Corticotropin-Releasing Factor Receptor Antagonism within the Dorsal Raphe Nucleus Reduces Social Anxiety-Like Behavior after Early-Life Social Isolation

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Social isolation of rats during the early part of development increases social anxiety-like behavior in adulthood. Furthermore, early-life social isolation increases the levels of corticotropin-releasing factor (CRF) receptors in the serotonergic dorsal raphe nucleus (dRN) of adult rats. Interactions between serotonin and CRF systems are thought to mediate anxiety behavior. Therefore, we investigated the effects of CRF receptor antagonism within the dRN on social anxiety-like behavior after early-life social isolation. Male rats were reared in isolation or in groups from weaning until midadolescence, and rehoused in groups and allowed to develop into adulthood. Adult rats underwent surgery to implant a drug cannula into the dRN. After recovery from surgery and acclimation to the testing arena, rats were infused with vehicle or the CRF receptor antagonist D-Phe-CRF₍₁₂₋₄₁₎ (50 or 500 ng) into the dRN before a social interaction test. Isolation-reared rats pretreated with vehicle exhibited increased social anxiety-like behavior compared with rats reared in groups. Pretreatment of the dRN with D-Phe-CRF₍₁₂₋₄₁₎ significantly reduced social anxiety-like behaviors exhibited by isolation-reared rats. Overall, this study shows that early-life social stress results in heightened social anxiety-like behavior, which is reversed by CRF antagonism within the dRN. These data suggest that CRF receptor antagonists could provide a potential treatment of stress-related social anxiety.

Introduction

Early-life stress is associated with the development of neuropsychiatric disorders in adulthood (Nemeroff, 2004). Isolation of rats from social counterparts during a critical period of development models chronic early-life stress and causes alterations to anxiety-like behavior that persist into adulthood, even after resocialization (Hall, 1998). Specifically, isolation of rats from preadolescence to midadolescence increases subsequent anxiety-like and fear behavior of midadolescent and adult rats within a social interaction test (van den Berg et al., 1999; Lukkes et al., 2009a).

Corticotropin-releasing factor (CRF) is an important mediator of anxiety-like and stress behavior (Bale, 2005). Central administration of CRF or CRF receptor agonists increases anxiogenic behavior in the open field (Takahashi et al., 1989), elevated plus maze (Pellemounter et al., 2002), and social interaction test (Spiga et al., 2006). Furthermore, ventricular infusion of CRF receptor antagonists produces anxiolytic effects in these behavioral tests (Spina et al., 2000). Increased serotonergic activity also modulates anxiety-like behavior (Lowry et al., 2005). For example, pretreatment of the basolateral amygdala with a serotonin

receptor agonist increases anxiety-like behavior in a social interaction test, whereas serotonin receptor antagonists have anxiolytic effects in this anxiety test (Gonzalez et al., 1996). In addition, CRF modulates limbic serotonin release via CRF receptor activation in the dorsal raphe nucleus (dRN) (Price and Lucki, 2001; Forster et al., 2006). Activation of CRF₂ receptors in the dRN increase serotonergic neuronal activity (Pernar et al., 2004) and increases serotonin release in areas of the limbic system, such as the nucleus accumbens and amygdala (Amat et al., 2004; Lukkes et al., 2008). Interestingly, adult rats that were reared in isolation during early life show prolonged CRF-mediated serotonin release in the nucleus accumbens and increased CRF₂ receptors in the dRN (Lukkes et al., 2009b).

Given the important role of CRF and serotonin in modulating anxiety-like behavior, elevated social anxiety-like behaviors observed in isolates could result from increased CRF receptor activation in the dRN. Certainly, CRF receptor antagonism within the dRN reduces the social deficits associated with social defeat of adult hamsters (Cooper and Huhman, 2007). Therefore, this study examined whether CRF receptor antagonism within the dRN before a social interaction test could reverse the anxiogenic profile of adult rats reared in isolation.

Materials and Methods

Animals and social isolation protocol. One hundred and twenty-two male Sprague Dawley rats (University of South Dakota Laboratory Animal Services, Vermillion, SD) were obtained at postnatal day 21 (P21) (day of weaning) and housed at a constant room temperature (22°C, 60% rela-

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Figure 1. Cannula placements in the dRN. Schematic representations of drug infusion cannulae placements in the dRN (gray dots) or outside the dRN (gray stars) for control pair-housed (**A**), group-reared (**B**), and isolation-reared (**C**) rats. Figures were adapted from Paxinos and Watson (1997).

tive humidity) with a reverse 12 h light/dark cycle (lights off at 10:00 A.M.). Food and water were available *ad libitum*. The following procedures were approved by the University of South Dakota Institutional Animal Care and Use Committee and were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

The current study used an early-life social isolation procedure that has been previously shown to increase CRF-mediated serotonin release, CRF receptor levels in the dRN, and social anxiety-like behavior (Lukkes et al., 2009a,b). On P21, male rats were housed either individually or in groups of three for a period of 3 weeks during preadolescent to midadolescent development (Andersen, 2003). After 3 weeks of isolation or group rearing, all rats were weighed and housed in groups of three (within the same treatment groups) for a resocialization period of 2 weeks (Lukkes et al., 2009a,b). At the end of the 5 week isolation/resocialization procedure, rats reached early adulthood (P56) and underwent stereotaxic surgery. A separate cohort of rats was used for control experiments to determine the effects of CRF receptor antagonism on locomotion in the absence of social interaction. Control rats were weaned at P21, reared in pairs, and underwent stereotaxic surgery at P56.

Stereotaxic surgery. Rats were anesthetized with a ketamine (80 mg/kg, i.p.; Met-Vet)/xylazine (6 mg/kg, i.p.; Rx Veterinary Products) mixture and placed in a stereotaxic frame (David Kopf Instruments) with the incisor bar set at -3.3 mm. A 22 gauge guide cannula (5 mm in length; Plastics One) was stereotaxically implanted 1 mm above the dRN (anteroposterior, -7.8 mm from bregma; mediolateral, -2.6 from midline) (Paxinos and Watson, 1997) at a 23° angle to avoid the cerebral aqueduct (Forster et al., 2006). Rats were administered the analgesic ketoprofen (5 mg/kg, i.m.; Fort Dodge Animal Health) at the conclusion of each surgery. All rats were allowed 2 d of recovery from surgery before acclimation to testing procedures.

Acclimation to the infusion procedure and to the testing arena. Acclimation procedures and behavioral testing were conducted during the dark phase of the light cycle from 11:00 A.M. to 5:00 P.M. Two days after surgery, all rats were acclimated to the infusion procedure and the testing arena over 3 consecutive days. The rat was gently restrained and a 30 gauge stainless-steel infusion cannula (1 mm longer than the guide) was inserted into the guide cannula. Artificial CSF (aCSF) (0.1005 g of KCl, 4.295 g of NaCl, 0.062 g of NaH_2PO_4 , 0.0995 g of Na_2HPO_4 , 0.1015 g of MgCl_2 , and 0.088 g of CaCl_2 in 500 ml; pH 7.4) was infused into the dRN (0.5 μl) over 1 min using a microinfusion pump (Stoelting). The cannula was left in place for an additional minute to allow for complete diffusion. On removal of the cannula, rats were returned to their home cages, and 20 min later, rats were placed into the testing arena for 30 min. The testing arena ($97.8 \times 70.17 \times 31.8$ cm) was within a dark experimental room illuminated by red light. Total distance moved (in centimeters) was recorded for

30 min over the 3 d acclimation period (Ethovision 3.1; Noldus Information Technology).

Social interaction test. After the 3 d acclimation period, both group- and isolation-reared rats were infused with aCSF (vehicle) or the CRF $_{1/2}$ receptor antagonist D-Phe-CRF $_{(12-41)}$ [[D-Phe 12 ,Nle 21,38 , α -Me-Leu 37]-CRF $_{(12-41)}$ (human, rat); 50 or 500 ng in 0.5 μl of aCSF; Bachem Americas] into the dRN 20 min before the 30 min social interaction test. The doses (50 or 500 ng) of D-Phe-CRF $_{(12-41)}$ used were based on those shown to reduce anxiety-like behavior after intracranial infusion (Erb and Stewart, 1999; Cooper and Huhman, 2007), and the volume (0.5 μl) infused into the dRN does not have nonspecific effects by diffusion into areas surrounding the dRN (Forster et al., 2006; Lukkes et al., 2008).

The social interaction test consisted of placing an unfamiliar pair-housed male rat, size matched with the experimental rat (± 20 g), at one end of the arena and a group- or isolation-reared rat in the other end of the arena. Behaviors scored were the latency of the group- or isolation-reared rat to first approach the unfamiliar conspecific (in seconds), the total duration (in seconds) of social contacts (sniffing, chasing, crawling over, or grooming the unfamiliar conspecific), and the total duration of freezing behavior (in seconds) for group- and isolation-reared rats (Spiga et al., 2006; Lukkes et al., 2009a). As for our previous experiments (Lukkes et al., 2009a), aggressive behavior between the experimental and unfamiliar conspecific was not observed. Behavior from all testing sessions was scored by one observer blind to treatment (J.L.) using Observer XT (Noldus Information Technology).

Locomotion test. Control pair-housed rats were infused with aCSF or D-Phe-CRF $_{(12-41)}$ (50 or 500 ng) into the dRN, 20 min before placement in the testing arena for 30 min. These rats were not exposed to the social interaction test. Instead, to determine whether D-Phe-CRF $_{(12-41)}$ had any nonspecific effects on locomotion, total distance moved (in centimeters) was recorded by Ethovision 3.1 and divided into 5 min time bins.

Histology. At the conclusion of each behavioral experiment, rats were administered a lethal dose of Fatal-plus (0.5 ml, i.p.; Vortech). Brains were removed and fixed in 10% formalin. Sections (60 μm) were cut at -12°C , and sections were analyzed under a light microscope by two experimenters blind to treatment for the location of drug infusion cannulae.

Data analysis. Significance levels for all statistical tests were set at $p \leq 0.05$ (SigmaStat, version 2.03; SPSS). A Grubb's outlier test was performed before all ANOVAs as previously described (Lowry et al., 2001). The effect of rearing treatment on locomotion over the 3 d acclimation period or the effect of drug treatment on locomotion over time, were analyzed using separate two-way ANOVAs with one repeated measure (time). To further examine significant effects of time, one-way repeated-measure ANOVAs were also used, followed by Dunnett's *post hoc* analysis. For the social interaction test, the effects of rearing and drug treatment on anxiety-like measures were analyzed using sepa-

Figure 2. Infusion of D-Phe-CRF into the dRN does not differentially affect locomotion. All rats, regardless of dRN drug infusion, showed reduced levels of distance moved over the testing session. $*p < 0.05$ compared with the first 5 min for all three drug treatments. The figure inset shows no significant difference in the total amount of distance moved between drug treatment groups. Error bars indicate SEM.

rate two-way ANOVAs, with significant effects further analyzed using Student–Newman–Keuls (SNK) *post hoc* comparisons.

Results

Drug infusion cannulae placement

Drug infusion cannulae were similarly located in the mid to posterior aspect of the dRN among control pair-housed ($n = 21$) (Fig. 1A), group-reared ($n = 28$) (Fig. 1B), and isolation-reared groups ($n = 32$) (Fig. 1C). Although infusions into the dRN were close to the cerebral aqueduct, cannulae were placed on a lateral-to-medial angle to minimize diffusion into the cerebral aqueduct (Forster et al., 2006; Lukkes et al., 2008). Since the dRN is a medial structure, the spread of the infusion most likely encompassed the majority of the bilateral dRN. Cannulae placements adjacent to the dRN were used as anatomical controls (Fig. 1B,C) to illustrate that the effects of $0.5 \mu\text{l}$ of D-Phe-CRF_(12–41) infused into the dRN were not attributable to spread to neighboring regions or into the ventricle (Forster et al., 2006; Lukkes et al., 2008).

Infusion of D-Phe-CRF into the dRN does not differentially affect locomotion

Total distance moved within the testing arena over the 3 d acclimation period before dRN drug treatment did not differ between pair-housed rats assigned to the different treatment groups ($F_{(2,32)} = 0.112$; $p = 0.894$). Furthermore, infusion of aCSF ($n = 7$) or D-Phe-CRF_(12–41) (50 or 500 ng; $n = 7/\text{group}$) within the dRN did not differentially affect the total distance moved ($F_{(2,8)} = 0.325$; $p = 0.732$) (Fig. 2, inset). To further analyze the effect of drug treatment on locomotion, distance moved was examined within 5 min time bins across the testing session (Fig. 2). A significant effect of time ($F_{(5,82)} = 25.644$; $p < 0.001$), but not of drug treatment ($F_{(2,17)} = 0.146$; $p = 0.865$), nor an interaction between drug treatment and time ($F_{(10,82)} = 0.803$; $p = 0.626$) was observed for control pair-housed rats infused with aCSF or D-Phe-CRF_(12–41) (50 or 500 ng) into the dRN. *Post hoc* analysis revealed that a significant decline in the amount of distance moved over time was observed in all three groups when compared with the first 5 min (Dunnett's $p < 0.05$) (Fig. 2).

Figure 3. CRF receptor antagonism within the dRN reduced social anxiety-like behavior of isolates. **A**, Isolation-reared rats exhibited increased latency to approach an unfamiliar conspecific (aCSF treatment) compared with group-reared rats during a 30 min social interaction test, which was significantly decreased after pretreatment of the dRN with 50 and 500 ng of D-Phe-CRF. **B**, Isolation-reared rats exhibited decreased duration of social contact compared with group-reared rats, which was increased to group-reared rat levels by pretreatment of the dRN with D-Phe-CRF. **C**, Isolation-reared rats also showed increased duration of fear behavior compared with group-reared rats, which was reduced to group-reared rat levels by pretreatment of the dRN with D-Phe-CRF. $*p < 0.05$ between group- and isolation-reared rats. $\#p < 0.05$ compared with aCSF infusion. $\delta p < 0.05$ compared with 50 ng of D-Phe-CRF infusion. Error bars indicate SEM.

Table 1. Behavior of rats infused with vehicle or D-Phe-CRF adjacent to the dRN

Behavior	dRN treatment	Group reared	Isolation reared	ANOVA results
Latency to approach (s)	aCSF	1.91 ± 0.45	6.12 ± 2.68	Rearing: $F_{(1,2)} = 8.799; p = 0.005$
	50 ng of D-Phe-CRF	1.85 ± 0.34	3.52 ± 0.48	Drug: $F_{(2,35)} = 1.833; p = 0.175$
	500 ng of D-Phe-CRF	3.64 ± 0.59	5.25 ± 1.45	Interaction: $F_{(2,35)} = 0.063; p = 0.939$
Duration of social contact (s)	aCSF	446.91 ± 60.48	233.77 ± 31.34	Rearing: $F_{(1,2)} = 17.432; p < 0.001$
	50 ng of D-Phe-CRF	383.17 ± 29.73	278.65 ± 34.33	Drug: $F_{(2,36)} = 1.636; p = 0.209$
	500 ng of D-Phe-CRF	305.44 ± 20.67	180.14 ± 55.79	Interaction: $F_{(2,36)} = 2.549; p = 0.092$
Duration of freezing (s)	aCSF	199.94 ± 32.92	421.22 ± 39.44	Rearing: $F_{(1,2)} = 25.647; p < 0.001$
	50 ng of D-Phe-CRF	241.23 ± 24.69	445.62 ± 55.86	Drug: $F_{(2,36)} = 0.644; p = 0.531$
	500 ng of D-Phe-CRF	116.39 ± 12.30	432.92 ± 149.07	Interaction: $F_{(2,36)} = 0.406; p = 0.669$

Shown are mean ± SEM ($n = 3–10$).

CRF antagonism within the dRN decreases anxiety-like behavior of isolation-reared rats in the social interaction test

Locomotion over the 3 d acclimation period did not differ between group- ($n = 28$) and isolation-reared ($n = 32$) rats ($F_{(1,159)} = 2.839; p = 0.094$). During the 30 min social interaction test, the latency to approach an unfamiliar conspecific was significantly altered by drug treatment ($F_{(2,45)} = 6.558; p = 0.003$), and an interaction between drug treatment and the day of testing was apparent ($F_{(2,45)} = 4.423; p = 0.018$), but an effect of rearing alone was not significant ($F_{(1,45)} = 0.162; p = 0.689$). Isolation-reared rats pretreated with vehicle ($n = 11$) exhibited a significant increase in the latency to approach an unfamiliar conspecific when compared with group-reared rats ($n = 11$) (SNK, $p < 0.05$) (Fig. 3A). However, pretreatment of the dRN with 50 ng of D-Phe-CRF_(12–41) significantly reduced the latency for isolation-reared rats ($n = 10$) to approach an unfamiliar conspecific when compared 50 ng D-Phe-CRF_(12–41)-pretreated group-reared rats ($n = 10$) and to vehicle-pretreated isolates (SNK, $p < 0.05$) (Fig. 3A). Furthermore, pretreatment of the dRN with 500 ng of D-Phe-CRF_(12–41) also reduced the latency for isolation-reared rats ($n = 11$) to approach an unfamiliar conspecific when compared with vehicle-pretreated isolates (SNK, $p < 0.05$), resulting in isolates exhibiting similar latency to approach as 500 ng D-Phe-CRF_(12–41)-pretreated group-reared rats ($n = 8$) (SNK, $p > 0.05$) (Fig. 3A). In contrast, the latency to approach was not different among D-Phe-CRF_(12–41) (50 or 500 ng) or vehicle-pretreated group-reared rats (Fig. 3A) (SNK, $p > 0.05$ for all comparisons).

A significant interaction between rearing and drug treatment ($F_{(2,51)} = 4.124; p = 0.022$) was observed for the total duration of social contacts, but there was no significant effect of rearing ($F_{(1,51)} = 0.346; p = 0.559$) or drug treatment alone ($F_{(2,51)} = 1.731; p = 0.187$). Isolation-reared rats pretreated with vehicle exhibited a significant decrease in the total duration of social contacts when compared with vehicle-treated group-reared rats (SNK, $p < 0.05$) (Fig. 3B). Pretreatment of the dRN with 500 ng of D-Phe-CRF_(12–41) significantly increased the duration of social contact of isolation-reared rats when compared with dRN pretreatment of isolates with either vehicle or 50 ng of D-Phe-CRF_(12–41) (SNK, $p < 0.05$) (Fig. 3B). Furthermore, isolation- and group-reared rats showed similar levels of social contact when pretreated with 50 or 500 ng of D-Phe-CRF_(12–41) (SNK comparisons between rearing groups at each dose $p > 0.05$) (Fig. 3B). In contrast to isolation-reared rats, there were no significant differences in the total duration of social contact between vehicle- and D-Phe-CRF_(12–41) (50 or 500 ng)-pretreated group-reared rats (Fig. 3B) (SNK, $p > 0.05$ for all comparisons).

A significant interaction between rearing and drug treatment ($F_{(2,52)} = 6.045; p = 0.004$) was observed in the total duration of freezing behavior, but there was no significant effect of rearing ($F_{(1,52)} = 3.549; p = 0.065$) or drug treatment alone ($F_{(2,52)} =$

1.489; $p = 0.235$). Isolation-reared rats pretreated with vehicle exhibited significantly greater total duration of freezing behavior when compared with vehicle-treated group-reared rats (SNK, $p < 0.05$) (Fig. 3C). Pretreatment of the dRN with 500 ng of D-Phe-CRF_(12–41) significantly decreased the duration of freezing behavior of isolation-reared rats when compared with isolates pretreated with either vehicle or 50 ng of D-Phe-CRF_(12–41) (SNK, $p < 0.05$) (Fig. 3C). Furthermore, isolation- and group-reared rats showed similar levels of freezing behavior when pretreated with 50 or 500 ng of D-Phe-CRF_(12–41) (SNK comparisons between rearing groups at each dose $p > 0.05$) (Fig. 3C). In contrast to isolates, there were no significant differences in the duration of freezing behavior between group-reared rats pretreated with vehicle or D-Phe-CRF_(12–41) (50 or 500 ng) (Fig. 3C) (SNK, $p > 0.05$ for all comparisons). In contrast to the effects of D-Phe-CRF_(12–41) infused into the dRN, infusion of D-Phe-CRF_(12–41) adjacent to the dRN (Fig. 1B,C) did not reverse social anxiety-like behavior of isolation-reared rats as evidenced by a significant effect of rearing condition on the behavioral measures accompanied by a lack of drug effect (Table 1).

Discussion

The current study demonstrates that early-life stress results in elevated social anxiety-like behavior in adulthood. To illustrate, isolates infused with vehicle exhibited increased latency to first approach an unfamiliar conspecific, decreased duration of social contact, and increased duration of freezing behavior when compared with group-reared rats in the social interaction test. These behaviors exhibited by isolation-reared rats are indicative of heightened social anxiety (Spiga et al., 2006; Lukkes et al., 2009a).

The elevated social anxiety-like behavior observed in isolation-reared rats was decreased by CRF receptor antagonism within the dRN. Pretreatment of the dRN with both 50 and 500 ng of D-Phe-CRF_(12–41) decreased the latency to approach an unfamiliar conspecific in isolation-reared rats when compared with pretreatment of the dRN with vehicle. In addition, infusion of 500 ng of D-Phe-CRF_(12–41) within the dRN decreased the duration of freezing behavior and increased the duration of social contact in isolates when compared with infusion of vehicle. The effects of D-Phe-CRF_(12–41) were specific to the dRN, since infusions adjacent to the dRN did not reduce the heightened social anxiety behaviors exhibited by isolation-reared rats. Moreover, these anxiolytic effects could not be accounted for by D-Phe-CRF_(12–41) infusions producing nonspecific effects on activity such as increased locomotion within the testing arena. Although previous studies have shown that CRF receptor antagonists administered centrally or within the basolateral amygdala increase social contact during a social interaction test (Dunn and File, 1987; Gehlert et al., 2005), this is the first demonstration that CRF receptor antagonism within the dRN reverses social anxiety-like behavior after early-

life stress. In line with the current study, 500 ng D-Phe-CRF_(12–41) pretreatment of dRN before social defeat increased the duration of social behavior and reduced submissive behavior of adult Syrian hamsters (Cooper and Huhman, 2007). Together, these data and the current study suggest that CRF receptors within the dRN are important components of the neural circuitry mediating social anxiety-like behavior.

In contrast to isolates, pretreatment of the dRN with D-Phe-CRF_(12–41) did not alter social or freezing behaviors of group-reared rats. Similar findings have been reported, in which CRF receptor antagonists, administered either systemically, intracerebroventricularly, or into the amygdala, reduce the anxiogenic effects of prestressors or CRF administration, but have little effect on the behavior of nonstressed animals (Heinrichs et al., 1992; Okuyama et al., 1999; Gehlert et al., 2005). This suggests that the ability of CRF receptor antagonists to reverse anxiety-like behaviors is dependent on previous stress experience. Specifically, the anxiolytic effects of CRF receptor antagonism for isolate rats in a social interaction test may be dependent on heightened CRF–serotonin interactions in the dRN as previously observed after social isolation (Lukkes et al., 2009b). Furthermore, the results imply that CRF receptors in the dRN play little role in mediating social or anxiety behaviors in group-reared or unstressed rats.

Although D-Phe-CRF_(12–41) antagonizes both CRF receptor subtypes, it exhibits 2–10 times greater affinity for the CRF₂ receptor compared with the CRF₁ receptor (Perrin et al., 1999). In general, there is a high density of CRF₂ receptors compared with CRF₁ receptors within the dRN (Chalmers et al., 1995). Furthermore, isolation-reared rats have elevated levels of CRF₂ receptors within the dRN compared with group-reared rats and show prolonged CRF₂-mediated increases in serotonergic activity compared with group-reared rats (Lukkes et al., 2009a,b). The exact role of CRF₁ or CRF₂ receptors in mediating anxiogenic behavior remains controversial. Clark et al. (2007) show a more robust anxiogenic effect of chronic CRF₁ receptor agonism in the dRN when compared with chronic CRF₂ receptor agonism. Furthermore, CRF₂ receptor knock-out mice display increased anxiety-like behavior in an elevated plus maze, suggesting that these receptors have an anxiolytic role (Bale, 2005). In contrast, CRF₂ receptor antagonism via ventricular injection (Takahashi et al., 2001) or within the lateral septum (Henry et al., 2006) have an anxiolytic effect during anxiety tests, suggesting a role for CRF₂ receptors in mediating anxiety-like behavior. To further elucidate the relationship between the CRF receptor subtypes and anxiety states, future studies should examine the role of dRN CRF₁ and CRF₂ receptor subtypes in mediating social anxiety-like behavior after social isolation using antagonists specific for each receptor subtype.

In summary, the current data suggest CRF receptor antagonism within the dRN reduces social anxiety-like behavior in rats exposed to early-life stress. Both early-life stress and increased CRF levels have been implicated in several mood disorders, including anxiety and depression (Nemeroff, 2004). Therefore, CRF receptor antagonism represents a possible therapeutic treatment for stress-related affective disorders.

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