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# Role of CRF<sub>1</sub> and CRF<sub>2</sub> receptors in fear and anxiety

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## Abstract

Fear and anxiety are common emotions that can be triggered by stress. This paper reviews the work examining the role played by specific corticotropin-releasing factor (CRF) receptors in mediating the expression of these emotions. Several lines of evidence taken from CRF<sub>1</sub> transgenic knockout mice, CRF<sub>1</sub> antisense oligonucleotide studies, and CRF<sub>1</sub> receptor antagonist work suggest that the anxiety inducing effects of CRF are mediated by the CRF<sub>1</sub> receptor. Of these three methodological approaches, the work using transgenic CRF<sub>1</sub> knockout mice appears to be the most consistent. In contrast, the work using specific CRF<sub>1</sub> antagonists has produced somewhat varied results that may be explained, in part, by the testing method. When animals are stressed prior to behavioral testing, CRF<sub>1</sub> receptor antagonists appear to have anxiolytic-like effects. In addition, chronic dosing with CRF<sub>1</sub> antagonists may have more potent anxiolytic-like effects, especially in animal models of spontaneous anxiety, than acute dosing procedures. Spontaneous anxiety is defined as behavior that is elicited entirely by the testing situation without current or prior aversive or explicitly induced stress. CRF<sub>1</sub> antisense oligonucleotide work is difficult to interpret because of potential toxicological side effects produced by the antisense oligonucleotide and, in some cases, the absence of verifiable reductions in CRF<sub>1</sub> receptor densities after treatment. Similar methods—CRF<sub>2</sub> knockouts, CRF<sub>2</sub> antisense oligonucleotides, and CRF<sub>2</sub> antagonists—were used to evaluate the function of CRF<sub>2</sub> receptors in emotionality. In comparison to the large number of CRF<sub>1</sub> receptor studies, fewer CRF<sub>2</sub> receptor investigations have been conducted and these studies have yielded mixed results. However, recent work demonstrating a robust reduction in CRF<sub>2</sub> receptors using a CRF<sub>2</sub> antisense oligonucleotide with minimal toxicity, and dose response studies using a peptide CRF<sub>2</sub> antagonist suggest that CRF<sub>2</sub> receptors play a role in stress-induced and spontaneous anxiety. Furthermore, inhibiting the actions of both CRF<sub>1</sub> and CRF<sub>2</sub> receptors produces a greater reduction in stress-induced behavior than inhibition of either receptor alone. Thus, current data suggest that CRF<sub>1</sub> and CRF<sub>2</sub> receptors are involved in the mediation of fear and anxiety behavior. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Corticotropin-releasing factor; CRF<sub>1</sub> receptor; CRF<sub>2</sub> receptor; Fear; Anxiety; Stress; Stress-induced anxiety; Spontaneous anxiety; CRF receptor antagonists

## 1. Introduction

Exposure to excessive or uncontrollable stress is a major factor associated with a variety of illnesses including psychopathology. Psychosocial stressors may trigger mood disorders or exacerbate the symptoms of schizophrenia as well as contributing to its relapse [1–3]. In early life, exposure to stress may increase the risk of developing behavioral problems and may sensitize the young to stressors elevating the risk for stress-induced psychopathology [4,5]. As a result, there is an intense effort to identify and unravel the key systems responsible for mediating the body's response to stress [6].

One system that has attracted considerable attention in the last two decades involves corticotropin-releasing factor (CRF). This 41-amino acid peptide is hypothesized to play an essential role in coordinating endocrine, autonomic,

immune, and behavioral responses to stress [7–11]. CRF and urocortin, a CRF-like peptide, are widely distributed throughout the mammalian brain [12–16]. These peptides exert their biological actions via two major G protein-coupled seven-transmembrane domain receptor subtypes known as CRF<sub>1</sub> [17–19] and CRF<sub>2</sub> [20,21]. The CRF<sub>2</sub> receptor has  $\alpha$ - and  $\beta$ -splice variants. In addition, a CRF<sub>2</sub> $\gamma$  receptor is found in human brain [22]. Studies demonstrate that CRF<sub>1</sub> and CRF<sub>2</sub> receptors have distinct pharmacological profiles and unique distribution patterns in brain and peripheral tissues [23,24]. For example, in the rat, high densities of CRF<sub>1</sub> receptors are found in the pituitary, brain stem, cerebellum, amygdala, and cortex whereas CRF<sub>2</sub> $\alpha$  receptors are found predominantly in the lateral septum, ventromedial hypothalamus, and olfactory bulb [25,26]. A somewhat similar receptor distribution pattern is found in the rhesus monkey brain with the exception of increased densities of CRF<sub>2</sub> receptors in the brain regions including the neocortex, amygdala, and hippocampal formation [27]. CRF<sub>2</sub> $\beta$  receptors occur in nonneuronal cells of the

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Table 1  
Summary of CRF<sub>1</sub> knockout and antisense oligonucleotide studies in relation to behavioral tests of fear and anxiety

Test methods	Major results	Reference
<i>CRF<sub>1</sub> knockout studies</i>		
Open-field	Increased locomotion	[40]
Light–dark box	Increased light box activity	[38,40]
Defensive-withdrawal	Increased open field activity	[39]
Elevated plus maze	Increased open arm activity	[38,39]
<i>CRF<sub>1</sub> antisense oligonucleotide studies</i>		
Defensive-withdrawal	Decreased latency to enter open field and increased open field time	[41]
Swim stress-induced anxiety in elevated plus maze	No effects	[41]
CRF-induced anxiety in elevated plus maze	Increased entries and open arm time	[42]
CRF-induced anxiety in open field	Increased open field activity	[42]
Social defeat induced anxiety in elevated plus maze	Increased entries and open arm time	[43,44]

brain such as the choroid plexis and in peripheral tissue including the heart, lung, and skeletal muscle [21,25,28]. In addition to CRF<sub>1</sub> and CRF<sub>2</sub> receptors, CRF and urocortin also bind to a CRF-binding protein [29].

The identification of CRF receptor subtypes has led to a number of studies addressing its functional properties. This paper examines the evidence for CRF<sub>1</sub> and CRF<sub>2</sub> mediation of fear and anxiety. Recent reviews discussing specific CRF receptor functions associated with other conditions including eating, gastrointestinal distress, substance abuse, and immunomodulation can be found elsewhere [30–34].

## 2. Animal testing methods

To evaluate alterations in fear and anxiety, various animal tests that generally involve psychological conflict are used to determine the unique contributions of CRF<sub>1</sub> and CRF<sub>2</sub> receptors. An important issue in interpreting the role of specific CRF receptors in emotional behavior is whether or not the animals were exposed to a form of stress prior to conflict testing. Therefore, to clarify this issue, emotional behavior elicited entirely by the testing situation without current or prior aversive or explicitly induced stress will be called spontaneous anxiety. Examples of spontaneous anxiety tests include the elevated plus maze, the defensive-withdrawal test, the open field, and light–dark test. Behavioral responses exhibited in tests involving unambiguous exposure to a stressor or its effects will be called stress-induced anxiety. For example, protocols involving electric foot shock, social defeat, or swim stress are typically used to induce an anxiogenic state prior to behavioral testing. In addition to these two general types of test procedures, some investigators have examined whether CRF receptor manipulations are effective in reversing the putative anxiety-stimulating effects of exogenous CRF.

In reviewing the literature, it is apparent that three major methodological approaches are used to evaluate the behavioral effects induced by targeted receptor alterations. These methods include knockout procedures, antisense oligonucleotide strategies, and administration of specific CRF

receptor antagonists. Advantages and limitations associated with these methods have been discussed previously (e.g. Refs. [35–37]).

## 3. Role of CRF<sub>1</sub> receptors in anxiety

### 3.1. CRF<sub>1</sub> receptor knockout studies

Three studies reported that mice lacking the CRF<sub>1</sub> receptor exhibited behavior suggestive of reduced anxiety (Table 1). In four different tests of spontaneous anxiety—open-field, light–dark box, defensive-withdrawal, elevated plus maze—which normally inhibits behavioral activity, mutant mice consistently exhibited elevated levels of locomotion consistent with an anxiolytic-like profile [38–40]. In these studies, analyses were also conducted to determine whether there was a general increase in activity following deletion of CRF<sub>1</sub> receptors. The investigators concluded that because there were no reliable increases in compartment or closed-arm entries made in the light–dark test and in the elevated plus maze, CRF<sub>1</sub> receptors play a role in the expression of anxiety without effects on general activity.

Independent tests conducted on stress-induced hormone secretion further showed that CRF<sub>1</sub> knockout mice had reduced levels of adrenocorticotropin (ACTH) secretion and corticosterone (CORT) after exposure to either restraint or swim stress [39,40]. Thus, these data provide strong evidence that CRF<sub>1</sub> receptors mediate spontaneous anxiety and stress-induced hormone activation.

### 3.2. CRF<sub>1</sub> receptor antisense oligonucleotide studies

Another strategy to evaluate the functional role of CRF<sub>1</sub> receptors is to downregulate its expression using antisense oligonucleotides (Table 1). In one study, administration of CRF<sub>1</sub> antisense oligonucleotides that produced a 10–12% knockdown of brain CRF<sub>1</sub> receptors induced anxiolytic-like behavioral effects in the defensive-withdrawal test [41]. However, in a stress-induced anxiety situation, antisense oligonucleotide-treated rats exhibited elevated levels of

anxiety behavior in the elevated plus maze similar to vehicle and missense-treated animals [41]. In this study, rats were tested in the elevated plus maze immediately after exposure to 90 s of swim stress, a procedure used to induce subsequent anxiogenic-like behavioral and endocrine effects. These data suggest that CRF<sub>1</sub> receptors may play a lesser role in mediating stress-induced anxiety states.

One aspect of this study that differs from the CRF<sub>1</sub> knockout mouse work is that CRF<sub>1</sub> antisense oligonucleotide administration failed to reduce the swim stress-induced increase in ACTH secretion. The basis underlying this difference is not known, however, whereas CRF<sub>1</sub> knockout strategies are highly effective in eliminating CRF-induced cellular actions [39,40], antisense oligonucleotide administration may be less effective and depend ultimately on the level of CRF<sub>1</sub> receptor knockdown that is produced.

In another study, CRF<sub>1</sub> antisense-treated rats were less responsive to the anxiety stimulating effects of exogenous CRF than control rats [42]. In the elevated plus maze and the open field test, CRF<sub>1</sub> antisense-treated animals exhibited increased levels of anxiolytic-like behavior (Table 1). In addition, primary pituitary and AtT-20 cells exposed to 72 h of antisense treatment exhibited a reduction in ACTH release after a CRF challenge. Receptor measurements revealed significant reductions in cortical and hypothalamic CRF<sub>1</sub> receptor binding after treatment with the CRF<sub>1</sub> antisense oligonucleotide. Whether the CRF<sub>1</sub> antisense oligonucleotide is also effective in producing anxiolytic-like behavior without prior CRF administration or reducing stress-induced ACTH release was not determined.

Another study conducted by the same group of investigators [43] showed that CRF<sub>1</sub> antisense oligonucleotide treatment reduced social defeat-induced anxiety in rats subsequently tested in the elevated plus maze (Table 1). This result is consistent with other data demonstrating that bilateral CRF<sub>1</sub> antisense oligonucleotide infusions in the central nucleus of the amygdala also reduced social defeat-induced anxiety measured in the elevated plus maze [44]. Although these two studies suggest that CRF<sub>1</sub> receptors play a major role in mediating stress-induced anxiety behavior, the interpretation of these results is not entirely clear. In one study, the reduction in CRF<sub>1</sub> receptor density was not provided [43] and in the amygdala study [44], the level of CRF<sub>1</sub> receptor knockdown in the amygdala after antisense treatment did not differ from the signal found in control groups. Hence, whether these stress-induced anxiolytic behavioral effects induced by the CRF<sub>1</sub> antisense oligonucleotide is a CRF<sub>1</sub> receptor mediated effect remains to be determined.

### 3.3. CRF<sub>1</sub> receptor antagonist studies

#### 3.3.1. CP-154,526

A number of specific CRF<sub>1</sub> receptor antagonists, have been developed, some of which are capable of penetrating the blood–brain barrier when administered in the periphery.

One of the first CRF<sub>1</sub> receptor antagonist to be developed and the most extensively tested in animals is CP-154,526 [45]. On the basis of the many studies summarized later, it is clear that pharmacological antagonism of CRF<sub>1</sub> receptors using CP-154,526 does not consistently produce an anxiolytic profile (Table 2).

Initial reports indicated that when administered to rats, this compound produced dose-dependent effects in blocking CRF-induced increases in acoustic startle as well as potentiated startle [45]. In addition, rats dosed with 32 mg/kg of CP-154,526 showed improved escape latency scores 1 day after exposure to inescapable shocks [46]. These results suggest that CRF<sub>1</sub> receptors are involved in the mediation of both pharmacologically, i.e. CRF-induced, and psychologically, i.e. stress conditioning, elicited behavior.

In one of the more comprehensive reports examining the behavioral effects of CP-154,526 on rats tested in a number of different animal models of anxiety, investigators reported that CRF<sub>1</sub> antagonism failed to reverse both shock-induced punished lever pressing and punished drinking [47]. In the elevated plus maze, a 5 mg/kg dose was reported to increase total arm entries. However, higher (10 and 20 mg/kg) doses produced no effects on total arm entries or on any of the number of other behavioral measures. Somewhat similar results were reported by other investigators using CP-154,526 [48]. In this study, a 1 mg/kg dose was highly effective in producing an anxiolytic-like effect in the elevated plus maze but higher doses of 3 and 10 mg/kg were without effects. The lack of anxiolytic-like effects at higher doses appears puzzling in light of data demonstrating that doses of CP-154,526 as high as 17.8 mg/kg were effective in reducing both CRF-induced and potentiated startle [45,49]. However, the modest behavioral effects noted in the elevated plus maze may be due, in part, to the absence of a pronounced CRF activated state prior to testing. When rats are pre-treated with CRF, CP-154,526 appears to produce more consistent anxiolytic-like effects in the elevated plus maze [50]. CP-154,526 doses of 3–10 mg/kg, but not lower doses, effectively reversed the CRF-induced attenuation on open-arm time.

Using other behavioral testing procedures, researchers demonstrated that CP-154,526 produced a dose-dependent reduction in the occurrence of ultrasounds emitted by neonatal rat pups during social isolation [51]. In adult rats, conditioned emission of ultrasonic vocalizations is also attenuated after administration of 30 mg/kg of CP-154,526 [52]. In contrast, the number of ultrasonic vocalizations emitted during the immediate post-shock period that began after exposure to nine 1.25 mA foot shocks delivered over a 3–7 min period was not significantly reduced by the CRF<sub>1</sub> antagonist. Other investigators using shock-induced stress procedures of lesser intensity (three 1.0 mA foot shocks) reported that i.c.v. delivery of CP-154,526 attenuated freezing, a measure of fear and anxiety occurring in the post-shock period [53].

In a report examining the long-term effects of

Table 2  
Summary of CRF<sub>1</sub> antagonist studies in relation to behavioral tests of fear and anxiety

Test methods	Major results	Reference
CP-154,526		
<i>Rat studies</i>		
CRF-induced startle	Reduced startle amplitude	[45]
Potentiated startle	Blocked potentiated startle	[45,49]
Uncontrollable shock-induced escape deficits	Improved escape behavior	[46]
Punished lever pressing	No effects	[47]
Punished drinking	No effects	[47]
Elevated plus maze	Increased total arm entries at low but not high doses	[47]
	Increased open arm activity at low but not high doses	[48]
CRF-induced anxiety in elevated plus maze	Increased open arm time	[50]
Shock-induced freezing	Decreased freezing	[53]
Defensive withdrawal	Decreased time in chamber after chronic but not acute treatment	[54]
Shock-induced ultrasounds	No effect	[52]
Conditioned ultrasounds	Decreased	[52]
Rat pup isolation-induced ultrasounds	Decreased	[51]
<i>Mouse studies</i>		
Light–dark box	Increased activity in light box	[47]
Swim stress-induced anxiety in light–dark box	Increased activity in light box	[50]
Free-exploration	Increase activity in novel compartments	[47]
Mouse defense battery	Decreased avoidance distance, pauses, biting, and escape	[47]
<i>Hamster studies</i>		
Conditioned social defeat	No effects	[55]
Antalarmin		
<i>Rat studies</i>		
Shock-induced freezing	Reduced freezing	[57]
Conditioned freezing	Reduced freezing	[57]
<i>Primate studies</i>		
Psychosocial stress	Reduced stress-induced fear and anxiety behavior	[58]
CRA1000 and CRA1001		
<i>Rat studies</i>		
CRF-induced anxiety in elevated plus maze	Reversed reduction in open arm time	[50]
Conspecific stress-induced feeding inhibition	Facilitated feeding	[60]
<i>Mouse studies</i>		
Swim stress-induced anxiety in light–dark box	Increased activity in light box	[50]
DPC904		
Conditioned freezing	Decreased rat freezing	[63]
R121919		
Elevated plus maze	Increased open arm activity in selectively bred high anxiety but not low anxiety rats	[62]

CP-154,526 in relation to spontaneous anxiety elicited by an unfamiliar environment, researchers found that chronic (3.2 mg/kg per day; 9–10 days), but not acute, treatment was more effective in producing anxiolytic-like effects in the defensive withdrawal test [54]. Chronic treatment was associated with a reduction in CRF<sub>1</sub> receptor binding occurring in the parietal cortex. Other CRF measures including CRF<sub>2</sub> binding, CRF<sub>1</sub> and CRF<sub>2</sub> mRNA and CRF content and mRNA and urocortin mRNA were unchanged in rats treated chronically with the behaviorally effective dose of CP-154,526. Because antagonism of CRF<sub>1</sub> receptors is likely to occur after acute and chronic CP-154,526 treatment, the long-term behavioral changes probably reflect an

involvement of other mediating mechanisms influenced by chronic exposure to CP-154,526.

In addition to the rat, a few studies have examined the effects of CP-154,526 on mouse emotional behavior (Table 2). In one study, doses of 5 mg/kg or greater produced anxiolytic-like behavior in several measures taken in a light–dark test, a free-exploration test, and stress-induced mouse defense test battery [47]. Other investigators reported that CP-154,526 produced no effects in the light–dark test, unless mice were exposed to stress prior to testing [50]. In the hamster, CP-154,526 failed to reverse the submissive–defensive effects of conditioned defeat on subsequent social interactions, albeit plasma

ACTH concentrations were reduced in comparison to vehicle-treated animals [55].

### 3.3.2. Antalarmin

A CRF<sub>1</sub> receptor antagonist chemically related to CP-154,526 [56] and called antalarmin (see Table 2) is reported to produce deficits in conditioned freezing [57]. In this study, rats were injected with either vehicle or antalarmin prior to shock exposure as well as prior to the conditioned test conducted 24 h after shock exposure. Antagonism of CRF<sub>1</sub> receptors both before shock exposure and before the conditioned test produced the least amount of conditioned freezing in comparison to rats that received antalarmin either before shock exposure or before testing for conditioned freezing. However, these latter two groups showed lower levels of freezing than rats treated only with vehicle throughout the experiments. These conditioned freezing data extend the work showing that CP-154,526 is effective in reducing the acute facilitating effects of shock on freezing [53].

In another study examining the role of CRF<sub>1</sub> receptors in a primate model of psychosocial stress, antalarmin administration produced a reduction in responses associated with fear and anxiety including body tremors, grimacing, teeth gnashing, urination, and defecation [58]. Furthermore, behavioral responses normally suppressed by stress such as sexual and exploratory behavior were promoted by antalarmin.

In addition to its anxiolytic-like behavioral effects, antalarmin is reported to blunt the endocrine and autonomic components of the stress response [57,58]; albeit under high stress conditions the antagonist was not effective in reducing either ACTH or CORT secretion [57].

### 3.3.3. CRA1000 and CRA1001

Other novel CRF<sub>1</sub> antagonists that were examined in animal models of anxiety (Table 2) include CRA1000 and CRA1001 [59]. These compounds were found to have anxiolytic-like effects in mice tested in the light–dark test and in rats tested in the elevated plus maze [50]. Notably, the anti-anxiety effects of CRA1000 and CRA1001 were reported in mice exposed to swim stress prior to light–dark testing and in rats pre-treated with CRF. When mice were tested in the light–dark test without prior stress exposure, CRA1000 and CRA1001 had no significant effects on anxiety behavior. The effectiveness of CRA1000 to attenuate stress-induced emotional effects was further investigated in rats exposed to conspecifics that received electric foot-shocks [60]. Rats dosed with 10 mg/kg of CRA1000 were more likely to engage in feeding after a 1 h emotional stress experience than vehicle-treated rats. This propensity to feed was not directly linked to CRF<sub>1</sub> receptor antagonism because nonstressed rats treated with CRA1000 did not exhibit alterations in food intake. On the basis of these studies, CRA1000 and CRA1001 appear to be

behaviorally effective especially in reversing stress-induced anxiety states and CRF activated effects.

### 3.3.4. Other CRF<sub>1</sub> antagonists

Two other specific CRF<sub>1</sub> antagonists reported to produce anxiolytic-like behavior are DPC904 [61] and R121919 [62] (Table 2). In a conditioned freezing test, rats treated with DPC904 showed a dose dependent reduction in freezing when re-exposed to the environment associated with foot-shock stress [63]. These results are consistent with the reported reduction in conditioned freezing produced by antalarmin [57].

The potential involvement of CRF<sub>1</sub> receptors in mediating stress-induced or pre-existing high anxiety states is highlighted in a study using R121919 [62]. In this study, rats bred for high and low anxiety behavior were tested in the elevated plus maze after treatment with the CRF<sub>1</sub> antagonist. In high anxiety rats, a 20 mg/kg dose but not lower doses (2.0 and 5.0 mg/kg) of R121919 increased open arm exploration. The investigators suggest that R121919 may be toning down an overly active CRF system in the locus coeruleus of high anxiety rats. In contrast, rats bred for low anxiety showed no change in behavior after treatment with R121919. This latter result, however, may be the result of ceiling effects or the innate propensity of low anxiety rats to readily explore the open arms of the elevated plus maze. Indeed, in other work, the benzodiazepine diazepam had lesser effects on elevated plus maze behavior of low anxiety rats in comparison to the high anxiety strain [64]. Perhaps, R121919 may have anxiolytic-like behavioral effects in low anxiety rats exposed to an intense anxiety inducing stressor.

## 4. Role of CRF<sub>2</sub> receptors in anxiety

### 4.1. CRF<sub>2</sub> receptor knockout studies

Unlike the reduction in anxiety behavior generally reported in CRF<sub>1</sub> knockout animals, CRF<sub>2</sub> deficient mice do not show a consistent change in anxiety behavior (Table 3). One study reported that CRF<sub>2</sub> deficiency produced no significant effects on anxiety responses in the elevated plus maze or in an open field test [65]. In another study, CRF<sub>2</sub> knockout mice showed no behavioral alterations in the light–dark test but appeared to exhibit increased anxiety in the elevated plus maze and open field test [66]. That is, CRF<sub>2</sub> knockout mice spent less time exploring the open arms of the elevated plus maze and less time in the inner portion of an open field [66]. These anxiogenic-like effects observed in the elevated plus maze and open field may be influenced by the reported increased in CRF mRNA occurring in the central amygdala nucleus of the CRF<sub>2</sub> knockout mouse [66]. The central amygdala plays an important role in the activation of diverse responses induced by stress [67–69]. Furthermore, in the rat central amygdala,

Table 3

Summary of CRF<sub>2</sub> knockout and antisense oligonucleotide studies in relation to behavioral tests of fear and anxiety

Test methods	Major results	Reference
<i>CRF<sub>2</sub> knockout studies</i>		
Elevated plus maze	No effects	[65]
	Decreased open arm activity	[66]
	Males, but not females, exhibit less open arm activity	[72]
Open field	No effects	[65]
	Decreased time in inner portions	[66]
	Males, but not females, exhibit increased center activity	[72]
Light–dark box	No effects	[66]
	Defensive-withdrawal	Males, but not females, spend more time in chamber
<i>CRF<sub>2</sub> antisense oligonucleotide studies</i>		
Defensive-withdrawal	No effects	[41]
Swim stress-induced anxiety in elevated plus maze	No effects	[41,43]
Shock-induced freezing	Decreased freezing	[63]
Conditioned freezing	Decreased freezing	[63]

stress elevates CRF mRNA [70] which is associated with an increase in anxiety behavior [71].

Some data suggest that emotional behavior mediated by the CRF<sub>2</sub> receptor may depend on the sex of the subject [72]. For example, in the elevated plus maze, male CRF<sub>2</sub> knockout mice spent less time in open arm activity than wild-type males. In contrast, female CRF<sub>2</sub> deficient mice did not differ from wild-type littermates in open-arm exploration. In addition, CRF<sub>2</sub> deletion had no effects on the behavior of females in both the light–dark emergence test and the open field, whereas male knockout mice spent more time in the dark and showed more center region open field activity.

In addition to behavioral differences, stress-induced hormone secretion differed in CRF<sub>2</sub> knockouts. Two studies reported increased ACTH secretion in response to stress [65,66], whereas another study found no effects on stress-induced ACTH and CORT secretion in both male and female knockout mice [72].

#### 4.2. CRF<sub>2</sub> receptor antisense oligonucleotide studies

Antisense oligonucleotides that target the CRF<sub>2</sub> receptor have also yielded mixed behavioral results (Table 3). In the defensive-withdrawal test, rats administered CRF<sub>2</sub> antisense oligonucleotides failed to exhibit alterations in anxiety behavior [41]. In addition, CRF<sub>2</sub> antisense treatment had no effects on anxiety behavior of rats tested in the elevated plus maze after exposure to swim stress or social defeat [41,43].

In contrast to these negative results, a recent study demonstrated that CRF<sub>2</sub> antisense oligonucleotide treatment attenuated freezing in rats during the immediate post-shock period as well as in a subsequent conditioned testing situation [63]. This reduction in freezing was not due to alterations in pain sensitivity produced by the CRF<sub>2</sub> antisense treatment.

Behavioral differences produced by CRF<sub>2</sub> antisense

oligonucleotides may be due to several factors including differences in anxiety testing and potential adverse side effects associated with particular CRF<sub>2</sub> antisense oligonucleotides, as suggested by body weight loss [41]. However, a key factor may be the effectiveness of the antisense oligonucleotide compound in reducing CRF<sub>2</sub> receptor levels. For example, in studies reporting no behavioral effects after CRF<sub>2</sub> antisense treatment, the reduction in brain CRF<sub>2</sub> receptor density appeared to be less than 20% in one study [41], and not reported in another [43]. In this context, marked suppression of CRF<sub>2</sub> receptors in the lateral septum may be essential in producing a significant change in emotional expression. In the study reporting a reduction in freezing, antisense oligonucleotide treatment reduced lateral septum CRF<sub>2</sub> receptor densities that approached 70% [63]. Lesser anxiolytic-like effects were found when CRF<sub>2</sub> receptors in the lateral septum were suppressed only 50% [63].

There is evidence that the lateral septum plays a role in anxiety behavior. For instance, pharmacological studies demonstrate that benzodiazepines injected into the lateral septum induce anxiolytic-like effects in the punished drinking test [73], the elevated plus maze [74] and the shock probe burying test [74]. In addition, CRF injected into the mouse lateral septum increased anxiety behavior in the elevated plus maze [75]. It should also be noted that antisense oligonucleotide treatments that produced pronounced reductions in lateral septum CRF<sub>2</sub> receptor density and freezing appeared to lack potential toxicological side effects on body weight [63].

#### 4.3. CRF<sub>2</sub> receptor antagonist studies

Attempts to determine the functional properties of CRF<sub>2</sub> receptors have been facilitated by the synthesis [76] and characterization [76,77] of a CRF<sub>2</sub> specific peptide antagonist called anti-sauvagine-30 (anti-Svg-30). Initial behavioral studies (Table 4) conducted in mice showed that

Table 4  
Summary of CRF<sub>2</sub> antagonist studies using anti-savagine-30 in relation to behavioral tests of fear and anxiety

Test strategies	Major results	Reference
<i>Mouse studies</i>		
Effects of lateral septum infusions on elevated plus maze behavior	No effects	[77]
Effects of lateral septum infusions on CRF-induced anxiety in elevated plus maze	Reversed reduction in open arm activity	[77]
Immobilization-induced anxiety in elevated plus maze	Reversed reduction in open arm activity	[77]
Effects of i.c.v. infusions on elevated plus maze activity behavior	Reduced open arm	[72]
Effects of hippocampal infusions on fear conditioning	No effects on freezing	[77]
Effects of lateral septum infusions on CRF-induced fear conditioning	Increases freezing	[77]
<i>Rat studies</i>		
Conditioned freezing	Reduced freezing	[78]
Elevated plus maze	Increased open arm activity	[78]
Defensive-withdrawal	Increased open field activity	[78]

infusion of 400 ng of anti-SVG-30 into the lateral septum had no anxiolytic-like behavioral effects in the elevated plus maze [75]. However, the CRF<sub>2</sub> antagonist was effective in reversing CRF-induced anxiogenic-like behavior in mice subsequently tested in the elevated plus maze [75]. In this study, anti-Svg-30 delivered to the lateral septum also attenuated immobilization-induced anxiety behavior in the elevated plus maze. These behavioral effects in mice may be specific to test conditions involving delivery of the peptide antagonist into the lateral septum. When anti-Svg-30 (400 ng) is delivered via i.c.v. procedures, mouse anxiety behavior is increased in the elevated plus maze [72,75]. In other work, intrahippocampal infusion of anti-Svg-30 had no effects on fear conditioning induced by either a tone or the shock environment whereas lateral septum infusions enhanced the conditioning process [75].

Recent dose-response studies [78] conducted in rats suggest that anti-Svg-30 has anxiolytic-like effects similar to those reported for CRF<sub>1</sub> antagonists (Table 4). Administration of anti-Svg-30 using i.c.v. methods produced a dose-dependent reduction (1–10 µg) in anxiety behavior occurring in the conditioned freezing test, the elevated plus maze, and the defensive-withdrawal test. In this study, a dose of anti-Svg-30 (10 µg) that consistently reduced anxiety behavior was further examined in rats tested in a locomotor activity box and found to have no significant locomotor activating effects. Therefore, the anti-Svg-30-induced reduction in freezing, and the increased in behavioral activity in opened, unprotected areas of the test environment does not appear to result from a general increase in locomotion.

## 5. Summary

Experiments focusing on the CRF<sub>1</sub> receptor have produced data that generally support its role in emotional behavior. In studies using CRF<sub>1</sub> antagonists, the bulk of the evidence suggests that various CRF<sub>1</sub> antagonists have prominent effects in normalizing stress-induced anxiety and lesser or variable effects on spontaneous anxiety

behavior. In addition, CRF<sub>1</sub> antagonists appear effective in reversing the anxiety producing effects of exogenous CRF. Hence, CRF<sub>1</sub> receptors may begin to have a predominant role under conditions involving pronounced stress when CRF neuropeptides are likely to be released.

Although the consistent anxiolytic-like profile of knockout mice tested in models of spontaneous anxiety suggests otherwise, it is possible that the CRF<sub>1</sub> knockout mouse has an emotional demeanor not altogether different from rats dosed chronically with a CRF<sub>1</sub> antagonist or the low anxiety bred rat. It is also possible that mice are more sensitive to the stimulating anxiogenic effects of endogenous CRF as indicated by reports of anxiolytic-like responsiveness in the light–dark box and free-exploration test after acute CRF<sub>1</sub> antagonist treatment.

To date, CRF<sub>1</sub> antisense oligonucleotide studies provide a mix of behavioral results suggesting that CRF<sub>1</sub> knockdown is associated with spontaneous and stress-induced anxiety. In addition, CRF<sub>1</sub> antisense treatments appear to reverse the anxiety provoking effects of exogenous CRF administration. A measurable reduction in CRF<sub>1</sub> mRNA using the antisense oligonucleotide approach in conjunction with a consistent reduction in anxiety behavior would strengthen the hypothesis that stress-induced states are mediated by the CRF<sub>1</sub> receptor.

At present, it is unclear whether the behavioral inconsistencies noted among studies using anti-Svg-30, the CRF<sub>2</sub> antagonist, represent a species effect, a dose effect, a delivery effect, or even a laboratory testing effect [79]. However, it is notable that on the basis of limited work in rats using potent CRF<sub>2</sub> antisense oligonucleotides and dose–response studies using anti-Svg-30, CRF<sub>2</sub> receptors appear to be involved in the expression of both stress-induced and spontaneous anxiety behavior. This reported involvement of CRF<sub>2</sub> receptors in emotional expression raises questions concerning the specificity of the CRF<sub>1</sub> receptor in playing a unique role in anxiety and stress-induced behavior. Studies using specific CRF<sub>2</sub> agonists [80,81], in combination with specific CRF<sub>2</sub> antagonists may provide further insights into the role of CRF<sub>2</sub> systems and anxiety behavior.

The intricate roles and neural systems and mechanisms

involved in the CRF<sub>1</sub> and CRF<sub>2</sub> mediation of spontaneous and stress-induced anxiety behavior remain to be defined. Of particular relevance are studies evaluating the behavioral actions of CRF<sub>1</sub> and CRF<sub>2</sub> receptors separately as well as together. For example, injecting rats with a dose of a CRF<sub>1</sub> antagonist, DPC904 [61] in combination with a CRF<sub>2</sub> antisense oligonucleotide resulted in lower levels of freezing than rats treated with only the CRF<sub>1</sub> antagonist or the CRF<sub>2</sub> antisense oligonucleotide [63]. Astressin, a CRF antagonist with high affinity for both CRF<sub>1</sub> and CRF<sub>2</sub> receptors and a potent blocker of stress-induced ACTH secretion [82] may also provide information on the relative roles of CRF receptor subtypes, especially when used in combination with specific CRF receptor antagonists. For example, a study using astressin and anti-Svg-30 reported differential roles of CRF<sub>1</sub> and CRF<sub>2</sub> receptors in fear conditioning and anxiety [75].

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