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ORIGINAL INVESTIGATION

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Conditioned increases in anxiogenic-like behavior following exposure to contextual stimuli associated with cocaine are mediated by corticotropin-releasing factor

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Abstract Although cocaine is a powerful reinforcer, it has been reported to produce anxiety in humans and anxiogenic-like behavior in animals. The goal of this study was three-fold: (1) to determine the doses of cocaine that induce anxiogenic-like behavior in the elevated plus-maze in rats, (2) to determine if cocaine-associated contextual cues are capable of eliciting anxiogenic-like behavior in the absence of the drug, and (3) to identify possible mechanisms through which cocaine-associated cues affect behavior in the elevated plus-maze. Measurement of the amount of time that the animals spend exploring the open arms of the maze provides a sensitive index of anxiogenic-like behavior in rats. In experiment 1, rats were injected with 10 mg/kg, 20 mg/kg, or 30 mg/kg cocaine HCl or saline for 6 days. On day 6, the rats were tested in the elevated plus-maze 25 min after injection with cocaine or saline. The animals chronically treated with the three doses of cocaine exhibited a dose-dependent increase in anxiogenic-like behavior in the elevated plus-maze, compared to the saline-treated group. In experiment 2, cocaine-induced (30 mg/kg) conditioning was achieved using a simple contextual design. On the final day of the experiment (day 6), after 5 days of conditioning, the rats were exposed for 25 min to the cocaine-associated contextual cues, then placed in the elevated plus-maze. Animals that had been exposed to cocaine-associated contextual cues prior to being placed in the elevated plus-maze exhibited a significant increase in anxiogenic-like behavior compared to the control groups. However, pretreatment of the rats with the CRF antagonist, ∝-helical $CRF_{9–41}$ (1 μg, ICV), on the test day, prior to exposure to cocaine-associated contextual cues, attenuated the subsequent anxiogenic-like behavioral response in the elevated plus-maze (experiment 3). The results suggest that contextual cues associated with repeated treatment with 30 mg/kg cocaine are capable of eliciting anxiogenic-like behavior in the absence of the drug and that CRF mediates the expression of anxiogenic-like behaviors in the elevated plus-maze following exposure to cocaine-associated cues. The conditioned anxiogenic action elicited by cocaine-associated cues may have relevance for understanding the complex addictive nature of this drug and some of the clinical phenomena related to its use.

Key words Anxiety · Elevated plus-maze · Classical conditioning · Drug abuse · CRF antagonist

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Introduction

Although cocaine is a powerful reinforcer in humans and other species (Wood and Lal 1987; Johanson and Fischman 1989), it may also increase anxiety. The anxiogenic-like actions of acutely administered cocaine have been revealed in numerous animal studies using a variety of different paradigms (Costall et al. 1989; Fontana and Commissaris 1989; Ettenberg and Geist 1991; Rogerio and Takahashi 1992a, b; Sarnyai et al. 1995). In humans, it is known that in addition to euphoria and a feeling of well-being, cocaine also produces a number of negative symptoms, including nervousness, anxiety, fear, depression, and irritability (Resnick and Resnick 1984; Spotts and Shontz 1984). Such anxiogenic effects also appear to accompany cocaine withdrawal in both animals and humans (Resnick and Resnick 1984; Gawin and Kleber 1986) and have been postulated to underlie, at least in part, recidivism (Resnick and Resnick 1984; Koob and Bloom 1988).

Cues associated with cocaine can acquire (through classical conditioning) the ability to elicit behavioral actions produced by the drug alone (Pavlov 1927; Stewart and Eikelbloom 1987; Pert et al. 1990). The classical conditioning of drug effects to external and interoceptive cues in animals may underlie the development of incen-

tive motivation, which is probably the principal behavioral substrate responsible for craving and drug-seeking behavior (Stewart et al. 1984; Pert 1994). Clinical studies also have revealed that drug-associated cues are capable of inducing increases in autonomic function (e.g. heart rate, blood pressure, skin temperature, respiration and galvanic skin response) as well as strong sensations of drug craving in addicts (Childress et al. 1987a, b).

Little is known regarding the classical conditioning of cocaine-induced negative symptoms to external and interoceptive cues. Findings from a clinical study indicate that cocaine addicts presented with cocaine-associated cues report increases in anxiety as well as drug craving (Berger et al. 1996). The primary purpose of this study was to evaluate possible anxiogenic-like effects in rats following exposure to cocaine or stimuli associated with cocaine and to compare such effects with those seen following abstinence from the drug. The unconditioned and conditioned anxiogenic effects of cocaine were assessed in the elevated plus-maze. The maze exploits the natural tendency of rats and mice to suppress exploration of the open arms when they are anxious (Pellow et al. 1985; Lister 1987). Measurement of the amount of time that the animals spend exploring the open arms of the maze provides a sensitive index of anxiogenic-like behavior. A secondary purpose of this study was to evaluate the role of corticotropin-releasing factor (CRF) in the conditioned anxiogenic effects of cocaine by assessing the ability of a CRF antagonist (∝-helical $CRF_{9–41}$) to prevent increases in anxiogenic-like behavior following exposure to cocaine-associated cues.

Materials and methods

Animals

Male Sprague-Dawley rats (Taconic Farms, Germantown, N.Y., USA) weighing between 250 and 300 g were group housed and maintained on a 12-h light-dark cycle (lights on at 0700 hours). Food and tap water were available ad libitum in the home cages. Animals were adapted to the vivarium for at least 1 week prior to commencement of the study. The animals were handled daily during the adaptation period. The behavioral testing was conducted between 1100 and 1600 hours.

Drug

Cocaine hydrochloride (Sigma, St Louis, Mo., USA) was prepared in sterile saline at a concentration of 10, 20 or 30 mg/kg. Animals were injected intraperitoneally (IP) with 1 ml/kg of the cocaine solution or sterile saline.

Apparatus and behavioral tests

Locomotor activity was assessed in Digiscan photocell activity monitors (Omnitech Electronics, Columbus, Ohio, USA). The apparatus were enclosed in individual sound attenuating compartments with one-way mirrors mounted in the doors of the chambers to allow observation of the animals during testing. The compartments were equipped with a 15-W florescent white light and ventilating fan that also provided masking noise. The monitors consisted of a clear Plexiglas box (30.5 cm high×42 cm long×42 cm wide) fitted inside a metal frame containing a series of 12 equally spaced infrared photocell detectors located 4 cm from the floor, along two adjacent walls of the chamber. Interruptions in the infrared light sources by the experimental animal were recorded and stored by an IBM AT computer. The room containing the locomotor chambers was located adjacent to the animal colony. Prior to each new session a drop of peppermint extract was applied to the wall of the chamber with a cotton swab. Locomotor behavior was assessed during 30-min sessions on the training days and a 25-min session on the test day.

The elevated plus-maze was constructed from opaque black Plexiglas. The maze was elevated 1 m above the floor. The arms of the maze were 45 cm long and 10 cm wide; the closed arms had walls on three sides that were 20 cm high, and the open arms did not have walls on any of the sides. The elevated plus-maze was located in the same room as the locomotor chambers. At the beginning of the behavioral test, the experimental animal was placed in the center of the maze facing one of the open arms. An observer seated approximately 1 m from the apparatus, at a height sufficient to observe the animal in all four arms of the maze, recorded the number of entries into the open and closed arms, the duration of time spent in the arms, the frequency of rearing, the number of fecal boli, and the amount of time spent autogrooming during the 5-min test. Autogrooming was defined as scratching, licking or nibbling of paws or fur. All behavioral scoring in the elevated plus-maze was performed using the Timer 1.3 program (developed at NIH 1989) and a Macintosh computer. Between tests, the apparatus was cleaned with dilute Alconox then wiped dry. All behavioral scoring was conducted by an observer who was unaware of group assignment.

Procedures

Experiment 1: unconditioned anxiogenic effects of cocaine

For 5 consecutive days, rats were injected IP with either saline (SAL; *n*=10), or one of three doses of cocaine: 10 mg/kg (*n*=9), 20 mg/kg (*n*=10) or 30 mg/kg (*n*=9). Immediately following injection, the rats were placed into the locomotor chambers for 30 min. On the final day of the experiment (day 6), the rats were injected with either cocaine or saline, placed into the locomotor chambers for 25 min, and then tested for 5 min in the elevated plus-maze. This procedure was adopted to allow comparisons to be made with experiment 2.

Experiment 2: conditioned locomotor and anxiogenic-like effects of cocaine

Cocaine-induced conditioning was achieved using a simple contextual design. Rats were randomly assigned to one of the following groups: Paired (*n*=8), Unpaired (*n*=7) or Control (*n*=7). On days 1–5, rats in the Paired group were injected with 30 mg/kg cocaine HCL and placed in the peppermint-scented locomotor chambers for 30 min. One hour after they were returned to their home cages they were injected with saline (1 ml/kg). Animals in the Unpaired and Control groups received an injection of saline prior to being placed in the peppermint scented locomotor chambers for 30 min on days 1–5. One hour after being returned to their home cages, the Unpaired animals received an IP injection of cocaine (30 mg/kg) and the Control animals received an IP injection of saline. On the test day (24 h after the last injection), the experimental animals were placed directly into the scented locomotor chambers without receiving an injection. Locomotor activity was recorded for 25 min, then the rats were removed from the chambers and placed directly on the elevated plus-maze. The behavior of the rats in the maze was recorded for 5 min, as described above.

Experiment 3: role of CRF in the conditioned locomotor and anxiogenic-like effects of cocaine

A 23 gauge stainless steel guide cannula (16 mm) was implanted 1.5 mm dorsal to the lateral cerebral ventricle (AP 7.9 mm, LAT 1.7 mm, DV 6.9 mm relative to interaural zero with the incisor bar at –3.5 mm) of anesthetized rats (chlorohydrate, 400 mg/kg IP) at least 10 days prior to the commencement of the study. The rats were assigned to the Paired, Unpaired or Control group and trained in the conditioning paradigm described above in experiment 2. On day 6 (test day), the rats in the three treatment groups received an ICV injection of either 1 μg α-helical $CRF_{9–41}$ in 5 μl sterile water (pH 6.7; Peninsula Labs) or the vehicle through a 30-gauge injector which extended 1.5 mm past the end of the guide cannula. Thirty minutes after the ICV injection, the rats were placed into the peppermint-scented locomotor chambers. After 25 min in the locomotor chambers, the animals were tested for 5 min in the elevated plus-maze, as described above. At the conclusion of the experiment, cannula patency and placement was verified via post-mortem injection of dilute cresyl violet. The brains were removed and cuts were made at four anterior-posterior levels parallel to the coronal plane. Staining was observed throughout the lateral ventricles for all experimental animals.

Statistical analysis

The data are presented as mean±SEM. The behavioral data were analyzed by analysis of variance (ANOVA). When the ANOVA indicated a significant difference among treatment groups ($P<0.05$), Fisher's PLSD was used to compare the Paired group with the Unpaired and Control groups.

Results

Experiment 1: unconditioned anxiogenic effects of cocaine

The data from two animals were excluded from the analysis because they fell or jumped off the elevated plus-maze during the test period. A one-way ANOVA revealed a significant treatment effect in the amount of time that the animals spent in the open arms of the elevated plus-maze [($F(3,34)=8.9$; $P<0.01$; Fig. 1A]. Post-hoc comparisons using Fisher's PLSD indicate that animals in each of the experimental groups that received chronic treatment with cocaine [10 mg/kg (n=9), 20 mg/kg (n=10), and 30 mg/kg (n=9)] spent significantly less time in the open arms of the maze than the saline group (SAL; n=10). The effect of cocaine on time spent in the open arms of the elevated plus-maze was dose-dependent. Although there was no significant treatment effect in the total number of arm entries [$F(3,34)=1.60$, $P>0.05$; Fig. 1B], the percentage of entries into the open arms was significantly lower in the group treated with 30 mg/kg cocaine than in the saline group [$F(3,34)=7.23$, $P<0.01$; Fig. 1C]. ANOVA also revealed a treatment effect in the time spent grooming in the elevated plus maze [$F(3,34)=7.04$; $P<0.01$]. Post-hoc comparisons using Fisher's PLSD indicate that animals in each of the experimental groups that received chronic treatment with cocaine spent less time autogrooming than the saline group. There were no group differences in frequency of rearing [$F(3,34)=1.37$; $P>0.05$] or number of fecal boli excreted [$F(3,34)=1.61$; $P>0.05$] in the elevated plus-maze.

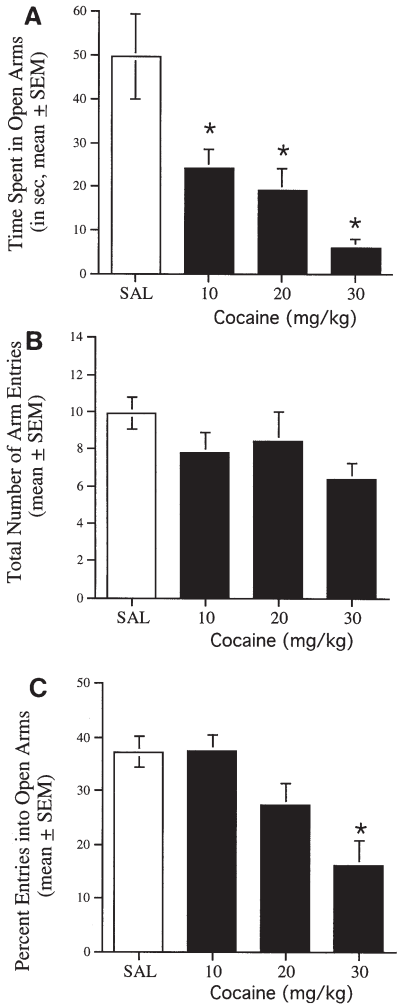

Fig. 1A–C Behavior in the elevated plus-maze 25 min after IP injection of saline (*SAL*), or 10, 20 or 30 mg/kg cocaine. The behavioral test lasted 5 min. Measures scored included: the duration of time spent in the open arms of the apparatus (mean ±SEM in s; **A**), total number of arm entries (mean ±SEM; **B**) and percentage of open arm entries (**C**). Data were analyzed via ANOVA. An *asterisk* (*) indicates a significant treatment effect compared to the SAL group at $P<0.05$

Experiment 2: conditioned locomotor and anxiogenic-like effects of cocaine

The elevated plus-maze data from one animal were excluded from the analysis because it fell from the apparatus during the test period. Injections of cocaine (30 mg/kg, IP) in the Paired rats during the 5-day training period produced a significant elevation in locomotor output compared to Unpaired and Control groups that received saline just before the training sessions (Fig. 2A). A two-way repeated ANOVA revealed that across the training period, the animals in the Paired group exhibited

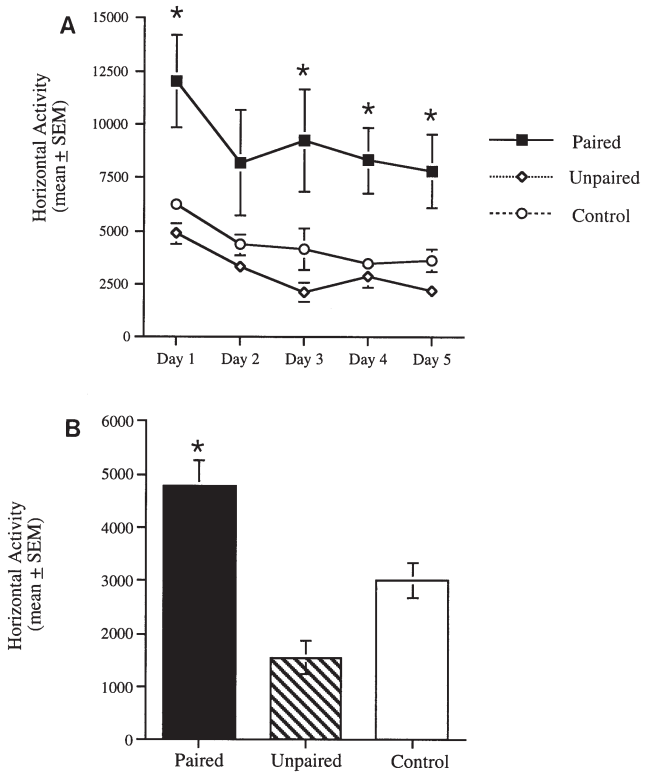

Fig. 2 **A** Horizontal locomotor activity during the five training sessions (mean±SEM; 30-min sessions). **B** Horizontal locomotor activity (mean±SEM; 25-min session) on the test day during exposure to locomotor apparatus cues which for the previous 5 days had been paired with cocaine injections in the Paired group and saline injections in the Unpaired and Control groups. Data were analyzed via ANOVA. An *asterisk* (*) indicates a significant treatment effect compared to saline-treated Control rats at $P<0.05$

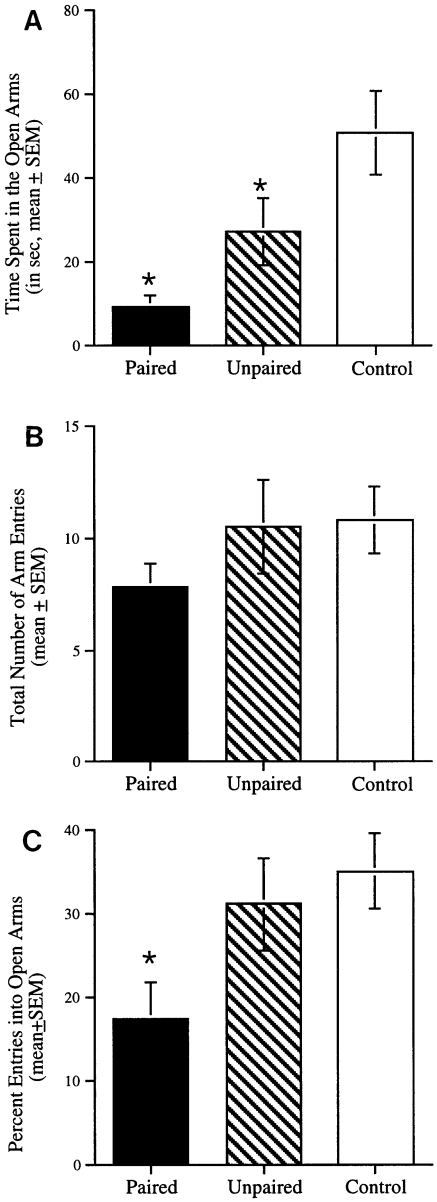

Fig. 3A–C Behavior in the elevated plus-maze after 25 min exposure to locomotor apparatus cues which for the previous 5 days had been paired with cocaine injections in the Paired group and saline injections in the Unpaired and Control groups. The behavioral test lasted 5 min. Measures scored included: the duration of time spent in the open arms of the apparatus (mean±SEM in s; **A**), total number of arm entries (mean±SEM; **B**) and percentage of open arm entries (**C**). Data were analyzed via ANOVA. An *asterisk* (*) indicates a significant treatment effect compared to the Control group at $P<0.05$

significantly greater horizontal locomotor activity than animals in the Unpaired and Control groups [$F(2,19)=10.68$, $P<0.01$]. Exposure to cocaine-associated contextual stimuli for 30 min over the 5-day training period was sufficient to produce conditioned increases in locomotor output and post-stimulus anxiogenic-like behavior in rats, which was revealed when the conditioned (Paired) and unconditioned (Unpaired and Control) rats were tested on day 6. ANOVA revealed a significant treatment effect on locomotor activity [$F(2,19)=18.05$, $P<0.01$; Fig. 2B]. Post-hoc comparisons using Fisher's PLSD indicated locomotor activity was significantly greater in the Paired group than the Unpaired and Control groups.

The Paired group ($n=8$) spent significantly less time in the open arms of the elevated plus-maze than the Unpaired ($n=7$) and Control ($n=7$) groups [$F(2,19)=8.18$, $P<0.01$; Fig. 3A]. Fisher's PLSD also revealed that the Unpaired animals spent significantly less time in the open arms than the Control group. Although the total number of arm entries did not differ among the treatment groups [$F(2,19)=1.9$, $P>0.05$; Fig. 3B], the percentage of entries that were into open arms was significantly lower in the Paired group than in the Unpaired and Control groups [$F(2,19)=3.9$, $P<0.05$; Fig. 3C]. The three treatment groups did not differ in other indices of anxiogenic-like behavior examined, including rearing frequency [$F(2,19)=0.74$; $P>0.05$], amount of time spent autogrooming [$F(2,19)=0.58$; $P>0.05$] and number of fecal boli excreted [$F(2,19)=0.37$; $P>0.05$] during the 5-min test in the elevated plus-maze.

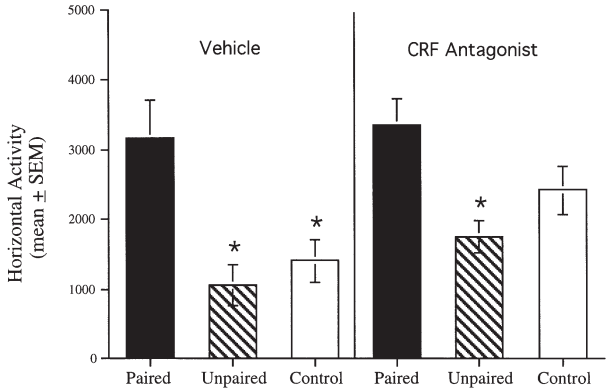

Fig. 4 Horizontal locomotor activity (mean±SEM) on the test day, during 25 min exposure to locomotor apparatus cues which for the previous 5 days had been paired with cocaine injections in the Paired group and saline injections in the Unpaired and Control groups. Thirty minutes prior to placement in the locomotor apparatus, animals were treated ICV with either a CRF antagonist (1 μg α-helical $CRF_{9–41}$) or the vehicle. Data were analyzed via ANOVA. An *asterisk* (*) indicates a significant treatment effect compared to the paired group at $P<0.05$

Experiment 3: role of CRF in the conditioned locomotor and anxiogenic-like effects of cocaine

Exposure to cocaine-associated contextual stimuli for 30 min over the 5-day training period was sufficient to produce conditioned increases in locomotor output on the test day in Paired rats that had been pretreated with either 1 μg α-helical $CRF_{9–41}$ or the vehicle. ANOVA revealed a significant treatment effect on locomotor activity in animals pretreated with α-helical $CRF_{9–41}$ [$F(2,25)=6.20$, $P<0.01$; Fig. 4). Post-hoc comparisons using Fisher's PLSD indicate that locomotor activity was significantly greater in the α-helical $CRF_{9–41}$-treated Paired ($n=9$) than Unpaired ($n=10$) group. Post-hoc analysis using Fisher's PLSD revealed a trend toward increased locomotor activity in the α-helical $CRF_{9–41}$-treated Paired group compared with the Control group ($n=9$; $P=0.056$). In the vehicle-treated groups, ANOVA followed by post-hoc comparisons using Fisher's PLSD indicates that locomotor activity was significantly greater in the Paired ($n=10$) than Unpaired ($n=8$) and Control ($n=10$) groups [$F(2,25)=7.40$, $P<0.01$; Fig. 4].

The data from five animals were excluded from the elevated plus-maze analysis because they fell or jumped off the apparatus during the test period. Among the animals pretreated with α-helical $CRF_{9–41}$, ANOVA did not reveal a significant treatment effect between the Paired ($n=9$), Unpaired ($n=8$) and Control ($n=8$) groups on the time spent in the open arms in the elevated plus-maze [$F(2,22)=0.79$, $P>0.05$; Fig. 5A], the total number of arm entries [$F(2,22)=0.46$, $P>0.05$; Fig. 5B], or the percent entries into the open arms [$F(2,22)=3.26$, $P>0.05$; Fig. 5C]. The three treatment groups did not differ in other indices of anxiogenic-like behavior examined, including rearing frequency [$F(2,22)=1.15$, $P>0.05$], amount of time spent autogrooming [$F(2,22)=0.28$, $P>0.05$], and number of fecal boli excreted [$F(2,22)=0.47$, $P>0.05$] during the 5-min test in the elevated plus maze.

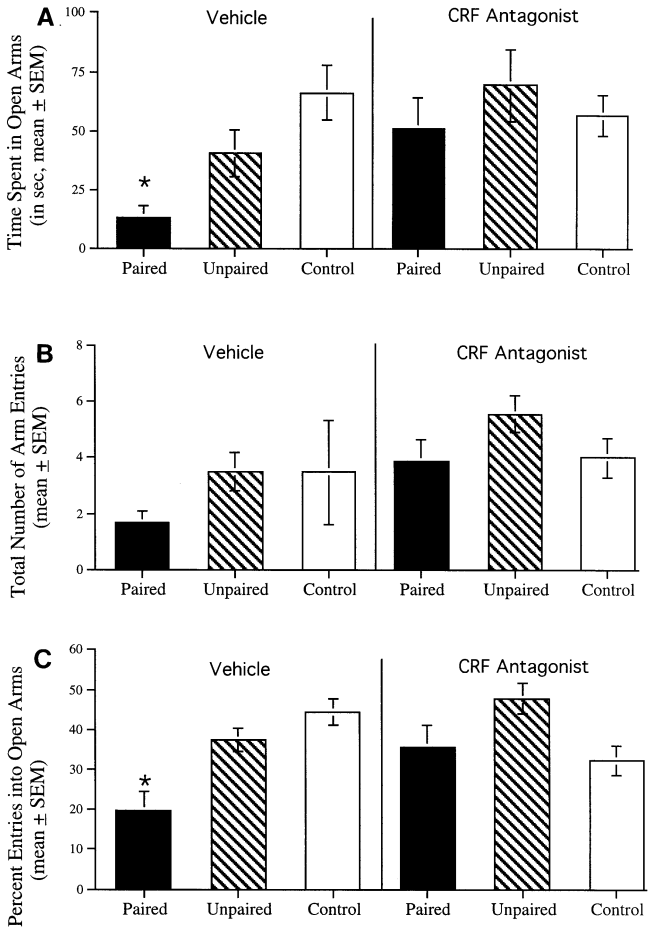

Fig. 5A–C Behavior in the elevated plus-maze after 25 min exposure to locomotor apparatus cues which for the previous 5 days had been paired with cocaine injections in the Paired group and saline injections in the Unpaired and Control groups. Thirty minutes prior to placement in the locomotor apparatus, animals were treated ICV with either a CRF antagonist (1 μg α-helical $CRF_{9–41}$) or the vehicle. The behavioral test in the elevated plus-maze lasted 5 min. Measures scored included: the duration of time spent in the open arms of the apparatus (mean±SEM in s; **A**), total number of arm entries (mean±SEM; **B**) and percentage of open arm entries (**C**). Data were analyzed via ANOVA. An *asterisk* (*) indicates a significant treatment effect compared to the Control group at $P<0.05$

Among the animals pretreated with the vehicle (ICV), the Paired group ($n=9$) spent significantly less time in the open arms of the elevated plus-maze than the Unpaired ($n=8$) and Control ($n=9$) groups [$F(2,23)=8.84$, $P<0.01$; Fig. 5A]. Although the total number of arm entries did not differ among the treatment groups [$F(2,23)=3.18$, $P>0.05$; Fig. 5B], the percentage of entries that were into the open arms was significantly lower in the Paired group than the Unpaired and Control groups [$F(2,23)=11.5$, $P<0.01$; Fig. 5C]. The three treatment groups did not differ in other indices of anxiogenic-like behavior examined, including rearing frequency

[$F(2,23)=0.03$, $P>0.05$], amount of time spent grooming [$F(2,23)=0.94$, $P>0.05$], and number of fecal boli excreted [$F(2,23)=1.07$, $P>0.05$] during the 5-min test in the elevated plus maze.

Discussion

The unconditioned anxiogenic-like actions of cocaine were revealed in experiment 1. Injections of cocaine as low as 10 mg/kg in cocaine-pretreated rats caused a significant decrease in the amount of time that the animals spent in the open arms of the elevated plus-maze when tested 25 min after drug administration. Such effects are generally thought to reflect increases in anxiogenic-like behavior (Lister 1987), and corroborate previous studies which indicate that cocaine can induce anxiogenic-like effects in mice and rats (Costall et al. 1989; Fontana et al. 1989; Ettenberg and Geist 1991; Rogerio and Takahashi 1992a, b; Sarnyai et al. 1995). However, the most intriguing finding to emerge from the present study is that cocaine-associated cues can elicit negative properties which persist and can be measured in a novel environment. Paired rats exposed to cocaine-associated stimuli in the activity chambers spent significantly less time in the open arms of an elevated plus-maze than the Unpaired and Control groups. Thus it appears that exposure to cocaine-associated cues for 25 min produces an anxiogenic-like effect that persists beyond exposure to the locomotor apparatus cues (Fig. 3). The Paired group also had significantly fewer open arm entries relative to closed arm entries, further supporting the robust anxiogenic-like effects of cocaine-associated cues in these rats. This measure has also been proposed to reflect increases in anxiety (Lister 1987). Since there was no significant difference in the total number of arm entries among the three groups, the effects reported above can not be attributed to group differences in locomotor output.

Thus, it appears that cocaine-associated cues can elicit two opposing effects. The predominant effect appears to involve activation of incentive motivational processes which energize behavior and propel an organism to engage in goal directed behavior (Pert 1994). An opposing action is the induction of a mild state of anxiety which may inhibit behavior under certain circumstances. Ettenberg and Geist (1991) have postulated the operation of similar opposing processes in determining goal directed behavior for cocaine infusions in a runway paradigm. The relative importance of either of these two opposing processes or their net balance may play an important role in determining individual vulnerability to cocaine addiction in animals and humans.

Operation of the energizing or incentive motivational action of cocaine-associated stimuli was revealed in the locomotor activity chambers on the test day (see Fig. 2). Exposure to contextual cues for 30 min over five daily sessions under the influence of cocaine produced strong behavioral conditioning which was revealed when all three experimental groups were tested without the drug on day 6. The conditioned increases in locomotor activity are evident when the performance of the Paired and Unpaired groups is compared on the test day. It should be noted that both of these groups were exposed to the same cocaine regimen, but in different contexts. Thus, the difference in behavior between them must be determined by contextual factors (i.e. conditioning). We have reported in a previous study that associative learning processes underlie the increased behavioral output of the Paired rats on the test day (Rothman and Pert 1994) and that such increases in behavior reflect the expression of incentive motivational processes (Pert 1994).

It is of interest to note that the Unpaired rats placed in the elevated plus-maze immediately following the 25-min session in the locomotor activity chambers also spent significantly less time in the open arms compared to the Control group (Fig. 3A). However, this effect was somewhat smaller in magnitude compared to that found in the Paired group. As noted above, such effects are generally thought to be reflective of increased anxiogenic-like behavior (Rogerio and Takahachi 1992a; Yang et al. 1992; Sarnyai et al. 1995). It is possible that the decrease in open arm time exhibited by the Unpaired group in experiment 2 may have been due to withdrawal from cocaine after 5 days of repeated injections. Termination of repetitive cocaine administration has been reported to precipitate negative symptoms indicative of anxiogenic-like behavior in a number of animal paradigms (Costall et al. 1989; Fontana et al. 1989; Harris and Aston-Jones 1993; Sarnyai et al. 1995; Barros and Miczek 1996). Furthermore, withdrawal from cocaine produces a stimulus state that generalizes to the anxiogenic drug pentylenetetrazol (Wood and Lal 1987; Wood et al. 1989). Several studies also have reported disruptions in operant behaviors maintained by food or a sweetened solution during cocaine withdrawal (Carroll and Lac 1987; Woolverton and Kleven 1988). In addition, termination of cocaine administration has increased thresholds for intracranial self-stimulation reward (Kokkinidis and McCarter 1990; Markou and Koob 1991), a behavior that is thought to be mediated predominantly through the mesoaccumbens dopamine system (Wise and Rompre 1987). Withdrawal effects must also contribute to the increase in anxiogenic-like behavior exhibited by the Paired group in this study. The anxiogenic-like effects achieved in this group, however, also include the conditioned component induced by exposure to cocaine-associated cues.

Acute cocaine injections have been found to increase ACTH and corticosterone release in rats following either systemic or intracerebroventricular injections (Moldow and Fischman 1987; Pilotte et al. 1990; Torres and Rivier 1992; Kling et al. 1993; Saphier et al. 1993; Carey et al. 1994). Such effects appear to be mediated through alterations in endogenous CRF. Cocaine stimulates hypothalamic CRF secretion in vitro (Calogero et al. 1989), while cocaine-induced increases in plasma ACTH and CORT, following systemic injections, are inhibited by in-

traventricular pretreatment with either CRF antiserum or the CRF antagonist, ∝-helical $CRF_{9–41}$ (Rivier and Vale 1987; Sarnyai et al. 1992). Using a design similar to the one used in this study, we have recently found that contextual cues associated with cocaine are effective in producing conditioned increases in plasma corticosterone in rats (DeVries et al. 1998).

CRF mediates, and CRF antagonists block, anxiogenic-like effects of several different types of stressors including novelty, physical restraint, ethanol or cocaine withdrawal and social conflict (reviewed in Dunn and Berridge 1990; Baldwin et al. 1991; Heinrichs et al. 1992, 1994; Sarnyai et al. 1995). The mechanism underlying the persistent anxiogenic-like effect that we observe in rats following exposure to cocaine-associated cues also appears to involve CRF. In experiment 3, rats in the Paired group that were pretreated on the test day with a CRF antagonist, ∝-helical $CRF_{9–41}$ continued to exhibit conditioned increases in locomotor activity during exposure to cocaine-associated contextual cues but did not exhibit conditioned anxiogenic-like behavior in the elevated plus-maze. These data suggest that conditioned increases in anxiogenic-like behavior, but not locomotor activity, are mediated by endogenous CRF. It is likely that exposure to cocaine-associated stimuli in the present study activated the HPA axis or enhanced extrahypothalamic CRF systems, and that such alterations in function may have persisted beyond the conditioned stimulus exposure and contributed to the anxiogenic-like effects observed in the elevated plus-maze test. It is also of interest to note that cocaine withdrawal-induced anxiogenic-like behavior has been demonstrated to involve extrahypothalamic-limbic CRF hypersecretion (Sarnyai et al. 1995). Exposure to conditioned stimuli in this study may have potentiated the anxiogenic effects of cocaine withdrawal through a similar mechanism. Indeed, human cocaine addicts presented with cocaine-associated cues report increases in anxiety and craving which are accompanied by increased HPA activity (Berger et al. 1996).

The conditioned anxiogenic-like effects elicited by cocaine-associated stimuli may have several clinical implications. For example, the induction of negative mood states (e.g. anger, depression, and anxiety) can elicit drug craving and relapse in cocaine and heroin addicts (Childress et al. 1987a, 1994; Wallace 1989). The induction of anxiety (and possibly other negative symptoms) by cocaine-associated cues may in some way further increase the motivational state that underlies craving during drug abstinence. The conditioned anxiogenic effects of cocaine also may be relevant for understanding the development and persistence of panic attacks in cocaine addicts (Anthony et al. 1989, Louie et al. 1989). It is possible that panic attacks could be triggered long after discontinuation of the drug by cocaine-associated cues.

In summary, both cocaine and cocaine-associated contextual cues increase anxiogenic-like behavior in the elevated plus-maze. Pretreatment with a CRF antagonist blocked conditioned increases in anxiogenic-like behavior following exposure to cocaine-associated cues, thereby suggesting that these behavioral effects may be mediated via the HPA axis. Additional research is needed to understand fully the neural circuitry through which the conditioned, as well as unconditioned, anxiogenic effects of cocaine are mediated.

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