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Sex differences in molecular and cellular substrates of stress

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Abstract

Women are twice as likely as men to suffer from stress-related psychiatric disorders, like unipolar depression and post-traumatic stress disorder. Although the underlying neural mechanisms are not well characterized, the pivotal role of stress in the onset and severity of these diseases has led to the idea that sex differences in stress responses account for this sex bias. Corticotropin-releasing factor (CRF) orchestrates stress responses by acting both as a neurohormone to initiate the hypothalamic-pituitary-adrenal (HPA) axis and as a neuromodulator in the brain. One target of CRF modulation is the locus coeruleus (LC)-norepinephrine system, which coordinates arousal components of the stress response. Hypersecretion of CRF and dysregulation of targets downstream from CRF, such as the HPA axis and LC-norepinephrine system, are characteristic features of many stress-related psychiatric diseases, suggesting a causal role for CRF and its targets in the development of these disorders. This review will describe sex differences in CRF and the LC-norepinephrine system that can increase stress-sensitivity in females, making them vulnerable to stress-related disorders. Evidence for gonadal hormone regulation of hypothalamic CRF is discussed as an effect that can lead to increased HPA axis activity in females. Sex differences in the structure of LC neurons that create the potential for hyperarousal in response to emotional stimuli are described. Finally, sex differences at the molecular level of the CRF₁ receptor that make the LC-norepinephrine system more reactive in females are reviewed. The implications of these sex differences for the treatment of stress-related psychiatric disorders also will be discussed.

Mood and anxiety disorders are debilitating mental illnesses that affect 20% population and are the leading cause of disability in the United States for younger adults (Mark et al. 2007; Bromet et al. 2011). They particularly present a challenge to women's health, because women are twice as likely to suffer from these disorders as men. Stress is a common factor in these disorders. Stressor exposure is associated with the onset and severity of the mood disorder, unipolar depression, and several anxiety disorders. In the case of the post-traumatic stress disorder (PTSD), the disease is precipitated by a traumatic event. There is some evidence to suggest that the sex bias in these diseases is due different types of stressors experienced by men vs. women. For example, men experience more physical attacks and serious accidents, while women are more likely to experience interpersonal assaults and sexual abuse (Breslau et al. 1998; Breslau 2002; Perkonig et al. 2000; Kessler et al. 1995; Olff et al. 2007; Tolin and Foa 2006). Although the type of stressor exposure likely plays a role the sex bias, there is also mounting evidence to suggest that women respond differently to stressful events than men. For example, when women and men are confronted with similar stressful events (e.g., car accidents) women are more likely to develop psychopathology (Iteke et al. 2011). Stressful life events also are more highly correlated with symptoms of anxiety and depression in women than men (Sandanger et al. 2004).

Importantly, the sex bias in PTSD remains after adjusting for the type of trauma, preexisting psychiatric disorders, and sex differences in reporting (Breslau 2009; Tolin and Foa 2006; Breslau et al. 1999). Together these data suggest that the increased incidence of stress-related psychiatric diseases in women is biologically determined.

Corticotropin releasing factor (CRF) is a 41 amino acid-containing neuropeptide that orchestrates the stress response by acting at the level of the pituitary to initiate the hypothalamic-pituitary-adrenal (HPA) axis response to stress, as well as centrally to modulate limbic and brain monoamine systems that are important in autonomic and behavioral components of the stress response (Owens and Nemeroff 1991; Valentino and Van Bockstaele 2008; Valentino and Commons 2005). Dysregulation of CRF has been linked to the pathophysiology of mood and anxiety disorders (Bremner et al. 1997; Nemeroff 1996). This review will focus on mechanisms by which sex differences in CRF function can lead to increased stress sensitivity in females. First, we will discuss how sex differences in CRF expression can lead to dysregulation of the HPA axis response to stress, an effect linked to certain types of depression and anxiety disorders. Second, sex differences in the morphology of locus coeruleus (LC) cells will be discussed as a potential determinant of emotional arousal. Finally, we describe sex differences in the function of the CRF₁ receptors in the LC-arousal system that can lead to hyperarousal, a core symptom of many stress-related diseases in females.

CRF dysregulation in stress-related psychiatric disorders

CRF is a key regulator of the HPA axis response to stress. Stressors initiate CRF release from neurons in the paraventricular nucleus of the hypothalamus (PVN), which is transported via the portal blood vessel system to the pituitary where it stimulates pituitary corticotropes to secrete adrenocorticotrophic hormone (ACTH) into the circulatory system. Circulating ACTH then initiates glucocorticoid release from the cortex of the adrenal glands. Glucocorticoids in turn act at the level of the pituitary and hypothalamus to terminate the stress response acting as negative feedback system. In addition to its role in the initiation of the HPA axis, CRF also acts centrally to regulate behavioral and autonomic responses to stress. This is thought to involve CRF-producing neurons in limbic regions such as the bed nucleus of the stria terminalis and central nucleus of the amygdala, regions which are known for mediating anxiety and emotional responses (Swanson et al. 1983; Walker et al. 2003). During stress, release of limbic CRF can modulate monoamine systems that have been implicated in mood and cognition (Rodaros et al. 2007; Wanat et al. 2008; Lodge and Grace 2005; Valentino and Van Bockstaele 2008; Valentino and Commons 2005). Although activation of both the HPA axis and central monoaminergic systems by CRF during acute stress is adaptive, the inappropriate or persistent activation of these systems can have adverse consequences leading to psychopathology.

There is evidence for hypersecretion or overactivity of CRF in patients suffering from stress-related disorders. Increased concentrations of CRF in the cerebrospinal fluid have been reported in depression and PTSD (Nemeroff et al. 1984; Baker et al. 1999; Bremner et al. 1997; Sautter et al. 2003; Banki et al. 1992). Effective treatments for depression, such as antidepressant medication and electroconvulsive shock therapy, decrease cerebrospinal fluid CRF levels, supporting the idea that increased CRF levels are responsible for depressive symptoms (Heuser et al. 1998; De Bellis et al. 1993; Nemeroff et al. 1991). Centrally, changes in CRF and CRF₁ receptor expression have been observed in the brains of patients with stress-related psychiatric disorders. Specifically, depressed subjects have a greater number CRF expressing neurons in the PVN, and higher PVN levels of CRF and CRF₁ receptor mRNA (Raadsheer et al. 1994; Wang et al. 2008). CRF expression is increased in the LC and dorsal raphe monoaminergic nuclei of depressed subjects (Austin et al. 2003;

Bissette et al. 2003). High levels of CRF also are found in the cortex of suicide victims, and this increase is accompanied by decreased CRF₁ receptor expression, which may be a compensatory response to cortical CRF hypersecretion (Merali et al. 2004). Multiple genomic studies have identified single nucleotide polymorphisms on the genes for CRF and the CRF₁ receptor that are linked to development and/or severity of depression, PTSD, and panic disorder (Smoller et al. 2003; Amstadter et al. 2011; Liu et al. 2006). Treatments for stress-related mental illnesses also support a central role for CRF in the etiology of these diseases. Anxiolytic treatments alter CRF expression in several brain regions, suggesting that this may be an important mechanism of action for these drugs (Owens et al. 1989; Owens et al. 1991). Moreover in a clinical trial, depressed patients treated with a CRF₁ receptor antagonist showed a reduction in symptoms of depression and anxiety (Zobel et al. 2000). Together these findings imply a causal role for CRF in the development of depression and certain anxiety disorders.

Sex differences in hypothalamic CRF function

In order to understand the biological basis for sex differences in stress-related psychiatric disorders, researchers have investigated sex differences in stress response systems. One of the most widely reported findings is that female rodents have higher levels of HPA axis hormones than males. Specifically, basal (i.e., unstressed) levels of the glucocorticoid, corticosterone, are elevated in female compared to male rats (Kitay 1961; Viau et al. 2005; Weinstock et al. 1998). There is a circadian rhythm to glucocorticoid release, and although both male and female rats display this rhythm, females have higher peak corticosterone levels and greater average corticosterone concentrations over a 24 h period than males (Critchlow et al. 1963). In response to stress, female rodents release more ACTH and corticosterone than males and their stress hormone levels remain elevated for a longer period of time (Livezey et al. 1985; Handa et al. 1994; Kitay 1961; Rivier 1999; Viau et al. 2005; Weinstock et al. 1998; Iwasaki-Sekino et al. 2009; Heinsbroek et al. 1991; Seale et al. 2004). In contrast to the reliable findings in rodents, reports of sex differences in ACTH and cortisol levels in humans are equivocal. Some studies find elevated stress-induced levels of these hormones in women, while others find the opposite (i.e., men are higher) or no difference (Uhart et al. 2006; Kirschbaum et al. 1999; Kirschbaum et al. 1995; Seeman et al. 2001; Kirschbaum et al. 1992; Kudielka and Kirschbaum 2005; Gallucci et al. 1993; Heuser et al. 1994; Friedmann and Kindermann 1989; Collins and Frankenhaeuser 1978). These discrepant findings are attributed to differences in the type and duration of stressor exposure, and other demographic factors such as the age of the subjects and their disease status (Kudielka and Kirschbaum 2005; Seeman et al. 2001; Paris et al. 2010).

Despite the discrepancies, the higher levels of HPA axis hormones found in female rodents and in some human studies could indicate greater activation of the HPA axis by CRF in females. In support of this, some studies report that female rodents have increased CRF expression in the PVN under basal conditions, particularly when estrogen levels are high (Viau et al. 2005; Iwasaki-Sekino et al. 2009; Duncko et al. 2001; but see Sterrenburg et al. 2012; Sterrenburg et al. 2011; Desbonnet et al. 2008). Certain stressors, such as early life stress, footshock, and psychological stress, increase CRF expression in the PVN selectively in female rats (Desbonnet et al. 2008; Iwasaki-Sekino et al. 2009). In contrast, restraint stress and chronic mild stress increase CRF mRNA in the PVN to a larger degree in males, however, this increase may not have been observed in females in some cases because of their high basal CRF expression (Sterrenburg et al. 2012; Sterrenburg et al. 2011; Duncko et al. 2001; Viau et al. 2005). Recently, sex-specific epigenetic modifications of the CRF gene have been identified following stress, underscoring the important contribution of epigenetic factors to differences in the CRF expression (Sterrenburg et al. 2012; Sterrenburg et al.

2011). Taken together these studies implicate greater CRF release from the PVN of females in their enhanced endocrine responses to stress.

Demographic data demonstrate that the higher rate of depression in women emerges after puberty and then decreases after menopause, suggesting a role for ovarian hormones (Kessler 2003). Because of this link, many studies have investigated gonadal hormone regulation of CRF expression. Gonadal steroid hormone receptors co-localize with CRF in neurons in PVN (Bao et al. 2005; Bao et al. 2006). The promoter region of the CRF gene contains both estrogen and androgen response elements, providing a means by which gonadal hormones can regulate CRF gene expression (Vamvakopoulos and Chrousos 1993; Bao et al. 2006). In rodents, estrogen stimulates CRF expression in the PVN, while androgens suppress this expression (Bohler et al. 1990; Lund et al. 2004; Haas and George 1988; Bingaman et al. 1994; Figueiredo et al. 2007). Although similar studies in humans are limited, Bao and Swaab (2007) found more hypothalamic CRF neurons in men with conditions that increased their estrogen levels above those of healthy male controls (i.e., estrogen therapy or estrogen producing tumors) (Bao and Swaab 2007). However, this same study failed to find the predicted decrease in CRF expressing neurons in women following menopause. This underscores the complexity of the interaction between sex hormones and CRF in humans.

Taken together, these studies suggest that increased hypothalamic levels of CRF in females, particularly during times of high estrogen secretion, can lead to sex differences in HPA axis hormone secretion and contribute to the sex bias in stress-related psychiatric disease. Sex differences in the HPA axis can also be attributed to other non-CRF related factors, such as increased glucocorticoid metabolism in males or impaired glucocorticoid negative feedback in females (Weiser and Handa 2009; Redei et al. 1994; Herbst et al. 1960; Troop 1959). Nonetheless, evidence for increased hypothalamic CRF expression in females certainly warrants further investigation, particularly in human studies, as a mechanism underlying female vulnerability to mood and anxiety disorders.

The LC-norepinephrine system and arousal

A core symptom of several stress-related psychiatric disorders is hyperarousal. Patients suffering from PTSD experience symptoms linked to hyperarousal, including hypervigilance (e.g., difficulty concentrating, sleeping, controlling anger, etc.) and re-experiencing (i.e., the replaying of the traumatic event) (O'Donnell et al. 2004; Southwick et al. 1999). Hyperarousal also is associated with ruminations that characterize melancholic depression (Gold et al. 1996; Gold and Chrousos 2002, 1999). The brain circuitry thought to mediate these arousal features is the LC-norepinephrine system (O'Donnell et al. 2004; Southwick et al. 1999; Gold et al. 1996; Gold and Chrousos 2002; Koob 1999).

The LC is a compact nucleus of norepinephrine-containing neurons located in the pons. A divergent efferent projection system arises from these neurons allowing for norepinephrine release throughout the neuraxis (Swanson and Hartman 1975). The LC serves as the major source of norepinephrine for the forebrain and the sole source of norepinephrine for the cortex and hippocampus (Abercrombie et al. 1988; Aston-Jones et al. 1995).

The physiological characteristics of LC neurons have led to predictions about the function of this system in behavior and in the response to stress. LC neurons discharge spontaneously (tonic) and their firing rate is positively correlated with electroencephalic (EEG) and behavioral indices of arousal (Aston-Jones and Bloom 1981a; Berridge and Foote 1991; Berridge et al. 1993). Relatively low firing rates are associated with slow wave sleep or quiet waking, and higher discharge rates are associated with more active waking (Aston-Jones and Bloom 1981a; Foote et al. 1980). A characteristic feature of LC neurons that has

suggested a role in vigilance is their activation by multimodal sensory stimuli. This results in a burst of synchronous discharge (phasic) that precedes orientation to the stimulus (Aston-Jones and Bloom 1981b; Foote et al. 1980). These observations have suggested a role of LC neurons in mediating arousal and facilitating attention towards discrete stimuli. More recent studies are refining our ideas about the role of the LC-norepinephrine system in behavior. In an elegant series of studies in which LC neurons were recorded in monkeys performing an oddball discrimination task, Aston-Jones and colleagues found that different modes of LC discharge are associated with distinct behaviors (Clayton et al. 2004; Aston-Jones and Cohen 2005). In the phasic discharge mode, LC neurons fire synchronously and are responsive to sensory stimuli. This mode of firing is associated with focused attention and staying on-task. In contrast, when LC neurons fire at a high spontaneous or tonic rate, they are less synchronous and less responsive to sensory stimuli. This mode of firing is associated with labile attention, behavioral flexibility and going off task, but also with poor performance on tasks requiring focused attention. These investigators suggested the compelling hypothesis that the LC is involved in decision processes and whether to stay on task or scan the environment in order to shift ongoing behavior to one that might be more adaptive.

The LC, CRF and stress

The LC-norepinephrine system is often activated in parallel with the HPA axis in response to stressors, and this activation is mediated at least in part by CRF (Aston-Jones et al. 1996; Page et al. 1993; Valentino and Van Bockstaele 2005). CRF axon terminals synaptically target norepinephrine neurons of the LC (Valentino and Van Bockstaele 2008; Van Bockstaele et al. 1996, 1998; Van Bockstaele et al. 1999). CRF microinfusion into the LC increases neuronal discharge rate and this is temporally correlated to an activation of cortical electroencephalographic activity (EEG), indicative of arousal (Curtis et al. 1997). That this is a direct effect of CRF on LC neurons is supported by the findings that CRF has similar effects in LC slice preparations even in the presence of tetrodotoxin (Jedema and Grace 2004). At the same time that CRF increases tonic LC discharge, it attenuates phasic sensory-evoked discharge, thereby biasing LC activity towards a high tonic–low phasic state that would favor scanning the environment and promote behavioral flexibility (Valentino and Foote 1988; Valentino and Foote 1987). Consistent with this, moderate doses of CRF administered directly into the LC increased cognitive flexibility in rodents performing the attentional set shifting task (Snyder et al. 2011). These effects of CRF to increase arousal and behavioral flexibility would be adaptive in a dynamic environment, as in acute stress.

Stressors mimic the effects of CRF on LC discharge and these can be prevented by local administration of CRF. For example, hypotensive stress increases LC tonic discharge rate, while inhibiting sensory-evoked phasic discharge, similar to the effects of CRF (Valentino and Wehby 1988; Curtis et al. 1997). Moreover, blocking CRF₁ receptors within the LC prevents these electrophysiological changes (Valentino and Wehby 1988; Valentino et al. 1991; Curtis et al. 1994). Similarly, non-noxious visceral stimuli, such as colon distention, increase tonic LC discharge via activation of CRF receptors, supporting the idea that stress-induced electrophysiological changes in LC activity are due to CRF (Lechner et al. 1997; Kosoyan et al. 2005).

Stress also alters downstream endpoints of LC activation through CRF-dependent mechanisms. For example, LC activation by hypotensive stress is temporally correlated to cortical EEG activation and this is prevented by intra-LC administration of a CRF antagonist (Page et al. 1993). This particular finding provided evidence that the arousal component of the stress response is mediated by local CRF actions within the LC. In addition to EEG endpoints, norepinephrine release and c-fos expression in forebrain targets are similarly

increased by both stress and intra-LC infusions of CRF (Page and Abercrombie 1999; Rassnick et al. 1998; Hajos-Korcsok et al. 2003). Finally, blocking CRF receptors in the LC prevents the effects of stress on several endpoints of LC activation, including expression of tyrosine hydroxylase (TH; the norepinephrine synthetic enzyme) and release of cortical norepinephrine (Melia and Duman 1991; Kawahara et al. 2000; Smagin et al. 1997).

The findings reviewed above support the concept that CRF released during stress acts as a neuromodulator to bias LC activity towards a high tonic mode of discharge. This in turn will increase arousal, decrease focused attention and promote behavioral flexibility, effects that would be adaptive in responding to an acute challenge. In this way, the LC plays an important role in mediating a cognitive limb of the stress response. However, if this system is engaged inappropriately or if the response continues long after the stress is terminated, these effects would be maladaptive.

CRF communication with the LC

The nuclear core of the LC where norepinephrine-containing cell bodies are clustered is only moderately innervated by CRF-containing fibers, which derive from Barrington's nucleus, the PVN, and the nucleus paragigantocellularis (Reyes et al. 2005; Valentino et al. 1992; Valentino et al. 1996). In contrast, a dense CRF terminal field lies within the dorsolateral peri-coerulear region (peri-LC) into which LC dendrites extend for several hundred microns (Shipley et al. 1996; Valentino et al. 1992). CRF projections from the PVN, as well as limbic regions, including the central nucleus of the amygdala and the bed nucleus of the stria terminalis, preferentially innervate the dendrites that extend into this peri-LC zone (Van Bockstaele et al. 1998; Van Bockstaele, 1999 #442). Because the limbic system is critical for coordinating emotional responses to stress, this limbic input into the peri-LC may be an anatomical substrate for an arousal response to emotional events. This contrasts with CRF regulation of the LC core which derives more from autonomically-related nuclei, including Barrington's nucleus and the nucleus paragigantocellularis (Valentino et al., 1992; 1996).

Ultrastructural studies revealed that CRF-immunoreactive axon terminals form synaptic specializations with LC dendrites in both the core and peri-LC regions (Van Bockstaele et al. 1996). The majority of these synapses are asymmetric, the type associated with excitatory neurotransmission (Van Bockstaele et al. 1996). Thus, it is not surprising that many CRF-containing axon terminals co-localize glutamate, although co-localization with enkephalin and GABA also is occasionally observed (Tjoumakaris et al. 2003; Valentino and Van Bockstaele 2001). Given the evidence for co-localization of CRF and GABA in the extended amygdala, it is possible that CRF-GABA terminals arise from there, whereas the CRF-glutamate axon terminals arise from other CRF-containing neurons (Day et al. 1999). Some CRF axon terminals are apposed to unlabeled terminals that form synaptic specializations with LC dendrites. This may be the structural basis for indirect presynaptic modulation of LC activity (Van Bockstaele et al. 1996).

Sexual dimorphism in LC structure

Given the role of the LC in mediating the arousal component of the stress response and that hyperarousal is a core symptom of stress-related disorders, sexual dimorphism of LC neurons could contribute to sex differences in these diseases. Some studies have found that LC neurons are more numerous in female compared to male rats (Guillamon et al. 1988; Garcia-Falgueras et al. 2006; Garcia-Falgueras et al. 2005). However, this did not generalize across rat strains (Garcia-Falgueras et al. 2006; Garcia-Falgueras et al. 2005). Convergent findings from our laboratory suggest that LC dendrites are more extensive, more complex and may process more information in female rats compared to males (Bangasser et al. 2011). An examination of TH immunoreactivity revealed that LC dendrites of female rats were

denser and extended further into the peri-LC region than those of male rats (Bangasser et al. 2011). Morphological analysis of individual juxtacellularly labeled LC neurons confirmed that LC dendritic trees of females vs. males were longer and had more branch points and ends (Bangasser et al. 2011). Furthermore, Sholl analysis revealed that LC dendrites of females had a more complex pattern of branching than dendrites of males. Immunoreactive labeling for synaptophysin, a synaptic vesicle protein, was also greater in females, particularly in the dorsolateral peri-LC where limbic afferents terminate, indicating that LC dendrites of females receive more input (Bangasser et al. 2011).

Sex differences in LC dendritic structure are particularly relevant to stress because of the topographical organization of LC afferents within the LC and peri-LC region described above. The enhanced extension of LC dendritic trees into the peri-LC and their greater complexity increases their probability of contacting limbic afferents that relay emotion-related information. Importantly, contacts between CRF terminals and LC dendrites occur predominantly in the peri-LC, so the receipt of CRF inputs would be greater within this zone. Enhanced synaptophysin expression also suggests that the LC of females receives more inputs from afferent structures. Thus, the LC of female rodents appears to be structured to process more information, particularly emotion-related information that may be carried in part by CRF. This structure would support a greater arousal response to emotional stimuli. Consistent with this idea, imaging has shown that noxious stimuli produce a greater activation of an emotional-arousal circuit that is comprised of the amygdala and LC in women compared to men (Labus et al. 2008).

Sex differences in CRF₁ receptor signaling

In addition to sex differences in LC structure, there are also functional sex differences in LC neuronal responses to CRF. A comparison of LC spontaneous and sensory-evoked discharge rates in male and female rats revealed no difference (Curtis et al., 2006). However, LC activation by hypotensive challenge, which activates LC neurons through CRF, was greater in female rats. Notably, this effect was unrelated to adult hormonal status of either males or females. Sex differences in LC responses to hypotension were attributed to differences in postsynaptic sensitivity to CRF because the CRF dose-response curve for LC activation was shifted to the left in females compared to males (Curtis et al. 2006).

A history of prior stress is an important determinant of LC responses to CRF. For example, prior CRF administration desensitizes LC neurons to subsequent CRF administration, and prior exposure to auditory or hypotensive stress produce cross-desensitization to a high dose of CRF (Conti and Foote 1995, 1996; Conti et al. 1997). In male rats, prior footshock or swim stress produce a complex change in the CRF dose-response curve for LC activation such that the linear portion of the curve is shifted to the left (indicative of sensitization) and the maximum is decreased (Curtis et al. 1995; Curtis et al. 1999; Curtis et al. 2006). Interestingly, this effect of prior stress is seen only in males. Thus, prior swim stress had little effect on the CRF dose response curve in females, perhaps because they were already at ceiling levels for CRF activation (Curtis et al. 2006).

The increased sensitivity of female LC neurons to CRF is a postsynaptic effect seen as a parallel shift to the left in the CRF dose-response curve in females compared to males and therefore is likely due to increased CRF receptor signaling. The complex shift in the CRF dose response curve seen only in males following stress, also suggested sex differences at the level of CRF receptor signaling. CRF activation of LC neurons is mediated by CRF₁ receptors (Jedema and Grace 2004; Schulz and Lehnert 1996). These are G-protein coupled receptor (GPCR) that preferentially signal through the cyclic adenosine monophosphate (cAMP) pathway by coupling to the GTP-binding protein Gs, although they can couple to

other G-proteins and activate other signaling cascades (Chen et al. 1986; Battaglia et al. 1987; De Souza 1995; Grammatopoulos et al. 2001). The CRF-induced increase in LC neuronal firing requires CRF₁ receptor activation of the cAMP signaling cascade, which is then thought to increase the firing rate by phosphorylating potassium channels (Jedema and Grace 2004). Therefore, the increased CRF sensitivity of LC neurons in females could be due to sex differences in cAMP signaling. In support of this, pretreatment with a cAMP antagonist (Rp-cAMP-S) revealed that the majority of the LC response to CRF in unstressed female rats was cAMP mediated, whereas in unstressed males only 50% of the LC response to CRF was cAMP-mediated (Bangasser et al. 2010). In stressed males, their sensitized response to CRF was completely dependent on cAMP (Bangasser et al. 2010). CRF₁ receptor immunoprecipitation studies from cortical tissue confirmed sex differences in CRF₁-Gs coupling that were suggested by the LC electrophysiology studies. Furthermore, they suggested that sex differences in LC responses to CRF were the result of differential coupling of the Gs protein to the CRF₁ receptor (Bangasser et al. 2010). Thus, in the unstressed condition CRF₁-Gs coupling, as determined by the amount of Gs pulled down with CRF₁ receptor, was greater in unstressed females compared to males and this was seen in both intact and ovariectomized females. Following swim stress, CRF₁-Gs coupling increased in males only and this increased to a magnitude that matched that of unstressed females. These sex differences in CRF₁-Gs coupling mirrored sex differences in LC electrophysiology and also were not dependent on adult hormonal status as they were seen with both ovariectomized and intact rats. It is noteworthy that these were the first data to link sex differences in physiology to sex differences in GPCR coupling and signaling.

Sex differences in CRF₁ receptor signaling could potentially play a role in the sexually dimorphic LC dendritic morphology described above. In addition to its effects on LC physiology, CRF can regulate the morphology of LC dendrites. For example, CRF increases neurite outgrowth in LC-like CATHa cells (Cibelli et al. 2001). Additionally, CRF accelerated the growth and branching of LC dendrites in organotypic slice cultures (Swinny and Valentino 2006). This effect was CRF₁ receptor mediated and required the activation protein kinase A (PKA), a downstream target of cAMP activation (Swinny and Valentino 2006). The greater CRF₁-Gs association in females is consistent with the more extensive and complex LC dendritic tree. However, direct evidence for causality between CRF and sex differences in LC dendritic morphology has yet to be established.

Sex differences in CRF₁ receptor trafficking

Excessive CRF is thought to occur in stress-related psychiatric diseases. However, cellular mechanisms can protect against excessive stimulation of the CRF₁ receptor. Homologous desensitization and internalization, a process by which receptors are trafficked from the cell membrane to the cytosol, enable CRF receptive cells to regulate their responses to excessive ligand or agonist binding (Krupnick and Benovic 1998; Premont 2005; Lefkowitz 1998). Desensitization of the CRF₁ receptor is initiated by GRK3 phosphorylation of a serine or threonine residue on the carboxy terminus of the receptor (Oakley et al. 2007; Teli et al. 2005). This promotes recruitment and binding of β -arrestin2, a protein that uncouples the receptor from its G-protein and targets it to clathrin-coated pits for endocytosis (Hauger et al. 2009; Holmes et al. 2006; Oakley et al. 2007). Once internalized the receptor can be recycled back to the membrane or degraded. Both swim stress and intra-LC CRF administration induce CRF₁ receptor internalization in LC dendrites of male rats (Bangasser et al. 2010; Reyes et al. 2006; Reyes et al. 2008). Receptor internalization is apparent at 1 and 24 hours after swim stress, and by 24 hours many receptors are associated with multivesicular bodies that target receptors for degradation (Reyes et al. 2008). This cellular event correlates with a decrease in the maximum magnitude of LC activation produced by CRF. CRF₁ receptor desensitization and internalization in LC neurons of male rats could

protect against excessive LC activation by CRF during conditions of repeated stress or CRF hypersecretion.

Notably, prior exposure to swim stress did not decrease the maximum magnitude of LC activation by CRF in females, suggesting that CRF₁ receptors fail to desensitize and/or internalize in females following stress (Curtis et al. 2006). An immunoelectron microscopy study confirmed differences in internalization (Bangasser et al. 2010). Unlike stressed male rats that had a high proportion of internalized receptors in their LC dendrites, in stress female rats the majority of CRF₁ receptors were associated with the plasma membrane. Interestingly, in unstressed females, a substantial proportion of CRF₁ receptors were cytoplasmic, suggesting that stress may actually recruit CRF₁ receptors to the plasma membrane in females. This sex difference in CRF₁ receptor trafficking could be attributed to sex differences in CRF₁ receptor association with β -arrestin2 (Bangasser et al. 2010). In the unstressed condition, CRF₁ receptor association with β -arrestin2 was comparable in both sexes. Swim stress increased CRF₁- β -arrestin2 association in male rats, consistent with the role of β -arrestin2 in the initiation of internalization. In contrast, swim stress did not affect CRF₁ association with β -arrestin2 in females, which remained comparable to the unstressed state. This explains the inability of CRF₁ receptors to internalize in stressed females. As for CRF₁-Gs coupling, the differences in association with β -arrestin2 were unrelated to adult hormonal status. Together the results suggested that the decreased ability of CRF₁ receptors in females to associate with β -arrestin2 compromises the process of CRF₁ receptor internalization and this is expressed physiologically as an inability to decrease the maximal CRF response after prior stress. The loss of this important cellular adaptation is another potential mechanism that confers vulnerability to excessive CRF in females and particularly one that could contribute to the core symptom of hyperarousal. It is noteworthy that the decreased ability of CRF₁ receptors in females to associate with β -arrestin2 also explains the enhanced association of CRF₁ receptors with Gs because β -arrestin2 sterically hinders binding of Gs to GPCRs.

In addition to sex differences in β -arrestin2 binding, sex differences in other downstream regulators of receptor trafficking and recycling may also critically establish sex-specific CRF₁ receptor localization. In cell culture, internalized human CRF₁ receptors are transited via the GTPase Rab5 to early endosomes and then rapidly recycled via a Rab4-mediated endosomal pathway (Holmes et al. 2006). Future studies investigating sex differences in these GTPases may demonstrate, for example, that decreased Rab4 activity in males accounts for the CRF₁ receptor internalization that persists 24 h after stressor exposure.

Differences in association of CRF₁ receptors with G proteins and β -arrestin2 may suggest structural differences in the receptor. These could include sex differences in agonist-induced receptor conformation or other post-translational modifications, such as phosphorylation of the carboxy tail. Although no sex differences were found in threonine phosphorylation, this does not preclude differences in serine or tyrosine phosphorylation or in other post-translational modifications (e.g., sulfation, glycosylation, etc.) (Bangasser et al. 2010). Alternatively, sex differences in G proteins and β -arrestin2 themselves or other intercellular regulators may establish these effects.

Sex Biased Signaling

The molecular and cellular sex differences described above would render females more sensitive to acute stress and less able to adapt to chronic stress or excess CRF. New knowledge about the function of β -arrestins in cells suggests even broader implications of the findings. In addition to its role in receptor internalization, β -arrestin2 can also activate several G-protein-independent signaling cascades by scaffolding signaling molecules,

including mitogen-activated protein kinase (e.g., ERK2, JNK3, and p38), tyrosine kinases (e.g., c-SRC, Hck, Fgr, Yes), as well as AKT, PI3 kinase and RhoA (for review see, Lefkowitz and Shenoy 2005; Violin and Lefkowitz 2007; DeWire et al. 2007; Shukla et al. 2011). The decreased ability of female CRF₁ receptors to associate with β -arrestin2 and increased ability to associate with Gs suggests that CRF₁ receptor signaling is sex biased, such that in female neurons there is a bias away from β -arrestin2-related signaling and towards Gs-related signaling. In male neurons the pattern is opposite. In this scenario, CRF released during stress would engage different signaling pathways in males and females, resulting in unique cellular functions that translate to distinct behavioral and/or pathological responses (Fig. 1). Consequences of these sex differences would be magnified when CRF is in excess as has been proposed in depression and post-traumatic stress disorder. In these conditions, sex differences in the signaling cascades engaged by CRF could underlie sex differences in the neurobehavioral expression of the disease.

Sex biased CRF₁ receptor signaling has important therapeutic implications for novel compounds that can shift the bias of CRF₁ receptor signaling from Gs- to β -arrestin2-related pathways. By shifting CRF signaling towards a β -arrestin2 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 towards specific pathways in an effort to promote efficacy for desired effects while diminishing adverse effects (Whalen et al. 2011). The angiotensin II receptor is being targeted in this way to take advantage of the anti-apoptotic effects that are mediated through β -arrestin, while at the same time hindering the G-protein mediated effects that result in hypertension (Ahn et al. 2004; Violin et al. 2006; Violin and Lefkowitz 2007; Revankar et al. 2004). Biased agonists such as these are currently available for the angiotensin II and β -adrenergic receptors (Wei et al. 2003; Ahn et al. 2004; Whalen et al. 2011; Drake et al. 2008). Because CRF₁ receptor mediated Gs-cAMP signaling increases LC firing rate and arousal, pharmaceuticals aimed at shifting signaling from G-protein mediated to β -arrestin-mediated may reduce the hyperarousal symptoms associated with stress-related psychiatric diseases.

A potential role for hormones in sex differences in the LC

Most sex differences are established by gonadal hormones. Although fluctuating gonadal hormones directly regulate CRF expression, they do not appear to modulate sex difference in CRF₁ receptor function or sex differences in LC dendritic morphology. Electrophysiological responses of LC neurons to endogenous CRF elicited by hypotensive stress were no different in intact vs. castrated males, suggesting no contribution of circulating testosterone to this effect (Curtis et al. 2006). In female rats, heightened electrophysiological responses to endogenous CRF were similar under several hormonal conditions tested: ovariectomy (removal of the ovaries), ovariectomy with estrogen plus progesterone replacement, and intact cycling females (Curtis et al. 2006). Consistent with this, there were no sex differences in CRF₁ receptor binding of Gs or β -arrestin2 in cycling vs. ovariectomized females, further indicating that circulating ovarian hormones do not contribute to these effects (Bangasser et al.). With respect to dendritic morphology, there was no change in LC dendritic density or the size of the LC dendritic field at different stages of the estrous cycle of females, suggesting that these effects are not linked to circulating estrogen and/or progesterone (Bangasser et al. 2011).

Although sex differences in CRF₁ receptor function and dendritic morphology appear unrelated to adult hormonal status, it remains possible that gonadal hormone surges that occur during the perinatal or pubertal period establish these effects. During the perinatal period, testosterone transiently rises masculinizing the male brain (Swerdlow et al. 1992).

This perinatal testosterone surge can establish sex difference in the HPA axis (Seale et al. 2005b; Seale et al. 2005a; McCormick et al. 1998). This surge also masculinizes the volume and neurochemistry of the bed nucleus of the stria terminalis and density of dendritic spines in the hippocampus, effects linked to sex differences in the modulation of learning by stress (del Abril et al. 1987; Han and De Vries 2003; Bangasser and Shors 2008; Leuner and Shors 2004; Dalla et al. 2009; Bangasser et al. 2005). In addition to the perinatal surge, a second protracted hormonal surge occurs during puberty that establishes sex differences in the brain and some behaviors (e.g., ingestive and sexual behaviors) (Swithers et al. 2008; Schulz et al. 2004; Schulz et al. 2009). Evuarherhe (2009) demonstrated that the pubertal androgen surge is required for programming HPA axis sensitivity to testosterone in adulthood, underscoring the importance of this second surge to the masculinization of stress responses (Evuarherhe et al. 2009). As sex differences in depression appear after puberty, it is likely that the pubertal increase in gonadal hormones determines the sex bias in this disease.

In some cases sex differences occur independent of gonadal hormones and instead arise from differential representation of genes on the X and Y chromosomes in non-gonadal cells (for review see, Arnold 2009; Arnold and Chen 2009). For example, sex differences in nociceptive responses are not related to surges in gonadal hormones or circulating hormone levels, but rather are established by genes on the X or Y chromosomes (Gioiosa et al. 2008). Similar direct genetic effects could also contribute to the sex differences in CRF₁ receptor function or LC dendritic morphology.

Sex differences in CRF function: Overview and implications

During a stressful event, the coordinated release of CRF in distinct brain regions affects multiple systems resulting in the integration of endocrine, behavioral and autonomic responses. Among the targets of CRF, the HPA axis and the LC-norepinephrine system mediate certain endocrine and arousal responses, respectively. Although short-term activation of these systems is adaptive in dealing with an immediate life-threatening challenge, their persistent activation is pathological and thought to underlie the dysregulated HPA axis and hyperarousal that are core components of several mood and anxiety disorders. Because these disorders occur twice as frequently in women as in men, here we detailed how sex differences CRF function at the level of the hypothalamus and LC contribute to the sex bias in these diseases. First, we described how estrogen can increase hypothalamic CRF expression resulting in greater HPA axis activation in females. Second, we focused on how sex differences at three levels of the LC-norepinephrine system could predispose females to hyperarousal, a core feature of several psychiatric diseases (Fig. 2). Specifically, the longer and more complex LC dendritic tree of females would allow for enhanced limbic input, forming a structural basis for heightened arousal in response to emotional events in females. At the level of the CRF₁ receptor, increased CRF₁ receptor coupling to Gs in females would result in female LC neurons that more sensitive to low levels of CRF. The lack of CRF₁ receptor association with -arrestin2 and compromised internalization following stress in females would render female LC neurons less adaptable to conditions of CRF hypersecretion, as are thought to occur during chronic stress, depression, and PTSD. Together, these sex differences would enhance CRF function in the hypothalamic and LC systems of females vs. males. Thus, when confronted with extreme or prolonged stressor exposure, CRF-mediated endocrine and arousal systems would be more likely to shift into a dysregulated state in females.

The focus of this review was on sex differences in the HPA and LC stress response systems. However, CRF has actions in other brain regions that are implicated in behavioral and autonomic aspects of the stress response and in symptoms of stress-related psychiatric disorders. It is possible that the sex differences described here for the LC extend, at least in

part, to these components of the CRF system to further contribute to sex differences in other aspects of the stress response.

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 is more likely in females, CRF antagonists may be more effective in women than men. However, sex differences in the CRF₁ receptor could translate to a sex difference in drug efficacy. The fact that CRF₁ receptors in females readily bind Gs, but fail to bind β -arrestin2, suggests a conformational change in the CRF₁ receptors of females vs. males. This difference in receptor conformation may alter the ability of CRF antagonists to bind, potentially affecting how these compounds would work in women. If this idea is supported, it would suggest that gender-based pharmacotherapies that consider sex differences in pharmacodynamics would be required to effectively treat stress-related psychiatric disorders in both men and women. Finally, these data would underscore the importance of including female as well as male subjects in preclinical pharmaceutical research.

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Figure 1.

This schematic depicts the predicted model of sex biased signaling. In males the CRF₁ receptor associates with β-arrestin2, which sterically hinders Gs association and biases signaling towards β-arrestin2-related pathways such as NFκB, AKT, ERK, and Src. In females, the CRF₁ receptor does not associate with β-arrestin2 as well and is more highly coupled to Gs, which signals through cAMP and sometimes ERK. Note that in both males and females CRF activates the receptor, but the signaling events are unique because of sex specific interactions of CRF₁ receptors with binding partners. The differential cellular responses can translate to different physiological and behavioral responses to the same stressor and different pathology.

Figure 2.

This schematic illustrates sex differences in the LC-arousal system. The images on the left depict LC neurons with dendrites (blue) receiving afferent inputs (in green). Because the LC dendritic tree is longer and more complex in females, they are more likely to receive projections from afferent structures that terminate in the peri-LC. The images on the right depict magnified views of LC dendrites in order to illustrate sex differences in the CRF₁ receptor coupling and trafficking. In males, CRF₁ receptors (red) associate with β -arrestin2 following ligand binding and become localized in the cytoplasm. CRF₁ receptors of females are highly coupled to Gs and following ligand binding they are predominantly found on the plasma membrane.