Pharmacology Biochemistry and Behavior, Vol. 60, No. 2, pp. 395–405, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00604-7

Cocaine Sensitization Can Accelerate the Onset of Peak Cocaine Behavioral Effects

ROBERT CAREY AND JUNMIN GUI

VA Medical Center and SUNY Health Science Center, 800 Irving Avenue, Research (151), Syracuse, NY

Received 9 May 1997; Revised 28 October 1997; Accepted 12 November 1997

CAREY, R. J. AND J. GUI. *Cocaine sensitization can accelerate the onset of peak cocaine behavioral effects.* PHARMACOL BIOCHEM BEHAV **60**(2) 395–405, 1998.—The development of sensitization to the behavioral effects of cocaine occurs with repeated intermittent usage. In the present study rats were given five daily IP injections of cocaine (10 mg/kg) immediately prior to placement in an open-field environment for 20 min to induce cocaine sensitization. Control groups received saline injections or cocaine injections (10 mg/kg) 30 min after testing in the home cage. One week later the animals were given a challenge test with 10 mg/kg cocaine. The animals that had received cocaine in the test environment exhibited a more rapid onset of cocaine-induced behavioral effects than either animals previously treated with saline or animals that had received cocaine in the home cage. In a second experiment, the same sensitization protocol was followed except that during the interval between the end of the cocaine/saline treatments and the challenge test, the animals were given six daily 20-min saline tests to assess the contribution of differential habituation and/or Pavlovian conditioning to the sensitization effect. Neither habituation or Pavlovian conditioning altered the more rapid onset of cocaine stimulant effects induced by repeated cocaine treatments. It is suggested that the faster onset of cocaine effects is another way in which cocaine sensitization contributes to cocaine abuse liability. © 1998 Elsevier Science Inc.

Cocaine Pavlovian conditioning Open field Locomotion Central zone Sensitization

TWO opposite but frequent effects of chronic drug use are the development of either (a) tolerance or, (b) sensitization to the initial drug-induced effect. In the case of the stimulant effects of cocaine, tolerance as well as sensitization can develop depending upon the schedule of drug administration. With a continuous cocaine treatment regimen tolerance occurs (25,43), whereas with discontinuous or intermittent cocaine usage sensitization typically is observed (41). The development of sensitization to cocaine with intermittent administration has attracted considerable attention as an important contributing factor in the addictive potency of cocaine (3,24,31,37). In view of this possible link to addictive processes, extensive preclinical research has been conducted in an effort to identify the mechanisms that underlie the development of sensitization to cocaine. Evidence obtained from a variety of methodologies implicate dopamine, opioid, and excitatory amino acid mechanisms (12,14–16,18–23,28–30,32,33,36,38,39) in the development of behavioral/neurochemical sensitization to cocaine. In addition, steroids such as corticosterone, estrogen, and testosterone also appear to contribute to the occurrence of cocaine sensitization effects (2,13,17,26,27,34,40). Interestingly, behavioral but not neuroendocrine effects of cocaine undergo sensitization (4). Altogether, these studies reveal the complexity of the cocaine sensitization process at the neurobiological level.

Although the possible mechanisms underlying cocaine sensitization are being intensively investigated, the behavioral dimensions of sensitization have received less attention. In a previous study, (7) we observed that the behavioral and pharmacokinetic effects of an IP cocaine injection are rapid with peak effects occurring within the first 5 min after the injection. Whereas most studies of behavioral sensitization to cocaine assess the overall behavioral drug response, we thought it would be useful to assess cocaine sensitization effects during the onset of the drug effect. A focus on the onset of the drug effect appeared relevant to issues relating cocaine sensitization to abuse liability because it is well known that the efficacy of a reinforcement upon behavior is strongly influenced by both the magnitude and the delay of the reinforcement. Thus, a potentially important behavioral impact of a sensitization mechanism upon drug reinforcement would be to shift the onset of peak drug effects to an earlier time after drug administration. Guided by this consideration, we examined whether repeated (IP) cocaine treatments (10 mg/kg) would lead to a

Requests for reprints should be addressed to Robert Carey, VA Medical Center, 800 Irving Avenue, Research (151), Syracuse, NY 13210.

shift in the onset of the locomotor stimulant effect to an earlier time after the cocaine injection.

METHOD

Animals

Naive male Sprague–Dawley rats from Taconic Farms (Germantown, NY), 6 months old and weighing approximately 500 g at the start of the experiments, were used. Upon arrival, the animals were housed in individual 25 × 17 ×17 cm wire mesh cages in a climate-controlled room at 22°C with a 12 D:12 L cycle. During the first week after arrival, all animals were handled and weighed daily for 7 days. During the second week the animals received three injections (IP) of 0.9% saline (1 ml/kg) to acclimate the animals to the injection procedure. All experiments occurred during the 12-h light cycle.

Drugs

Cocaine hydrochloride (Mallinckrodt Specialty Chemical, St. Louis, MO) was dissolved in sterile distilled H_2O in a concentration of 10 mg/ml. All injections were IP.

Apparatus

All of the behavioral tests were conducted in a square open-field compartment that was 60 × 60 × 45 cm. A closed-circuit video camera (RCA TC7011U) was mounted 50 cm above the open-field box. All signals were analyzed by a video tracking system, the Videomex-V from Columbus Instruments (Columbus, OH), and the data was imported into a PC-compatible computer. The walls of the chamber were white, and the floor of the open-field box was covered by plain white paper that was changed after each animal. Ambient white noise (80 dB) was provided by an audio tape player and was turned on immediately prior to placement of the animal in the test chamber and turned off upon removal from the test chamber. Testing was conducted under conditions of red-light illumination to enhance the contrast between the subject and background and to reduce the animal's shadow. To be sure that penetration of the central zone had occurred, the animal's head was blackened by a marker pen and the camera only tracked this feature of the rat's body. A central zone (CZ) comprising 1/9 of the floor area was programmed to be monitored by the video analyzer independently from the rest of the open field. During each session, data was collected every 2.5 min or in some instances 30 s by the computer. A dot matrix printer (Epson FX-286e) was placed outside the test room and was connected to the image analyzer by a parallel cable and the computer screen tracings of the animal's movement were printed out either every 2.5 min or every 30 s, depending upon the experiment. The complete test procedure was conducted automatically without the presence of the experimenter in the test room. In addition, a VHS VCR was also connected to the camera for the purpose of recording supplementary behavioral data and providing the ability for one to review and reinput the video tape signal to the image analyzer in case of a malfunction of either the analyzer or the printer during the experiments.

Design and Procedures

Two separate experiments were performed. In each experiment, there were two phases: (a) a sensitization induction phase, and (b) a sensitization challenge test phase. In both experiments the induction phase was the same. Initially, the animals received a 10-min test in the open-field environment following a saline injection. This test was used to match groups upon the two dependent variables used in the experiment, namely locomotion distance and entries into the central zone. Three days after the completion of this nondrug test the animals were assigned to one of three treatment conditions: (a) saline (S), (b) 10 mg/kg cocaine before (Coc-B), and (c) 10 mg/kg cocaine after (Coc-A). The Coc-B and Coc-A groups had $n = 7$, respectively, whereas the S group had $n = 14$, one-half of which was given cocaine in the sensitization challenge test and the other half was given saline. The S and the Coc-B treatments were administered immediately prior to placement of the animals in the test environment. The Coc-A treatment group received the cocaine injection 30 min after testing in its homecage. All animals received five daily treatments during the induction phase. In Experiment 1, the animals were given a sensitization challenge test 7 days after completion of the in-

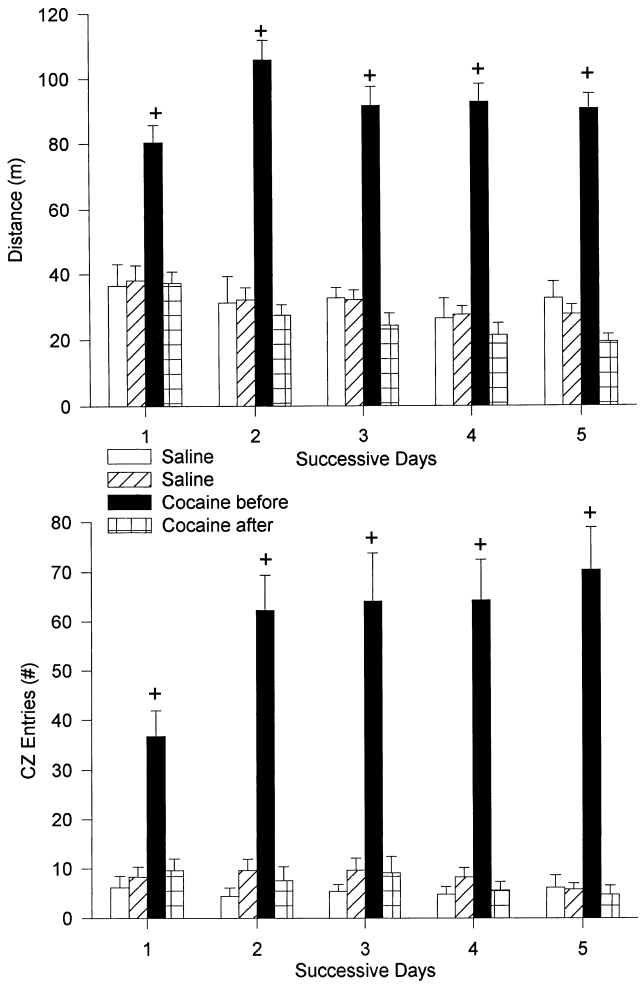

FIG. 1. Means and SEMs for locomotion distance (upper panel) and central zone entries (lower panel) during 20-min test sessions on 5 successive treatment days. The cocaine-before group received cocaine (10 mg/kg) immediately before testing and the cocaine-after group received cocaine (10 mg/kg) 30 min after testing in the home cage. The two saline groups received saline immediately before testing. $^{+}$Denotes $p < 0.01$ for the difference between the group that received cocaine before testing vs. all other groups.

duction phase. In the challenge test, the Coc-B, Coc-A and one-half of the S group received 10 mg/kg cocaine immediately prior to testing. One-half of the original S group received saline on this last test day to have a baseline nondrug reference group. The test lasted for 20 min. In Experiment 2 the induction protocol was the same (the Coc-B and Coc-A groups had $n = 7$ and the S group had $n = 14$), but the sensitization challenge test procedures were modified. Prior to the challenge test for sensitization the animals were given six daily saline injections. This testing was performed to extinguish possible cocaine conditioned effects that could add to the sensitization effects. If the sensitization effects were the result of conditioned effects adding to the drug-induced effects then the extinction procedure should serve to reduce the cocaine response in the Coc-B group to that of the Coc-A and S groups when they are given the cocaine challenge test after the extinction procedure. This latter test was conducted the day after the sixth extinction test. Because the sensitization effects in Experiment 1 were most evident within the first 5 min after the cocaine injection, this challenge test lasted 5 min with behavior recorded every 30 s. In addition, the animals were sacrificed immediately after testing to measure brain and plasma levels of cocaine.

Statistical Analyses

Multivariate Analysis of Variance (MANOVA) was used to analyze the behavioral data to determine the group effects, repeated measurement effects, within-session effects, as well as the interaction between these three variables. Subsequently, more specific comparisons were needed using one-way and two-way ANOVA for locomotion distance (meters) and CZ entries. To make specific group comparisons, post hoc Duncan's multiple range tests were performed because this test incorporates the variability at all treatments. $p < 0.05$ was used as the criterion for statistical significance. One-way ANOVA was employed to assess the biochemical data.

Biochemical Procedures

Immediately following completion of the behavioral testing in Experiment 2, animals were placed in a plastic restraining cone (Braintree Products, Inc.) and sacrificed by decapitation. Trunk blood was collected in tubes containing 200 μl of 0.5% sodium fluoride and centrifuged for 15 min at 2,500 rpm. The plasma was frozen at −70° and subsequently assayed for cocaine. The brain was rapidly removed and dissected on a chilled glass plate. Under magnification, three brain samples were collected; a frontal cortex sample (a 2 × 2 mm bilateral section of the medial frontal pole), a bilateral striatal and a bilateral limbic sample. The limbic tissue included nucleus accumbens, olfactory tubercle, and overlying pyriform cortex. Following dissection, the samples of brain tissue were weighed, placed in tubes containing 0.5 ml of 0.1 M perchloric acid and 4.5 μl of 10 μm/ml dihydroxybenzylamine (DHBA) as an internal standard, and then homogenized and centri-

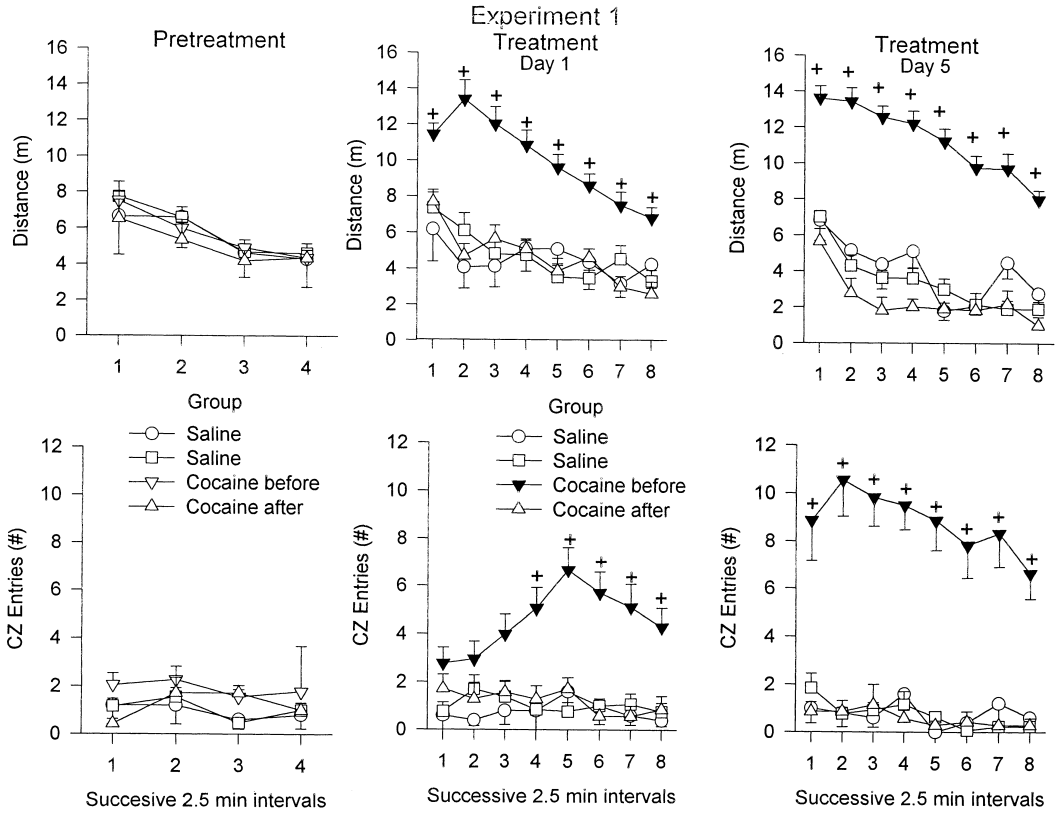

FIG. 2. Within-session means and SEMs for distance (upper panel) and central zone entries (lower panel) on the pretreatment test (10 min) and day 1 and day 5 treatment days (20 min). All groups received saline prior to the pretreatment test. $^{+}$Denotes $p < 0.01$ for the difference between the group that had received cocaine-before testing in the treatment phase vs. all other groups.

fuged. The resulting supernatant was filtered through 0.2 μm pore filters and the extracts were stored at −70°C until the HPLC-EC analysis, which was completed within 24–72 h. The tissue samples were analyzed for dopamine (3-hydroxytyramine), the dopamine metabolites, DOPAC (3,4-dihydroxyphenyl-acetic acid), 5-HT (5-hydroxytryptamine), and the metabolite 5-HIAA (5-hydroxyindole-3-acetic acid). A BAS biophase C18 reverse phase column (4.6 × 250 mm, 5 μm) was used. The buffer was 0.15 M monochloroacetic acid, pH 3.1, 2 mM EDTA, and 0.86 mM SOS (sodium octyl sulfate). This was added to 35 ml acetonitrile to make 1 l. This solution was then filtered and degassed and 18 ml tetrahydrofuran (THF) was added. The mobile phase flow rate was 1.2 ml/min and a BAS 4B EC detector was set at 0.8 V.

After the blood samples were centrifuged to separate plasma from blood cells, a solid phase extraction procedure was followed to prepare the plasma sample for analysis. The extraction column was a Narc2, 3 ml (125 mg) column (J. T. Baker, Phillipsburg, NJ). 0.5 to 1.0 ml of serum was used, depending upon availability. One hundred percent acetonitrile was added to the serum (3:1 acetonitrile to serum) and centrifuged for 5 min at 2,500 RPM. The supernate was decanted and added to it 0.1 M sodium phosphate buffer, pH 6.1 (2:5 buffer to serum). Using 0.1 M HCl, the final pH of the sample was between 4 and 6. Under vacuum, the column was first conditioned with 2 ml methanol followed by 2 ml 0.1 m sodium phosphate buffer (pH 6.1). Before the column could dry the prepared sample was passed through the column and this was immediately followed with 3 ml HPLC grade water, 3 ml 0.1 m HCl followed by 9 ml 100% methanol. Finally, the sample was eluted with 2 1.0 ml Methylene chloride/Isopropanol/Ammonium hydroxide (77:19:4) and then dried under a stream of nitrogen. Mobile phase was added to the dried sample and directly injected into the HPLC column.

For cocaine analyses in plasma and brain tissue a 100 × 4.6 mm 3 μm Adsorbosphere catecholamine column (Alltech, Deerfield, IL) was used in conjunction with a 76% 0.02 M Potassium phosphate, pH 3.0 buffer, and 24% acetonitrile mobile phase. Column temperature was maintained at 25°C, with a flow rate of 0.5 ml/min. The samples were detected with a Bioanalytical Systems (West Lafayette, IN) variable wavelength UV detector. The setting was 235 nm (8).

RESULTS

An overall statistical analysis was performed upon the results obtained in Experiment 1 during the 5 days of cocaine treatment. Separate MANOVA analyses were performed for the distance and central zone entry data using group, day, and within-session interval as the three variables. For distance, there was a highly significant group effect, $F(3, 24) = 188.9$, $p < 0.001$, interval effect $F(4,24) = 128.1$, $p < 0.001$ and group interval interaction, $F(12, 96) = 10.7$, $p < 0.001$. No other main effects or interactions were statistically significant ($p > 0.05$). In the case of central zone entries, there was a statistically significant group effect, $F(3, 24) = 79.5$, $p < 0.001$, and a statistically significant group interval interaction, $F(12, 96) = 6.4$, $p < 0.001$. No other differences were statistically significant ($p > 0.05$). Figure 1 presents the session totals for distance and CZ entries over the 5 days of treatment. One-way ANOVAs performed on group differences for each day yielded F-values that were statistically significant at the $p < 0.001$ level, and on each day the cocaine before group had higher scores than the other three groups, which did not differ from each other. The three-way MANOVA statistical analysis indicated that there was a marked treatment by within-session interval relationship. Accordingly, Fig. 2 presents the within-session results for the pretreatment test and for treatment day 1 and treatment day 5. On the pretreatment test, there were no group differences for distance, $F(3, 24) = 0.34$, $p > 0.05$, or central zone entries, $F(3, 24) = 0.61$, $p > 0.05$. There was a statistically significant within-session effect for distance, $F(3, 24) = 16.8$, $p < 0.001$, but not for CZ entries, $F(3, 24) = 1.19$, $p > 0.05$. The within-session decline in distance is consistent with a within-session habituation effect. In that we have previously noted the absence of habituation (9) for CZ entries, the absence of a within-session effect for central zone entries in the this study is consistent with this previous observation. It can be seen that the effects of the cocaine treatment are readily apparent in Fig. 2 for both treatment day 1 and treatment day 5. As can be seen in the upper half of Fig. 2, the effects of the cocaine treatment were evident in the first interval and the increases in locomotion occurred at each within-session interval. Inspection of day 1 vs. day 5 indicates that the onset of peak locomotion shifted from the second to the first interval. In the lower half of Fig. 2 the CZ entry data is presented. Inspection of day 1 vs. day 5 indicates a more dramatic change in behavior with repeated treatments oc-

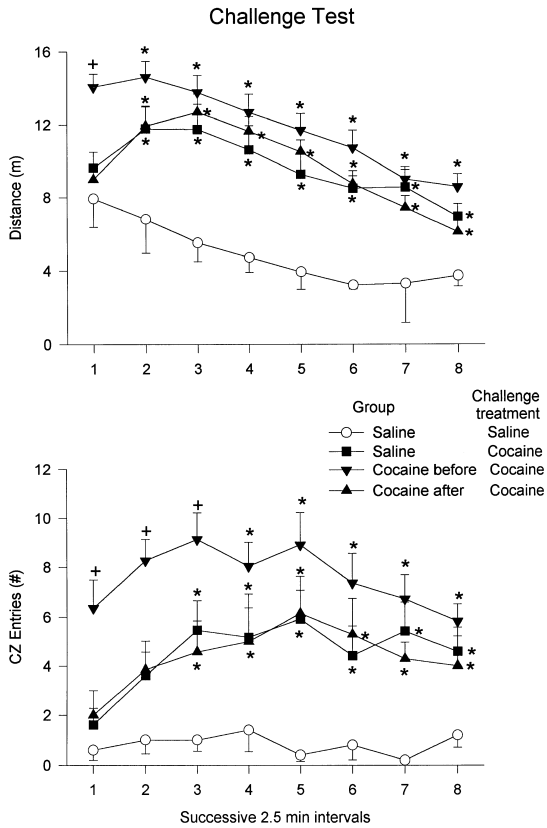

FIG. 3. Within-session means and SEMs on a 20-min challenge test conducted 1 week after the treatment phase of the experiment. In this test three groups received cocaine (10 mg/kg) immediately prior to testing (the cocaine-before group, the cocaine after-group, and one saline group). +Denotes $p < 0.01$ for the difference between the cocaine-before group vs. all other groups. *Denotes $p < 0.01$ for the difference between the three groups that received cocaine vs. the group that received saline prior to testing.

curred for CZ entries than was the case for locomotion distance. On day 1 the animals in the initial session intervals did not exhibit an increase in CZ entries. In fact, there is a lack of correspondence between CZ entries and locomotion distance in that the animals entered the CZ more frequently in the latter part of the session when their locomotion was decreasing. In session 5, however, the highest rates of CZ entry occurred early in the test session. Thus, the CZ entry results were consistent with a more rapid onset of cocaine effects with repeated treatments.

To more directly evaluate the suggestion that repeated cocaine treatments shifted the onset of peak effects to a time closer to the injection, a second phase of testing was conducted. This testing was undertaken 1 week after the completion of the five successive daily cocaine treatments. On this test day, three groups were given cocaine (10 mg/kg) immediately before the 20-min test, and one group received saline. The three cocaine groups included (a) the group that had received the cocaine treatments in the test environment (Coc-B), (b) the group that had received the five cocaine treatments in the home cage after testing (Coc-A), and (c) one of the two groups that received saline during the first five sessions. The other saline group again received saline on this challenge test day. The overall statistical analysis indicated that for distance there was a statistically significant group effect, $F(3, 24) = 7.2$, $p < 0.001$, and a group interval interaction, $F(27, 96) = 2.9$, $p < 0.001$. Similarly, for CZ entries there was a statistically significant group effect, $F(3, 24) = 7.0$, $p < 0.001$, interval effect, $F(7, 24) = 2.7$, $p < 0.001$, but not a statistically significant group interval interaction, $F(27, 96) = 1.1$, $p > 0.05$. The results are shown graphically in Fig. 3. As can be seen in the upper half of Fig. 3, the group that had previously received cocaine in the test environment exhibited an enhanced locomotion response to cocaine in the first interval compared to the other two cocaine treatment groups. The one-way ANOVA for the first interval was $F(3, 24) = 9.6$, $p < 0.001$, with the Coc-B group having a significantly higher locomotion level than all other groups. In the subsequent seven intervals, all three cocaine treatment groups had elevated locomotion levels above the saline treatment $p < 0.001$, but the three groups were not different from each other. In the lower half of Fig. 3, it can be seen that the Coc-B group that had received cocaine previously in the test environment had a greater initial response to the cocaine treatment. The one-way ANOVAs for intervals 1–3 yielded F-values of 5.8, $p < 0.001$, 8.7, $p < 0.001$ and 3.7, $p < 0.01$, respectively, with the Coc-B group significantly higher than all other groups. By the fourth interval, however, the three cocaine treatment groups were statistically indistinguishable, but yet all exhibited a statistically reliable increase in CZ entries over the level of the saline group ($p < 0.01$).

The finding in the first experiment that exposure to cocaine in the test environment but not in the home cage enhanced the onset of peak cocaine effects indicated that some type of associative process was involved in this effect. One seemingly straightforward way to assess this possibility was to subject animals to an extinction process and then determine if the effect was eliminated. Accordingly, in the second experiment, the same initial protocol used in Experiment 1 was followed in which animals received five daily 20-min tests. One group received cocaine (10 mg/kg) immediately prior to test-

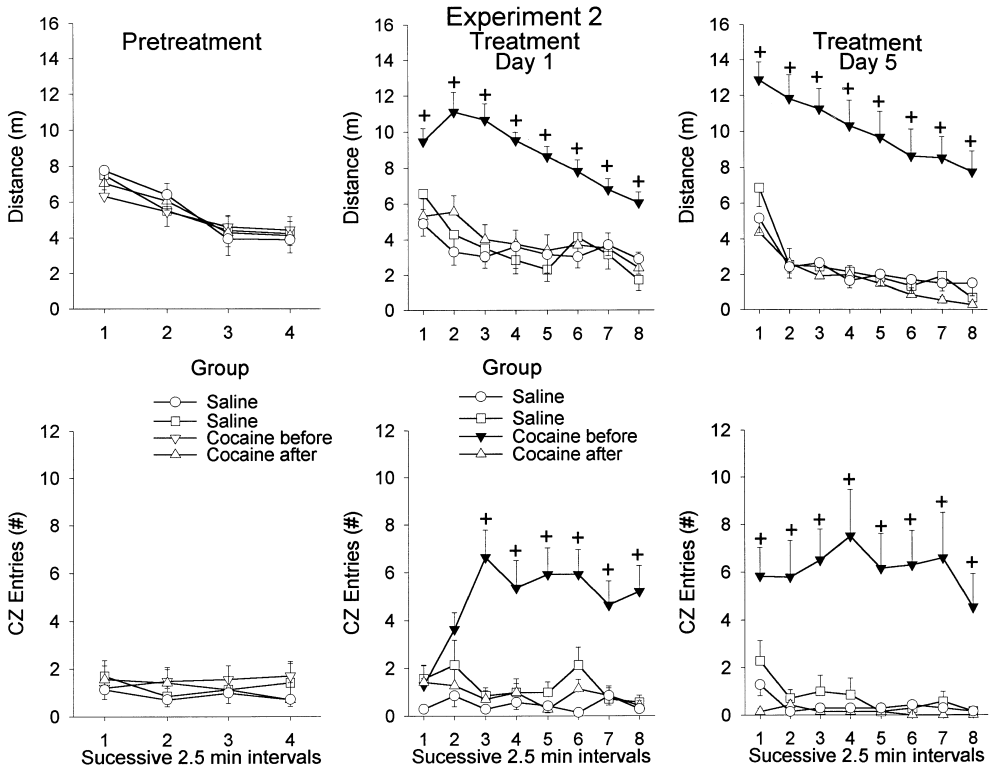

FIG. 4. Means and SEMs for locomotion distance (upper panel) and central zone entries (lower panel) on a pretreatment test (10 min) and day 1 and day 5 treatment test sessions (20 min) in Experiment 2. All groups received saline prior to the pretreatment test. $^{+}$Denotes $p < 0.01$ for the differences between the group that received cocaine (10 mg/kg) immediately before testing in sessions 1 and 5 vs. all other groups.

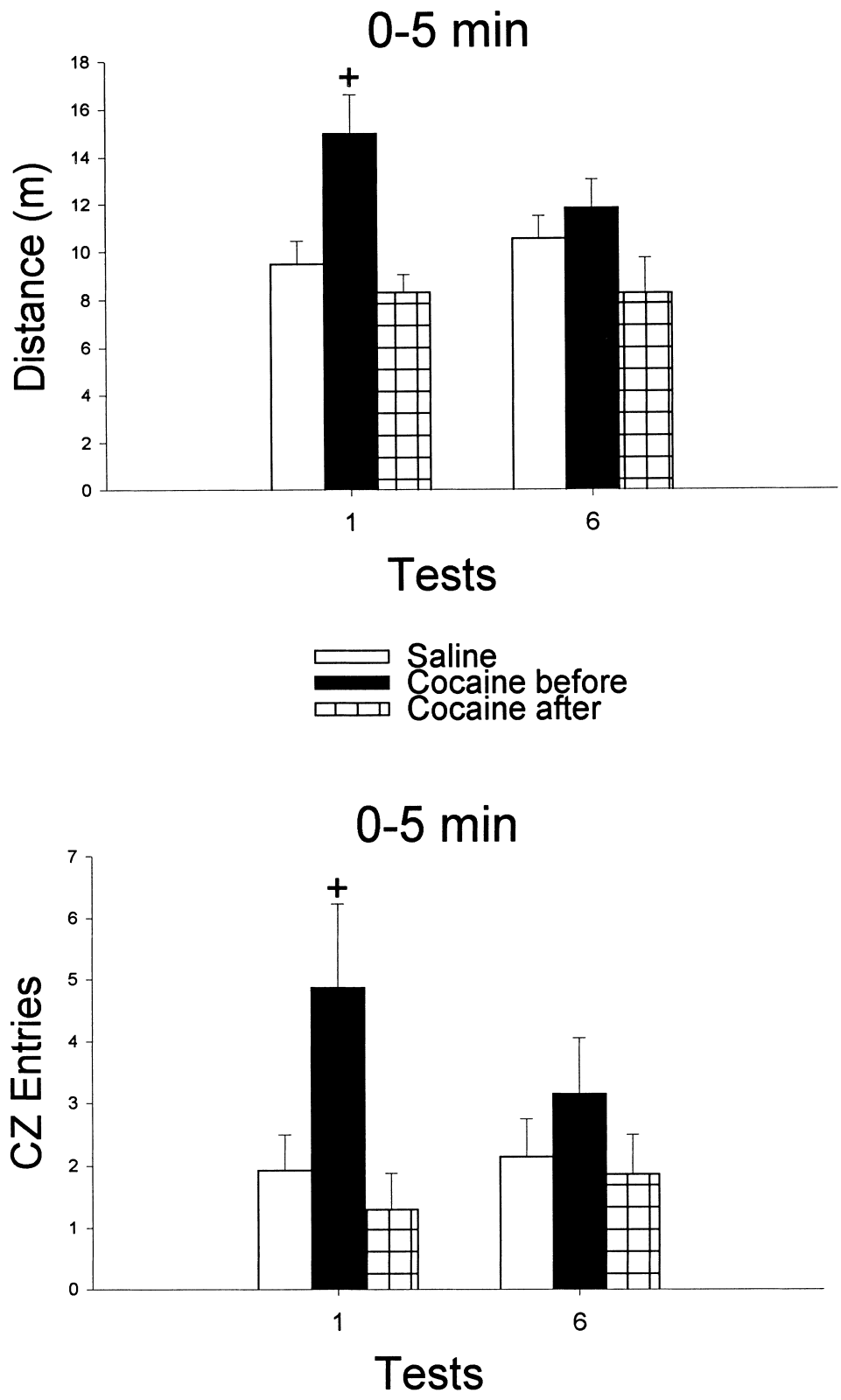

FIG. 5. Means and SEMs for distance and central zone entries on the first and sixth extinction tests in Experiment 2. The first 5 min of the 20-min test sessions are presented. $^{+}$Denotes $p < 0.01$ for the difference between the cocaine-before group vs. all other groups.

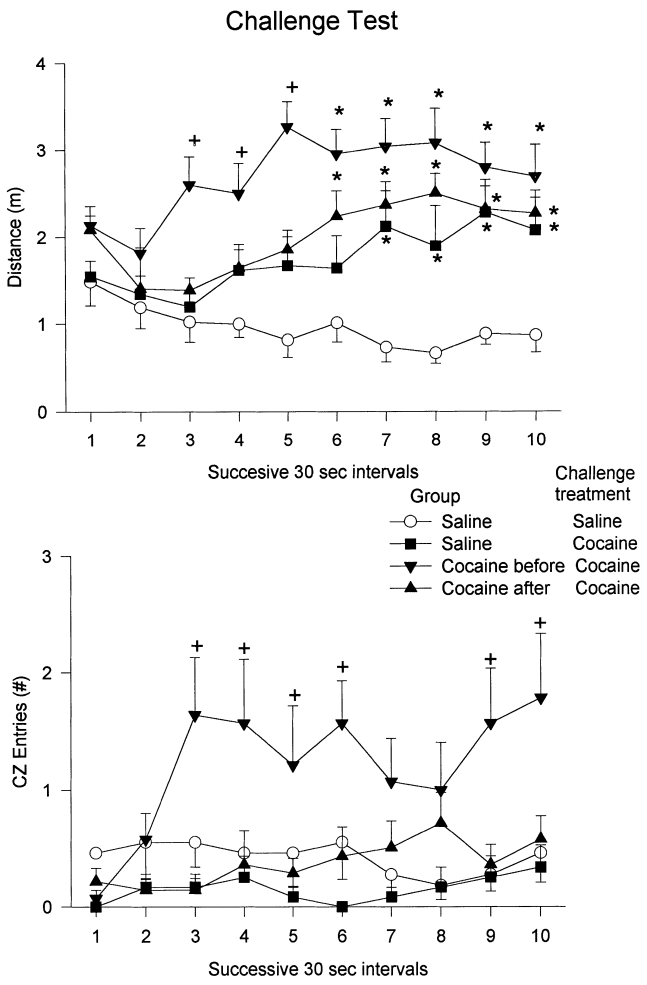

FIG. 6. Within-session means and SEMs for distance and central zone entries on the 5-min challenge test in Experiment 2. This test was conducted 1 day after the extinction phase of the experiment. Three groups received cocaine (10 mg/kg) immediately prior to testing (cocaine-before group, cocaine-after group, and one saline group). The remaining saline group received saline prior to testing. $^{+}$Denotes $p < 0.01$ for the difference between the cocaine-before group vs. all other groups, and *denotes $p < 0.01$ for the difference between the three groups that received cocaine vs. the saline group.

ing (Coc-B), another group received the same cocaine treatment in its home cage 30 min after testing (Coc-A), and the remaining two groups received saline prior to testing (S). After completion of this treatment phase, all of the groups received six successive daily tests of 20 min in which they received saline immediately before testing. This was the extinction treatment. As was the case in the first experiment, the groups did not differ from each other on the pretreatment test, $F(3, 25) = 0.07, p > 0.05$, and $F(3, 25) = 0.25, p > 0.05$ for distance and CZ entries, respectively. For the distance measure there was a statistically significant interval effect, $F(3, 25) = 17.1, p < 0.001$, but for CZ entries there was not a statistically significant interval effect, $F(3, 25) = 0.49, p > 0.05$. The difference between distance and CZ entries as related to the within session interval results indicates the occurrence of habituation for the distance measure but not the CZ entry measure. This result is similar to Experiment 1 and to previous studies (9).

For the 5 days of treatment, however, there were statistically significant group differences, $F(3, 25) = 43.0, p < 0.001$, and $F(3, 25) = 34.8, p < 0.001$, for distance and CZ entries, respectively. For both measures, the group that received cocaine prior to testing had higher scores than all of the other groups that did not differ from each other. There were no other statistically significant effects, and the day effect was not significant $p > 0.05$. Figure 4 presents the results of the pretreatment test and the first and fifth cocaine treatments for each group across eight successive 2.5-min intervals. As can be seen in the far left panel, the groups were closely matched prior to the initiation of the cocaine treatments. As was the case for Experiment 1, the repeated cocaine treatments led to a shift to peak behavioral effects induced by cocaine to occur in the initial 2.5-min period following the cocaine injection. On the extinction tests there were also statistically significant effects. Figure 5 presents the results obtained in the first and last days of extinction (days 1 and 6, respectively). In that the critical effects in Experiment 1 occurred shortly after the injection, the data for the first 5 min of each session is presented. The statistical results for the 20-min session were similar to those shown for the initial 5 min of testing. As can be seen in Fig. 5, the cocaine before treatment resulted in higher levels of locomotion, $F(2, 25) = 12.2, p < 0.001$, and CZ entries, $F(2, 25) = 7.9, p < 0.001$, on the first extinction test. On the last extinction day, however, there were no statistically significant group differences, $F(2, 25) = 0.3, p > 0.05$, and $F(2, 25) = 0.44, p > 0.05$ for distance and CZ entries, respectively. Thus, these results indicate that the cocaine before treatment induced a conditioned cocaine response that was subsequently extinguished. One day after the completion of the extinction protocol, three groups were given the cocaine treatment (10 mg/kg) immediately before testing (a) the Coc-B group, (b) the Coc-A group, and (c) one of the S groups. The remaining S group was given saline. This test lasted 5 min, and behavior was recorded every 30 s to attempt to determine if the onset of the cocaine response had been modified by the prior cocaine treatments. The results are presented in Fig. 6. As can be seem in Fig. 6, the Coc-B group again exhibited a more rapid onset of the behavioral response than the other two groups that received cocaine in the challenge test. For distance, there was a group effect, $F(3, 24) = 9.3, p < 0.001$, interval effect, $F(9, 24) = 6.8, p < 0.001$, and group interval interaction $F(3, 96) = 3.8, p < 0.001$. For CZ entries there was a similar set of statistical findings, $F(3, 24) = 5.2, p < 0.001$, for group differences, $F(9, 24) = 2.8, p < 0.05$, for interval effects, and $F(3, 96) = 2.7, p < 0.01$, for the group interval interaction. For locomotion distance the one-way ANOVAs for intervals 3–5 yielded F-values of 7.6, $p < 0.001$, 5.9, $p < 0.001$, and 13.9, $p < 0.001$, respectively, with the Coc-B group having a higher level of locomotion than all other groups, $p < 0.05$. On intervals 6–10 the locomotion distance score of all three cocaine groups were higher than the saline group, $p < 0.05$, but not different from each other, $p > 0.05$. In the lower half of Fig. 6, it can be seen that the Coc-B group has more CZ entries than all other groups. One-way ANOVAs indicated that these differences were statistically significant at intervals 3–6 and 9–10. Thus, the extinction procedure that was sufficient to eliminate the cocaine-conditioned response to saline and test environment cues was insufficient to eliminate the enhanced onset of the Coc-B animal's response to cocaine. Immediately after completion of the 5-min challenge test, all the animals were sacrificed and ex vivo measurements were performed on limbic brain tissue. The results obtained for dopamine, DOPAC/DA turnover ratio, 5-HT, 5-HIAA/5-HT turnover ratios, and cocaine concentrations in limbic tissue samples

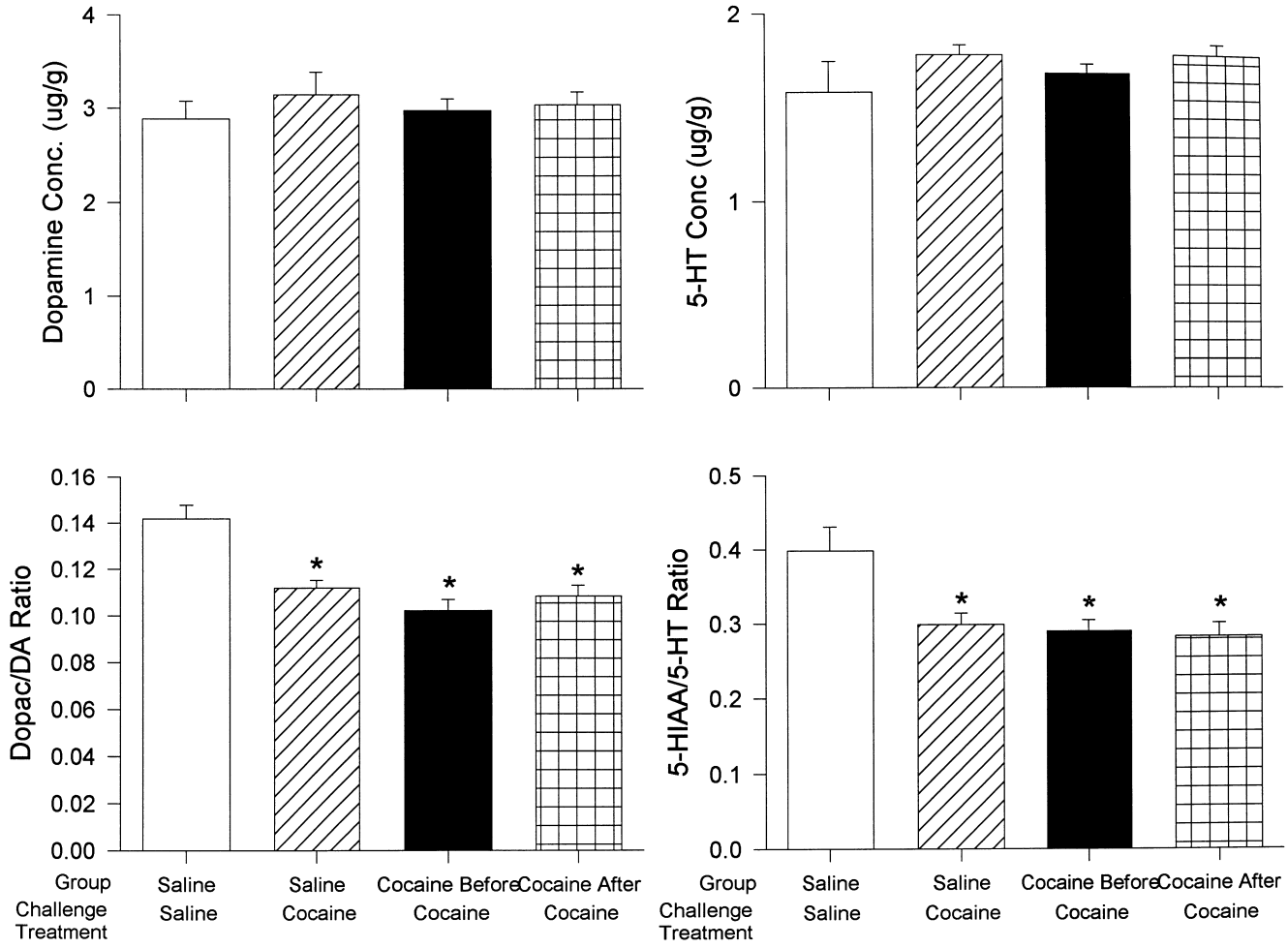

FIG. 7. Means and SEMs for the ex vivo brain measurements obtained upon animals sacrificed immediately after the 5-min challenge test in Experiment 2. The measures were obtained from limbic brain samples. *Denotes $p < 0.01$ for each of the cocaine-treated groups vs. saline-treated animals.

are presented in Figs. 7 and 8. As can be seen in Fig. 7, the cocaine treatments had no effect upon dopamine concentration, $F(3, 24) = 0.35, p > 0.05$, or 5-HT concentration, but had a substantial effect upon DOPAC/DA ratios, $F(3, 24) = 13.8, p < 0.001$, and 5-HIAA/5-HT ratios, $F(3, 24) = 7.4, p < 0.001$. The three cocaine treatment groups did not differ from each other, but all were different from the saline treatment ($p < 0.01$). In cortical and striatal tissue samples there were no statistically significant effects on any of these neurotransmitter measurements. Figure 8 shows the cocaine concentrations in the limbic tissue brain sample for the three groups that received cocaine. The saline group had no detectible cocaine. The three cocaine treatment groups did not differ statistically, $F(2, 18) = 0.004$, $p > 0.05$. These findings indicate that the cocaine treatments had an equivalent neurochemical impact regardless of prior treatment. It also was found that there were no differences in cocaine concentration in plasma, cortex, or striatum among the three groups that received cocaine for the challenge test.

DISCUSSION

Cocaine-induced sensitization effects have been extensively studied and are considered to be an important component of the abuse liability of cocaine (37). One straightforward implication of cocaine sensitization effects relevant to drug abuse issues is that sensitization, by increasing the magnitude of the cocaine behavioral effects, would also increase the magnitude of cocaine reinforcement effects. In addition to reinforcement magnitude, another important variable that determines reinforcement efficacy is the delay of reinforcement. That is, the shorter the delay, the more effective the reinforcer. In the case of cocaine, this would be the length of the delay from drug taking to the onset of peak reinforcement effects. The findings of the present experiments, which showed that repeated cocaine treatments advanced the onset of peak cocaine stimulant effects to a time closer to the cocaine injection, suggests that sensitization effects may also accelerate the onset of cocaine reinforcement effects. Thus, by both increasing the magnitude of cocaine reinforcement and decreasing the delay in the onset of peak cocaine reinforcement effects, sensitization processes may substantially enhance the abuse liability of cocaine.

In addition to providing an additional facet to the behavioral characterization of the sensitization phenomena, namely the onset of cocaine stimulant effects, the present study also undertook to assess the potential contribution of habituation

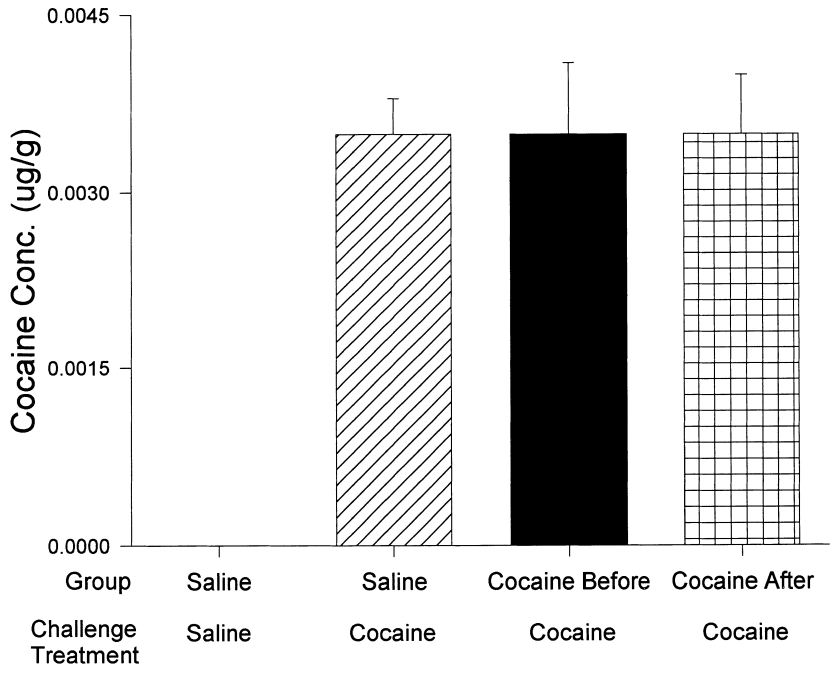

FIG. 8. Means and SEMs for cocaine concentrations obtained from limbic brain tissue samples. There was no detectible cocaine in the saline treatment group, and the three groups that received cocaine (10 mg/kg) did not differ statistically from each other.

and conditioning to this sensitization effect. In that the sensitization effects observed in our experiments are context specific, it is apparent that some type of associative process is implicated. Using the more rapid onset of the cocaine stimulant effects as an index of sensitization, we assessed whether this effect could be accounted for by the general associative processes of habituation and/or Pavlovian conditioning. Animals exposed to the test environment without cocaine undergo habituation to the test environment cues. If animals are treated with cocaine, however, it is possible that habituation effects may be blocked by cocaine (10). One obvious consequence of this potential habituation differential is that the behavioral differences between the cocaine-treated and saline-treated groups would increase as a consequence of greater habituation in the saline group than the cocaine group. This habituation-dependent difference could then be mislabelled as sensitization. Somewhat less apparent is the possible contribution of habituation when the comparison is made between cocaine and saline groups on a cocaine challenge test comparison. In this circumstance, the habituation effect developed by the saline control group during the sensitization treatment phase may subtract from the cocaine treatment effect in this group in the challenge test. As a consequence, the occurrence of a greater response to cocaine by cocaine-sensitized animals on the cocaine challenge test may be attributable to a subtractive effect of habituation in the control group rather that to a sensitization effect. Importantly, the habituation differential would be applicable to context specific sensitization in that the habituation would be context specific. The present study is pertinent to this issue because behavioral sensitization to cocaine was observed using two behavioral measures of cocaine efficacy: distance and central zone entries. Critically, central zone entries did not exhibit habituation in the control groups so that a habituation differential could not account for the behavioral sensitization to cocaine observed using central zone entries as the dependent variable.

Another potential contributing factor to the occurrence of context specific behavioral sensitization to cocaine stimulant effects is that conditioning can develop to the cocaine associated cues (11,35). Thus, when animals previously treated with cocaine are compared with animals who have either not previously received cocaine or received cocaine out of context then any differential behavioral effects induced by the cocaine treatment may be explicable by the conditioning differential. That is, all groups could have the same unconditioned response to cocaine, but the group previously exposed to the test cues under the influence of cocaine may also have the added effect of the cocaine-conditioned response. Thus, the conditioning explanation could account for the context specific sensitization in terms of an added effect of conditioning. This contrasts with a habituation explanation that accounts for the context-specific sensitization by a subtractive effect of habituation upon the unconditioned cocaine response. Seemingly, both such effects could occur together with conditioning adding to the unconditioned cocaine response in the cocaine group and habituation subtracting from the unconditioned cocaine response in the control group. The present study directly assessed the conditioning factor by subjecting animals that received cocaine in the test environment to an extinction procedure. The extinction testing revealed that indeed a conditioned cocaine response did develop to the test environment cues but, that with repeated non-cocaine exposures to the test environment, the conditioned response did extinguish. In addition, the extinction procedure also provided the opportunity for habituation to develop in this cocaine-treated group. Importantly, when these animals were treated with cocaine they still exhibited an enhanced onset of the cocaine response compared with the control groups. Thus, the present study suggests that this component of cocaine sensitization effects are not explicable in terms of habituation and/or conditioning factors (1,42). In the case of drug conditioning, however, the

drug not only induces the unconditioned response but it also provides interoceptive drug cues. As we have shown in previous studies (5,6), interoceptive drug cues can activate a conditioned drug response even after the response has been extinguished in the nondrug state to the exteroceptive cues. Possibly then, following repeated cocaine treatments, the initial postinjection cocaine concentrations that have detectable stimulus effects but lack behavioral activating effects could function as Pavlovian-conditioned stimuli and activate the full cocaine response before behaviorally activating brain concentrations of cocaine are achieved. This latter possibility, however, is merely speculative at this time. Thus, the underlying mechanism that mediates context-specific behavioral sensitization of cocaine effects remains elusive.

ACKNOWLEDGEMENTS

This research was supported by NIDA Grant RO1DA05366-11 and the technical work of Gail DePalma in the performance of the biochemical measurements is acknowledged.

REFERENCES

1. Anagnostaras, S. G.; Robinson, T. E.: Sensitization to the psychomotor stimulant effects of amphetamine: Modulation by associative learning. Behav. Neurosci. 110:1397–1414; 1996.
2. Badiani, A.; Morano, M. I.; Akil, H.; Robinson, T. E.: Circulating adrenal hormones are not necessary for the development of sensitization to the psychomotor activating effects of amphetamine. Brain Res. 673:13–24; 1995.
3. Berridge, K. C.; Robinson, T. E.: The mind of an addicted brain: Neural sensitization of wanting vs. liking. Curr. Direct. Psychol. Sci. 4:71–76; 1995.
4. Borowsky, B.; Kuhn, C. M.: Chronic cocaine administration sensitizes behavioral but not neuroendocrine responses. Brain Res. 543:301–306; 1991.
5. Carey, R. J.: An examination of the use of stimulant drugs as conditioned and unconditioned stimuli in a classical conditioning paradigm. Drug Dev. Res. 16:305–315; 1989.
6. Carey, R. J.: Pavlovian conditioning between co-administered drugs: Elicitation of an apomorphine-induced antiparkinsonian response by scopolamine. Psychopharmacology (Berlin) 104:463–469; 1991.
7. Carey, R. J.; Damianopoulos, E. N.; DePalma, G.: Differential temporal dynamics of serum and brain cocaine: Relationship to behavioral, neuroendocrine and neurochemical cocaine induced response. Life Sci. 55:1711–1716; 1994.
8. Carey, R. J.; DePalma, G.: A simple, rapid HPLC method for the concurrent measurement of cocaine and catecholamines in brain tissue samples. J. Neurosci. Methods 58:25–28; 1995.
9. Carey, R. J.; Gui, J.: A simple and reliable method for the positive identification of Pavlovain conditioned cocaine effects in open-field behavior. J. Neurosci. Methods 73:1–8; 1997.
10. Damianopoulos, E. N.; Carey, R. J.: Conditioning, habituation and behavioral reorganization factors in chronic cocaine effects. Behav. Brain Res. 49:149–157; 1992.
11. Damianopoulos, E. N.; Carey, R. J.: A new method to assess Pavlovian conditioning of psychostimulant drug effects. J. Neurosci. Methods 53:7–17; 1994.
12. De Montis, M. G.; Devoto, P.; Meloni, D.; Gambarana, C.; Giorgi, G.; Tagliamonte, A.: NMDA receptor inhibition prevents tolerance to cocaine. Pharmacol. Biochem. Behav. 42:179–182; 1992.
13. Deroche, V.; Piazza, P. V.; Le Moal, M.; Simon, H.: Repeated corticosterone administration sensitizes the locomotor response to amphetamine. Brain Res. 584:309–313; 1992.
14. Di Lullo, S. L.; Martin-Iverson, M. T.: Presynaptic dopaminergic neurotransmission mediates amphetamine-induced unconditioned but not amphetamine-conditioned locomotion and defecation in the rat. Brain Res. 568:45–54; 1991.
15. Druhan, J. P.; Jakob, A.; Stewart, J.: The development of behavioral sensitization to apomorphine is blocked by MK-801. Eur. J. Pharmacol. 243:73–77; 1993.
16. Farfel, G. M.; Kleven, M. S.; Woolverton, W. L.; Seiden, L. S.; Perry, B. D.: Effects of repeated injections of cocaine on catecholamine receptor binding sites, dopamine transporter binding sites and behavior in rhesus monkey. Brain Res. 578:235–243; 1992.
17. Haaren, F. V.; Meyer, M. E.: Sex differences in locomotor activity after acute and chronic cocaine administration. Pharmacol. Biochem. Behav. 39:923–927; 1991.
18. Heidbreder, C.; Goldberg, S. R.; Shippenberg, T.: Inhibition of cocaine-induced sensitization by the α-opioid receptor antagonist naltrindole. Eur. J. Pharmacol. 243:123–127; 1993.
19. Heidbreder, C. A.; Shippenberg, T. S.: U-69593 prevents cocaine sensitization by normalizing basal accumbens dopamine. Neuroreport 5:1797–1800; 1994.
20. Henry, D. J.; White, F. J.: Repeated cocaine administration causes persistent enhancement of D_1 dopamine receptor sensitivity within the rat nucleus accumbens. J. Pharmacol. Exp. Ther. 258:882–890; 1991.
21. Henry, D. J.; White, F. J.: The persistence of behavioral sensitization to cocaine parallels enhanced inhibition of nucleus accumbens neurons. J. Neurosci. 15:6287–6299; 1995.
22. Itzhak, Y.: Blockade of sensitization to the toxic effects of cocaine in mice by nitric oxide synthase inhibitors. Pharmacol. Toxicol. 74:162–166; 1994.
23. Itzhak, Y.; Stein, I.: Sensitization to the toxic effects of cocaine in mice is associated with the regulation of *N*-methyl-D-aspartate receptors in the cortex. J. Pharmacol. Exp. Ther. 262:464–470; 1992.
24. Jodogne, C.; Marinelli, M.; Le Moal, M.; Piazza, P. V.: Animals predisposed to develop amphetamine self-administration show higher susceptibility to develop contextual conditioning of both amphetamine-induced hyperlocomotion and sensitization. Brain Res. 657:236–244; 1994.
25. Johansson, E. K.; Tucker, S. M.; Ginn, H. B.; Martin, B. R.; Aceto, M. D.: Functional and dispositional tolerance develops during continuous cocaine exposure. Eur. J. Drug Metab. Pharmacokinet. 17:155–162; 1992.
26. Kalivas, P. W.; Duffy, P.: Similar effects of daily cocaine and stress on mesocorticolimbic dopamine neurotransmission in the rat. Biol. Psychiatry 25:913–928; 1989.
27. Kalivas, P. W.; Stewart, J.: Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16:223–244; 1991.
28. Kalivas, P. W.; Striplin, C. D.; Steketee, J. D.; Klitenick, M. A.; Duffy, P.: Cellular mechanisms of behavioral sensitization to drugs of abuse [review]. Ann. NY Acad. Sci. 654:128–135; 1992.
29. Karler, R.; Calder, L. D.; Chaudhry, I. A.; Turkanis, S. A.: Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. Life Sci. 45:599–606; 1989.
30. Koff, J. M.; Shuster, L.; Miller, L. G.: Chronic cocaine administration is associated with behavioral sensitization and time-dependent changes in striatal dopamine transporter binding. J. Pharmacol. Exp. Ther. 268:277–282; 1994.
31. Koob, G. F.: Drugs of abuse: Anatomy, pharmacology and function of reward pathways [review]. Trends Pharmacol. Sci. 13:177–184; 1992.
32. LeDuc, P. A.; Mittleman, G.: Interactions between chronic haloperidol treatment and cocaine in rats: An animal model of intermittent cocaine use in neuroleptic treated populations. Psychopharmacology (Berlin) 110:427–436; 1993.
33. Peris, J.; Boyson, S. J.; Cass, W. A.; Curella, P.; Dwoskin, L. P.; Larson, G.; Lin, L. H.; Yasuda, R. P.; Zahniser, N. R.: Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. J. Pharmacol. Exp. Ther. 253:38–44; 1990.
34. Peris, J.; Decambre, N.; Coleman-Hardee, M. L.; Simpkins, J. W.: Estradiol enhances behavioral sensitization to cocaine and

amphetamine-stimulated striatal [^{3}H]dopamine release. Brain Res. 566:255–264; 1991.
35. Pert, A.; Post, R. M.; Weiss, S. R. B.: Conditioning as a critical determinant of sensitization induced by psychomotor stimulants. NIDA Res. Mono. Series 97:208–240; 1990.
36. Ping, H. X.; Xie, L.; Gong, X. J.; Liu, G. Q.; Wu, H. Q.: Effect of dizocilpine maleate on monoamines and their metabolites in rat brain. Acta Pharmacol. Sin. 13:206–208; 1992.
37. Robinson, T. E.; Berridge, K. C.: The neural basis of drug craving: An incentive-sensitization theory of addiction [review]. Brain Res. Rev. 18:247–291; 1993.
38. Segal, D. S.; Kuczenski, R.: Repeated cocaine administration induces behavioral sensitization and corresponding decreased extracellular dopamine responses in caudate and accumbens. Brain Res. 577:351–355; 1992.
39. Shimosato, K.; Marley, R. J.; Saito, T.: Differential effects of NMDA receptor and dopamine receptor antagonists on cocaine toxicities. Pharmacol. Biochem. Behav. 51:781–788; 1995.
40. Sorg, B. A.: Mesocorticolimbic dopamine systems: Cross-sensitization between stress and cocaine. Ann. NY Acad. Sci. 654:136–144; 1992.
41. Stewart, J.; Badiani, A.: Tolerance and sensitization to the behavioral effects of drugs. Behav. Pharmacol. 4:289–312; 1993.
42. Stewart, J.; Vezina, P.: Extinction procedures abolish conditioned stimulus control but spare sensitized responding to amphetamine. Behav. Pharmacol. 2:65–71; 1991.
43. Zeigler, S.; Lipton, J.; Toga, A.; Ellison, G.: Continuous cocaine administration produces persisting changes in brain neurochemistry and behavior. Brain Res. 552:27–35; 1991.