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Evidence for Pavlovian conditioning of cocaine-induced responses linked to emotional behavioral effects

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Abstract

The pairing of cocaine treatments with a specific test environment typically leads to cocaine-conditioned drug effects. In this study, we first pre-exposed rats 10 times to an open-field environment to establish an habituation asymptote in locomotor activity prior to the initiation of cocaine treatments. Two groups (N=10) equated for locomotion, grooming, central zone penetrations and rearing behavior were used. One group received five pairings of cocaine (10.0 mg/kg) and the second group five pairings of saline injections with placements in the open-field environment. Subsequently, both groups received a saline test to detect possible cocaine-conditioned behavioral effects. During the cocaine treatment phase, cocaine enhanced locomotion and central zone penetrations but decreased rearing and grooming. On the conditioning test, the cocaine group exhibited enhanced central zone penetrations and decreased grooming as compared to the saline group. There were no group differences in locomotion or rearing. When within group comparisons were performed between behavioral responses on the pre-conditioning test vs. the conditioning test, the saline group scores were essentially unchanged. In contrast, the cocaine group exhibited higher central zone penetrations and decreased grooming without changes in locomotion or rearing. In that a cocaine conditioning test can also be viewed as a cocaine withdrawal test, two additional experiments were conducted using an unpaired conditioning protocol to test for withdrawal effects without conditioning. These results indicated that the central zone and grooming effects observed in the conditioning protocol were not withdrawal effects. Altogether, these findings provide support for Pavlovian conditioning of cocaine-induced changes in emotion-related behavioral responses.
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1. Introduction

Cocaine is a potent stimulant drug, which can be highly addictive. In addition to its direct unconditioned effects upon behavior, cocaine also can induce conditioned drug effects. In fact, there is now a well developed clinical literature, which has shown that stimuli associated with cocaine usage acquire cocaine-conditioned stimulant properties. Cocaine-conditioned stimuli not only can evoke cocaine-like effects but during drug abstinence can evoke craving (Newlin, 1992; O'Brien et al., 1993; Childress et al., 1999). Cocaine-conditioned effects have been extensively studied using animal models. Perhaps, the simplest animal behavior model used is one in which cocaine is administered to an animal prior to its placement in an open-field. Typically, cocaine-treated animals are hyperactive relative to non-drug controls. Following several pairings of cocaine with a test environment placement, it has been frequently reported (Franklin and Druhan, 2000; Carey et al., 2003) that the cocaine treatment group is more active than the control when both groups receive a vehicle injection prior to placement in the open-field. This effect is generally considered to be a cocaine-conditioned hyperactivity.

In several papers, we (Carey et al., 2003) and others (Ahmed et al., 1996) have pointed up some of the problems embedded in the use of an open-field environment as a conditioned stimulus and locomotor activity as indicative of

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a psychostimulant-conditioned response. One major complication is that locomotor activity is sensitive to habituation effects. Thus, the open-field-conditioned stimulus is not stable and neutral but rather has a changing unconditioned effect independent of the drug-induced behavioral response. Thus, with repeated testing in a conventional open-field environment, rats become less active as the environment becomes increasingly familiar. As a consequence, when a control group is given a vehicle treatment, its baseline activity level decreases with repeated exposure to the test environment, which is serving as the conditioned stimulus. Subsequently, when the psychostimulant drug group and control groups are both tested with a vehicle treatment, the psychostimulant drug group is typically more active. It is indeterminate, however, as to whether the drug group is manifesting a conditioned drug response of hyperactivity or that the drug group did not habituate as effectively to the test environment as the control group. Another complication in terms of a conditioning analysis is that the drug groups' conditioned activity level is lower than its activity level prior to the initiation of drug treatment, or to a control groups matched for initial activity level but subsequently not tested (Carey et al., 2003). Unlike classical Pavlovian conditioning in which the response to the conditioned stimulus is greater than the original baseline response elicited by the conditioned stimulus prior to conditioning (Pavlov, 1927), the cocaine-conditioned locomotor response is a response, which is diminished as compared to the activity level elicited by the open-field environment stimulus prior to the conditioning protocol. Rather, the conditioned locomotor response for the cocaine group is a response, which has declined less than that of the control group.

In the present study, we undertook to further examine the issue of cocaine conditioning of locomotor activity in an open-field. Our approach was to habituate animals extensively to a test environment such that cocaine treatments were initiated at a point at which the activity level of the control group was stabilized and was no longer changing. Under this arrangement, the cocaine treatments would induce hyperlocomotion but the baseline locomotor activity level of the reference control group would remain stable. The question to be answered was whether the cocaine unconditioned response (UCR) of hyperlocomotion generates a conditioned response (CR) of hyperlocomotion when habituation was no longer changing in the control group. In addition to using this experimental manipulation of familiarity upon the cocaine locomotor response, we also evaluated the effects of cocaine on other open-field behaviors, namely, central zone activity and grooming (Cooper and Van der Hoek, 1993; Carey and Gui, 1997; Druhan and Wilent, 1999). These are measures of interest in that central zone activity and grooming in an open-field environment are generally considered to be indices of emotional behavioral processes. In that a cocaine conditioning protocol includes repeated cocaine treatments followed by a non-cocaine test for conditioning, these same elements also constitute a withdrawal test. In that withdrawal effects are typically dysphoric, changes in emotionally linked behavior might occur in withdrawal and be mislabeled as conditioned effects. To assess this possibility, we conducted two additional experiments in which we administered repeated cocaine treatments and then conducted a non-cocaine test to assess for possible withdrawal effects. In these studies, the repeated cocaine treatments were administered unpaired to the test environment so that withdrawal effects could be evaluated in the absence of conditioned effects.

2. Materials and methods

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 and 12 h 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 (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 (6 a.m.–6 p.m.). This 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 i.p.

2.3. Apparatus

All of the behavioral tests were conducted in 60×60×40 cm and 68 cm diameter×40 cm open-field compartments of approximately equal area. Testing was conducted in two similar subdivisions of the testing room with a circular and square chamber in each subdivision. 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 criteria of 2 cm for a movement to be scored (Ethovision 3.0 from Noldus Information Technology, Leesburg, VA). A central zone (CZ) comprising one-ninth of the floor area was monitored independently from the rest of the area and is only distinguished by the computer software. The accuracy of the system for the measurement of distance was validated by moving objects 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 in the test chamber and turned off upon removal from the test chamber. Each chamber was illuminated by two overhead 12 V projection lamps placed 50 cm above the chamber adjacent to the video camera. Testing was 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 animal's head was blackened with a non-toxic marker and the Ethovision system only tracked this feature of the rat's body. During each session, data was calculated every 2.5 min and the computer screen tracings of the animal patterns of locomotion were recorded. In previous reports (Dai and Carey, 1995; Carey and Gui, 1997), we have presented the tracing of the locomotion patterns generated by animals in this test environment. In the present study, the tracings of the locomotion patterns were similar to those previously reported (Carey and Gui, 1997). From the tracings, one can readily identify repetitive stereotypic movements (Dai and Carey, 1995). Such movement patterns were infrequently observed in occasional isolated instances in the present studies and had no statistical impact upon the total distance scores. In addition, a VHS VCR was also connected to each camera to videotape sessions. The videotapes were always reviewed after each session in order to validate the recording of the tracking of the animal and to validate that patterns generated by the tracking system mirrored the locomotor behavior of the animal. The videotapes of the last habituation test or pre-conditioning test, the drug treatment test sessions and the conditioning test 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 experiments were undertaken only after experimenters established intra- and inter-experimenter reliability coefficients of $r \geq 0.9$ on scoring 2 successive days of videotapes of open-field test sessions. The occurrence and frequency of other atypical stereotypical behaviors such as circling were also scored and recorded (Carey et al., 2002). None of these behavioral responses were reliably observed and none reached statistical significance ($P > 0.05$).

2.4. Behavioral testing

2.4.1. Experiment 1

Following acclimation to handling and injection procedures, 20 rats received ten 20-min tests in the open-field environment (five per week). Immediately prior to placement in the test environment, the animals received a saline injection (i.p.). Although we have previously observed no group differences in activity related to the open-field shape or subsection of the test room in which testing was conducted, the 20 animals were equally distributed to each test environment and always tested in the same location. We observed no statistically reliable difference related to the test environment shape or location ($P > 0.05$). After completion of the 10 habituation sessions, 2 groups ($N=10$) were formed equally distributed between each test environment and test location ($P > 0.05$) and equated for means and S.E.M.s in terms of locomotion scores ($P > 0.05$).

After completion of the habituation protocol, the two groups were given an additional five daily test sessions designed to induce a cocaine-conditioned behavioral effect. One group received saline immediately prior to each test session and the other group was administered cocaine (10 mg/kg) immediately prior to testing. Two days after completion of the cocaine treatment phase, both groups were given one more open-field test session in which both groups were injected with saline immediately prior to testing. The final test served as the cocaine conditioning test.

2.4.2. Experiment 2

Another consideration pertinent to the administration of repeated cocaine treatments and the subsequent administration of a saline treatment for a conditioning test is that the saline test could also be viewed as a cocaine withdrawal test. In consideration of this possibility, we conducted a second experiment in which withdrawal from cocaine treatment would be the variable and not conditioning. To accomplish this objective, cocaine was never administered in the test environment but rather was always administered in the homecage. In order to be able to assess the possible withdrawal effects using behaviorally equated groups, all animals ($N=40$) were first given a 20-min test in the open-field environment. Immediately prior to testing, all animals ($N=40$) received a saline injection (i.p.). On the basis of scores on this pretest, two groups ($N=20$) were formed with statistically equivalent ($P > 0.05$) scores on the behavioral dependent variables of locomotor distance, central zone entries and grooming. The groups received 10 daily injections of either saline or cocaine (10 mg/kg) depending upon group assignment. The treatments were administered in the homecage. Two days after completion of this treatment regimen, the groups were again administered saline and retested in the open-field environment. This test was conducted to assess possible withdrawal effects related to repeated cocaine treatments. In order to evaluate directly in the same experiment whether a paired cocaine treatment protocol would yield similar findings, a second phase of the experiment was conducted. In this phase, 10 animals from the cocaine group and 10 from the saline group were given 5 daily cocaine treatments immediately before placement in

the test environment. This was the paired cocaine treatment. The remaining 20 animals from the cocaine and saline groups were given saline injections immediately prior to 5 placements in the test environment. Two days after completion of this protocol, all animals were given a saline test to assess possible cocaine-conditioned effects.

2.4.3. Experiment 3

While the first two experiments provided consistent evidence that cocaine-induced changes in central zone behavior were conditioned rather than withdrawal effects, the results for grooming were inconclusive. In order to more directly evaluate the role of conditioning in grooming effects observed in a cocaine conditioning tests, a third experiment was conducted in which only grooming behavior was evaluated. In this experiment, three groups of animals (N=10) were used. Initially, all groups were given a 20-min saline pretest in the test environment and grooming behavior was scored. On the basis this test, three groups of animals were formed equated for grooming (P>0.05). Subsequently, the groups received 10 additional tests in the test environment, followed 2 days later by a saline test for conditioning. One group always received saline before and after testing (saline paired, S-P). The second group (cocaine paired group, C-P) received cocaine (10.0 mg/kg) before and saline after testing. The third group (cocaine unpaired, C-UP) received saline before and cocaine after testing. In this way, the C-P and C-UP groups would be equated for withdrawal effects on the conditioning test.

2.5. Statistical analyses

Two-way analysis of variance (ANOVA) was used to analyze the behavioral data to determine the between group effects, repeated treatment effects, as well as the interaction between variables. Subsequently, to make more specific comparisons, one-way ANOVAs were used. In order to make specific group comparisons, post-hoc Duncan's multiple range tests were performed. Comparisons of mean differences between two groups for session total scores were made using independent t-tests. P<0.05 was used as the criterion for statistical significance.

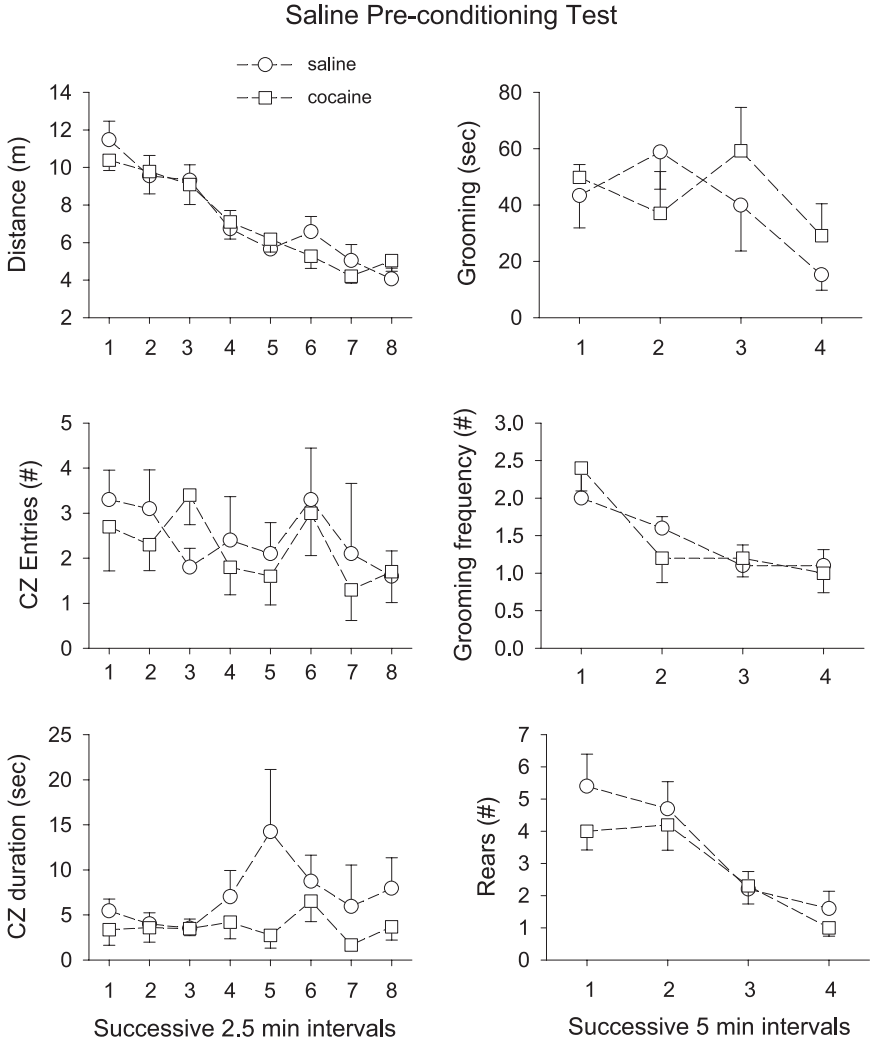

Fig. 1. Means and S.E.M.s for saline and cocaine groups on a saline pre-conditioning test. No group differences were statistically significant. P>0.05.

3. Results

3.1. Experiment 1

Fig. 1 presents the behavioral results on the final day of the habituation protocol. As can be seen in Fig. 1, the groups were closely matched ($P>0.05$) for each of the behavioral measures. Fig. 2 presents the behavioral effects observed over the course of the five cocaine/saline treatment tests. As is apparent in Fig. 2, the cocaine treatment had a substantial impact upon behavior. It can be seen in the left panel in Fig. 2 that cocaine increased the distance traversed in the open-field as well as activity in the central zone of the open-field ($F_{(1,18)}$=9.1, 13.9, 7.2; $P<0.01$ for distance, central zone entries and central zone duration, respectively). The right panel in Fig. 2 presents the effect of cocaine upon grooming and rearing behavior. Cocaine decreased all of these behaviors ($F_{(1,18)}$=69.9, 11.5, 9.4; $P<0.01$ for grooming duration, grooming frequency and rearing, respectively).

After completion of the series of cocaine treatments, the groups were given a saline test to assess possible cocaine-conditioned effects. As can be seen in Fig. 3, cocaine did induce behavioral effects consistent with conditioning on some but not all behavioral measures. As in apparent in the upper left panel in Fig. 3, the cocaine and saline groups had virtually equivalent locomotor distance scores ($P>0.05$). On the other hand, the cocaine group still exhibited higher levels of central zone activity than the saline group ($F_{(1,18)}$=7.3, 4.8; $P<0.05$ for entries and duration, respectively). The right panel in Fig. 3 shows that the cocaine group also exhibited less grooming and rearing than the saline group. While overall rearing was less, this difference was not statistically significant ($P>0.05$). Grooming duration as well as frequency were significantly less in the

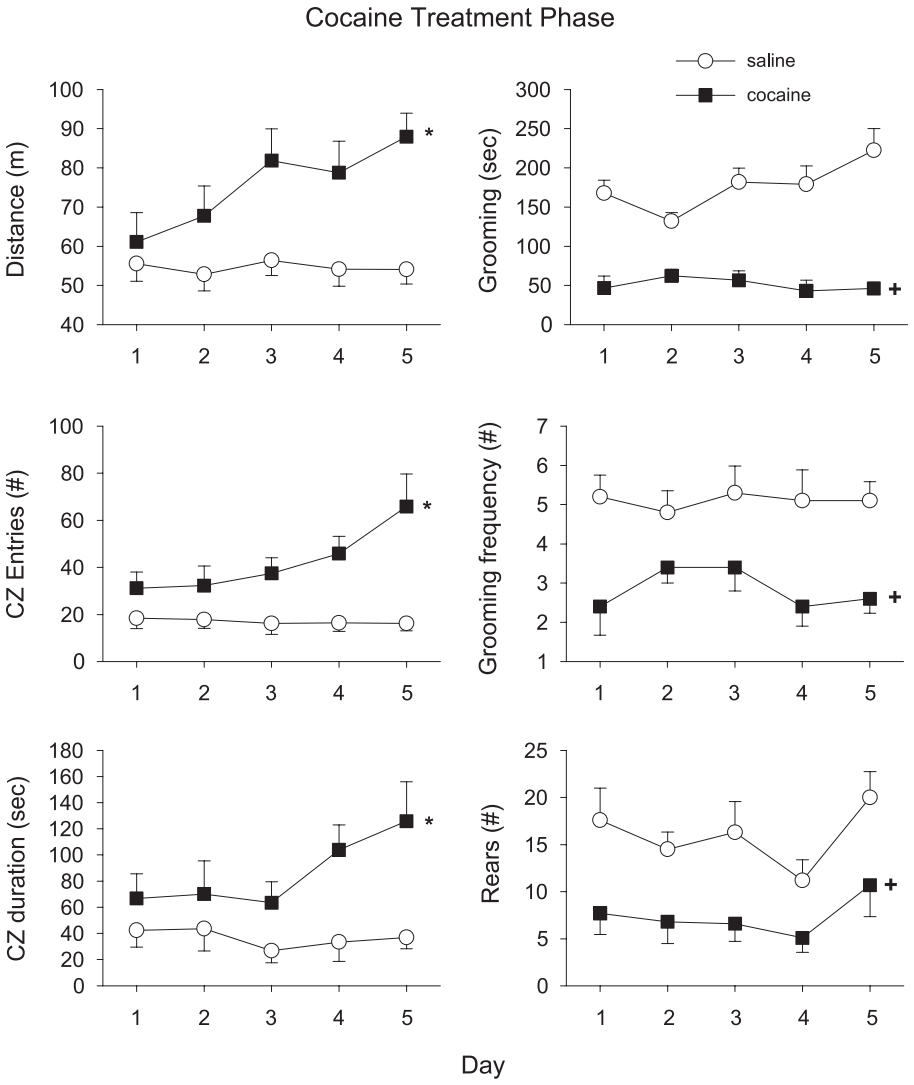

Fig. 2. Means and S.E.M.s for cocaine and saline groups over the course of five successive cocaine conditioning induction tests. The saline group received saline prior to each test and the cocaine group 10.0 mg/kg cocaine. * denotes $P<0.01$ for scores higher than the saline group and + denotes $P<0.01$ for scores lower than the saline group.

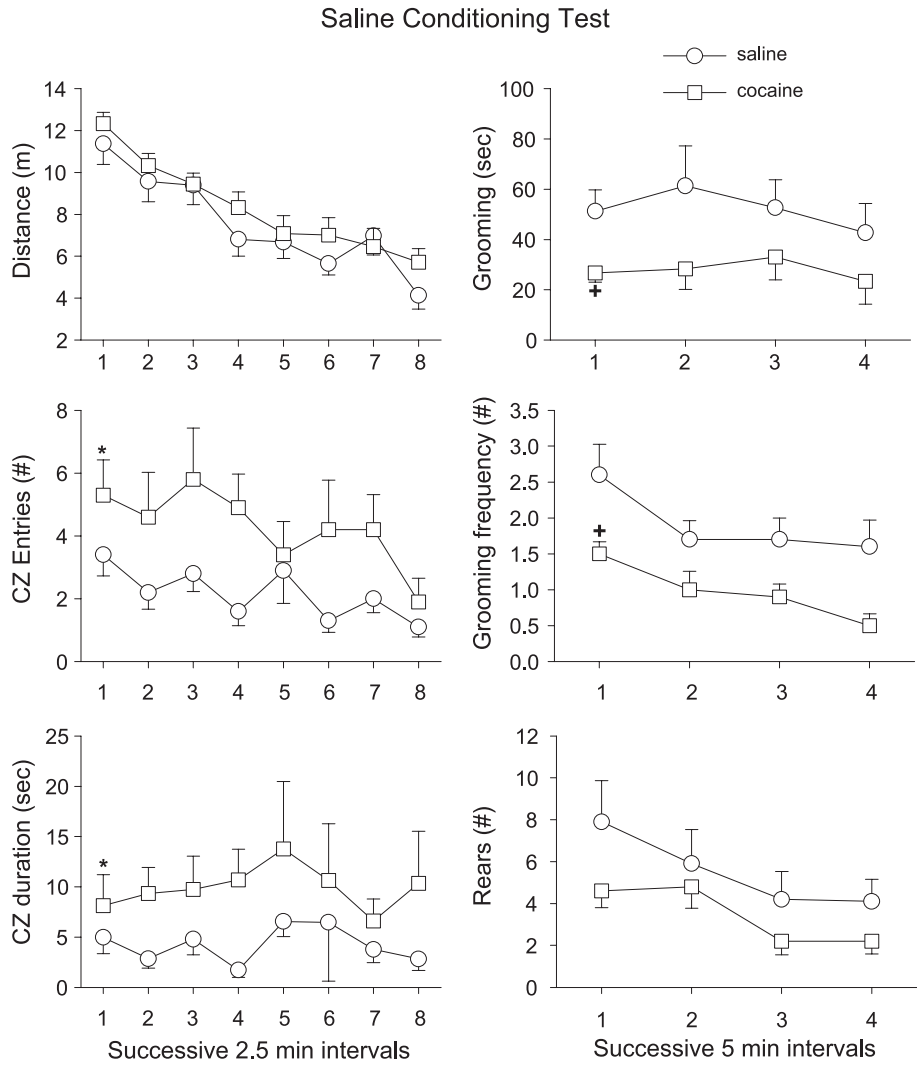

Fig. 3. Means and S.E.M.s for cocaine and saline groups on the saline conditioning test. * denotes $P<0.05$ for scores greater than the saline group and + denotes $P<0.01$ for scores lower than the saline group.

cocaine-treated group ($F_{(1,18)}$=10.8 and 14.4; $P<0.01$, respectively).

3.2. Experiment 2

In that both groups had virtually identical pretest scores, the post-test results which followed the 10 days of repeated cocaine/saline treatments unpaired to the test environment are presented in Fig. 4. Consistently for all behavioral measures, the cocaine group had decreased mean scores. While some of the mean differences approached statistical significance ($P<0.07$, grooming), none of the differences reached the criterion $P<0.05$ level of significance. Fig. 5 presents the results for the cocaine paired treatment phase of Experiment 2. As can be seen in Fig. 5, the cocaine treatment had a profound effect upon behavior and that the two cocaine treatment subgroups and the two saline treatment subgroups had very similar behavioral performances. The differences between the cocaine and saline treatment groups were highly significant statistically for all of the behavioral measures ($F_{(3,36)}$=13.3, 10.3, 4.1, 4.3 and 15.9; $P<0.01$ for distance, CZ entries, CZ duration, CZ/meter and grooming, respectively). In addition, all group×drug interactions were statistically significant ($F_{(5,180)}$=5.5, 5.7, 2.7, 3.3 and 6.5; $P<0.001$ for distance, CZ entries, CZ duration, CZ/meter and grooming, respectively). The conditioning test results are presented in Fig. 6. To simplify the data presentation, the data for the two cocaine treatment groups were combined as were the data for the saline groups. There were no statistical differences between either of these pairs of groups ($P>0.05$). As can be seen in Fig. 6, the paired cocaine group had higher distance and central zone scores. In view of the difference in locomotor distance scores between the cocaine and saline groups, an additional central zone measure is included (central zone entries per meter, CZ/m). This was done to adjust the central zone entries for differences in locomotor distance. As in all previous testing, the cocaine paired group had lower grooming scores than

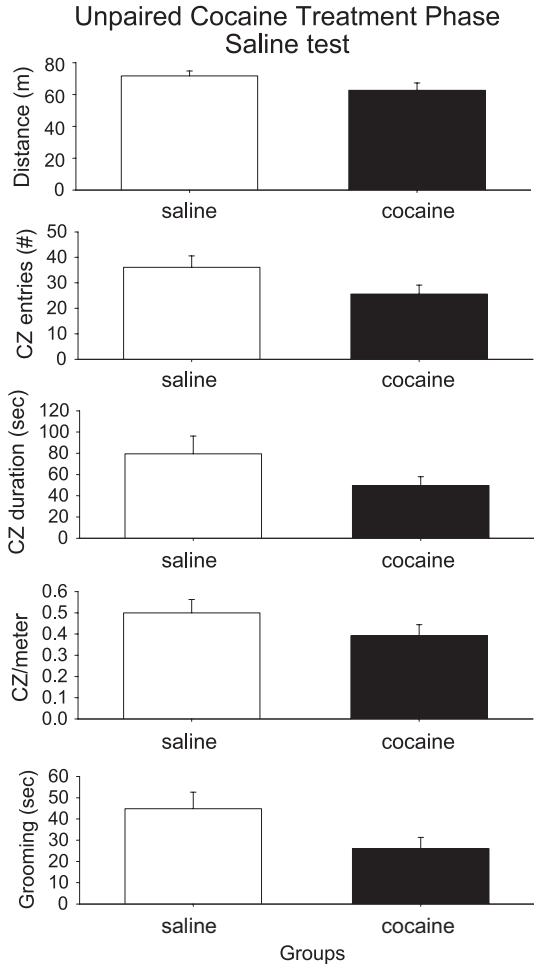

Fig. 4. Means and S.E.M.s for distance, central zone entries, central zone duration, central zone entries/meter and grooming duration during a 20-min saline test 2 days after a 10-day regimen of either saline or cocaine (10 mg/kg) injections in the homecage.

the saline paired group (t=4.0, 3.4, 2.2, 2.4, 4.4; df=38; P<0.05 for distance, CZ entries, CZ duration, CZ/meter and grooming, respectively). It is also important to observe that in this experiment the saline groups had only one exposure to the test environment prior to initiation of the conditioning protocol as compared to 10 exposures in Experiment 1. As a consequence, the saline group exhibited substantial changes in open-field behavior during the conditioning phase (see Fig. 5) including decreased locomotion and increased grooming. These are behavioral changes consistent with habituation to the test environment. This effect is also evident in comparing Figs. 4 and 6 in which it can be seen that the saline group had lower locomotor distance scores but higher grooming scores on the conditioning test (Fig. 6) as compared to their locomotor distance scores and grooming scores on their first test in the open-field when the environment was novel.

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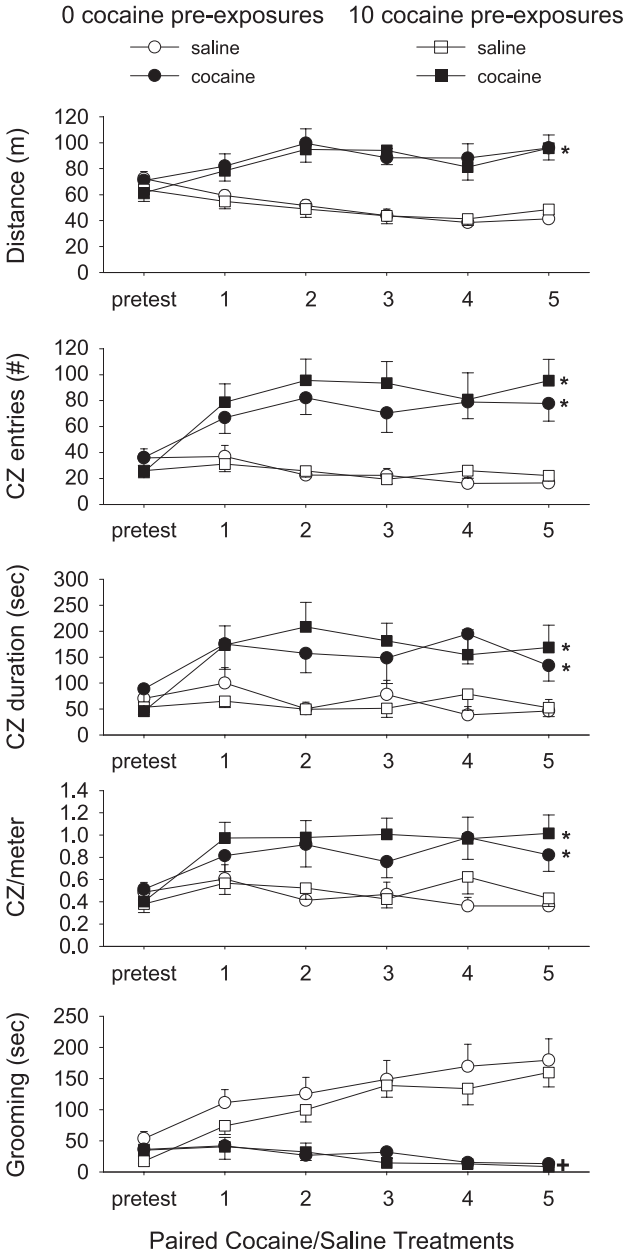

Fig. 5. Means and S.E.M.s for distance, central zone entries, central zone duration central zone entries/meter and grooming for two sets of groups, which received either saline or cocaine (10 mg/kg) injections on 5 successive test days. One pair of saline and cocaine groups had received 10 daily homecage saline injections and the other pair of saline and cocaine groups had previously received 10 homecage injections of cocaine (10 mg/kg). All groups received saline on the pretest. * denotes scores higher than saline groups (P<0.01). + denotes scores lower than saline groups (P<0.01).

grooming scores on the conditioning test (Fig. 6) as compared to their locomotor distance scores and grooming scores on their first test in the open-field when the environment was novel.

3.3. Experiment 3

Experiment 3 was conducted to assess the effect of cocaine upon grooming when cocaine exposure and withdrawal were held constant but the cocaine conditioning was varied using a cocaine paired/unpaired protocol. Fig. 7A presents the effects of the cocaine paired/unpaired treatment on grooming behavior. As can be seen in Fig. 7A, the groups were closely matched in the pretest, but the cocaine paired treatment was highly effective in grooming suppression whereas the unpaired treatment had no apparent effect upon grooming as compared to the saline paired group. The statistical analysis indicated that the cocaine paired treatment effect upon grooming was highly significant statistically ($F_{(2,27)}$=33.1, P<0.001). The cocaine unpaired and saline paired groups did not differ statistically (P>0.05). The results of the saline conditioning test are presented in Fig. 7B. As can be seen in Fig. 7B, the cocaine paired group exhibited substantially less grooming than either the saline paired or the cocaine unpaired groups. The one-way ANOVA was statistically significant ($F_{(2,27)}$=14.4, P<0.001) and comparisons among the specific groups using Duncan's multiple range test indicated that the cocaine paired group had statistically significant lower grooming scores that either the saline paired or the cocaine unpaired group (P<0.01) but that the saline paired and the cocaine unpaired groups did not differ (P>0.05).

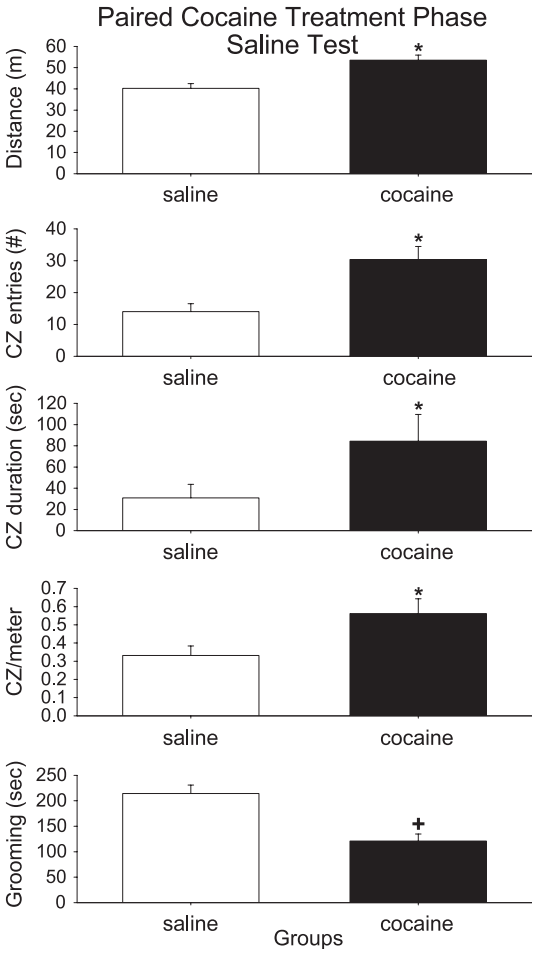

Fig. 6. Means and S.E.M.s for distance, central zone entries, central zone duration, central zone entries/meter and grooming on a saline test conducted 2 days after completion of the 5 days of paired cocaine/saline treatments. * denotes scores higher than the saline paired group (P<0.05). + denotes scores lower than the saline paired group (P<0.05).

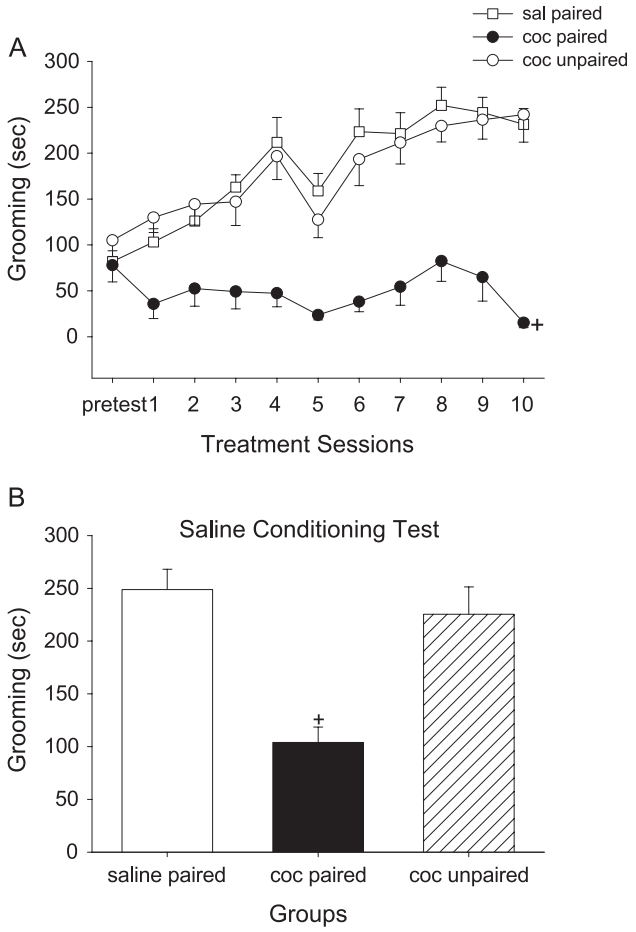

Fig. 7. (A) Means and S.E.M.s of grooming duration in ten 20-min tests in which separate groups received either saline or cocaine paired to the test environment or cocaine unpaired to the test environment. On the pretest, all groups received saline. + denotes grooming scores lower than the saline and cocaine unpaired groups (P<0.001). (B) A saline test conducted 2 days after the 10-day treatment regimen. + denotes grooming scores lower than the saline and cocaine unpaired groups (P<0.001).

4. Discussion

The basic strategy of the first experiment was to initiate cocaine conditioning treatment after animals were well habituated to a test environment so that the effects of the

cocaine treatment would occur against a stable unchanging control group baseline. The control group fulfilled this objective as its behavioral measures remained stable. For the cocaine treatment group, the cocaine treatment induced a robust drug effect. Locomotion and central zone penetration were substantially increased by cocaine, but grooming and rearing were decreased. In that the cocaine treatment had a potent unconditioned effect against a background of an essentially unchanging control group baseline, the question addressed was whether or not conditioned cocaine effects could be observed. We were particularly interested in effects upon locomotion in that conditioned cocaine effects upon locomotion are generally reported (Damianopoulos and Carey, 1992; Druhan and Wilent, 1999). Previously, we have suggested that these cocaine-conditioned effects on locomotion are difficult to differentiate from possible effects of cocaine upon habituation processes in that the reference control group(s) tend to continue to exhibit habituation-related decreases in locomotion (Carey et al., 2003). The results from the present study showed that in well-habituated animals, cocaine did not induce a conditioned locomotion effect. This result is consistent with the proposition that cocaine-conditioned hyperlocomotion can be impacted by habituation effects. If we had only found negative evidence for cocaine conditioning, however, this line of argument would be rather weak since negative effects by themselves are unconvincing. Importantly, the absence of a conditioned locomotor effect occurred in the presence of cocaine-conditioned effects manifested in increased central zone activity and decreased grooming behavior. These behavioral measures, grooming and central area penetration, are frequently linked to what are considered emotional behavioral effects. There is an extensive literature in which central zone behavior has been related to anxiogenic/anxiolytic processes (Ramos et al., 1998; Ramos et al., 2003; Vendruscolo et al., 2003). We (Carey and Gui, 1997) and others (Druhan et al., 1996) have shown previously that cocaine increases central zone entries and time spent in the central zone during cocaine treatment and in conditioning tests. When such effects are observed during the cocaine treatment phase or in a conditioning test in which the cocaine group is more active than the control group, one could argue that these effects are somehow disinhibitory and secondary to the increased level of locomotor activity. In the conditioning test in the present study, however, the cocaine and control groups did not differ in terms of locomotion but yet differed in terms of central zone penetrations and grooming behavior.

The nature of cocaine-induced hyperlocomotion effects in an open-field environment are not well understood. Recently (Carey et al., 2004), we have reported that the cocaine effects upon locomotion in an open-field are strongly influenced by the degree of familiarity with the open-field environment. That is, the more novel the environment, the more pronounced the locomotor stimulant effects of cocaine. Thus, in part, cocaine can be seen as enhancing exploratory activity. Considered from this perspective, it is tempting to speculate that interoceptive cues generated by cocaine have some similarity to the interoceptive cues activated by exposure to a novel environment (Piazza et al., 1990). With repeated cocaine treatments, these cues, through associative processes, can become linked to test environment cues which elicit exploratory activity. In this way, the test environment cues can enhance exploratory activity in a conditioning test following a prior cocaine treatment protocol. In contrast, for a saline paired treatment, the interoceptive cues activated by the novel environment fade with repeated treatments possibly replaced with cues linked to inhibitory response mechanisms in the frontal cortex and hippocampus (Thiel et al., 1998; Schildein et al., 2000; De Souza Silva et al., 2002) which mediate the dynamic inhibitory processes which underlie habituation.

If the cocaine conditioning protocol is initiated after the inhibitory habituation processes are well established, then the cocaine exploratory-related interoceptive cues, which become associated with the test environment cues may be less able to transfer to the non-cocaine state in that the inhibitory habituation cues had already been associated with the test environment cues following a saline treatment. In our second experiment in which the cocaine treatment was initiated after only partial habituation to the test environment, evidence for a cocaine-conditioned locomotor effect was observed. This finding appears consistent with the above proposition, which suggests that cocaine cues reinforce environmental stimuli that elicit exploratory activity. These contrasts with Experiment 1 in which test environment cues had become strongly linked to inhibitory habituation mechanisms and essentially eliminated the opportunity for an association of cocaine cues to the exploratory activation cues generated by the test environment. Clearly, the present limited observations make this proposition only suggestive.

Our findings that repeated pairings of cocaine to a test environment led to effects consistent with conditioned increased central zone penetrations and decreased grooming behavior regardless of prior habituation to the test environment suggested another dimension to cocaine conditioning. In that these behavioral responses are frequently induced by anxiogenic/anxiolytic stimuli, the issue arose as to whether these behavioral changes might represent withdrawal effects from the repeated cocaine treatment response rather than conditioned cocaine effects. In our second experiment, we undertook to determine if testing in an open-field following repeated cocaine treatments unpaired to the test environment would lead to increased central zone penetrations and decreased grooming. All of the behavioral trend effects were consistent with a withdrawal phenomena (i.e., decreased locomotor activity, decreased central zone penetration and decreased grooming). In that the directional effects of decreased central zone penetrations (adjusted for activity level) were opposite to the putative cocaine-conditioned increases in central zone penetrations, the

findings in this experiment indicated that the increased central zone penetrations induced by a paired cocaine treatment regimen were not secondary to withdrawal phenomena. In contrast, the directional effects of withdrawal upon grooming behavior were the same as for the paired cocaine-conditioned grooming effects. In order to clarify the contribution of possible withdrawal effects to the possible cocaine-conditioned effects on grooming behavior, a third experiment focused upon grooming behavior and used a paired/unpaired cocaine conditioning protocol. In this study, the cocaine paired and unpaired groups received the same cocaine exposures and the contribution of withdrawal to the conditioning test, therefore would presumably be the same. In that decreased grooming behavior on the conditioning test was observed in the cocaine paired group and not the cocaine unpaired group, the findings in this study are consistent with grooming suppression representing a cocaine-conditioned effect. While there may also be cocaine withdrawal-induced suppression of grooming behavior, the repeated exposure to the test environment during the cocaine unpaired treatment phase appears to effectively eliminate this effect.

The absence of a conditioned hyperlocomotion effect following habituation to the test environment in the present study differs somewhat from the observation of Ahmed et al. (1996) (i.e., photobeam interruption in the Ahmed study vs. specific behavioral responses in the present study). Furthermore, the Ahmed study used a within subject analysis and two test environments (paired vs. unpaired). Although the use of two test environments can offer additional analytic power, these authors did not counterbalance the test sequence such that the tests were conducted first in the drug paired environment and secondly in the non-drug environment. These considerations make it difficult to directly compare the present results with this study. Consistent with the present results, the amphetamine paired treatment (in the Ahmed study) did not appear to enhance the non-drug activity level above the activity level prior to the amphetamine conditioning protocol.

In general (Stefanski et al., 1992; Osborn et al., 1998; Angrini et al., 1998; Escorihuela et al., 1999; Ramos et al., 2003), pharmacological and genetic manipulations designed to modulate anxiety have reported that anxiolytic/anxiogenic effects upon open-field behavior are manifested by changes in central zone penetration and grooming. Of course, inferences regarding specific emotional state based upon open-field behavioral measures such as grooming and central zone penetrations need to be treated cautiously (File and Andrews, 1991). One could argue that such behavioral effects relate to alterations in sensory processes such as thigmotactic scanning (Fornaguera et al., 1995) or behavioral inhibitory processes, etc. It is also difficult to categorically define cocaine effects in terms of such properties as anxiogenic effects or diminished inhibition in that such effects are dependent upon a variety of factors such as environmental test conditions and cocaine dose level. Regardless of these difficult issues, the present findings do point to some type of emotional response induced by cocaine which become conditioned to environmental cues. It is also of critical importance that the cocaine conditioning protocol changed the baseline level of these responses (central zone penetrations and grooming) from what they were prior to the initiation of conditioning and changed the responses in the same direction as they were modified by cocaine. This result is consistent with Pavlovian conditioning.

It is well established that systemic injections of cocaine (5–20 mg/kg) induces a conditioned place preference (CPP) (Shippenberg and Heidbreder, 1995; Bedingfield et al., 1998; Rademacher and Steinpreis, 2002; Zavala et al., 2003; Skoubis and Maidment, 2003). This cocaine effect is considered to represent conditioning of the positive hedonic effects generated by cocaine which underlie its addictive potency. At the same time cocaine is known to induce anxiogenic effects (Paine et al., 1999; Blanchard and Blanchard, 1999). Furthermore, these cocaine anxiogenic effects occur during cocaine withdrawal and can be induced by exposure to cocaine-conditioned stimuli (Sarnyai et al., 1995; De Vries and Pert, 1998; Sarnyai, 1998). Conversely, exposure to anxiogenic or stressful stimuli can reactivate cocaine CPP effects (Sanchez and Sorg, 2001; Lu et al., 2002). While considerable attention has been given to cocaine-induced increases in dopamine, particularly in the nucleus accumbens (Koob, 1992; Woolverton and Johnson, 1992; Pontieri et al., 1995; Wise, 1998), it is also the case that cocaine is equally efficacious in increasing brain 5-hydroxytryptamine (5-HT) and norepinephrine (NE) (Koe, 1976; Ritz et al., 1990). In that 5-HT and NE are linked to emotional states, such as anxiety (Fontana et al., 1999; Owens et al., 2000; Salchner and Singewald, 2002; Hajos-Korcsok et al., 2003), it is perhaps not surprising that cocaine elicits multiple emotional effects. It is also the case that serotonergic agonists tend to decrease grooming and increase central zone penetration in the open-field (Kostowski et al., 1989; Bagdy et al., 2001; Graf et al., 2003; Nic Dhonnchadha et al., 2003). It appears plausible that it is these serotonergic aspects of cocaine which are conditioned. In fact, it has been reported that selective blockade of the dopamine transporter by GBR 12909 (Van der Hoek and Cooper, 1994) does not simulate the effects of cocaine upon grooming behavior. Interestingly, when a straight runway behavioral test is used and cocaine is administered in the goal box, strong approach conditioning is induced (Geist and Ettenberg, 1997; Raven et al., 2000). This effect is consistent with a positive hedonic effect similar to food reward. Unlike food rewarded animals, however, cocaine rewarded animals exhibit approach to the goal box coupled with avoidance of the goal box as proximity to the goal box increases. This is analogous to the approach-avoidance effects originally described by Miller and Murray (1952) when food reward is accompanied by an aversive electric shock. The cocaine runway studies highlight the duality of the apparent

combined reward and aversive properties of cocaine. Interestingly, aversive stimuli are able to reactivate cocaine CPP effects (Sanchez and Sorg, 2001; Lu et al., 2002).

Clearly, the study of the behavioral effects of cocaine and cocaine-conditioned effects requires the recognition of the multiplicity of neurochemical and behavioral changes induced by cocaine and how these effects are modified by repeated cocaine treatments. The behavioral contribution of non-dopaminergic effects of cocaine, namely effects upon norepinephrine and serotonin and their roles in conditioning processes have received much less attention than cocaine effects upon dopamine. Seemingly, it is the effects upon norepinephrine and serotonin, which are important contributors to the anxiogenic/anxiolytic effects of cocaine. It is also important to recognize that, in the present study, open-field environment testing was conducted under red light conditions which favors a low stress environment (Nasello et al., 1998). Perhaps under such circumstances, the potential anxiogenic effects of increased norepinephrine are less important and provide an opportunity for the anxiolytic serotonergic effects to dominate. It would be of substantial importance to compare the unconditioned and conditioned effects of cocaine obtained in the open-field under different, more stressful conditions than used in the present study, such as with aversive white light illumination of the open-field. It may be that environmental conditions interact with the norepinephrine (anxiogenic) and serotonergic (anxiolytic) effects of cocaine and in this way influence dysphoric/euphoric effects which impact upon the addictive liability of cocaine use.

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