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Sensitization to Stereotypy in DBA/2J but Not C57BL/6J Mice With Repeated Cocaine

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TOLLIVER, B. K. AND J. M. CARNEY. *Sensitization to stereotypy in DBA/2J but not C57BL/6J mice with repeated cocaine.* PHARMACOL BIOCHEM BEHAV **48**(1) 169–173, 1994.—The present study investigated the effects of seven repeated daily injections of 32 mg/kg cocaine on stereotyped behaviors (repetitive locomotion, rearing, and head bobbing) in two genetically distinct strains of mice. An initial injection of cocaine induced no stereotypy in either DBA/2J or C57BL/6J mice. Following the fourth daily injection, cocaine induced stereotypies in DBA/2J mice (11.33 ± 4.40) compared to saline controls (2.67 ± 1.54). Cocaine-induced stereotypy in DBA/2J mice was further enhanced following the seventh daily injection (19.83 ± 4.39) as compared to saline controls (0.67 ± 0.54). No cocaine-induced stereotypy was observed in C57BL/6J mice following any injection. Eighth day saline challenges of cocaine-sensitized mice did not induce stereotypy. Eighth day cocaine challenges of saline-treated mice induced no stereotypy in either strain. The current study demonstrates that sensitization to cocaine can be influenced by genotype and suggests that genetically defined animals may be useful in elucidating mechanisms underlying sensitization and tolerance to cocaine.

Cocaine Stereotypy DBA/2J C57BL/6J Sensitization Tolerance

INBRED mice provide a unique and powerful tool in characterizing the determinants of responsiveness to drugs of abuse. Inbred strains are the result of at least 20 successive brother–sister matings and consist of genetically identical individuals which are homozygous at all gene loci (5). When environmental factors are held constant, interstrain differences in behavioral and physiological responses to a given drug are attributable to differences in the genotype between strains. Genotype-dependent variation in responsiveness to a number of drugs of abuse, including cocaine, has been reported (3, 4,7). Robust differences in the effects of acute cocaine doses on heart rate (16), monoamine uptake inhibition (2), and locomotor activity (6,19) have been demonstrated across a range of inbred mouse strains. Moreover, adaptive responses to repeated cocaine administration are also under genetic control (17). Recent studies in our laboratory have demonstrated interstrain differences in responsiveness to the locomotor stimulant effects of acute and repeated cocaine in inbred mice (18). For example, an initial injection of 32 mg/kg cocaine stimulates locomotion to a greater degree in DBA/2J mice than in C57BL/6J mice. However, upon repeated daily injections, the locomotor stimulation produced by 32 mg/kg cocaine is diminished to near saline levels in DBA/2J mice, while C57BL/6J mice remain consistently responsive to each of the seven daily cocaine injections. An important consideration in interpreting this interstrain difference in tolerance development is the possible emergence of stereotyped behavior with repeated cocaine injections. While acute moderate doses of cocaine elevate locomotor activity, acute high doses and repeated moderate doses of cocaine result in abnormal, repetitive movements and disrupted locomotion (8). Cocaine-induced stereotypy and cocaine-induced locomotor activity are thought to be mediated by separate neural pathways (9), and may be regulated differently following long-term exposure to cocaine. Sensitization to stereotypy with repeated cocaine administration has been consistently observed in several species including mice (8). Because locomotor stimulation and stereotypy may be mutually incompatible behaviors, the tolerance to locomotor stimulation in DBA/2J mice may, therefore, be attributable to the emergence of stereotypy with

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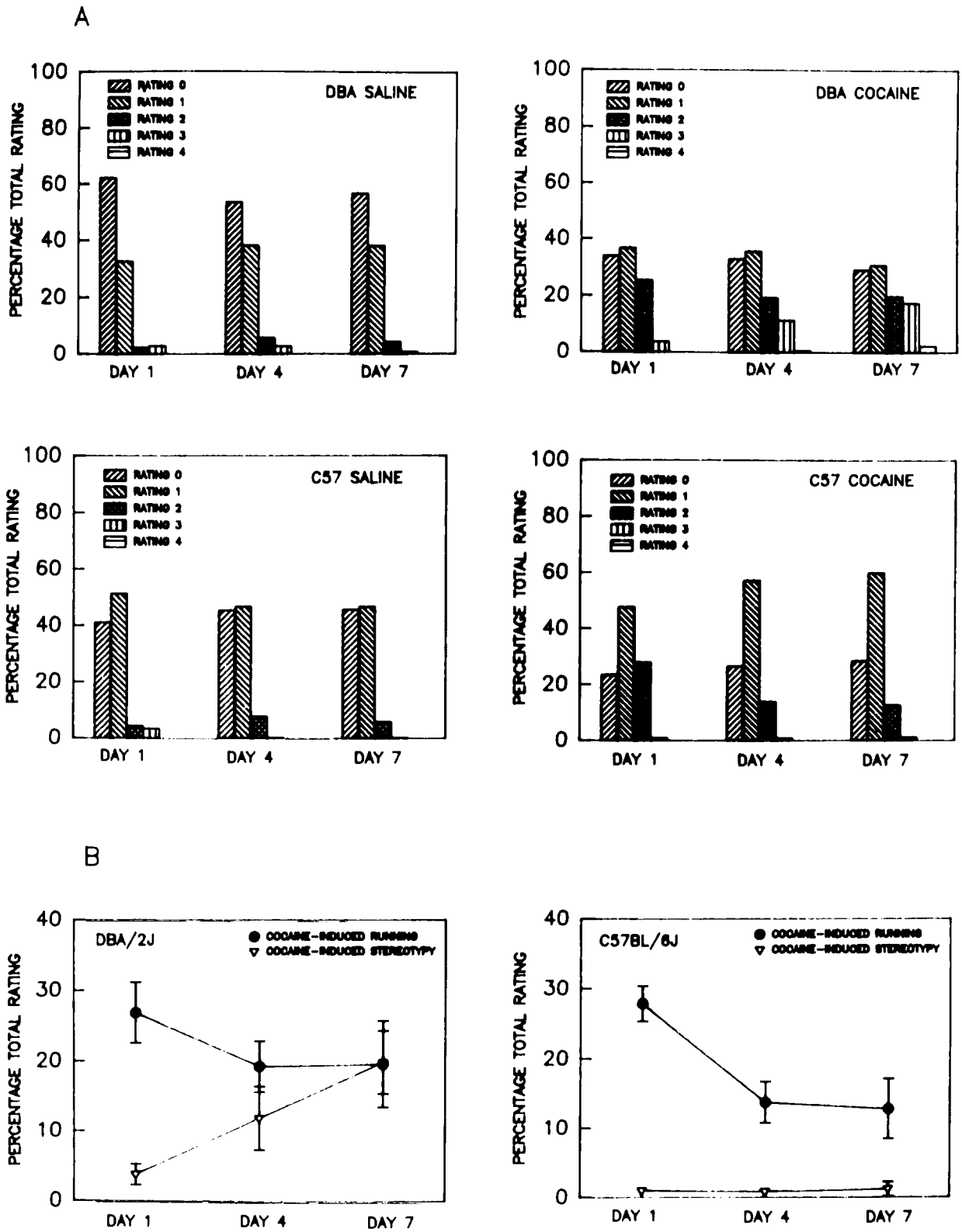

FIG. 1. Relative frequencies of motor behaviors in 10 30 s scoring periods in the 50 min following acute or repeated injections of saline or 32 mg/kg cocaine. (A) Data represent mean (n = 6 each strain per treatment) frequencies of each rating as a percentage of the total behavioral score. Intense stereotypy (rating 4) was observed only in cocaine-treated DBA/2J mice. (B) Changes in cocaine-induced running and stereotypy in DBA/2J and C57BL/6J mice with repeated injections. Data represent mean ± SEM, n = 6 each strain per treatment.

repeated cocaine. The purpose of the present study is to determine whether any interstrain differences exist in the development of sensitization to cocaine-induced stereotypy, which may correlate with the differences in development of locomotor tolerance in DBA/2J and C57BL/6J mice as a result of repeated cocaine administration.

METHOD

Animals and Drugs

Adult male DBA/2J and C57BL/6J mice (22-30 g) were purchased from the Jackson Laboratory (Bar Harbor ME) and housed for at least 1 week on a 12 L : 12 D cycle with food and water available ad lib prior to testing. Mice were housed three per cage throughout the test period.

Cocaine hydrochloride (Sigma, St. Louis, MO) was dissolved in isotonic saline at a concentration of 3.2 mg/ml and was injected IP in a volume of 10 μl per g of b.wt. The final cocaine HCl dosage was 32 mg/kg/day.

Behavioral Scoring

Stereotypy testing was conducted following the first, fourth, and seventh daily injections of saline or 32 mg/kg cocaine in lighted circular locomotor chambers with aluminum grid floors and wire screen covers. The procedure used is a modification of that described previously (10). Each animal was rated by an observer for 30 s periods every 5 min, beginning 10 min before and continuing until 50 min after an injection of saline or 32 mg/kg cocaine (n = 6 each strain per drug treatment). Initial scoring was conducted by an observer not blind to the treatment conditions; subsequent scoring by a second observer blind to the treatment conditions was highly consistent with initial scores. The following rating scale was used: 0—normal quiet behavior, no locomotion, with or without grooming; 1—normal exploratory behavior, slow ambulation, sniffing; 2—rapid locomotion, running, no grooming; 3— stereotyped behavior, repetitive rearing, and locomotion within a more restricted area of cage; 4—intense stereotypy, disrupted locomotion with repetitive gnawing, and head bobbing in a restricted area of cage. Mice were exposed to the chambers only on test days and were returned to the home cages after injections on all other days.

In addition to the test sessions described above, two experiments were conducted to address the possible influence of conditioning factors on the induction of stereotyped behavior by cocaine. In the first experiment, mice from each strain were challenged with saline or cocaine on the eighth day following the 7 day injection regimen described above. All subjects receiving 8 day challenges had previously received seven injections and three test chamber exposures. Mice that had received seven daily injections of saline or 32 mg/kg cocaine were challenged with 32 mg/kg cocaine or saline (n = 3 each strain per drug treatment), respectively, and tested as before. In the second experiment, DBA/2J mice (n = 3) received six daily injections of 32 mg/kg cocaine but received no test chamber exposures after any of these injections. On the seventh day these mice were challenged with 32 mg/kg cocaine and were tested as before.

Data Analysis

Stereotypy data were analyzed using two-way and three-way analyses of variance for between-subject and mixed-factorial experiments as appropriate. Student-Newman-Keuls analysis was used for all post hoc multiple comparisons and interpretation of interactions.

RESULTS

Repeated injections of 32 mg/kg cocaine produced overall changes in motor behavior across the 7 day treatment period, $F(1, 60) = 17.98$, $p < 0.0001$ (Fig. 1). Cocaine induced stereotypy to a greater extent on the seventh day than on the first or fourth days [drug × day interaction: $F(2, 60) = 8.04$, $p < 0.001$]. A main effect of strain, $F(1, 60) = 27.09$, $p < 0.0001$, and a significant strain × drug, $F(1, 60) = 21.12$, $p < 0.0001$, interaction were also found, with DBA/2J mice much more susceptible to cocaine-induced stereotypy than C57BL/6J mice across the 7 day treatment period (Fig. 2). In addition, interstrain differences were found in the development of sensitization to cocaine-induced stereotypy with repeated injections [strain × drug × day interaction: $F(2, 60) = 3.88$, $p < 0.03$]. When analyzed by strain, a main effect of cocaine, $F(1, 30) = 21.69$, $p < 0.0001$, and a drug × day interaction, $F(2, 30) = 6.33$, $p < 0.01$, were present in DBA/2J mice. Cocaine did not induce stereotypy at any day in C57BL/6J mice [overall $F(5, 30) = 2.33$, $p = 0.067$]. Saline had no effects at any day in either strain, $F(5, 30) = 1.75$, $p = 0.154$.

The emergence of stereotyped behavior in DBA/2J mice with repeated injections is cocaine dependent and is not explained solely by conditioning factors (Table 1). Cocaine does not induce stereotypy in mice of either strain that have received seven daily saline injections, $F(3, 14) = 2.10$, $p = 0.146$, not significantly different from cocaine effects in naive mice. An 8th day challenge injection of saline does not induce stereotypy in mice of either strain that have received seven daily injections of 32 mg/kg cocaine, $F(3, 14) = 1.19$, $p = 0.348$, not significantly different from saline effects in naive mice. Furthermore, pairing of the cocaine injection with the test chamber in which stereotypy is measured is not necessary for the emergence of stereotypy. DBA/2J mice receiving repeated injections of 32 mg/kg cocaine but no previous test chamber exposures exhibited stereotypy upon a seventh injection of cocaine (Table 1).

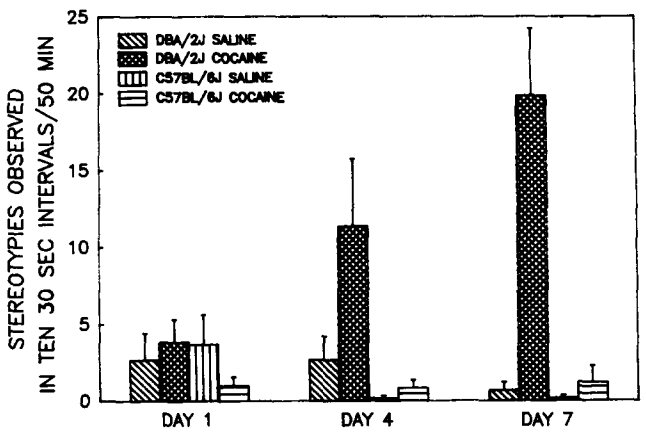

FIG. 2. Frequency of stereotyped behaviors in DBA/2J and C57BL/6J mice following repeated injections of saline or 32 mg/kg cocaine. Data are presented as mean ± SEM, n = 6 each strain per treatment. Significant main effect of strain, $F(1, 60) = 27.09$, $p < 0.0001$, and significant strain × day interaction, $F(2, 60) = 5.47$, $p < 0.01$, are present as determined by three-way mixed factorial ANOVA.

DISCUSSION

Both sensitization and tolerance have been reported to develop to cocaine-induced stereotypy (13) and locomotor stimulation (15). Conditioning factors (12), dose (14), and schedule of administration (11) are all known to influence sensitization and tolerance to cocaine. The present study demonstrates that genotype is another important determinant of the adaptive responses to repeated cocaine. We have recently shown that significant tolerance to the locomotor stimulant effects of cocaine develops in DBA/2J but not C57BL/6J mice with repeated daily injections (18). As determined in automated photocell testing chambers, cocaine-induced locomotion is decreased almost 300% in DBA/2J mice over the 7 day testing period. Because these locomotor activity experiments were conducted in darkened opaque testing chambers, it was not possible to determine whether stereotypy and disrupted locomotion began to emerge with repeated injections.

The current results demonstrate that upon repeated administration, decreases in cocaine-induced running per se in DBA/2J mice, while present, are relatively small (Fig. 1). Rapid locomotion in cocaine-treated mice decreased only from 25% total rating on day 1 to 19% total rating on days 4 and 7, and normal exploration decreased only from 36% total rating to 30% total rating over this period. It is unlikely then that the tolerance we reported previously is attributable only to decreases in cocaine-induced running with repeated injections; this "tolerance" may actually be the result of sensitization to stereotyped behavior in DBA/2J mice. Stereotypy is induced in DBA/2J mice by the fourth day of repeated daily injections of 32 mg/kg cocaine (Fig. 1). Stereotypy was significantly increased in frequency and intensity in DBA/2J mice by the seventh daily injection, with some intense (rating 4) stereotypy beginning to emerge. The time course of this sensitization to cocaine-induced stereotypy is consistent with the previously reported time course for the development of tolerance to locomotor stimulation in DBA/2J mice (18).

In C57BL/6J mice, which did not develop locomotor tolerance to 32 mg/kg cocaine as measured in the previous study, no cocaine-induced stereotypy was observed during any test session (Fig. 2). The decrease in cocaine-induced running, from 28% total rating on day 1 to 12% total rating on day 7, was offset by a concomitant increase in exploratory locomotion, from 47% total rating on day 1 to 60% total rating on day 7, in C57BL/6J mice with repeated cocaine. These data indicate that cocaine-induced running can decrease upon repeated injections without the emergence of stereotypy in C57BL/6J mice, and support the idea that cocaine-induced locomotor stimulation and stereotypy may be regulated separately. Altogether, the results in Figs. 1 and 2 confirm that the adaptive responses to repeated cocaine are under genetic control and suggest that DBA/2J and C57BL/6J mice may be useful in identifying the mechanisms underlying sensitization and tolerance to cocaine.

Because only one dose of cocaine was used for all pretreatment and challenge injections, the dose dependency of sensitization to stereotypy cannot be determined from the present study. Consequently, the possibility that C57BL/6J mice become sensitized to cocaine-induced stereotypy with repeated injections of a higher dose of cocaine cannot be ruled out. In fact, previous studies in our laboratory indicate that some locomotor tolerance develops in C57BL/6J mice with repeated daily injections of 56 mg/kg cocaine (18). Whether this tolerance is attributable to the emergence of stereotypy at the higher dose is unknown. Similarly, it is unknown whether doses of cocaine lower than 32 mg/kg produce sensitization to stereotypy in either strain when administered repeatedly. However, locomotor responses to 1.0, 3.2, and 10.0 mg/kg doses of cocaine were not altered with repeated daily injections of the same dose in either strain (18). The potential importance of dose in the development of cocaine-induced stereotypy in the two strains may be addressed in the future by employing a range of doses for both pretreatment and challenge injections.

Our previous studies of cocaine-induced locomotor activity in the two strains have suggested that conditioning is an important variable in the development of locomotor tolerance with repeated cocaine. Environmental context of cocaine exposure is also known to influence sensitization to stereotypy in rats (12). In the present study we evaluated the possible

TABLE 1

ROLE OF CONDITIONING AND ENVIRONMENTAL CONTEXT ON COCAINE-INDUCED STEREOTYPY IN DBA/2J AND C57BL/6J MICE

		Stereotypies Observed	
Pretreatment	Challenge	DBA/2J	C57BL/6J
Naive	Saline (6)	2.67 ± 1.74	3.67 ± 1.93
Cocaine × 7	Saline (3)	0.00 ± 0.00*	0.00 ± 0.00*
Naive	Cocaine (6)	3.83 ± 1.48	1.00 ± 0.57
Saline × 7	Cocaine (3)	5.67 ± 4.02†	1.00 ± 1.22†
Cocaine × 6, test chamber × 2	Cocaine (6)	19.83 ± 4.39	1.20 ± 1.08
Cocaine × 6, home cage	Cocaine (3)	11.00 ± 6.04	

Effects of repeated cocaine or saline on cocaine-induced stereotypy. All cocaine pretreatment and challenge injections were given at a dose of 32 mg/kg. All mice challenged on the eighth day previously received seven injections and three test chamber exposures. Naive and home cage mice were not previously exposed to test chambers. Values are mean ± SEM for the number of animals listed in parentheses.

*Not different from saline effects in naive mice, $F(3, 14) = 1.19, p = 0.348$.

†Not different from cocaine effects in naive mice, $F(3, 14) = 2.10, p = 0.146$.

role of environment and conditioning on the emergence of stereotypy with repeated cocaine. Sensitization to stereotypy in DBA/2J mice was dependent on the history of cocaine dosing in that mice treated with seven daily injections of saline did not exhibit stereotypy upon an eighth day challenge with 32 mg/kg cocaine. Additionally, sensitization to stereotypy is not simply a function of conditioning because DBA/2J mice previously shown to be sensitized to cocaine (i.e., those that exhibited stereotypy on day 7 of repeated daily cocaine injections) did not exhibit stereotypy after an injection of saline on day 8. We have recently found that in DBA/2J mice that have received six daily injections of 32 mg/kg cocaine paired with six test chamber exposures, a cocaine challenge on the seventh day elicits only 28% of the locomotor activity elicited by an initial injection. In contrast, in DBA/2J mice that have received six daily home-cage cocaine injections with no test chamber exposures, a cocaine challenge induces 80% of the locomotor activity induced by an initial injection (18). If locomotor tolerance in DBA/2J mice is wholly attributable to the emergence of stereotypy, then stereotypy should be greatly reduced or absent in cocaine-treated mice that are naive to the test chamber. However, chamber-naive DBA/2J mice that have received six daily injections of 32 mg/kg cocaine do exhibit stereotyped behavior upon a seventh day cocaine challenge (Table 1), with intermediate frequency between drug/chamber-naive mice and drug/chamber-exposed mice. Thus, the emergence of stereotypy with repeated injections does not appear to be as dependent upon environmental context as does the development of locomotor tolerance. Although further study is warranted, this discrepancy suggests that the previously reported locomotor tolerance in DBA/2J mice is not solely a result of sensitization to stereotypy.

The present study further characterizes the significant differences that exist in the behavioral responsiveness to repeated cocaine in the genetically distinct DBA/2J and C57BL/6J strains of mice. Previous studies in our laboratory suggest that these differences are not attributable to pharmacokinetic factors because brain cocaine levels following a single injection or the last of seven daily injections are not different between strains. These results imply that interstrain differences in CNS sensitivity to cocaine exist in DBA/2J and C57BL/6J mice. The number of genes that may contribute to these differences in unknown. It has been suggested, on the basis of experiments with other inbred strains, that the genetic determinants for cocaine-induced running and cocaine sensitization are likely to segregate independently (17). A potential tool for investigating the genetic determinants of cocaine responsiveness is the extensive B × D recombinant inbred series developed from the DBA/2J and C57BL/6J progenitor strains (1). This series consists of 24 lines with over 400 marker loci mapped, and may allow determination of single or multiple gene loci that associate with these traits of cocaine sensitivity. Future experiments using the B × D RI lines in conjunction with the progenitor strains may suggest candidate genes as potential determinants of sensitization and tolerance to repeated cocaine.

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