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Sex-Biased Stress Signaling: The Corticotropin-Releasing Factor Receptor as a Model

Rita J. Valentino, Debra Bangasser, and Elisabeth J. Van Bockstaele

The Children's Hospital of Philadelphia (R.J.V.), Temple University (D.B.), and Thomas Jefferson University School of Medicine (E.J.V.), Philadelphia, Pennsylvania

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ABSTRACT

Sex differences in the prevalence or severity of many diseases and in the response to pharmacological agents are well recognized. Elucidating the biologic bases of these differences can advance our understanding of the pathophysiology of disease and facilitate the development of treatments. Despite the importance to medicine, this has been an area of limited research. Here, we review physiologic, cellular, and molecular findings supporting the idea that there are sex differences in receptor signaling and trafficking that can be determinants of pathology. The focus is on the receptor for corticotropin-releasing factor (CRF), the orchestrator of the stress response, which has been implicated in diverse stress-related diseases that show a female prevalence. Data are reviewed that show sex differences in the association of the CRF receptor

(CRF1) with the Gs protein and β -arrestin 2 that would render females more responsive to acute stress and less able to adapt to chronic stress as a result of compromised CRF1 internalization. Because β -arrestin 2 serves to link CRF1 to Gs-independent signaling pathways, this sex-biased signaling is proposed to result in distinct cellular responses to stress that are translated to different physiologic and behavioral coping mechanisms and that can have different pathologic consequences. Because stress has been implicated in diverse medical and psychiatric diseases, these sex differences in CRF1 signaling could explain sex differences in a multitude of disorders. The possibility that analogous sex differences may occur with other G-protein-coupled receptors underscores the impact of this effect and is discussed.

Introduction

Many diseases exhibit sex differences, such that their prevalence and/or severity are greater in one sex. Identifying the biologic bases for sex differences in specific diseases can advance our understanding of their etiology and lead to the development of more effective individualized therapies. Sex differences are particularly prominent in neuropsychiatric diseases (Table 1). For example, autism, substance abuse, and attention deficit disorder are more prevalent in males (Table 1) (Gaub and Carlson, 1997; Ramtekka et al., 2010). In contrast, affective disorders and many anxiety disorders are nearly twice as prevalent in females, compared with males (Table 1) (Kessler et al., 1994; Kessler, 2003). Similarly, the incidence of posttraumatic stress disorder (PTSD) is greater in females despite males being exposed to more traumatic events (Breslau, 2001, 2002). These mood and anxiety disorders are debilitating mental illnesses and are a leading cause of disability in the United States for younger adults (Alonso et al.,

2011; Bromet et al., 2011). A common underlying feature of the psychiatric disorders that are more prevalent in females is an association with stress. Stressor exposure is associated with the onset and severity of depression and several anxiety disorders (Kendler et al., 1995). For PTSD, stress is the precipitating event. Of importance, the sex bias in PTSD remains after adjusting for the type of trauma, preexisting psychiatric disorders, and sex differences in reporting (Breslau et al., 1999; Tolin and Foa, 2006; Breslau, 2009). Together, these data support the notion that the increased incidence of stress-related psychiatric diseases in females is biologically determined.

In addition to an association with stress, anxiety disorders, PTSD, and depression share a core symptom of hyperarousal, which is a defining feature of the diseases and is characterized by sleep disturbances, inability to concentrate, restlessness, and increased vigilance (Southwick et al., 1999; Gold and Chrousos, 2002). To elucidate the biologic bases for the female prevalence of these diseases, we examined a major point of intersection between stress and arousal systems. This was the interaction between the orchestrator of the stress response, corticotropin-releasing factor (CRF) and a major arousal

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ABBREVIATIONS: BDNF, brain-derived neurotrophic factor; CRF, corticotropin-releasing factor; CRF-OE, CRF overexpressing mice; CRF1, corticotropin-releasing factor receptor subtype 1; ERK, extracellular signal-regulated kinase; GPCR, G protein-coupled receptors; LC, locus coeruleus; peri-LC, peri-coeruleus; PKA, protein kinase A; PTSD, post-traumatic stress disorder.

TABLE 1
Lifetime Prevalence of Psychiatric Disorders by Sex (>9000 Subjects)
(NCS-R, <http://www.hcp.med.harvard.edu/ncs/>).

Disorder	Prevalence		Female:Male Ratio
	Females	Males	
	%		
Panic	6.2	3.1	2.0
Generalized anxiety	7.1	4.2	1.7
Any anxiety disorder	36.4	25.4	1.4
PTSD	9.7	3.6	2.7
Major depression	20.2	13.2	1.5
Any affective disorder	24.4	17.5	1.4
Bipolar	4.5	4.3	1.0
Conduct disorder	7.1	12.0	0.6
ADHD	6.4	9.8	0.7
Alcohol abuse	7.5	19.6	0.4
Drug abuse	4.8	11.6	0.4

system, the locus coeruleus (LC)–norepinephrine system (Valentino and Van Bockstaele, 2008). Here, we describe the role of these systems in stress and how their interaction is an important cognitive limb of the stress response but can become dysfunctional and result in stress-related pathology. We then describe sex differences in functional end points of CRF-LC interactions and parallel differences in CRF receptor signaling and trafficking as identified in cortical tissue. The global implications of the discovery that sex can determine the direction of receptor signaling are discussed with respect to clinical and therapeutic applications.

Corticotropin-Releasing Factor and Stress-Related Psychiatric Disorders

The hypothalamic neuropeptide that orchestrates the stress response is CRF, which functions as a neurohormone that is released from paraventricular hypothalamic neurons into the hypophyseal portal system to elicit secretion of adrenocorticotropin (ACTH) from anterior pituitary corticotrophs (Vale et al., 1981). This is the initial step in a cascade that results in adrenal corticosteroid release, which is considered to be a hallmark of stress. Synaptically released CRF in extrahypophyseal neural circuits that innervate the limbic forebrain, midbrain and pontine monoamine nuclei, and autonomic-related brainstem nuclei facilitates the coordination of autonomic, behavioral, and cognitive responses to stress with the endocrine limb (de Kloet et al., 2005). Although coordinated CRF release in these circuits would be well designed for coping in a dynamic environment with potential life-threatening challenges, the initiation of these responses would be maladaptive in the absence of stressors or if the responses persisted after stress termination. In this regard, inappropriate or excessive CRF is thought to be a pathophysiological factor in the stress-related psychiatric disorders that are more prevalent in females, including PTSD and depression. For example, CRF levels are elevated in the cerebrospinal fluid of patients with PTSD or depression (Nemeroff et al., 1984; Bremner et al., 1997; Arborelius et al., 1999; Baker et al., 1999; Sautter et al., 2003). CRF protein and mRNA are elevated in numerous brain regions of depressed suicide victims (Leake et al., 1990; Austin et al., 2003; Bissette et al., 2003). Finally, many of the symptoms in a subset of individuals with severe depression resemble over-activity of the stress axis, including

increased basal cortisol secretion, blunted rhythm, and resistance to dexamethasone suppression (Gold et al., 1988a,b; Gold and Chrousos, 2002).

CRF effects are mediated through two CRF receptor subtypes, CRF1 and CRF2, in which genes have been cloned (Perrin et al., 1993; Lovenberg et al., 1995; Chen et al., 2005). These receptors have been well characterized with respect to their distribution and pharmacological specificity (Chalmers et al., 1996; Hauger et al., 2003, 2006; Bale and Vale, 2004). In addition, many details related to signaling and trafficking of the receptors have been identified (Hauger et al., 2006, 2009; Hillhouse and Grammatopoulos, 2006). The present review focuses on CRF1, because this is the subtype through which CRF acts to elicit ACTH release and to initiate many of the central effects associated with the stress response, including arousal and anxiogenic effects. As such, novel therapeutics for stress-related disorders are being developed to target CRF1 (Holsboer, 1999; Grammatopoulos and Chrousos, 2002). In addition, this is the CRF receptor subtype for which sex differences in signaling have been described (Bangasser et al., 2010). In contrast, the role of CRF2 in stress is less well defined, with some studies suggesting stress-protective effects and others providing evidence for pro-depressive effects mediated by this receptor (Bale et al., 2000; Hammack et al., 2003; Valentino and Commons, 2005). Reviews of CRF2 structure, signaling, and function can be found elsewhere (Hauger et al., 2006; Hillhouse and Grammatopoulos, 2006).

Corticotropin-Releasing Factor and the Locus Coeruleus-Norepinephrine System

The norepinephrine nucleus LC in the pons is a primary target of CRF during stress (Valentino and Van Bockstaele, 2008). The LC is the major source of norepinephrine in forebrain and has long been implicated in arousal and directing the mode of attention (Aston-Jones et al., 1995; Waterhouse et al., 1998; Berridge and Waterhouse, 2003). CRF axon terminals synapse with LC dendrites and also are apposed to afferent axon terminals in the LC, suggesting that CRF has both direct and indirect actions on LC neurons (Van Bockstaele et al., 1996). Consistent with this, CRF increases the spontaneous discharge rates of LC neurons in vivo when directly applied onto LC neurons and in vitro in the presence of tetrodotoxin (Curtis et al., 1997; Jedema and Grace, 2004). This action involves cyclic AMP-mediated inhibition of potassium conductance (Jedema and Grace, 2004). In addition, CRF decreases the signal-to-noise ratio of LC responses to sensory stimuli (Valentino and Foote, 1987; Valentino and Foote, 1988). Related to this, it is noteworthy that at least one-third of CRF axon terminals in the LC colocalize glutamate, the neurotransmitter that mediates LC activation by many stimuli (Valentino et al., 2001).

The net effect of CRF on LC neurons is to shift discharge toward a high tonic state that favors increased tonic and decreased phasic, stimulus-evoked activity (Valentino and Foote, 1987; Valentino and Foote, 1988). This mode of discharge has been associated with a shift from focused attention to a scanning mode that would promote increased arousal and behavioral flexibility (Aston-Jones et al., 1999; Aston-Jones and Cohen, 2005). Supporting this, CRF microinfusion into the LC desynchronizes cortical electroencephalographic activity, a sign

of elevated arousal, and promotes behavioral flexibility in an attentional set shifting task (Curtis et al., 1997; Snyder et al., 2012). Stressors mimic the electrophysiological effects on LC neurons, and this can be prevented by microinfusion of CRF antagonists into the LC providing evidence for CRF neurotransmission in the LC during stress (Valentino et al., 1991; Curtis et al., 2001, 2012).

In addition to its electrophysiological effects, CRF has enduring effects on LC dendritic morphology, as seen in cultured slices from neonatal rats or in CATH.a cells, immortalized norepinephrine cells that resemble LC neurons (Cibelli et al., 2001; Swinny and Valentino, 2006). In the cultured explants, CRF increases dendritic growth, and in CATH.a cells, it promotes neurite outgrowth; these effects are CRF1 mediated. Both actions require cyclic AMP-dependent protein kinase and mitogen-activated protein kinase (MAPK) pathways. The morphologic effects of CRF on LC dendrites in cultured slices were also shown to require activation of the small GTPase Rac, which promotes and stabilizes dendritic branches (Swinny and Valentino, 2006). This was the first evidence linking CRF receptor actions to actin cytoskeletal regulation through Rho GTPases and suggested molecular mechanisms for stress-induced remodeling of the actin cytoskeleton.

The LC system is designed in a way that would allow CRF effects on LC dendritic growth to have a profound impact on how the LC-norepinephrine system is regulated. LC dendrites extend for hundreds of microns outside the LC core (Shipley et al., 1996), and afferents to these peri-coerulear (peri-LC) regions are functionally distinct from afferents to the nuclear core. The majority of CRF afferents to the nuclear core of the LC are related to autonomic function. These include Barrington's nucleus, which regulates the parasympathetic neurons that control pelvic visceral function, and the nucleus paragigantocellularis, which regulates sympathetic preganglionics involved in cardiovascular function (Valentino et al., 1992). In contrast, CRF afferents terminating outside of the nuclear core, in peri-LC regions into which LC dendrites extend, derive from limbic regions, such as the central amygdalar nucleus (Van Bockstaele et al., 1998, 1999, 2001). These afferents convey emotion-related information, and their links to the LC provide an anatomic substrate for emotional arousal. The further LC dendrites extend into the peri-LC space, the higher the probability that they will contact limbic afferents relaying emotion-related information to the LC arousal system and the more likely the system will be regulated by emotion-related stimuli. By promoting LC dendritic growth, CRF shapes a structural basis for emotional arousal. One speculation from these data is that early life stressors create a foundation for higher emotional arousal through the process of CRF release in the LC. Potential sex differences in this effect are discussed below.

In addition to these morphologic effects, CRF acting at CRF1 potentiates the ability of tropomyosin receptor kinase B (TrkB) agonists, brain-derived neurotrophic factor (BDNF), and neurotrophin-4, to induce the norepinephrine phenotype of LC neurons in culture (Traver et al., 2006). In this preparation, BDNF increases the number of tyrosine hydroxylase-expressing neurons, and although CRF has no effect on its own, it enhances this effect of BDNF. These CRF1 actions have been associated with activation of adenylyl cyclase, induction of cyclic AMP, and activation of Epac (see below).

Despite the electrophysiological and immunohistochemical evidence for CRF1 in LC neurons, many *in situ* hybridization studies have failed to detect CRF1 mRNA in LC neurons of rodents, although LC neurons of humans and nonhuman primates express CRF1 mRNA (Sanchez et al., 1999; Van Pett et al., 2000; Hiroi et al., 2001). Although difficult to reconcile, this could be attributed to distribution of the mRNA in the extensive LC dendritic system, which could dilute the signal, making it difficult to detect. Of note, a relatively recent *in situ* hybridization study provided evidence for CRF1 mRNA in rat LC (Zeng et al., 2003). In addition, CRF1 mRNA (but not CRF2 mRNA) was detected in LC neurons cultured from embryonic rats (day 14) in the aforementioned study (Traver et al., 2006).

Sex Differences in Corticotropin-Releasing Factor Regulation of the Locus Coeruleus

The first evidence suggesting sex differences in CRF receptor-mediated effects came from electrophysiological comparisons of LC neuronal activity in male and female rats (Curtis et al., 2006). The electrophysiological characteristics of LC neurons are mostly comparable between sexes. For example, LC firing rate and the magnitude of sensory-evoked responses that are mediated by glutamatergic inputs are similar in male and female rats. However, the magnitude of LC activation by hypotensive challenge, which activates LC neurons through CRF release in the LC, was greater in female than in male rats. Of note, this effect was unrelated to adult hormonal status of either males or females. These sex differences in the magnitude of LC activation by hypotensive stress could be attributed to differences in postsynaptic sensitivity to CRF. Thus, the CRF dose-response curve for LC activation was shifted to the left in females, compared with males, and doses of CRF that were below threshold for increasing LC discharge rate in male rats were effective in females. In addition to sex differences in the acute effect of CRF, sex differences were apparent in the manner in which a history of stress regulated LC sensitivity to CRF (Curtis et al., 2006). For example, in male rats, a history of shock or swim stress shifts the CRF dose-response curve for LC activation to the left, with a decrease in the maximal response, such that neurons are more sensitive to low doses of CRF and less sensitive to higher doses. In contrast, in female rats, the CRF dose-response curve for LC activation is not altered by a history of stress.

With respect to the morphologic effects of CRF on LC neurons, it is noteworthy that LC dendrites of female rats are longer and more complex, having more branch points and extending further into the peri-LC than LC dendrites of male rats (Bangasser et al., 2011). This is reminiscent of the effects of CRF on LC dendrites and would result in a greater magnitude of arousal in response to emotion-related stimuli by favoring more contacts with limbic terminals that convey emotion-related information (Fig. 1). Nonetheless, the role of CRF in these sex differences has yet to be established.

CRF1 Signaling and Trafficking

The aforementioned sex differences suggest distinctions in CRF1 signaling. CRF1 is of the Class B family of seven transmembrane G protein-coupled receptors (GPCR). In brain, the primary mode of signaling is through Gs α , which binds to

the third intracellular loop (Hauger et al., 2006, 2009). This results in activation of adenylyl cyclase, with consequent formation of cyclic AMP and phosphorylation of protein kinase A (PKA). Activated PKA phosphorylates diverse cellular proteins, including potassium ion channels, and inactivation of potassium channels through phosphorylation has been implicated in the ability of CRF to increase LC neuronal discharge rates (Jedema and Grace, 2004). Phosphorylation of cyclic AMP response element-binding protein by PKA provides a potential mechanism whereby stress can affect cell transcription via CRF. Evidence suggests that BDNF is upregulated through this pathway (Bayatti et al., 2005). PKA signaling through CRF1 has also been linked to activation of the serine threonine kinase SGK-1, which is associated with synaptic plasticity and cell survival (Sheng et al., 2008). CRF1 signaling through cyclic AMP that is independent of PKA results in activation of the guanine nucleotide exchange factor Epac (Traver et al., 2006). This proceeds to activate extracellular signal-regulated kinase-mitogen-activated protein kinase (ERK-MAPK) pathways that regulate BDNF signaling. This cascade is involved in the ability of CRF to potentiate the phenotypic determination of LC neurons by BDNF (Traver et al., 2006). CRF1 has been linked to other signaling pathways, particularly in other tissues or cells in culture, and these are reviewed elsewhere (Hillhouse and Grammatopoulos, 2006).

Similar to other G protein-coupled receptors, CRF1 is internalized after agonist binding. Both agonist and stress-induced CRF1 internalization have been described in vivo in rat LC neurons (Reyes et al., 2006, 2008). In male rats, CRF microinfusion in the LC causes CRF1 internalization in early endosomes in LC dendrites that is apparent by 5 minutes after injection and is more pronounced by 30 minutes after injection (Reyes et al., 2006). Evidence for CRF1 internalization in male rat LC dendrites is also apparent 1 and 24 hours after a single

15-minute swim stress, and this is completely prevented by pretreatment with a selective CRF1 antagonist (Reyes et al., 2008). At both time points, CRF1 is associated with early endosomes in the cytoplasm. However, by 24 hours after swim stress, significantly more CRF1 is associated with multivesicular bodies, compared with 1 hour after stress, indicative of degradation and downregulation of the receptor. This effect would be consistent with the decreased maximum response of the CRF dose-response curve for LC neuronal activation seen in male rats 24 hours after swim stress.

The molecular mechanisms guiding agonist-induced CRF1 internalization have been well characterized in cell preparations, including primary cortical neurons and HEK293 cells (Holmes et al., 2006; Oakley et al., 2007). After agonist binding, CRF1 undergoes sequential phosphorylation steps by G protein receptor kinases on both the carboxyl tail and the third intracellular loop. This promotes the recruitment and binding of β -arrestin 2 and internalization by a dynamin-dependent process.

Sex Differences in Corticotropin-Releasing Factor Signaling

The sex differences observed in LC neuronal responses to CRF could be attributed to differences in CRF1 signaling. Consistent with this, in females, LC activation by CRF was almost completely prevented by the PKA antagonist Rp-cAMP-S, whereas only a fraction (~50%) of the CRF-elicited LC activation was prevented by the PKA antagonist in males (Bangasser et al., 2010). To examine the possibility of sex differences in CRF1 signaling, CRF1 was immunoprecipitated from rat cortex (Bangasser et al., 2010), a tissue of high CRF1 expression and lacking CRF2 (Van Pett et al., 2000). Although the cortex exhibits one of the highest densities of CRF1 expression in brain, relatively few studies have examined the effects of activating these receptors on behavior, presumably because the region's greater area makes it less amenable to microinjection experiments, compared with more discrete regions, such as the amygdala. Nonetheless, several animal studies provide evidence for a role of cortical CRF1 in stress-related behaviors and pathology (Jaferi and Bhatnagar, 2007; Magalhaes et al., 2010; Bijlsma et al., 2011). In addition, chronic stress increases in CRF1 mRNA in cortex in rodents, and CRF1 mRNA is decreased in the cortex of depressed suicide victims, possibly in response to increased CRF peptide (Merali et al., 2004; Anisman et al., 2007).

Immunoprecipitated CRF1 from the cortex of unstressed female rats was associated with approximately three times more Gs, compared with unstressed male rats (Bangasser et al., 2010). In contrast, there were no sex differences in Go or Gq/11 association with CRF1. Similar to the sex differences in LC responses to CRF, adult hormonal status was not a determinant of the sex differences in CRF1-Gs coupling, because similar results were obtained with intact and ovariectomized females. Of importance, in rats with a history of swim stress, CRF₁-Gs association increased in males to a magnitude that matched that of unstressed females, but the same stress history had no effect in females. Although it is not feasible to perform similar receptor immunoprecipitation studies with LC tissue, these sex differences in CRF₁-Gs coupling determined in cortical tissue mirrored sex differences in LC sensitivity to CRF and provide potential molecular mechanisms for these physiologic differences.

Fig. 1. Schematic depiction of how sex differences in LC dendritic morphology could affect emotional arousal. LC neurons of female rats have longer and more complex dendrites than do neurons of males. Thus, the probability that LC dendrites will contact limbic afferents that convey emotion-related information and terminate in the peri-LC rather than the core is greater in females than in males. This would be predicted to result in a greater magnitude of arousal in response to emotion-related stimuli. BNST, bed nucleus of the stria terminalis; CNA, central nucleus of the amygdala; PGI, paraventricular nucleus of the hypothalamus. Reproduced with permission from Bangasser et al. (2011).

Of note, these were the first data, to our knowledge, to link sex differences in physiology to sex differences in the coupling of G proteins to receptors.

Sex Differences in CRF₁ Receptor Trafficking

In contrast to its ability to induce CRF₁ internalization in male LC neurons, swim stress does not result in CRF₁ internalization in LC neurons of female rats (Bangasser et al., 2010). Indeed, the cellular localization of CRF₁ is opposite in female and male LC neurons. In the unstressed male rats, ~50% CRF₁ immunogold labeling is on the plasma membrane of LC neurons, and 50% is cytoplasmic. Swim stress shifts the distribution of CRF₁ immunolabeling, such that ~70% is cytoplasmic, indicative of internalization. This is consistent with the decreased maximal activation of LC discharge produced by CRF. In contrast, in female LC neurons in the unstressed condition, CRF₁ is predominantly cytoplasmic and swim stress shifts the distribution to the plasma membrane. In this case, it is not clear whether swim stress actually recruits CRF₁ to the plasma membrane or whether there is a decrease in the synthesis of CRF₁ that would render relatively less in the cytoplasm, compared with the plasma membrane. The inability of CRF₁ to be internalized in female LC neurons by 24 hours after swim stress is consistent with the lack of change in the maximum effect of CRF.

The compromised ability to internalize CRF₁ in female LC neurons is likely the result of an inability of β -arrestin 2 to associate with CRF₁, a critical molecular step in the process of receptor internalization. In the unstressed condition, CRF₁ immunoprecipitated from cortical tissue of male and female rats pulled down an equivalent amount of β -arrestin 2 (Bangasser et al., 2010). After swim stress, the association of β -arrestin 2 with CRF₁ greatly increased in males, consistent with stress-induced CRF₁ internalization. However, in females, stress did not alter CRF₁– β -arrestin 2 association. The deficit in β -arrestin 2 association with CRF₁ in females relative to males can account for the compromised ability to internalize CRF₁ after stress, and this molecular mechanism may account for the inability of stress to decrease the maximal CRF response of female LC neurons. As for CRF₁–Gs coupling, the differences in β -arrestin 2 association were unrelated to adult hormonal status. Of note, the decreased ability of CRF₁ receptors in females to associate with β -arrestin 2 could also account for an enhanced association of CRF₁ with Gs, because β -arrestin 2 sterically hinders binding of Gs to GPCRs (Kohout and Lefkowitz, 2003).

Sex differences in receptor association with G proteins and β -arrestins are unique and suggest structural differences in CRF₁, perhaps as a result of post-translational modifications. CRF₁ is glycosylated at many points; however, differences in glycosylation would be predicted to alter molecular weight, and there was no indication of sex differences in the molecular weight of the receptor (Grigoriadis and De Souza, 1989). Sex differences in GRK phosphorylation at one or more of several sites at which CRF₁ is phosphorylated after agonist binding could account for differences in Gs or β -arrestin 2 association.

Sex Differences in the Consequences of CRF Overexpression

The aforementioned sex differences in CRF₁ signaling would render female neurons more sensitive to CRF and less

able to adapt to excessive CRF. However, sex differences in CRF₁ signaling and trafficking have little consequence in the absence of CRF and, thus, are unlikely to be manifest in the absence of stress. Instead, the presentation of a stressor that releases CRF is necessary to reveal the consequences of these molecular differences. Sex differences in CRF signaling and trafficking would have the greatest impact in conditions of excessive CRF, as has been proposed to occur in the same stress-related psychiatric disorders that are more prevalent in females, PTSD and depression. Different strains of CRF-overexpressing mice (CRF-OE) have been used to model the pathologic condition of excessive CRF (Stenzel-Poore et al., 1994; Dirks et al., 2002; Groenink et al., 2003; Lu et al., 2008). The best characterized of these is a transgenic line in which CRF expression is under control of the metallothionein promoter (Stenzel-Poore et al., 1994). These mice have elevated CRF expression in brain neurons in most regions that normally express CRF. They exhibit evidence of hypothalamic-pituitary-adrenal axis overactivity, including adrenal hypertrophy and elevated plasma adrenocorticotropin and corticosterone. In addition, these mice show anxiogenic effects in many animal models. Conditional CRF-OE mice have been generated in which CRF can be overexpressed throughout the entire brain (Lu et al., 2008). A disadvantage of the conditional model is that CRF is expressed ubiquitously in both neurons and glia in brain, and unlike the CRF transgenic mice, which exhibit CRF overexpression primarily in brain regions where CRF is typically expressed, the pattern of CRF expression in the conditional line is substantially different, with the highest expression being in the olfactory bulb, cortex, and hippocampus.

Sex differences were apparent in CRF-OE transgenic mice (metallothionein promoter) with respect to LC activity recorded from slice preparations *in vitro* and CRF₁ cellular localization (Bangasser et al., 2012). The characteristics of LC neuronal activity were comparable in male and female wild-type mice. Despite a greater CRF innervation of LC neurons of male CRF-OE mice, compared with wild-type mice, LC discharge rates were similar. In contrast, LC neurons of female CRF-OE mice had discharge rates that were nearly three times greater than those of wild-type mice or male CRF-OE mice, suggesting that neurons of male mice have mechanisms to adapt to excessive CRF that are not present in females. CRF₁ trafficking could account for the sex differences in LC activity of CRF-OE mice. The cellular distribution of CRF₁ in LC neurons of male wild-type and CRF-OE mice were analogous to CRF₁ distribution in unstressed and stressed male rats, respectively. In wild-type mice CRF₁ was equally distributed in the cytoplasm and on the plasma membrane, and in CRF-OE mice, 80% of CRF receptor labeling was in the cytoplasm, consistent with internalization and accounting for the lack of effect of excess CRF on male LC neurons. The situation was opposite for female wild-type and CRF-OE mice, such that CRF₁ was predominantly cytoplasmic in wild-type mice and on the plasma membrane in CRF-OE mice, where it would be available to be activated by excessive levels of CRF. The inability of neurons in female CRF-OE mice to internalize CRF₁, an effect that may be in part attributed to diminished CRF₁– β -arrestin 2 association, results in an over-activated LC-norepinephrine system.

Because overactivation of the LC-NE system translates to the hyperarousal symptoms that define stress-related psychiatric disorders, it is not surprising that these disorders are

more prevalent in females. Although the molecular sex differences described here may bias toward stress-related pathology, they may also be programmed for evolutionary advantages in species in which the female must remain vigilant and exhibit behavioral flexibility in performing multiple tasks, including foraging for food while protecting from predators.

Although sex differences in the behavioral phenotype of CRF-OE mice have not been systematically explored, some differences have been noted. Female CRF-OE mice gain more weight and have larger adrenals, compared with their wild-type counterparts, whereas these differences are not apparent in males (Bangasser et al., 2012). Female CRF-OE mice also have enhanced pelvic visceral responses to stressors and show more behavioral inhibition in novel environments, compared with males (Million et al., 2007). Finally, female CRF-OE mice exhibit impaired social interaction with males, whereas male CRF-OE mice do not show this reaction (Heinrichs et al., 1997).

Sex-Biased CRF Signaling

Although sex differences in CRF1 trafficking and signaling have important implications for stress-related disorders, new perspectives about β -arrestin function suggest even broader implications of the findings. In addition to its role in receptor internalization, β -arrestin 2 can engage G protein-independent signaling cascades by scaffolding receptors to signaling molecules, including mitogen-activated protein kinase (e.g., ERK2, JNK3, and p38), tyrosine kinases (e.g., c-SRC), and AKT, PI3 kinase, and RhoA (Lefkowitz and Shenoy, 2005; Violin and Lefkowitz, 2007). A compromised ability of female CRF₁ receptors to associate with β -arrestin 2 would shunt CRF1 signaling down Gs-related pathways. In contrast, in male neurons β -arrestin 2, G-protein-independent pathways would be favored, at least relative to CRF1 signaling in females. By engaging sex-specific signaling pathways, CRF released during stress could have sex-specific cellular consequences that translate to distinct physiologic and/or behavioral responses and distinct pathology (Fig. 2).

The consequences of CRF1 sex-biased signaling would be magnified when CRF is in excess, as has been proposed to occur in stress-related psychiatric disorders that are more prevalent in females. In these conditions, sex differences in signaling cascades engaged by CRF could underlie sex differences in the pathologic presentation of stress. Because Gs-protein and β -arrestin 2 signaling regulate phosphorylation dynamics in cells, the excessive CRF would be predicted to result in sex-specific phosphoprotein profiles. Keys to understanding sex differences in stress-related psychiatric disorders may lie in the differences between these profiles. This is currently being examined by performing a deep phosphoproteomic analysis of cortex of male and female CRF-OE mice with use of stable isotope labeling of whole mouse and high-resolution mass spectrometry (Valentino et al., 2012). The initial results confirm the model of sex-biased signaling, because ~15% of the phosphopeptides that could be quantified in both groups were significantly enriched in either the female (10%) or male (5%), based on a false discovery rate of 1%, which translated to a 1.52-fold difference. Ingenuity pathway analysis supported the concept of sex-biased CRF1 signaling and indicated that kinases (including PKA) were prominent in the top five canonical pathways in which phosphopeptides that were enriched in the female

CRF-OE phosphoproteome were overrepresented. In contrast, phosphopeptides that were enriched in male CRF-OE cortex were overrepresented in pathways related to Rho signaling, which has been linked to β -arrestin. Of interest, phosphopeptides enriched in the female CRF-OE were overrepresented in an amyloid processing pathway and included enzymes that process amyloid precursor protein to neuropathological amyloid β and kinases that phosphorylate tau, which leads to the formation of fibrillary tangles. Because Alzheimer's disease is more prevalent in females and has been linked to stress (Ruitenber et al., 2001; Csernansky et al., 2006; Wilson et al., 2006; Figueira and Ouaknin, 2010), these findings suggest a mechanism by which sex-biased CRF1 signaling favors pathways that increase vulnerability to this disease in females.

Sex-biased CRF₁ receptor signaling has important therapeutic implications for novel compounds that can shift the bias of CRF₁ receptor signaling. By shifting CRF signaling toward a β -arrestin 2 pathway, such compounds could potentially make females more resilient to the pathophysiological consequences of stress, particularly hyperarousal. Biased agonists are currently being designed to direct receptor-mediated cellular events toward specific pathways to promote efficacy for desired effects and diminish adverse effects (Bohn and McDonald, 2010; Rajagopal et al., 2011; Whalen et al., 2011). One example of this is the angiotensin II receptor, which may be targeted to take advantage of the anti-apoptotic effects mediated through β -arrestin and to avoid G-protein-mediated hypertensive effects (Rajagopal et al., 2011). Pharmaceuticals aimed at shifting CRF1 signaling from G-protein mediated to β -arrestin mediated may reduce the hyperarousal symptoms associated with stress-related psychiatric diseases.

Overview and Implications

This review integrates convergent findings supporting the novel concept of sex differences in receptor signaling and trafficking, using CRF1 as a model. A caveat is that much of the evidence for sex-biased CRF1 signaling derives from studies using cortical tissue, and there may be region-specific effects of CRF1 signaling. Even post-translational modifications, such as receptor glycosylation, can be region specific (Grigoriadis and De Souza, 1989). Nonetheless, the finding that sex differences in CRF1 signaling identified in cortex were consistent with sex differences in CRF1 trafficking and physiologic sensitivity in LC neurons supports the notion that these differences generalize to other regions expressing CRF1. Sex differences in Gs coupling would confer differences in agonist sensitivity and, in the case of CRF, differences in acute responses to stressors. Of note, small-molecule non-peptide CRF1 antagonists bind to transmembrane domains of the receptor that are in proximity to the Gs-protein coupling site in the third intracellular loop. Potential sex-specific phosphorylation of sites in this loop could confer sex differences in the ability of these antagonists to bind (Hoare et al., 2004; Hauger et al., 2006). Differences in receptor association with β -arrestin influence receptor trafficking and the ability to adapt to the excessive CRF that is predicted to be present in diseases related to severe or chronic stress. For females, this would translate to an enhanced sensitivity to acute stress and decreased ability to adapt to chronic or repeated stress. The broader implication of this model, given evidence for

Sex Biased CRF1 Signaling

Fig. 2. Schematic depicting the predicted model of sex-biased signaling. In females, the decreased ability of CRF₁ to associate with β -arrestin 2 biases signaling through Gs-related pathways. In contrast, in males, CRF₁ receptor can associate with β -arrestin 2, which results in a relative bias toward β -arrestin 2-related pathways. As a result of differential signaling, stress-induced release of CRF can produce sex-specific cellular responses that can translate to different physiologic and behavioral responses. Because CRF1-Gs signaling is linked to tonic LC activation and increased LC dendritic length, this is predicted to be greater in females and to result in hyperarousal in response to stress. At a clinical pathologic level, this would predispose to stress-related psychiatric disorders, such as depression and PTSD, in which hyperarousal is a significant component. Consequences of CRF1- β -arrestin2 signaling are less well known, but these may lead to more adaptive responses and, perhaps, pathology that is more prevalent in males, as indicated in Table 1. The question marks underscore the speculation here.

β -arrestin 2/Gs-protein-independent signaling, is that sex can be a determinant of the coping responses and pathology elicited by stress (Fig. 2). Identifying the consequences of sex-biased CRF1 signaling will help us to understand why females are more vulnerable to certain stress-related diseases.

Sex differences in CRF function have important implications for drug development. Currently, CRF antagonists are being designed to treat mood and anxiety disorders. Because the data reviewed here suggest that CRF hypersecretion has a greater impact in females than in males, CRF antagonists may be more effective in women than in men. Alternatively, sex differences in the CRF₁ receptor could affect the ability of these drugs to prevent CRF1 signaling. Pharmacotherapies designed with sex differences in mind may prove to be necessary in treating psychiatric consequences of stress.

Although this review focused on CRF1 signaling, given many of the shared characteristics of different GPCRs, it is unlikely that sex-biased signaling is limited to CRF1. Recently, sex differences were reported in phosphorylation of cannabinoid receptors that could lead to differences in trafficking (Xing et al., 2011). Sex differences have also been reported in ERK and AKT pathways and their regulation in a model of schizophrenia, the neonatal ventral hippocampal lesion model, suggesting mechanisms by which altered dopamine signaling may underlie sex differences in vulnerability to schizophrenia (Bychkov et al., 2011). This unexplored area of sex differences in receptor signaling has the potential to greatly impact our knowledge of pathophysiology of diverse diseases and to transform their related therapeutics.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Valentino, Bangasser, Van Bockstaele.

References

Alonso J, Petukhova M, Vilagut G, Chatterji S, Heeringa S, Üstün TB, Alhamzawi AO, Viana MC, Angermeyer M, and Bromet E et al. (2011) Days out of role due to common physical and mental conditions: results from the WHO World Mental Health surveys. *Mol Psychiatry* 16:1234–1246.

Anisman H, Prakash P, Merali Z, and Poulter MO (2007) Corticotropin releasing hormone receptor alterations elicited by acute and chronic unpredictable stressor

challenges in stressor-susceptible and resilient strains of mice. *Behav Brain Res* 181:180–190.

Arborelius L, Owens MJ, Plotsky PM, and Nemeroff CB (1999) The role of corticotropin-releasing factor in depression and anxiety disorders. *J Endocrinol* 160:1–12.

Aston-Jones G and Cohen JD (2005) An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci* 28:403–450.

Aston-Jones G, Rajkowski J, and Cohen J (1999) Role of locus coeruleus in attention and behavioral flexibility. *Biol Psychiatry* 46:1309–1320.

Aston-Jones G, Shipley MT, and Grzanna R (1995) The locus coeruleus, A5 and A7 noradrenergic cell groups, in *The Rat Brain* (Paxinos G, ed, ed) pp 183–213, Academic Press, San Diego, CA.

Austin MC, Janosky JE, and Murphy HA (2003) Increased corticotropin-releasing hormone immunoreactivity in monoamine-containing pontine nuclei of depressed suicide men. *Mol Psychiatry* 8:324–332.

Baker DG, West SA, Nicholson WE, Ekhtor NN, Kasckow JW, Hill KK, Bruce AB, Orth DN, and Geraciotti TD, Jr (1999) Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. *Am J Psychiatry* 156:585–588.

Bale TL, Contarino A, Smith GW, Chan R, Gold LH, Sawchenko PE, Koob GF, Vale WW, and Lee KF (2000) Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. *Nat Genet* 24:410–414.

Bale TL and Vale WW (2004) CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu Rev Pharmacol Toxicol* 44:525–557.

Bangasser DA, Curtis A, Reyes BA, Bethea TT, Parastatidis I, Ischiropoulos H, Van Bockstaele EJ, and Valentino RJ (2010) Sex differences in corticotropin-releasing factor receptor signaling and trafficking: potential role in female vulnerability to stress-related psychopathology. *Mol Psychiatry* 15:877–896–904.

Bangasser DA, Reyes BA, Piel D, Garachh V, Zhang XY, Plona ZM, Van Bockstaele EJ, Beck SG, and Valentino RJ (2012) Increased vulnerability of the brain norepinephrine system of females to corticotropin-releasing factor overexpression. *Mol Psychiatry* doi: 10.1038/mp.2012.24. [Epub ahead of print].

Bangasser DA, Zhang X, Garachh V, Hanhauser E, and Valentino RJ (2011) Sexual dimorphism in locus coeruleus dendritic morphology: a structural basis for sex differences in emotional arousal. *Physiol Behav* 103:342–351.

Bayatti N, Hermann H, Lutz B, and Behl C (2005) Corticotropin-releasing hormone-mediated induction of intracellular signaling pathways and brain-derived neurotrophic factor expression is inhibited by the activation of the endocannabinoid system. *Endocrinology* 146:1205–1213.

Berridge CW and Waterhouse BD (2003) The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Brain Res Rev* 42:33–84.

Bijlsma EY, van Leeuwen ML, Westphal KG, Olivier B, and Groenink L (2011) Local repeated corticotropin-releasing factor infusion exacerbates anxiety- and fear-related behavior: differential involvement of the basolateral amygdala and medial prefrontal cortex. *Neuroscience* 173:82–92.

Bissette G, Klimek V, Pan J, Stockmeier C, and Ordway G (2003) Elevated concentrations of CRF in the locus coeruleus of depressed subjects. *Neuropsychopharmacology* 28:1328–1335.

Bohn LM and McDonald PH (2010) Seeking ligand bias: assessing GPCR coupling to beta-arrestins for drug discovery. *Drug Discov Today Technol* 7:e37–e42.

Bremner JD, Licinio V, Darnell A, Krystal JH, Owens MJ, Southwick SM, Nemeroff CB, and Charney DS (1997) Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. *Am J Psychiatry* 154:624–629.

Breslau N (2001) The epidemiology of posttraumatic stress disorder: what is the extent of the problem? *J Clin Psychiatry* 62 (Suppl 17):16–22.

- Breslau N (2002) Gender differences in trauma and posttraumatic stress disorder. *J Gen Med Specif Med* 5:34–40.
- Breslau N (2009) The epidemiology of trauma, PTSD, and other posttrauma disorders. *Trauma Violence Abuse* 10:198–210.
- Breslau N, Chilcoat HD, Kessler RC, and Davis GC (1999) Previous exposure to trauma and PTSD effects of subsequent trauma: results from the Detroit Area Survey of Trauma. *Am J Psychiatry* 156:902–907.
- Bromet E, Andrade LH, Hwang I, Sampson NA, Alonso J, de Girolamo G, de Graaf R, Demyttenaere K, Hu C, and Iwata N et al. (2011) Cross-national epidemiology of DSM-IV major depressive episode. *BMC Med* 9:90.
- Bychkov E, Ahmed MR, and Gurevich EV (2011) Sex differences in the activity of signalling pathways and expression of G-protein-coupled receptor kinases in the neonatal ventral hippocampal lesion model of schizophrenia. *Int J Neuropsychopharmacol* 14:1–15.
- Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, and De Souza EB (1996) Corticotropin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol Sci* 17:166–172.
- Chen A, Perrin M, Brar B, Li C, Jamieson P, Digrucchio M, Lewis K, and Vale W (2005) Mouse corticotropin-releasing factor receptor type 2alpha gene: isolation, distribution, pharmacological characterization and regulation by stress and glucocorticoids. *Mol Endocrinol* 19:441–458.
- Cibelli G, Corsi P, Diana G, Vitiello F, and Thiel G (2001) Corticotropin-releasing factor triggers neurite outgrowth of a catecholaminergic immortalized neuron via cAMP and MAP kinase signalling pathways. *Eur J Neurosci* 13:1339–1348.
- Csernansky JG, Dong H, Fagan AM, Wang L, Xiong C, Holtzman DM, and Morris JC (2006) Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia. *Am J Psychiatry* 163:2164–2169.
- Curtis AL, Bello NT, and Valentino RJ (2001) Endogenous opioids in the locus coeruleus function to limit the noradrenergic response to stress. *J Neurosci* 21:RC152.
- Curtis AL, Bethae T, and Valentino RJ (2006) Sexually dimorphic responses of the brain norepinephrine system to stress and corticotropin-releasing factor. *Neuropsychopharmacology* 31:544–554.
- Curtis AL, Lechner SM, Pavlovich LA, and Valentino RJ (1997) Activation of the locus coeruleus noradrenergic system by intracoeular microinfusion of corticotropin-releasing factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. *J Pharmacol Exp Ther* 281:163–172.
- Curtis AL, Leiser SC, Snyder K, and Valentino RJ (2012) Predator stress engages corticotropin-releasing factor and opioid systems to alter the operating mode of locus coeruleus norepinephrine neurons. *Neuropharmacology* 62:1737–1745.
- de Kloet ER, Joëls M, and Holsboer F (2005) Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 6:463–475.
- Dirks A, Groenink L, Bouwknicht JA, Hijzen TH, Van Der Gugten J, Ronken E, Verbeek JS, Veening JG, Dederen PJ, and Korosi A et al. (2002) Overexpression of corticotropin-releasing hormone in transgenic mice and chronic stress-like autonomic and physiological alterations. *Eur J Neurosci* 16:1751–1760.
- Figueira ML and Ouaknin S (2010) Gender-related endocrinological dysfunction and mental disorders. *Curr Opin Psychiatry* 23:369–372.
- Gaub M and Carlson CL (1997) Gender differences in ADHD: a meta-analysis and critical review. *J Am Acad Child Adolesc Psychiatry* 36:1036–1045.
- Gold PW and Chrousos GP (2002) Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol Psychiatry* 7:254–275.
- Gold PW, Goodwin FK, and Chrousos GP (1988a) Clinical and biochemical manifestations of depression: relationship to the neurobiology of stress (Part 1). *N Engl J Med* 316:348–353.
- Gold PW, Goodwin FK, and Chrousos GP (1988b) Clinical and biochemical manifestations of depression. Relation to the neurobiology of stress (2). *N Engl J Med* 319:413–420.
- Grammatopoulos DK and Chrousos GP (2002) Functional characteristics of CRH receptors and potential clinical applications of CRH-receptor antagonists. *Trends Endocrinol Metab* 13:436–444.
- Grigoriadis DE and De Souza EB (1989) Heterogeneity between brain and pituitary corticotropin-releasing factor receptors is due to differential glycosylation. *Endocrinology* 125:1877–1888.
- Groenink L, Pattij T, De Jongh R, Van der Gugten J, Oosting RS, Dirks A, and Olivier B (2003) 5-HT1A receptor knockout mice and mice overexpressing corticotropin-releasing hormone in models of anxiety. *Eur J Pharmacol* 463:185–197.
- Hammack SE, Schmid MJ, LoPresti ML, Der-Avakian A, Pellymounter MA, Foster AC, Watkins LR, and Maier SF (2003) Corticotropin releasing hormone type 2 receptors in the dorsal raphe nucleus mediate the behavioral consequences of uncontrollable stress. *J Neurosci* 23:1019–1025.
- Hauger RL, Grigoriadis DE, Dallman MF, Plotsky PM, Vale WW, and Dautzenberg FM (2003) International Union of Pharmacology. XXXVI. Current status of the nomenclature for receptors for corticotropin-releasing factor and their ligands. *Pharmacol Rev* 55:21–26.
- Hauger RL, Risbrough V, Brauns O, and Dautzenberg FM (2006) Corticotropin releasing factor (CRF) receptor signaling in the central nervous system: new molecular targets. *CNS Neurol Disord Drug Targets* 5:453–479.
- Hauger RL, Risbrough V, Oakley RH, Olivares-Reyes JA, and Dautzenberg FM (2009) Role of CRF receptor signaling in stress vulnerability, anxiety, and depression. *Ann N Y Acad Sci* 1179:120–143.
- Heinrichs SC, Min H, Tamraz S, Carmouché M, Boehme SA, and Vale WW (1997) Anti-sexual and anxiogenic behavioral consequences of corticotropin-releasing factor overexpression are centrally mediated. *Psychoneuroendocrinology* 22:215–224.
- Hillhouse EW and Grammatopoulos DK (2006) The molecular mechanisms underlying the regulation of the biological activity of corticotropin-releasing hormone receptors: implications for physiology and pathophysiology. *Endocr Rev* 27:260–286.
- Hiroi N, Wong ML, Licinio J, Park C, Young M, Gold PW, Chrousos GP, and Bornstein SR (2001) Expression of corticotropin releasing hormone receptors type I and type II mRNA in suicide victims and controls. *Mol Psychiatry* 6:540–546.
- Hoare SR, Sullivan SK, Schwarz DA, Ling N, Vale WW, Crowe PD, and Grigoriadis DE (2004) Ligand affinity for amino-terminal and juxtamembrane domains of the corticotropin releasing factor type I receptor: regulation by G-protein and non-peptide antagonists. *Biochemistry* 43:3996–4011.
- Holmes KD, Babwah AV, Dale LB, Poulter MO, and Ferguson SS (2006) Differential regulation of corticotropin releasing factor 1alpha receptor endocytosis and trafficking by beta-arrestins and Rab GTPases. *J Neurochem* 96:934–949.
- Holsboer F (1999) The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. *J Psychiatr Res* 33:181–214.
- Jaferi A and Bhatnagar S (2007) Corticotropin-releasing hormone receptors in the medial prefrontal cortex regulate hypothalamic-pituitary-adrenal activity and anxiety-related behavior regardless of prior stress experience. *Brain Res* 1186:212–223.
- Jedema HP and Grace AA (2004) Corticotropin-releasing hormone directly activates noradrenergic neurons of the locus ceruleus recorded in vitro. *J Neurosci* 24:9703–9713.
- Kendler KS, Kessler RC, Walters EE, MacLean C, Neale MC, Heath AC, and Eaves LJ (1995) Stressful life events, genetic liability, and onset of an episode of major depression in women. *Am J Psychiatry* 152:833–842.
- Kessler RC (2003) Epidemiology of women and depression. *J Affect Disord* 74:5–13.
- Kessler RC, McGonagle KA, Nelson CB, Hughes M, Swartz M, and Blazer DG (1994) Sex and depression in the National Comorbidity Survey. II: Cohort effects. *J Affect Disord* 30:15–26.
- Kohout TA and Lefkowitz RJ (2003) Regulation of G protein-coupled receptor kinases and arrestins during receptor desensitization. *Mol Pharmacol* 63:9–18.
- Leake A, Perry EK, Perry RH, Fairbairn AF, and Ferrier IN (1990) Cortical concentrations of corticotropin-releasing hormone and its receptor in Alzheimer type dementia and major depression. *Biol Psychiatry* 28:603–608.
- Lefkowitz RJ and Shenoy SK (2005) Transduction of receptor signals by beta-arrestins. *Science* 308:512–517.
- Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, and Oltsdorf T (1995) Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc Natl Acad Sci USA* 92:836–840.
- Lu A, Steiner MA, Whittle N, Vogl AM, Walser SM, Ableitner M, Refojo D, Ekker M, Rubenstein JL, and Stalla GK et al. (2008) Conditional mouse mutants highlight mechanisms of corticotropin-releasing hormone effects on stress-coping behavior. *Mol Psychiatry* 13:1028–1042.
- Magalhaes AC, Holmes KD, Dale LB, Comps-Agrar L, Lee D, Yadav PN, Drysdale L, Poulter MO, Roth BL, and Pin JP et al. (2010) CRF receptor 1 regulates anxiety behavior via sensitization of 5-HT2 receptor signaling. *Nat Neurosci* 13:622–629.
- Merali Z, Du L, Hrdina P, Palkovits M, Faludi G, Poulter MO, and Anisman H (2004) Dysregulation in the suicide brain: mRNA expression of corticotropin-releasing hormone receptors and GABA(A) receptor subunits in frontal cortical brain region. *J Neurosci* 24:1478–1485.
- Million M, Wang L, Stenzel-Poore MP, Coste SC, Yuan PQ, Lamy C, Rivier J, Bufington T, and Taché Y (2007) Enhanced pelvic responses to stressors in female CRF-overexpressing mice. *Am J Physiol Regul Integr Comp Physiol* 292:R1429–R1438.
- Nemeroff KB, Widerlöv E, Bissette GT, Walléus H, Karlsson I, Elkdung K, Kilts CD, Loosen PT, and Vale W (1984) Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 226:1342–1344.
- Oakley RH, Olivares-Reyes JA, Hudson CC, Flores-Vega F, Dautzenberg FM, and Hauger RL (2007) Carboxyl-terminal and intracellular loop sites for CRF1 receptor phosphorylation and beta-arrestin-2 recruitment: a mechanism regulating stress and anxiety responses. *Am J Physiol Regul Integr Comp Physiol* 293:R209–R222.
- Perrin MH, Donaldson CJ, Chen R, Lewis KA, and Vale WW (1993) Cloning and functional expression of a rat brain corticotropin releasing factor (CRF) receptor. *Endocrinology* 133:3058–3061.
- Rajagopal S, Ahn S, Rominger DH, Gowen-MacDonald W, Lam CM, Dewire SM, Violin JD, and Lefkowitz RJ (2011) Quantifying ligand bias at seven-transmembrane receptors. *Mol Pharmacol* 80:367–377.
- Ramtekkar UP, Reiersen AM, Todorov AA and Todd RD (2010) Sex and age differences in attention-deficit/hyperactivity disorder symptoms and diagnoses: implications for DSM-V and ICD-11. *J Am Acad Child Adolesc Psychiatry* 49:217–228, e211–213.
- Reyes BA, Fox K, Valentino RJ, and Van Bockstaele EJ (2006) Agonist-induced internalization of corticotropin-releasing factor receptors in noradrenergic neurons of the rat locus coeruleus. *Eur J Neurosci* 23:2991–2998.
- Reyes BA, Valentino RJ, and Van Bockstaele EJ (2008) Stress-induced intracellular trafficking of corticotropin-releasing factor receptors in rat locus coeruleus neurons. *Endocrinology* 149:122–130.
- Ruitenberga A, Ott A, van Swieten JC, Hofman A, and Breteler MM (2001) Incidence of dementia: does gender make a difference? *Neurobiol Aging* 22:575–580.
- Sánchez MM, Young LJ, Plotsky PM, and Insel TR (1999) Autoradiographic and in situ hybridization localization of corticotropin-releasing factor 1 and 2 receptors in nonhuman primate brain. *J Comp Neurol* 408:365–377.
- Sautter FJ, Bissette G, Wiley J, Manguno-Mire G, Schoenbachler B, Myers L, Johnson JE, Cerbone A, and Malaspina D (2003) Corticotropin-releasing factor in posttraumatic stress disorder (PTSD) with secondary psychotic symptoms, non-psychotic PTSD, and healthy control subjects. *Biol Psychiatry* 54:1382–1388.
- Sheng H, Sun T, Cong B, He P, Zhang Y, Yan J, Lu C, and Ni X (2008) Corticotropin-releasing hormone stimulates SGK-1 kinase expression in cultured hippocampal neurons via CRH-R1. *Am J Physiol Endocrinol Metab* 295:E938–E946.
- Shipley MT, Fu L, Ennis M, Liu WL, and Aston-Jones G (1996) Dendrites of locus coeruleus neurons extend preferentially into two pericoerulear zones. *J Comp Neurol* 365:56–68.

- Snyder K, Wang WW, Han R, McFadden K, and Valentino RJ (2012) Corticotropin-releasing factor in the norepinephrine nucleus, locus coeruleus, facilitates behavioral flexibility. *Neuropsychopharmacology* **37**:520–530.
- Southwick SM, Bremner JD, Rasmusson A, Morgan CA, 3rd, Arnsten A, and Charney DS (1999) Role of norepinephrine in the pathophysiology and treatment of post-traumatic stress disorder. *Biol Psychiatry* **46**:1192–1204.
- Stenzel-Poore MP, Heinrichs SC, Rivest S, Koob GF, and Vale WW (1994) Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *J Neurosci* **14**:2579–2584.
- Swinny JD and Valentino RJ (2006) Corticotropin-releasing factor promotes growth of brain norepinephrine neuronal processes through Rho GTPase regulators of the actin cytoskeleton in rat. *Eur J Neurosci* **24**:2481–2490.
- Tolin DF and Foa EB (2006) Sex differences in trauma and posttraumatic stress disorder: a quantitative review of 25 years of research. *Psychol Bull* **132**:959–992.
- Traver S, Marien M, Martin E, Hirsch EC, and Michel PP (2006) The phenotypic differentiation of locus coeruleus noradrenergic neurons mediated by brain-derived neurotrophic factor is enhanced by corticotropin releasing factor through the activation of a cAMP-dependent signaling pathway. *Mol Pharmacol* **70**:30–40.
- Vale W, Spiess J, Rivier C, and Rivier J (1981) Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* **213**:1394–1397.
- Valentino RJ, Bangasser D, Plona Z, Ding H, and Seeholzer SH (2012) The sex biased phosphoproteome: a novel approach towards understanding the molecular basis for sex differences in neuropsychiatric diseases. *Neuropsychopharmacology* **38**: S272–273. DOI: 10.1038/npp.2012.220
- Valentino RJ and Commons KG (2005) Peptides that fine-tune the serotonin system. *Neuropeptides* **39**:1–8.
- Valentino RJ and Foote SL (1987) Corticotropin-releasing factor disrupts sensory responses of brain noradrenergic neurons. *Neuroendocrinology* **45**:28–36.
- Valentino RJ and Foote SL (1988) Corticotropin-releasing hormone increases tonic but not sensory-evoked activity of noradrenergic locus coeruleus neurons in unanesthetized rats. *J Neurosci* **8**:1016–1025.
- Valentino RJ, Page M, Van Bockstaele E, and Aston-Jones G (1992) Corticotropin-releasing factor innervation of the locus coeruleus region: distribution of fibers and sources of input. *Neuroscience* **48**:689–705.
- Valentino RJ, Page ME, and Curtis AL (1991) Activation of noradrenergic locus coeruleus neurons by hemodynamic stress is due to local release of corticotropin-releasing factor. *Brain Res* **555**:25–34.
- Valentino RJ, Rudoy C, Saunders A, Liu XB, and Van Bockstaele EJ (2001) Corticotropin-releasing factor is preferentially colocalized with excitatory rather than inhibitory amino acids in axon terminals in the peri-locus coeruleus region. *Neuroscience* **106**:375–384.
- Valentino RJ and Van Bockstaele E (2008) Convergent regulation of locus coeruleus activity as an adaptive response to stress. *Eur J Pharmacol* **583**:194–203.
- Van Bockstaele EJ, Bajic D, Proudfit H, and Valentino RJ (2001) Topographic architecture of stress-related pathways targeting the noradrenergic locus coeruleus. *Physiol Behav* **73**:273–283.
- Van Bockstaele EJ, Colago EE, and Valentino RJ (1996) Corticotropin-releasing factor-containing axon terminals synapse onto catecholamine dendrites and may presynaptically modulate other afferents in the rostral pole of the nucleus locus coeruleus in the rat brain. *J Comp Neurol* **364**:523–534.
- Van Bockstaele EJ, Colago EEO, and Valentino RJ (1998) Amygdaloid corticotropin-releasing factor targets locus coeruleus dendrites: substrate for the co-ordination of emotional and cognitive limbs of the stress response. *J Neuroendocrinol* **10**: 743–757.
- Van Bockstaele EJ, Peoples J, and Valentino RJ (1999) A.E. Bennett Research Award. Anatomic basis for differential regulation of the rostralateral peri-locus coeruleus region by limbic afferents. *Biol Psychiatry* **46**:1352–1363.
- Van Pett K, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, Prins GS, Perrin M, Vale W, and Sawchenko PE (2000) Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. *J Comp Neurol* **428**:191–212.
- Violin JD and Lefkowitz RJ (2007) Beta-arrestin-biased ligands at seven-transmembrane receptors. *Trends Pharmacol Sci* **28**:416–422.
- Waterhouse BD, Devilbiss D, Fleischer D, Sessler FM, and Simpson KL (1998) New perspectives on the functional organization and postsynaptic influences of the locus coeruleus efferent projection system. *Adv Pharmacol* **42**:749–754.
- Whalen EJ, Rajagopal S, and Lefkowitz RJ (2011) Therapeutic potential of β -arrestin- and G protein-biased agonists. *Trends Mol Med* **17**:126–139.
- Wilson RS, Arnold SE, Schneider JA, Kelly JF, Tang Y, and Bennett DA (2006) Chronic psychological distress and risk of Alzheimer's disease in old age. *Neuroepidemiology* **27**:143–153.
- Xing G, Carlton J, Zhang L, Jiang X, Fullerton C, Li H, and Ursano R (2011) Cannabinoid receptor expression and phosphorylation are differentially regulated between male and female cerebellum and brain stem after repeated stress: implication for PTSD and drug abuse. *Neurosci Lett* **502**:5–9.
- Zeng J, Kitayama I, Yoshizato H, Zhang K, and Okazaki Y (2003) Increased expression of corticotropin-releasing factor receptor mRNA in the locus coeruleus of stress-induced rat model of depression. *Life Sci* **73**:1131–1139.

Address correspondence to: Rita J. Valentino, The Children's Hospital of Philadelphia, 402D Abramson Pediatric Research Center, Civic Center Blvd., Philadelphia, PA 19104. E-mail: rjv@mail.med.upenn.edu