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Corticotropin-releasing Factor in the Dorsal Raphe Nucleus: Linking Stress Coping and Addiction

Rita J. Valentino,

The Children's Hospital of Philadelphia, Philadelphia, PA 19104

Irwin Lucki, and

The University of Pennsylvania, Philadelphia, PA 19104

Elisabeth Van Bockstaele

Thomas Jefferson University, Philadelphia, PA 19107

Abstract

Addiction and stress are linked at multiple levels. Drug abuse is often initiated as a maladaptive mechanism for coping with stress. It is maintained in part by negative reinforcement to prevent the aversive consequences of stress associated with abstinence. Finally, stress is a major factor leading to relapse in subjects in which drug seeking behavior has extinguished. These associations imply overlapping or converging neural circuits and substrates that underlie the processes of addiction and the expression of the stress response. Here we discuss the major brain serotonin (5-HT) system, the dorsal raphe nucleus (DRN)-5-HT system as a point of convergence that links these processes and how the stress-related neuropeptide, corticotropin-releasing factor (CRF) directs this by a bimodal regulation of DRN neuronal activity. The review begins by describing a structural basis for CRF regulation of the DRN-5-HT system. This is followed by a review of the effects of CRF and stress on DRN function based on electrophysiological and microdialysis studies. The concept that multiple CRF receptor subtypes in the DRN facilitate distinct coping behaviors is reviewed with recent evidence for a unique cellular mechanism by which stress history can determine the type of coping behavior. Finally, work on CRF regulation of the DRN-5-HT system is integrated with literature on the role of 5-HT-dopamine interactions in addiction.

Keywords

serotonin; dorsal raphe nucleus; corticotropin-releasing factor; stress; receptor trafficking; drug abuse

I. Anatomical Basis for CRF Regulation of the DRN-5-HT System

CRF was initially characterized as the primary neurohormone that initiates release of adrenocorticotropin from the anterior pituitary in response to stress, an effect often considered to be the hallmark of the stress response (Vale et al., 1981). Since that critical discovery, the presence of CRF-containing axon terminals and receptors outside of the pituitary, as well as

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Corresponding Author: Rita J. Valentino, The Children's Hospital of Philadelphia, 402D Abramson Bldg., Civic Ctr. Blvd., Philadelphia, PA 19104, 215-590-0650 (voice), 215-590-3364 (fax), rjv@mail.med.upenn.edu.

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the ability of centrally administered CRF to mimic many of the autonomic and behavioral aspects of the stress response, has led to the idea that CRF coordinates multiple limbs of the stress response (Bale and Vale, 2004; Owens and Nemeroff, 1991). The DRN-5-HT system is compelling as a target of CRF given the established role of this system in stress-related psychiatric disorders (Cowen, 1993; Heils et al., 1997; Lesch, 1991; Lesch, 2001; Mann, 1999; Nordstrom and Asberg, 1992; van Praag, 1984).

A. CRF innervation of the DRN

Note that the evidence described below is from rat studies except where noted. CRF cells that are both intrinsic and extrinsic to the DRN regulate the 5-HT system. CRF-immunoreactive fibers densely innervate the DRN in a topographically organized manner (Kirby et al., 2000; Sakanaka et al., 1987; Swanson et al., 1983). In the caudal DRN, CRF innervation is greatest dorsally (adjacent to the ventricle and in lateral aspects of the DRN). Proceeding rostrally, CRF innervation becomes more ventral and medial and at the most rostral levels of the DRN, CRF-immunoreactive fibers are most dense in the interfascicular region. This topographical innervation is of interest because DRN subregions have specific afferents and efferent projections that may confer functional distinctions (Jacobs et al., 1978; O'Hearn and Molliver, 1984; Steinbusch, 1981). The cellular targets of CRF axon terminals differ between DRN subregions (Valentino et al., 2001). Thus, CRF axon terminals synapse more frequently with dendrites in the dorsal and lateral DRN compared to the ventromedial DRN, where CRF terminals are more often apposed to other axon terminals, suggestive of presynaptic actions. Although CRF axon terminals synapse with both GABA- and 5-HT-containing dendrites, GABAergic processes are more frequently targeted by CRF axon terminals in the DRN (Waselus et al., 2005). This is consistent with electrophysiological studies described below, suggesting that one mode of action of CRF on 5-HT neurons is indirectly mediated inhibition. Notably, the vesicular glutamate transporter type 2 co-localizes with CRF in a substantial proportion of axon terminals and there is relatively little co-localization of CRF with the vesicular glutamate transporter type 1 (Waselus and Van Bockstaele, 2007). Vesicular glutamate transporter type 2 axon terminals are thought to derive from neurons in subcortical regions, in contrast to the vesicular glutamate transporter type 1 terminals, which originate from cortical areas and hippocampus (Freneau et al., 2001; Herzog et al., 2001). Nuclei containing neurons that are immunoreactive for the vesicular glutamate transporter type 2 as well as CRF-immunoreactive neurons that are potential sources of afferents to the DRN include the paraventricular hypothalamic nucleus, bed nucleus of the stria terminalis and central nucleus of the amygdala (Hur and Zaborszky, 2005; Lin et al., 2003; Poulin et al., 2008; Ziegler et al., 2002).

The precise source of CRF afferents to the DRN is unknown because there are no studies that combine CRF immunohistochemistry with retrograde labeling from the DRN. Therefore one can only speculate on this based on the documented localization of CRF neurons in rat brain and afferent inputs to the DRN that have been identified from retrograde tracing studies. Based on retrograde tracing from specific DRN subdivisions, potential CRF inputs to the ventromedial DRN include the bed nucleus stria terminalis, lateral hypothalamus and paraventricular hypothalamic nucleus (Lee et al., 2003). Potential inputs to the lateral wing region would include the above as well as Barrington's nucleus.

Although much of what we know regarding CRF regulation of the DRN is based on rodent studies, the CRF innervation of the human DRN is well documented (Austin et al., 1997; Ruggiero et al., 1999). Dual labeling suggests that at least some CRF fibers directly interact with 5-HT-containing processes in the human DRN, although this has not been examined at the ultrastructural level (Ruggiero et al., 1999). CRF-immunoreactivity is elevated in the DRN of depressed suicide subjects (Austin et al., 2003), in line with hypotheses that CRF is

upregulated in depression (Gold and Chrousos, 2002; Holsboer, 1999; Nemeroff, 1996) and suggesting that CRF in the DRN may be particularly important in the pathophysiology of affective disorders.

B. CRF receptors in the DRN

Two CRF receptor subtypes, CRF₁ and CRF₂, mediate the actions of CRF in brain (Chalmers et al., 1995; Dautzenberg and Hauger, 2002). Whereas CRF₁ predominates in most brain regions, the DRN is somewhat unique in its relatively high expression of CRF₂ (Chalmers et al., 1995). CRF₂ mRNA is present at caudal levels of the DRN where it is expressed in both GABA and 5-HT neurons and at mid levels where it is primarily in 5-HT neurons (Day et al., 2004). Although CRF₁ mRNA is low in the DRN and virtually undetectable in some studies, its presence is detected by immunohistochemical techniques and its function is evidenced by physiological and behavioral effects. Immunohistochemical localization reveals distinct differences between CRF₁ and CRF₂ that suggest that activating the two receptors will have different functional consequences (Waselus et al., 2009). For example, CRF₂ staining is more punctate and is observed in both dendrites and axon terminals although it is more prominent in dendrites. At the light microscopic level CRF₂ appears localized within the cytoplasm whereas CRF₁ is localized to the periphery of neuronal profiles. Using electron microscopy to visualize immunogold labeled receptors this differential distribution is readily apparent as a much higher percentage of CRF₂ labeling (85%) within the cytoplasm compared to CRF₁ labeling (55%). The differential cellular distribution of CRF receptor subtypes plays an important role in stress-related behavioral plasticity (see below).

C. CRF cells in the DRN

In addition to being targeted by CRF-containing axon terminals, the DRN contains a small population of neurons that co-localize CRF with 5-HT and are primarily in the dorsomedial subnucleus (Commons et al., 2003). CRF that is co-localized with 5-HT in DRN neurons could act through local circuits to regulate activity of nearby DRN neurons or local 5-HT release. Notably, CRF/5-HT neurons of the DRN project to the CRF-containing cells in the central nucleus of the amygdala (Commons et al., 2003). CRF may function presynaptically in this pathway to regulate 5-HT release in the central nucleus of the amygdala. In this regard, a number of stressors alter 5-HT release in the amygdala (Kirby et al., 1995; Kirby et al., 1997) and this may be presynaptically modulated by CRF. Alternatively, CRF from the dorsomedial DRN could act as a co-neurotransmitter with 5-HT to fine-tune postsynaptic effects on CRF-containing amygdala neurons. CRF inhibits activity of neurons of the central nucleus of the amygdala *in vitro*, although it is not known whether the amygdalar neurons that are inhibited by CRF are also CRF-containing (Rainnie et al., 1992). The CRF projection from DRN to the central nucleus of the amygdala may be relevant to withdrawal from drugs of abuse such as alcohol and central nervous system stimulants as this is associated with robust CRF release in the central nucleus of the amygdala, an effect that is thought to contribute to the aversive aspects of withdrawal (Richter and Weiss, 1999).

II. CRF Regulation of DRN Neuronal Activity and 5-HT Release

Because of the neurochemical heterogeneity of the DRN and the presence of multiple CRF receptor subtypes on both 5-HT and non-5-HT neurons, the regulation of the DRN-5-HT system by CRF has been difficult to unravel. From diverse electrophysiological, microdialysis and behavioral studies a scheme has emerged of complex regulation whereby CRF acting at different receptor subtypes can have opposing effects on 5-HT activity that each facilitate contrasting behavioral strategies (i.e. active vs. passive) for coping with challenges. Moreover, the predominant effect of CRF, and therefore the predominant strategy, may be determined by a dynamic mechanism of differential receptor trafficking in DRN neurons.

A. Acute Effects of CRF on DRN-5-HT Activity

An initial study of CRF effects on DRN neurons using in vitro slice preparations reported that CRF activated a small percentage (approximately 30%) of neurons in the ventral aspect of the caudal DRN (Lowry et al., 2000). In vivo electrophysiological and microdialysis studies, some that used agonists and antagonists for selective CRF receptor subtypes, established that CRF has biphasic effects on DRN neurons that can be attributed to multiple CRF receptors expressed by both 5-HT and non-5-HT DRN neurons. Low doses of CRF that are more selective for CRF₁ receptors inhibit neuronal activity and 5-HT extracellular levels in many forebrain targets. (Kirby et al., 2000; Price et al., 1998; Price and Lucki, 2001). This inhibition is engaged during swim stress, which also decreases 5-HT release in certain forebrain terminal regions such as the lateral septum and amygdala (Kirby et al., 1995; Price et al., 2002; Roche et al., 2003b). Functional anatomical studies suggest that inhibition of the DRN-5-HT system by swim stress is mediated by recruitment of CRF afferents that target and activate GABA neurons in the dorsolateral DRN via CRF₁ receptors (Roche et al., 2003a). Consistent with a role for GABA in CRF₁-mediated inhibition, whole cell patch clamp recordings indicate that activation of CRF₁ increases GABA release onto 5-HT neurons in the DRN and causes an inward current in non-5-HT neurons (Kirby et al., 2008).

With increasing CRF concentrations, the inhibitory effect on both neuronal activity and 5-HT release in forebrain are lost and neuronal inhibition is replaced by excitation (Kirby et al., 2000; Lukkes et al., 2008; Price and Lucki, 2001). This has been attributed to engagement of CRF₂ receptors. Electrophysiological consequences of selectively activating CRF₂ receptors, which are more abundant than CRF₁ in the DRN, are complex. Because urocortin II, a member of the urocortin family of CRF-like peptides, has selective actions at CRF₂ receptors it has been used to probe the function of this receptor (Reyes et al., 2001). Although relatively low doses of the selective CRF₂ agonist, urocortin II, produced a short-lived inhibition of DRN neurons, a higher dose excited many neurons (Pernar et al., 2004). Juxtacellular labeling studies revealed that neurons that were activated by urocortin II were 5-HT-containing, whereas non-5-HT neurons were primarily inhibited (Pernar et al., 2004). This suggested that excitation of 5-HT neurons by CRF₂ activation may occur through direct activation and/or disinhibition. Together, the results support a scheme whereby CRF₁ and CRF₂ regulate the DRN-5-HT system in opposing manners. This is apparent at the level of neuronal activity as well as 5-HT release in forebrain regions where low doses of ovine CRF that are more selective for CRF₁ decrease, and higher doses that begin to activate CRF₂ receptors or selective CRF₂ agonists increase, 5-HT extracellular levels in forebrain (Amat et al., 2004; Lukkes et al., 2008; Price et al., 1998; Price and Lucki, 2001). The effects of CRF on 5-HT release in the nucleus accumbens are particularly relevant to substance abuse because 5-HT influences dopamine release in this region (see below). Similarly, effects of CRF on 5-HT release in the amygdala may contribute to withdrawal-associated aversion.

B. Plasticity of CRF Regulation of the DRN-5-HT System

The opposing regulation of the DRN-5-HT system by CRF₁ and CRF₂ allows for flexibility in how stressors affect the system. For example, stressors that release relatively low concentrations of CRF in the DRN should inhibit the system and those that release high concentrations should activate the system. Because DRN inhibition and excitation facilitate different behaviors (see below) this may be a means for eliciting behavioral responses that are appropriate for a specific stressor. This is well illustrated in the comparison of responses to a single vs. repeated swim stress, a stressor that has been used to examine behavioral plasticity that is sensitive to antidepressant agents (Detke et al., 1997; Porsolt et al., 1978). In response to an initial exposure to swim stress, the DRN-5-HT system is inhibited by endogenous CRF and this is associated with active escape behaviors (Price et al., 2002). Upon a second exposure to swim stress, when the active coping strategy is replaced by more passive behavior (i.e.,

immobility), this inhibition is absent (Price et al., 2002). Importantly, antidepressant administration that shifts the passive behavioral strategy back to an active strategy, reinstates the inhibition (Kirby and Lucki, 1997).

We recently identified differential trafficking of CRF receptor subtypes as a likely cellular mechanism whereby DRN neurons can have qualitatively different responses to CRF (or to stressors that release CRF) and as a result, can facilitate alternate behavioral strategies (Waselus et al., 2009). As depicted in Figure 1, CRF₁ and CRF₂ are differentially localized within DRN neurons. In unstressed animals CRF₁ is evenly distributed on the plasma membrane and within the cytoplasm, whereas CRF₂ is primarily cytoplasmic. The predominance of CRF₁ on the plasma membrane favors the inhibitory effect of CRF with an initial exposure to swim stress. However, following a single exposure to swim stress, CRF₁ tends to internalize while CRF₂ is recruited to the plasma membrane, thereby resulting in a shift in the predominant CRF receptor subtype. This phenomenon requires CRF₁ activation because it is greatly attenuated by prior administration of a selective CRF₁ antagonist. This cellular effect qualitatively changes the neuronal response to CRF from inhibition to excitation and can account for the shift from active to passive behavior in response to an initial vs. subsequent swim stress. This novel cellular mechanism that allows cells to have distinct responses to CRF depending on the history of stress may serve to promote alternate coping strategies if the original response is not appropriate or sufficient in dealing with a persistent or repeated stress.

Both CRF₁ and CRF₂ are coupled to the GTP-binding protein, Gs, and transducer activation of adenylate cyclase so the question arises as to how differential trafficking of the receptors in the same cell would alter the electrophysiological effect. However, recent evidence for coupling of these receptors to diverse signaling pathways suggests that this can occur (Hillhouse and Grammatopoulos, 2006). It is also possible that the receptors are in different neuronal populations, e.g., GABA vs. 5-HT and that could explain differential effects on 5-HT activity. Even in this scenario, increasing the influence of CRF₂ by recruitment to the plasma membrane would still change the general neuronal response to CRF.

Given the differential effects of activating CRF₁ and CRF₂ in the DRN, an intriguing speculation is that certain stressors may release one of the urocortins (urocortin II or urocortin III) that is selective for CRF₂ receptors, rather than CRF. However, the localization of axon terminals containing these peptides is as yet unknown so that the potential for this model it is not clear.

III. CRF Regulation of the DRN-5-HT System: Translation to Behavior

The ability of stress history to produce a qualitative change in the response to CRF is meaningful only if this translates to changes in behavior. The concept that CRF₁-mediated inhibition and CRF₂-mediated excitation of the DRN-5-HT system facilitate active and passive coping strategies, respectively, is mentioned above. Here we review the literature leading to this view and also present new findings in support of this concept. Note that the terms “active” and “passive” are in reference to whether a response involves motor activity.

A. Learned Helplessness and Forced Swim Test

Learned helplessness refers to the loss of an active escape response in animals that have been previously exposed to uncontrollable shock (Maier, 1984). It has been used to understand the passive behavior that characterizes depression. Because learned helplessness is associated with increased expression of the immediate early gene, c-fos, in DRN-5-HT neurons and increased 5-HT extracellular levels in projections regions, it has been attributed to activation of DRN-5-HT neurons (Maier et al., 1995; Maier and Watkins, 2005). Additionally, pharmacological manipulation of the DRN has verified that DRN neuronal activation is both necessary and

sufficient for learned helplessness. A role for CRF₂ mediated excitation of the DRN-5-HT system in this phenomenon was revealed by demonstrating that administration of selective CRF₂ agonists into the DRN produce the behavioral deficit, increase c-fos expression in 5-HT neurons and increase 5-HT release in projection areas (Hammack et al., 2003b). Conversely, selective CRF₂, but not CRF₁, antagonists block the ability of inescapable shock to produce learned helplessness. This well supports the notion that CRF₂ mediated activation of DRN-5-HT neurons promotes a passive behavioral strategy. Consistent with the concept that CRF₁-mediated inhibition of the DRN-5-HT promotes active coping, administration of low doses of CRF into the DRN that inhibit this system prevent learned helplessness produced by inescapable shock and facilitate an active escape response (Hammack et al., 2003a).

The association between CRF-mediated inhibition and active coping is best seen with changing behavioral responses to swim stress. When rats are first exposed to swim stress, active escape behaviors such as climbing and swimming are more prevalent and this is associated with CRF-mediated inhibition of the 5-HT release in forebrain regions (Kirby and Lucki, 1998; Price et al., 2002). With subsequent exposures, swim stress-induced inhibition is lost and this is associated with a shift from active to passive coping (Kirby and Lucki, 1998; Price et al., 2002). Administration of antidepressants that reinstate the ability of swim stress to inhibit 5-HT release corresponds to a return to active escape behaviors (Kirby and Lucki, 1997). Notably, a correlation has been demonstrated between active behaviors and decreases in 5-HT release in lateral septum (Kirby and Lucki, 1997).

B. Behavioral Effects Associated with CRF₁ and CRF₂ ligands in the DRN

More recently we have begun to directly investigate the behavioral consequences of activating CRF receptors in the DRN (see Supplemental Information for detailed methods). Either vehicle (artificial cerebrospinal fluid; ACSF), ovine CRF (3 or 30 ng in 100 nl), urocortin 2 (30 or 100 ng in 100 nl) or antisauvagine-30 (50 ng in 100 nl) were directly administered into the DRN of adult male rats prior to exposure to a 15 min swim stress performed as previously described (Detke et al., 1997). The ovine form of CRF is more potent at CRF₁ receptors. Urocortin 2 is selective for CRF₂ receptors and antisauvagine-30 is a selective CRF₂ antagonist (Reyes et al., 2001). Administration of the low dose of CRF decreased the incidence of immobility (Fig. 2A1). In contrast to CRF, urocortin 2 (30 or 100 ng in 100 nl) had no effect on behavior in response to swim stress (Fig. 2A2,A3). This may be a reflection of relatively few CRF₂ receptors on the plasma membrane. Interestingly, like the low dose of CRF, administration of the selective CRF₂ antagonist, antisauvagine-30 (50 ng in 100 nl) decreased immobility and increased active escape behaviors (Fig. 2A4). Together the results suggest that CRF released during swim stress interacts with CRF₁ receptors that are prominent on the plasma membrane to facilitate active escape behaviors and also binds to the relatively small number of CRF₂ receptors. Blocking CRF₂ receptors allows more CRF to be available to interact with CRF₁, further promoting active coping. These findings further support the working model that engaging CRF₁ and CRF₂ in the DRN facilitate active and passive coping behaviors, respectively.

Finally, effects of local infusion of CRF in DRN in defensive burying are also consistent with the model. Using a model of defensive burying of a shock probe (Lapiz-Bluhm et al., 2008), the same dose of CRF that decreased immobility in response to swim stress (3 ng in 100 nl) also decreased the latency to bury in response to a shock probe and increased burying duration, whereas urocortin 2 was ineffective (Fig. 2B).

IV. CRF Regulation of the DRN-5-HT System: Potential Link to Addiction

A. Initiation of substance abuse

The DRN-5-HT system plays a complex role in addiction through its ability to regulate discharge activity of dopaminergic neurons and dopamine release in targets (See for reviews (Bubar and Cunningham, 2008; Di Matteo et al., 2008; Weiss et al., 2001)). The multiplicity of 5-HT receptors and their presence on different neurons within reward circuitry suggests a complex regulation of the dopamine release that ultimately drives drug-seeking behavior. Most 5-HT receptor subtypes including the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT₃ and 5-HT₄, have excitatory effects on dopamine release in the nucleus accumbens and/or discharge activity of dopamine neurons in the ventral tegmental area that project to the nucleus accumbens (Di Matteo et al., 2008). Particularly, a role for 5-HT₃ receptors in the regulation of accumbens dopamine has suggested that this may be a pharmacological target for treating aspects of substance abuse (Di Matteo et al., 2008; McBride et al., 2004; Weiss et al., 2001). In contrast to most 5-HT receptors, 5HT_{2C} receptors exert tonic inhibitory control over dopamine release in the nucleus accumbens, although enhancing effects that are indirectly mediated through actions in the medial prefrontal cortex have also been reported (Bubar and Cunningham, 2008; Leggio et al., 2009). Certain drugs of abuse, such as cocaine, increase 5-HT as well as dopamine in the nucleus accumbens (Andrews and Lucki, 2001; Parsons et al., 1995). These studies have mostly focused on ethanol or cocaine-induced dopamine release and suggest a role for this system in abuse and/or addiction to these classes of drugs.

Stress can influence different aspects of substance abuse and addiction through effects on multiple neuronal systems. The actions of CRF on the DRN-5HT system described above suggest mechanisms by which stress can influence substance abuse through this system. One means by which this can occur is through CRF₁ mediated inhibition of the DRN-5-HT that occurs during acute stress. The hyposerotonergic state that results from CRF₁ mediated inhibition could contribute to the initiation of substance abuse, perhaps by promoting impulsive behavior. For example, decreased serotonergic function is a defect in several animal models of voluntary alcohol drinking such as alcohol preferring rats, high alcohol drinking rats and Fawn Hooded rats that prefer alcohol (LeMarquand et al., 1994b; McBride and Li, 1998). This translates to humans in that decreased serotonergic function has been identified as a biological factor that is linked to early onset alcoholism (Johnson, 2000; Johnson and Ait-Daoud, 2000; LeMarquand et al., 1994a). In line with our hypothesis that serotonergic deficits produced by acute stress and CRF₁ activation promote active behavior, a hyposerotonergic state has been linked to the increased impulsivity that is a component of drug seeking behavior (Virkkunen and Linnoila, 1990). One may speculate the decreased accumbal 5-HT negatively affects DA release and substance abuse is facilitated in an effort to elevate DA levels. A similar hyposerotonergic/hypodopaminergic state is seen in withdrawal and can contribute to relapse. Interestingly, recent human genetic studies revealed an association between alcohol drinking and polymorphisms in CRF₁ receptor genes (Treutlein et al., 2006). One may speculate that these polymorphisms affect alcohol consumption by determining the response of the DRN-5-HT system to stress and the magnitude of 5-HT release in accumbens.

B. Relapse after extinction

Convergent evidence implicates a role for CRF inhibition of median raphe neurons in stress-induced relapse. Local injections of CRF here reinstate alcohol-seeking behavior in rats that have undergone extinction (Le et al., 2002). Because these doses were comparable to doses that inhibit 5-HT activity when injected into the DRN and because the 5-HT_{1A} agonist, 8-hydroxy-DPAT had similar effects, it was concluded that this is a result of inhibition of 5-HT function. Additionally, administration of a CRF antagonist into the median raphe nucleus prevented the ability of footshock stress to reinstate alcohol-seeking behavior. Surprisingly,

this study did not examine the effects of CRF or CRF antagonist injections into the DRN on the basis that 8-hydroxy-DPAT was ineffective when administered into the DRN. Therefore, a site of action for CRF in the DRN is certainly possible. Importantly, the findings are consistent with the concept that acute stress promotes active drug-seeking behavior in subjects that have undergone extinction via CRF-mediated inhibition of raphe-5HT.

IV. Conclusions and Future Directions

The DRN-5-HT system has been a focal point of numerous investigations into the neurobiology of stress-related disorders including substance abuse. Here we reviewed findings that link stress to this system via the neuropeptide, CRF. The regulation of this system is finely tuned by CRF to facilitate different responses that are appropriate for specific conditions. This occurs as a result of differential cellular localization and trafficking dynamics of multiple CRF receptor subtypes. Most findings support a model whereby acute stress releases CRF in the DRN that acts on CRF₁ receptors that are more prominent on the plasma membrane. This decreases 5-HT function in forebrain and promotes active coping styles. CRF-related deficits in serotonin activity produced by acute stress may promote the impulsive behavior involved in the initiation of substance abuse, particularly for ethanol. Similarly, these deficits may come into play in stress-induced relapse once the behavior is extinguished. In this regard it would be of interest to examine selective CRF₁ antagonists in these two stages of addiction.

A history of stress promotes opposing trafficking of CRF receptors such that CRF₂ becomes more prominent on the membrane and this switches the serotonergic response to CRF to an excitation that promotes passive behavior. This may be a mechanism by which stress leads into depression that is characterized by passivity. This model suggests a potential for CRF₂ related antagonists or mixed CRF₁/CRF₂ antagonists as antidepressant agents.

Future studies designed to elucidate behavioral functions of distinct DRN subnuclei and how CRF affects cells in these subnuclei are necessary to understand the precise role of CRF-5-HT interactions in psychiatric disease and substance abuse. The development of pharmacological and genetic tools that can be used to specifically manipulate CRF in neuronal populations in the DRN will advance our knowledge of the system and may also provide therapeutic agents for the treatment of psychiatric disorders and addiction. Investigations that provide detail on CRF receptor signaling that directs trafficking dynamics will allow us ultimately to manipulate this process and can provide novel therapeutic targets. Finally, determining the functional relevance of recently identified polymorphisms in the human CRF₁ receptor to serotonin function and ultimately behavior may allow us to predict individual vulnerability to depression and addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

ACSF	artificial cerebrospinal fluid
CRF	corticotropin-releasing factor
DRN	dorsal raphe nucleus
GABA	gamma amino butyric acid
5-HT	serotonin

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Figure 1.

Schematic depicting how differential trafficking of CRF₁ and CRF₂ results in different physiological and behavioral responses. Ovals represent DRN neurons, blue symbols are CRF₁ and red symbols are CRF₂. In the unstressed state approximately 50% of CRF₁ receptors are on plasma membrane, whereas 10% of CRF₂ are on plasma membrane. In this state, CRF acts at CRF₁ receptors to inhibit the DRN-5-HT system. This is associated with active coping. With repeated stress, the receptors are trafficked in opposing directions. Particularly, CRF₂ is recruited to the plasma membrane. The result is that CRF can interact with CRF₂ receptors and the neuronal response switches from inhibition to excitation. This is associated with passive coping behavior.

Figure 2.

Effects of intra-DRN administration of CRF receptor agonists and antagonists on behavior in the forced swim test and defensive burying. A1–4. Effects of agents on the incidence of immobility in 3 5-min epochs during forced swim. A1. The lower dose of CRF (3 ng, n=8), but not higher dose (10 ng, n=8) decreases the incidence of immobility. For ACSF n=10. A two-way ANOVA revealed a statistically significant effect of drug ($F(2,77)=8.5$, $p<0.001$) and time ($F(2,77)=57$, $p<0.001$) and no interaction. A2. Urocortin 2 (30 ng, n=7) did not change the incidence of immobility compared to ACSF injection (n=8). A3. Urocortin 2 (100 ng, n=10) did not change the incidence of immobility compared to ACSF injection (n=9). A4. Injection of the selective CRF2 antagonist, antisauvagine-30 (50 ng, n=9) decreased the incidence of

immobility compared to ACSF injection (n=10). A two way ANOVA revealed an effect of drug treatment ($F(1,59)=6.4$, $p=0.01$) and time ($F(2,59)=57$, $p<0.001$) and no interaction. B. Effects of intra-DRN CRF (3 ng, n=13) or urocortin 2 (30 ng, n=9) on the latency to bury and the duration of burying in the defensive burying test. The matched ACSF groups are n=13 and n=8 for CRF and urocortin 2, respectively. * $p<0.05$, ** $p=0.01$, Student's t-test.