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Stimulus gated cocaine sensitization: Interoceptive drug cue control of cocaine locomotor sensitization

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Abstract

Repeated cocaine treatments typically generate sensitization effects which are environment specific. In this study, we investigated whether drug treatments with highly selective receptor specificity can also function as contextual cues to control the expression of cocaine sensitization effects. Two experiments were conducted in which separate groups of rats ($N=10$) received ten paired or unpaired cocaine (10.0 mg/kg) treatments. In the experiments, autoreceptor preferring low doses of either the 5-HT_{1A} agonist, 8-OHDPAT (8OH) (0.05 mg/kg) or the D_1/D_2 agonist apomorphine (APO) (0.05 mg/kg) were administered 20 min prior to cocaine administration and test environment placement (paired treatment). Under these conditions, the drug cues generated by the 8OH/APO treatments were associated with the cocaine stimulant effect in the test environment. The unpaired treatment groups received the same drug treatments but the cocaine was administered after testing, in the homecage. Consequently, for these groups, the 8OH/APO drug cues generated by the drug treatments would *not* become associated with the cocaine stimulant effect in the test environment. Critically, both 8OH and APO pretreatments elicited equivalent unconditioned response effects which were opposite to the cocaine unconditioned response effects; that is, behavioral inhibition vs. behavioral stimulation. Initially, the 8OH and APO pretreatments prevented the locomotor stimulant effects of cocaine; but, these inhibitory effects were reversed in the paired groups with repeated cocaine treatments, consistent with the emergence of cocaine sensitization effects. In the unpaired 8OH and APO pretreatment groups, behavioral suppression persisted throughout the treatment protocol. Subsequently, paired and unpaired groups were compared in four conditioning/sensitization tests. The conditioning tests included: a saline/saline test; and a 8OH/saline test (Experiment 1); and, a saline/saline test and a APO/saline test (Experiment 2). There were no paired/unpaired group differences in these conditioning tests. The sensitization tests included: a saline/cocaine test; and a 8OH/cocaine test (Experiment 1); and, a saline/cocaine test and a APO/cocaine test (Experiment 2). There were no paired/unpaired group differences in the saline/cocaine test for sensitization but paired/unpaired group differences were found in both the 8OH/cocaine and APO/cocaine sensitization tests. In these tests the paired but not the unpaired groups exhibited cocaine locomotor sensitization effects. Critically when, in an additional test, the pretreatments in the cocaine tests were reversed (i.e., 8OH paired group received APO and APO paired group received 8OH prior to cocaine), then there was no evidence for cocaine sensitization. Since the 8OH/APO pretreatments had equivalent inhibitory response effects, it was the stimulus properties of these drugs which controlled the expression of the cocaine locomotor sensitization effects. These findings support the critical role of associative processes in the stimulus-gating of psychostimulant drug sensitization. Importantly, this report incorporates a new methodology in which context can be specified in terms of highly specific brain receptor targets rather than in terms of global environmental situational cues.
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In the study of drug conditioning, it has been shown that drugs can function as conditioned stimuli (CS) as well as unconditioned stimuli (UCS). The fact that drugs can serve as CS as well as UCS is not surprising in that the stimulus properties of centrally active drugs are well known (Overton et al., 1999). The use of drugs as CS in operant as well as Pavlovian conditioning is well-established (Bevins and Peterson, 2004; Jarbe et al., 1981; Lal and Bennet, 1989; Overton, 1977; Siegel, 1977, 1988). Furthermore, a drug cue can be an effective CS

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when another drug is used as an UCS (Carey, 1989, 1991; Greeley et al., 1984; Revusky, 1985; Revusky and Reilly, 1990a,b; Taukulis, 1986, 1996). Drugs have also been used as a CS in open-field test paradigms in which drugs as diverse as buspirone, dizocilpine (MK-801) (Carey et al., 1999) and ±8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OHDPAT) (Carey et al., 2002) seemingly acquire CS properties for cocaine locomotor stimulant effects. The present study was undertaken to expand upon these latter observations by using two centrally active drug treatments as contextual cues for cocaine locomotor stimulant effects. Importantly, we used drug treatments which elicited unconditioned locomotor responses (UCR) opposite to the cocaine locomotor UCR. In separate experiments, we used doses of apomorphine and 8-OHDPAT which inhibited locomotor behavior to an equivalent degree and paired these drugs with cocaine. In our prior experimentation, we have shown that low autoreceptor preferring doses (0.01–0.05 mg/kg) of 8OH and APO elicit equivalent degrees of behavioral suppression (Carey et al., 2004a,b).

With a paired treatment protocol, the drug stimuli generated by the low dose 8-OHDPAT and apomorphine treatments, which otherwise would be linked to the inhibitory UCR evoked by these drug treatments, are associated with the cocaine UCR. If the drug cues generated by 8-OHDPAT (Cunningham et al., 1987; Glennon, 1986; Schreiber et al., 1995; Schreiber and De Vry, 1993) and by apomorphine (Bristow et al., 1998; Yamaguchi et al., 1991; Zuch and Cory-Slechta, 2001) can function as cocaine contextual stimuli then, the emergence of cocaine sensitization effects should be linked to these drug cues. To assess this possibility, paired/unpaired conditioning protocols were used to equate total drug exposures. With repeated pairings of 8-OHDPAT or apomorphine with cocaine, cocaine sensitization effects emerged. Critically, this sensitization was stimulus-bound to either the 8-OHDPAT or to the apomorphine drug cues.

1. Materials and methods

1.1. Animals

Naive male Sprague–Dawley rats from Taconic Farms (Germantown, NY), 4 months old and weighing approximately 400 g at the start of the experiments were used. Upon arrival, the animals were housed in individual 48 × 27 × 20 cm clear polycarbonate cages in a climate-controlled room at 22–24 °C with a 12-h dark and 12-h light cycle. During the 1st week after arrival, all animals were handled and weighed daily for 7 days. During the second week the animals received three injections (I.P.) of 0.9% saline (1.0 ml/kg) in order to acclimate the animals to the injection procedure. All experiments occurred during the 12-h light cycle (6AM–6PM). This protocol (IACUC 4-E) was approved by the Veterans Administration Medical Center's Subcommittee for Animal Studies.

1.2. Drugs

Cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile distilled H_2O to a concentration of 10.0 mg/ml. Cocaine injections were administered I.P. Apomorphine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.4 mg/ml ascorbic acid in sterile water to concentrations of 0.02, 0.05 and 0.1 mg/ml. 8-OHDPAT (±8-hydroxy-2-(di-*n*-propylamino) tetralin) (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile distilled H_2O to concentrations of 0.02, 0.05 and 0.1 mg/ml. Apomorphine and 8-OHDPAT injections were administered s.c. in a volume of 0.5 ml/kg. Cocaine injections were administered I.P. in a volume of 1.0 ml/kg. Saline injections (0.9% sodium chloride) were administered in a volume of 1.0 ml/kg (I.P.) as a treatment or 0.5 ml/kg (s.c.) as a pretreatment.

1.3. Apparatus

All of the behavioral tests were conducted in square 60 × 60 × 40 cm or round (68 cm: diameter × 40 cm: height) open-field compartments of approximately equal area. Testing was conducted in two similar subsections of the testing room with a circular and square chamber in each subsection. While we had previously found that there were no differences in activity levels related to chamber shape or room section, we always equated these factors across groups and treatments to eliminate the possibility that chamber shape or room section could be potential uncontrolled variables. In the present experiments, neither test room subsection nor test chamber shape was statistically significant variables on any behavioral measure ($P > 0.05$). Closed-circuit video cameras (Sanyo VCB-5123B) were mounted 50 cm above the open-field enclosures. All signals were analyzed by a video tracking system using a distance criterion of 2 cm for a movement to be scored (Ethovision, Noldus Information Technology, Inc, Leesburg, VA). The accuracy of the system for the measurement of distance was validated by moving objects at a fixed distance and confirming that the tracking system generated the same distances. The walls of the chamber were white and the floor of the open-field was covered by plain white paper, which was changed after each animal. Masking sound (75 dB) was provided by a white noise generator (San Diego Instruments, San Diego, CA) and was turned on immediately prior to placement of the animal into the test chamber and turned off upon removal from the test chamber. Each chamber is illuminated by two overhead 12 V projection lamps placed 50 cm above the chamber adjacent to the video camera. Each lamp contains a red filer so that testing could be conducted under conditions of red light illumination to avoid the possible aversive quality of white light and to enhance the contrast between the subject and background as well as to reduce the animal's shadow. The testing under red light conditions is less stressful and also favors locomotor activation as the rats are transferred from the ambient light of the vivarium to the red light of the testing room (Nasello et al., 1998). The animal's head was blackened with a non-toxic marker and the camera tracked only this feature of the rat's body while the animal was being tested in the open-field environment. During each session, data were calculated every 2.5 min by the software. The computer screen tracings of the animal's patterns of

locomotion were constantly present on monitors outside of the test room and saved by the software. In previous reports (Carey and Gui, 1997; Dai and Carey, 1995), we have presented the tracing of the locomotion patterns generated by animals in this test environment. In the present study, the locomotion patterns were similar to those previously presented (Carey and Gui, 1997; Dai and Carey, 1995). The tracings recorded by the tracking system could readily be used to identify small repetitive movements. Such tracings occurred infrequently and idiosyncratically. The complete test procedure was conducted automatically without the presence of the experimenter in the test room. In addition, a VHS VCR was connected to each camera to videotape sessions. The videotapes were always reviewed after each session in order to validate that the recording of the tracings represented the animals' locomotor patterns.

1.4. Testing protocols

1.4.1. Experiments 1 and 2

1.4.1.1. Validation of inhibitory effects of 8OH and APO pretreatments. While we have previously demonstrated pretest behavioral inhibitory effects of low dose 8OH and APO pretreatment upon locomotion (Carey et al., 2004a,b), we again validated these effects in the present experiments. Following acclimation to handling and injections, separate sets of 20 animals in each experiment were given six 20 min tests in the open-field environment. Immediately prior to each test, the animals were administered a saline injection (1 ml/kg). This protocol was used to establish a stable baseline for saline locomotor response levels prior to the initiation of the autoreceptor drug pretreatments. In fact, there were no statistically reliable changes in locomotor distance scores over the final three saline tests which preceded the autoreceptor drug tests ($P>0.05$). The next three tests were designed to assess the effects of low doses of 8-OHDPAT (Experiment 1) or apomorphine (Experiment 2). All animals were administered two injections. The first injection (pretreatment) was administered subcutaneously (S.C.) in the homecage 20 min prior to testing and the second injection (treatment) was administered intraperitoneally (I.P.) immediately before testing. The pretreatments for the 20 animals used in Experiment 1 were 8-OHDPAT injections of 0.01, 0.025 and 0.05 mg/kg, respectively, and the treatments were all saline injections. For Experiment 2, the pretreatments were apomorphine injections of 0.01, 0.025 and 0.05 mg/kg. Two days after the completion of these tests, all groups were given a saline test in which saline injections were the pretreatments as well as the treatments. After this pre-conditioning test, the animals were subdivided into paired and unpaired groups ($N=10$) equated in terms of means and SEMs ($P>0.05$) for their behavioral response to 8-OHDPAT in Experiment 1 or to apomorphine in Experiment 2 prior to the initiation of the conditioning protocol.

1.4.1.2. Sensitization induction phase. Each group received ten sensitization induction sessions. The paired groups received either 0.05 mg/kg 8-OHDPAT or 0.05 mg/kg apomorphine 20 min prior to a cocaine injection (10 mg/kg). The cocaine was administered immediately prior to placement in the open-field environment for a 20 min test session. Upon return to the homecage, this group received a saline injection. The unpaired groups received either a 0.05 mg/kg 8-OHDPAT or a 0.05 mg/kg apomorphine injection 20 min prior to testing and a saline injection immediately prior to placement in the open-field test environment. After completion of this test session, the animals were returned to their homecage and administered cocaine (10.0 mg/kg). Thus, over the course of the ten conditioning sessions, all groups received the same total number of drug treatments. For the paired groups, the test environment was associated with a cocaine injection; whereas, for the unpaired group, placement in the test environment was associated with a saline injection.

1.4.1.3. Tests for conditioning/sensitization effects. One day after completion of the ten sensitization induction sessions, the groups were given four tests on successive days. In the first test, all groups were given a saline injection as a pretreatment followed 20 min later by a second saline injection and a 20 min placement in the test environment. This test was conducted to assess whether the drug treatment protocol led to altered behavioral baselines possibly attributable to a conditioned cocaine response evoked by injection and test environment cues. In the second test, the paired and unpaired groups received either 0.05 mg/kg 8-OHDPAT (Experiment 1) or 0.05 mg/kg apomorphine (Experiment 2) as a pretreatment and 20 min later were administered a saline injection and were then placed in the test environment. This test was necessary to determine if the paired treatments altered the inhibitory efficacy of the pretreatments. In the third test, both the paired and unpaired groups in Experiments 1 and 2 were pretreated with saline and then given cocaine (10 mg/kg) immediately prior to testing. This test was important in order to determine if the responsivity of the groups to the cocaine treatment had been altered by the paired vs. unpaired protocol. In the fourth test, the paired and unpaired groups received either the 0.05 mg/kg 8-OHDPAT (Experiment 1) or the 0.05 mg/kg apomorphine (Experiment 2) pretreatment 20 min prior to testing and then all groups were administered cocaine (10 mg/kg) immediately before placement in the test environment. This test was conducted to determine if the paired treatment protocol selectively induced a cocaine sensitization effect.

1.4.2. Reversal tests

After completion of the three tests, the paired groups in Experiments 1 and 2 were given two additional tests. The first test was a saline test in which each of the paired groups received saline injections as both the pretreatment (20 min prior to testing) and treatment (immediately prior to testing). On the next day, the groups were given a cocaine test; this time, however, the pretreatments used in Experiments 1 and 2 were reversed. For the paired 8-OHDPAT group in Experiment 1, the pretreatment was 0.05 mg/kg apomorphine (20 min prior to

testing) and the treatment was 10 mg/kg cocaine (administered immediately prior to testing). For the paired apomorphine group in Experiment 2, the pretreatment was 0.05 mg/kg 8-OHDPAT instead of 0.05 mg/kg apomorphine. These tests were conducted to determine if the cocaine sensitization acquired under one drug state during the paired treatment regimen would transfer to a different drug treatment which had an equivalent response suppression effect.

1.5. Statistical analyses

One and two-way Analysis of Variance (ANOVA) were used to assess possible drug treatment effects upon the behavioral responses. In order to make specific group comparisons, post hoc Duncan's multiple range tests were performed. $P<0.05$ was used as the criterion for statistical significance. Paired *t*-tests were used for within group test comparisons ($P<0.05$, two tailed *t*-tests).

2. Results

2.1. Experiment 1

2.1.1. Validation of behavioral inhibition of 8OH pretreatment

In the pre-conditioning tests, 8-OHDPAT (8OH) pretreatments progressively suppressed open-field locomotor behavior with increasing dose levels ($F_{(3,57)}=20.2$, $P<0.001$) consistent with our previous reports (Carey et al., 2004a,b).

2.1.2. Sensitization induction phase

Fig. 1 presents the results obtained during the sensitization induction protocol in which the groups received either cocaine paired or unpaired to the test environment placement. Fig. 1 presents the saline/saline pretest and the first and last sensitization induction sessions. As can be seen in Fig. 1, the groups were closely matched on the pretest but diverged on the cocaine tests in terms of locomotion. Not surprisingly, the cocaine treatment enhanced locomotion ($F_{(1,18)}=19.9$, $P<0.00$, for group differences) and the group × session interaction was also significant ($F_{(2,36)}=16.9$, $P<0.001$). Two salient effects of the 8OH paired pretreatment upon the cocaine effects were that: (a) on the first cocaine treatment, the locomotion scores did not increase above the saline pretest scores for the paired group; and (b), by the final cocaine treatment session, the locomotion scores in the paired cocaine group were substantially higher than their saline pretest and their initial cocaine paired treatment scores, respectively (paired *t*-tests, $P<0.05$). Furthermore, the 8OH unpaired group had sustained reductions in locomotion throughout in comparison to saline pretest scores (paired *t*-tests $P<0.05$).

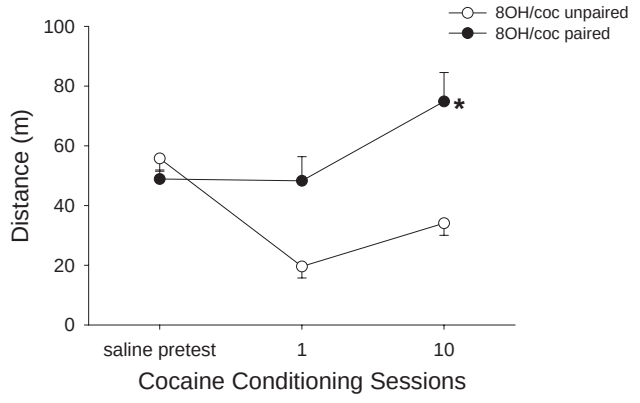

Fig. 1. Means and SEMs for locomotion distance scores in meters (m) during the 20 min tests in the sensitization induction phase of Experiment 1 (8OH experiment). During the saline pretest, both groups received saline as a pretreatment 20 min prior to testing and saline immediately before testing. In the cocaine tests, the paired group received 0.05 mg/kg 8OH 20 min before testing and 10.0 mg/k cocaine immediately prior to testing. The unpaired groups received 0.05 mg/kg 8OH 20 min prior to testing, saline immediately before testing and 10.0 mg/kg cocaine after returning to homecage. *Denotes $P<0.001$ in paired vs. unpaired group comparisons.

2.1.3. Conditioning/sensitization tests

In order to evaluate overall paired vs. unpaired group comparisons on the four conditioning/sensitization tests, a 2-way ANOVA was performed. The analysis indicated statistically significant treatment group differences ($F_{(1,18)}=4.7$, $P<0.05$); test effects ($F_{(3,54)}=16.2$, $P<0.001$) and a test × by by group interaction ($F_{(3,54)}=6.0$, $P<0.001$). In view of the statistically significant group × test interaction, we compared treatment groups on each test. Fig. 2A,B,C,D presents the within-session paired vs. unpaired group comparisons on each test. As can be seen in Fig. 2A, the paired and unpaired groups had virtually identical distance scores on the saline/saline test ($F_{(1,18)}=0.54$, $P>0.05$). Also, there was no statistically significant group difference in the 8OH/saline test (B) ($F_{(1,18)}=1.5$, $P>0.05$) and in the saline/cocaine test (C) ($F_{(1,18)}$ 0.01 $P>0.05$). However, in the test in which the pretreatment was 8OH followed by cocaine (D), there was a statistically significant difference between groups ($F_{(1,18)}=26.2$, $P<0.001$), indicative of a cocaine sensitization effect selectively in the paired group.

2.1.4. Reversal tests

Fig. 3 presents the reversal test results. Within-session results for locomotion distance are presented for the 8OH paired group. In Fig. 3A, the within-group comparisons are presented for the 8OH paired group on its locomotor behavior on the saline test day in which it received saline as both pretreatment and treatment vs. the test day in which it received 0 005 mg/kg 8OH as the pretreatment and 10.0 mg/kg cocaine as the treatment. As is evident in Fig. 3A, the 8OH pretreatment/cocaine treatment combination substantially enhanced locomotor behavior above the saline baseline level ($F_{(1,18)}=23.1$, $P<0.001$). Fig. 3B compares the within-session locomotion scores on the test in which the 8OH paired group received 0.05 mg/kg APO in place of 0.05 m/kg 8OH as the pretreatment 20 min prior to the cocaine treatment. As is evident in Fig. 3B, the APO pretreatment effectively blocked the cocaine locomotion stimulant effect ($F_{(1,18)}=1.6$, $P>0.05$). Indeed, the overall locomotion distance scores for the cocaine treatment session were below the saline baseline.

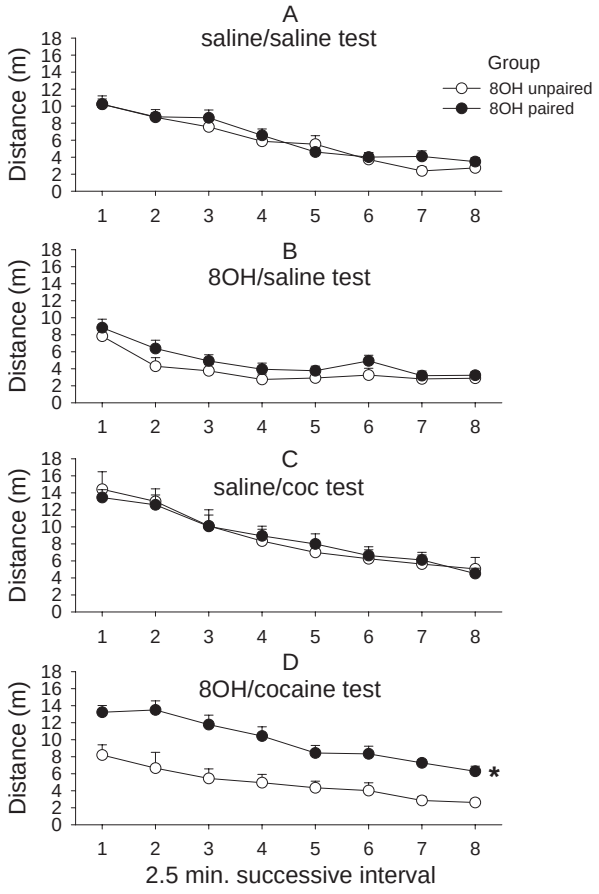

Fig. 2. Means and SEMs presents within-session locomotion distance scores in meters (m) in eight successive 2.5 min intervals in four tests for conditioning/sensitization in Experiment 1 (8OH experiment). In (A), both groups (paired and unpaired) received saline injections 20 min before and saline immediately before testing. In (B), both groups received 0.05 mg/kg 8OH 20 min prior to testing and saline immediately prior to testing. In (C), both groups received saline 20 min before testing and 10 mg/kg cocaine immediately prior to testing; and, in (D), both groups received 0.05 mg/kg 8OH 20 min before testing and 10.0 mg/kg cocaine immediately prior to testing. *$P<.01$ in paired vs. unpaired group comparisons.

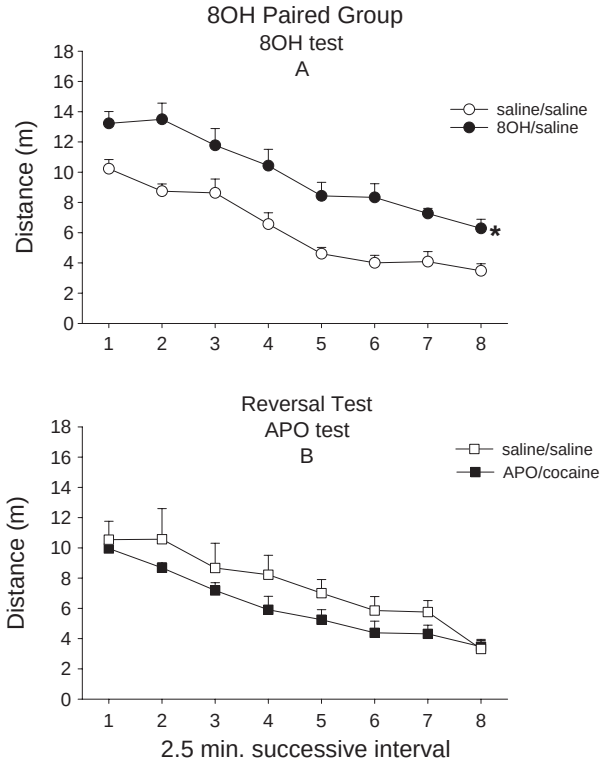

Fig. 3. Means and SEMs of within-session locomotion distance scores in meters (m) in the reversal test phase of Experiment 1 (8OH experiment). In (A), the 8OH paired group within session locomotion distance scores are compared on the saline/saline vs. the 0.05 mg/kg 8OH/cocaine (10 mg/kg) tests. In (B), the reversal test, the 8OH paired group received a saline/saline test and a 0.05 mg/kg APO/cocaine (10 mg/kg) test. *Denotes $P<0.01$ in saline/saline vs. 8OH/cocaine test comparisons.

2.2. Experiment 2

2.2.1. Validation of behavioral inhibition of APO pretreatment

In the pre-conditioning test results of the second experiment in which all animals were treated with apomorphine (APO) (0.01–0.05 mg/kg) 20 min prior to saline, APO progressively suppressed locomotion with increasing dose levels ($F_{(3,57)}=22.3$, $P<0.001$) similar to findings obtained in our earlier study (Carey et al., 2004b).

2.2.2. Sensitization induction phase

The effects of the paired vs. unpaired cocaine treatments upon behavior in the saline pretest and in the first and last sensitization induction sessions are presented in Fig. 4. The saline pretest scores for both groups on the saline/saline test were closely matched but substantial group differences occurred with the repeated paired/unpaired cocaine treatments ($F_{(1,18)}=10.8$, $P<0.01$). In addition, the group × test session interaction was statistically significant ($F_{(2,36)}=42.8$, $P<0.001$). As can be seen in Fig. 4, the locomotion distance scores for the unpaired group were reduced throughout apomorphine testing.

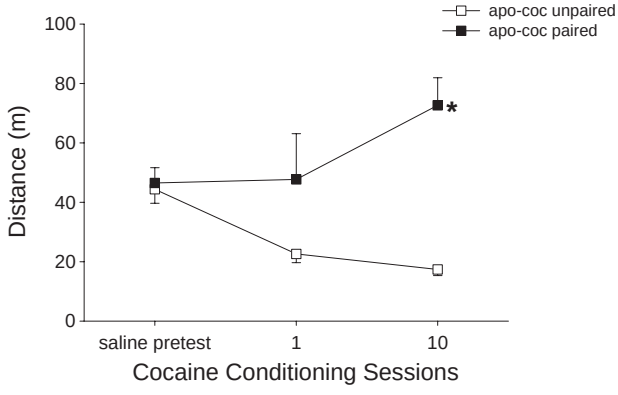

Fig. 4. Means and SEMs for locomotion distance scores in meters (m) in the saline pretest and in the first and last cocaine test sessions during the sensitization induction phase of Experiment 2 (APO experiment). In the saline pretest, both groups (paired and unpaired) received saline 20 min before testing and saline immediately prior to testing. In the first and last cocaine tests, the paired group received 0.05 mg/kg APO 20 min before testing and 10.0 mg/kg cocaine immediately prior to testing. The unpaired group received 0.05 mg/kg APO 20 min before testing, saline immediately prior to testing and 10.0 mg/kg cocaine after testing in the homecage. *Denotes $P<0.001$ in paired vs. unpaired group comparisons.

For the paired group, the cocaine treatment on the first cocaine treatment day did not change locomotion from its pretest saline/saline level; but, by the final cocaine treatment, locomotion increased to well above the pretest saline/saline scores as well as compared to initial APO/cocaine distance scores (paired t-tests, $P<0.05$).

2.2.3. Conditioning/sensitization tests

In order to compare the paired/unpaired groups across the four conditioning/sensitization tests, a 2-way ANOVA was performed on the distance scores. Although the group differences did not achieve statistical significance ($F_{(1,18)}=2.1$, $P>0.05$) there was a statistically significant test effect ($F_{(3,54)}=24.9$, $P<0.001$) and a group × test session interaction ($F_{(3,54)}=5.1$, $P<0.005$). In light of the statistically significant interaction, the treatment groups were compared on each test. Fig. 5A presents the within-session locomotion distance scores for the APO paired vs. unpaired groups on the saline test. As is evident in Fig. 5A. the group locomotion scores following saline were similar ($F_{(1,18)}=0.04$, $P>0.05$). The results of the tests in which the groups received 0.05 mg/kg APO/saline are presented in Fig. 5B. In the APO/saline test, the groups did not differ ($F_{(1,18)}=1.9$, $P>0.05$). In Fig. 5C, the results of the saline/cocaine test are presented. As can be seen in Fig. 5C, the groups exhibited equivalent locomotion stimulant responses to cocaine ($F_{(1,18)}=0.16$, $P>0.05$). However, in the APO/cocaine test, the locomotion scores for the paired group were substantially higher than the unpaired group ($F_{(1,18)}=8.7$, $P<0.01$), indicative of a cocaine sensitization effect.

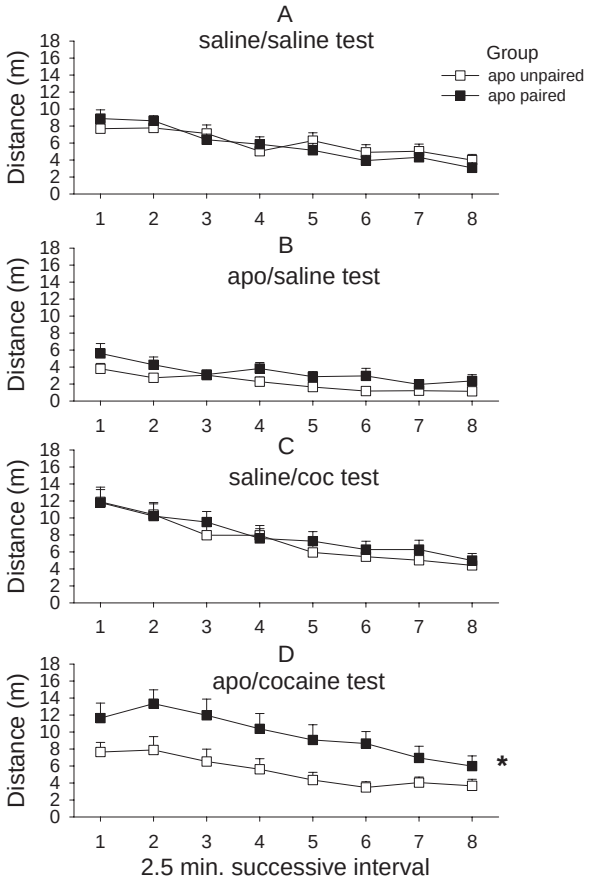

Fig. 5. Means and SEMs for locomotion distance scores in meters (m) in the four conditioning/sensitization tests in Experiment 2. In (A) the within-session locomotion distance scores are presented. Both groups (paired and unpaired) received saline 20 min before testing and saline immediately prior to testing. In (B), both groups received APO 20 min before testing and saline immediately prior to testing. In (C), both groups received saline 20 min before testing and 10.0 mg/kg cocaine immediately prior to testing. In (D), the cocaine conditioning test, both groups received APO 20 min before testing and 10.0 mg/kg cocaine immediately prior to testing. *Denotes $P<0.001$ in paired vs. unpaired group comparisons.

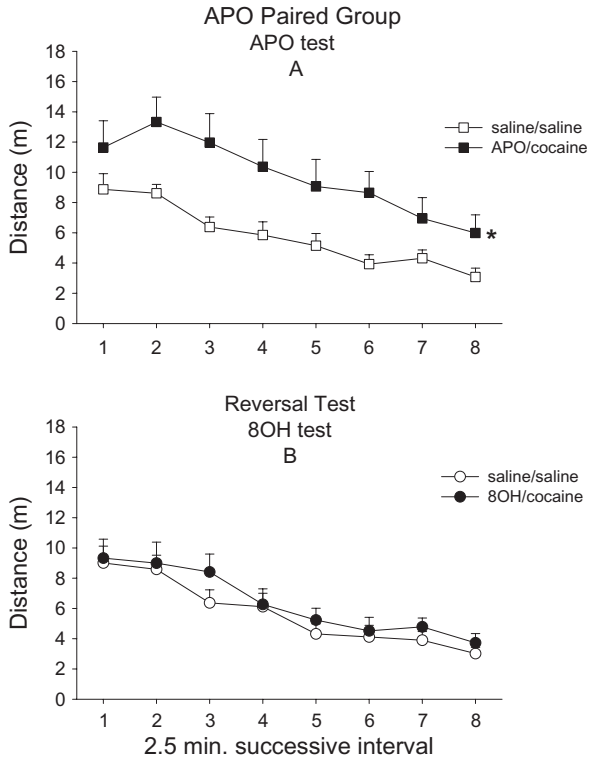

Fig. 6. Means and SEMs of within-session locomotion distance scores in meters (m) in the reversal test phase of Experiment 2 (APO experiment). In (A), the paired group within-session locomotion distance scores are compared for the saline/saline vs. the APO/cocaine tests. In (B), the reversal test, the APO paired group within-session locomotion distance scores are presented for the saline/saline vs. the 8OH/cocaine tests. *Denotes $P<0.01$ in the saline/saline vs. APO/cocaine test comparisons.

Fig. 6A,B presents the locomotion distance scores for the APO paired group on the reversal test. Fig. 6A shows the within-session locomotion distance scores of the APO paired group on the saline test vs. the APO/cocaine test. As is apparent in Fig. 6A, the cocaine treatment induced a substantial locomotor stimulant effect even though the APO paired group had received the inhibitory 0.05 mg/kg APO pretreatment ($F_{(1,18)}=8.4$, $P<0.01$). When the same group received cocaine but with 0.05 mg/kg 8OH as the pretreatment in the conditioning test, then the cocaine locomotion stimulant effect was blocked ($F_{(1,18)}=0.46$, $P>0.05$). As was the case in Experiment 1, the specific drug used in the paired treatment regimen was the critical determinant as to whether a cocaine stimulant effect occurred.

3. Discussion

Context specific sensitization is a well known phenomenon with respect to repeated usage of several psychostimulant drugs (Bedingfield et al., 1996; Cornish and Kalivas, 2000; Cromberg et al., 2000; Erb et al., 2004; Pert et al., 1990; Zavala et al., 2000). Context has typically been manipulated using complex environmental stimuli. The findings obtained in the present studies indicate that the drug state in which the psychostimulant drug is experienced can also be a critical contextual cue. In investigating this question of drug state dependent sensitization, we employed low dose levels of drug treatments with different but selective receptor targets (i.e., $5HT_{1A}$ receptors for 8-OHDPAT and D_1 and D_2 receptors for apomorphine) but, yet, which have behaviorally equivalent response suppression effects (Carey et al., 2004a,b). Using a paired vs. unpaired inhibitory pretreatment/stimulant treatment protocol made it possible to track and detect possible changes in the inhibitory drug treatment. Our findings showed that the paired protocol led to enhancement of the cocaine stimulant effects with repeated pairings and that these changes were not explicable as wearing-off effects of the inhibitory drug treatment in that the inhibitory effects were maintained in the unpaired groups. Furthermore, we found that the sensitization effects were not explicable as environmental cue conditioning or as a diminution of the inhibitory effect of the 8OH and APO pretreatments as a consequence of being paired with cocaine. These inferences are made on the basis of our finding that the paired/unpaired groups did not differ on the post-sensitization saline tests in which either saline, 8OH or APO was given as pretreatments. In addition, we found that both paired and unpaired groups responded similarly to cocaine treatment when saline was administered as pretreatment. This result indicates that responsivity to cocaine had not been altered by the paired/unpaired protocol. Rather, these negative findings highlight the critical role of drug cues in exerting stimulus control over the expression of cocaine sensitization effects.

While the present findings are important for extending the scope of contextual stimuli from exteroceptive situational stimuli to interoceptive drug stimuli, they also add an additional and important methodological innovation. That is, by using different drug treatments with different mechanisms of action but, yet, having equivalent behavioral effects upon the dependent variable of locomotion, we could then perform a critical reversal experiment. Specifically, we were able to conduct the cocaine tests with the pretreatments reversed (i.e., APO paired received 8OH and 8OH paired received APO). If the evidence for drug state specific sensitization we obtained had represented drug response effects only, then the switching of the equivalent response inhibitory drug treatments should not have prevented the expression of the cocaine locomotor stimulant effect. In fact, we found that when the drug pretreatments were reversed, then the locomotor stimulant effects of cocaine were completely blocked. It is also important to note that the 8OH/cocaine and APO/cocaine paired treatments were equally effective in inducing the cocaine sensitization effects. Thus, the critical component for the emergence of sensitization with repeated pairings was not a response domain phenomenon nor an effect of the repeated 8OH/cocaine or APO/cocaine treatments per se. Evidently, the 8OH and APO drug stimuli were reconfigured into either 8OH/cocaine or APO/cocaine stimulus complexes critical to the expression of cocaine sensitization. In this respect, it also needs to be recognized that for the 8OH and APO paired groups, the initial cocaine treatment did not elicit a stimulant effect as compared to the saline baseline; but, yet, it was, nonetheless, still a stimulant treatment as compared to the 8OH or APO/saline treatments. Thus, the Pavlovian prerequisite was present (i.e., UCS effect > CS effect) in that the cocaine stimulant effect was dominant over the inhibitory 8OH/APO effect in the sensitization induction phase. Seemingly, the behavioral dominance of the cocaine treatment permitted the emergence of cocaine sensitization effects in the paired groups. When, however, the drug pretreatments were reversed, this acquired cocaine effect would not be elicited because it was dependent on the interoceptive drug stimulus characteristics of the respective 8OH and APO pretreatments. The fact that the cocaine sensitization effects were completely abolished with the reversal of the 8OH/APO pretreatments unmistakably links the emergence of the cocaine sensitization effects in the paired treatment protocol to associative processes. The dependence of the presence or absence of the cocaine sensitization effects on the drug stimulus underscores the critical role of associative processes in cocaine sensitization. Indeed, these findings highlight the importance of behavioral and associative learning processes in controlling the behavioral expression of cocaine sensitization effects generated by neurobiological mechanisms (Grignaschi et al., 2004; Hu et al., 2004; Javaram and Steketee, 2004, 2005; Nasif et al., 2005; Szumblinsky et al., 2004; Williams and Steketee, 2005). This gating of the behavioral expression of cocaine sensitization by a highly selective brain receptor target offers a new behavioral model to investigate the critical role of contextual stimuli in psychostimulant sensitization phenomena.

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