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Chronic Cocaine Administration Is Associated with Behavioral Sensitization and Time-Dependent Changes in Striatal Dopamine Transporter Binding[1]

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ABSTRACT

Chronic cocaine administration has been associated with sensitization (an increase in drug effect) rather than the tolerance observed with many psychotropic compounds. Because cocaine acts at the presynaptic dopamine transporter, we evaluated sensitization and striatal dopamine transporter binding *in vivo* in several mouse strains. All strains of mice evaluated showed increased activity after cocaine compared with after saline injections. BALB/cByJ, DBA/2J, B6AF1/J and C57BL6/J mice exhibited sensitization when assayed 72 hr after five daily injections of cocaine at 20 and 40 mg/kg/day, whereas B6AF1/J mice showed sensitization at 20 but not at 40 mg/kg/day. CD-1 mice did not exhibit sensitization at either dose. Striatal dopamine transporter binding *in vivo* was increased in DBA/2J and B6AF1/J mice when determined 72 hr after five injections of 40 mg/kg/day cocaine. In contrast, a continuous infusion of cocaine at the same dose and duration did not produce sensitization or binding changes in DBA/2J mice. The time course of transporter binding alterations after intermittent cocaine exposure indicated no change at 1 day, increased binding at 3 days, a return to control levels at 7 days and decreased binding at 14 days. These data indicate that both sensitization and alterations in dopamine transporter binding occur after chronic cocaine injection but that these changes are unlikely to be directly related.

Chronic cocaine administration has been associated with behavioral sensitization—an increase in drug effect over the course of exposure. Sensitization to cocaine has been demonstrated in a number of species, including mice, dogs, rats and monkeys (Post and Contel, 1983). In mice, sensitization to cocaine is commonly associated with motor hyperactivity. Substantial differences occur among mouse strains in degree and type of behavioral sensitization, in other pharmacodynamic effects such as heart rate responses and in pharmacokinetic parameters such as brain concentrations of cocaine and metabolites (Carney and Tolliver, 1993; George and Ritz, 1990; Ruth *et al.*, 1988; Shuster *et al.*, 1977; Wiener and Reith, 1990).

Behavioral sensitization depends on a number of variables, including mode of drug administration, duration of administration, assay methodology and genetics of the animal model. Studies have indicated that prolonged sensitization occurs after four daily cocaine injections (Shuster *et al.*, 1977) or, alternatively, that only one injection can produce sensitization in a novel environment (Jackson and Nutt, 1993). King *et al.* (1992) recently reported that intermittent *vs.* chronic drug administration differentially affected subsequent sensitization. Finally, several investigators have emphasized the importance of genetic differences in predisposition toward cocaine sensitization (George and Goldberg, 1989; Shuster, 1991).

The neurochemical mechanism for sensitization to cocaine remains uncertain. Cocaine acts by blocking the reuptake of dopamine, norepinephrine and serotonin, with additional effects on cholinergic muscarinic and *sigma* receptors (Carroll *et al.*, 1992). The striatum and nucleus accumbens appear to be the locus of cocaine action because cocaine inhibits dopamine uptake into the nerve terminals, and these regions house the terminals of the nigrostriatal and mesolimbic pathways. Recently, Kuhar *et al.* developed an *in vivo* binding assay for the dopamine transporter (Boja *et al.*, 1990; Kuhar *et al.*, 1990; Pogun *et al.*, 1991; Scheffel *et al.*, 1991). This technique allows determination of dopamine transporter binding without the potential confounding effects of tissue preparation, buffers and assay conditions.

To examine the relationship between behavioral sensitization and changes in the dopamine transporter, we exposed five different mouse strains (three inbred, one outbred and one hybrid) to chronic intermittent cocaine and determined sensitization and *in vivo* specific uptake of the high-affinity cocaine analog [^{3}H]WIN 35,428. A second experiment examined behavioral sensitization and specific uptake of [^{3}H]WIN 35,428 after continuous infusion compared with intermittent injection of cocaine. Finally, we evaluated the time course of specific uptake of [^{3}H]WIN 35,428 after chronic intermittent cocaine administration.

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Methods

Experiment I: Sensitization by Strain

Materials. Male mice 6 to 8 weeks old were obtained of the following strains: CD-1 (Charles River Laboratories, Wilmington, MA) and DBA/2J, BALB/cByJ, C57BL6/J, and B6AF1/J (Jackson Laboratories, Bar Harbor, ME). Mice were maintained on a 12-hour light-dark cycle with food and water *ad libitum*. [^{3}H]WIN 35,428 (specific activity, 83 Ci/mmol) and Solvable were obtained from Dupont-New England Nuclear (Boston, MA). Cocaine hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were obtained from standard commercial sources.

Drug administration. The mice received one of three protocols: saline (control), 20 mg/kg/day cocaine and 40 mg/kg/day cocaine. These doses were chosen based on a report (Shuster *et al.*, 1977) that indicated proportional increases in activity occurred with increasing the pretreatment dose from 10 to 40 mg/kg. Cocaine hydrochloride was dissolved in saline and administered *via* subcutaneous injection on five consecutive days.

Open-field activity. Activity was determined with a Digiscan activity monitor (Omnitech, Columbus, OH). Open-field activity was measured for 30 min after the first injection in the chronic course, and again following a challenge dose given 72 hr after the last chronic injection. Determinations included total distance traveled (in cm) and stereotypic movements. Activity was defined as total distance traveled (in cm). All monitoring took place between 9:00 A.M. and 12:00 noon.

Dopamine transporter binding *in vivo*. Binding, as previously described (Kuhar *et al.*, 1990; Pogun *et al.*, 1991; Scheffel *et al.*, 1991), was performed 72 hr after the last subcutaneous injection. Briefly, mice were injected *via* the tail vein with 2 μCi [^{3}H]WIN 35,428. After 30 min, the animals were killed, and the cerebellum and striatum were dissected on ice. Tissue was weighed and solubilized overnight at 40°C with Solvable. Radioactivity was determined by scintillation spectrometry. Total and nonspecific binding values were determined from striatal and cerebellar tissue, respectively. Specific binding was defined as striatal binding (in cpm)/weight (total) minus cerebellar binding (in cpm)/weight (nonspecific). As previously described, no displaceable binding was present in cerebellum (Byrnes *et al.*, 1993). It is unlikely that binding was affected by the presence of cocaine or its metabolites because the approximate half-life of cocaine in plasma and brain is 0.25 hr in the mouse, and the approximate half-life of benzolyecgonine is 1 hr (Benuck *et al.*, 1987).

Experiment II: Discontinuation after Continuous Infusion

CD-1 and DBA/2J mice were used in this experiment. Osmotic pumps were obtained from Alza (model 2001, Palo Alto, CA). Mice were injected with 40 mg/kg cocaine, and open-field activity was determined over 30 min as described. Twenty-four hours later, osmotic pumps with either 40 mg/kg/day cocaine or saline were implanted subcutaneously with the mice under brief ether anesthesia. After 5 days, the pumps were removed. Seventy-two hours after pump removal, mice were challenged with cocaine (40 mg/kg), and open-field activity was recorded for 30 min.

Dopamine transporter binding. Binding was conducted as described for experiment I at 72 hr after pump removal.

Experiment III: Time Course of Cocaine Discontinuation after Intermittent Dosage

DBA/2J mice were used for this experiment. Cocaine (40 mg/kg/day) and saline were delivered as described for experiment I. Dopamine transporter binding was determined at 1, 3, 7 and 14 days after the last cocaine injection as described.

Data Analysis

Data were analyzed by analysis of variance with Dunnett's or Newman-Keuls test. A value of $P < .05$ was considered significant.

Results

Experiment I: Sensitization by Strain

Open-field activity. All strains of mice exhibited significantly higher levels of activity after acute cocaine injections compared with after saline injections. In addition, all strains showed significantly increased activity when challenged 72 hr after a chronic course of cocaine (sensitization) (fig. 1). BALB/cByJ, DBA/2J, B6AF1/J and C57BL6/J mice demonstrated significant increases in activity when challenged 72 hr after a 20-mg/kg/day chronic course of cocaine. In addition, CD-1, BALB/cByJ, DBA/2J and C57BL6/J mice showed significant increases in activity after a 40-mg/kg/day chronic course.

Dopamine transporter binding. Specific uptake (striatal binding minus cerebellar binding) of [^{3}H]WIN 35,428 was significantly increased in both DBA/2J and B6AF1/J mice when determined 72 hr after a 40-mg/kg/day chronic course of cocaine (fig. 2). Binding in these strains after chronic administration of 20 mg/kg/day cocaine and binding in all other strains at both 20 and 40 mg/kg/day cocaine were similar to that obtained with saline. Nonspecific binding was similar to that of controls for all strains at both doses of cocaine.

Experiment II: Discontinuation after Continuous Infusion

Open-field activity. Activity did not differ between cocaine- and saline-exposed mice in either CD-1 and DBA/2J in response to challenge doses of cocaine at 3 days after discontinuation following 5 days of continuous infusion (fig. 3).

Dopamine transporter binding. Specific uptake (striatal binding minus cerebellar binding) of [^{3}H]WIN 35,428 did not differ in either CD-1 or DBA/2J mice when determined 72 hr after continuous administration of cocaine or saline (fig. 4). Nonspecific binding was similar to that of controls in both continuous and intermittent administration groups.

Experiment III: Time Course of Cocaine Discontinuation

Dopamine transporter binding. After chronic cocaine administration, specific uptake (striatal binding minus cerebellar binding) of [^{3}H]WIN 35,428 was unchanged from that of mice receiving saline at 1 day after discontinuation. However, at 3 days after discontinuation, binding was significantly increased in cocaine-exposed mice. At day 7 after discontinuation, binding had returned to control levels, and at day 14, binding in cocaine-exposed mice was significantly decreased compared with controls (fig. 5). Nonspecific binding was similar to that of controls at each time point.

Discussion

The results of this study confirm the differences among mouse strains in cocaine sensitization, as previously reported (Shuster *et al.*, 1977). Furthermore, these data indicate that strains differ in effects on dopamine transporter binding 3 days after a sensitizing course of cocaine and that changes in binding in different strains do not parallel the development of sensitization. Second, this study indicates that both sensitization and changes in binding are dependent on the schedule of drug administration: in DBA/2J mice, intermittent administration produced sensitization and receptor upregulation, whereas continuous administration produced neither behavioral nor neurochemical effects. Third, receptor alterations in DBA/2J mice after chronic intermittent cocaine administration followed a

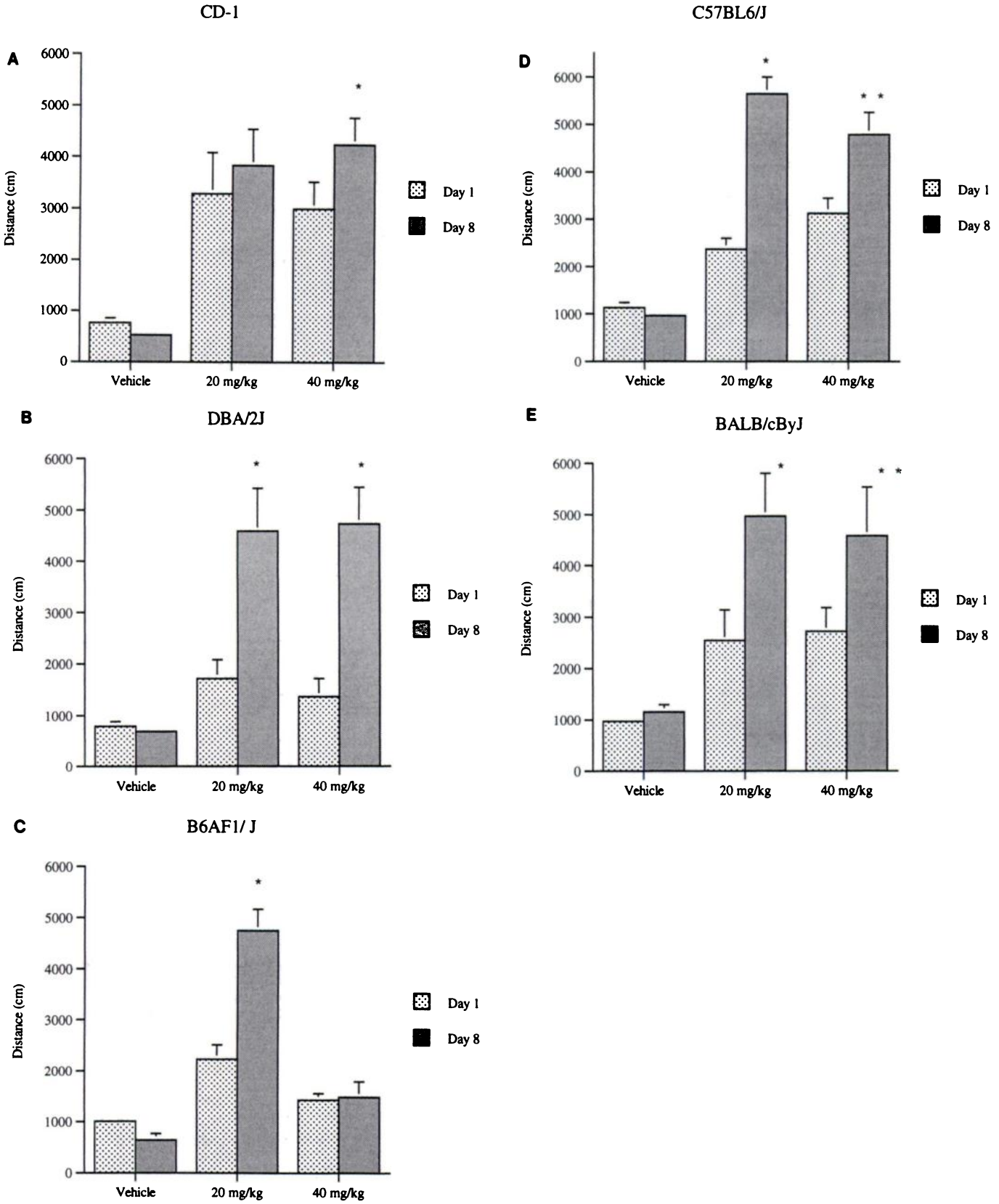

Fig. 1. Open-field activity after chronic cocaine administration (experiment I). Mice were treated for 5 days with injections of either cocaine (20 mg/kg/day or 40 mg/kg/day) or saline (vehicle). Activity was recorded for a 30-min period immediately after the first injection (day 1) and after a challenge dose 3 days after the last injection (day 8). Results are mean + S.E.M.; $n = 7$ to 15 at each point. A, CD-1; B, DBA/2J; C, B6AF1/J; D, C57BL6/J; E, BALB/cByJ. * $P < .01$ *vs.* day 1. ** $P < .05$ *vs.* day 1.

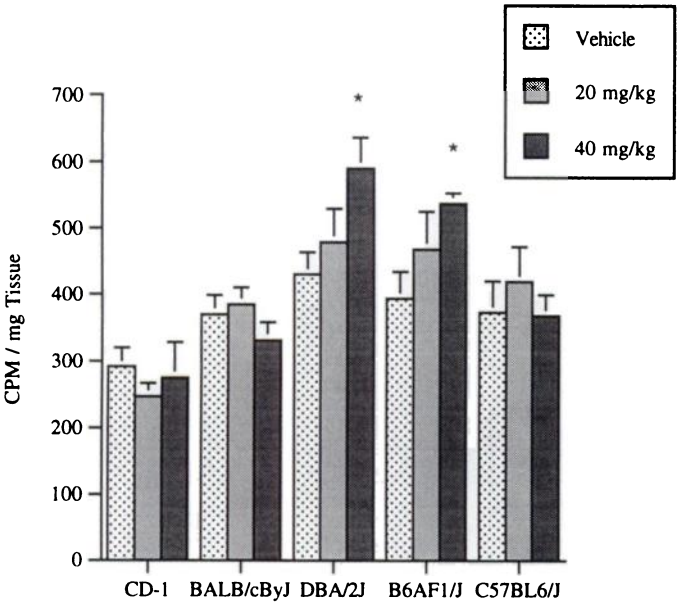

Fig. 2. Dopamine transporter binding after chronic cocaine administration (experiment I) at 3 days after the last injection. Mice were treated for 5 days with injections of either cocaine (20 mg/kg/day or 40 mg/kg/day) or saline (vehicle). Specific uptake of [^{3}H]WIN 35,428 was determined as described in the text. Results are mean + S.E.M.; n = 7 to 10 at each point. * $P < .05$ *vs.* vehicle.

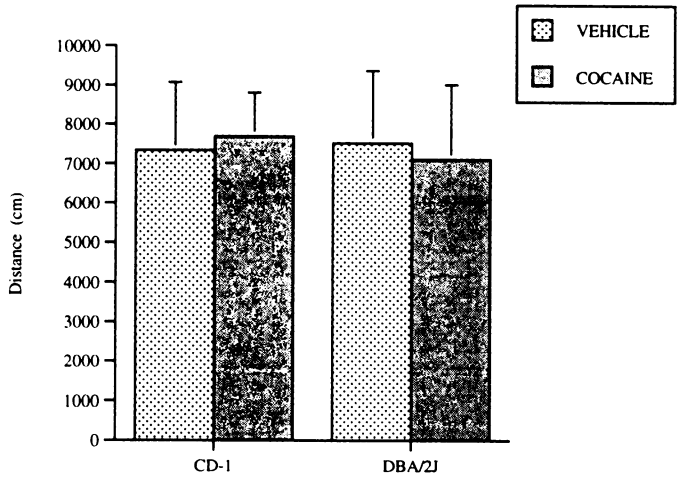

Fig. 3. Open-field activity after continuous infusion of cocaine (experiment II). CD-1 and DBA/2J mice were treated for 5 days with continuous infusion of either cocaine (40 mg/kg/day) or saline (vehicle). Activity was recorded for a 30-min period after cocaine injections (40 mg/kg) 1 day before implantation of osmotic pump and 3 days after pump removal (day 8). Graph represents data for day 8 only. Results are mean + S.E.M.; n = 6 at each point. There were no significant differences between cocaine- and saline-treated groups.

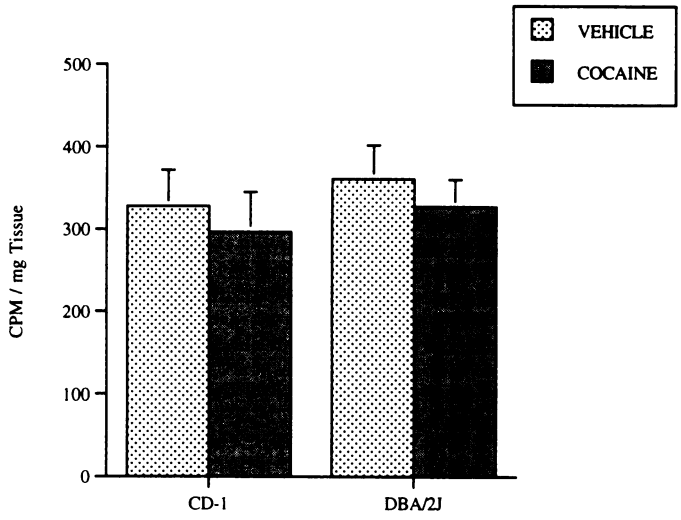

Fig. 4. Dopamine transporter binding after continuous infusion of cocaine (experiment II). CD-1 and DBA/2J mice were treated for 5 days with continuous infusion of either cocaine (40 mg/kg/day) or saline (vehicle). Specific uptake of [^{3}H]WIN 35,428 as described in the text was determined 3 days after osmotic pump removal. Results are mean + S.E.M.; n = 3 to 6 at each point. There were no significant differences between cocaine- and vehicle-treated groups.

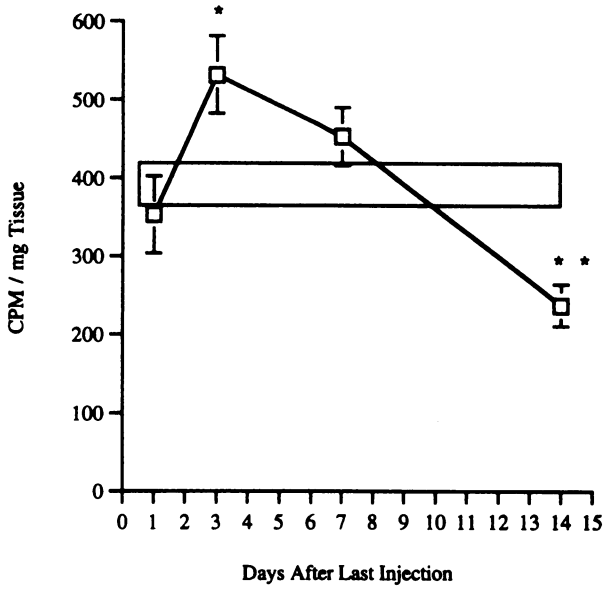

Fig. 5. Time course of dopamine transporter binding after chronic cocaine discontinuation (experiment III). DBA/2J mice were treated for 5 days with injections of either cocaine (40 mg/kg/day) or saline (vehicle). Specific uptake of [^{3}H]WIN 35,428 was determined at 1, 3, 7 and 14 days after the last injection. Results are mean ± S.E.M.; n = 6 to 20 at each point. Open bar represents saline results ± S.E.M.. * $P < .01$ *vs.* day 1 and day 14 and $P < .05$ *vs.* vehicle. ** $P < .05$ *vs.* day 1, day 3 and vehicle.

biphasic pattern characterized by an initial increase in binding, a subsequent return to baseline and an eventual reduction in binding.

With regard to strain differences, at 20 mg/kg/day, DBA/2J mice exhibited the greatest increase in activity with sensitization (167%). C57BL6/J, B6AF1/J and BALB/cByJ mice were sensitized by increases of 138%, 112% and 95%, respectively. In contrast, CD-1 mice did not increase their activity significantly after chronic cocaine exposure. These data are consistent with the report of Shuster *et al.* (1977), which indicated higher levels of activity in C57BL6/J mice compared with in BALB/cByJ mice on the fourth day of a chronic intermittent cocaine course.

At 40 mg/kg/day, DBA/2J mice showed the greatest degree of sensitization, increasing the total distance traveled by 245%. BALB/cByJ, C57BL6/J and CD-1 mice increased their activity by 68%, 53% and 41%, respectively. The relative reductions in sensitization at 40 mg/kg/day compared with 20 mg/kg/day and the lack of apparent sensitization of B6AF1/J mice at the higher dose may be due to excessive stimulation rather than

the lack of a dose-response effect. All mouse strains, and the B6AF1/J strain in particular, exhibited increased stereotypic movements at a dosage of 40 mg/kg/day compared with 20 mg/kg/day, and in the B6AF1/J strain, little locomotor activity appeared to result.

Specific uptake of [^{3}H]WIN 35,428 was significantly increased 3 days after cocaine discontinuation in the DBA/2J and B6AF1/J mice receiving 40 mg/kg/day *vs.* saline. Although DBA/2J mice had greater binding overall, the differences between these strains were not significant. Specific uptake was not significantly different from that for saline at a dosage of 20 mg/kg/day cocaine or in any other strain at either 20 or 40 mg/kg/day. Prior data are conflicting concerning the effects of chronic cocaine on dopamine transporter or related site binding. Hanbauer *et al.* (1984) reported an increased density of [^{3}H]cocaine binding sites in rat striatum 48 hr after discontinuation of 21 days of cocaine. George and Ritz (1990) found that differences in locomotor activity between Long Sleep and Short Sleep mice induced by single injections of cocaine were not accompanied by differences in *in vitro* [^{3}H]mazindol binding in striatum. Reith and Selmeci (1991) reported that although a chronic cocaine course resulted in strain-dependent differences in sensitization, these were not accompanied by differences in *in vitro* [^{3}H]WIN 35,428 binding in striatum. The discrepancies between these results and the results reported in the present study may be due to the use of different mouse strains, doses of cocaine, binding methodologies and intervals after cocaine discontinuation. The *in vivo* binding methods used in the present study avoid potential limitations induced by tissue preparation, buffers and assay conditions (Miller *et al.*, 1987). In addition, as demonstrated in experiment III, the interval between cocaine discontinuation and determination of binding appears to be important: no change was observed at 24 hr after discontinuation, but a significant increase was observed by 72 hr.

Experiment II demonstrated that responses to chronic cocaine varied with the schedule of administration. CD-1 and DBA/2J mice were used in this study based on results from experiment I: both strains showed behavioral sensitization at 40 mg/kg/day, but DBA/2J mice exhibited receptor up-regulation, whereas CD-1 mice did not. In the continuous-infusion paradigm, neither strain became sensitized to cocaine. In addition, neither strain developed receptor up-regulation, in contrast to the results observed with DBA/2J mice receiving intermittent administration. As noted, sensitization was more robust in the DBA/2J mice. These findings corroborate data from Reith *et al.* (1987) as well as King *et al.* (1993), who showed that continuous infusion of cocaine resulted in a syndrome that resembled tolerance more than sensitization. Although these studies present similar findings, it should be noted that Reith *et al.* (1987) treated BALB/cBy mice for 18 days (25 mg/kg/day), whereas King *et al.* (1993) treated Sprague-Dawley rats for 14 days (40 mg/kg/day). Both studies tested for behavioral sensitization on days 1 and 7 of discontinuation.

Finally, results from the discontinuation study performed in experiment III indicate that changes in dopamine transporter binding are related to cocaine discontinuation and follow a course of up-regulation with subsequent down-regulation. One day after discontinuation of chronic cocaine (40 mg/kg/day), dopamine transporter binding did not differ from that of mice receiving saline. However, 3 days after discontinuation, specific binding was significantly elevated. At 7 days after discontinuation, binding was significantly decreased from that of day 3 and was not different from that of mice receiving saline. At 14 days after discontinuation, binding was significantly decreased compared with that of mice receiving saline. These results are consistent with those of Farfel *et al.* (1992), who found decreased *in vitro* [^{3}H]GBR12935 dopamine transporter binding 2 weeks after the last injection of a four-times-a-day, 14-day cocaine course in rhesus monkeys. In addition, Cline *et al.* (1992) demonstrated decreased *in vivo* specific uptake of [^{3}H]WIN 35,428 at 10 days after cocaine discontinuation, and Sharpe *et al.* (1991) reported decreased *in vitro* [^{3}H]mazindol binding in several regions in rats 10 days after discontinuation of 10 days of cocaine.

Thus, chronic intermittent administration of cocaine is associated with behavioral sensitization and dopamine transporter alterations. Our data indicate that it is unlikely that behavioral and receptor effects are directly related because strains such as BALB/cByJ and CD-1 exhibited sensitization but not receptor changes, which is in contrast to the DBA/2J strain in which both effects occurred. However, an indirect relation is suggested by the parallel occurrence of sensitization and increased binding in DBA/2J mice after intermittent cocaine administration and the resolution of these changes in mice treated by chronic infusion. Such a link might be related to dopamine transporter gene expression, although in a recent abstract, Tolliver *et al.* argued against such an effect of chronic cocaine (1993).

It is possible that both sensitization and receptor changes are related to an additional phenomenon, such as a structural change in the dopamine transporter or an effect on another receptor system. Similar discrepancies between binding changes and behavior have been observed in other systems. For example, chronic administration of an "inverse agonist" benzodiazepine compound, compared with an antagonist, leads to receptor up-regulation but not necessarily behavioral hyperactivity (Pritchard *et al.*, 1991). Also in this model, results from behavioral and binding assays vary with intermittent *vs.* continuous infusion paradigms.

Additional explanations for sensitization and receptor changes may be related to changes in postsynaptic D1 and D2 receptors. Mayfield *et al.* (1992) demonstrated that 6 days of cocaine followed by 7 days of discontinuation yielded no differences in striatal or nucleus accumbens D1 receptors as assayed by [^{3}H]SCH 23390 quantitative autoradiography. In contrast, Henry and White (1991), using single-cell electrophysiological and microiontophoretic techniques, found that 14 days of twice-daily cocaine (10 mg/kg) increased the inhibitory response of nucleus accumbens neurons to SKF 38393. D1 receptor sensitivity was increased 7 days and 1 month after final cocaine injection. Peris *et al.* (1990) reported that in rats, an 8-day course of cocaine (10 mg/kg/day) resulted in behavioral sensitization and an increase in postsynaptic D2 receptors in the nucleus accumbens at 24 hr after the final cocaine dose. However, sensitization persisted at 1 week, whereas D2 receptor density had returned to control levels at this time.

In summary, these data indicate that chronic intermittent but not continuous cocaine administration is associated with behavioral sensitization and, in some strains, dopamine transporter upregulation. Receptor changes after intermittent administration are characterized by an initial increase in binding followed by a subsequent decrease. Results in different strains suggest that sensitization and receptor changes are not directly

related. Additional studies evaluating dopamine transporter mRNA may elucidate the mechanisms for alterations in dopamine transporter binding.

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