; that is, SCH 23390 prevented cocaine-induced activation in DBA mice.

Previous biochemical studies (8) have shown that there is no significant between strain difference in D_1 receptor binding characteristics using [^{3}H]SCH 23390 in brain regions from C57BL and DBA and only modest genetic differences in B_{max} values for D_2 selective ligands, [^{3}H]sulpiride and [^{125}I]epidepride. These results lead us to speculate that the mechanism for differential inhibition of cocaine activation may be due to efficiency of receptor coupling to effector molecules, differential synaptic DA release mechanisms, or crosstalk between second messenger systems.

ACKNOWLEDGEMENTS

This work was supported, in part, by U.S.P.H.S. grants DA 01717, AA 00079, and AA 08125.

REFERENCES

1. Baldo, B. A.; Kelley, A. E. Cross-sensitization between cocaine and GBR 12909, a dopamine uptake inhibitor. Brain Res. Bull. 27:105–108; 1991.
2. Benuck, M.; Lajtha, A.; Reith, M. E. Pharmacokinetics of systemically administered cocaine and locomotor stimulation in mice. J. Pharmacol. Exp. Ther. 243:144–149; 1987.
3. Boehme, R. E.; Ciaranello, R. D. Genetic control of dopamine and serotonin receptors in brain regions of inbred mice. Brain Res. 266:51–65; 1983.
4. Bosy, T. Z.; Ruth, J. A. Differential inhibition of synaptosomal accumulation of [^{3}H]-monoamines by cocaine, tropacocaine, and amphetamine in four inbred strains of mice. Pharmacol. Biochem. Behav. 34:165–172; 1989.
5. Boyer, C. S.; Petersen, D. R. Pharmacokinetic analysis of the metabolism of cocaine to norcocaine and *n*-hydroxynorcocaine in mice. Drug Metab. Dispos. 20:863–868; 1992.
6. Chagraoui, A.; Vasse, M.; Protais, P. Effects of chronic treatments with amineptine and desipramine on motor responses involving dopaminergic systems. Psychopharmacology (Berlin) 102: 201–206; 1990.
7. Darney, K. J., Jr.; Lewis, M. H.; Brewster, W. K.; Nichols, D. E.; Mailman, R. B. Behavioral effects in the rat of dihydrexidine, a high-potency, full-efficacy D_1 dopamine receptor agonist. Neuropsychopharmacology 5:187–195; 1991.
8. Erwin, V. G.; Womer, D. E.; Campbell, A. D.; Jones, B. C. Pharmacogenetics of cocaine: II. Mesocorticolimbic and striatal dopamine and cocaine receptors in C57BL and DBA mice. Pharmacogenetics 3:189–196; 1993.
9. Filibeck, U.; Cabib, S.; Castellano, C.; Puglisi-Allegra, S. Chronic cocaine enhances defensive behaviour in the laboratory mouse: Involvement of D_2 dopamine receptors. Psychopharmacology (Berlin) 96:437–441; 1988.
10. Heikkila, R. E.; Manzino, L. Behavioral properties of GBR 12909, GBR 13069 and GBR 13098: Specific inhibitors of dopamine uptake. Eur. J. Pharmacol. 103:241–248; 1984.
11. Hoffman, D. C.; Beninger, R. J. The D_1 dopamine receptor antagonist, SCH 23390 reduces locomotor activity and rearing in rats. Pharmacol. Biochem. Behav. 22:341–342; 1985.
12. Jatlow, P. I.; Van Dyke, C.; Barash, P.; Byck, R. Measurement of benzoylecgonine and cocaine in urine, separation of various cocaine metabolites using reversed-phase high-performance liquid chromatography. J. Chromatogr. 152:115–121; 1978.
13. Jones, B. C.; Campbell, A. D.; Radcliffe, R. A.; Erwin, V. G. Cocaine actions, brain levels and receptors in selected lines of mice. Pharmacol. Biochem. Behav. 40:941–948; 1991.
14. Jones, B. C.; Campbell, A. D.; Radcliffe, R. A.; Erwin, V. G. Psychomotor stimulant effect of cocaine is affected by genetic makeup and experimental history. Ann. NY Acad. Sci. 654:456–458; 1992.
15. Jones, B. C.; Radcliffe, R. A.; Erwin, V. G. Pharmacogenetics of cocaine: I. Locomotor activity and self-selection in C57BL and DBA mice. Pharmacogenetics 3:182–188; 1993.
16. Kebabian, J. W.; Calne, D. B. Multiple receptors for dopamine. Nature 277:93–96; 1979.
17. Kilty, J. E.; Lorang, D.; Amara, S. G. Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science 254:578–579; 1991.
18. Kuhar, M. J.; Ritz, M. C.; Boja, J. W. The dopamine hypothesis of the reinforcing properties of cocaine. Trends. Neurosci. 14: 299–302; 1991.
19. Lau, C. E.; Imam, A.; Ma, F.; Falk, J. L. Acute effects of cocaine on spontaneous and discriminative motor functions: Relation to route of administration and pharmacokinetics. J. Pharmacol. Exp. Ther. 257:444–456; 1991.
20. Molloy, A. G.; O'Boyle, K. M.; Pugh, M. T.; Waddington, J. L. Locomotor behaviors in response to new selective D_1 and D_2 dopamine receptor agonists, and the influence of selective antagonists. Pharmacol. Biochem. Behav. 25:249–253; 1986.
21. Reith, M. E.; Meisler, B. E.; Lajtha, A. Locomotor effects of cocaine, cocaine congeners, and local anesthetics in mice. Pharmacol. Biochem. Behav. 23:831–836; 1985.
22. Reith, M. E.; Meisler, B. E.; Sershen, H.; Lajtha, A. Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. Biochem. Pharmacol. 35:1123–1129; 1986.
23. Reith, M. E.; Selmeci, G. Cocaine binding sites in mouse striatum, dopamine autoreceptors, and cocaine-induced locomotion. Pharmacol. Biochem. Behav. 41:227–230; 1992.
24. Reith, M. E.; Wiener, H. L.; Fischette, C. T. Sertraline and cocaine-induced locomotion in mice. I. Acute studies. Psychopharmacology (Berlin) 103:297–305; 1991.
25. Richfield, E. K. Zinc modulation of drug binding, cocaine affinity states, and dopamine uptake on the dopamine uptake complex. Mol. Pharmacol. 43:100–108; 1993.
26. Rothman, R. B.; Grieg, N.; Kim, A.; De Costa, B. R.; Rice, K. C.; Carroll, F. I.; Pert, A. Cocaine and GBR 12909 produce equivalent motoric responses at different occupancy of the dopamine transporter. Pharmacol. Biochem. Behav. 43:1135–1142; 1993.
27. Ruth, J. A.; Ullman, E. A.; Collins, A. C. An analysis of cocaine effects on locomotor activities and heart rate in four inbred mouse strains. Pharmacol. Biochem. Behav. 29:157–162; 1988.
28. Severson, J. A.; Randall, P. K.; Finch, C. E. Genotypic influences on striatal dopaminergic regulation in mice. Brain Res. 210: 201–215; 1981.
29. Sibley, D. R.; Monsma, F. J., Jr. Molecular biology of dopamine receptors. Trends. Pharmacol. Sci. 13:61–69; 1992.
30. Slotnick, B. M.; Leonard, C. M. Stereotaxic atlas of the albino mouse forebrain. DHEW Publication (ADN)75-100; 1975.

31. van den Boss, R.; Cools, A. R.; Ogren, S. O. Differential effects of the selective D_2-antagonist raclopride in the nucleus accumbens of the rat on spontaneous and *d*-amphetamine-induced activity. Psychopharmacology (Berlin) 95:447–451; 1988.

32. Zahniser, N. R.; Peris, J. Neurochemical mechanisms of cocaine-induced sensitization. In: Lakoski, J. M.; Galloway, M. P.; White, F. J., eds. Cocaine pharmacology, physiology, and clinical strategies. Boca Raton, FL: CRC Press, Inc.; 1992: 229–260.