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Simultaneous Monitoring of Conditioned Place Preference and Locomotor Sensitization Following Repeated Administration of Cocaine and Methamphetamine

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SHIMOSATO, K. AND S. OHKUMA. *Simultaneous monitoring of conditioned place preference and locomotor sensitization following repeated administration of cocaine and methamphetamine.* PHARMACOL BIOCHEM BEHAV **66**(2) 285–292, 2000.—The paradigm of conditioned place preference has been widely used to demonstrate the rewarding properties of psychomotor stimulants. Such drugs also stimulate locomotor activity. Repeated administration of low doses of psychomotor stimulants causes progressive increases in the locomotor stimulating effect, a phenomenon termed behavioral sensitization. Using a new activity monitor (SCANET MV-10LD) that simultaneously measures the amount of time spent and the distance traveled in each side of a two-compartment chamber, the present study assessed place preference conditioning and locomotor sensitization following repeated administration of cocaine or methamphetamine (MAP) in mice. We examined the effect of environmental factors on these activities using two different types of chamber: one having a single cue, and the other having dual cues for the discrimination of compartments. In both types of chamber, cocaine (5–20 mg/kg) and MAP (1–2 mg/kg) similarly produced conditioned place preference. However, repeated cocaine administration caused the development of locomotor sensitization only in the single-cue chamber. On the other hand, repeated administration of MAP resulted in the development of sensitization in both types of chamber. The findings indicate that environmental factors differentially affect the development of locomotor sensitization, but not place preference conditioning, following repeated administration of cocaine or methamphetamine. The advantages of this new system will be discussed. © 2000 Elsevier Science Inc.

Conditioned place preference Locomotor activity Behavioral sensitization Environmental factor Cocaine Methamphetamine

A WIDE variety of addictive drugs have rewarding properties in humans and animals. The paradigm of place preference conditioning has been widely used to examine the rewarding properties of psychomotor stimulants and opiates in animals (3,7). Addictive drugs also produce a locomotor stimulating effect. It has been proposed that both locomotor stimulation and the rewarding effects of psychomotor stimulants are associated with the activation of the mesolimbic dopaminergic mechanism, originating from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) (6,34). Nonetheless, the previous paradigm for conditioned place preference (CPP) did not measure locomotor activity during the conditioning and test sessions. Recently, a few computerized systems have successfully monitored both place preference conditioning and locomotor activity during the conditioning and test sessions (4,17,19).

Repeated administration of low doses of psychostimulants results in progressive increases in the locomotor stimulating effect produced by a subsequent dose of the drug; the phenomenon is referred to as behavioral sensitization. Not only behavioral sensitization but sensitization to the rewarding properties of addictive drugs have been proposed to develop following repeated administration of the drug (25). Animals repeatedly pretreated with cocaine or amphetamines showed increased self-administration of the drug, as well as behavioral sensitization (8,21,35). Prior exposure to cocaine, amphetamine, or morphine was found to increase the rewarding

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effect of the drug as measured by a CPP method (16,28,29). Given that the neural basis of sensitization to both locomotor stimulation and the rewarding properties of psychomotor stimulants lies in the mesolimbic dopaminergic mechanism (25), it is expected that CPP may proceed as the behavioral sensitization develops following repeated administration of psychomotor stimulants or opiates. Thus far, however, reports have been contradictory as to whether CPP and behavioral sensitization concurrently develop following repeated drug administration (17) or not (4,19).

The aim of this report is to describe a new method that concurrently determines the development of CPP and behavioral sensitization following repeated administration of cocaine or methamphetamine (MAP) in mice. The present study examined the effect of environmental factors on these behavioral phenomena using two different types of CPP chamber, having single or dual cues for the discrimination of compartments. The results obtained suggest that the mechanisms underlying behavioral sensitization are probably separable from those underlying the sensitization to the rewarding properties following repeated administration of the psychomotor stimulants. In addition, we will outline the advantages of this new methodology.

METHOD

Animals

Male Slc:ddY mice (Japan SLC, Inc., Hamamatsu, Shizuoka) at the age of 5 weeks were acclimated to the animal facilities for at least 10 days before starting the experiment. Mice were maintained on a constant light–dark cycle (illumination 0700–2100 h), with food and water freely available except during behavioral sessions. Subjects were randomly assigned to each treatment group. Behavioral sessions were conducted 5 days per week between 1000 and 1700 h. Prior to behavioral sessions, animals were placed in a quiet, air-conditioned room for at least 60 min. The experimental protocol was approved by the Animal Reseach Committee of the Kawasaki Medical School; the procedures were compliance with the Guidelines on the Care and Use of Laboratory Animals issued by the Kawasaki Medical School.

Apparatus

An activity monitor (SCANET MV-10LD, Toyo Sangyo Co. Ltd., Nakaniikawa-Gun, Toyama), equipped with 72 × 72 infrared photosensor systems, concurrently measured the distance traveled and the amount of time spent in each side of an acrylic two-compartment chamber. The chamber (40 × 30 × 30 cm), consisting of transparent walls, was divided into two equal-sized compartments by a black partition that was penetrable by infrared light. This study used two types of partition; one had a passageway (10 × 5 cm) to allow a mouse to move into either compartment, and the other did not, to restrain animal's movement to one of the two compartments. Behavioral sessions were conducted in two different types of chamber; single- and dual-cue chambers. In the single-cue chamber, animals distinguished the two compartments by floor color: one was black and the other was white. Both compartments had black ceilings. In the dual-cue chamber, animals discriminated the two compartments by the difference in floor and ceiling color. The ceiling of the black-floored room was painted white and the ceiling of the white-floored room was black. The light intensity on the floor of the single- and dual-cue chambers was 76–86 and 73–90 lx, respectively.

Place Preference Conditioning

Subjects were randomly assigned to each group with respect to stimulant dose, type of CPP chamber, and compartment pairing. In a preconditioning session, animals were given free access to both compartments of a chambers for 15 min. With the activity monitors, locomotor activity (distance traveled) and the amount of time spent in each compartment were determined during the preconditioning session. Table 1 summarizes the amounts of time spent (in seconds) in the white-floored compartment during the preconditioning session for each group.

During conditioning sessions, mice were intraperitoneally (IP) injected once a day with either, 5, 10, or 20 mg/kg cocaine HCl or 1 or 2 mg/kg methamphetamine (MAP) HCl. Immediately after cocaine and MAP injection, animals were confined in one of the two compartments for 30 and 60 min, respectively. On alternate days, the groups of mice were injected with saline and confined in the other compartment of the chamber. Animals received drug or saline injections four times each during the conditioning sessions. Locomotor activity was measured in each session. Mice that received only saline were used as controls.

The CPP test was conducted the day after the last conditioning session. Mice, initially placed in the saline-paired compartment, were allowed free access to both compartments for 15 min, during which the amount of time spent in each compartment was measured. The CPP score was determined by the difference between the amounts of time spent in the drug-paired compartment before and after the conditioning sessions.

Behavioral Sensitization

During each conditioning session, locomotor activity was measured for 30 and 60 min after administration of cocaine and MAP, respectively. The development of behavioral sensitization was assessed by progressive increases in locomotor activity across the conditioning sessions. Seven days after the place preference test, cocaine- and MAP-treated groups and their controls were challenged with IP injection of 10 mg/kg

TABLE 1

THE AMOUNTS OF TIME (SECONDS; ± SEM) SPENT IN THE WHITE-FLOORED COMPARTMENT DURING THE PRECONDITIONING SESSION FOR EACH TREATMENT GROUP

Drug	Dose (mg/kg)	Paired w/Black-Floored Compartment		Paired w/White-Floored Compartment	
		Single-Cue	Dual-Cue	Single-Cue	Dual-Cue
Cocaine	0	359 ± 28	435 ± 26	399 ± 26	452 ± 38
	5	444 ± 8	449 ± 37	397 ± 25	435 ± 26
	10	413 ± 36*	493 ± 18	400 ± 35	463 ± 26
	20	395 ± 27†	532 ± 33	414 ± 39	467 ± 36
MAP	0	399 ± 18	398 ± 29	404 ± 15†	469 ± 11
	1	414 ± 16	453 ± 36	375 ± 36	394 ± 21
	2	375 ± 27*	464 ± 23	393 ± 25	416 ± 27

Three-way ANOVA demonstrated a significant effect of chamber type for cocaine- and methamphetamine (MAP)-treatment groups, $F(1, 130) = 16.16$, $p < 0.001$, and $F(1, 84) = 7.35$, $p < 0.01$, respectively. On the other hand, there was no significant effect of dose, paired compartment, or interaction among the three factors for either treatment group. * : $p < 0.05$ and †0.01, respectively, vs. dual-cue (post hoc comparison with Scheffe's *S*-test).

cocaine or 1 mg/kg MAP and placed in the drug-paired compartment for 30 and 60 min, respectively. During these intervals, the levels of drug-induced locomotion were determined as an index for the expression of behavioral sensitization.

To examine whether behavioral sensitization was dependent upon associative learning or nonassociative processes, other groups of mice were conditioned with either 20 mg/kg cocaine, 2 mg/kg MAP, or saline in the single- and dual-cue chambers as described above. Six days after CPP test, animals in each group were challenged with saline and placed in the drug-paired compartment for 30 or 60 min, dependent upon cocaine- or MAP-treatment, respectively. Locomotor activity elicited by the saline challenge was used as an index of conditioned locomotor activity. On the following day, drug- and saline-treated mice received a challenge injection of 10 mg/kg cocaine or 1 mg/kg MAP dependent upon the treatment regimen. Subsequently, half the animals in each group were placed in the drug-paired compartment, and the other half of them in the saline-paired compartment for 30 or 60 min, during which locomotor activity was measured.

Statistical Analysis

Statistical analyses of the data for the amounts of time spent in the white-floored compartment during the preconditioning session and the data for the CPP scores were conducted using three- and two-way analysis of variance (ANOVA), respectively, followed by post hoc comparison with Scheffe's *S*-test. Locomotor activity across the conditioning sessions was statistically analyzed using repeated-measures ANOVA with dose as a between factor and session day as a within factor. When session effect and session × dose interaction were significant, further analysis was conducted on each dose group with session day as a within factor. Locomotor activity elicited by the saline and drug challenge was analyzed with two- or three-way ANOVA. Post hoc comparison among the means was conducted with Scheffe's S-test. Linear correlation between the CPP score and locomotor activity elicited by the stimulant challenge was analyzed with simple regression test using the data from the drug-treated groups.

RESULTS

Conditioned Place Preference

The conditioning treatments with cocaine induced CPP for the drug-paired compartment (Fig. 1a), while there was no effect of chamber type. A significance of difference in the CPP score was confirmed by ANOVA with a significant main effect of cocaine dose, $F(3, 138) = 13.59$, $p < 0.0001$, but not chamber type, $F(1, 138) = 0.06$, $p > 0.05$, NS. No interaction between cocaine dose and chamber type was observed, $F(3, 138) = 1.17$, NS. The conditioning treatments also significantly increased the CPP score in the MAP-treated group (Fig. 1b), $F(2, 90) = 41.17$, $p < 0.0001$; two-way ANOVA, while the type of chamber had no effect on it. There was no interaction between MAP dose and chamber type.

Cocaine-Induced Behavioral Sensitization

In the single-cue chamber, cocaine dose-dependently increased locomotor activity (Fig. 2a), as indicated by a significant main effect of cocaine dose, $F(3, 60) = 51.15$, $p < 0.0001$, repeated measures ANOVA. Repeated administration of cocaine resulted in progressive increases in locomotor activity across the conditioning sessions. This was confirmed by the ANOVA with a significant session effect, $F(3, 180) = 7.46$, $p < 0.0001$, and an interaction between session and dose, $F(9, 180) = 3.32$, $p < 0.001$. When compared with locomotor activity at the first session, 10 mg/kg cocaine produced a significantly higher locomotor activity at the fourth session, as indicated by a significant session effect $F(3, 45) = 4.88$, $p < 0.01$, repeated measures ANOVA, followed by post hoc comparison ($p < 0.05$). Also, 20 mg/kg cocaine produced significantly higher locomotor activity at the third and fourth sessions than that at the first session, as shown by a significant session effect, $F(3, 45) = 5.36$, $p < 0.01$, followed by post hoc comparison ($p < 0.05$).

In the dual-cue chamber, cocaine produced dose-dependent increases in locomotor activity (Fig. 2b), as indicated by a significant main effect of cocaine dose, $F(3, 78) = 13.57$, $p < 0.0001$, repeated-measures ANOVA. However, repeated injections of cocaine failed to cause a progressive increase in locomotor activity across the conditioning sessions. This was

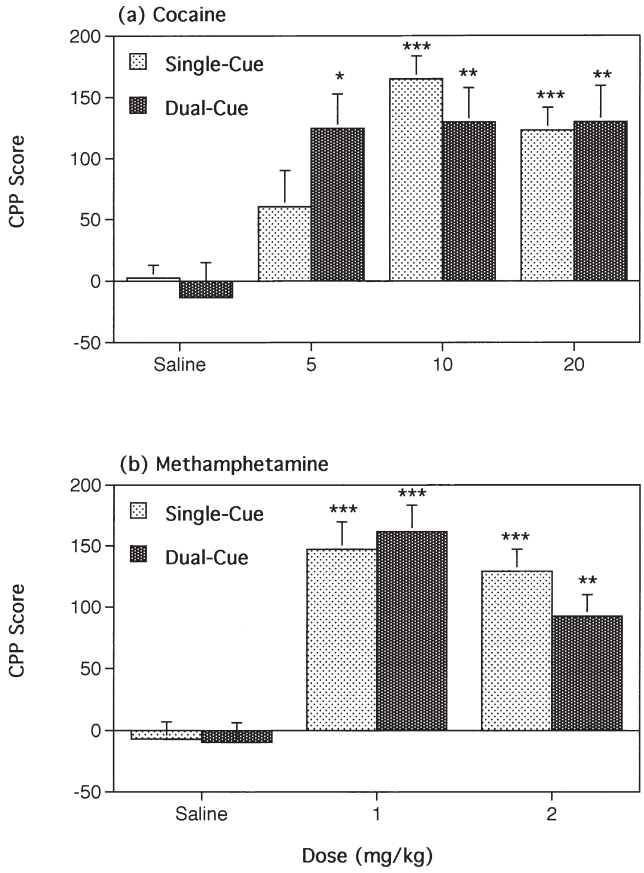

FIG. 1. Conditioned place preference following repeated administration of cocaine (a) or methamphetamine (MAP; b) in mice. Animals were conditioned with either cocaine (0, 5, 10, or 20 mg/kg) or MAP (0, 1 or 2 mg/kg) within one of two compartments of the single-cue and dual-cue chambers four times each. Control injections were given with saline in the other compartment on alternate days. Two-way ANOVA revealed significant main effects of cocaine, $F(3, 138) = 13.59$, $p < 0.0001$, and MAP, $F(2, 90) = 41.1$, $p < 0.0001$, but neither effect of chamber type nor interaction between treatment and chamber type. The numbers of animals were 16 or 22 in each cocaine-treated group, and 16 in each MAP-treated group. Asterisks *, **, and *** denote significant differences in the CPP score compared to that in the respective control groups ($p < 0.05$, 0.01, and 0.001, respectively; Scheffe's *S*-test).

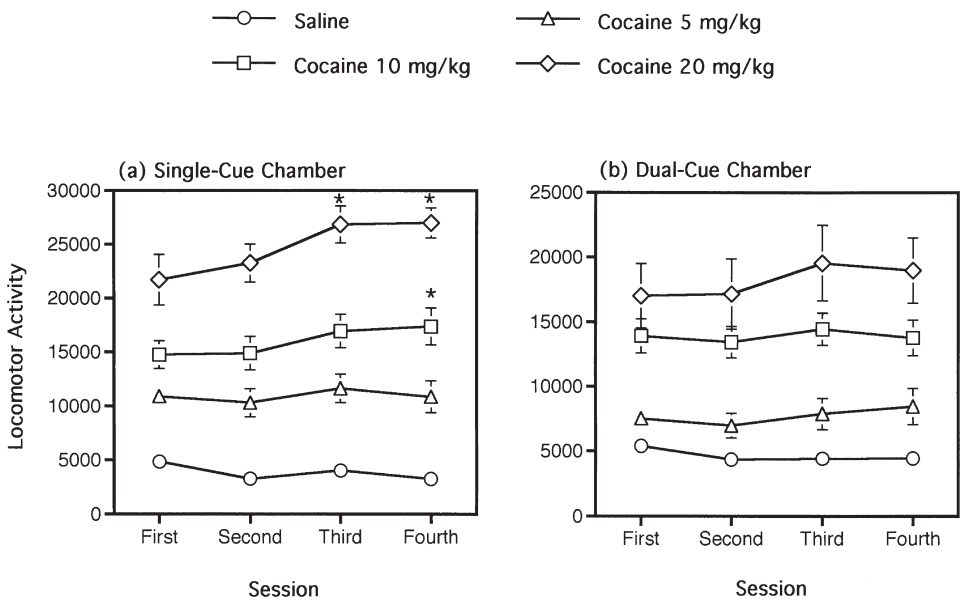

FIG. 2. The development of behavioral sensitization to 10 and 20 mg/kg, but not 5 mg/kg of cocaine across the conditioning sessions in the single-cue chamber (a). The same treatment at any dose failed to induce the sensitization in dual-cue chambers (b). Repeated measures ANOVA for the single-cue groups revealed significant main effects of cocaine dose, $F(3, 60) = 51.15$, $p < 0.0001$, and session, $F(3, 180) = 7.46$, $p < 0.0001$, as well as an interaction between them, $F(9, 180) = 3.32$, $p < 0.001$. Each asterisk denotes a significant difference in locomotor activity compared to that at the first session in the same treatment group ($p < 0.05$; Scheffe's S-test).

confirmed by ANOVA with neither session effect, $F(3, 234) = 2.03$, NS, nor session × dose interaction, $F(9, 234) = 1.00$, NS.

Seven days after the CPP test, the expression of behavioral sensitization was examined by the challenge injection of 10 mg/kg cocaine. Locomotor activity after the cocaine challenge in the single-cue chamber was significantly higher in the cocaine-treated group than in the controls, while there was no difference among the groups in the dual-cue chamber (Fig. 3). This was confirmed by two-way ANOVA with significant main effects of chamber type, $F(1, 138) = 5.25$, $p < 0.05$, and treatment, $F(3, 138) = 12.81$, $p < 0.0001$, and significant interaction between them, $F(3, 138) = 4.36$, $p < 0.01$. Post hoc analysis conducted on the groups in the single-cue chamber demonstrated higher locomotor activity in all of the cocaine-treated groups compared to the control, $F(3, 60) = 15.34$, $p < 0.0001$, one-way ANOVA; $p < 0.01$, 0.001, and 0.0001 for 5, 10, and 20 mg/kg cocaine-treated groups, respectively. Simple regression tests revealed no significant linear correlation between the CPP score and locomotor activity elicited by the cocaine challenge in the single-cue chamber ($r = 0.18$, NS). Further, the simple regression test, conducted on each dose, observed no correlation between the two parameters.

Methamphetamine-Induced Behavioral Sensitization

MAP dose dependently increased locomotor activity in the single- and dual-cue chambers (Fig. 4), as shown by repeated-measures ANOVA with significant main effect of MAP dose, $F(2, 45) = 115.39$ and 91.60 for single- and dual-cue chamber, respectively, $p < 0.0001$. With repeated administration, animals showed progressive increases in locomotor activity across the conditioning sessions. This was confirmed by the ANOVA with significant session effect, $F(3, 135) = 18.99$ and 29.70 for single- and dual-cue chambers, respectively, $p < 0.0001$, and interaction between session and dose, $F(6, 135) = 7.46$ and 9.43 for single- and dual-cue chambers, respectively, $p < 0.0001$. Post hoc comparison noted significant increases in locomotor activity at the third and fourth sessions compared to that at the first session in both the single- and dual-cue chambers; the levels of significance are represented by asterisks in Fig. 4.

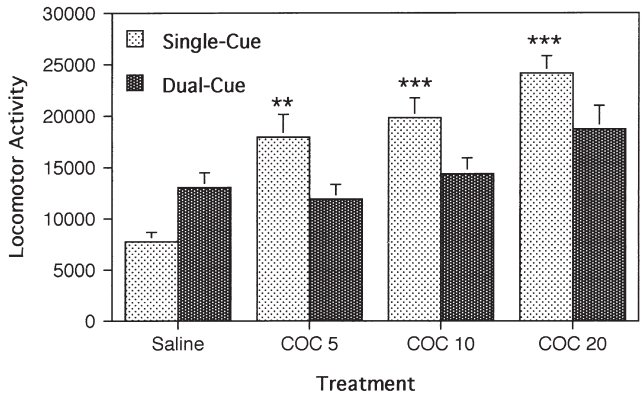

FIG. 3. The expression of behavioral sensitization elicited by the challenge injection with 10 mg/kg cocaine in the single-cue chamber (a), but not in the dual-cue chamber (b). Two-way ANOVA revealed significant main effects of chamber type, $F(1, 138) = 5.25$, $p < 0.05$, and treatment, $F(3, 138) = 12.81$, $p < 0.0001$, as well as an interaction between them, $F(3, 138) = 4.36$, $p < 0.01$. Asterisks ** and *** denote significant differences in locomotor activity compared to that in the control group ($p < 0.01$, and 0.001, respectively; Schefe's S-test).

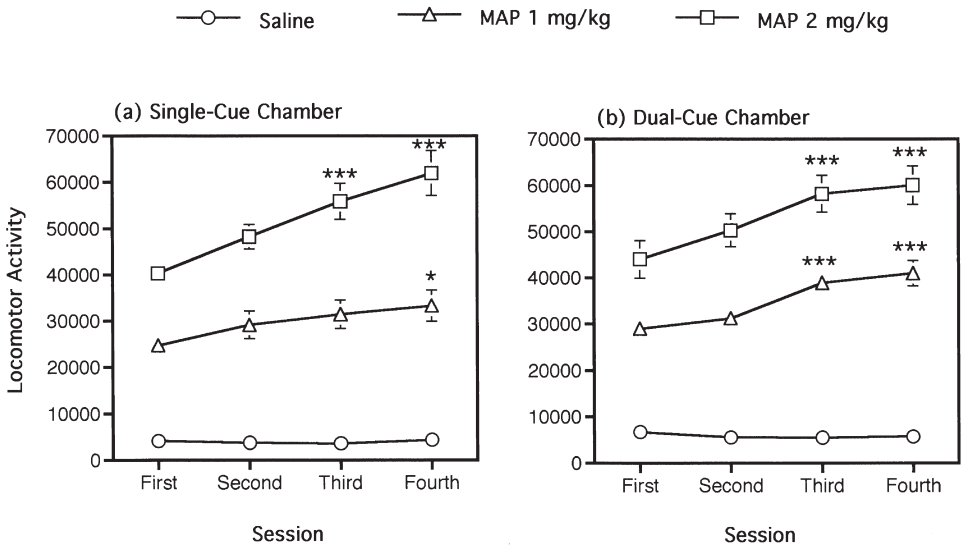

FIG. 4. The development of behavioral sensitization to 1 and 2 mg/kg of methamphetamine (MAP) across the conditioning sessions in the single-cue (a) and dual-cue chambers (b). Repeated-measures ANOVA revealed significant main effects of MAP dose, $F(2, 45) = 115.39$ and 91.60, $p < 0.0001$, and session, $F(3, 135) = 18.99$ and 29.70, $p < 0.0001$, as well as an interaction between them, $F(6, 135) = 7.46$ and 9.43, $p < 0.001$, for the single-cue and dual-cue groups, respectively. Asterisks * and *** denote significant differences in locomotor activity compared to that at the first session in the same treatment group ($p < 0.05$ and 0.001, respectively; Scheffe's S-test).

Seven days after the CPP test the expression of behavioral sensitization was examined by a challenge injection of 1 mg/kg MAP. The challenge injection produced significantly higher locomotor activity in the MAP-treated groups than in the control group, while chamber type had no effect of (Fig. 5). This was confirmed by two-way ANOVA with a significant main effect of treatment, $F(2, 90) = 42.99$, $p < 0.0001$, but neither effect of chamber type nor interaction between them. Simple regression tests revealed no significant linear correlation between the CPP score and locomotor activity elicited by the MAP challenge in the single- and dual-cue chambers ($r = 0.26$, and -0.29, respectively, NS). Further, the simple regression test, conducted on each dose, showed no correlation between the two parameters.

Associative Learning and Nonassociative Processes of Sensitization

Other mice, conditioned with 20 mg/kg cocaine, 2 mg/kg MAP or saline, were challenged with saline 6 days after a CPP test, and placed in the drug-paired compartment. On the following day, the subjects received the respective challenge dose of the drug; then half of each group were placed in the drug-paired compartment, and the other half in the saline-paired compartment.

The saline challenge showed no difference in locomotor activity between the cocaine- and saline-treated groups (Fig. 6a). After the cocaine challenge, locomotor activity in the single-cue chamber was significantly higher in the cocaine-treated groups than in the control, while no difference was observed in the dual-cue chamber (Fig. 6b). However, there was no difference in activity between the subgroups placed into the drug- and saline-paired compartments. This was confirmed by three-way ANOVA with significant main effect of drug, $F(1, 44) = 13.13$, $p < 0.001$, and interaction between drug and chamber type, $F(1, 44) = 3.95$, $p = 0.05$, followed by post hoc comparison ($p < 0.05$ and 0.001 for the drug- and saline-paired compartment, respectively).

The saline challenge produced significantly higher locomotor activity in the MAP-treated group compared to the control in the dual-cue chamber, while no difference was observed in the single-cue chamber (Fig. 7a). The finding was confirmed by two-way ANOVA with a significant main effect of drug, $F(1, 55) = 11.89$, $p < 0.01$, and interaction between

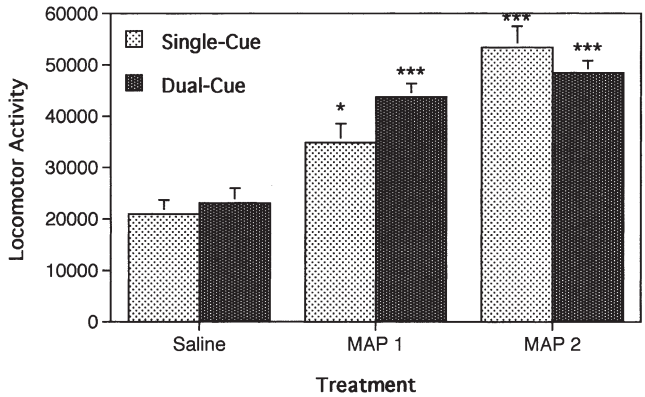

FIG. 5. The expression of behavioral sensitization elicited by the challenge injection with 1 mg/kg MAP in the single-cue (a) and dual-cue chambers (b). Two-way ANOVA revealed a significant main effect of treatment, $F(2, 90) = 42.99$, $p < 0.0001$, but neither effect of chamber type nor interaction between treatment and chamber type. Asterisks * and *** denote significant differences in locomotor activity compared to that in the respective control groups ($p < 0.05$ and 0.001, respectively Scheffe's S-test).

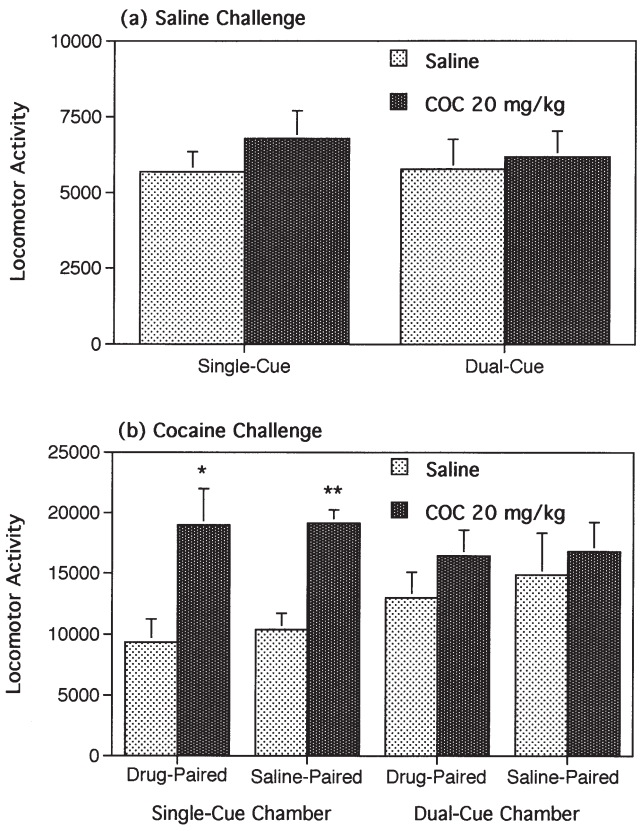

FIG. 6. The expression of behavioral sensitization elicited by the challenge injection with 10 mg/kg cocaine, without evidence for conditioned locomotor activity in the group treated with 20 mg/kg cocaine in the single-cue chamber. However, neither conditioned locomotor activity nor the expression of the challenge-induced sensitization was observed in the group treated in the dual-cue chamber. Three-way ANOVA revealed a significant main effect of drug on locomotor activity elicited by the cocaine challenge, $F(1, 44) = 13.13$, $p < 0.001$, and an interaction between drug and chamber type, $F(1, 44) = 3.95$, $p = 0.05$. Asterisks * and ** denote significant differences in locomotor activity compared to that in the saline control group ($p < 0.05$ and 0.01, respectively; Scheffe's *S*-test). The number of animals in each treatment group was 13.

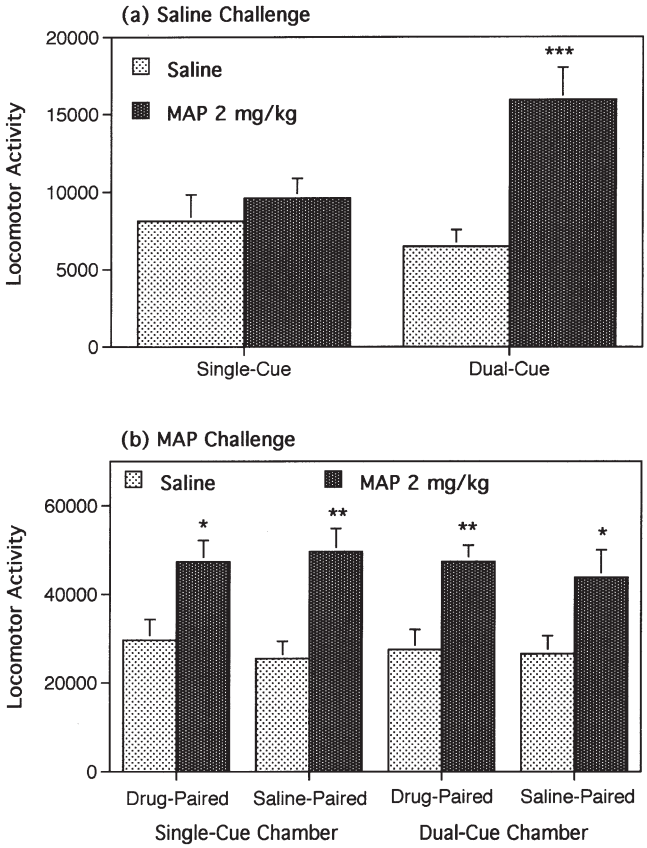

FIG. 7. The expression of behavioral sensitization elicited by the challenge injection with 1 mg/kg MAP, without evidence for conditioned locomotor activity in the group treated with 2 mg/kg MAP in the single-cue chamber. On the other hand, both conditioned locomotor activity and the challenge-induced sensitization were observed in the group treated in the dual-cue chamber. Two-way ANOVA revealed a significant main effect of drug on locomotor activity elicited by the saline challenge, $F(1, 55) = 11.89$, $p < 0.01$ and an interaction between drug and chamber type, $F(1, 55) = 6.30$, $p < 0.05$. In addition, three-way ANOVA revealed only significant main effect of drug on locomotor activity elicited by the MAP challenge, $F(1, 51) = 34.92$, $p < 0.0001$. Asterisks *, **, and *** denote significant differences in locomotor activity compared to that in the saline control group ($p < 0.05$, 0.01 and 0.001, respectively; Scheffe's *S*-test). The numbers of animals in each treatment group were 14–16.

drug and chamber type, $F(1, 55) = 6.30$, $p < 0.05$, followed by post hoc comparison ($p < 0.001$). After the MAP challenge, all the MAP-treated groups showed significantly higher locomotor activity than the control group, as indicated by three-way ANOVA, with only a significant main effect of drug, $F(1, 51) = 34.92$, $p < 0.0001$ (Fig. 7b). There was no difference in the activity between the subgroups placed into the drug- and saline-paired compartments.

DISCUSSION

The present study found the simultaneous development of CPP and locomotor sensitization across conditioning sessions with cocaine and MAP. This is consistent with the previous finding that CPP and behavioral sensitization concurrently developed during repeated cocaine administration (17). Based on these findings, it appears as if the mechanisms underlying sensitization to the rewarding properties of addictive drugs might share common neural systems with those underlying behavioral sensitization (25). However, the cocaine treatment within the dual-cue chamber caused CPP but not the development of behavioral sensitization. The simple regression tests showed no correlation between the CPP score and the degree of behavioral sensitization to cocaine or MAP. Further, other investigations have also demonstrated that repeated treatment with cocaine or amphetamine produced CPP without the development of behavioral sensitization (4,19). Therefore, the present results, together with the previous findings, indicate that the mechanisms underlying behavioral sensitization are probably separable from those underlying the development of place preference conditioning following repeated administration of cocaine and MAP. Supporting this issue, it has recently been shown that the core region of the NAc is implicated in the mechanisms underlying conditioned stimulus-reinforcement association or learning,

whereas the shell region contributes to the potentiative effects of amphetamine on locomotor activity and lever responding with conditioned reinforcement (11,20).

In the single-cue chamber, repeated injections of MAP caused robust, progressive increases in locomotor activity during the conditioning sessions. The same treatments with higher doses of cocaine also produced slight but significantly progressive increases in locomotion during the conditioning sessions. It has been proposed that behavioral sensitization results from associative learning processes such as drug-environment conditioning (22,33), nonassociative processes (24,27), or their combination (2,32). After the cocaine or MAP challenge, the expression of behavioral sensitization was similarly observed in both the drug- and saline-paired compartments in each group. However, the saline challenge in the drug-paired compartment revealed no evidence for conditioned locomotor activity in either group treated with cocaine or MAP. Therefore, these results suggests that behavioral sensitization observed in the single-cue chamber predominantly involves the mechanism of nonassociative processes (24,27).

On the other hand, repeated treatments with cocaine in the dual-cue chamber failed to induce progressive increases in locomotor activity during the conditioning sessions. Further, the cocaine challenge revealed no differences in locomotor activity among the cocaine- and saline-treated groups, although augmented locomotor activity in the saline control group might, at least in part, account for this result. These findings were inconsistent with the results obtained in the single-cue chamber and the fact that when animals were repeatedly injected with amphetamine in their respective distinct environments, the development of behavioral sensitization was observed in each environment (2,26). One possible explanation for this discrepancy involves the emergence of stereotypy. However, this is unlikely because cocaine dose-dependently increases locomotor activity up to a 40 mg/kg dose, and because even 40 mg/kg cocaine produces no stereotyped behaviors that prevent locomotor activity in ddY mice (preliminary experiments). Another possible explanation is to argue differences in treatment regimen. In the previous studies, animals received the drug in the test environment or a "third world" and saline in their home cages (2,22,26,32). On the other hand, the present animals received cocaine in one compartment of the dual-cue chamber and saline in the other compartment; these were "upside-down" environments relative to each other. Accordingly, it is conceivable that repeated treatments with saline in the "upside-down" environment may retard the development of behavioral sensitization to cocaine, although the mechanism remains unclear.

In contrast to cocaine, repeated treatments with MAP in the dual-cue chamber produced robust, progressive increases in locomotor activity during the conditioning sessions. After the MAP challenge, the expression of behavioral sensitization was similar in both the drug- and saline-paired compartments. Furthermore, the saline challenge produced a significantly higher locomotor activity in the MAP-treated group than in the saline control group. Therefore, it is conceivable that both associative and nonassociative processes may contribute to the sensitization to MAP in the dual-cue chamber. The reason for these differences in the profile of behavioral sensitization between MAP and cocaine is unclear. These stimulants have been shown to produce locomotor stimulation via activation of the mesolimbic dopamine system; nevertheless, differences in the actions have been demonstrated between the drugs. Amphetamines increase dopamine levels in the synapse by releasing the amine through dopamine transporters (9,14), whereas cocaine inhibits the reuptake of dopamine released (12,23). Acute and sensitized locomotor responses to cocaine and amphetamines are differentially suppressed by dopamine receptor antagonists (15) or the D_2 receptor agonist quinpirole (31). Further, repeated injection of amphetamine into the VTA induces sensitized response to a subsequent challenge dose (5,10), whereas repeated intra-VTA injections of cocaine did not (30). These differences, combined with effects of repeated drug and saline treatments in the "upside-down" environments, may account for the differences in the profile of behavioral sensitization between cocaine and MAP in the dual-cue chamber.

Medications for the treatment of drug abuse are expected to have few undesirable side effects such as emesis, depression, and dysphoria, and to have little effect on motor activity (18). Accordingly, it is important that preclinical research examines the effect of candidate compounds not only on the rewarding properties of addictive drugs but also on locomotor activity in animals. Recently, a few studies investigated the effects of potential agents for the treatment of cocaine abuse on reinforcing and locomotor stimulant properties of cocaine (1,13). These studies, however, measured the rewarding and stimulant properties in separate experiments. In contrast, the procedure described here can simultaneously monitor rewarding properties and locomotor stimulation effects of psychostimulants. Thus, the present new procedure provides a useful strategy for preclinical screening of potential medications for the treatment of drug addiction.

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