Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Pharmacol Biochem Behav. Author manuscript; available in PMC 2016 November 01.

Published in final edited form as:

Pharmacol Biochem Behav. 2015 November ; 138: 148–155. doi:10.1016/j.pbb.2015.10.002.

CRF antagonism within the ventral tegmental area but not the extended amygdala attenuates the anxiogenic effects of cocaine in rats

Aaron Ettenberg, **Samuel W. Cotten**, **Michael A. Brito**, **Adam K. Klein**, **Tatum A. Ohana**, **Benjamin Margolin**, **Alex Wei**, and **Jennifer M. Wenzel**

Behavioral Pharmacology Laboratory, Department of Psychological & Brain Sciences University of California, Santa Barbara, CA 93106-9660

Abstract

In addition to its initial rewarding effects, cocaine has been shown to produce profound negative/anxiogenic actions. Recent work on the anxiogenic effects of cocaine has examined the role of corticotropin releasing factor (CRF), with particular attention paid to the CRF cell bodies resident to the extended amygdala (i.e, the central nucleus of the amygdala [CeA] and the bed nucleus of the stria terminalis [BNST]) and the interconnections within and projections outside the region (e.g., to the ventral tegmental area [VTA]). In the current study, localized CRF receptor antagonism was produced by intra-BNST, intra-CeA or intra-VTA application of the CRF antagonists, D-Phe CRF $_{(12\text{-}41)}$ or astressin-B. The effect of these treatments were examined in a runway model of i.v. cocaine self-administration that has been shown to be sensitive to both the initial rewarding and delayed anxiogenic effects of the drug in the same animal on the same trial. These dual actions of cocaine are reflected in the development of an approach-avoidance conflict ("retreat behaviors") about goal box entry that stems from the mixed associations that subjects form about the goal. CRF antagonism within the VTA, but not the CeA or BNST, significantly reduced the frequency of approach-avoidance retreat behaviors while leaving start latencies (an index of the positive incentive properties of cocaine) unaffected. These results suggest that the critical CRF receptors contributing to the anxiogenic state associated with acute cocaine administration may lie outside the extended amygdala, and likely involve CRF projections to the VTA.

Keywords

Cocaine; anxiety; operant runway; corticotropin-releasing factor; extended amygdala; drug self-administration

Send all correspondence to: Dr. Aaron Ettenberg, Department of Psychological & Brain Sciences, University of California, Santa Barbara, CA 93106-9660, aaron.ettenberg@psych.ucsb.edu, Voice: (805) 893-3682, FAX: (805) 893-4303.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

1. INTRODUCTION

Human users of cocaine report that the initial euphoric “high” produced by the drug’s ingestion is soon followed by an aversive “crash” characterized by intense feelings of anxiety, dysphoria, irritability, and drug craving (Gawin and Kleber, 1986; Kosten, 1989; Resnick et al., 1977; Rohsenow et al., 2007; Williamson et al., 1997). The onset of this aversive state is not likely a simple consequence of drug clearance since human self-reports indicate that the negative subjective response to the drug occurs even while plasma (and hence presumably brain) levels of the drug remain high (Van Dyke and Byck, 1982). Animal studies have likewise confirmed these dual and opposing actions of cocaine; while animals will readily self-administer the drug (Koob and Goeders, 1989; Porrino et al., 2004; Woolverton, 1992) and develop learned preferences for places associated with its administration (Bardo et al., 1995; Carr et al., 1989; Mucha et al., 1982; Spyraki et al., 1987), they also exhibit cocaine-induced decreased entries into the center of an open field (Simon et al., 1994; Yang et al., 1992), into a brightly illuminated area of a light-dark box (Costall et al., 1989), and into the open arms of an elevated plus maze (Costall et al., 1990; Paine et al., 2002; Rogerio and Takahashi, 1992) – all indices of heightened anxiety. In our own laboratory, we have shown that while animals develop learned preferences for places paired with the immediate positive effects of cocaine, they come to avoid places associated with the affective state present 15-min after an intravenous injection (Ettenberg et al., 1999; 2004). Additionally, animals trained to run a straight alley for a reward of i.v. cocaine develop a unique behavior in which they leave the start box faster and faster over trials (an indication of the drug’s positive incentive properties) but then stop at the threshold of the goal box and retreat back toward the start box (Ettenberg et al., 1999, Knackstedt et al., 2002). The frequency of this approach-avoidance “retreat” behavior increases with trials and has been shown to result from mixed positive and negative associations that the animals form with the cocaine-paired goal box (see reviews by Ettenberg 2004, 2009). The runway test therefore uniquely provides information about both the positive (start latency) and negative (retreat frequency) aspects of the goal-box experience in the same animal on the same trial.

Given the mixed consequences of cocaine administration, it seems likely that an organism’s decision to self-administer the drug must entail an assessment of the relative valence of its dual and opposing effects. A complete understanding of the neuronal systems underlying cocaine self-administration must therefore include the identification of the mechanisms responsible for both the positive/rewarding and negative/anxiogenic states produced by the drug. In this regard, a considerable body of research from both human and animal studies has implicated central mesocorticolimbic dopamine (DA) pathways in the development and maintenance of cocaine self-administration. Cocaine is a potent indirect agonist at DA synapses by way of its inhibition of the DA transporter (Koe, 1976; Reith et al., 1986) and lesions or pharmacological incapacitation of the DA system prevent the rewarding effects of cocaine as measured in both conditioned place preference (Morency and Beninger, 1986; Spyraki et al., 1987; Wenzel et al., 2013) and in operant self-administration studies (Ettenberg et al., 1982; Pettit et al., 1984; Roberts and Koob, 1982; Wise, 1984). In humans, brain imaging studies have identified a correlation between DA neuronal activity and the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

"high" reported by cocaine users (Breiter et al., 1997; Volkow et al., 1996; 1997) and the administration of DA antagonist drugs has been reported to dose-dependently reduce the self-reported ratings of the drug's rewarding/euphoric impact (Romach et al., 1999).

In contrast to the strong consensus regarding the role of DA in cocaine reward, the neurobiological systems underlying the drug's negative/anxiogenic effects remain unclear. One system that has received considerable recent attention is the "extended amygdala", which is comprised of several interconnected regions including the central nucleus of the amygdala (CeA), the posterior portion of the nucleus accumbens shell, and the bed nucleus of the stria terminalis (BNST) (Alheid, 2003; Alheid and Heimer 1988). The extended amygdala projects to several hypothalamic and brainstem nuclei that modulate the hypothalamic-pituitary-adrenal (HPA) axis and can thereby alter the organism's response to stress (Gray, 1993). It has also been implicated in fear conditioning, in the aversive state associated with withdrawal from chronic drug exposure, and in stress-induced reinstatement of drug-seeking behavior (e.g., Aston-Jones et al., 1999; Koob and LeMoal, 2008; Erb et al., 2001; Walker et al., 2009). Our own work has demonstrated that inactivation of the CeA or BNST dramatically reduces the aversive effects of cocaine as measured in both the conditioned place test and the runway self-administration test (Wenzel et al., 2011; 2014). What remains unclear is which neurons within the BNST and/or CeA might be critical for the anxiogenic effects of cocaine. A reasonable candidate in response to this question would be corticotropin releasing factor (CRF).

Cocaine has been shown to increase circulating plasma levels of CRF (Goeders, 1997; Rivier and Vale, 1987; Sarnyai et al., 2001) and produce changes in CRF peptide or mRNA levels within the extended amygdala (Maj et al., 2003; Richter et al., 1995; Zhou et al., 1996). Indeed, both the CeA and the BNST contain CRF-immunoreactive cells bodies, terminals and receptors (Cassel and Gray, 1989; Sakanaka et al., 1986; Swanson et al., 1983) and CRF signaling within the extended amygdala has been correlated with behavioral responses to fearful and/or anxiogenic stimuli (Funk et al., 2006). Infusions of CRF or a CRF agonist into the BNST elicits anxiogenic responses in both the elevated plus-maze and the social interaction test (Lee et al., 2008; Sahuque et al., 2006; Shepard et al., 2000) and produces aversions for places associated with its administration (Sahuque et al., 2006). In contrast, antagonism of CRF function in the CeA or BNST has been reported to attenuate the anxiogenic response to bright light or the stress inherent to the social defeat test (de Jongh et al., 2003; Heinrichs et al., 1992; Liebsch et al., 1995; Swiergiel et al., 1993; Walker et al., 2009) and Erb and Stewart (1999) reported that CRF antagonism within the BNST (but not the CeA) completely blocked reinstatement of cocaine-seeking induced by presentation of foot-shock stress. Finally, as stated above, we have reported that reversible lesions of either the CeA or BNST reduce the approach-avoidance anxiogenic response to cocaine in the self-administration runway (Wenzel et al., 2011; 2014).

Of course it remains possible that while the CRF cell bodies contributing to the negative subjective response to cocaine lie within the extended amygdala, the critical target of those cells lie outside the region in areas to which the CRF cells project. Of particular relevance in this regard is the CRF innervation of the DA cell bodies of the mesocorticolimbic system in the ventral tegmental area (VTA)(e.g., Rodaros et al., 2007; Tagliaferro and Morales, 2008;

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Wang and Morales, 2008). In fact several recent studies have suggested that aversive stimuli and aversive states (e.g., stress, drug withdrawal) might produce their behavioral effects via CRF modulation of VTA-DA neurons (e.g., Blacktop et al., 2011; Boyson et al., 2014; Grieder et al., 2014; Twining et al., 2014; Wang et al., 2007). The current study was therefore devised to further examine the relative roles of CRF receptors in the extended amygdala and the VTA in the anxiogenic response to cocaine as measured in the runway self-administration test.

2. METHODS

2.1 Subjects

A total of 91 adult male Sprague-Dawley rats (Charles River Laboratories, Hollister, California, USA) weighing 275–300g at the time of surgery served as subjects for the current experiments. Rats were pair-housed in hanging plastic cages within a temperature-controlled (23° C) vivarium maintained under a reverse 12-hour light-dark cycle (lights off at 0800h). Subjects were provided *ad libitum* access to food (Purina Rat Chow) and water throughout the duration of the study. All animal handling and procedures adhered to the PHS *Guide for the Care and Use of Laboratory Animals* and were reviewed and approved by University of California at Santa Barbara's Institutional Animal Care and Use Committee.

2.2 Surgery

The surgical procedures employed here were as previously described (e.g., Wenzel et al., 2011, 2014). Briefly, subjects were gentled through daily handling for one week after which each was individually fitted with a chronic indwelling jugular catheter (13 mm of polyethylene tubing, 0.3 mm inner diameter, 0.64 outer diameter; Dow Corning Corporation, Midland, MI, USA) inserted under deep anesthesia induced by an injection of a combined solution of ketamine and xylazine (56.5 and 7.5 mg/kg, i.m. respectively). During the same surgical session each subject was stereotaxically implanted with bilateral guide cannula (Item 313G; Plastics One) aimed either at the bed nucleus of the stria terminals (BNST), the central nucleus of the amygdala (CeA), or the ventral tegmental area (VTA). Stereotaxic coordinates were based upon the brain atlas of Paxinos and Watson (2005) and were aimed 1 mm above the target to account for the internal injection cannula (which protruded 1 mm below the end of the guide cannula). Coordinates were: BNST = A/P −0.3 mm from bregma, M/L +3.5 mm from midline with a lateral inclination of 15°, D/V −6.2 mm below skull surface; CeA = A/P −2.1 mm, M/L +4.0 mm, D/V −6.4mm from skull surface; and VTA = A/P −6.0 mm, M/L +0.75 mm, D/V −7.0 mm. During surgery each subject received a 2.0 mg/kg s.c. injection of the non-opiate analgesic, flunixin meglumine (FluMeglumine; Phoenix Pharmaceuticals, Belmont, California, USA) to reduce post-surgical pain and 3ml of 0.9% physiological saline s.c. to prevent dehydration.

All subjects were allowed a minimum of one week to recover from surgery before experimental procedures began. During this time, i.v. catheters were flushed once daily with 0.1 ml of Timentin antibiotic (25 mg/kg) followed by 0.1 ml of heparinized 0.9% physiological saline. Prior to the start of the experiment, and every seven days thereafter, i.v.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

catheter patency was confirmed by observing the behavioral impact of an i.v. injection of the fast-acting barbiturate, methohexital sodium (Brevital; 2.0 mg/kg in 0.1 ml filtered nanopure water). Those animals that were unresponsive to the Brevital (i.e. did not exhibit the loss of their righting reflex) when examined prior to testing were re-implanted with a new catheter using the left jugular vein and given additional days for recovery. If catheter patency failed during the course of behavioral testing, that animal was removed from the data analysis (n = 8).

2.3 Drugs

Cocaine hydrochloride (provided by the National Institute on Drug Abuse) was dissolved in 0.9% physiological saline and sterile filtered. For runway trials, a reinforcing dose of 1.0 mg/kg i.v. was delivered in a volume of 0.1 ml over 4.3 seconds via a 10 ml syringe nested in a motorized syringe pump (Razel Scientific Instruments, St. Albans, Vermont, USA). This dose was selected on the basis of prior work in our laboratory demonstrating it to reliably produce both approach and avoidance behavior in the runway and to produce consistent conditioned place preferences and aversions in response to immediate and delayed effects of the drug, respectively (Ettenberg et al., 1999; Ettenberg and Bernardi, 2006; 2007; Knackstedt et al., 2002; Raven et al., 2000).

The experiments described herein were conducted in two phases. An initial study was completed to investigate the effects of CRF antagonism within the extended amygdala (Wenzel, 2013; unpublished data) followed more recently by a second study of CRF antagonism within the VTA. For the “extended amygdala” experiment the nonselective CRF receptor 1 (CRF-R1) and 2 (CRF-R2) antagonist D-Phe $CRF_{(12-41)}$ (Sigma Aldrich, St. Louis, MO) was dissolved in sterile saline to produce three dose concentrations (0.0, 5.0, and 25.0 ng/ 0.5µl). These doses were selected from research demonstrating that the 25 ng dose infused into the BNST significantly decreased stress-induced cocaine reinstatement in rats (Erb and Stewart, 1999). For the VTA, astressin B, a newer, and slightly longer-acting nonselective CRF R1/R2 antagonist (Rivier and River, 2014) was dissolved in sterile saline and centrally infused in doses of 0.0, 0.25, 0.5 and 1.0 µg /0.5µl). Note that both drugs have been shown to be potent antagonists at the CRF-1 and CRF-2 receptor sites (Rivier and Rivier, 2014). The astressin B was generously provided by Dr. Jean Rivier, at the The Salk Institute, La Jolla, California. All i.c. injections were administered bilaterally in volumes of 0.5 µl over 90 s via a 10 µl Hamilton syringe seated in a motorized Razel syringe pump.

2.4 Apparatus

2.4.1 Self-Administration Runway—Two straight-arm runways (155 cm L× 15 cm W × 40 cm H) served as the test apparatus. On opposite ends of the straight alley were identically-sized start and goal boxes (24 × 25 × 40 cm) separated from the runway by retractable doors. Imbedded in the walls of each alley were 13 equally-spaced infrared photodetector-emitter pairs the input from which was fed through a custom ANY-maze interface (Stoelting Co., Wood Dale, IL) to a desktop computer that recorded the subjects’ location in the runway in real time. Above and running along the entire length of each runway were two magnetic tracks placed between which was a flow-through swivel (model 375-22PS; Instech Laboratories Inc., Plymouth Meeting, PA) that was connected via PE20

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

tubing to the animal's i.v. catheter on one end, and to a cocaine-filled 10 ml syringe seated in a syringe pump on the other end. The swivel was fitted with a plastic disc that prevented it from falling through the opening between the tracks. A pot magnet affixed to the underside of the disc was aligned to create a magnetic repulsion between the swivel and the tracks so that the swivel floated slightly above the tracks thereby providing a low-friction mechanism that allowed the rat to move freely throughout the alley (for a more detailed description of the runway apparatus, see Geist and Ettenberg, 1990).

2.4.2 Locomotor Activity Chambers—Locomotor activity was measured in 12 identical Plexiglas chambers each measuring 20 cm L × 40 cm W × 20 cm H (Kinder Scientific, San Diego, California, USA). Each chamber contained a set of 15 infrared photo emitter-detector pairs located 8 cm above the floors and evenly spaced along the long axis; seven more emitter-detector pairs were distributed along the narrow axis of each chamber. A subject's movement within the chamber was detected via interruption of the infrared-photobeams. A desktop computer running custom software (Kinder Scientific) recorded the animals' movements in real time during two 1-h test sessions. Activity was measured as distance traveled in cm during 5-min intervals.

2.5 Procedures

2.5.1 Runway Self-administration—Rats were habituated to the runway on a single 10-min trial with the goal door closed. Runway testing began 24 h later. Each rat was bilaterally infused with either i.c. saline (vehicle) or an assigned dose of one of the two nonselective CRF receptor antagonists. Final sample sizes for the treatment groups (after histological analyses; see below) were as follows: or the CeA n=5, n= 6 and n=10 for high dose, low dose and vehicle groups, respectively; for the BNST n=5, n=6 and n=6 for the high, low and vehicle groups respectively; for the VTA n=8, n=8, n=7 and n=9 for the high, medium, low dose and vehicle groups, respectively). Infusions were accomplished with a 28-gauge internal cannula (Plastics One) that was inserted into and protruded 1.0 mm beyond the tip of the indwelling guide cannula. The infusion cannula was connected by PE tubing to a 10µl syringe seated in a Razel syringe pump. The pump was then activated and infusions were slowly applied over a 90 sec period. An additional 60 sec was provided to permit the solutions to diffuse away from the cannula tip before the internal cannula were removed. Each animal was then returned to its home cage for 10 min, followed by a single runway trial each day.

For runway testing, a rat was connected to the drug delivery system and then placed into the start box for 5-s after which the start door was opened and the trial thereby initiated. Once the rat traversed the runway and entered the goal box, the goal door was closed (to prevent retracing) and a single i.v. infusion of cocaine (1.0 mg/kg) was administered. After 5 min in the goal box, the animal was removed from the apparatus and returned to its home cage. Testing consisted of 15 single daily trials during which three dependent measures were recorded. *Start latency*—the time required for the animal to leave the start box once the start door opened; *run time*—the time required for the rat to enter the goal box after it had left the start box; and the frequency of approach-avoidance *retreats*—the number of times an animal

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

halted its forward motion, turned and retreated back at least the length of two photocells in the runway (a minimum distance of approximately 30 cm).

2.5.2 Locomotor Activity Test—Forty-eight hours following the final runway trial, the impact of CRF antagonist infusions on spontaneous locomotor behavior was assessed to determine whether or not group differences in runway behavior could be accounted for by treatment-induced changes in the motor capacity of the animals. Since the effects of astressin-B (relative to vehicle controls) tended to strengthen with repeated administration as testing continued, it was important to examine the effects of the drug (and of D-Phe CRF $_{(12\text{-}41)}$) in subjects having had prior exposure to the antagonists, as opposed to naïve animals. A subset of subjects was therefore randomly drawn from the antagonist groups in the runway portion of the study to form a drug and vehicle group from each of the three "brain target" conditions (CeA, BNST and VTA). For this purpose, and based on the results observed in the runway test, only the high dose of each antagonist was examined (and compared to a saline control group). Testing began with a 60-min habituation period in which the animals were placed into an assigned activity chamber and allowed to acclimate to the environment for 60-min. Each animal was then removed and bilaterally infused with either a CRF antagonist (25ng of D-Phe CRF $_{(12\text{-}41)}$ or 1.0 μg of astressin-B) or vehicle, followed 10-min later by a return to their assigned activity chamber where the distance travelled over the next 15 min was collected in three 5-min bins. The data thus reflect locomotor activity for six groups of animals: rats that received pretreatment with vehicle (n=11) or the high dose of D-Phe CRF $_{(12\text{-}41)}$ delivered to the CeA (n=11), the high dose of D-Phe CRF $_{(12\text{-}41)}$ (n=12) or vehicle (n=12) delivered into the BNST (n=12), or the high dose of astressin-B (n=6) or vehicle (n=6) delivered into the VTA.

2.6 Histology

At the conclusion of behavioral testing, animals were euthanized with a lethal i.v. dose of sodium pentobarbital and phenytoin sodium solution (Euthasol®, Virbac, Fort Worth, Texas, USA). Brains were removed and stored in 10% Formalin and subsequently sliced on a cryostat (Leica CM 1800) into 40 μm frozen coronal sections that were mounted on 1.5% gelatin-coated slides and stored at −20° C prior to staining. Slides were stained in cresyl violet solution and viewed under magnification to determine cannula tip placement within the CEA, BNST, or BLA.

3. RESULTS

3.1 Histology

The regions within which successful cannula implants were confirmed for each brain target (BNST, CeA and VTA) are schematically provided in Figure 1. Subjects were excluded from the final data analyses in cases where one or both of the bilateral cannula in a given subject missed their intended targets. A total of 13 animals were removed from the data set because they did not meet this criterion.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3.2 Runway Self-Administration

Figure 2 illustrates the mean (+SEM) performance of animals running an alley for i.v. cocaine in the presence of CRF-antagonist challenge to the CeA, BNST or VTA. Note that the data were averaged over 3 trial bins to increase the clarity of the results by reducing some of the inherent trial-to-trial variability common in the use of one-trial/day test protocols. Separate two-factor (Group × Trial) ANOVAs (with repeated measures over Trials) were utilized to evaluate group performance on each of the three dependent measures (i.e., on the data depicted in each of the panels of Fig 2).

3.2.1 Start Latency—The time required to leave the start box once the start door opened is depicted in the top three panels of Figure 2. In general, start latency tended to decrease as trials progressed for subjects in all groups. While this main effect of Trial was only marginally significant in the CeA animals [$F(4,92) = 2.22$, $p=0.07$], it reached statistical significance in both the BNST [$F(4,92)=2.58$, $p<.05$] and VTA groups [$F(4,112)=8.35$, $p<.01$]. There were no statistically reliable effect of Group and no Group × Trial interaction for subjects in all three brain regions, indicating that across all groups animals left the start box faster as testing continued.

3.2.2 Run Time—Mean Run Times for each group of subjects are depicted in the middle panels of Figure 2. For both the CeA and BNST, repeated testing resulted in longer times to enter the goal box; a main effect of Trials [CeA -- $F(4,92)=4.74$, $p<.01$; BNST – $F(4,92) = 3.87$, $p<.01$]. However, all groups behaved comparably relative to each other and across trials, as reflected in the fact that there were no significant differences for Group nor a Group × Trial interaction for either brain area. In contrast, animals in the VTA groups behaved differently from one another [a main effect of Group; $F(3,28)=2.91$, $p<.05$), an effect clearly seen in the Figure where animals treated with the two higher doses of the CRF antagonist into the VTA entered the goal box sooner than those treated with either the low dose or the vehicle. For the VTA, the analyses identified no significant effect of Trials nor a Group × Trial interaction; results likely attributable to the fact that when averaged across all groups and across trials, the elevated response in two of the groups was offset by the decreased responding in the other two groups

3.2.3 Retreat Frequency—Of particular interest for the current study was the effect of CRF antagonist treatments on the approach-avoidance behavior of animals running for i.v. cocaine. Those data are depicted in the bottom three panels of Figure 2. For both the CeA and BNST groups, when averaged across all groups, animals emitted more retreat behaviors as testing progressed [main effect of Trial; CeA-- $F(4,92)=10.76$, $p<.001$; BNST -- $F(4,92)=9.345$, $p<.001$] but the groups did not differ from one another nor was there a reliable Group X Trial interaction. Once again, in contrast to the lack of any treatment effects in the extended amygdala groups, intra-VTA administration of astressin-B produced dose-dependent effects similar to those observed in the run time data. Animals treated with the low dose of the antagonist behaved comparably to those treated with vehicle, while the two higher doses of the antagonist produced dramatic reductions in the frequency of approach-avoidance behaviors. These results were reflected in the data analyses which produced statistically significant effects for both Trial and Group [$F(4,112)=3.57$, $p<.01$ and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

F(3,28)=3.00, p<.05, respectively]. The Group × Trial interaction approached but failed to reach statistical significance [F(12,112)=1.69, p=.07].

3.3 Locomotor Activity Test

Figure 3 illustrates the mean (±SEM) spontaneous locomotor response of subjects (measured as distance traveled in mm) during a 15-min test session beginning 10-min after i.c. treatment with either the vehicle or the high dose of the CRF antagonist into the CeA (left panel), BNST (middle panel) or VTA (right panel). Mixed two-factor (Group × Time) ANOVAs were computed on the data depicted in each of the panels of Figure 3. As the illustration suggests, in each treatment condition there was a dramatic decrease in activity over the course of testing [main effect of Trial for the CeA, BNST and VTA respectively: F(2,44)=122.11, p<.001; F(2,40)=93.54, p<.001; and F(2,30)=30.15, p<.001]. There were no differences between vehicle and CRF treatment groups in any of the three brain regions nor were there any statistically reliable Group × Trial interactions. Clearly, CRF antagonist pre-treatment did not alter the locomotor response of animals relative to vehicle pre-treatment.

4. DISCUSSION

Non-selective CRF receptor antagonism within the VTA, but not the extended amygdala (i.e., the CeA or BNST) produced dose-dependent decreases in the approach-avoidance conflict behavior of rats running an alley once daily for i.v. cocaine administration (Fig 2). The lack of any significant effects of treatment in the extended amygdala is unlikely to be a result of an inadequate dose of D-Phe CRF $_{(12\text{-}41)}$ since the intracranial doses employed here are within the range used to successfully block shock-induced reinstatement of cocaine seeking in prior studies (e.g., Erb and Stewart, 1999). Additionally, the differential effects of CRF antagonism within the VTA and extended amygdala cannot easily be accounted for by the different antagonists employed in the two regions since both have been shown to be potent at both the CRF-1 and CRF-2 receptors (Rivier and Rivier, 2014). Additionally, the reduction in retreat behaviors observed following CRF antagonism within the VTA cannot be explained by a nonspecific impairment in motoric function since the treatments produced no significant alterations in the spontaneous locomotor response of subjects in the absence of cocaine (Fig 3). It would seem then that the most parsimonious explanation for the current results is that CRF receptor antagonism within the VTA, but not the CeA or BNST, attenuates the anxiogenic response to cocaine. Thus, the current results suggest that CRF release within the VTA likely contributes to the anxiogenic response of animals experiencing acute cocaine administration.

Of course the lack of significant behavioral effects during CRF antagonism within the extended amygdala should not be taken to suggest that the structures of this brain region play no role in the negative or aversive effects of cocaine. Indeed, our own previous work has shown that reversible blockade of cells within either the CeA or BNST reduces the approach-avoidance conflict of animals running an alley for i.v. cocaine (Wenzel et al., 2011). Furthermore, we have shown that this effect appears to involve norepinephrine release within the extended amygdala from terminals originating in the cells bodies of the ventral noradrenergic bundle (Wenzel et al., 2014; see also Cecchi et al., 2002a; 2002b; Pacak et al., 1995). Indeed, others have similarly shown that post-synaptic noradrenergic

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

receptors are located on CRF immunoreactive cells of the CeA (Rudoy et al., 2009) and the BNST (Phelix et al., 1992; 1994), and binding of norepinephrine to its receptors has been shown to increase CRF release (Tsagarakis et al., 1988). The current results need not be inconsistent with these results if in fact the critical terminals of the CRF cell bodies within the extended amygdala reside outside the region. In that regard, note that both the CeA and BNST send CRF projections to the VTA (Rodaros et al., 2007), and cells of the VTA express type 1 and type 2 CRF receptors (Sauvage and Steckler, 2001; Ungless et al., 2003). CRF levels have been shown to increase in the VTA during footshock-induced reinstatement of cocaine seeking (Wang et al., 2005) and intra-VTA CRF receptor antagonism reportedly prevents such reinstatement from occurring (Blacktop et al., 2011; Wang et al., 2005, Wang et al., 2007). In other studies, the administration of CRF itself directly into the VTA has been shown to reinstate cocaine-seeking behavior in rats, an effect thought to result from either direct or indirect activation of DA cell bodies (Hahn et al., 2009). These authors suggest that, during stressor presentation, CRF acts to excite DA mesolimbic “reward” circuitry that thereby promotes drug seeking behavior (Wise and Morales, 2010). However, in the current study, CRF antagonism within the VTA produced no evidence of enhanced motivation to seek cocaine; i.e., start latencies were unaffected by the treatment. Thus, the reduction in retreat behaviors observed following intra-VTA treatment would appear to be a consequence of a selective reduction in the anxiogenic response to cocaine rather than an increase in the positive incentive properties of the drug.

A plausible explanation for the differences between the authors’ interpretation of CRF’s action and that stemming from researchers conducting response-reinstatement studies may relate to differences in the degree of drug exposure experienced by the subjects in the two behavioral paradigms. Animals in the runway received only 15 single daily injections of a moderate dose of cocaine (1 mg/kg i.v.), while typical lever-press self-administration studies involve the repeated ingestion of cocaine over prolonged test sessions that result in much larger doses of daily cocaine exposure. Given that chronic cocaine administration has been shown to cause neuroplastic changes in several addiction-related brain systems including the extended amygdala (for review, see Corominas et al., 2010; Everitt et al., 2008; Koob and Volkow, 2010; Szumlinksi et al., 2008), the neural mechanisms mediating cocaine experience likely differ in animals with varying degrees of drug history. Thus, while CRF appears to be integral to withdrawal-related anxiety and stress-induced reinstatement in animals with chronic cocaine experience, the nature and impact of CRF release within the VTA may be different in animals without the same record of chronic drug exposure. Indeed, Wang and colleagues (2005) have shown that footshock stress increased CRF release in the VTA of both chronically cocaine-treated and cocaine naïve rats, however, but only increased VTA DA and glutamate levels in rats with a history of chronic cocaine administration. These neurochemical effects were blocked by pretreatment with a CRF antagonist, suggesting that following repeated cocaine exposure, changes in the sensitivity of CRF systems may influence the subjects’ susceptibility to stress. In contrast, the current study was devised to examine the state of the organism during the initial stages of drug use, i.e., prior to the development of many of the neuroadaptations that occur during and/or following more extended drug administration. We hypothesize that once exposed to cocaine, it is the balance between the acute positive and negative properties of the drug that greatly

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

determines the likelihood that the organism will seek the drug again (Ettenberg et al., 2015). In this context, CRF release within the VTA appears to contribute to the anxiogenic response to acute cocaine and in that way factors into the organism's decision whether or not to self-administer the drug after initial exposure.

Acknowledgments

The research described herein was made possible by a grant from the National Institute on Drug Abuse (DA033370) awarded to AE.

References

Alheid GF. Extended amygdala and basal forebrain. Ann NY Acad Sci. 2003; 985:185–205. [PubMed: 12724159]

Alheid GF, Heimer L. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience. 1988; 27:1–39. [PubMed: 3059226]

Aston-Jones G, Delfs JM, Druhan J, Zhu Y. The bed nucleus of the stria terminalis. A target site for noradrenergic actions in opiate withdrawal. Ann NY Acad Sci. 1999; 877:486–98. [PubMed: 10415666]

Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev. 1995; 19:39–51. [PubMed: 7770196]

Blacktop JM, Seubert C, Baker DA, Ferda N, Lee G, Graf EN, Mantsch JR. Augmented cocaine seeking in response to stress or CRF delivered into the ventral tegmental area following long-access self-administration is mediated by CRF receptor type 1 but not CRF receptor type 2. J Neurosci. 2011; 31:11396–403. [PubMed: 21813699]

Boyson CO, Holly EN, Shimamoto A, Albrechet-Souza L, Weiner LA, DeBold JF, Miczek KA. Social stress and CRF-dopamine interactions in the VTA: role in long-term escalation of cocaine self-administration. J Neurosci. 2014; 34:6659–67. [PubMed: 24806691]

Breiter HC, Gollub RL, Weisskoff RM, Kennedy DN, Makris N, Berke JD, Goodman JM, Kantor HL, Gastfriend DR, Riorden JP, Mathew RT, Rosen BR, Hyman SE. Acute effects of cocaine on human brain activity and emotion. Neuron. 1997; 19:591–611. [PubMed: 9331351]

Carr, DB.; Fibiger, HC.; Phillips, AG. Place preference as a measure of drug reward. In: Liebman, JM.; Cooper, SJ., editors. The neuropharmacological basis of reward. Oxford: Clarendon Press; 1989. p. 264-319.

Cassell MD, Gray TS. Morphology of peptide-immunoreactive neurons in the rat central nucleus of the amygdala. J comp neurol. 1989; 281:320–333. [PubMed: 2468696]

Cecchi M, Khoshbouei H, Javors M, Morilak DA. Modulatory effects of norepinephrine in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. Neuroscience. 2002a; 12:13–21. [PubMed: 12044468]

Cecchi M, Khoshbouei H, Morilak DA. Modulatory effects of norepinephrine, acting on alpha 1 receptors in the central nucleus of the amygdala, on behavioral and neuroendocrine responses to acute immobilization stress. Neuropharmacology. 2002b; 43:1139–1147. [PubMed: 12504920]

Corominas M, Roncero C, Casas M. Corticotropin releasing factor and neuroplasticity in cocaine addiction. Life Sci. 2010; 86:1–9. [PubMed: 19914260]

Costall B, Domeney AM, Gerrard PA, Horovitz ZP, Kelly ME, Naylor RJ, Tomkins DM. Effects of captopril and SQ29,852 on anxiety-related behaviours in rodent and marmoset. Pharmacol Biochem Behav. 1990; 36:13–20. [PubMed: 2112256]

Costall B, Kelly ME, Naylor RJ, Onaivi ES. The actions of nicotine and cocaine in a mouse model of anxiety. Pharmacol Biochem Behav. 1989; 33:197–203. [PubMed: 2780777]

Erb S, Shaham Y, Stewart J. Stress-induced relapse to drug seeking in the rat: role of the bed nucleus of the stria terminalis and amygdala. Stress. 2001; 4:289–303. [PubMed: 22432148]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Erb S, Stewart J. A role for the bed nucleus of the stria terminalis, but not the amygdala, in the effects of corticotropin-releasing factor on stress-induced reinstatement of cocaine seeking. J Neurosci. 1999; 19:RC35. [PubMed: 10516337]

de Jongh R, Groenink L, van Der Gugten J, Olivier B. The light-enhanced startle paradigm as a putative animal model for anxiety: effects of chlordiazepoxide, flesinoxan and fluvoxamine. Psychopharmacology. 2002; 159:176–180. [PubMed: 11862346]

Ettenberg A. Opponent process properties of self-administered cocaine. Neurosci Biobeha Rev. 2004; 27:721–728.

Ettenberg A. The runway model of drug self-administration. Pharmacol Biochem Behav. 2009; 91:271–277. [PubMed: 19032964]

Ettenberg A, Fomenko V, Kaganovsky K, Shelton K, Wenzel JM. On the positive and negative affective responses to cocaine and their relation to drug self-administration in rats. Psychopharmacology (Berl). 2015; 232:2363–75. [PubMed: 25662610]

Ettenberg A, Bernardi RE. Effects of buspirone on the immediate positive and delayed negative properties of intravenous cocaine as measured in the conditioned place preference test. Pharmacol Biochem Behav. 2007; 87:171–8. [PubMed: 17524462]

Ettenberg A, Bernardi RE. Anxiolytic-like actions of buspirone in a runway model of intravenous cocaine self-administration. Pharmacol Biochem Behav. 2006; 85:393–9. [PubMed: 17064759]

Ettenberg A, Pettit HO, Bloom FE, Koob GF. Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacology. 1982; 78:204–9. [PubMed: 6296898]

Ettenberg A, Raven MA, Danluck DA, Necessary BD. Evidence for opponent-process actions of intravenous cocaine. Pharmacol Biochem Behav. 1999; 64:507–12. [PubMed: 10548263]

Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW, Robbins TW. Review Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. Philosophical Transactions - Royal Soc Bio Sci. 2008; 363:3125–35.

Funk D, Li Z, Lê AD. Effects of environmental and pharmacological stressors on c-fos and corticotropin-releasing factor mRNA in rat brain: Relationship to the reinstatement of alcohol seeking. Neuroscience. 2006; 138:235–43. [PubMed: 16359808]

Gawin FH, Kleber HD. Abstinence symptomatology and psychiatric diagnosis in cocaine abusers: Clinical observations. Arch Gen Psychiatry. 1986; 43:107–13. [PubMed: 3947206]

Geist TD, Ettenberg A. A simple method for studying intravenous drug reinforcement in a runaway. Pharmacol Biochem Behav. 1990; 36:703–6. [PubMed: 2377672]

Goeders NE. A neuroendocrine role in cocaine reinforcement. Psychoneuroendocrinol. 1997; 22:237–59.

Gray TS. Amygdaloid CRF pathways. Role in autonomic, neuroendocrine, and behavioral responses to stress. Ann NY Acad Sci. 1993; 697:53–60. [PubMed: 8257022]

Grieder TE, Herman MA, Contet C, Tan LA, Vargas-Perez H, Cohen A, et al. VTA CRF neurons mediate the aversive effects of nicotine withdrawal and promote intake escalation. Nat Neurosci. 2014; 17:1751–8. [PubMed: 25402857]

Hahn J, Hopf FW, Bonci A. Chronic cocaine enhances corticotropin-releasing factor-dependent potentiation of excitatory transmission in ventral tegmental area DA neurons. J Neurosci. 2009; 29:6535–6544. [PubMed: 19458224]

Heinrichs SC, Pich EM, Miczek KA, Britton KT, Koob GF. Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. Brain Res. 1992; 581:190–7. [PubMed: 1327398]

Knackstedt LA, Samimi M, Ettenberg A. Evidence for opponent-process actions of intravenous cocaine and cocaethylene. Pharmacol Biochem Behav. 2002; 72:931–6. [PubMed: 12062583]

Koe BK. Molecular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. J Pharmacol Exp Ther. 1976; 199:649–61. [PubMed: 994022]

Koob, GF.; Goeders, NE. Neuroanatomical substrates of drug self-administration. In: Liebman, JM.; Cooper, SJ., editors. The Neuropharmacological Basis of Reward. New York: Oxford Univ Press; 1989. p. 214-63.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Koob GF, Le Moal M. Addiction and the brain antireward system. Ann Rev Psychol. 2008; 59:29–53. [PubMed: 18154498]

Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacol. 2010; 35:217–38.

Kosten TR. Pharmacotherapeutic interventions for cocaine abuse: Matching patients to treatments. J Nerv Mental Dis. 1989; 177:379–389.

Lee Y, Fitz S, Johnson PL, Shekhar A. Repeated stimulation of CRF receptors in the BNST of rats selectively induces social but not panic-like anxiety. Neuropsychopharmacol. 2008; 33:2586–94.

Liebsch G, Landgraf R, Gerstberger R, Probst JC, Wotjak CT, Engelmann M, Holsboer F, Montkowski A. Chronic infusion of a CRH1 receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. Regul Pept. 1995; 59:229–39. [PubMed: 8584759]

Maj M, Turchan J, Smialowska M, Przewlocka B. Morphine and cocaine influence on CRF biosynthesis in the rat central nucleus of amygdala. Neuropeptides. 2003; 3:105–10. [PubMed: 12747942]

Morency MA, Beninger RJ. DAergic substrates of cocaine-induced place conditioning. Brain Res. 1986; 399:33–41. [PubMed: 3026573]

Mucha RF, van der Kooy D, O'Shaughnessy M, Bucenieks P. Drug reinforcement studied by the use of place conditioning in rat. Brain Res. 1982; 243:91–105. [PubMed: 6288174]

Pacak K, McCarty R, Palkovits M, Kopin IJ, Goldstein DS. Effects of immobilization on in vivo release of norepinephrine in the bed nucleus of the stria terminalis in conscious rats. Brain Res. 1995; 688:242–6. [PubMed: 8542318]

Paine TA, Jackman SL, Olmstead MC. Cocaine-induced anxiety: Alleviation by diazepam, but not buspirone, dimenhydrinate, or di-phenhydramine. Behav Pharmacol. 2002; 13:511–23. [PubMed: 12409990]

Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Elsevier Academic Press; 2005.

Pettit HO, Ettenberg A, Bloom FE, Koob GF. Destruction of DA in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology. 1984; 84:167–173. [PubMed: 6438676]

Phelix CF, Liposits Z, Paull WK. Monoamine innervation of bed nucleus of stria terminalis: an electron microscopic investigation. Brain Res Bull. 1992; 28:949–65. [PubMed: 1379113]

Phelix CF, Liposits Z, Paull WK. Catecholamine-CRF synaptic interaction in a septal bed nucleus: afferents of neurons in the bed nucleus of the stria terminalis. Brain Res Bull. 1994; 33:109–19. [PubMed: 7903902]

Porrino LJ, Daunais JB, Smith HR, Nader MA. The expanding effects of cocaine: studies in a nonhuman primate model of cocaine self-administration. Neurosci Biobehav Rev. 2004; 27:813–20. [PubMed: 15019430]

Raven MA, Necessary BD, Danluck DA, Ettenberg A. Comparison of the reinforcing and anxiogenic effects of intravenous cocaine and cocaethylene. Exp Clin Psychopharmacol. 2000; 8:117–24. [PubMed: 10743912]

Reith ME, Meisler BE, Sershen H, Lajtha A. Structural requirements for cocaine congeners to interact with DA and serotonin uptake sites in mouse brain and to induce stereotyped behavior. Biochem Pharmacol. 1986; 35:1123–9. [PubMed: 3964292]

Resnick RB, Kestenbaum RS, Schwartz LK. Acute systemic effects of cocaine in man: a controlled study by intranasal and intravenous routes. Science. 1977; 195:696–8. [PubMed: 841307]

Richter RM, Pich EM, Koob GF, Weiss F. Sensitization of cocaine-stimulated increase in extracellular levels of corticotropin-releasing factor from the rat amygdala after repeated administration as determined by intracranial microdialysis. Neurosci Lett. 1995; 187:169–72. [PubMed: 7624019]

Rivier C, Vale W. Cocaine stimulates adrenocorticotropin (ACTH) secretion through a corticotropin-releasing factor (CRF)-mediated mechanism. Brain Res. 1987; 422:403–6. [PubMed: 2823980]

Rivier JE, Rivier CL. Corticotropin-releasing factor peptide antagonists: Design, characterization and potential clinical relevance. Front Neuroendocrinol. 2014; 35:161–70. [PubMed: 24269930]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Roberts DC, Koob GF. Disruption of cocaine self-administration following 6-hydroxyDA lesions of the ventral tegmental area in rats. Pharmacol Biochem Behav. 1982; 17:901–4. [PubMed: 6817350]

Rodaros D, Caruana DA, Amir S, Stewart J. Corticotropin-releasing factor projections from limbic forebrain and paraventricular nucleus of the hypothalamus to the region of the ventral tegmental area. Neuroscience. 2007; 150:8–13. [PubMed: 17961928]

Rogerio R, Takahashi RN. Anxiogenic action of acute but not repeated cocaine administration in handling-habituated mice in the plus-maze test. Brazilian J Med Bio Res. 1992; 25:713–716.

Rohsenow DJ, Martin RA, Eaton CA, Monti PM. Cocaine craving as a predictor of treatment attrition and outcomes after residential treatment for cocaine dependence. J Stud Alcohol Drugs. 2007; 68:641–8. [PubMed: 17690796]

Romach MK, Glue P, Kampman K, Kaplan HL, Somer GR, Poole S, et al. Attenuation of the euphoric effects of cocaine by the DA D1/D5 antagonist ecopipam (SCH 39166). Arch Gen Psychiat. 1999; 56:1101–6. [PubMed: 10591286]

Rudoy CA, Reyes AR, Van Bockstaele EJ. Evidence for beta1-adrenergic receptor involvement in amygdalar corticotropin-releasing factor gene expression: implications for cocaine withdrawal. Neuropsychopharmacology. 2009; 34:1135–1148. [PubMed: 18596687]

Sahuque L, Kullberg EF, McGeehan AJ, Kinder JR, Hicks MP, Blanton MG, Janak PH, Olive MF. Anxiogenic and aversive effects of corticotropin-releasing factor (CRF) in the bed nucleus of the stria terminalis in the rat: role of CRF receptor subtypes. Psychopharmacology. 2006; 186:122–32. [PubMed: 16568282]

Sakanaka M, Shibasaki T, Lederis K. Distribution and efferent projections of corticotropin-releasing factor-like immunoreactivity in the rat amygdaloid complex. Brain Res. 1986; 382:213–38. [PubMed: 2428439]

Sarnyai Z, Shaham Y, Heinrichs SC. The role of corticotropin-releasing factor in drug addiction. Pharmacol Rev. 2001; 53:209–44. [PubMed: 11356984]

Sauvage M, Steckler T. Detection of corticotropin-releasing hormone receptor 1 immunoreactivity in cholinergic, DArgic and noradrenergic neurons of the murine basal forebrain and brainstem nuclei--potential implication for arousal and attention. Neuroscience. 2001; 104:643–52. [PubMed: 11440798]

Shepard JD, Barron KW, Myers DA. Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. Brain Res. 2000; 861:288–95. [PubMed: 10760490]

Simon P, Dupuis R, Costentin J. Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. Behav Brain Res. 1994; 61:59–64. [PubMed: 7913324]

Spyraki C, Nomikos GG, Varonos DD. Intravenous cocaine-induced place preference: attenuation by haloperidol. Behav Brain Res. 1987; 26:57–62. [PubMed: 3675835]

Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology. 1983; 36:165–186. [PubMed: 6601247]

Swiergiel AH, Takahashi LK, Kalin NH. Attenuation of stress-induced behavior by antagonism of corticotropin-releasing factor receptors in the central amygdala in the rat. Brain Res. 1993; 623:229–34. [PubMed: 8221104]

Szumlinski K, Ary AW, Lominac KD. Homers regulate drug-induced neuroplasticity: implications for addiction. Biochem Pharmacol. 2008; 75:112–133. [PubMed: 17765204]

Tagliaferro P, Morales M. Synapses between corticotropin-releasing factor-containing axon terminals and dopaminergic neurons in the central tegmental area are predominantly glutametergic. J Comp Neurol. 2008; 506:616–26. [PubMed: 18067140]

Tsagarakis S, Holly JM, Rees LH, Besser GM, Grossman A. Acetylcholine and norepinephrine stimulate the release of corticotropin-releasing factor-41 from the rat hypothalamus *in vitro*. Endocrinology. 1988; 123:1962–9. [PubMed: 2970959]

Twining RC, Wheeler DS, Ebben AL, Jacobsen AJ, Robble MA, Mantsch JR, Wheeler RA. Aversive stimuli drive drug seeking in a state of low dopamine tone. Biol Psychiatry. 2014; 77:895–902. [PubMed: 25442790]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Ungless MA, Singh V, Crowder TL, Yaka R, Ron D, Bonci A. Corticotropin-releasing factor requires CRF binding protein to potentiate NMDA receptors via CRF receptor 2 in DA neurons. Neuron. 2003; 39:401–7. [PubMed: 12895416]

Van Dyke C, Byck R. Cocaine Sci Am. 1982; 246:128–41. [PubMed: 7043731]

Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Ding YS, Logan, et al. Relationship between psychostimulant-induced "high" and DA transporter occupancy. Proc Nat Acad Sci USA. 1996; 93:10388–92. [PubMed: 8816810]

Volkow ND, Wang GJ, Fischman MW, Foltin RW, Fowler JS, Abumrad NN, et al. Relationship between subjective effects of cocaine and DA transporter occupancy. Nature. 1997; 386:827–30. [PubMed: 9126740]

Walker DL, Miles LA, Davis M. Selective participation of the bed nucleus of the stria terminalis and CRF in sustained anxiety-like versus phasic fear-like responses. Prog Neuropsychopharmacol Bio Psychiatry. 2009; 33:1291–308. [PubMed: 19595731]

Wang HL, Morales M. Corticotropin-releasing factor binding protein within the central tegmental area is expressed in a subset of dopaminergic neurons. J Comp Neurol. 2008; 509:302–18. [PubMed: 18478589]

Wang B, Shaham Y, Zitzman D, Azari S, Wis RA, You ZB. Cocaine experience establishes control of midbrain glutamate and DA by corticotropin-releasing factor: a role in stress-induced relapse to drug seeking. J Neurosci. 2005; 25:5389–96. [PubMed: 15930388]

Wang B, You ZB, Rice KC, Wise RA. Stress-induced relapse to cocaine-seeking: roles for the CRF(2) receptor and CRF-binding protein in the ventral tegmental area of the rat. Psychopharmacology. 2007; 19:283–94. [PubMed: 17437087]

Wenzel JM, Cotton SW, Dominguez HM, Lane JE, Shelton K, Su ZI, Ettenberg A. Noradrenergic β-receptor antagonism within the central nucleus of the amygdala or bed nucleus of the stria terminalis attenuates the negative/anxiogenic effects of cocaine. J Neurosci. 2014; 34:3467–3474. [PubMed: 24599448]

Wenzel JM, Su ZI, Shelton K, Dominguez HM, von Furstenberg VA, Ettenberg A. The dopamine antagonist cis-flupenthixol blocks the expression of the conditioned positive but not the negative effects of cocaine in rats. Pharmacol Biochem Behav. 2013; 114–115:90–96.

Wenzel JM, Waldroup SA, Haber ZM, Su ZI, Ben-Shahar O, Ettenberg A. Effects of lidocaine-induced inactivation of the bed nucleus of the stria terminalis, the central or the basolateral nucleus of the amygdala on the opponent-process actions of self-administered cocaine in rats. Psychopharmacology. 2011; 217:221–30. [PubMed: 21487661]

Wenzel, JM. Doctoral Dissertation. University of California; Santa Barbara: 2013. An Investigation of the Neural Substrates Underlying the Anxiogenic Effects of Cocaine.

Williamson S, Gossop M, Powis B, Griffiths P, Fountain J, Strang J. Adverse effects of stimulant drugs in a community sample of drug users. Drug Alcohol Depend. 1997; 44:87–94. [PubMed: 9088780]

Wise RA. Neural mechanisms of the reinforcing action of cocaine. NIDA Res Mon. 1984; 50:15–33.

Wise RA, Morales M. A ventral tegmental CRF-glutamate-DA interaction in addiction. Brain Res. 2010; 1314:38–43. [PubMed: 19800323]

Woolverton WL. Determinants of cocaine self-administration by laboratory animals. Ciba Found Symp. 1992; 166:149–61. [PubMed: 1638910]

Yang XM, Gorman AL, Dunn AJ, Goeders NE. Anxiogenic effects of acute and chronic cocaine administration: neurochemical and behavioral studies. Pharmacol Biochem Beha. 1992; 41:643–50.

Zhou Y, Spangler R, LaForge KS, Maggos CE, Ho A, Kreek MJ. Corticotropin-releasing factor and type 1 corticotropin-releasing factor receptor messenger RNAs in rat brain and pituitary during "binge"-pattern cocaine administration and chronic withdrawal. J Pharmacol Exp Ther. 1996; 279:351–8. [PubMed: 8859013]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Highlights

- Rats running an alley for i.v. cocaine develop an approach-avoidance conflict about goal-box entry
- Start latencies were unaffected by CRF receptor antagonism within the VTA, BNST or CeA
- Locomotor behavior was also unaffected by CRF antagonist treatments
- Approach-avoidance conflict behavior was reduced only by CRF antagonism within the VTA
- Cocaine's anxiogenic effects were reduced by CRF antagonism of the VTA but not the BNST or CeA

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

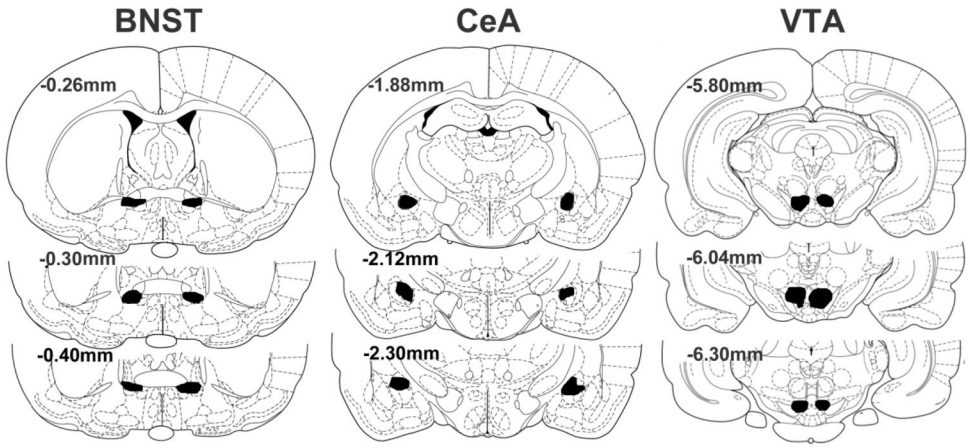

Figure 1.

Schematic representation of the target regions (outlined in black) within which the cannula placements were located within the bed nucleus of the stria terminal (BNST), the central nucleus of the amygdala (CeA) or the ventral tegmental area (VTA). Numbers represent mm posterior to bregma. The figure was redrawn from Paxinos and Watson (2005).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

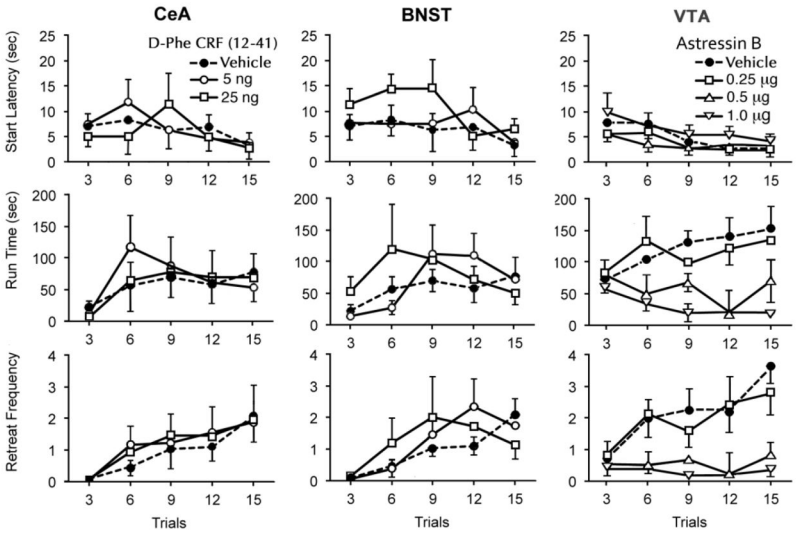

Figure 2.

The runway performance of each group (±SEM) of animals following intracranial administration of varying doses of the CRF antagonist D-Phe CRF $_{(12\text{-}41)}$ delivered into the CeA (left column of panels) or the BNST (middle column of panels) or astressin B delivered into the VTA (right column of panels). The top three panels (horizontally) reflect start latencies, the middle three horizontal panels show run times, and bottom three panels depict approach-avoince retreat behaviors.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

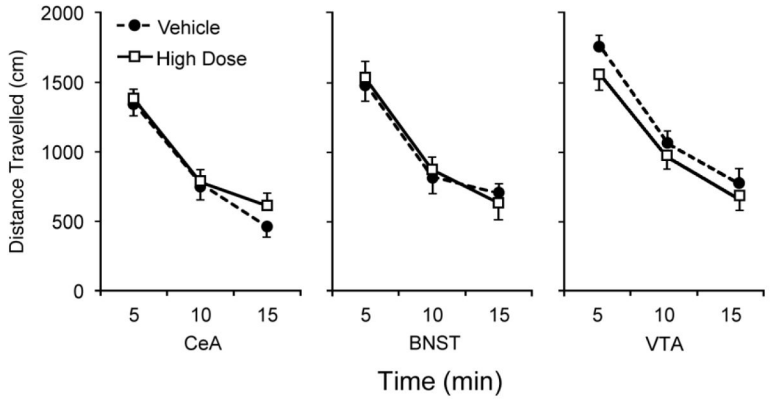

Figure 3.

Mean (±SEM) locomotor activity during a 15-min test beginning 10-min after intra-cranial administration of the high dose of the CRF antagonist D-Phe CRF $_{(12\text{-}41)}$ delivered into the CeA or the BNST (left and middle panels, respectively) or astressin B delivered into the VTA (right panel).