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A study of the lasting effects of cocaine pre-exposure on anxiety-like behaviors under baseline conditions and in response to central injections of corticotropin-releasing factor

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Abstract

Anxiety-like behaviors emerge with repeated exposure to and short-term withdrawal from cocaine. The stress-related neuropeptide, corticotropin-releasing factor (CRF), has been implicated in the anxiogenic effects of cocaine withdrawal, as well as in some of the long-lasting effects of cocaine. One objective of the present experiments was to determine whether repeated exposures to cocaine, under conditions that induce anxiety in the initial withdrawal period, would induce longer-lasting anxiogenic responses. A second objective was to determine whether any such effects would be potentiated by CRF. In Experiment 1, animals were injected once daily for 7 days with cocaine (30 mg/kg, i.p.) or saline in the home cages and, after a 10-day drug-free period, were given an i.c.v. injection of CRF (0.5 or 5.0 μg) or vehicle, followed by a 5-min test for anxiety in the elevated plus maze or light–dark transition apparatus. In Experiment 2, animals were given the cocaine or saline injections in a distinct environment. At test, they were placed in the distinct environment after the CRF (0.5 μg) or vehicle injection and were subsequently tested for anxiety. Cocaine produced enhanced levels of anxiety when pre-exposures were given in a distinct environment, but not when they were given in the home cage. In neither case did cocaine differentially alter anxiety-like responses to CRF. The results suggest that a "reminder" of the drug experience, such as re-exposure to cocaine-paired contextual cues, may be necessary to induce elevated levels of anxiety after the initial withdrawal period. In addition, although the results do not rule out a role for endogenous CRF in lasting cocaine-induced anxiogenic responses, they suggest that an increased sensitivity of CRF receptors to the peptide is not responsible for the effect.

Keywords: Cocaine; Anxiety; Corticotropin-releasing factor (CRF); Withdrawal; Elevated plus maze; Light–dark transition test; Rat

1. Introduction

Anxiety is considered one of the hallmark symptoms of cocaine withdrawal, particularly in the initial withdrawal or so-called "crash" phase (Gawin, 1991). It has been postulated that anxiety also persists into the later withdrawal period and is accompanied by intense craving that may lead to relapse (Richter and Weiss, 1999; Weiss et al., 2001). However, a relationship between anxiety and the long-term effects of cocaine withdrawal has been difficult to demonstrate in humans and has been the subject of surprisingly limited study in laboratory animals. While there are numerous reports that animals exhibit enhanced anxiety during the first several days of withdrawal from cocaine (Basso et al., 1999; DeVries and Pert, 1998; Mutschler and Miczek, 1998; Sarnyai et al., 1995), the extent to which anxiety-like responses persist over extended drug-free periods is unclear. In some studies, cocaine pre-exposed animals exhibited anxiety-like behaviors after drug-free periods of up to 7 days (e.g., Fontana and Commissaris, 1989; Gordon and Rosen, 1999), whereas in other studies, cocaine pre-exposure was ineffective in elevating anxiety beyond the first 2–3 days of withdrawal (e.g., Mutschler and Miczek, 1998).

In laboratory studies, the stress-related neuropeptide, corticotropin-releasing factor (CRF), has been implicated in the anxiogenic effects of early cocaine withdrawal. For example, i.c.v. injections of CRF-receptor antagonists have been found to attenuate anxiety-like behavior in the defensive burying paradigm 48 h after cessation of chronic cocaine treatment (Basso et al., 1999). Likewise, central antagonism of CRF receptors has been

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found to interfere in anxiety-like behavior in the elevated plus maze when animals are re-exposed to the environment in which cocaine was administered, 48 h following cessation of treatment (DeVries and Pert, 1998). In addition, it has been reported that behavioral anxiety exhibited 48 h after withdrawal from cocaine is accompanied by reduction in tissue levels of CRF immunoreactivity in the amgydala, hypothalamus and basal forebrain, suggesting increased release of the peptide in these regions (Sarnyai et al., 1995).

Although there is mounting evidence that CRF systems play an important role in some of the long-lasting effects of cocaine, including stress-induced reinstatement of cocaine seeking (Shaham et al., 2000) and behavioural sensitization (Erb and Brown, 2006), the possible role of CRF in lasting anxiogenic responses induced by cocaine is not clear. Most reports, however, are consistent with the idea that increases in the activity of CRF systems that accompany anxiety during the early withdrawal phase do not persist for more than 2–3 days (e.g. Ambrosia et al., 1997; Zhou et al., 1996). Thus, to the extent that anxiety does in fact persist beyond the early withdrawal period, it is unlikely that it can be attributed to sustained increases in the basal activity of CRF systems, as it can in the early withdrawal period.

There is, however, mounting evidence that prior, repeated exposure to cocaine produces changes in the *responsivity* of the central nervous system to CRF following prolonged drug-free periods, raising the possibility that animals pre-exposed to cocaine may show lasting, potentiated anxiety-like behaviors in response to CRF. For example, we recently reported that animals pre-exposed to cocaine exhibit a potentiated locomotor response to i.c.v. injections of CRF up to 28 days post-treatment and that this potentiated behavioral response is accompanied by enhanced c-*fos* mRNA expression in the central nucleus of the amygdala (CeA) (Erb et al., 2003, 2005). Likewise, Wang et al. (2005) recently reported that injections of CRF into the ventral tegmental area (VTA) selectively increased local dopamine and glutamate release in cocaine-experienced, but not naïve, rats.

The objectives of the present experiments were two-fold. The first was to determine whether cocaine pre-exposure conditions, similar to those shown previously to produce anxiety-like behaviour in the initial withdrawal period (e.g. DeVries and Pert, 1998; Yang et al., 1992) and potentiated locomotor and neuronal responses to CRF following extended drug-free periods (Erb et al., 2003, 2005), would produce elevations in anxiety-like behaviors after an extended drug-free period of 10 days. The second objective was to determine whether cocaine pre-exposed animals, relative to those pre-exposed to saline, would exhibit differential anxiety-like behaviors in response to i.c.v. injections of CRF.

2. Materials and methods

2.1. Subjects

A total of 120 male Wistar rats (275–300 g at the beginning of experimentation) were used in these experiments. Animals were housed singly in plastic cages and maintained on a regular light–dark schedule (lights on 0730–1930), in a temperature- and humidity-controlled vivarium. Free access to standard laboratory rat chow and water was given throughout the experiment. All procedures were performed in accordance with the guidelines of the Canadian Council of Animal Care and were approved by the animal care committee at the University of Toronto.

2.2. Surgery

Before surgery, rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and injected with atropine sulfate (0.12 mg, s.c.). Using standard aseptic stereotaxic techniques, a 23 gauge guide cannula (Plastics One, Roanoke, VA) was implanted in the right lateral ventricle, aimed 1 mm above the injection site. The final coordinates for the injector cannula tip were: anterior/posterior: −1.0 mm from bregma; lateral/medial: −1.4 mm from bregma; dorsal/ventral: −3.7 mm from dura (Paxinos and Watson, 1997). The cannula was affixed to the skull using jewellers' screws and dental acrylic. After surgery, a stainless steel blocker was inserted into the intracranial cannula.

Cannula placements were verified at least 3 days prior to any experimental manipulations by injecting angiotensin (Sigma-Aldrich, Oakville, ON) i.c.v. and observing subsequent drinking behaviour. Angiotensin was prepared in distilled water and injected in a concentration of 50 ng/2 μl (see below for i.c.v. injection procedures). Placements were considered to be accurate if a rat drank water within 1 min of the infusion and sustained drinking over 2–3 min (Sakai et al., 1995).

2.3. Intraventricular microinjection procedures

CRF and its vehicle were injected in a volume of 4 μl, with a 10 μl Hamilton syringe connected to an injector (30 gauge) that extended 1 mm beyond the tip of the intraventricular cannula to the infusion site. Infusions were made over 60 s, after which the injectors were left in place for an additional 45–60 s. During the infusion the animal was allowed to move freely in the cage.

2.4. Drugs

Cocaine HCl (Medisca Pharmaceuticals, St. Laurant, QC) was dissolved and sterile filtered in physiological saline. CRF (Sigma-Aldrich, Oakville, ON) was dissolved in sterile physiological saline and was injected i.c.v. in a volume of 4 μl.

2.5. Apparatus

2.5.1. Elevated plus maze

The 4-arm maze (each arm 50 cm long × 10 cm wide) was constructed of Plexiglas. The arms extended at 90° angles from a centre square platform (10 cm × 10 cm). The entire floor surface was covered in a flat black rubber material. Two arms, positioned 180° from each other, were surrounded by 40 cm high dark opaque walls; the remaining two arms were not surrounded by walls. The entire apparatus was elevated off the ground with 115 cm poles, one attached to the end of each arm.

A covered lamp illuminated with a 40 W bulb was placed beneath the centre of the plus maze; otherwise, the room was dark during testing.

2.5.2. Light–dark transition box

The apparatus was a rectangular chamber constructed of Plexiglas and comprised of two adjacent and abutting sections (each 60 cm long × 30 cm wide × 25 cm high); the sections were separated by a black Plexiglas partition containing a 12 cm × 12 cm opening. The walls and floor of one section were black (i.e., "dark" side) and the walls and floor of the other section were clear (i.e., "light" side). The light side was illuminated by a 20-W light located 20 cm above the floor on the end wall; otherwise, the room was dark during testing.

2.6. Procedures

2.6.1. Exp 1A and B: CRF-induced anxiety following previous home cage injections of cocaine

2.6.1.1. Cocaine pre-exposure. Animals were given daily injections of cocaine (30 mg/kg, i.p.) or saline (i.p.) for 7 days. Injections were given in the home cage at approximately 10:00 a.m. Following a 10- to 12-day drug-free period animals were tested for anxiety in either the elevated plus maze (Exp 1A) or light–dark transition apparatus (Exp 1B).

2.6.1.2. Exp 1A: Elevated plus maze test. On each of 2 days prior to testing, rats were habituated to the transport and injection procedures to be used during testing. Specifically, rats were transported in their home cages to a temporary holding room. After 30 min, they were given a sham i.c.v. injection. About 20 min later, they were transferred to the testing room where they remained for 2 h under the lighting conditions in which testing was conducted.

During testing, which occurred over a 2-day period (i.e., each animal was tested on 1 of 2 days), rats were transported to the temporary holding room. After 30 min, they were given an injection of CRF (0.5, or 5.0 μg, i.c.v.) or vehicle (i.c.v.). About 20 min later they were carried to the adjacent testing room where they were immediately placed in the centre compartment of the elevated plus maze. Time spent on the open arms and number of open and closed arm entries and exits were recorded over a 5-min test period. Entries were defined by an animal crossing onto an arm with both front paws and at least 1 hind paw; exits were defined by an animal placing 2 paws outside of an arm. Behavior was recorded by an experimenter positioned approximately 1 m from and behind a closed arm of the maze; the experimenter was blind to the treatment condition.

2.6.1.3. Exp 1B: Light–dark transition test. One day prior to testing, rats were habituated to the transport and injection procedures to be used during testing. Specifically, rats were transported in wire hanging cages to an adjacent temporary holding room where they were given a sham i.c.v. injection. About 20 min later, they were transferred to the testing room where they remained for 2 h under the lighting conditions in which testing was conducted.

During testing, which occurred over a 3-day period (i.e., each animal was tested on 1 of 3 days), rats were transported to the temporary holding room where they were given an injection of CRF (0.5, or 5.0 μg, i.c.v.) or vehicle (i.c.v.). About 20 min later they were carried to the testing room where they were immediately placed in the dark chamber of the light–dark transition apparatus. Latency to emerge from the dark chamber (defined by an animal having both front paws and at least one hind paw in the light chamber) and total time spent in the light chamber were recorded over a 5-min test period. Behaviour was observed and recorded via a monitor connected to a video-camera in an adjacent room; behavior was recorded by an experimenter blind to the treatment conditions.

2.6.2. Exp 2A and B: CRF-induced anxiety following re-exposure to a distinct environment previously paired with cocaine

In Experiment 1, cocaine pre-exposures, given in the home cage, did not produce elevated levels of anxiety in either test, nor did it differentially alter the response to an i.c.v. injection of CRF. Experiment 2 was conducted, therefore, to determine whether administering the cocaine in a distinct environment (rather than the home cage), and subsequently testing animals for anxiety following re-exposure to that environment, would alter anxiety-like behaviours. There is some evidence (see Discussion), that in order for cocaine pre-exposure to induce elevated levels of anxiety after a drug-free period that exceeds the initial withdrawal, the drug may need to be given in a distinct environment and the animal may need to be re-exposed to that environment before testing.

2.6.2.1. Cocaine pre-exposure. Animals were given daily injections of cocaine (30 mg/kg, i.p.) or saline (i.p.) for 7 days in a distinct environment. The distinct environment was a standard operant chamber, constructed of Plexiglas walls and a steel rod floor. Each day, animals were transferred to a room adjacent to the holding room that housed the chambers. Once transferred, the animals were injected with cocaine or saline, and placed for 30 min in the chamber. Injections were given at approximately 10:00 a.m.

Following a 10- to 12-day drug-free period animals were tested for anxiety in the elevated plus maze or light–dark transition apparatus.

2.6.2.2. Exp 2A: Elevated plus maze test. On each of 2 days prior to testing, rats were habituated to the transport and injection procedures to be used during testing. Rats were transported to the room housing the distinct environment and were given a sham i.c.v. injection in this room. After 30 min they were transferred to the testing room where they remained for 2 h under the lighting conditions in which testing was conducted.

During testing, which occurred over a 2-day period (i.e., each animal was tested on 1 of 2 consecutive days), rats were transported to the room with the distinct environment where they were given an injection of CRF (0.5 μg, i.c.v.) or vehicle (i.c.v.). Immediately following the injection, they were placed in the distinct environment. After 30 min, they were transported to the test room where they were immediately placed in the

centre chamber of the elevated plus maze and behavior was observed and recorded as described for Experiment 1A.

2.6.2.3. Exp 2B: Light–dark transition test. One day prior to testing, rats were habituated to the transport and injection procedures, as described in Experiment 2A.

During testing, which occurred over a 3-day period (i.e., each animal was tested on 1 of 3 days), rats were transported to the room housing the distinct environment where they were given an injection of CRF (0.5 μg, i.c.v.) or vehicle (i.c.v.). Immediately following the injection, they were placed in the distinct environment. After 30 min, they were carried to an adjacent testing room where they were immediately placed in the dark chamber of the light–dark transition apparatus and behaviour was observed and recorded as described for Experiment 1B.

2.7. Statistical analyses

The dependent measures that were analyzed for the elevated plus maze test were time spent in the open arms (in s) and total number of open and closed arm entries and exits. The dependent measures for the light–dark transition test were time spent in the light chamber (in s) and latency to enter the light chamber (in s). In all cases, the dependent variables were analyzed using ANOVAs for the between subjects factors of Pre-treatment (vehicle or CRF dose) and Pre-exposure (Cocaine, Saline). Significant effects were followed up with Fisher's LSD post-hoc comparisons ($p<.05$), as appropriate.

3. Results

3.1. Exp 1A and B: CRF-induced anxiety following previous home cage injections of cocaine

3.1.1. Exp 1A: Elevated plus maze

Fig. 1A shows, for cocaine and saline pre-exposed rats, the mean percent time (±S.E.M.) spent in the open arms (i.e., time in open arms/[time spent in open+closed arms]) of the elevated plus maze during the 5 min test following pre-treatment with vehicle or CRF. A 2×3 ANOVA for time spent on the open arms revealed a significant effect of CRF Pre-treatment ($F[2,40]=4.33$, $p<.03$), but no effect of Pre-exposure or interaction between the two factors. As indicated in the figure, post-hoc comparisons between the various Pre-treatment conditions revealed significant differences between the vehicle and 5.0 μg CRF groups and between the two CRF dose groups. Thus, the higher dose of CRF was associated with reduced time spent in the open arms of the maze. A similar ANOVA for number of entries into the open arms (data not shown), also an index of anxiety-like behavior, paralleled the outcome of the ANOVA for time spent in the open arms (Pre-treatment: $F[2,40]=3.74$; $p<.05$). Furthermore, a correlation between the two measures was highly significant ($r=69$, $p<.001$).

Fig. 1B shows, for cocaine and saline pre-exposed rats, total number of entries (±S.E.M.) and exits into and out of the four arms of the elevated plus maze. The ANOVA on these data did not reveal any significant effects. Thus, there were no differences between Pre-treatment and Pre-exposure groups in overall level of activity.

3.1.2. Exp 1B: Light–dark transition test

Fig. 2A shows, for cocaine and saline pre-exposed rats, the mean time (±S.E.M.) spent in the light chamber (i.e., time in light/[time in light+dark]) of the light–dark apparatus during the 5 min transition test following pre-treatment with vehicle or CRF. Consistent with the outcome of the analysis for time spent in the open arms of the plus maze, this analysis revealed a significant effect of CRF Pre-treatment ($F[2,66]=28.47$, $p<.001$), but no effect of Pre-exposure or Pre-treatment×Pre-exposure interaction. As indicated in the figure, post-hoc comparisons of the Pre-treatment effect revealed significant differences between the vehicle and both CRF dose groups and between the two CRF dose groups. Thus, pre-treatment with CRF was dose-dependently associated with reduced time spent in the light chamber, in both cocaine and saline pre-exposed rats.

Fig. 2B shows, for cocaine and saline pre-exposed rats, the mean (±S.E.M.) latency to enter the light chamber during the 5 min test following pre-treatment with vehicle or CRF. Consistent with the analysis for time spent in the light chamber, an ANOVA on the dependent measure of latency to enter the

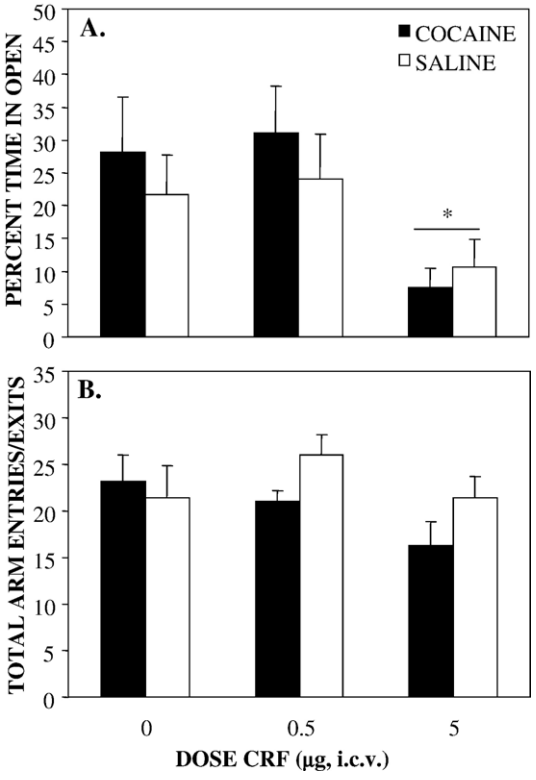

Fig. 1. Exp 1A: (A) Mean percent time (±S.E.M.) spent in the open arms (i.e., time in open arms/[time in open+closed arms]) of the elevated plus maze during a 5 min test; (B) Total number (±S.E.M.) of open and closed arm entries and exits. Animals ($n=7$–9 per group) had been pre-exposed to cocaine or saline 10–12 days before testing and were pre-treated with vehicle (0 μg, i.c.v.) or CRF (0.5 or 5.0 μg, i.c.v.) 5 min before testing. *Different from other Pre-treatment conditions, $p<.05$.

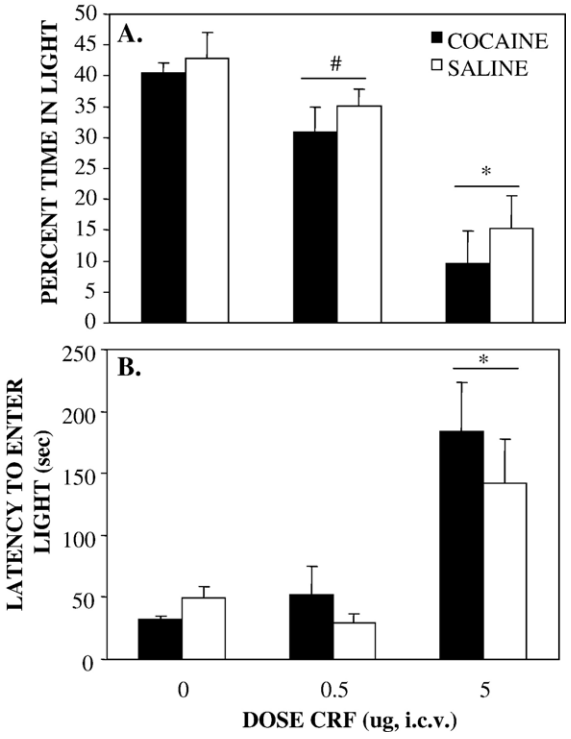

Fig. 2. Exp 1B: (A) Mean percent time (±S.E.M.) spent in the light chamber (i.e., time in light/[time in light+dark]) of the light–dark apparatus during a 5-min transition test; (B) Latency (±S.E.M.) to enter the light chamber during the 5-min test. Animals (n=10–14 per group) had been pre-exposed to cocaine or saline 10–12 days before testing and were pre-treated with vehicle (0 μg, i.c.v.) or CRF (0.5 or 5.0 μg, i.c.v.) 5 min before testing. *Different from other Pre-treatment conditions, $p<.05$. #Different from the vehicle condition, $p<.05$.

light chamber revealed only a significant effect of Pre-treatment ($F[2,66]=19.98$, $p<.001$). In this case, post-hoc comparisons revealed significant differences between the vehicle and 5.0 μg CRF dose groups and between the 0.5 and 5.0 μg dose groups. Thus, pre-treatment with the relatively higher dose of CRF (5.0 μg) was associated with an increased latency to enter the light chamber.

3.2. Exp 2A and B: CRF-induced anxiety following re-exposure to a distinct environment previously paired with cocaine

3.2.1. Exp 2A: Elevated plus maze

Fig. 3A shows, for cocaine and saline pre-exposed rats, the mean percent time (±S.E.M.) spent in the open arms (i.e., time in open arms/[time spent in open+closed arms]) of the elevated plus maze during the 5-min test following pre-treatment with vehicle or CRF. A 2×2 ANOVA for time spent on the open arms revealed a significant effect of CRF Pre-treatment ($F[2,40]=4.33$, $p<.03$) and a significant effect of Pre-exposure ($F[1,29]=6.415$, $p<.03$), but no interaction between the two factors. It can be seen in the figure that, consistent with the outcome in Experiment 1A, CRF reduced time spent in the open arms of the plus maze in both cocaine and saline pre-exposed animals. In contrast to Experiment 1A, however, cocaine pre-exposed rats spent less time in the open arms of the plus maze when compared with saline pre-exposed rats, regardless of whether or not they had been pre-treated with CRF. Thus, re-exposure to a distinct environment previously paired with cocaine was associated with an increase in anxiety-like behaviour, as was pre-treatment with CRF.

As was the case in Experiment 1A, a similar ANOVA for number of entries into the open arms (data not shown) paralleled the outcome of the ANOVA for time spent in the open arms (Pre-treatment: $F[1,29]=8.32$; $p<.01$; Pre-exposure: $F[1,29]=5.80$, $p<.03$). In addition, a correlation between the two measures was highly significant ($r=90$, $p<.001$).

Fig. 3B shows, for cocaine and saline pre-exposed rats, total number of entries and exits into and out of the four arms of the elevated plus maze. The ANOVA on these data did not reveal any significant effects. Thus, there were no differences between Pre-treatment and Pre-exposure groups in overall level of activity.

3.2.2. Exp 2B: Light–dark transition test

Fig. 4A shows, for cocaine and saline pre-exposed rats, the mean percent time (±S.E.M.) spent in the light chamber (i.e., time in light/[time in light+dark]) of the light–dark apparatus during the 5-min transition test following pre-treatment with vehicle or CRF. Consistent with the outcome of the analysis for time spent in the open arms of the plus maze in Experiment 2A, this analysis revealed a significant effect of CRF Pre-treatment

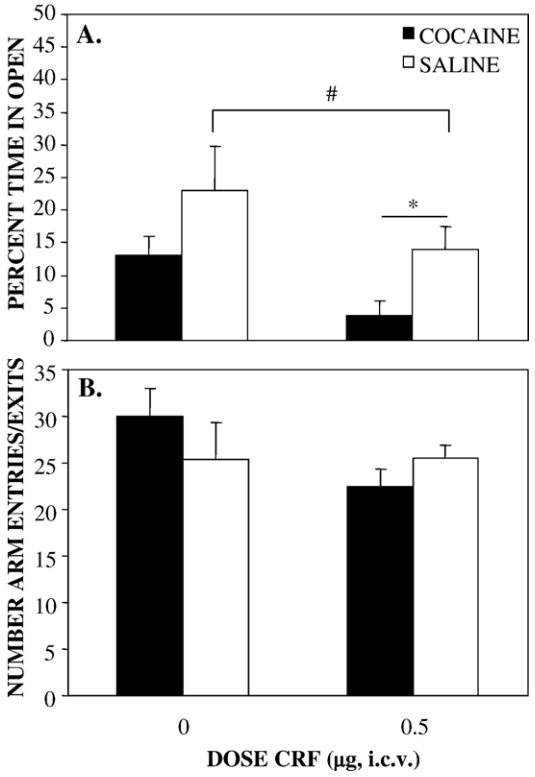

Fig. 3. Exp 2A: (A) Mean percent time (±S.E.M.) spent in the open arms (i.e., time in open arms/[time in open+closed arms]) of the elevated plus maze during a 5 min test; (B) Total number (±S.E.M.) of open and closed arm entries and exits. Animals (n=7–9 per group) had been pre-exposed to cocaine or saline in a distinct environment, 10–12 days before testing. On test day, they were pre-treated with vehicle (0 μg, i.c.v.) or CRF (0.5 μg, i.c.v.), placed in the distinct environment for 30 min, and subsequently tested. *Different from the "0-dose" group, $p<.05$. #Different from the Cocaine Pre-exposure condition, $p<.05$.

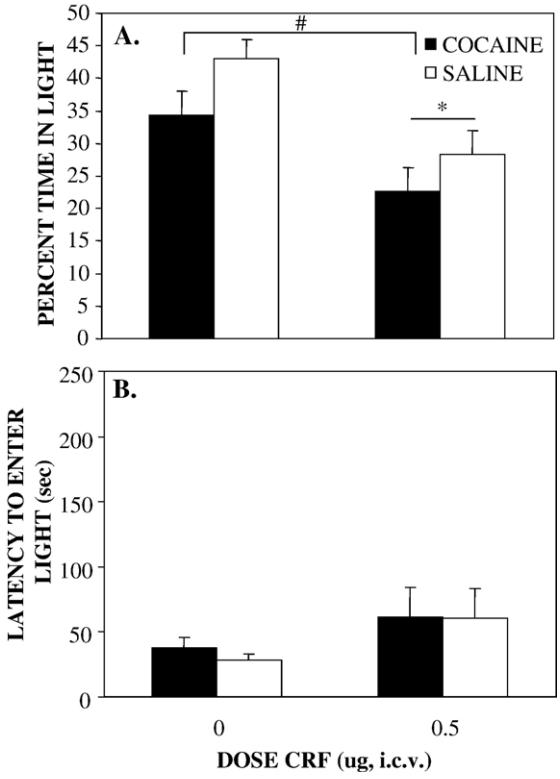

Fig. 4. Exp 2B: (A) Mean percent time (±S.E.M.) spent in the light chamber (i.e., time in light/[time in light+dark]) of the light–dark apparatus during a 5-min transition test; (B) Latency (±S.E.M.) to enter the light chamber during the 5-min test. Animals (n=12 per group) had been pre-exposed to cocaine or saline in a distinct environment, 10–12 days before testing. On test day, they were pre-treated with vehicle (0 μg, i.c.v.) or CRF (0.5 μg, i.c.v.), placed in the distinct environment for 30 min, and subsequently tested. *Different from the "0-dose" group, $p<.05$. #Different from the Saline Pre-exposure condition, $p<.05$.

($F[1,44]=14.37$, $p<.001$) and Pre-exposure ($F[1,44]=4.13$, $p<.05$), but no interaction between the two factors. It can be seen in the figure that, consistent with the outcome in Experiment 1B, CRF reduced time spent in the light chamber in both cocaine and saline pre-exposed animals. However, in contrast to Experiment 1B, but consistent with Experiment 2A, cocaine pre-exposed rats spent less time in the light chamber when compared with saline pre-exposed rats, regardless of whether or not they had been pre-treated with CRF. Thus, re-exposure to a distinct environment previously paired with cocaine was associated with an increase in anxiety-like behaviour in the light–dark transition test, as was pre-treatment with CRF.

Fig. 4B shows, for cocaine and saline pre-exposed rats, the mean (±S.E.M.) latency to enter the light chamber during the 5 min test following pre-treatment with vehicle or CRF. In this case, the ANOVA did not reveal any significant effects.

4. Discussion

Two findings emerge from the present series of experiments. First, re-exposure to a distinct environment previously paired with cocaine resulted in enhanced levels of anxiety in the elevated plus maze and light–dark transition tests, when animals were re-exposed to the environment after a drug-free period of at least 10 days. Home cage injections of cocaine, on the other hand, failed to produce long-lasting changes in anxiety-like behaviors. Second, in contrast to our predictions, i.c.v. injections of CRF prior to testing failed to potentiate anxiety-like behaviors in cocaine relative to saline pre-exposed animals, whether animals had been given the cocaine or saline treatments in the home cage or distinct environment. Thus, cocaine pre-exposure conditions which have been previously shown to induce a potentiated locomotor response to i.c.v. injections of CRF, after a drug-free period of 10 days (Erb et al., 2003, 2005), do not induce changes in anxiety-like behaviours.

As mentioned, relatively few studies have explored the effects of prior, repeated exposure to cocaine on anxiety-like behaviors beyond the first 48 h of withdrawal. Together with the present findings, however, what data there are seem to be consistent with the idea that cocaine pre-exposure can produce anxiogenic responses after the initial withdrawal period, if the pre-exposures are explicitly paired with distinct contextual cues and if animals are re-exposed to those cues at the time of testing. For example, Gordon and Rosen (1999), using a very similar cocaine pre-exposure regimen (20 mg/kg, i.p., for 7 days) to that used in the present experiments (30 mg/kg, i.p., for 7 days), showed that cocaine pre-exposed rats exhibited enhanced acoustic and fear-potentiated startle responses after a 1-week drug-free period, so long as the cocaine injections were given in the context of the startle chamber; rats pre-exposed to cocaine in the home cage and tested one week later did not show elevated startle responses. These results are consistent with the outcome of the present experiments and are supported by the collective outcomes of other studies in which, arguably, animals either were or were not exposed at test to an environment that had been explicitly paired with cocaine (e.g., Fontana and Commissaris, 1989; Mutschler and Miczek, 1998). Thus, a "reminder" of the drug experience, such as re-exposure to contextual cues previously paired with cocaine, may be necessary to reactivate a cocaine-induced anxiety-like state after the initial withdrawal period. Whether other "reminders", such as discrete cues explicitly paired with cocaine or a cocaine challenge, might have similar effects on anxiety to those induced by contextual cue awaits investigation.

It is interesting to note that in the first 48 h of withdrawal from cocaine, animals exhibit enhanced levels of anxiety, irrespective of whether they are re-exposed to a distinct context previously paired with cocaine at the time of testing (e.g., DeVries and Pert, 1998). The effects of withdrawal on anxiety during this early period following the termination of cocaine has been attributed, at least in part, to enhanced activity of CRF systems within the CeA; during this period, CRF mRNA expression (Erb et al., 2004; Zhou et al., 1996) and peptide levels (Richter and Weiss, 1999) are enhanced in the CeA and CRF protein levels (Sarnyai et al., 1995) and receptor binding (Ambrosia et al., 1997) are reduced in this region, suggesting increased release of peptide. After the first 48–72 h of withdrawal, however, the preponderance of evidence suggests that the activity of CRF systems returns to baseline (Ambrosia et al., 1997; Erb et al., 2004; Zhou et al., 1996; Zorrilla et al., 2001). Thus, to the extent that CRF plays a role in cocaine-induced anxiety following more prolonged periods of withdrawal, the

present findings suggest that the system needs to be reactivated, such as by re-exposure to contextual cues previously paired with the drug, in order to facilitate an anxiogenic response.

Although in the present experiments endogenous release of CRF in response to cocaine-paired contextual cues cannot be ruled out as a mechanism mediating cocaine-induced anxiety after a 10-day withdrawal period, such a role for CRF would not appear to be mediated by an enhanced sensitivity of CRF receptors to the peptide per se. That is, the present findings failed to reveal an effect of repeated cocaine exposure on anxiety-like responses to a low dose of CRF, under the same pre-exposure and withdrawal conditions previously found to produce a potentiated locomotor response to the same dose of CRF (Erb et al., 2003, 2005). CRF did, however, non-differentially enhance anxiety-like responses in cocaine and saline pre-exposed animals.

Together with our earlier related work assessing locomotor activity, these results suggest that the mechanisms mediating the effects of cocaine pre-exposure on CRF-induced locomotor activity are not the same as those that mediate CRF-induced anxiety. Such speculation does not seem particularly bold given that largely dissociable neuronal systems are thought to mediate the locomotor-activating and anxiety-inducing effects of both cocaine and CRF. For example, the mesocorticolimbic system, comprised of DA and glutamatergic interconnections between the VTA, NAcc and prefrontal cortex, is primarily responsible for the locomotor-sensitizing effects of cocaine (e.g., Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000). Likewise, local injections of CRF in the VTA induce behavioral activation (Kalivas et al., 1987) and we have recently demonstrated that antagonism of CRF receptors interferes in the expression of cocaine sensitization (Erb and Brown, 2006), a result that points to an interaction between CRF and DA systems in the mediation of cocaine-induced locomotor sensitization. In contrast, elevated levels of CRF in the CeA have been related to anxiety-like responses in the initial 48 h of withdrawal from cocaine (see Sarnyai et al., 2001) and local CRF injections in this region and the closely associated limbic forebrain region, the bed nucleus of the stria terminalis, potentiate fear and anxiety-related behaviours (Jasnow et al., 2004; Liang et al., 1992).

It should be noted, however, that although the primary neuronal mechanisms mediating locomotion and anxiety are largely dissociable, there is reason to think that the mechanisms may also overlap and that regions of overlap may in fact be functionally important in mediating both behavioral effects. For example, we have recent evidence demonstrating a strong correlation in cocaine pre-exposed animals between potentiated CRF-induced locomotor responsivity and c-*fos* mRNA expression in the CeA (Erb et al., 2005). To the extent that this correlation reflects a functional relationship between the two measures, and to the extent that the known role of CRF in the CeA on cocaine-induced anxiety in short-term withdrawal extends to longer withdrawal periods, one might have expected a potentiating effect of cocaine on CRF-induced anxiety in the present experiments. From this perspective, one possible explanation for the present null effects with respect to the CRF manipulations is that different populations of neurons in the CeA mediate CRF-induced locomotion versus CRF-induced anxiety, and that those neurons responsible for locomotor activity become more rapidly sensitized by cocaine. It is conceivable, for example, that in order for a potentiating effect of cocaine pre-exposure on CRF-induced anxiety to be revealed, a more intensive cocaine dosing regimen than that used in the present experiments is required. Regardless, the present findings demonstrate that under cocaine pre-exposure and withdrawal conditions sufficient to induce a potentiated locomotor response to a low dose of CRF and enhanced baseline levels of anxiety, anxiety-like responses to CRF are unaltered.

Finally, it should be noted that although we did not find an effect of cocaine pre-exposure on CRF-induced anxiety responses, this is not to say that cocaine might not, after similar drug-free periods, affect anxiety responses induced by other stressors. For example, Blatchford et al. (2005) recently reported that a history of opiate exposures produced elevated levels of anxiety in response to restraint stress, up to 7 days after the last exposure. Whether the differential effect between this and the present study is attributable to the type of drug pre-exposure or the type of stressor is a question that warrants investigation in the future.

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