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Central CRF_2 receptor antagonism reduces anxiety states during amphetamine withdrawal

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Abstract

Increased depressive and anxiety-like behaviors are exhibited by rats and humans during withdrawal from psychostimulants. Anxiety-like behaviors observed during amphetamine withdrawal are mediated by increased expression and activity of corticotropin releasing factor type 2 (CRF_2) receptors in the dorsal raphe nucleus (dRN). Anxiety-like behavior of rats during withdrawal can be reversed by CRF_2 receptor antagonism in the dRN, but the efficacy of global central CRF_2 receptor antagonism is unknown. Rats were treated with amphetamine (2.5 mg/kg, ip.) or saline daily for 2 weeks, and were tested for anxiety-like behaviors during withdrawal. Rats undergoing withdrawal showed increased anxiety-like behavior, which was reduced by ventricular infusion of the CRF_2 antagonist antisauvagine-30 (ASV 2 μg/2μl). Surprisingly, ventricular ASV increased anxiety-like behavior in rats pre-treated with saline, but had an anxiolytic effect in un-treated rats. Western blots were performed to determine whether differences in CRF receptor densities could explain ASV-induced behavioral results. Saline pre-treated rats showed reduced CRF_1 receptor expression in the lateral septum compared to amphetamine pre-treated and un-treated rats. Overall, these results suggest that central CRF_2 antagonism reduces anxiety states during amphetamine withdrawal, and that behavioral effects may be dependent upon the balance of CRF_1 and CRF_2 receptor activity in anxiety-related regions.

Keywords

Anxiety; Stress; Corticotropin-releasing factor; Amygdala; Lateral Septum; Antisauvagine-30; Psychostimulant

Introduction

Anxiety and depression are major components of maintaining the cycle of addiction, and are responsible for the negative reinforcement of drug seeking behaviors (Sarnyai, et al., 1995;

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Koob & Le Moal, 2008). Both rats and humans exhibit increased anxiety states during withdrawal from amphetamine (Cantwell & McBride, 1998; Schuckit et al., 1999; Srisurapanont, 1999a,b; Srisurapanont, 2001; Romanelli et al., 2006; Shoptaw et al., 2009; Barr et al., 2010; Vuong et al., 2010). There is currently no FDA-approved treatment for the negative affect and increased anxiety states exhibited during psychostimulant withdrawal, thus there is great need to identify potential pharmacological targets.

Corticotropin-releasing factor (CRF) is strongly implicated in negative affect and anxiety states (Radulovic, et al., 1999; Takahashi, 2001; Bale and Vale, 2004; Bale, 2005; Lukkes et al., 2009b). Activation of the CRF_2 receptors within the serotonergic cell body region, the dorsal raphe nucleus (dRN), increases serotonin neuronal activity and release of serotonin in the limbic system (Pernar et al., 2004; Forster et al., 2006; 2008; Scholl et al., 2010). Furthermore, CRF_2 receptors are up-regulated in the dRN of rats for up to 6 weeks of amphetamine withdrawal, with no change in expression of dRN CRF_1 receptors (Pringle et al., 2008). Infusions of CRF into the dRN augments serotonin release in the central nucleus of the amygdala (CeA) of amphetamine pre-treated rats, as compared to CRF-infused controls, via CRF_2 receptor-dependent mechanisms (Scholl et al., 2010). Importantly, anxiety-like behaviors of rats undergoing amphetamine withdrawal can be reduced by CRF_2 receptor antagonism directly within the dRN (Vuong et al., 2010). Therefore, CRF_2 receptor antagonists may be important in the treatment of withdrawal-induced anxiety to prevent the ongoing cycle of addiction.

However, it is not known whether widespread antagonism of central CRF_2 receptors throughout the entire brain will be effective in reducing anxiety states during amphetamine withdrawal. This is important to understand, given that any potential therapeutics targeting CRF_2 receptors will not be directly infused into the dRN, but instead will access CRF_2 receptors throughout the entire brain as a result of systemic administration. The global brain distribution of CRF_2 receptors is limited (as compared to CRF_1 receptors), but expression is highest in regions relevant to fear and anxiety-like behaviors, including the hypothalamus, amygdala, lateral septum (LS) and bed nucleus of the stria terminalis (BNST; Chalmers et al., 1995; Radulovic et al., 1999; Liu et al., 2004; Henry et al., 2006; Skorzewska et al., 2011; Takahashi et al., 2011; Lebow et al., 2012; Ventura-Silva et al., 2012; Elharrar et al., 2013). Therefore, in addition to the dRN, centrally-administered CRF_2 receptor antagonism will affect multiple brain regions that have the potential to affect anxiety states during amphetamine withdrawal.

Whether activation of central CRF_2 receptors enhances or reduces anxiety-like behaviors has been tested using genetic models and pharmacological manipulation, with conflicting results. For example, CRF_2 receptor knockout mice show hypersensitivity to swim stress and displayed increased anxiety behaviors compared to wild-type mice (Bale, et al., 2000; Kishimoto et al., 2000), suggesting that central CRF_2 antagonism would increase anxiety behaviors. Conversely, ventricular administration of the CRF_2 receptor antagonist, antisauvagine-30 (ASV) decreases anxiety-like behaviors of rats when compared to vehicle infusion (Takahashi, 2001; Takahashi, et al., 2001). Given the conflicting findings, and the fact that previous research has not tested the central effects of CRF_2 receptor antagonism in a pre-stressed model, the current studies determined whether central CRF_2 receptor

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antagonism is effective at reducing anxiety-like behaviors of rats during amphetamine withdrawal. We also examined whether expression of CRF_1 and CRF_2 receptors in the CeA, LS and BNST is altered by either amphetamine or saline pre-treatment, in order to elucidate which regions aside from the dRN may facilitate behavioral changes following central CRF_2 receptor antagonism.

Materials and Methods

Animals

Eighty-one male Sprague-Dawley rats were obtained from the University of South Dakota Animal Research Center (Vermillion, SD) at weaning age (3 weeks old). Rats were housed in pairs and had free access to food and water. The holding room was kept at a temperature of 22°C and humidity of 60%. The lights in the room were set on a reverse 12 hr light-dark cycle with lights off at 10 a.m. Rats were used in the experiments described below when they reached early adulthood (8 weeks old). All procedures were approved by the University of South Dakota's Institutional Animal Care and Use Committee, and carried out under the National Institutes of Health Guide for the Care and Use of Laboratory Animals, with all efforts made to reduce the number of animals used and potential suffering.

Experiment 1: Effects of icv. CRF_2 Receptor Antagonism on Anxiety-like Behavior during Amphetamine Withdrawal

Amphetamine Pre-treatment: Rats were injected with amphetamine (2.5 mg/kg, ip., N = 16) or saline (N = 14) daily for 14 days during the dark phase of the light cycle. This injection schedule results in increased anxiety-like behaviors as measured on the elevated plus maze (EPM) at 24 hours, 2 weeks and 4 weeks withdrawal (Barr, et al., 2010; Vuong, et al., 2010). Rats underwent a 2 week withdrawal period with no injections, during which they were weighed on a weekly basis (Vuong et al., 2010).

Surgery: During the second week of the withdrawal period, aseptic stereotaxic surgery was performed to implant a guide cannula to allow intracerebroventricular (icv.) infusion. Anesthesia was induced with 4% isoflurane in 3.0 L/min O_2, and the rat was placed into a stereotaxic instrument (David Kopf Instruments, Tujunga CA) with the nose bar was set at −3.3 mm. Throughout the surgery, anesthesia was maintained at 2–2.5% isoflurane with body temperature kept constant at 37 °C using an isothermic heating pad (Braintree Scientific, Braintree MA). A 22-gauge, 1.5 mm long stainless steel guide cannula (Plastics One, Roanoke, VA) was stereotaxically implanted 2 mm above the lateral ventricle (AP: −1.0mm from bregma; ML: −1.5 from midline; Paxinos and Watson, 1997) and held in place with dental cement (Plastics One) anchored by 3 screws in the skull. At the conclusion of surgery, the analgesic Ketoprofen (5 mg/kg, im.; Met-Vet, Mettawa IL) was administered. All rats were allowed 3 days of recovery before being acclimated to the icv. infusion procedure.

Acclimation, Infusion, and Elevated Plus Maze Testing: All acclimations, infusions and testing were conducted at least 1 hour following the onset of the dark phase of the light cycle, in a dark room illuminated by red lighting. Rats were acclimated to the handling and

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infusion process over three days prior to testing on the EPM (Vuong et al., 2010; Bledsoe et al., 2011). On day 14 of withdrawal, a 30 gauge cannula (2 mm longer than guide cannula) was inserted into the lateral ventricle, and rats were infused with the CRF_2 receptor antagonist ASV-30 (2 μg/2μl; Tocris Bioscience; Minneapolis, MN; Takahashi, et al., 2001; Vuong et al., 2010) or vehicle (2 μl; 2% EtOH/2% camphor in artificial cerebrospinal fluid; Vuong et al., 2010; Bledsoe et al., 2011) at a rate of 0.5 μl/minute using a microdrive pump (Stoelting, Wood Dale IL), 20 minutes prior to EPM testing. The rat was placed in the center of the EPM (Noldus Information Technology, Wageningen, Netherlands), facing a closed arm and allowed to explore the maze for 5 minutes. An infrared camera above the maze recorded the test and Ethovision XT v5 software (Noldus Information Technology) was used to calculate the total distance moved, latency to enter open arms, entries into the open arms, and time spent in the open arms.

Histology: After testing was completed, animals were euthanized with sodium pentobarbital (Fatal Plus, Vortech, Dearborn MI,). Brains were removed and fixed in 10% buffered formalin (Fisher Scientific) and sectioned at 60 μm on a freezing microtome. Sections were stained with cresyl violet and analyzed for cannula placement by two experimenters, one blind to treatment and results. Only data from rats with correct cannula placements were included in the analysis.

Experiment 2: Effects of icv. CRF_2 Receptor Antagonism on Anxiety-like Behavior in Rats not Exposed to Pre-treatment—Given that the CRF_2 receptor antagonist ASV-30 (2 μg) increased anxiety-like behavior in saline pretreated rats (Fig. 2), opposite to the effect observed in rats without pre-treatment in other studies (e.g., Takahashi, et al., 2001), a second experiment was conducted to determine the effects of icv. ASV-30 (2 μg/2 μl) on anxiety-like behaviors in rats that did not undergo any injection pre-treatment. Male rats (N = 18) at the same age as those tested for Experiment 1 underwent surgery, acclimation, infusion, EPM testing and histological analysis as described for Experiment 1, in the absence of any prior saline or amphetamine injections.

Experiment 3: Expression of CRF_1 and CRF_2 Receptors in Brain Regions Associated with Anxiety States—A third experiment was performed to determine whether differences in CRF receptor densities could underlie the differences in ASV-induced behavioral results between un-treated, saline or amphetamine pre-treated rats.

Pre-treatment and Tissue Collection: Amphetamine and saline pre-treatment was performed as described for experiment 1. Rats comprising the un-treated group were matched for age with the pre-treated rats, and were housed in the same holding room as pre-treatment groups but not handled except for twice-weekly cage changes. At the 2 week withdrawal time point, rats (N = 11 per group) were decapitated and brains rapidly removed. Brains were frozen and stored at −80 °C, and sectioned frozen (300 μm) within a cryostat (Lecia Jung CM 1800; North Central Instruments, Plymouth MN) at −12 °C. Tissue was dissected from frozen sections on a freezing stage (Physiotemp; North Central Instruments) from regions that have high CRF_2 receptor expression and are involved in mediating anxiety-like behaviors: the lateral septum (LS), CeA and bed nucleus of the stria terminalis

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(BNST) as according to Paxinos and Watson (1997), and homogenized in 40μl of HEPES buffer (1.19%, pH 7.4) containing 14μl/ml protease inhibitor stock “complete” (Roche Diagnostics, IN, USA) and stored at −80°C until processing.

Western Immuno-blots: Procedures for measuring CRF receptor levels follow those used by us in the past (Lukkes et al., 2009a). Briefly, protein concentrations were determined within 5 μl sample duplicates using a Bradford Kit (BioRad Laboratories, Hercules, CA, USA) and a microplate reader (Bio-Tek Instruments, Winooski, VT, USA). Samples were processed with a 1.5M Tris loading buffer containing 1× SDS/β-mercaptoethanol, vortexed and boiled for 3 min prior to separation by 10% SDS-PAGE. Following electrophoresis (BioRad Laboratories), proteins were transferred to an Immuno-Blot PVDF membrane (0.2 μm, BioRad Laboratories). The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBS-T) for 2 hours at RT and incubated with primary polyclonal antibodies to CRF_1 receptors (1:25; Santa Cruz Biotech, Santa Cruz, CA, USA, #sc-12381) or CRF_2 receptors (1:50; Santa Cruz Biotech, #sc-20550) in 5% nonfat dry milk in TBS-T overnight 4 °C. The membranes were rinsed three times for 5 min at RT in TBS-T. After the rinsing procedure, the membranes were incubated for 2 hr at RT in IRDye 800-conjugated affinity purified anti-goat IgG (H & L) (Rockland Inc., Gilbertsville, PA, USA #605-732-125) at 1:2000 in 5% nonfat dry milk in TBS-T. Control for protein loading was achieved by using primary antibodies to actin (1:2000; #MAB1501R; Chemicon International, USA) and secondary antibodies to actin at 1:5000 for IRDye 80–conjugated affinity-purified anti-mouse IgG (H&L; #610-132-121; Rockland Inc.) in 5% nonfat dry milk in TBS-T. Proteins were detected using the Odyssey infrared imaging system (excitation/emission filters at 780 nm/820 nm range, LI-COR Biosciences, Lincoln, NE, USA). Optical density of each protein band was obtained using Odyssey software (LI-COR Biosciences), and normalized against background. Optical density for each of the CRF_1 and CRF_2 receptors from each individual sample was then corrected against actin levels, and percentage difference from un-treated control samples were calculated per individual membrane (Noshita et al., 2002; Perrotti et al., 2004; Mantsch et al., 2007; Griesbach et al., 2012).

Statistical Analysis

Separate two-way ANOVA were used to analyze each EPM variable for experiment 1 (pre-treatment x infusion), while separate one-way ANOVA were used to determine differences in EPM measures between infusion treatments for experiment 2, and for western blot data of experiment 3. Significant main effects or interactions were followed by Student–Newman–Keul’s (SNK) *post hoc* tests for multiple comparisons. Sigma Stat v3.5 was used for all analyses, with significance set at $P<0.05$.

Results

Infusion Cannula Placement

Cannula placements in the lateral ventricle (Fig. 1) ranged from 0.26 mm to 1.40 mm posterior from bregma and 0.8 mm to 2.2 mm lateral from midline. Cannula placements did not differ among saline and amphetamine pretreated rats and un-treated rats.

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Effects of icv. Infusion of CRF_2 Antagonist on Anxiety Behaviors during Amphetamine Withdrawal

For latency to enter open arms, there was a significant interaction between pre-treatment and icv. infusion ($F_{(1,31)} = 9.535$, P = 0.004; Fig. 2A). Vehicle-infused amphetamine pre-treated rats took significantly longer to enter open arms compared both to saline pre-treated rats infused with vehicle (P = 0.017) and to amphetamine pre-treated ASV-infused rats (P = 0.002; Fig. 2A). There were no significant differences between saline pre-treated vehicle and saline pre-treated ASV-infused rats (P = 0.296) or between saline and amphetamine pre-treated rats that received ASV infusions (P = 0.073) in latency to enter open arms (Fig. 2A).

There was also a significant interaction between pre-treatment and icv. infusion for the number of entries into open arms ($F_{(1,31)} = 10.400$, P = 0.003; Fig. 2B). The number of entries into the open arms for amphetamine pre-treated vehicle-infused rats were significantly lower than for saline pre-treated vehicle-infused rats (P = 0.012) and as compared to amphetamine pre-treated rats infused with ASV (P = 0.022; Fig. 2B). However, ASV infusions in saline pre-treated rats resulted in a significant decrease in entries into open arms when compared to saline pre-treated rats infused with vehicle (P = 0.039; Fig. 2B). There was no significant difference in open arm entries between amphetamine and saline pre-treated rats that received ASV (P = 0.069).

Similarly, there was a significant interaction between pre-treatment and icv. infusion in time spent in open arms ($F_{(1,31)} = 21.440$, P = < 0.001; Fig. 2C). For rats infused with vehicle, amphetamine pre-treated rats spent significantly less time in the open arms than saline pre-treated rats (P = 0.002; Fig. 2C), and as compared to amphetamine pre-treated rats infused with ASV (P = 0.001; Fig. 2C). However, saline pre-treated rats infused with ASV spent significantly less time in open arms than saline pre-treated vehicle infused rats (P = 0.006) and as compared to amphetamine pre-treated rats infused with ASV (P = 0.003; Fig. 2C).

Intracerebroventricular infusions of ASV did not affect the total distance moved within the maze, as there was no significant main effect of pre-treatment group ($F_{(1,31)} = 0.661$, P = 0.422; Fig. 2D), infusion ($F_{(1,31)} = 0.178$, P = 0.676), nor an interaction between pre-treatment and icv infusion ($F_{(1,31)} = 0.915$, P = 0.346).

Effects of icv. Infusion of ASV on Anxiety-like Behaviors in Un-treated Rats

Infusion of ASV into the lateral ventricle of rats not exposed to pre-treatment resulted in decreased latency to enter the open arms ($F_{(1,14)} = 7.669$, P = 0.015; Fig. 3A), increased number of entries into the open arms ($F_{(1,15)} = 4.952$, P = 0.042; Fig. 3B) and increased time spent in the open arms of the EPM ($F_{(1,15)} = 5.795$, P = 0.029; Fig. 3C) as compared to vehicle-infused rats. However, icv. infusion of ASV did not affect the total distance moved within the maze, ($F_{(1,15)} = 3.297$, P = 0.089; Fig. 3D).

CRF Receptor Expression

A significant effect of pre-treatment was observed for CRF_1 receptor expression in the LS ($F_{(2,25)} = 5.385$, P = 0.011; Fig. 4A), with saline pre-treated rats showing reduced CRF_1 receptor expression when compared to un-treated (P = 0.019) and amphetamine pre-treated

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($P = 0.011$) rats. However, CRF_2 receptor expression within the LS was not altered by pre-treatment ($F_{(2,30)} = 0.150$, $P = 0.861$; Fig. 4B). There was also a significant effect of pre-treatment on CRF_1 receptor levels in the CeA ($F_{(2,21)} = 8.117$, $P = 0.002$; Fig. 4C), with both saline ($P = 0.004$) and amphetamine ($P = 0.004$) pre-treatment reducing the level of CeA CRF_1 receptor expression. Again, pre-treatment had no effect on CRF_2 receptor expression in the CeA ($F_{(2,27)} = 0.234$, $P = 0.793$; Fig. 4D). There was no effect of pre-treatment on either CRF_1 ($F_{(2,30)} = 2.121$, $P = 0.138$; Fig. 4E) or CRF_2 receptor expression ($F_{(2,30)} = 0.604$, $P = 0.553$; Fig. 4F) in the BNST.

Discussion

Consistent with previous reports (Barr et al., 2010; Vuong et al., 2010), the current study demonstrates that rats treated with amphetamine exhibit heightened anxiety states during withdrawal. Furthermore, icv. infusion of a CRF_2 receptor antagonist decreased anxiety-like behaviors of rats in the EPM during amphetamine withdrawal, without affecting locomotion. Intra-dRN infusion of a CRF_2 receptor antagonist attenuates anxiety-like behaviors of rats during amphetamine withdrawal and also following early life stress, with CRF_1 receptor antagonism having little effect (Vuong et al., 2010; Bledsoe et al., 2011). The current study adds to this by demonstrating that global central blockade of CRF_2 receptors also reduces anxiety-like behaviors in an animal model of heightened anxiety, highlighting the therapeutic potential of CRF_2 receptor antagonism. Given that CRF_2 receptors are found in the periphery, specifically in the heart, gastrointestinal (GI) tract, lung, skeletal muscle, and vasculature (as reviewed by Bale & Vale, 2004), future research should assess the effects of systemically-administered CRF_2 receptor antagonists to further determine the feasibility of targeting CRF_2 receptors in the treatment of anxiety.

In contrast to amphetamine pre-treated rats, icv. infusion of ASV had an anxiogenic effect on saline pre-treated rats. This observation is consistent with findings from CRF_2 knockout mice, which exhibit increased anxiety behavior (Bale et al., 2000), but is in contrast to pharmacological findings. Takahashi et al. (2001) show that icv. infusion of ASV (in a concentration range used by the current study) decreases anxiety-like behaviors in rats not subjected to any pre-treatment. Similarly, we demonstrate here that rats not exposed to pre-treatment injection procedures exhibit decreased anxiety-like behaviors following icv. infusion of ASV. The most likely explanation for our results is that the mild stress induced by prior handling and injection was sufficient to alter behavioral responses of saline-treated rats to CRF_2 receptor antagonism in mildy anxiogenic environments. Combined, previous and current findings suggest that while CRF_2 receptors mediate production of anxiety-like behaviors in rodents, central pharmacological blockade will have differential effects on behavior depending on prior handling experience. These findings emphasize the idea that there are occasions in which a non-handled control group might need to serve as a ‘control’ for the manipulated vehicle-treated animals to assist in interpretation of pharmacological and behavioral findings. Furthermore, these results suggest that the ability of mild pre-stress to induce anxiety-like behavior when combined with CRF_2 receptor antagonism may increase drug seeking behavior and relapse, a possibility that requires further testing.

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One of the potential mechanisms by which ASV has differential effects on behavior as a function of handling experience could be related to changes in regionally-specific CRF receptor expression. In contrast to icv. infusion, direct infusion of CRF_1 or CRF_2 receptor antagonists into the dRN has no effect on anxiety-like behaviors of saline pre-treated or control rats (Vuong et al., 2010; Bledsoe et al., 2011). This suggests that the dRN was not the locus of the ASV-induced anxiogenic effect in saline pre-treated rats. Therefore, we examined CRF receptor expression in three other brain regions (LS, CeA and BNST) in which CRF_2 receptors are associated with fear or anxiety-like reponses (Radulovic et al., 1999; Liu et al., 2004; Henry et al., 2006; Skorzewska et al., 2011; Takahashi et al., 2011; Lebow et al., 2012; Ventura-Silva et al., 2012; Elharrar et al., 2013).

One of the major differences in CRF receptor expression in saline pre-treated rats as compared to both un-treated and amphetamine pre-treated rats was observed as a decrease in CRF_1 receptors within the LS. However, a role for LS CRF_1 receptors in mediating anxiety is not clear as CRF_2 receptors in the LS have been more often implicated in increased anxiety-like behaviors (e.g. Radulovic et al., 1999; Henry et al., 2006; Bakshi et al., 2002). Interestingly, activation of CRF_1 and CRF_2 receptors have opposing effects on glutamatergic transmission in the LS (Liu et al., 2004). Specifically, glutamatergic transmission in the LS is facilitated by CRF_1 receptor activity but dampened by CRF_2 receptor activation (Liu et al., 2004). Thus, when CRF_2 receptors are blocked by ASV as in the current study, CRF actions on CRF_1 receptors in the LS would be attenuated in saline pre-treated rats as compared to un-treated and amphetamine pre-treated rats, potentially reducing glutamatergic transmission.

Converging evidence suggests that reduced glutamate transmission in the LS is linked to increased anxiety-like behaviors (Radulovic et al., 1999; Henry et al., 2006; Bakshi et al., 2002; Liu et al., 2004). Therefore, attenuated CRF_1 receptor-mediated excitatory transmission in the LS following ASV-treatment of saline rats may disrupt the overall balance of activity in anxiety-regulating circuits to have an anxiogenic effect. Future work should directly test this and other possibilities. For example, it is also possible that other mechanisms related to changes in CRF receptors underlie the anxiolytic effects of ASV in saline pre-treated rats which were not detected by the current study. This could be due to the limitations of not being able to distinguish surface from cytosolic receptor expression, not differentiating CRF receptor expression in the subregions of each region studied, or simply that effects occurred in regions that were not studied here.

In conclusion, the current study demonstrates that central antagonism of CRF_2 receptors attenuates anxiety-like behaviors of rats during amphetamine withdrawal. Opposing effects of CRF_2 receptor antagonism on anxiety-like behavior of amphetamine and saline pre-treated rats appear to be due to a combination of blocking elevated levels of CRF_2 receptors in the dRN of amphetamine pre-treated rats (Pringle et al., 2008; Vuong et al., 2010) and CRF_2 receptor antagonism in the LS unmasking the effects of decreased CRF_1 receptors in saline pre-treated rats. Overall, the findings highlight the effectiveness of central CRF_2 antagonism as a possible pharmacological strategy to treat anxiety during abstinence in psychostimulant-dependent individuals, to reduce the risk of relapse.

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Abbreviations

5-HT	serotonin
BNST	bed nucleus of the stria terminalis
aCSF	artificial cerebrospinal fluid
CeA	central nucleus of the amygdala
CRF	corticotropin-releasing factor
dRN	dorsal raphe nucleus
EPM	elevated plus maze
GABA	γ-aminobutyric acid
LS	lateral septum
icv	intracerebroventricular

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Highlights

- The central effects of CRF_2 receptor antagonism in anxiety were tested.
- CRF_2 antagonist reduces anxiety during amphetamine withdrawal.
- CRF_2 antagonist increases anxiety of saline controls.
- Difference in CRF_2 antagonist effects may be due to altered CRF expression.

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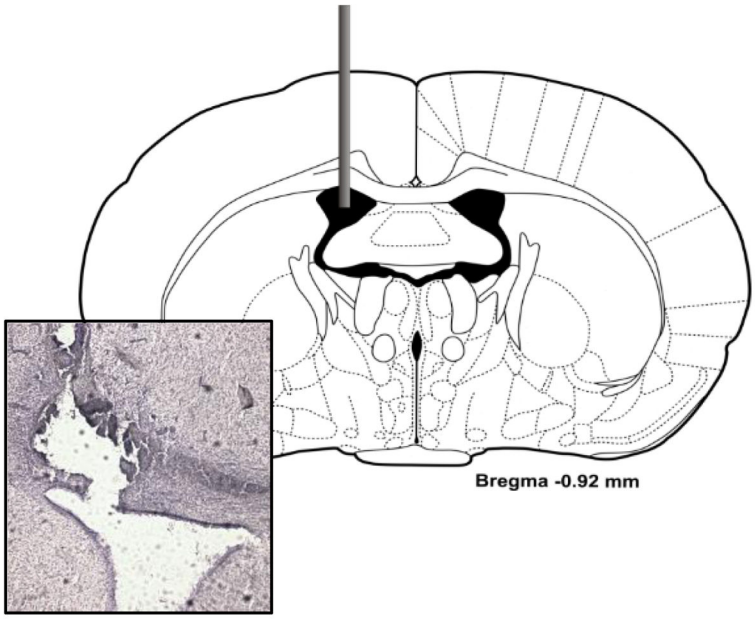

Fig. 1.

Representative diagram and photomicrograph illustrating infusion cannula placement into the lateral ventricle (schematic adapted from Paxinos and Watson, 1997).

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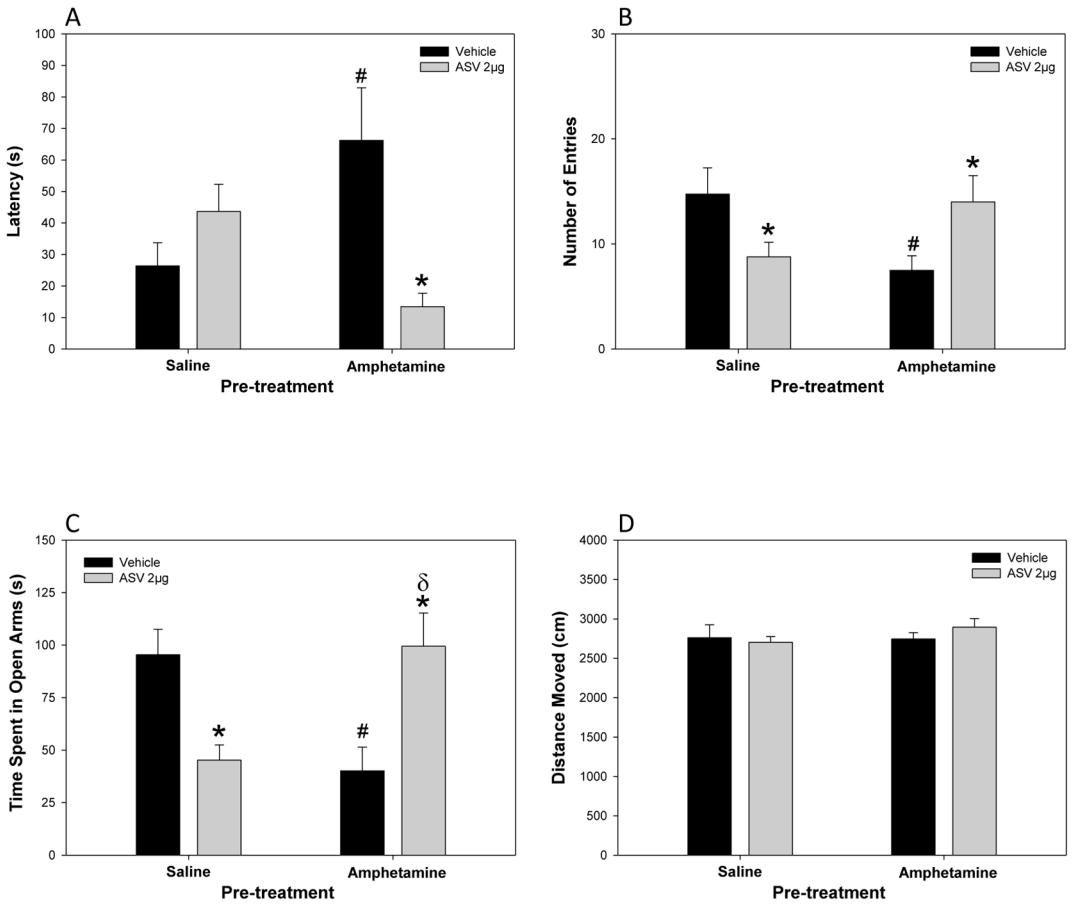

Fig. 2.

(A) Latency to enter open arms, (B) number of entries into open arms, (C) time spent in open arms and (D) total distance moved in the elevated plus maze (EPM) for amphetamine and saline pre-treated rats infused with either ASV or vehicle. Data represent mean ± SEM. $N = 6–8$ per treatment group. $^{\#}P < 0.05$ between vehicle-infused saline and amphetamine pre-treatment groups; $^{*}P < 0.05$ between vehicle and ASV-30, within pretreatment groups; and $^{\delta}P < 0.05$ between ASV-infused saline and amphetamine pre-treatment groups.

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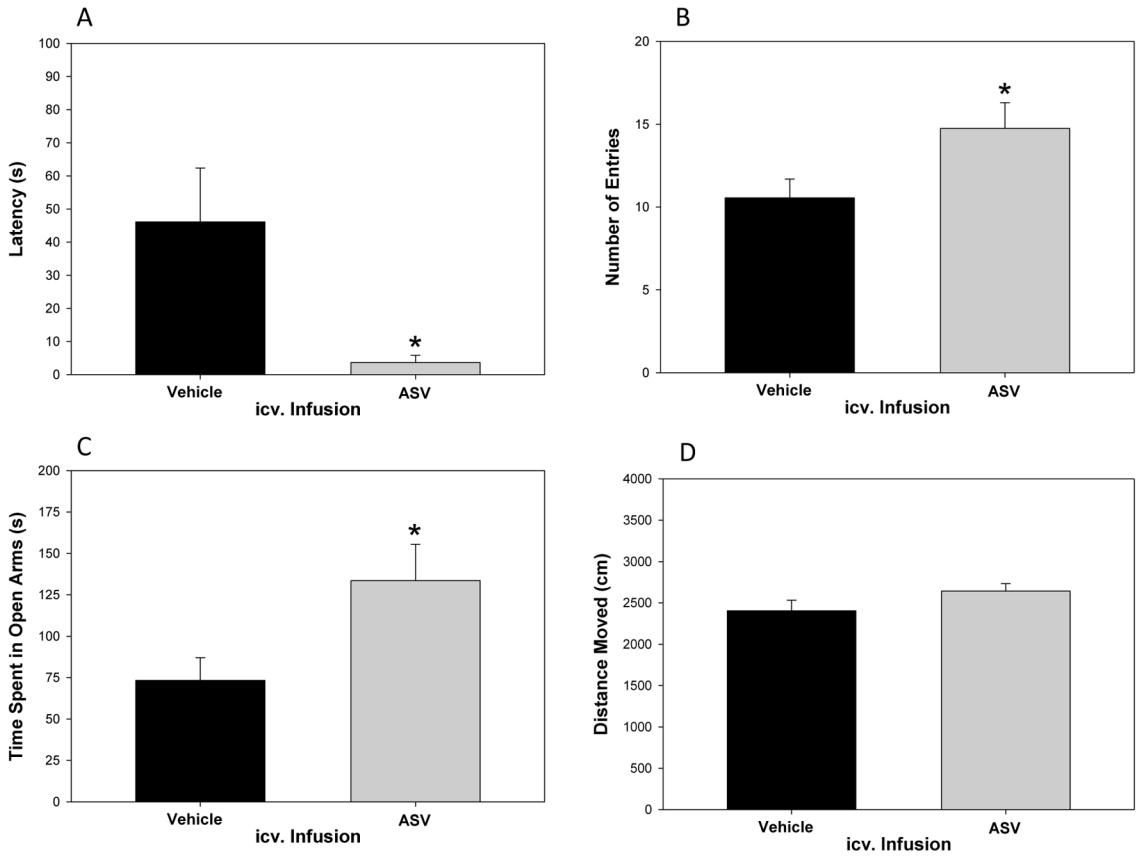

Fig. 3.

(A) Latency to enter open arms, (B) number of entries into open arms, (C) time spent in open arms and (D) total distance moved in the elevated plus maze (EPM) for rats not exposed to pre-treatment that were infused with either ASV or vehicle. Data represent mean ± SEM. $N = 9$ per treatment group. *$P < 0.05$ between vehicle and ASV.

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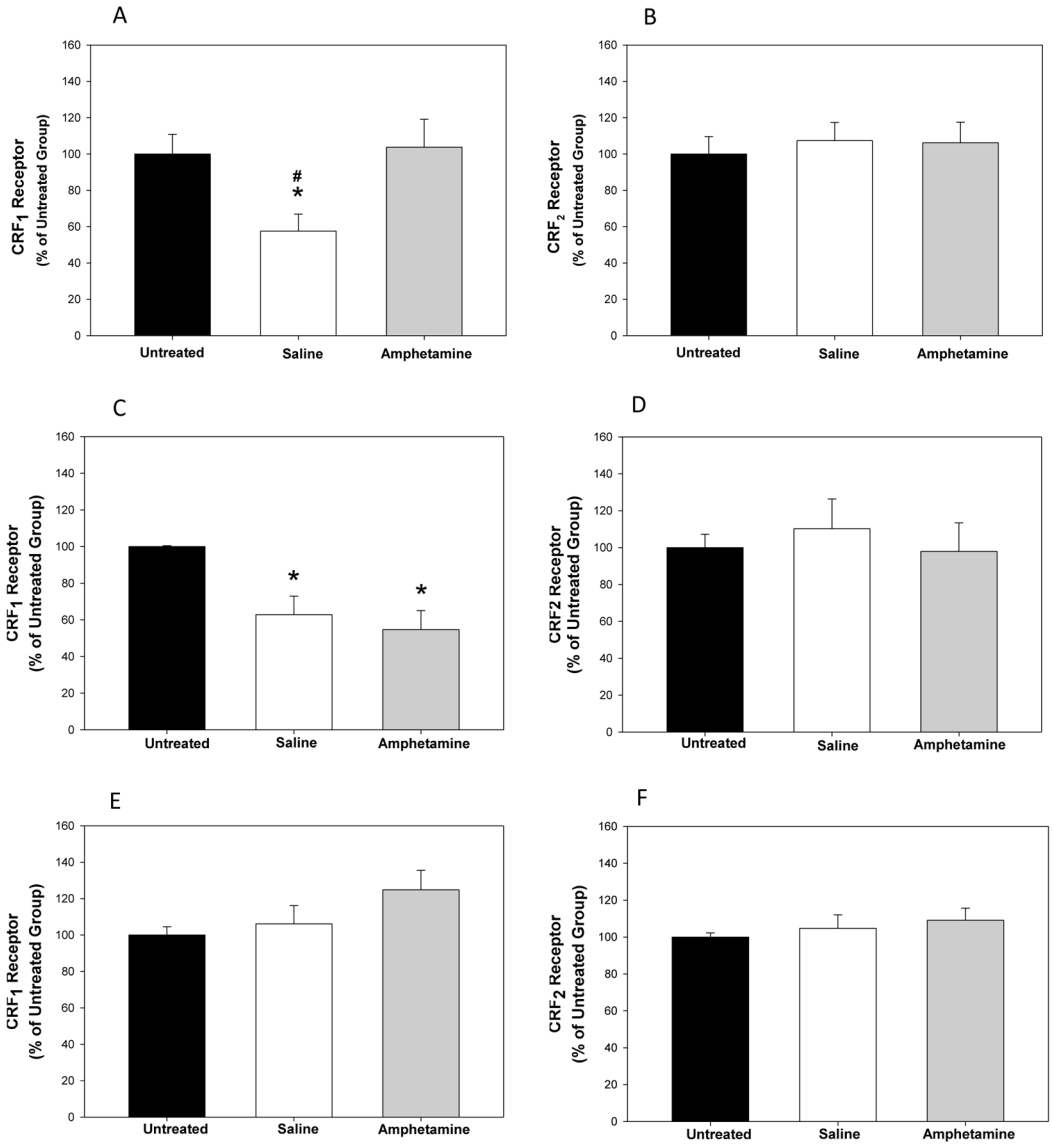

Fig. 4.

CRF_1 receptor expression in the (A) lateral septum (LS), (C) central nucleus of the amygdala (CeA) and (E) bed nucleus of the stria terminalis (BNST), and CRF_2 receptor expression in the (B) lateral septum (LS), (D) central nucleus of the amygdala (CeA) and (F) bed nucleus of the stria terminalis (BNST) in rats not exposed to pre-treatment (un-treated), amphetamine and saline pre-treated rats. Data represent mean ± SEM. $N = 11$ per treatment group. $^{\#}P < 0.05$ between saline and amphetamine pre-treatment groups, $^{*}P < 0.05$ compared to un-treated rats.