

Interactive report

# A role for corticotropin releasing factor and urocortin in behavioral responses to stressors<sup>1</sup>

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## Abstract

Corticotropin-releasing factor (CRF) and CRF-related neuropeptides have an important role in the central nervous system to mediate behavioral responses to stressors. CRF receptor antagonists are very effective in reversing stress-induced suppression and activation in behavior. An additional CRF-like neuropeptide, urocortin, has been identified in the brain and has a high affinity for the CRF-2 receptor in addition to the CRF-1 receptor. Urocortin has many of the effects of CRF but also is significantly more potent than CRF in decreasing feeding in both meal-deprived and free-feeding rats. In mouse genetic models, mice over-expressing CRF show anxiogenic-like responses compared to wild-type mice, and mice lacking the CRF-1 receptor showed an anxiolytic-like behavioral profile compared to wild-type mice. Results to date have led to the hypothesis that CRF-1 receptors may mediate CRF-like neuropeptide effects on behavioral responses to stressors, but CRF-2 receptors may mediate the suppression of feeding produced by CRF-like neuropeptides. Brain sites for the behavioral effects of CRF include the locus coeruleus (LC), paraventricular nucleus (PVN) of the hypothalamus, the bed nucleus of the stria terminalis (BNST), and the central nucleus of the amygdala. CRF may also be activated during acute withdrawal from all major drugs of abuse, and recent data suggest that CRF may contribute to the dependence and vulnerability to relapse associated with chronic administration of drugs of abuse. These data suggest that CRF systems in the brain have a unique role in mediating behavioral responses to diverse stressors. These systems may be particularly important in situations where an organism must mobilize not only the pituitary-adrenal system, but also the central nervous system in response to environmental challenge. Clearly, dysfunction in such a fundamental brain-activating system may be the key to a variety of pathophysiological conditions involving abnormal responses to stressors such as anxiety disorders, affective disorders, and anorexia nervosa. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Corticotropin-releasing factor; Urocortin; Stress; Anxiety; Drug addiction

## 1. Introduction

The neuropeptide corticotropin releasing factor (CRF) has a central role in the response of the body to stressors, and its activation is a critical part of the state of stress. Stress can be defined generally as responses to demands (usually noxious) upon the body [93], or in a definition more focused on the central nervous system and behavioral responses, as alterations in psychological homeostatic processes [16]. A state of stress is associated with various external and internal challenges to the body and brain, usually termed stressors, and the construct of stress may

represent the extreme pathological continuum of over-activation of the normal activation (arousal) or emotional systems of the body [48] (Fig. 1). Historically, the state of stress has been defined biologically by various physiological changes that include an activation of the pituitary-adrenal axis and release of glucocorticoids into the bloodstream. The activation of the hypothalamic-pituitary-adrenal axis by stress long has been known to involve the action of CRF liberated into the pituitary portal system of the median eminence to trigger the release of adrenocorticotrophic hormone (ACTH) from the pituitary. CRF via a neurotropic action in the pons and brainstem activates the sympathetic nervous system [38,119]. Adaptive behavioral changes are another major component of the response to bodily demands or challenges to homeostasis, and these changes may involve extrahypothalamic CRF systems [57]. Evidence demonstrating a neurotropic role for CRF in the

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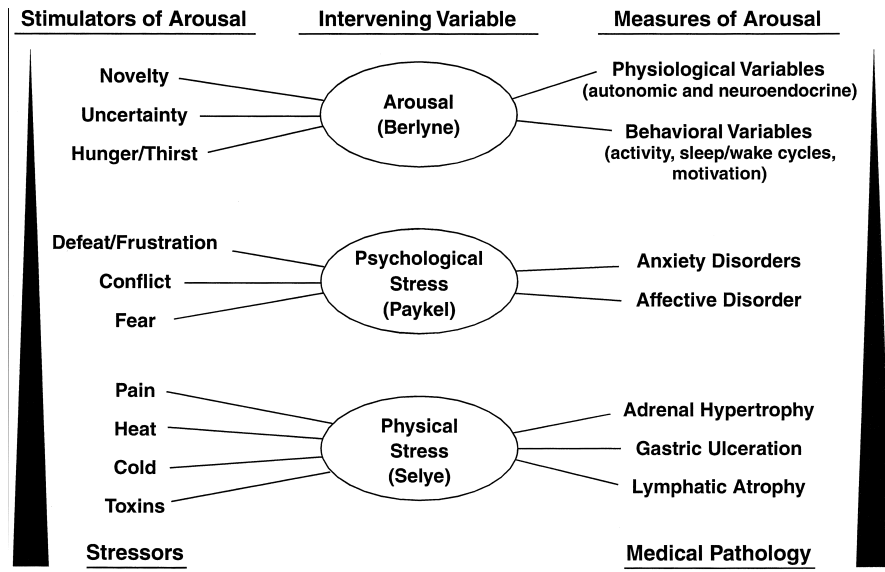


Fig. 1. Continuum describing the relationship between the constructs of arousal, psychological stress, physical stress, and pathology. While arousal leads to autonomic and behavioral changes, when arousal increases to stress-like levels, psychiatric and physical disorders result. The corticotropin-releasing factor and norepinephrine systems contribute to both arousal and stress responses (modified with permission from Ref. [48]).

central nervous system outside the pituitary-adrenal axis suggests a parallel means for mediating behavioral responses to stressors and a contribution to the behavioral state of stress in addition to the classic activation of adrenal steroids.

The hypothesis of a neurotropic role for CRF in the central nervous system is supported by substantial neurobiological evidence. Immunoreactivity for CRF has been localized in the central nervous system both in the hypothalamus and in extrahypothalamic structures [13,108]. CRF-stained cells and fibers are found in high concentrations in the central nucleus of the amygdala, parabrachial area and the substantia innominata, BNST, LC and olfactory bulb.

Another CRF-related neuropeptide, urocortin, that is structurally and pharmacologically similar to members of the CRF family of peptides (including CRF, sauvagine, and urotensin I) was discovered in 1995 [122]. Urocortin (Ucn) derived its name by its sequence similarity to fish urotensin I (63%; 'uro') and mammalian CRF (45%; 'cort'). Again, substantial neurobiological evidence points to a neurotropic role for Ucn in the central nervous system. High levels of Ucn mRNA have been observed in the Edinger–Westphal (EW) nucleus and the lateral superior olive, and Ucn mRNA also has been seen in several somatomotor nuclei, a subset of magnocellular neurons in the supraoptic nucleus and, caudally, in the lateral hypothalamus (LH). Projections of Ucn-IR are observed throughout the brain and spinal cord, the lateral septum, the supraoptic nucleus and PVN, the central and periaqueductal grey and the EW nucleus [60]. In contrast to CRF, levels of Ucn-IR are not high in the median eminence,

suggesting that Ucn is not an important endocrine factor in the regulation of ACTH or beta-endorphin release [60].

CRF receptors have been cloned, and to date there are two different receptor types, CRF-1 and CRF-2. Both are Gs-coupled receptors, and the distribution of CRF neurons, the CRF binding sites, and in situ hybridization of CRF receptor mRNA [82] show good correspondence [31]. CRF-1 receptors are expressed mainly in the medial septum, pituitary, cortex, cerebellum, hindbrain and olfactory bulb, whereas CRF-2 receptors are found in the lateral septum, ventral medial hypothalamus, and choroid plexus [81,19]. CRF and Ucn both bind with high affinity to the CRF-1 receptor, but only Ucn binds with high affinity to the CRF-2 receptor leading to the hypothesis that Ucn may be an endogenous ligand for the CRF-2 receptor [122].

## 2. Behavioral effects of CRF and urocortin

CRF and Ucn have dramatic behavioral and physiological effects when administered directly into the central nervous system. Administration of CRF and Ucn into the central nervous system intracerebroventricularly (i.c.v.), or intracerebrally at specific brain sites, produces a wide variety of behavioral effects, and the behavioral pharmacological profile resulting from exogenous administration of these neuropeptides depends on the baseline state of arousal and stress of the animal (see Table 1 and reviews [2,6,33]). In non-stressed animals under low arousal conditions, CRF and Ucn administered i.c.v. produce a dose-dependent behavioral activation that includes increases in locomotor activity, rearing and grooming when rats are tested in a

Table 1  
Behavioral effects of centrally administered CRF peptides

CRF receptor agonist	Paradigm	CRF receptor antagonist
Suppresses exploration of unfamiliar environment	Elevated plus-maze	Reverses stress-, drug-, and genotypically induced suppression of exploration
Facilitates startle	Acoustic startle	Blocks fear-potentiated startle
Induces conditioned fear	Conditioned emotional response	Blocks acquisition of conditioned emotional response
Enhances stress-induced freezing	Cued electric shock	Attenuates stress-induced freezing
Decreases food intake	Deprivation-induced eating	Reverses stress- and drug-induced anorexia
Produces aversion	Taste/Place conditioning	Weakens drug-induced place aversion
Enhances sensitization	Amphetamine stereotypy	Attenuates stress-induced sensitization
Enhances defensive burying	Shock-probe	Reduces defensive burying

familiar environment [33,58,95,107]. This activation is not observed following systemic administration of CRF and is not blocked by hypophysectomy or pretreatment with dexamethasone, suggesting that this effect of CRF is mediated by actions in the central nervous system independent of the pituitary-adrenal axis [15,34].

Electrophysiologically, CRF and Ucn have excitatory properties. CRF injected i.c.v. in doses of 0.01–0.10  $\mu$ g produced electroencephalographic activation characteristic of arousal [35], and at higher doses CRF produced seizure-like activity [35,72]. At sufficiently high doses Ucn [37], like CRF, elicits limbic seizures, an effect that appears to be mediated by CRF-1 receptors [8]. CRF can facilitate learning and memory, enhancing retention at low doses and impairing performance at higher doses [52]. Recent studies have shown that the CRF-induced facilitation of memory may be mediated by CRF-1 receptors [86]. Injection of CRF into the hippocampus enhanced learning and this was blocked by a CRF-1, but not CRF-2, antagonist. Ucn also is similarly potent to CRF in its memory-modulating effects, facilitating performance in tests of learning and memory, such as passive avoidance and the Morris water maze at low doses, but decreasing performance at high doses [123].

When animals are exposed to a more stressful environment, the profile of the behavioral effects of exogenously administered CRF and Ucn changes to reflect an enhanced behavioral response to stress. The same i.c.v. doses that produce marked behavioral activation in a familiar environment produce behavioral suppression in a novel, presumably stressful environment. Rodents pretreated with CRF show decreases in behavior in an open field [107,113] (with or without food [14]), decreased exploration in a multi-compartment chamber [10], and decreased exploration in an elevated plus-maze [7]. Evidence that this anxiogenic-like effect is mediated by CRF-1 receptors has been obtained by differential antistress effects of CRF-1 antisense oligonucleotides versus CRF-2 antisense oligonucleotides [70]. Ucn also shares the anxiogenic-like properties of CRF, a putative CRF-1-mediated effect, as shown by behavior in several paradigms, including the open-field, the elevated plus-maze, and the light–dark test [49,77]. The behavioral suppression observed in these exploration

tests is consistent with other studies showing that CRF and Ucn have an ‘anxiogenic-like’ or stress-like effect. CRF enhances the acoustic startle response [110], increases conditioned fear in a conditioned suppression test [24], and enhances stress-induced freezing behavior [96]. Such anxiogenic-like effects of CRF and Ucn injection appear to prime rats for anxiogenic-like responses to sodium lactate administration [91].

Other behavioral actions that resemble a state of stress include decreases in food intake [4,61,30,90], decreases in alcohol intake [9], decreases in sexual behavior [97,98], and increases in defensive burying in habituated rats [32] (see Table 1). The stress-like effects of CRF clearly have aversive properties in that CRF at high doses can produce both taste aversions and place aversions [18,41]. Thus, exogenously administered CRF and Ucn produce behavioral activation, enhance behavioral responses to stress, and produce a behavioral state that is aversive and resembles a state of stress (for detailed reviews, see [2,33]).

Exogenous Ucn administered i.c.v. elicits many of the same behavioral effects as other members of the CRF peptide family. However, Ucn more potently reduces food intake than CRF under both fasted and *ad libitum* feeding conditions in mice, rats and sheep [25,80,105], and this has led to the hypothesis that the CRF-2 receptor is involved in the anorectic effects of CRF-related compounds, a hypothesis supported by some preliminary antisense studies [102]. Ucn also suppresses food intake following peripheral administration in mice [5] (Fig. 2).

### 3. Behavioral effects of CRF receptor antagonists

More compelling evidence for a role of endogenous CRF-like neuropeptides in behavioral responses to stressors comes from the demonstration of antistress actions of CRF antagonists. Evidence using competitive CRF receptor antagonists, such as alpha-helical CRF<sub>9–41</sub> and [D-Phe<sup>12</sup>, Nle<sup>21,38</sup>, C<sup>α</sup>-MeLeu<sup>37</sup>] CRF<sub>12–41</sub> (D-Phe CRF<sub>12–41</sub>) [27,116], has provided strong support for the hypothesis that brain CRF systems play a role in mediating behavioral responses to stress (see Table 1). These two neuropeptide antagonists have high affinity for both the CRF-1 and

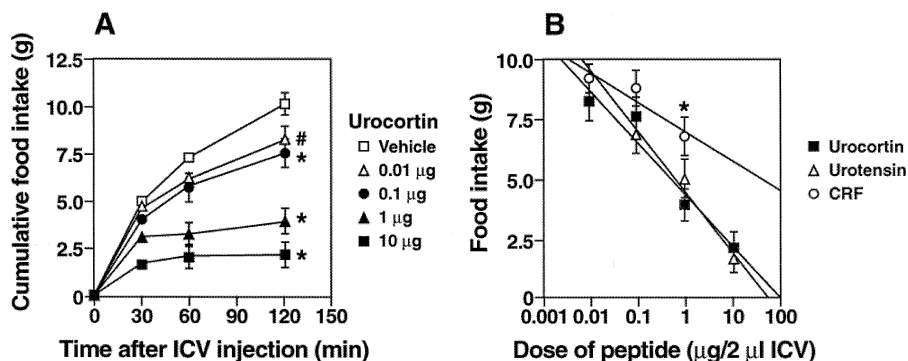


Fig. 2. (A) Effects of various doses of urocortin (Ucn) on food intake in rats previously food-deprived for 24 h. Ucn at doses greater than 0.1 μg significantly attenuated food consumption (\*  $p < 0.05$  compared to vehicle value, Newman–Keuls test; #  $p < 0.05$ , Student's  $t$ -test, vehicle versus treatment). (B) Comparison of the effects of various doses of Ucn, urotensin, and CRF on food intake as measured 120 min after peptide i.c.v. infusion. Regression analysis and test for parallelism indicates that CRF was significantly ( $p < 0.05$ ) less effective than Ucn and urotensin in suppressing food intake after 24 h food deprivation (taken with permission from Ref. [105]).

CRF-2 receptors [122]. Alpha-helical CRF<sub>9–41</sub> injected i.c.v. was shown to reverse the attenuation of feeding induced by stress in rats [61], and to attenuate stress-induced fighting in rats [114], suggesting that both the suppression and activation in behavior associated with stressors may involve endogenous CRF systems. In mice, alpha-helical CRF<sub>9–41</sub> reversed the suppression in exploratory behavior produced by restraint stress [11], and in rats produced a more rapid emergence from a small dark enclosure into a large open field and more exploration of the unfamiliar open-field [113]. Subsequent studies have shown that CRF receptor antagonists are very effective in reversing the decrease in exploration of the open arms of an elevated plus-maze caused by exposure to a variety of stressors including restraint, swim stress, ethanol withdrawal and social stress [44,47,74]. Alpha-helical CRF<sub>9–41</sub>

or D-Phe CRF<sub>12–41</sub> administered i.c.v. just prior to the placement in the elevated plus-maze reversed the stress (resident exposure)-induced inhibition of exploration on the open arms of the elevated plus-maze (Fig. 3).

Alpha-helical CRF<sub>9–41</sub> inhibited stress-induced freezing behavior in rats [50] and fear potentiation of the acoustic startle response [109]. Consistent with these observations, alpha-helical CRF<sub>9–41</sub> also attenuated the acquisition of the conditioned suppression test [22]. Other effects of CRF receptor antagonists consistent with an antistress effect include a reduction in defensive burying [59], reversal of the decrease in food intake produced by 17-beta-estradiol [29], and reversal of the 'anxiogenic-like' effects of cholecystokinin in rats [12].

The observed extrahypothalamic antistress actions of CRF receptor antagonists do not appear to depend on the

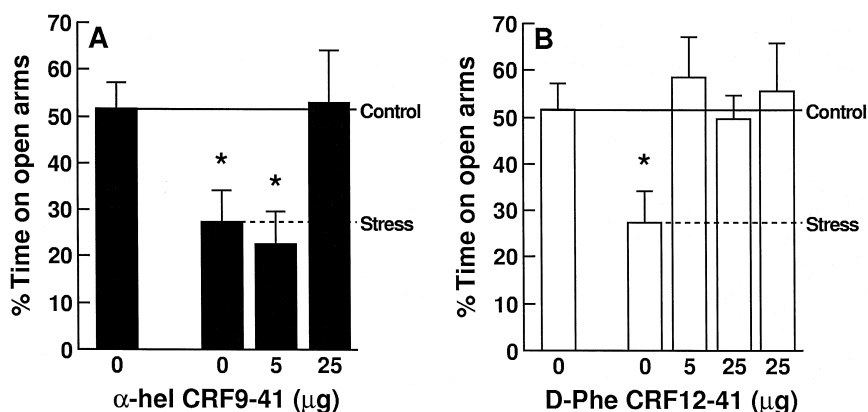


Fig. 3. Effects of intracerebroventricular administration of CRF receptor antagonist alpha-helical CRF<sub>9–41</sub> and D-Phe CRF<sub>12–41</sub> 5 min before testing on the elevated plus-maze. Subjects were either taken from the home cage (control) and infused with vehicle (0) or defeated socially and exposed to conspecific aggression for 30 min (stress) and administered with vehicle (0) or CRF antagonists (alpha-helical CRF<sub>9–41</sub>: 1 and 5 nM or 5 and 25 μg; D-Phe CRF<sub>12–41</sub>: 0.2, 1 and 5 nM or 1, 5 and 25 μg). The data are expressed as the percentage of total time spent on all four arms (mean ± SEM). Each group contained 8–10 rats. Asterisks (\*) indicate significantly different from control group ( $p < 0.05$  by Newman–Keuls test). CRF, corticotropin-releasing factor (modified with permission from Ref. [74]).

nature of the stressor but more on how the stressor is delivered over time. CRF receptor antagonists are very effective in reversing the decrease in exploration on the elevated plus-maze produced by a variety of stressors [47,74]. Social stress [47,74], restraint stress [44], swim stress [44] and ethanol withdrawal as the stressor [7] all produce a suppression in exploration on the plus-maze that can be reversed with treatment with the CRF receptor antagonist alpha-helical CRF<sub>9–41</sub> when the treatment is just prior to the test. Administration of the CRF receptor antagonist just prior to the stressor also blocks the subsequent suppression of behavior normally observed on the plus-maze, but the dose-effect function is shifted to the right [46].

The CRF receptor antagonist alpha-helical CRF<sub>9–41</sub> has similar effects in reversing the changes in food intake associated with different stressors. Alpha-helical CRF<sub>9–41</sub> reverses the decrease in food intake produced by restraint stress [61], reverses the decrease in familiar food consumption observed in nutritionally deficient subjects [43], and increases feeding induced by administration of neuropeptide Y and tail-pinch in rats [42]. Corticotropin-releasing factor also appears to have a role in the interaction of stressors with drug responses, particularly those of psychomotor-stimulants [54], and activation of brain CRF may be involved in the behavioral cross-sensitization between stress and psychostimulant drugs [23].

However, CRF receptor antagonists are ineffective in certain operant tests such as the Geller–Seifter conflict test (Koob and Britton, unpublished observations). Here, the behavioral situation involves a highly trained response where the response to punishment already has been learned. The conflict procedures are very sensitive to drugs acting on the brain GABAergic systems including benzodiazepines, barbiturates and ethanol [94]. CRF receptor antagonists, in contrast, appear to be more effective in behavioral situations where the response to stress requires a change in behavior. How these distinctions translate to other stress situations and to the psychopathology of anxiety and stress disorders in humans remains to be determined.

#### 4. Mouse genetic models and CRF

Based on the results from animal models using a CRF receptor antagonist, overactivity of CRF can be hypothesized to be involved in behavioral responses to stressors and, by extrapolation to the human condition, a number of psychiatric disorders involving a high level of stress including anxiety and affective disorders. Another approach is to use molecular genetic methodology to produce a model of chronic CRF overactivation. A transgenic mouse model of CRF overproduction was developed where a CRF transgene was composed of rat genomic CRF gene and 3' and 5' substitutions [106]. The CRF transgenic mice

exhibited endocrine abnormalities including elevations of ACTH and corticosterone, but also enhanced responsiveness to novelty and an 'anxiogenic-like' response on the elevated plus-maze. These behavioral effects were reversed by central administration of the CRF antagonist alpha-helical CRF<sub>9–41</sub>.

Similarly, removal of the CRF-1 receptor using a knockout approach produces the opposite phenotype [104]. Mice with the CRF-1 nullmutation showed less of an anxiogenic-like response in the elevated plus-maze as well as a decreased secretion of ACTH and corticosterone following restraint stress [104]. An independently generated CRF-1 knockout model also showed less of a stress-like response in the light–dark test [115]. Others have shown that treatment with a CRF-1 antisense oligodeoxynucleotide directly into the brain reduced anxiogenic-like responses [99–101]. These models lend support to the hypothesis that extrahypothalamic CRF plays an important role in behavioral responses to stressors.

#### 5. Neurobiological sites of action for CRF-related neuropeptides

The behavioral effects of CRF, specifically with regard to the role of CRF in arousal and behavioral responses to stress, may be mediated by a number of brain sites linked by specific neurochemical and functional circuits. The LC, the PVN of the hypothalamus, the BNST, and the central nucleus of the amygdala seem to be predominantly implicated in the behavioral actions of CRF (Fig. 4). All of these regions interact functionally to mediate behavioral and hormonal responses to stressors and also interact with more classic stress/arousal neurotransmitters such as the forebrain norepinephrine projections. The nucleus LC is a major source of norepinephrine projections to the BNST and the central nucleus of the amygdala. However, most of the evidence for CRF/norepinephrine interactions to date have been focused on the LC.

Physiological (internal) and environmental (external) stressors activate the LC in the pons, the major source of norepinephrine projections to the forebrain [119]. These include such stimuli as hypotensive stress, bladder distension, and footshock, and significant evidence exists to show that CRF plays an important role in this activation. CRF injected i.c.v. or directly into the LC increases the firing rate of the LC [28] and increases release of norepinephrine in LC projection areas [103]. Perhaps most compelling is evidence that activation of the LC by certain stressors is prevented by administration of CRF receptor antagonists directly into the region of the LC [27,65,120].

In addition, stressors increase the concentration of CRF in the region of the LC [20], and central administration of CRF has been shown to activate the LC [118]. Noradrenergic antagonists can reverse some of the stress-like effects of i.c.v. CRF [24]. Also, CRF receptor antagonists microin-

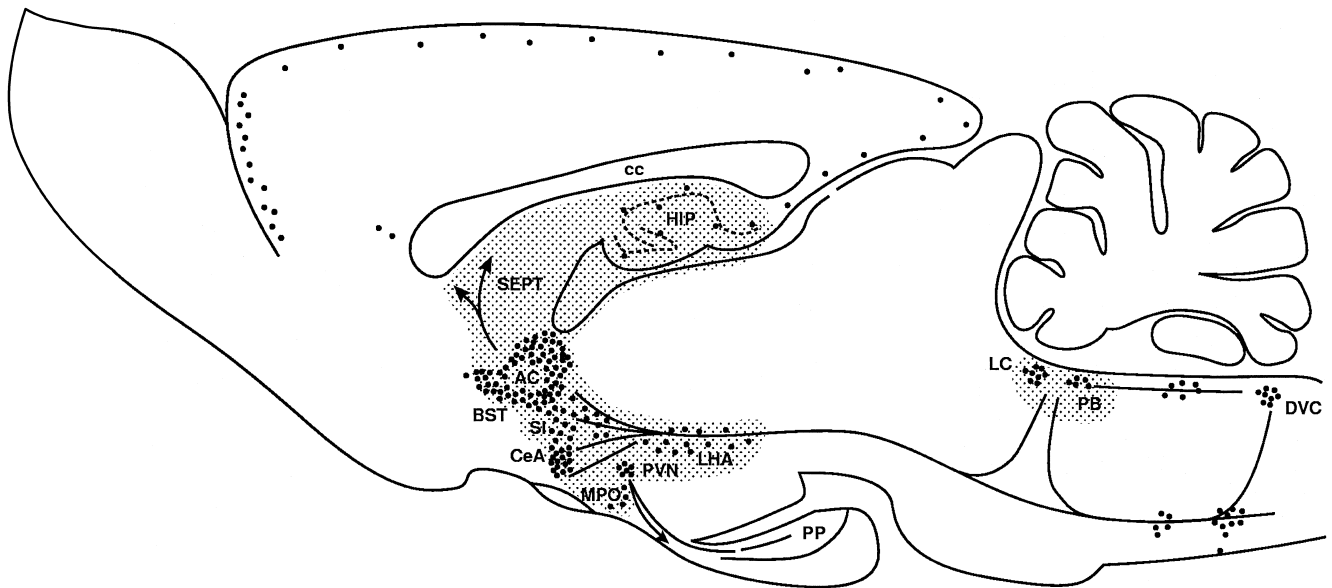


Fig. 4. Sagittal section through the brain of a rat depicting the location of brain corticotropin-releasing factor cell bodies and pathways. Shaded areas represent sites identified as mediating the effects of CRF in behavioral responses to stressors. This includes the paraventricular nucleus of the hypothalamus (PVN), the locus coeruleus (LC), the hippocampus (HIP), the bed nucleus of the stria terminalis/substantia innominata (BNST/SI), and the central nucleus of the amygdala (CeA) (taken with permission from Ref. [55]).

jected directly into the LC region reduced the duration of shock-induced freezing [112]. Microinjections of CRF directly into the region of the LC enhanced retention latency in an inhibitory avoidance task, and this effect did not occur in rats treated with the norepinephrine neurotoxin 6-hydroxydopamine [21]. In addition, the LC is particularly sensitive to the suppression of exploratory behavior produced by central administration of CRF [17].

The PVN of the hypothalamus also may be involved in the suppression of behavior produced by stressors, particularly the suppression of food intake. CRF is effective in decreasing food intake when injected into the PVN [62]. Perhaps more importantly, the CRF receptor antagonist alpha-helical CRF<sub>9-41</sub>, when injected i.c.v. or directly into the PVN, reverses stress-induced suppression of feeding and facilitates tail-pinch and neuropeptide Y (NPY)-induced feeding [42,45], an action that could involve CRF-1 or CRF-2 receptor actions. Immunotargeting of CRF neurons in the PVN using local administration of a CRF monoclonal antibody/toxin mixture also enhanced the orexigenic effects of NPY [73]. In addition, food intake occurring in response to a physiological stressor such as nutritional imbalance normally may be limited by endogenous CRF systems because the decrease in familiar food intake produced by this stressor is reversed by alpha-helical CRF<sub>9-41</sub> [43]. The exact role of the pituitary adrenal axis in these PVN effects is not clear, but these actions of CRF may reflect not only the projection of the PVN to the median eminence but also the projection of the PVN to brainstem autonomic systems [40]. Thus, CRF-like peptides in the hypothalamus may have an endogenous action to limit food intake that would be expressed following

stress exposure such as novel food stuffs, novel environment, or nutritional imbalance [42,43].

There also may be CRF norepinephrine interactions in the hypothalamus. Stress induces norepinephrine release in the PVN of the hypothalamus as measured by *in vivo* microdialysis [79], and there is evidence that norepinephrine in the PVN stimulates release of CRF [3]. Anatomical evidence also exists for norepinephrine-CRF synaptic connections in the PVN of the hypothalamus [71].

Another potential site of action of CRF and potential CRF/norepinephrine interaction is the BNST. CRF injected directly into the BNST enhances the startle response, and neurotoxin cell-body-specific lesions of the BNST or microinfusion of a CRF receptor antagonist blocked the effects of i.c.v. CRF on the startle response [66]. Stress induces norepinephrine release in the BNST as measured by *in vivo* microdialysis [78]. Norepinephrine terminals appear to form synapses with dendrites of CRF neurons in the ventrolateral BNST [83]. Together, these results suggest the possibility that CRF itself in the BNST may mediate certain behavioral responses to stress, and the neurocircuitry is present for a norepinephrine-CRF interaction similar to that observed in the PVN of the hypothalamus.

A number of studies support a role for the amygdala in the behavioral effects of CRF. CRF injected i.c.v. potentiates the acoustic startle response [69], and lesions of the central nucleus of the amygdala, but not the PVN, block the CRF potentiation of the acoustic startle response [117]. CRF microinjected into the amygdala also stimulates locomotor and exploratory behavior in mice [67,68]. Perhaps more compelling evidence for a role of endogenous CRF

systems in the amygdala in behavioral responses to stressors is the observation that microinjection into the central nucleus of the amygdala of the CRF receptor antagonist alpha-helical CRF<sub>9–41</sub> reverses social stress-induced suppression of behavior in the plus-maze at doses 100 times lower than those effective by the i.c.v. route [47]. Injections of similar doses of alpha-helical CRF<sub>9–41</sub> into the central nucleus of the amygdala also blocked the suppression in exploration of the open arms of the plus-maze resulting from withdrawal from chronic ethanol [87] (see below). Consistent with these results, similar doses of alpha-helical CRF<sub>9–41</sub> injected into the central nucleus of the amygdala attenuated stress-induced freezing [111]. Together, these results suggest that endogenous CRF in the central nucleus of the amygdala has an important role in the suppression of behavior associated with stress.

Evidence for CRF in the amygdala having a role in behavioral responses to stressors also is supported by the effects of both acute and chronic stressors on extracellular levels of CRF in the amygdala in freely moving animals as measured by in vivo microdialysis [84]. Rats subjected to an acute (30 min) restraint stress showed increases in CRF significantly above baseline levels during the stressor administration [75,84]. Even more compelling is the observation that rats withdrawn from several drugs of abuse show increased extracellular levels of CRF in the amygdala [26,76,89] (see below).

Potential CRF/norepinephrine interactions are demonstrated by evidence that norepinephrine in the central nucleus of the amygdala stimulates release of CRF [85]. In addition, neurocircuitry appears to be present for a norepinephrine-CRF interaction similar to that observed in the PVN of the hypothalamus and the BNST [121]. These divergent sites for the behavioral actions of CRF and the common element of a CRF/noradrenergic interaction have led to the hypothesis that this system may provide a foundation for a feed-forward activational system that is particularly important in situations where an organism must mobilize both hormonal and behavioral responses to environmental challenge [51]. However such a feed-forward system may be particularly vulnerable to allostatic-like adjustments that lead to psychopathology such as anxiety and affective disorders [51].

## 6. The role of CRF in drug addiction

Stressors and the state of stress contribute significantly to various aspects of drug addiction, particularly in the components of addiction such as acute withdrawal, protracted abstinence and vulnerability to relapse [36]. Acute withdrawal from drugs of abuse long has been associated with somatic signs, and the manifestation of these somatic signs varies with each drug of abuse. However, there are several common neurobiological changes associated with acute withdrawal that may have motivational significance

in contributing to the maintenance of drug addiction, and one of these actions may involve activation of brain stress systems. Acute withdrawal is associated with a negative affective state including various negative emotions such as dysphoria, depression, irritability and anxiety. For example, cocaine withdrawal in humans in the outpatient setting is characterized by severe depressive symptoms combined with irritability, anxiety, and anhedonia lasting several hours to several days (i.e., the ‘crash’) and may be one of the motivating factors in the maintenance of the cocaine dependence cycle [39]. Opiate withdrawal is characterized by severe dysphoria, and ethanol withdrawal produces dysphoria and anxiety.

While neuroadaptive mechanisms that reflect changes in reward function have been hypothesized to be decreases in neurochemical function associated with the same neurotransmitters activated to produce the acute reinforcing

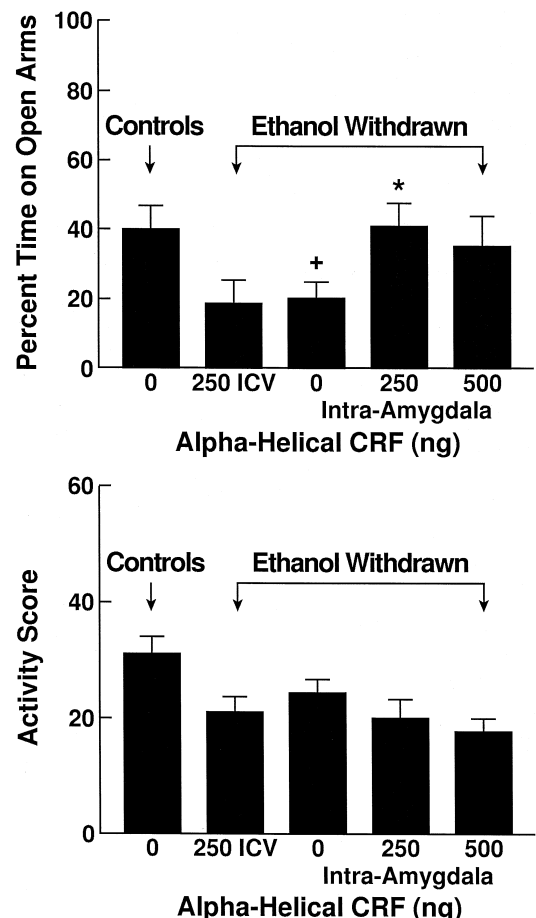


Fig. 5. Effects of microinfusion of CRF receptor antagonist alpha-helical CRF into the central nucleus of the amygdala and alpha-helical CRF<sub>9–41</sub> administered i.c.v. in the elevated plus-maze during ethanol withdrawal. The top panel shows percentage time exploring the open arms of the maze. The lower panel shows the activity score on the maze. The data are expressed as mean ( $\pm$ SEM) of percentage time exploring the open arms and are plotted as a function of the dose of alpha-helical CRF<sub>9–41</sub>. Asterisk (\*) indicates significant difference ( $p < 0.05$ ) compared to vehicle treatment. Plus sign (+) indicates significant difference ( $p < 0.05$ ) compared to pair-fed controls (taken with permission from Ref. [87]).

effects of drugs [53], another common adaptation to repeated administration of drugs of abuse that may not be involved in the acute reinforcing effects of the drugs may be the activation of brain and pituitary stress systems (Table 1). Pituitary-adrenal function in humans is activated during drug dependence and acute withdrawal, and dysregulation can persist even past acute withdrawal [63,64]. Brain corticotropin-releasing factor (CRF) function, outside of the pituitary adrenal axis, also appears to be activated during acute withdrawal from cocaine, ethanol, opiates, and tetrahydrocannabinol and thus may mediate behavioral aspects of stress associated with abstinence [46,55,88,89].

Following cessation of chronic administration of drugs of abuse, rats treated repeatedly with cocaine and ethanol show significant anxiogenic-like responses. These anxiogenic-like responses are reversed with i.c.v. administration of a CRF receptor antagonist [87,92]. Intracerebroventricular administration of a CRF receptor antagonist or microinjections of low doses of the CRF receptor antagonist into the central nucleus of the amygdala also reversed the anxiogenic-like effects of ethanol withdrawal [87] (Fig. 5). Similar doses of the CRF receptor antagonist injected into the amygdala were active in reversing the aversive effects of opiate withdrawal [46] (Fig. 6).

Additional evidence supporting a role for activation of brain CRF systems during acute withdrawal are studies showing an increase in extracellular levels of CRF in the region of the central nucleus of the amygdala during acute withdrawal from drugs of abuse. Animals exposed to a chronic ethanol liquid diet to induce ethanol dependence showed a time-related increase in CRF in the amygdala as

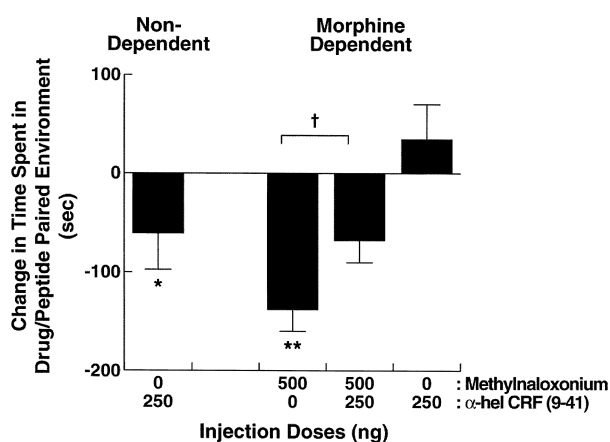


Fig. 6. Mean ( $\pm$ SEM) difference between pre- and post-conditioning scores before and after pairing of a particular environment with intramygdala administration of the opioid receptor antagonist methylnaloxonium (MN) and/or the CRF receptor antagonist alpha-helical CRF<sub>9-41</sub> in non-dependent ( $n = 15$ ) and morphine-dependent rats (MN alone,  $n = 12$ ; MN + alpha-helical CRF<sub>9-41</sub>,  $n = 17$ ; alpha-helical CRF<sub>9-41</sub> alone,  $n = 12$ ). \*  $p < 0.05$ , \*\*  $p < 0.005$ , pre- vs. post-conditioning scores within group; †  $p < 0.05$  (taken with permission from Ref. [46]).

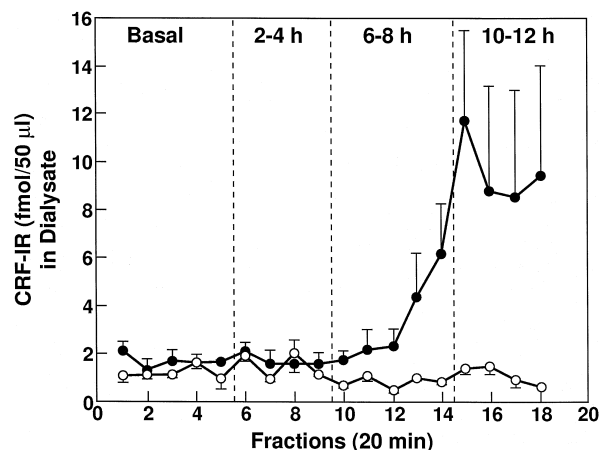


Fig. 7. Effects of ethanol withdrawal on corticotropin-releasing factor immunoreactivity (CRF-IR) levels in the rat amygdala as determined by microdialysis. Dialysate was collected over four 2-h periods regularly alternated with non-sampling 2 h periods. The four sampling periods correspond to the basal collection (before removal of ethanol), and 2–4 h, 6–8 h, and 10–12 h after withdrawal. Fractions were collected every 20 min. Data are represented as mean  $\pm$  SEM ( $n = 5$ /group). ANOVA confirmed significant differences ( $p < 0.05$ ) between the two groups over time (taken with permission from Ref. [76]).

measured by in vivo microdialysis [76] (Fig. 7). Similar results have been observed during withdrawal from cocaine [88] and precipitated cannabinoid withdrawal [89].

Drug addiction not only involves acquisition of drug-taking and maintenance of drug-taking, but also functions as a chronic relapsing disorder with reinstatement of drug-taking after detoxification and abstinence. Stress — and by association CRF — may contribute to the reinstatement of drug-taking after abstinence and, by extrapolation to the human condition, to relapse in multiple ways. Increasing evidence suggests that chronic exposure to drugs of abuse can change the ‘set point’ for drug reward. Animals with prolonged access to cocaine will continue to increase their cocaine intake on a daily basis and show enhanced intake of cocaine at all doses tested. Indeed, the dose-effect function appears to shift upward instead of to the right (tolerance) or left (sensitization) [1]. Such a change in drug reward set point may reflect an allostatic rather than homeostatic adaptation. Allostasis can be defined as marshalling all the parameters of the internal milieu to match perceived and anticipated environmental demands in order to maintain stability. Maintenance of this stability at a new pathological set point can provide the basis by which a small challenge can lead to breakdown, or in drug addiction, relapse [56]. The hypothesis suggested here is that the neurobiological bases for this complex syndrome of protracted abstinence may involve subtle molecular and cellular changes in the stress system circuitry associated with a contribution from CRF. Elucidation of the exact role for this neuropeptide and other stress-related neuropeptides such as neuropeptide Y will be the challenge of future research on the neurobiology of drug addiction.

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