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Sex Differences in the Locus Coeruleus-Norepinephrine System and its Regulation by Stress

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Abstract

Women are more likely than men to suffer from post-traumatic stress disorder (PTSD) and major depression. In addition to their sex bias, these disorders share stress as an etiological factor and hyperarousal as a symptom. Thus, sex differences in brain arousal systems and their regulation by stress could help explain increased vulnerability to these disorders in women. Here we review preclinical studies that have identified sex differences in the locus coeruleus (LC)-norepinephrine (NE) arousal system. First, we detail how structural sex differences in the LC can bias females towards increased arousal in response to emotional events. Second, we highlight studies demonstrating that estrogen can increase NE in LC target regions by enhancing the capacity for NE synthesis, while reducing NE degradation, potentially increasing arousal in females. Third, we review data revealing how sex differences in the stress receptor, corticotropin releasing factor 1 (CRF₁), can increase LC neuronal sensitivity to CRF in females compared to males. This effect could translate into hyperarousal in women under conditions of CRF hypersecretion that occur in PTSD and depression. The implications of these sex differences for the treatment of stress-related psychiatric disorders are discussed. Moreover, the value of using information regarding biological sex differences to aid in the development of novel pharmacotherapies to better treat men and women with PTSD and depression also is highlighted.

Keywords

corticotropin releasing hormone; sex differences; arousal; post-traumatic stress disorder; major depression; noradrenergic

1. Introduction

Women are roughly twice as likely as men to suffer from certain psychiatric disorders, such as posttraumatic stress disorder (PTSD) and major depression (Breslau, 2002; Freedman et al., 2002; Kessler, 2003; Kessler et al., 2012; Tolin and Foa, 2006). In addition to their

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shared sex bias, PTSD and depression are characterized by symptoms of hyperarousal that include: irritability, restlessness, agitation, sleep disturbance, and an inability to concentrate (American Psychiatric Association, 2013). There is some evidence that these hyperarousal symptoms are more pronounced in women. For example, women with these disorders suffer from insomnia more often than men (Hall et al., 2000; Kobayashi and Mellman, 2012). Additionally, ruminations, which are associated with high arousal states (Pedersen et al., 2011; Thomsen et al., 2003), are more common in depressed women than men (Mezo and Baker, 2012; Nolen-Hoeksema et al., 1999). One possible explanation for these data is that sex differences in brain arousal centers predispose women to disorders with hyperarousal as a core symptom. One candidate region for such sex differences is the locus coeruleus (LC), which regulates levels of arousal by releasing norepinephrine (NE) into forebrain regions (Aston-Jones and Cohen, 2005; Berridge and Waterhouse, 2003). A focus of this review will be to highlight preclinical literature revealing mechanisms by which estrogens can increase NE levels in LC target regions, perhaps contributing to hyperarousal in females.

This review also will detail the literature revealing sex differences in LC regulation by stress. Clinically, stress is of interest because it is linked to the etiology of PTSD and depression. In particular, the development of these disorders is attributed, at least in part, to the hypersecretion of the stress-related neuropeptide, corticotropin releasing factor (CRF; Hauger et al., 2009; Hauger et al., 2012; Nemeroff, 1996). Although CRF is primarily known for its role in initiating the hypothalamic pituitary adrenal axis in response to stress (Vale et al., 1981), it is the central effects of CRF that are thought to regulate behavioral stress responses and contribute to the symptoms of these “stress-related” psychiatric disorders (Bale and Vale, 2004; Bangasser and Kawasumi, 2015; Hauger et al., 2009; Owens and Nemeroff, 1991; Valentino and Van Bockstaele, 2002). There is evidence that the LC, in particular, is a target of CRF hypersecretion in depressed patients (Austin et al., 2003). Moreover, it has been proposed that subtypes of depression with symptoms of hyperarousal are attributable to a combined high CRF and high NE state (Gold and Chrousos, 1999; Gold and Chrousos, 2002; Koob, 1999). These studies, along with higher rates of stress-related psychiatric disorders in women, have prompted basic research revealing increased female LC neuronal sensitivity to CRF and the mechanisms underlying this effect (Bangasser et al., 2010; Bangasser et al., 2013; Curtis et al., 2006). These studies will be discussed, as will their implications for facilitating the treatment of stress-related psychiatric disorders in both men and women.

2. Sex differences in LC structure

2.1 Sex difference in LC neuronal number

The LC comprises a cluster of noradrenergic containing neurons located in the rostral rhombencephalic tegmental area within the pons (Aston-Jones, 2004). Despite its relatively small size (~2200 neurons in the adult rat), the LC has a wide efferent projection system that allows for regulation of brain regions throughout the neuroaxis (Aston-Jones, 2004; Guillemon et al., 1988; Swanson and Hartman, 1975). In fact, the LC is the major source of NE for the forebrain and the only NE source for the cortex and hippocampus (Abercrombie et al., 1988; Aston-Jones, 2004; Morrison et al., 1978).

It has been reported that the LC of adult female rats is larger than that of adult males (Guillamon et al., 1988; Luque et al., 1992; Pinos et al., 2001), a difference that may affect the regulation of target regions (Fig. 1). This increase in size occurs because female rats have more NE containing neurons than male rats (Guillamon et al., 1988; Luque et al., 1992; Pinos et al., 2001). This sex difference emerges during development and, unlike sex differences in other structures (e.g., the sexually dimorphic nucleus of the preoptic area and the bed nucleus of the stria terminalis; (Chung et al., 2000; Davis et al., 1996), it is not attributable to a sex difference in apoptosis (Pinos et al., 2001). Instead, it is primarily attributed to LC neurogenesis that continues during puberty in females but not in males (Pinos et al., 2001). It is not fully understood how this sex difference is established or why the neurogenesis stops after puberty in females, but the presence of ovarian hormones throughout development is thought to play a role (Guillamon et al., 1988; Pinos et al., 2001). There is evidence for a different mechanism that masculinizes the male LC, involving prenatal testosterone (Pinos et al., 2001; Rodriguez-Zafra et al., 1993). Future studies are needed to further clarify the role of gonadal hormones in establishing sex differences in LC development.

The sex difference in rodent LC size and neuronal number is dependent on strain (Garcia-Falgueras et al., 2005; Garcia-Falgueras et al., 2006). Specifically, sex differences in LC size are observed in the ancestral Wistar strain, but not in the Long-Evans strain, an effect attributed to a loss of the sex difference as a result of inbreeding for other traits (Garcia-Falgueras et al., 2005; Garcia-Falgueras et al., 2006). Currently, behavioral data on the consequences of sex differences in LC size in Wistar, but not Long-Evans rats is lacking, but future studies addressing this issue could illuminate the consequences of a larger LC. Other studies suggest that the sex difference in LC size may be subregion specific, as the dorsal ascending projection zone of the LC is larger in male than female Sprague–Dawley rats (Babstock et al., 1997)

The implications of these nuanced sex differences in LC rodent size for human psychiatric diseases are unclear. Although, to our knowledge, no one has directly compared LC size in humans, two studies conducted separately by the same group reported the LC size as ~15,700 neurons in men and ~18,300 neurons in women, revealing a structural LC sex difference in humans (Busch et al., 1997; Ohm et al., 1997). These data suggest that the sex difference in LC size found in at least some rodent strains does, in fact, correspond to a similar phenomenon in humans. While the consequences of a larger LC in females is not entirely clear, it could increase female's capacity for NE production and release.

2.2 Sex differences in LC dendritic morphology

In addition to sex differences in LC neuronal number, sex differences in the morphology of LC dendrites have also been identified. The majority of LC inputs innervate dendrites that extend far into the pericoerulear region (peri-LC; Shipley et al., 1996; Van Bockstaele et al., 2001). These dendrites, which expand into the ventromedial and dorsolateral peri-LC regions of female rats are denser than those of males (Bangasser et al., 2011). Further evidence for the complexity of female LC dendrites arises from morphological analysis of individual LC neurons, revealing that the LC dendritic trees of females are longer and more

complex than those of males (Fig. 1; Bangasser et al., 2011). The more extensive dendrites in females appear to receive more synaptic input, as immunoreactivity for synaptophysin, a synaptic vesical protein, was greater in the LC of female rats than males, particularly in the dorsolateral peri-LC region (Bangasser et al., 2011). The sex difference in LC dendrites could bias the type of afferent information that the female LC receives. Although the information from the relatively few afferents that innervate the dendrites in the nuclear core of the LC (e.g., Barrington's nucleus and the nucleus paragigantoceullaris; Aston-Jones et al., 1986; Valentino et al., 1996) is predicted to be similar in males and females, the longer and more complex dendritic trees found in females could increase contacts with afferents that synapse in the peri-LC region. This could translate into an increase in the amount of autonomic and nociceptive information coming into the ventromedial peri-LC from the nucleus of the solitary tract and the periaqueductal gray, respectively, that females receive (Van Bockstaele et al., 2001). Additionally, the sex difference in dendritic structure in the dorsolateral peri-LC could bias females to receive more CRF-containing afferents from limbic regions, including the central nucleus of the amygdala and the bed nucleus of the stria terminalis, which terminate in this region (Van Bockstaele et al., 1998; Van Bockstaele et al., 2001). This structure may be particularly relevant for the sex bias in stress-related psychiatric disorders, as it could provide a substrate for increased arousal in response to emotional events in females. In support of this idea, an imaging study found that noxious stimuli produced greater activation of the emotional-arousal circuit, which includes the amygdala and LC, in women when compared to men (Labus et al., 2008). Imaging studies examining the activation of this circuit following exposure to emotional stimuli in women and men could be designed to further test this hypothesis.

3. Regulation of NE synthesis, degradation, and receptors by estrogens

3.1 Estrogens increase NE in LC terminal regions

As noted, the LC has a wide efferent projection system through which it can release NE into the forebrain (Aston-Jones, 2004; Swanson and Hartman, 1975). It is through regulation of forebrain target regions that the LC alters states of arousal, attention, and vigilance (Chamberlain and Robbins, 2013; Sara, 2009; Szabadi, 2013). Thus, sex differences in LC-induced NE release could result in sex differences in these functions. There is evidence from microdialysis studies that ovarian hormones regulate NE levels in LC terminal regions (Fig. 2). For example, in the hypothalamus, NE levels change across the rat estrous cycle such that they are increased during the proestrus phase, when levels of ovarian hormones are higher (Selmanoff et al., 1976). This effect may be mediated by the LC as well as the A1 and A2 noradrenergic cell groups in the medulla that densely project to the hypothalamus (Aston-Jones, 2004). Estrogens appear to be key players in NE regulation because estradiol treatment increases norepinephrine levels in the ventral hippocampus, cortex, and hypothalamus of ovariectomized female rats (Alfinito et al., 2009; Lubbers et al., 2010). Effects in the hippocampus and cortex are mimicked by activation of either estrogen receptor subtype (ER), ER α and ER β (Lubbers et al., 2010). Because both subtypes are expressed in the LC (Mitra et al., 2003; Shughrue et al., 1997), the increase of NE in target regions could result from direct estrogenic effects on this nucleus.

3.2 Estrogens increase NE synthesis and decrease NE degradation

Ovarian hormones have been reported to increase NE levels in LC target regions via presynaptic modulation of NE release (Vathy and Etgen, 1988). This can occur through estrogen regulation of NE biosynthetic enzymes (Serova et al., 2002). For example, the expression of tyrosine hydroxylase (TH), which catalyzes the conversion of tyrosine to L-dihydroxyphenylalanine (DOPA), a precursor to NE, is increased by estradiol treatment of gonadectomized animals (Fig. 2; Maharjan et al., 2005; Pau et al., 2000; Serova et al., 2002; Serova et al., 2004). Given that TH is the rate limiting enzyme for catecholamine synthesis, estradiol-induced increases in TH could enhance the capacity for NE production (Serova et al., 2004). The effects of estrogens on TH appear to be limited to short-term estradiol treatments that mimic the preovulatory surge, as chronic estradiol treatment fails to alter TH expression in the LC (Serova et al., 2004; Tseng et al., 1997). This result then suggests that it is the cyclical nature of estrogen release that is critical, which is consistent with the finding that female rats have greater activation of TH neurons in the proestrus phase of their cycle when estrogens are higher (Maharjan et al., 2005). Although less studied, there also is evidence that estradiol treatment in ovariectomized female rats can increase the expression of dopamine β -hydroxylase (DBH), the enzyme that catalyzes the hydroxylation of dopamine into NE (Fig. 2; Serova et al., 2002). The mechanism by which estrogens regulate both TH and DBH is transcriptional (Maharjan et al., 2005; Serova et al., 2002; Serova et al., 2011). This regulation can occur through direct genomic effects of estrogens on estrogen responsive elements (EREs) found on the TH and DBH promoters (Maharjan et al., 2005; Serova et al., 2004). There is also evidence that estrogens act on plasma membrane ERs to initiate signaling cascades, which increase TH and DBH by regulating other elements on their promotor regions (Maharjan et al., 2005; Maharjan et al., 2010; Serova et al., 2011).

Another mechanism that could contribute to higher levels of NE in LC target regions involves gonadal hormone regulation of enzymes that degrade NE. Much of the research on this topic has focused on catechol-*O*-methyltransferase (COMT) because single nucleotide polymorphisms (SNPs) on the COMT gene have been associated with a range of psychiatric disorders that have sex differences in their prevalence and/or disease progression, including anxiety disorders (Domschke et al., 2007; Harrison and Tunbridge, 2008; Lewinsohn et al., 1998). Notably, there is a sex difference in COMT activity in human postmortem prefrontal cortex tissue such that men have higher COMT activity than women (Chen et al., 2004). Preclinical studies indicate that estrogen regulation could account for this sex difference (Fig. 2). In female rats, COMT levels are lowest in the proestrus cycle phase, indicating an inverse relationship between circulating estrogens and COMT (Parvez et al., 1978). In cell culture, estrogens decrease COMT activity and protein levels (Jiang et al., 2003), purportedly through the EREs on the COMT promotor (Xie et al., 1999). However, mRNA levels of COMT in human cortex are comparable between the sexes (Bray et al., 2003; Chen et al., 2004), suggesting that the sex difference in COMT activity in humans is not simply transcriptional, but may involve novel isoforms, cofactors, or interacting proteins (Harrison and Tunbridge, 2008). As noted, SNPs on the COMT gene are found in psychiatric disorders, and several studies have identified sex by genotype interactions (for review see, (Harrison and Tunbridge, 2008). The most common SNP implicated is the Val¹⁵⁸Met polymorphism that alters COMT activity so that it is reduced in Met¹⁵⁸ versus Val¹⁵⁸

homozygous carriers (Chen et al., 2004). Several studies have found that the Met¹⁵⁸ allele is associated with anxiety-related traits and disorders in women, particularly of Caucasian descent, but not men (Domschke et al., 2004; Domschke et al., 2007; Eley et al., 2003; Enoch et al., 2003; Olsson et al., 2005; Stein et al., 2005). As COMT degrades catecholamines in general, the specific neurotransmitter that contributes to anxiety in women with the Met¹⁵⁸ allele is unclear. However, it is tempting to speculate that, at least in some women, the lower activity of COMT observed in females is exacerbated in carriers of the Met¹⁵⁸ allele, and this potentiated reduction in activity increases NE in the prefrontal cortex to levels that contribute to greater hyperarousal symptoms.

3.3 Estrogens alter adrenergic receptors

In addition to estrogens' regulation of NE synthesis and degradation, estrogens also can regulate adrenergic receptors. Most of the work on this topic has been conducted by Etgen and colleagues in the context of NE regulation of hypothalamic-mediated female reproductive behavior in rats (for review see, Etgen et al., 2001). In brief, estrogens shift the balance of NE receptor signaling away from β -adrenergic activation of the cAMP pathway, which inhibits luteinizing hormone (LH) and female reproductive behaviors, towards α 1-adrenergic PLC-mediated signaling, which facilitates the LH surge and reproductive behaviors (Etgen et al., 1992; Etgen et al., 2001). Aside from its role in reproduction, estrogen regulation of adrenergic receptors in LC target regions implicated in arousal, attention, and vigilance, has received less attention. Yet, there is evidence that ovariectomized females treated with estrogen have reduced cortical postsynaptic α 2-adrenoceptor binding and mRNA relative to their untreated counterparts (Ansonoff and Etgen, 2001; Karknias et al., 1997, but see Shansky et al., 2009). Additionally, in cycling females, compared to ovariectomized females and intact males, β -adrenergic receptor binding is decreased in cortical membranes and there is less β -adrenergic receptor stimulated cAMP (Wagner and Davies, 1980). In the striatum—which primarily receives innervation from the A1 and A2 noradrenergic cell group (Aston-Jones, 2004)—conditions that reduce estrogens, such as menopause in women and ovariectomy in rats, increase β 1-adrenergic receptor expression, while estrogen treatment of striatal neurons down-regulates β 1-adrenergic receptor action (Meitzen et al., 2013). Taken together, these studies suggest that estrogens typically decrease adrenergic receptor expression and function in the cortex and striatum (Fig. 2). However, the consequences of this effect, and whether similar receptor regulation by estrogens occurs in other LC target regions, remain unclear.

Collectively, these studies reveal that estrogens can increase NE synthesis and decrease NE degradation, effects that can account for higher NE levels observed in LC target regions following estrogen treatment. However, in parallel with these effects, estrogens also reduce postsynaptic adrenergic receptors, decreasing the ability of NE to affect downstream structures. A similar pattern of high NE levels and decreased α 2 adrenergic receptors is observed in the Flinders Sensitive Line, which is a genetic model of depressive-like behavior (Landau et al., 2015; Zangen et al., 1999). In this model, the decreased receptors are thought to reflect receptor downregulation following a persistently high NE tone (Landau et al., 2015). Estrogen-induced reductions in adrenergic receptors could similarly be compensatory

in females, allowing them to maintain NE function at a level comparable to males under normal circumstances. Clearly, more studies are needed to test this hypothesis.

In stress-related psychiatric disorders, hyperarousal symptoms are likely caused by an imbalance between NE release and postsynaptic adrenergic receptors, resulting in too much NE tone. While this imbalance can occur in both males and females, perhaps the dynamic regulation of this system by estrogens in females increases the risk that this balance will be shifted into a high NE state. For example, a high NE state could occur if NE synthesis fails to decrease when estrogen levels are reduced, or if adrenergic receptors become insensitive to estrogen-induced downregulation. Such effects could increase the risk of hyperarousal symptoms in women, particularly during their reproducing years when estrogen levels fluctuate regularly. The data indicating that the rates of depression in women exceed that of men only following puberty and prior to menopause (Angold and Worthman, 1993; Kessler et al., 1993; Kessler, 2003), support the role of ovarian hormones in contributing to the pathophysiology of this disorder. However, more research assessing these effects throughout the female lifespan would help elucidate this issue.

4. Sex differences in CRF regulation of LC neurons and underlying mechanisms

4.1 CRF regulation of LC physiology

Changes in the electrophysiological responses of LC neurons shift states of arousal and attention. In awake animals, LC neurons discharge in a *tonic* fashion, and the rate of this tonic firing is positively correlated with electroencephalographic (EEG) and arousal (Aston-Jones and Bloom, 1981b; Berridge and Foote, 1991; Berridge et al., 1993). In addition to tonic firing, salient sensory stimuli can evoke a burst of synchronous discharge known as a *phasic response* (Aston-Jones and Bloom, 1981a; Foote et al., 1980). This phasic response precedes changes in behavior, implicating LC neurons in attentional processes (Aston-Jones and Bloom, 1981a; Foote et al., 1980). Further studies have demonstrated that phasic firing is associated with focused attention (Aston-Jones and Cohen, 2005; Clayton et al., 2004). In contrast, high tonic firing is associated with labile attention, behavioral flexibility, and losing focus (Aston-Jones and Cohen, 2005). Thus, the ability of LC neurons to switch between tonic and phasic firing can facilitate shifting between scanning the environment or focusing on a specific stimulus, allowing the animal to adapt to changing attentional demands (Aston-Jones and Cohen, 2005).

Stress shifts the mode of LC activity by increasing tonic firing, while decreasing sensory-evoked phasic responses (Curtis et al., 1997; Valentino and Wehby, 1988), and this effect is mediated by CRF. Specifically, local blockade of CRF₁ receptors prevents stress-induced changes in LC activity (Curtis et al., 1994; Valentino and Wehby, 1988; Valentino et al., 1991). Moreover, administration of CRF directly into the LC mimics the effect of stress by increasing tonic firing, while attenuating phasic firing (Valentino and Foote, 1987; Valentino and Foote, 1988). One downstream consequence of CRF in the LC is an increase in cortical EEG, indicative of a higher arousal state (Curtis et al., 1997). Stress via activation of CRF receptors in the LC also increases cortical EEG, as well as NE release (Kawahara et al.,

2000; Page et al., 1993; Smagin et al., 1997). When considered together, these studies reveal that stress via CRF activation of the LC regulates target regions to increase arousal and shift attention towards scanning the environment, responses that could help the organism identify and cope with threatening stimuli during a stressful event.

4.2 Sex differences in CRF regulation of LC physiology

The aforementioned studies that initially characterized the effects of stress and CRF on LC physiology were conducted exclusively in male rats. Curtis et al. (2006) extended these findings to females and found interesting sex differences. Although the physiology of LC neurons is similar in unstressed male and female rats, hypotensive stress—a physiological stressor elicited by a nitroprusside-induced drop in blood pressure—increased LC tonic firing more in females than males. This sex difference in physiology was mimicked by CRF. Specifically, the CRF dose-response curve for LC activation in females was shifted to the left, such that a low dose of CRF that failed to activate the LC in males increased firing in females (Curtis et al., 2006). The role of adult circulating gonadal hormones in this effect was investigated, and surprisingly, there was no effect of estrogens or any other circulating gonadal hormone on stress and CRF regulation of LC physiology (Curtis et al., 2006). These data suggest that this sex difference is attributable to hormonal surges during development or sex chromosome genes (Arnold, 2009; Schulz et al., 2009; Swerdloff et al., 1992).

Another physiological effect initially identified in males is that acute stressor exposure alters subsequent LC neuronal responses to CRF. Specifically, prior (24 h earlier) exposure to footshock, swim stress, and even CRF itself, desensitizes male LC neurons to high doses of CRF (Conti and Foote, 1995; Conti and Foote, 1996; Curtis et al., 1995; Curtis et al., 1999; Curtis et al., 2006). Exposure to these same stressors also increases LC neuronal sensitivity to low CRF doses in males when compared to their unstressed male counterparts (Curtis et al., 1995; Curtis et al., 1999; Curtis et al., 2006). In other words in males, a history of stressor exposure changes the CRF-dose response curve for LC activation so that it shifts to the left and has a lower plateau. Unlike males, a history of swim stress has no effect on the CRF-dose response curve for LC activation in females (Bangasser et al., 2010; Curtis et al., 2006). This is perhaps because female LC neurons are already very sensitive to low levels of CRF, even in the unstressed state, and they are less adaptable to high levels of CRF as will be detailed below.

4.3 Sex differences in CRF₁ receptor signaling and coupling

Given that sex differences in LC neuronal responses to CRF were observed following CRF administration, the underlying mechanism was predicted to be postsynaptic. Further investigation confirmed this by linking the physiological findings to sex differences in CRF₁ receptor signaling. This receptor is a G-protein coupled receptor that preferentially couples to the G_s protein to activate the cAMP signaling pathway, although promiscuous coupling and initiation of additional signaling pathways is possible (Chen et al., 1986; Grammatopoulos et al., 2001; Hillhouse and Grammatopoulos, 2006). LC neuronal firing in response to CRF is thought to be primarily mediated by the cAMP signaling cascade, which when activated, purportedly increases neuronal firing via phosphorylation of a potassium channel (Jedema and Grace, 2004). To determine whether sex differences in neuronal

responses to CRF were linked to sex differences in cAMP signaling, pretreatment with a cAMP antagonist was utilized. This manipulation revealed that LC neuronal responses to CRF in males were only partially cAMP mediated (Bangasser et al., 2010). In contrast, LC neuronal responses to CRF were completely cAMP mediated in females (Bangasser et al., 2010). Prior swim stressor exposure altered the signaling in males, such that their sensitized responses to CRF were completely cAMP mediated. This manipulation failed to sensitize the LC neuronal responses to CRF in females or alter the contribution of cAMP signaling (Bangasser et al., 2010). Together these studies suggest that in unstressed rats, CRF induces greater cAMP signaling in the LC of females than males (Fig. 3). In contrast, stressor exposure alters signaling in male, but not female rats. Specifically, 24 h after swim stressor exposure, male LC neuronal responses to CRF were completely cAMP mediated. Prior stressor exposure had no effect on the contribution of cAMP signaling to LC neuronal firing in females, which remained high. Given that cAMP signaling increases LC tonic firing (Jedema and Grace, 2004), these effects on cAMP signaling can account for the heightened sensitivity of LC neurons to CRF in male rats with a history of stress and females.

cAMP signaling is initiated by activation of the Gs protein, so sex differences in binding of the Gs protein to the CRF₁ receptor could result in sex differences in the amount of cAMP signaling. Indeed, such sex differences are found in cortical tissue, where the CRF₁ receptor of female rats is more highly coupled to Gs than in males (Bangasser et al., 2010). Prior stress increases coupling of Gs to the CRF₁ receptor in males, but causes no further increase in females. Although technical limitations (i.e., small protein yield) prevented coupling from being assessed specifically in the LC, the sex difference in coupling in the cortex supports cAMP signaling results in the LC. Collectively, these findings support a model wherein increased CRF₁ receptor-Gs coupling leads to greater cAMP signaling, thereby heightening tonic firing. Given that rapid tonic LC firing is correlated with high arousal, this sex differences in CRF₁ receptor coupling and signaling could heighten arousal in females in response to stress. Perhaps there are situations where this is adaptive. However, during extremely stressful or prolonged stressful events, this physiology is predicted to predispose females towards shifting into the dysregulated state of hyperarousal that, as noted, characterizes certain psychiatric disorders.

4.4 Sex differences in CRF₁ receptor trafficking

As noted, stress-related psychiatric disorders are characterized by CRF hypersecretion. (Hauger et al., 2009; Hauger et al., 2012; Nemeroff, 1996). Fortunately, there are some compensatory mechanisms that can protect neurons from excessive CRF signaling in an effort to maintain homeostasis. One such mechanism is internalization, where CRF₁ receptors on the plasma membrane are trafficked into the cytosol in response to high concentrations of CRF. *In vitro* studies have revealed that the process of desensitization and subsequent internalization is typically initiated by GRK3 phosphorylation of serine and threonine residues on the carboxy terminus of the receptor, which promotes the recruitment and translocation of β -arrestin2 (Hauger et al., 2009; Oakley et al., 2007; Teli et al., 2005). Upon binding, β -arrestin2 uncouples the receptor from its cognate G protein and targets it for internalization (Hauger et al., 2009; Holmes et al., 2006; Oakley et al., 2007). These

internalized receptors can either be recycled or degraded (Hauger et al., 2009; Reyes et al., 2006).

CRF₁ receptor internalization has been demonstrated in the LC dendrites of male rats following the central administration of CRF and acute exposure to swim stress (Bangasser et al., 2010; Reyes et al., 2006; Reyes et al., 2008). Similarly, internalization is observed in male CRF overexpressing mice (Bangasser et al., 2013). CRF₁ receptor internalization is apparent as soon as 5 minutes after receptor activation, but persists for at least 24 hours (later time points have not been tested; (Bangasser et al., 2010; Reyes et al., 2006; Reyes et al., 2008). Overtime, the proportion of internalized CRF₁ receptors increases, as does their incorporation into multivesicular bodies, an effect indicative of receptor downregulation (Reyes et al., 2008). These studies indicate that, similar to the *in vitro* studies, the CRF₁ receptor in male LC dendrites internalizes following high concentrations of ligand (Fig. 3). Remarkably, the same effect does not occur in females. Female rats exposed to acute swim stress and female CRF overexpressing mice have a larger proportion of CRF₁ receptors on the plasma membrane of the LC dendrites than their unstressed counterparts (Bangasser et al., 2010; Bangasser et al., 2013). This result suggests that excessive ligand binding actually recruits CRF₁ receptors to the plasma membrane in females. Physiologically, this sex difference in trafficking can account for the fact that, despite high levels of CRF in the LC, male CRF overexpressing mice maintain their tonic LC firing rate at wild type levels (Bangasser et al., 2013). In contrast, the LC neurons of CRF overexpressing female mice fire roughly three times faster than wild type mice. As LC neuronal firing translates into arousal (Aston-Jones and Bloom, 1981a; Berridge and Foote, 1991), a lack of CRF₁ receptor internalization in females may predispose them to hyperarousal symptoms under conditions of CRF hypersecretion, as occur in stress-related psychiatric disorders (Hauger et al., 2009; Hauger et al., 2012; Nemeroff, 1996).

The mechanisms underlying the sex difference in internalization are thought to be linked to sex differences in β -arrestin2 recruitment to the CRF₁ receptor, which, as previously mentioned, initiates receptor internalization. In cortical tissue, acute swim stress promotes β -arrestin2 binding to the CRF₁ receptor in male rats (Bangasser et al., 2010). However, this increase in β -arrestin2 binding was not observed following the same stressor in females (Fig. 3). If these same mechanisms are occurring in the LC, it would explain the sex difference in CRF₁ trafficking.

The sex difference in β -arrestin2 binding may have further implications for cellular function. In addition to its role in initiating internalization, β -arrestin2 can also activate its own suite of signaling cascades that are often distinct, and sometimes opposed to those activated by G proteins (Ahn et al., 2004; Lefkowitz and Shenoy, 2005; Violin and Lefkowitz, 2007). This has led to the proposal that the signaling of the CRF₁ receptor is sex biased, such that it signals more through β -arrestin2 mediated pathways in males (Bangasser and Valentino, 2012; Valentino et al., 2012; Valentino et al., 2013a; Valentino et al., 2013b). As detailed above, the female CRF₁ receptor signals more through the Gs-cAMP pathway (Bangasser et al., 2010). As the activation of different signaling cascades can lead to different cellular events, sex biased signaling may be an important way that sex differences in physiology,

behavior, and even pathology are established (Bangasser and Valentino, 2012; Valentino et al., 2012; Valentino et al., 2013a; Valentino et al., 2013b).

5. Implications of sex differences in LC-NE system and its regulation by stress

Taken together, these studies reveal that the LC-NE system is regulated by estrogens and stress in a way that, under certain conditions, can increase susceptibility to states of high arousal in females relative to males. Despite this body of knowledge, the majority of studies have focused on understanding sex differences in the LC-NE system were conducted in adult rodents. It will be important to extend these findings to early development and aging to determine if, and how, sex differences in this important arousal system are established across the lifespan. Another goal of future studies should be to assess whether similar mechanisms occur in humans. Although some of the aforementioned approaches are currently restricted to preclinical models, neuroimaging data on sex differences in LC activation and further behavioral data on sex differences in stress-induced arousal would be valuable. Ultimately, if similar mechanisms are found to occur in humans, they could help explain the sex bias in psychiatric disorders that have hyperarousal as a key feature.

The identification of similar sex differences in the human LC-NE system would have implications for the treatment of certain stress-related psychiatric disorders. For example, if increased NE release is more common in women with these disorders, then they may respond better than men to a β -adrenergic receptor antagonist, such as propranolol, which has been used to reduce the consolidation (i.e. stabilization) and reconsolidation (i.e., the stabilization of a reactivated memory) of emotional memories (Cahill et al., 1994; Mueller and Cahill, 2010; Nader et al., 2000; Pitman et al., 2002). Corroborating this idea, a recent meta-analysis found that propranolol was more effective at reducing the reconsolidation of traumatic memories in women than men (Lonergan et al., 2013). Also, tricyclic antidepressants which increase the amount of NE in the synapse work better in men and post-menopausal women than in cycling women (Kornstein et al., 2000).

Sex differences in the CRF₁ receptor also have implications for therapeutics, because CRF₁ receptor antagonists are being developed to treat stress-related diseases (Holsboer and Ising, 2008; Ising et al., 2007; Nemeroff, 1996; Zobel et al., 2000). If increased sensitivity to CRF is driving hyperarousal symptoms, particularly in women, then they may better respond to treatments aimed at regulating CRF than men. Furthermore, the molecular studies suggest that there may be a conformational sex difference in the CRF₁ receptor of females compared to males that biases it to bind Gs instead of β arrestin2. If true, this sex difference in conformation could extend to the extracellular domain where antagonist bind, thereby altering their efficacy. In support of this, sex differences in the efficacy of a CRF₁ receptor antagonist in the dorsal raphe have been reported, such that the antagonist was effective at mitigating the effects of stress in male, but not female mice (Howerton et al., 2014). If this is a wider phenomenon, it might suggest this class of drug might have a reduced efficacy for women.

Perhaps even more importantly, the studies detailed here highlight that, by comparing males and females, new pharmacological targets can be identified. For example, biased ligands that shift the signaling of receptors away from G-protein mediated signaling and towards β arrestin2-mediated signaling have been developed for other receptors (Violin and Lefkowitz, 2007; Whalen et al., 2011). If such a compound could be developed for the CRF₁ receptor, it may be particularly effective at treating hyperarousal symptoms in women (Bangasser and Valentino, 2012; Valentino et al., 2013a; Valentino et al., 2013b). As more researchers focus on investigating sex differences in the LC, it is expected that novel therapeutic targets will be identified that can lead to the development of better treatments for stress-related psychiatric disorders for both men and women.

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Highlights

- Psychiatric disorders linked to stress and hyperarousal are more prevalent in women.
- There are structural sex differences in the locus coeruleus (LC) arousal center.
- Estrogens increase norepinephrine levels in LC target regions.
- Female LC neurons are more sensitive to the stress neuropeptide CRF.
- Collectively, these effects may contribute to sex biases in psychiatric disorders.

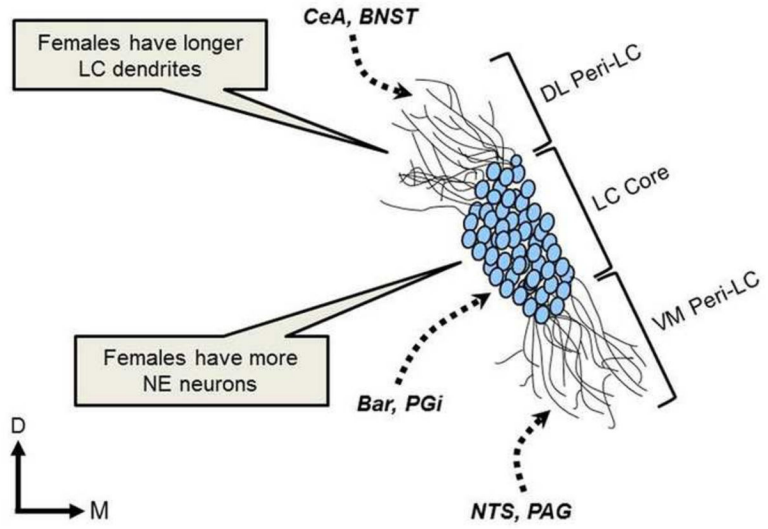


Figure 1. This image depicts the LC core and peri-LC regions, along with important LC afferents and structural sex differences in the rat LC. It has been reported that the female LC contains more noradrenergic neurons than the male LC. Additionally, the dendrites of female LC neurons are larger and more complex, extending further in the peri-LC region. This structure increases the probability that female LC dendrites will contact afferents that terminate in the peri-LC regions. Bar, Barrington’s nucleus; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; DL Peri-LC; dorsolateral pericoerulear locus coeruleus; LC Core, locus coeruleus core; NTS, nucleus of the solitary tract; PAG, periaqueductal gray; PGI, nucleus paragigantoceullaris; VM Peri-LC, ventromedial pericoerulear locus coeruleus

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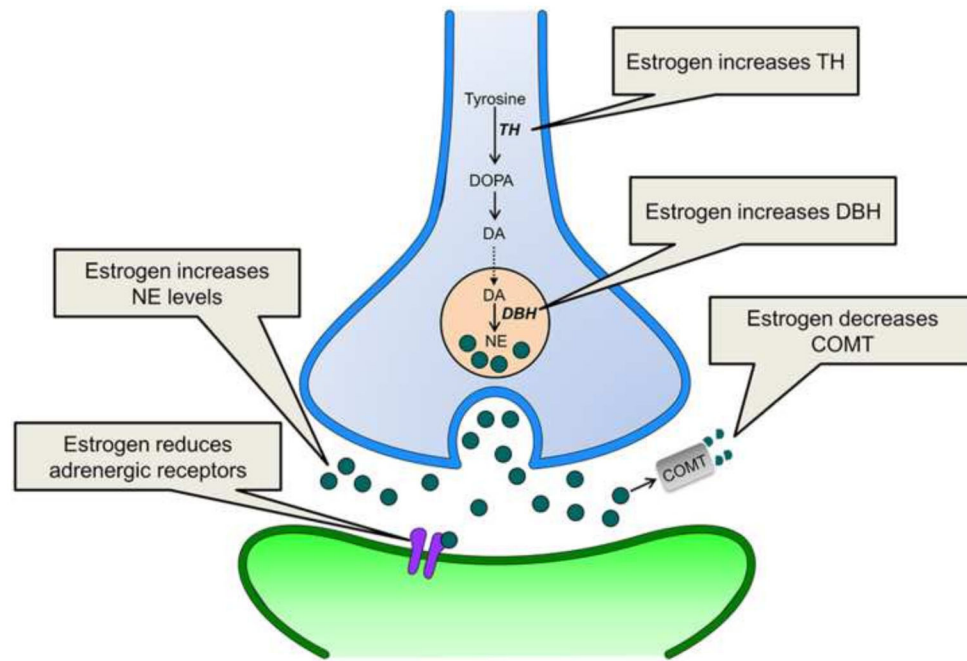


Figure 2.

This schematic illustrates how estrogen can regulate the NE system. Estrogen can increase NE levels by increasing NE synthesis within the LC neuron (blue) and reducing NE degradation. Estrogen also decreases adrenergic receptor (purple) levels on postsynaptic neurons (green), an effect that may be compensatory. DA, dopamine; DBH, dopamine β -hydroxylase; DOPA, L-dihydroxyphenylalanine; COMT, catechol-*O*-methyltransferase; NE, norepinephrine

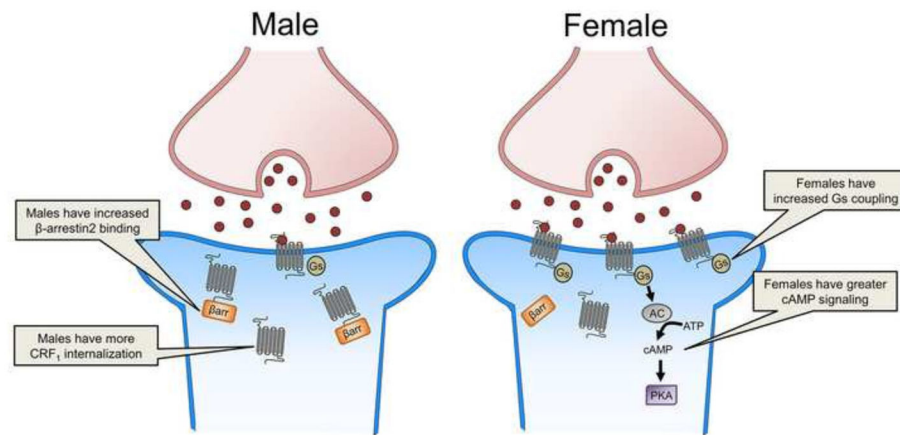


Figure 3.

This schematic depicts sex differences in the CRF₁ receptor. The image on the *left* depicts increased β-arrestin2 binding to the CRF₁ receptor (gray) and internalization in a male LC neuron (blue). The image on the *right* depicts CRF (red circle) binding to the CRF₁ receptor (gray), which increases Gs coupling and signaling through the cAMP pathway in a female LC neuron (blue). AC, adenylyl cyclase; ATP, adenosine triphosphate; βarr., β-arrestin2; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A