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Cocaine-conditioned behavioral effects: a role for habituation processes

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Abstract

Cocaine has potent locomotor stimulant effects in rodents, which seemingly can become conditioned to test environment cues. In two experimental protocols, we measured the effects of cocaine on locomotor activity and grooming behavior, and subsequently tested whether these cocaine effects became conditioned to contextual cues. In the first experiment, three groups of rats received 14 injections of either saline or cocaine (10 mg/kg) paired or unpaired to the test environment. Cocaine increased locomotion and decreased grooming during treatment and on the conditioning test. Over the course of the treatment phase, however, the saline- and cocaine-unpaired groups but not the cocaine paired group developed progressively lower locomotion and higher grooming scores indicative of substantial habituation effects. To examine whether the cocaine may have impaired the acquisition of habituation effects rather than induce a Pavlovian cocaine conditioned response, an additional experiment was conducted in which two additional non-habituation saline and cocaine control groups were added to the experimental design. On a conditioning test, the two non-habituation control groups were equivalent in activity and grooming behavior to the cocaine-paired group. The findings were consistent with a failure by cocaine-paired animals to acquire habituation effects, which could transfer to the non-cocaine state. The connection between cocaine and novelty/habituation may have substantial importance for understanding cocaine effects.
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Keywords: Cocaine; Locomotion; Pavlovian conditioning; Habituation; Open-field; Grooming; Novelty

1. Introduction

Pavlov (1927) first demonstrated that a drug effect could be conditioned to situational cues. This dimension of drug action has received increasing recognition as an important aspect of drug use (Eikelboom and Stewart, 1982; Poulos and Cappell, 1991; Siegel, 1989; Stewart and Eikelboom, 1987; Stewart and Vezina, 1988). One long standing focus of drug conditioning has been the conditioning of drug-induced autonomic nervous system effects (Eikelboom and Stewart, 1982; Pavlov, 1927). More recently, Pavlovian drug conditioning has expanded to include psychostimulant-induced motoric and reward effects (Barr et al., 1983; Beninger and Hahn, 1983; Carey and Damianopoulos, 1994; Matsuzaki et al., 1989; Pickens and Dougherty, 1971; Stewart and Eikelboom, 1987; Stewart and Druhan, 1993).

There are many variables which can impact upon psychostimulant drug conditioning effects (Damianopoulos and Carey, 1992; Post et al., 1992; Sarter, 1991; Schiff, 1982; Siegel, 1989; Stewart and Eikelboom, 1987; Stewart and Vezina, 1988) and include variables related to drug treatment regimen (i.e., continuous vs. intermittent schedules), dose, route of administration, time between injections and test environment exposure, duration of exposure, interval between drug treatment and tests for conditioning, as well as other organismic variables such as gender and developmental stage (Haaren and Meyer, 1991; Johansson et al., 1992; Zeigler et al., 1991). Overall, such variables pertain to the modulation of the drug as an unconditioned stimulus (UCS). In order to demonstrate that the drug UCS did in fact induce conditioning, an integral component of a drug conditioning study is the inclusion of an unpaired drug treatment control group. Such a control group has an equivalent drug history to the paired drug group but lacks the contiguity relationship to the conditioned stimulus (CS). This paired/unpaired design is a satisfactory paradigm to rule out behavioral effects on a conditioning test, which could be attributed to persistent organismic changes induced

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by exposure to the drug treatment. While one can precisely specify the unconditioned drug stimulus through specification of the drug, the dose (mg/kg) and the route of administration (subcutaneous, intraperitoneal or intramuscular), the more complex problem, however, is the specification of the unconditioned response (UCR) in observational terms (Schwarting et al., 1993). In drug conditioning of autonomic nervous system responses, the drug induced response can be readily identified as a perturbation in the homeostatic response system and, importantly, the conditioned response can be identified against a background of a stable homeostatic response baseline (e.g., body temperature). In contrast, a psychostimulant drug effect is behaviorally expressed as a modulation of spontaneous on-going motoric behaviors involving changing response baselines subject to the influence of behavioral processes such as attention and familiarization/habituation in addition to the drug treatment.

Critically, a psychostimulant drug treatment may not only affect the changing baseline rate of motoric activity directly but it may also interact with the complex behavioral processes related to familiarization/habituation. As a consequence, equivalent behavioral effects in post-treatment tests for conditioning might be observed following a psychostimulant drug treatment either through its possible interaction with non-drug behavioral processes related to novelty/familiarization or by Pavlovian conditioning of the repeated psychostimulant drug induced motoric activation effect (Carey and Damianopoulos, 1993; Damianopoulos and Carey, 1993).

The locomotor stimulant effects of cocaine have been extensively studied (Heidbreder and Shippenberg, 1994; Koob, 1992; Mattingly et al., 1994) and there is evidence that theses effects can become conditioned to associated contextual cues (Franklin and Druhan, 2000a,b; Carey and Damianopoulos, 1994; Damianopoulos and Carey, 1995; Druhan and Wilent, 1999). While these studies provide support for the Pavlovian conditioning of the locomotor stimulant effects of cocaine in the context of the paired vs. unpaired conditioning paradigm, the use of open-field behavior presents additional complexity. In Pavlovian conditioning, the CS is assumed to be neutral with respect to the UCS (i.e., a tone does not elicit salivation). An open-field, however, is an UCS, which elicits unconditioned behavior such as locomotion. A moderate cocaine dose (e.g., 10 mg/kg) may modulate this unconditioned behavioral response. In a Pavlovian conditioning framework, however, the open-field is designated as the CS. The cocaine treatment is the UCS and the behavior, the UCR. The open-field, however, is not a neutral CS but is in fact an UCS. Furthermore, the open-field does not generate a stable UCR in that with repeated exposure to the same environment, non-drug animals undergo significant habituation to the environment such that the behavioral UCR can become substantially attenuated (Cerbone and Sadile, 1994).

Since the control treatment groups, in drug conditioning studies using open-field behavior, experience the test environment in the non-drug state, their initial behavioral response to the novel stimuli of the test environment undergoes an inhibitory/familiarization/habituation process resulting in decreased behavioral responding. What is unknown under these circumstances, however, is whether the habituation effects occur in a parallel but latent manner in the drug treated group and whether this effect transfers to the nondrug state when the animals are given posttreatment nondrug tests for conditioning (Damianopoulos and Carey, 1994). Seemingly, the behavioral effects of retarded or blocked habituation can be behaviorally equivalent to conditioned psychostimulant drug effects (i.e., decreased inhibition can result in a behavioral outcome that is equivalent to increased facilitation). This is an issue of fundamental importance to the interpretation of psychostimulant drug-conditioning studies.

In the present report, we conducted several studies, which have relevance to the issue of habituation processes to cocaine conditioning of open-field behavior. In the first experiment, we used a conventional paired/unpaired conditioning design and tracked the behavioral effects of a series of paired/unpaired cocaine treatments. We used locomotion and rearing as response measures of locomotor stimulation and included grooming behavior as another response measure affected by cocaine (Cooper and Van der Hoek, 1993), which is not reliably correlated with locomotor stimulant effects (Damianopoulos and Carey, 1994). Prior to initiation of the conditioning paradigm, we matched groups on the dependent behavioral measures to provide the opportunity for within group assessments of the effects of the repeated paired/unpaired cocaine exposures to the open-field environment. The objective was to provide a detailed measurement of the changes in behavior, which occur with repeated open-field testing in order to more effectively address processes such as habituation as a possible contributor to conditioned cocaine behavioral effects in a saline test for conditioning. In an additional directly related experiment, we included unpaired control groups, which not only received cocaine or saline unpaired to the test environment cues but also were not tested in the open-field after the initial matching test. These controls served as parallel non-habituated controls to permit a between group assessment of the comparative effects of non-habituation to the test environment vs. exposure to the test environment paired to a cocaine treatment.

2. Method

2.1. 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/light 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 (intraperitoneally) 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 (6 a.m.–6 p.m.). The experimental protocol (IACUC 4-E) was approved by the Veterans Administration Medical Center's Subcommittee for Animal Studies.

2.2. Drugs

Cocaine hydrochloride (Sigma, St. Louis, MO) was dissolved in sterile distilled H_2O to a concentration of 10 mg/ml. All injections were administered intraperitoneally.

2.3. Apparatus

All of the behavioral tests were conducted in square open-field compartments which were 60 × 60 × 45 cm. Closed-circuit video cameras (RCA TC7011U) were mounted 50 cm above the open-field enclosures. All signals were analyzed by a video tracking system using a criteria of 2 cm for a movement to be detected (Videomex-V from Columbus Instruments, Columbus, OH). The data was imported into a PC compatible computer. 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 noise (80 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 in the test chamber and turned off upon removal from the test chamber. Testing was conducted under conditions of red light illumination to avoid the 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 animal's head was blackened with a nontoxic marker and the camera only tracked this feature of the rat's body. During each session, data was collected every 2.5 min by the computer. Dot matrix printers (Epson FX-286e) were placed outside the test rooms and were connected to the image analyzers by a parallel cable and the computer screen tracings of the animal's movement were printed out every 2.5 min. 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 each camera to video tape selected sessions. The videotapes of all pretreatment tests, all drug treatment sessions in Experiment 1, the first and last drug treatment sessions in Experiment 2 and all conditioning tests were scored for rearing and grooming behavior every 5 min. Three experimenters uninformed of the drug treatments scored the videotapes for grooming and rearing. Rearing responses were scored each time the animal reared up on its hindlimbs and raised its forelimbs off the floor onto the wall or into the air. Grooming was timed in seconds and included both facial and flank grooming behavior. All experimenters underwent training prior to data collection using videotapes from other experiments. Scoring for the present experiments were undertaken only after experimenters established intra- and inter-experimenter reliability coefficients of $r > .9$ on scoring 2 successive days of videotapes of open-field test sessions.

2.4. Behavioral testing

2.4.1. Experiment 1

Initially, 39 animals received 10 days of daily handling including 3 days of saline injections to acclimate the animals to manipulation and injection procedures. Next, all animals were given a 20-min pretreatment test immediately following a saline injection in the test environment in order to form three groups, which were statistically equivalent with respect to the dependent variable of locomotion distance. One day after the completion of the matching protocol, the three matched groups received 14 additional 20-min treatment sessions in which spontaneous behavior was recorded. Altogether, these 14 20-min test sessions were conducted over 3 weeks (five per week). All animals received two injections. The first was administered immediately prior to testing in the open-field and the second was administered 2 h after testing in the homecage. The treatment groups were saline–saline ($n = 19$), cocaine–saline (10 mg/kg, $n = 10$) and saline–cocaine (10 mg/kg, $n = 10$). The first treatment specified in each injection pair was the one administered immediately prior to testing and the second treatment was the one administered 2 h after testing in the homecage. These treatment sessions served as the acquisition phase designed to establish a conditioned drug response to test environment cues. Four days after the last drug treatment session, a test for conditioning was conducted. On the conditioning test, all groups received saline injections immediately prior to testing. Thus, the injection protocol for the conditioning test was the same as the treatment injection protocol except that only saline treatments were administered. This treatment procedure was a conventional paired/unpaired Pavlovian conditioning protocol.

2.4.2. Experiment 2

The same handling and acclimation procedures were followed in the second set of experiments. After completion of the adaptation measures, all animals were given a 20-min test in the open-field test environment. Using the results from this test, the animals were subdivided into five groups matched for locomotion distance. Subsequently, three groups [saline/saline ($n = 15$), saline/cocaine ($n = 14$) and cocaine/saline ($n = 14$)] received nine additional 20-min tests (three per week) in the open-field environment. The remaining two groups were not tested but received either saline ($n = 15$) or cocaine (10 mg/kg, $n = 15$) on the same days in the

homecage. Four days after the completion of this treatment protocol, all five groups received saline immediately before a 20-min test in the open-field. This test served as the conditioning test.

The strategy underlying this experimental design was to assess the possible antihabituation effects of cocaine by determining if the cocaine treatments given during open-field testing were the equivalent of not being exposed to the open-field environment. The groups, which received saline or cocaine in their homecage, served as the non-habituation control groups. If cocaine given prior to testing in the open-field blocked habituation to the test environment then this effect would be the functional equivalent of not being placed in the test environment.

2.5. Statistical analyses

One-way, two-way and three-way analysis of variance (ANOVA) was used to analyze the behavioral data to determine possible group effects and interactions. In order to make specific group comparisons, post-hoc Duncan's multiple range tests were performed. Pearson's correlational analysis was used to assess the relationship between drug treatment behavior and conditioning test performance and between dependent variables. $P<.05$ was used as the criterion for statistical significance.

3. Results

3.1. Experiment 1

Three-way ANOVAs were performed for the drug treatment phase of the experiment: Group × Treatment Day × Interval. None of the three-way interactions were statistically significant [$F(14,182)=1.1$, $P>.05$; $F(6,76)=0.8$, $P>.05$; $F(6,76)=1.1$, $P>.05$ for distance, grooming and rearing, respectively]. In the absence of a three-way interaction, the presentation of this facet of the results focused on two-way ANOVAs to evaluate the Group × Treatment Day effects. Fig. 1 presents the Group × Treatment Day for the initial pretreatment test and the 14 days of treatment. As is evident in Fig. 1, the groups were closely matched on the pretreatment test [$F(2,36)=0.34$, $P>.05$; $F(2,36)=1.1$, $P>.05$; $F(2,36)=0.62$, $P>.05$ for distance, grooming and rearing, respectively]. On the treatment days, cocaine had marked effects on locomotion distance and on grooming behavior, but not on rearing scores [$F(2,36)=40.3$, $P<.001$; $F(2,36)=35.9$, $P<.001$; $F(2,36)=0.86$, $P>.05$ for distance, grooming and rearing, respectively]. The Group × Treatment day interaction was statistically significant only for grooming [$F(26,468)=3.2$, $P<.001$]. The Group × Treatment Day interactions for distance and rearing were $F(26,468)=1.0$, $P>.05$ and $F(26,468)=0.9$, $P>.05$, respectively. As can be seen in Fig. 1, the saline/saline and saline/cocaine groups were virtually equivalent in their behavior across treatment days and that the

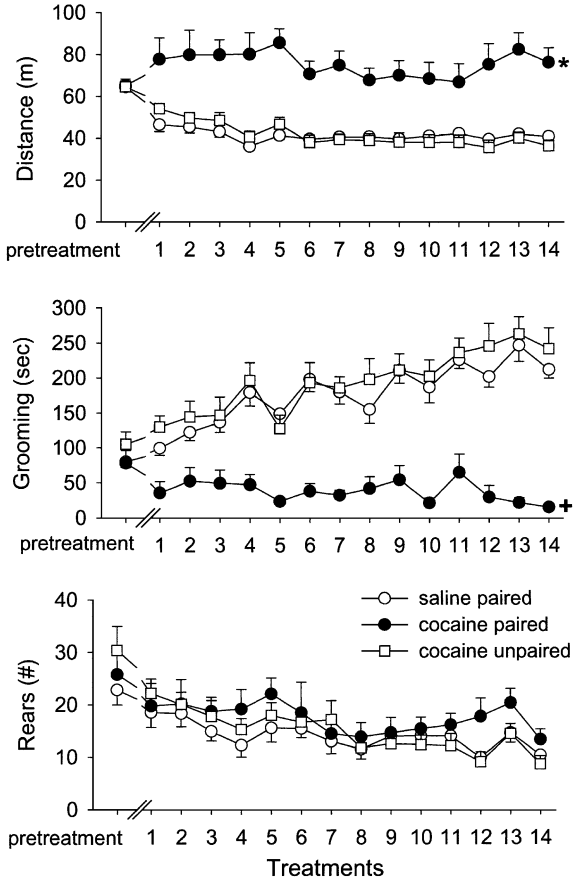

Fig. 1. Means and S.E.M. for distance (upper), grooming (middle) and rearing (bottom) over the course of a series of 20-min open-field tests for three treatment groups. On the pretreatment day, all groups received saline. On the subsequent 14 treatment days, the saline-paired group received saline immediately before testing and 2 h after testing; the cocaine-paired group received cocaine (10 mg/kg) immediately before testing and saline 2 h after testing; the cocaine-unpaired group received saline immediately before testing and cocaine (10 mg/kg) 2 h after testing in the homecage. * Denotes $P<.001$ vs. saline- and cocaine-unpaired. $^{+}$ Denotes $P<.001$ vs. saline- and cocaine-unpaired.

group differences in locomotion distance and grooming behavior were attributable to cocaine effects in the cocaine/saline group. Group comparisons using Duncan's multiple range test indicated that the cocaine/saline group differed statistically ($P<.01$) from the saline/saline and saline/cocaine groups, which did not differ from each other, $P>.05$. Inspection of Fig. 1 (Panel B) indicates that the Group × Treatment Day interaction for grooming behavior was attributable to the progressive increase in grooming behavior in the saline/saline and saline/cocaine groups across test sessions. The strong interaction effect for grooming but not locomotion distance implies that these two measures are not simply mirror images (i.e., the higher the locomotion score, the less time grooming). As is shown in Fig. 1 (Panel B), the grooming times for the saline/saline and the saline/cocaine groups by the last injection averaged over 200 s. This time, however, needs to be placed in the context of the total test session which was 1200 s leaving a large proportion of the

session for other nongrooming behaviors (e.g., locomotion). To examine this matter further, correlation coefficients were determined for treatment days 1 and 14. The correlation coefficients between distance and grooming for the saline/saline, saline/cocaine and cocaine/saline groups were $r = -.39$, $-.01$ and $-.29$, all $P > .05$ for treatment day 1; $r = -.06$, $-.35$ and .05 for treatment day 14, all $P > .05$. While overall there were predominantly negative correlations between distance scores and grooming times, none of the correlation coefficients approached the $P < .05$ level for statistical significance.

Another consideration relevant to the cocaine induced behavioral responses is the effect of cocaine on within session performance. In that statistically significant cocaine effects were only observed for locomotion distance and grooming behaviors, the within session results for these behaviors are presented in Fig. 2. On the pretreatment day, the groups were closely matched and there were no statistically significant group differences [$F(2,36) = 0.13$, $P > .05$ and $F(2,36) = 1.1$, $P > .05$, for distance and grooming, respectively], or Group × Interval interactions [$F(14,252) = 0.37$, $P > .05$ and $F(14,252) = 0.52$, $P > .05$]. There were statistically significant interval effects [$F(7,14) = 40.2$, $P < .001$ and $F(3,6) = 3.6$, $P < .05$ for distance and grooming, respectively] indicative of within session changes in behavior. For treatment days 1 and 14, there were statistically significant group differences for distance [$F(2,36) = 8.6$ and 26.0, $P < .001$ treatment days 1 and 14, respectively] and grooming [$F(2,36) = 10.1$ and 43.2, $P < .001$ for treatment days 1 and 14, respectively] but the Group × Interval interactions were not statistically significant (all F values < 1.3, $P > .05$). For the distance measure, there were highly significant interval effects [$F(7,14) = 23.8$ and 41.7, $P < .001$ for days 1 and 14, respectively], but there were not statistically significant interval effects for grooming behavior [$F(3,6) = 0.18$ and 1.6, $P > .05$ for treatment days 1 and 14, respectively]. In addition to indicating differences between grooming and locomotion behavior as previously observed in the correlational analyses, the within session data also shows that the onset of cocaine effects occurred rapidly and in the first session. The presence of a strong interval effect for locomotion coupled with the absence of

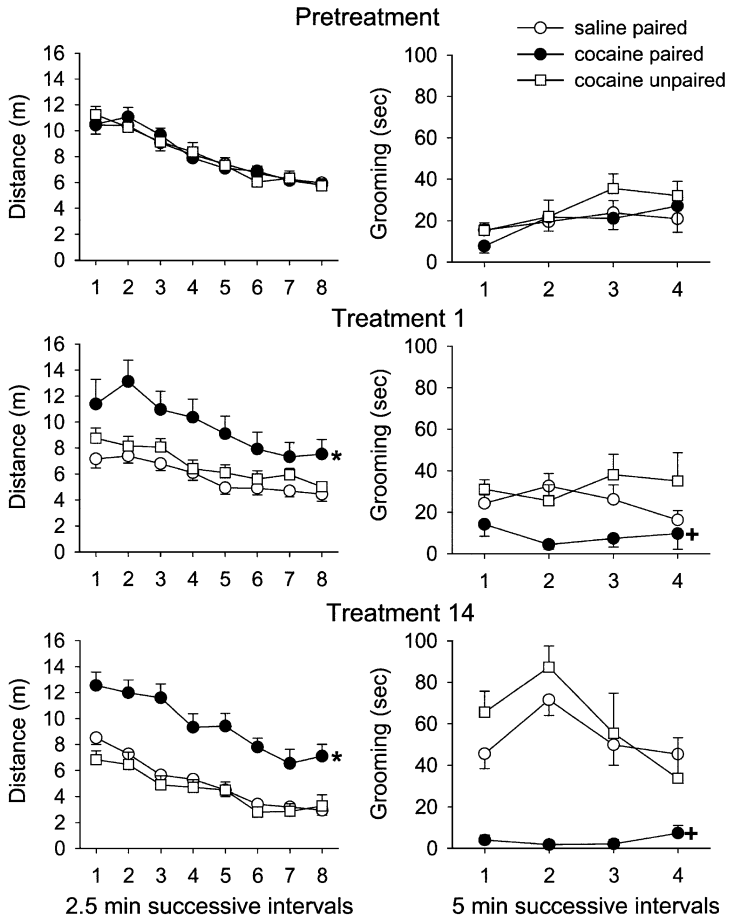

Fig. 2. Means and S.E.M. for saline-paired, cocaine (10 mg/kg)-paired and cocaine (10 mg/kg)-unpaired on open-field behavior on three 20-min tests: pretreatment, treatment days 1 and 14. The panels on the left side of the figure present the within-session distance scores in eight successive 2.5-min intervals. The panels on the right side depict the within session grooming times in four successive 5-min intervals. * Denotes $P < .001$ vs. saline- and cocaine-unpaired. $^{+}$ Denotes $P < .001$ vs. saline- and cocaine-unpaired.

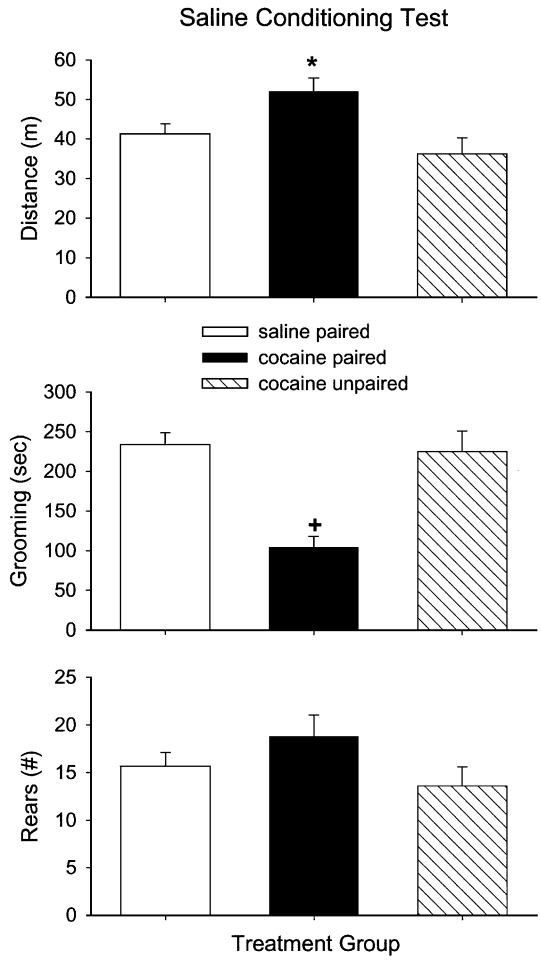

Fig. 3. Means and S.E.M. for the saline-paired, cocaine-paired and cocaine-unpaired groups on a 20-min saline test for conditioned cocaine behavior. The upper panel presents the mean distance scores, the middle panel the mean grooming times and the bottom panel, the mean number of rears. * Denotes $P<.05$ vs. saline-paired and cocaine-unpaired groups. $^{+}$ Denotes $P<.01$ vs. saline-paired and cocaine-unpaired groups.

a Group × Interval interaction indicated that the cocaine treatment did not alter within session habituation but rather elevated the response level overall.

The results for the conditioning test are presented in Fig. 3. In that none of the Group × Interval interactions were statistically significant [$F(14,252)=1.3$, $P>.05$; $F(6,108)=1.5$, $P>.05$; $F(6,108)=1.1$, $P>.05$ for distance, grooming and rearing, respectively] only the session total scores are presented. There were statistically significant group differences obtained on the conditioning test for distance [$F(2,36)=5.4$, $P<.01$] and grooming [$F(2,36)=13.6$, $P<.001$] but not for rearing [$F(2,36)=0.8$, $P>.05$]. As was the case for the drug treatment phase, there was a statistically significant interval effect for locomotion distance [$F(14,252)=36.9$, $P<.001$] but not for grooming [$F(3,6)=1.4$, $P>.05$]. The results for the conditioning test are presented in Fig. 3 as bar graphs for session totals. As is evident in Fig. 3, the saline/saline and saline/cocaine groups were statistically equivalent but the cocaine/saline group had elevated locomotion distance scores and decreased grooming times ($P<.01$, Duncan's multiple range tests). These findings are consistent with Pavlovian conditioning studies of cocaine behavioral effects in that the unpaired cocaine treatment had no effect relative to saline treatments and the behaviors affected by cocaine during the treatment phase were the ones for which statistically significant effects were obtained on the conditioning test. In order to put these effects in perspective, however, it is relevant to recognize that the conditioning test for the cocaine/saline group was its second saline test. The pretreatment test was its first saline test. For the saline/saline and saline/cocaine groups, however, the conditioning test was their 16th saline test. In order to match the groups for saline tests, we next compared the cocaine/saline group scores on the conditioning test with the saline/saline and saline/cocaine group scores on their second saline test (i.e., treatment day 1). As can be seen in Fig. 4,

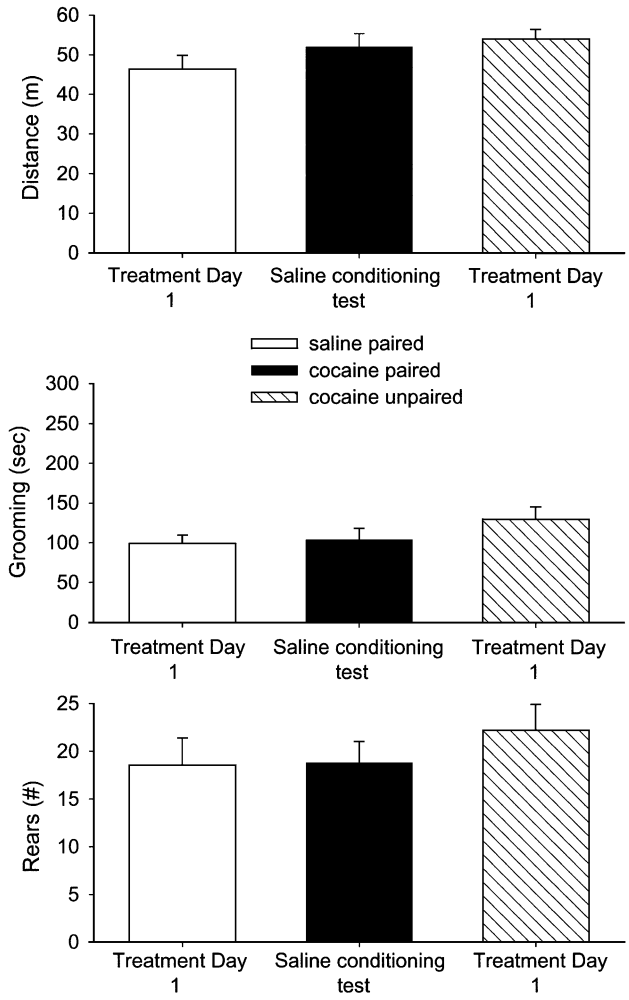

Fig. 4. Means and S.E.M. for the saline-paired, cocaine-paired and cocaine-unpaired groups on the 20-min tests, which were the second saline tests for each group. For the cocaine-paired group, this test was the saline conditioning test and for the saline-paired and cocaine-unpaired groups, it was the first treatment test. All groups had received a saline pretreatment test, which was their first saline test.

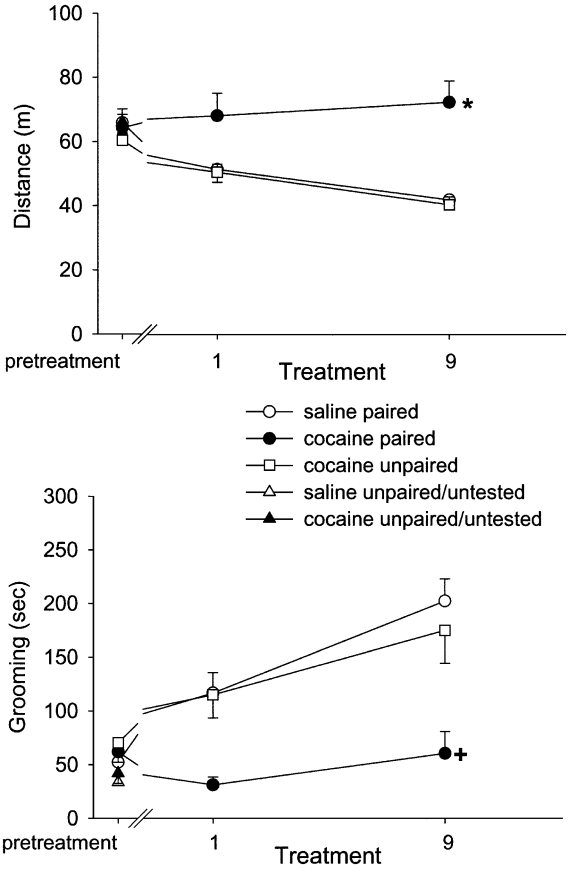

Fig. 5. Means and S.E.M. for the saline-paired, cocaine-paired, cocaine-unpaired, saline-untested and cocaine-untested groups on the 20-min open-field pretreatment test and the first and ninth treatment tests. All five groups received the pretreatment test but only the saline-paired, cocaine-paired and cocaine-unpaired groups received the subsequent nine 20-min tests. The upper panel presents the distance scores and the lower panel the grooming times for the respective groups. * Denotes $P<.001$ vs. saline- and cocaine-unpaired. $^{+}$ Denotes $P<.001$ vs. saline- and cocaine-unpaired.

the three groups appear closely matched when the second saline test is used as a way to compare groups. A statistical comparison of the group differences using one-way ANOVA indicated that none of the mean differences were statistically significant [$F(2,37)=1.2$, 1.1 and 0.4, all $P>.05$ for distance, grooming and rearing, respectively]. Another pertinent analysis of the conditioning test results was conducted by performing a correlational analysis between distance and grooming scores on the last treatment day and on the conditioning test for the saline/saline, saline/cocaine and cocaine/saline groups. For the saline/saline groups, the correlation coefficient between these two test days were $r=.80$, $P<.001$ and $r=.61$, $P<.005$ for distance and grooming, respectively; for the saline/cocaine group, $r=.73$, $P<.01$ and $r=.6$, $P<.05$ for distance and grooming, respectively, and for the cocaine/saline group, $r=.22$, $P>.05$ and $r=.29$. $P>.05$ for distance and grooming, respectively. Thus, the cocaine UCR did not reliably predict the putative cocaine CR.

The results for the first phase of Experiment 2 are presented in Fig. 5, which shows the pretreatment and treatment days 1 and 9 distance and grooming scores. As in Experiment 1, rearing scores were not significantly affected by the treatments ($P>.05$) and, therefore, are not included. The pretreatment test not only includes saline/saline, saline/cocaine and cocaine/saline groups similar to Experiment 1, but also contains the saline and cocaine unpaired and untested treatment groups, which received no testing after the pretreatment matching test and received all of their injections in the homecage during the treatment phase. On the pretreatment test, the five groups were closely matched and there were no statistically significant group differences [$F(4,72)=0.38$ and 1.2, $P>.05$ for distance and grooming, respectively]. For the three groups tested in the treatment phase, the overall pattern of cocaine treatment effects is similar to Experiment 1 [$F(2,40)=12.9$ and 13.5, $P<.001$ distance and grooming, respectively]. Duncan's multiple range test indicated that the saline/saline and saline/cocaine groups were not statistically different ($P>.05$),

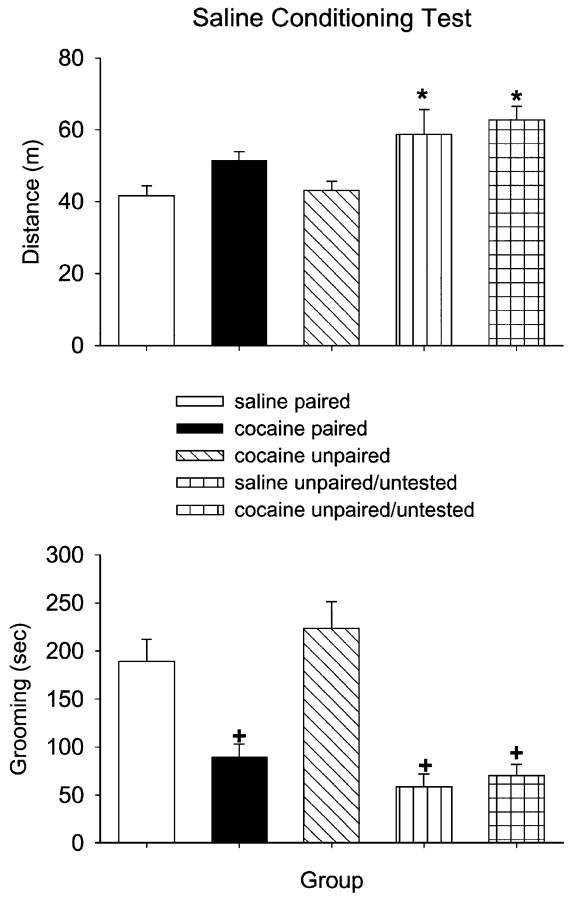

Fig. 6. Means and S.E.M. for the five groups on the 20-min saline conditioning test. For the saline- and cocaine-unpaired/untested groups, this was their second test in the open-field, whereas for the saline-paired, cocaine-paired and cocaine-unpaired groups, this was their 11th 20-min test in the open-field environment. * Denotes $P<.05$ vs. saline-paired and cocaine-unpaired groups. $^{+}$ Denotes $P<.01$ vs. saline-paired and cocaine-unpaired groups.

but that the cocaine/saline group was different from the other two groups on both response measures ($P < .01$). The changes from the pretreatment test through treatment test 9 were evaluated using a two-way ANOVA. As is apparent in Fig. 5, there were statistically significant interaction effects for both distance and grooming when the pretreatment level is included in the analysis [$F(4,80) = 6.3$ and 6.2, $P < .001$ for distance and grooming, respectively].

The conditioning test results are presented in Fig. 6. As was observed in Experiment 1, there were statistically significant group differences obtained for distance and grooming scores [$F(4,72) = 5.2$, $P < .01$ and 15.8, $P < .001$], respectively. A comparison of group differences using Duncan's multiple range test indicated that the saline and cocaine unpaired and untested groups had higher distance scores than the saline/saline and saline/cocaine groups ($P < .05$) but were not higher than the cocaine/saline group ($P > .05$). For grooming behavior, the saline and cocaine unpaired/untested groups and the cocaine/saline group had statistically equivalent as well as significantly lower grooming scores than the saline/saline and saline/cocaine groups ($P < .01$).

4. Discussion

One approach to the issue of Pavlovian conditioning of cocaine effects to the test environment cues of an open-field environment is to posit the test environment as the CS and the cocaine treatment as the UCS which elicits a UCR. With repeated pairings, the expectation is that the test environment cues will come to elicit a fractional cocaine UCR as the CR. The first experiment results were consistent with this formulation in that the test environment cues elicited a cocaine CR, which was fractional response of the cocaine UCR. That is, the cocaine paired group exhibited higher locomotor distance scores and lower grooming scores than the two control groups on the cocaine treatment tests and similar but smaller differences were observed on the saline conditioning test. The analysis of the behavioral response of the treatment groups over the course of the treatment phase also revealed behavioral changes from the first test in the open-field to the last test in the open-field for the groups which did not receive cocaine paired to placement in the open-field. For those animals, the open-field was not simply a neutral CS but rather was an UCS, which elicited behaviors. Furthermore, the effect on the UCR elicited by the open-field changed substantially with repeated testing such that locomotor activity decreased by approximately one third and grooming more than doubled. In contrast, the animals, which received cocaine, changed little in comparison to their initial exposure to the test environment prior to the cocaine treatment. While it is appropriate to characterize the cocaine treatment a drug UCS, it also seems appropriate to characterize the open-field as an UCS. Thus, the cocaine paired group appears to have both the cocaine and the open-field as dual UCS treatments and their interaction generates the behavioral effects in the open-field. Rather than labeling the behavior of the cocaine-paired group in the open-field as a cocaine UCR, it would appear valid to consider this behavior as a modified open-field UCR. From this perspective, the open-field UCR in the non-drug group is modified by habituation processes, whereas the cocaine paired group is modified by cocaine and by habituation processes. Instead of the conditioning test representing a cocaine CR, this test can be conceived of as an altered open-field UCR. For the control groups, this UCR is altered by habituation processes and in the cocaine paired group by the combined effect of cocaine and possibly habituation processes. Prior to the conditioning test, the cocaine paired group had experienced the test environment once following a saline injection, whereas the control group had received 15 saline tests. When the cocaine-paired and the control groups were compared with open-field saline tests equated among groups, then there were no effects of the paired cocaine treatments. This analysis lends support to the importance of the contribution of habituation effects to results obtained on the saline conditioning test.

In the second experiment, additional control groups were added to the basic cocaine-paired/unpaired experimental design. These control groups were saline and cocaine unpaired groups, which were not tested. In the conditioning test, it was observed that the unpaired and untested cocaine and saline groups were equivalent in their performance and were statistically indistinguishable from the cocaine paired group. Thus, on the cocaine conditioning test, the cocaine paired treatment was functionally equivalent to non-testing. The saline and cocaine non-tested groups differed from the saline paired and cocaine unpaired tested groups only in terms of exposure to the test environment. The untested groups received one saline test exposure prior to the conditioning test, whereas the saline- and cocaine unpaired tested groups received 10 exposures. The cocaine paired group also had received one exposure to the test environment without cocaine prior to the conditioning test. Thus, the untested groups and the cocaine paired group had the same non-cocaine exposure with the test environment. Again, when non-cocaine exposure to the test environment was held constant, then the cocaine paired treatment had no effect on a saline conditioning test. Previously, we (Damianopoulos and Carey, 1994) and others (Ahmed et al., 1995, 1998) have drawn attention to the similarities in behavioral effects between drug induced antihabituation effects and putative Pavlovian conditioned drug response in studies using open-field behavior to assess psychostimulant drug conditioning. The present study is consistent with a cocaine induced anti-habituation effect.

Seemingly, the most prosaic way to account for a cocaine antihabituation effect is to consider it a drug state dependent effect. There is substantial evidence that cocaine (Callahan and Cunningham, 1993; Carey et al., 2001, 2002; De La

Garza et al., 1998; Geter-Douglass and Riley, 1996; Glennon, 1986; Schreiber and De Vry, 1993; Schreiber et al., 1995; Witkin et al., 1991; Przegalinski and Filip, 1997) has drug stimulus properties. The case can be made therefore, that the cocaine interoceptive drug stimuli together with the exteroceptive test environment stimuli compromise the stimulus complex of the environment, which the animals experience during the paired drug treatment phase of the experiment. Subsequently, when a saline test is conducted and the interoceptive drug stimuli are no longer present, the cocaine-treated animals experience the environment as relatively more novel than animals, which have repeatedly experienced the test environment in a non-drug state. According to this formulation, the cocaine acting as a stimulus source creates a somewhat more complex or different environment from the saline treated animals. While drug stimuli may be a necessary condition for drug state dependent effects to occur, it has not been established that it is also a sufficient condition for the induction of a drug state dependent effect. The case for such an interpretation would be supported if the cocaine treated groups demonstrated a habituation effect during the cocaine treatment phase. Considering cocaine simply as an additional stimulus, one would expect that the animals treated with cocaine would undergo habituation during the drug treatment phase to the test environment, since they would be gaining increasing familiarity with the test environment plus the drug stimuli. When subsequently tested without cocaine, this habituation would be lost to some extent and an increase in behavior would occur as a dishabituation effect. When the changes in locomotor activity of the cocaine-treated groups across treatment days were compared, however, no habituation effect to repeated testing was observed. In that there was no habituation to the test environment evident over the cocaine treatments, a drug state dependent habituation effect appears to be a less tenable interpretation of the saline conditioning test findings. In terms of locomotor activity, the cocaine group exhibited within session habituation, which was equivalent to the saline group. Thus, the habituation process did not appear to be affected by cocaine but rather it was the carry over from one session to another, which was affected. It appears plausible to argue that the cocaine treatment interfered with the retention of the habituation process. We have previously reported (Damianopoulos et al., 1999) that 5-HT_{1A} agonists and antagonists can interfere with habituation to a novel environment. Interestingly, there is evidence that hippocampal serotonin has an important role in processes relevant to habituation (Bidzinski et al., 1998). In view of the importance of the hippocampus in habituation to a novel environment, it is of interest that we have recently reported that cocaine (Muller et al., 2002a) has substantial effects upon serotonin in the hippocampus. Perhaps, these effects on serotonin interfere with information storage in the hippocampus pertinent to habituation (Lemaire et al., 1999; Patacchioli et al., 1989; Sadile et al., 1992).

Another way to consider the absence of between session habituation effects in the cocaine paired treated animals is as if a two factor process in which both sensitization and habituation processes occur. Many studies have indicated that cocaine can induce sensitization effects (Kalivas et al., 1992; Koff et al., 1994; Pert et al., 1990; Segal and Kuczenski, 1992). At a 10-mg/kg dose level, cocaine sensitization effects appear to be modest and it is possible that the habituation effects generated by repeated testing can balance the sensitization effects induced by repeated cocaine treatments. One could then argue that on a conditioning test, the habituation effects are present but are partially counteracted by cocaine conditioned stimulant effects. The challenge is to experimentally validate the presence of a masked or latent habituation effect in the paired cocaine treatment group. In the present experiments, all animals experienced one 20-min session in the test environment so that matched groups could be formed and the baseline response of all animals to the novel environment could be determined. The marked decrease in locomotion in non-cocaine-treated animals from the pretreatment session to the first treatment test session indicated that substantial habituation occurred to this one test session experience. Considering cocaine effects on a conditioning test in terms of habituation effects, one would predict that a maximal cocaine effect on a conditioning test relative to unpaired control animals would occur if the cocaine-paired group only experienced the test environment under the influence of cocaine, whereas the controls received a series of tests in which they became well habituated to the environment. If the comparison group was one which received saline or cocaine injections in the homecage but never tested, then the results of the present study suggest that the paired cocaine treatments would not have a statistically significant effect relative to nonhabituated controls. Seemingly, cocaine paired treatment effects can be matched by unpaired and untested control groups. This type of comparison of a cocaine paired treatment vs. an unpaired untested control would appear to be germane to other situations in which cocaine conditioned effects are considered to be prominent but which entail comparison groups, which undergo habituation to the test environment cues such as context specific sensitization (Anagnostaras and Robinson, 1996; Pert et al., 1990) conditioned place preference (CPP) (Bardo et al., 1995; Bedingfield et al., 1998; Khroyan et al., 1999; Mucha et al., 1982; Shippenberg and Heidbreder, 1995). It has been shown that in a place preference test animals will exhibit a preference for the more novel environment (Bardo and Bevins, 2000; Bevins and Bardo, 1999; Carr et al., 1988; Klebaur and Bardo, 1999; Scoles and Siegel, 1986). Thus, drug treatments which interfere with habituation to an environment in a CPP protocol would make that environment relatively more novel than the saline associated environment in a saline CPP test. Possible habituation differences may contribute to some of the CPP effects induced by cocaine as well as other drugs. While there have been efforts to cope

with this issue in CPP paradigms by the use of an additional environment to which the animals are less habituated (Cador et al., 1992; Hand et al., 1989; Parker, 1992), this type of manipulation, however, still leaves a potential imbalance in that on the CPP test the cocaine-paired group could be viewed as being exposed to one more relatively novel environment in the conditioning test than the saline group. That is, the cocaine paired environment could be considered as an additional novel environment for the cocaine-paired group on the saline conditioning test. Employing an unpaired and untested control group, which receives saline in the same environment as the paired cocaine group but is not tested in the environment in which the cocaine paired group is tested, is needed in this type of experiment. It is certainly of importance to determine if there is any arrangement of paired cocaine treatments in which spontaneous exploratory behavior is used as the dependent variable that a cocaine-paired treatment results in facilitation of behavioral activity as compared to non-exposure to the test environment. Another interesting attempt to circumvent the anti-habituation issue includes instituting the drug conditioning protocol after animals are well habituated to the environment (Ahmed et al., 1996). This type of approach rests on the assumption that habituation quickly reaches a stable plateau. As indicated in our first experiment, the amount of grooming behavior continued to increase over a large number of exposures to the test environment suggesting that habituation may not reach a steady state. Even using a prehabituation protocol, however, it would be necessary to incorporate an unpaired untested control group during the drug conditioning phase to control for both the potential loss of habituation by non-exposure to the test environment as might be expected for the cocaine paired group, as well as a possible increase in habituation effects in the saline control group, which undergoes additional testing.

In the study of open-field behavior, the locomotor stimulant effects of cocaine are primarily studied. While such behaviors can readily be automated, the present study also points up the utility of studying grooming behavior. While this behavior is less amenable to automatic scoring, it is a behavior reliably affected by cocaine (Cooper and Van der Hoek, 1993) and, as is shown in the present study, yields robust effects on saline tests for conditioning. Furthermore, the correlational analysis indicated that the grooming scores and locomotion scores were not reliably correlated indicating that the measures were not mirror images. Thus, the present study indicates the utility of the measurement of grooming behavior for the study of cocaine as well as habituation effects. The further observation that the behavioral scores of an animal on the cocaine treatment test did not reliably correlate with scores on the saline-conditioning test argues against the conditioning test results being an indication of a Pavlovian conditioned response in that the conditioning model would predict a substantial and statistically reliable correlation between the unconditioned drug response and the conditioned drug response. The grooming measures also appear useful to an examination of the effects of repeated testing in the open-field environment. With locomotor behavior, the largest effect appeared to be the decrease from the first to the second exposure to the test environment. Grooming behavior, however, exhibited a progressive increase over a number of test sessions suggesting that a dynamic interaction with the environment can be occurring when little change is evident in locomotor behavior. In addition, reductions in locomotion with repeated treatments only provide a negative measure of habituation, whereas the increase in grooming behavior provides a positive measure of habituation. The use of both positive and negative changes in behavior offer a more effective and complete behavioral analysis pertinent to habituation than is provided by negative changes in locomotor behavior alone.

On a more general level, the present study is relevant to the development of an understanding of the contribution of habituation processes to the study of drug conditioning. There are many drugs including cocaine, which can function as discriminative stimuli. Seemingly then, many drugs would generate equivalent effects to cocaine on a conditioning test if drug stimulus effects were the primary variable accounting for cocaine conditioned drug effects. It would be misleading, however, to equate drug stimulus properties to an exteroceptive stimulus, such as a tone. Rather, drug stimuli can create an altered internal state, such that external stimuli are processed in this altered context. In its most pronounced manifestation, a drug state can be used to promote drug state dependent learning in which there is no apparent transfer of information between the drug state and the non-drug state (Overton, 1977). The conventional drug discrimination training procedure does not directly bear on this issue since drug cuing does not necessarily imply an absence of information transfer between the drug vs. non-drug state. A drug discrimination is a necessary but not a sufficient condition for an absence of information transfer between the drug state and the non-drug state. For cocaine specifically, a case can be developed that information processing in a cocaine state of behavioral activation involving altered neurotransmission in brain including in the hippocampus (Muller et al., 2002b) does not completely transfer to the non-cocaine state. Although the external environment is the same for the cocaine and non-cocaine state, an environment experienced repeatedly under the cocaine state may appear less familiar when subsequently experienced under the non-cocaine state than if the environments had been experienced repeatedly under the non-cocaine state. A conceptualization of the effects of cocaine pairing to a test environment in terms of drug state dependent effects implies that the effects of cocaine dose level would be poorly related to effects observed on a conditioning test. That is, a dose level, which is sufficient to induce a drug state dependent effect, would induce the maximum effect in terms of habituation transfer so that any increase above this threshold dose would have little or no additional effect. An alternative possibility consistent with habituation effects and conditioning would be to consider the cocaine treatment

as inducing an experiential state of novelty. From this perspective, the cocaine treatments are analogous to repeatedly testing animals in novel environments. This hypothesis would be consistent with the presence of within session but not between session habituation in the cocaine paired group. In that this experiential effect could become conditioned to the test environment, it is also consistent that the cocaine paired animals performed similarly to the unpaired/untested animals on the conditioning test. In view of the potent reinforcing properties of novelty (Berlyne, 1955), this interpretation of the cocaine effect would also be consistent with the established reinforcing properties of cocaine (Koob, 1992) as well as the suggested linkage of cocaine abuse to novelty seeking behavior (Dellu et al., 1996). Unlike a drug state dependent hypothesis, the novelty hypothesis points to an interaction between cocaine dose level and environmental novelty. That is, as the dose level of cocaine is increased, the intensity of the novelty effects may shift from positive reinforcement to an anxiogenic/aversive property. Such a consideration suggests that these hypotheses are amenable to experimental analyses. Thus, experimental manipulation of habituation processes may be a valuable approach to the development of an improved understanding of cocaine effects.

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