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Sex differences in circuits activated by corticotropin releasing factor in rats

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Abstract

Women are more likely than men to suffer from psychiatric disorders characterized by corticotropin releasing factor (CRF) hypersecretion, suggesting sex differences in CRF sensitivity. In rodents, sex differences in the sensitivity of specific brain regions to CRF have been identified. However, regions do not work in isolation, but rather form circuits to coordinate distinct responses to stress. Here we examined whether CRF activates different circuits in male and female rats. Following central administration of CRF or artificial cerebrospinal fluid (aCSF), neuronal activation in stress-related areas was assessed using cFOS. Functional connectivity was gauged by correlating cFOS-positive cells between regions and then identifying differences in correlations for aCSF-treated and CRF-treated groups within sex. This analysis revealed that CRF altered different circuits in males and females. As an example, CRF altered correlations involving the dorsal raphe in males and the bed nucleus of the stria terminalis in females, suggesting sex differences in stress-activated circuits controlling mood and anxiety. Next, plasma estradiol and progesterone levels were correlated with cFOS counts in females. Negative correlations between estradiol and neuronal activation in the regions within the extended amygdala were found in CRF-treated, but not aCSF-treated females. This result suggests that estrogens and CRF interact to modulate the fear and anxiety responses mediated by these regions. Collectively, these studies reveal sex differences in the way brain regions work together in response to CRF. These differences could drive different stress coping strategies in males and females, perhaps contributing to sex biases in psychopathology.

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Keywords

corticotropin releasing hormone; estradiol; progesterone; sexual dimorphism; anxiety; circuit; connectome

Corticotropin releasing factor (CRF) is a neuropeptide that orchestrates responses to stress. It initiates the endocrine limb of the stress response by activating the hypothalamic pituitary adrenal axis (Owens and Nemeroff, 1991; Vale et al., 1981). Additionally, it works as a neuromodulator regulating many brain regions to coordinate different behavioral responses to stress (Bale and Vale, 2004; Owens and Nemeroff, 1991; Valentino and Commons, 2005). Clinically, CRF hypersecretion is thought to contribute to the pathophysiology of stress-related psychiatric disorders, including major depression and posttraumatic stress disorder (Austin et al., 2003; Bremner et al., 1997; De Bellis et al., 1993; Heuser et al., 1998; Nemeroff, 1996; Raadsheer et al., 1994; Wang et al., 2008). Another shared feature of these disorders is their higher prevalence in women than in men (Breslau, 2002; Kessler et al., 2012). Thus, researchers have begun to investigate sex differences in responses to CRF in rodent models to identify mechanisms that may explain female sensitivity to disorders characterized by CRF hypersecretion (reviewed in, Bangasser, 2013; Bangasser and Valentino, 2014).

Studies exploring sex differences in responses to CRF in rodent models typically focus on the effect of CRF in a specific brain area. For example, our previous research found that noradrenergic neurons in the locus coeruleus (LC)-arousal system are more sensitive to CRF in female compared to male rats (Bangasser et al., 2010; Curtis et al., 2006). This increased sensitivity in females is associated with increased coupling of the CRF₁ receptor to the GTP-binding protein, Gs, and greater signaling through the cAMP-PKA pathway (Bangasser et al., 2010; Bangasser et al., 2016). In contrast to the LC, CRF administration in the dorsal raphe (DR) increased anxiety-related behaviors more in male than in female mice, an effect linked to sex differences in the distribution of CRF receptors on different cell types within the DR (Howerton et al., 2014). Together these studies reveal sex differences in sensitivity to CRF, and highlight how this differential sensitivity can be region specific.

Stress responsive regions do not work in isolation, but rather coordinate with other areas forming circuits to mediate different physiological and behavioral responses. If specific brain regions respond differently to CRF in males and females, then the circuits that they form with other areas to coordinate stress responses are also likely to be affected by CRF in a sex-specific manner. Our laboratory began to test this idea by assessing whether central administration of CRF altered stress networks differently in male and female rats in two different phases of the estrous cycle: diestrus, when levels of ovarian hormones are lower, and proestrus when levels of ovarian hormones are higher (Wiersielis et al., 2016). The regions examined as part of the network were chosen for their role in regulating stress responses and because they contain CRF receptors (Van Pett et al., 2000; Wiersielis et al., 2016). Additionally, CRF activates neurons in these regions, as measured with the immediate early gene cFOS (Wiersielis et al., 2016). To statistically assess functional connectivity, we used a previously developed technique where cFOS counts are correlated

between brain regions and statistical comparisons are made to test for differences in the strengths of these correlations (Dwyer and Leslie, 2016; Maras et al., 2014). This approach revealed that CRF-activated networks differed most between males and females in proestrus (Wiersielis et al., 2016). This result suggests that ovarian hormones can alter the effect of CRF.

The present study expands on our previous findings to assess how CRF changes network activation relative to vehicle infused controls. The data are compared within each sex to evaluate how CRF infusions alter networks in male and female rats. Then we extend our previous work, which indicated that ovarian hormones can alter the effect of CRF, by correlating plasma levels of estradiol and progesterone in females to neuronal activation in the stress network. This analysis allows us to begin to gauge if and where ovarian hormones play a role in regulating the effect of CRF.

Methods

Subjects

Adult (> 70 day old) male (n = 23) and female (n = 42) Sprague Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were used. All rats were individually housed on a 12 h reversed light/dark cycle (dark onset at 9:00 am) with *ad libitum* food and water. All studies were conducted in accordance with the National Institutes of Health guidelines and were approved by Temple University's Institutional Animal Use and Care Committee. Rats were handled for at least 3 min per day for 3 days prior to the start of the studies to minimize the stress of the infusion procedure. Data regarding behavioral responses evoked by CRF and estrous cycle modulation of the CRF-activated network were described in a previous study by Wiersielis et al. (2016). Here we performed new analyses, not previously reported, on the subjects detailed in that paper to assess sex differences in network activation between vehicle-treated and CRF-treated rats. In the Wiersielis et al. (2016) paper, the estrous cycle was tracked in the female rats and effects of estrous cycle were reported. Here we added measures of estradiol and progesterone, as detailed below, to begin to assess the individual influences of each ovarian hormone on network activation.

Stereotaxic surgery

Rats were anesthetized and then maintained on a 2% isoflurane-in-air mixture administered via nose cone and positioned using a stereotaxic instrument. A guide cannula aimed at the lateral ventricle (−1.1 mm A/P and −1.5 mm M/L from Bregma, −4.4 mm D/V from the surface of the skull) was surgically implanted as previously described (Bangasser et al., 2013; Wiersielis et al., 2016). A dummy cannula was inserted to prevent blockage of the guide cannula. Rats were given at least 7 days after surgery to recover before subsequent testing.

Microinfusions, tissue and plasma collection, and tissue processing

Rats were infused using a microinfusion pump (Harvard Apparatus) at a rate of 1µl/min with artificial cerebrospinal fluid (aCSF, 3µl) or ovine CRF (American Peptides) 0.3µg in 3µL of aCSF as described (Howard et al., 2008; Wiersielis et al., 2016). This dose of CRF was

chosen because it induces physiological effects similar to stressor exposure, mimicking, for example, the magnitude of stress-induced activation of LC neurons (Bangasser and Valentino, 2012; Curtis et al., 2006; Curtis et al., 1993; Curtis et al., 1997; Zitnik et al., 2014) and behavior effects similar to those observed during stressor exposure (Conti et al., 2002; Sherman and Kalin, 1987; Wiersielis et al., 2016). Following infusion (~75 min), rats were deeply anesthetized with Euthasol (0.2 ml/kg, i.p.), and transcardially perfused. Trunk blood was collected and spun in Heparin coated tubes at $3,000\times g$ at 4° C. Plasma was separated and frozen for later analysis (see hormone assays). Tissue was stored until sectioned (30µm) as previously detailed (Wiersielis et al., 2016). Cannula placement sections were verified and animals with poor cannula placements were dropped from analysis (Wiersielis et al., 2016). To assess neuronal activation, every 4th brain tissue section was processed for cFOS using an anti-cFOS antibody (1:1000, Santa Cruz H-125) as previously detailed (Wiersielis et al., 2016). The antibody was visualized with an avidin-biotin reaction (ABC Vectastain, Burlingame, CA) and diaminobenzadine (DAB; Vector Laboratories, Inc. SK-4100) as described (Wiersielis et al., 2016).

cFOS analysis

cFOS immunoreactive cells were analyzed in stress-related regions containing CRF receptors (Van Pett et al., 2000). This analysis has been detailed previously (Wiersielis et al., 2016), but in brief, images were acquired at 10× magnification with a camera (Leica DFC450) affixed to a brightfield microscope (Leica DM5500). Pictures of two brain sections per rat for smaller regions and four brain sections per rat for larger regions were used for analysis. cFOS cell counting was conducted using ImageJ software (NIH) by a researcher blind to the experimental conditions.

Hormone assays

Plasma progesterone concentrations were measured using MPBiomedicals Progesterone double antibody kit (07170102) with an I-125 tracer. All reagents were used at ½ volume and plasma samples were at ½ or ¼ volume. This kit detects plasma progesterone in the 0.2-50ng/ml range. This progesterone antibody cross-reacts 100% with Progesterone, 5.41% with 20 α-Dihydroprogesterone, 3.8% with Desoxicorticosterone, 0.70% with Corticosterone, 0.67% with 17α-Hydroxyprogesterone, 0.41% with Pregnenolone, 0.23% with Androstenedione, 0.16% with Testosterone. The Intra-assay variability was 3.6 and the Inter-assay variability was 6.7%.

Plasma 17β-estradiol concentrations was measured using an MPBiomedicals 17β-Estradiol double antibody kit (07138102) with an I-125 tracer. All reagents and samples were used at ½ volume. This kit detects plasma 17β-Estradiol in the 10-3,000 pg/ml range. This 17B-estradiol antibody cross-reacts 100% with Estradiol-17β, 20% with Estrone, 1.51% with Estriol, and 0.68% with 17β-Estradiol. The Intra-assay variability was 4.7% and the Inter-assay variability was 9.1%.

Statistical analyses

For each sex and treatment (i.e., aCSF or CRF), cFOS activation was correlated between brain regions using Pearson product moment correlations. These correlations were used for a

community structure analysis, which was conducted with an open-source brain connectivity toolbox (Rubinov and Sporns, 2010) for Matlab (Matlab R2017a, MathWorks, Natick, MA). This analysis was used to determine community structure by quantitatively segregating the broader stress network into modules of dense functional interconnectivity. Negative correlations were asymmetrically weighted for this analysis (Rubinov and Sporns, 2011). To determine how connected a region was to other regions within the same module, within-module degree z-scores were calculated for each region. Participation coefficients were calculated to determine between-module connectivity, or the degree to which each region is connected across the modules (Guimera and Nunes Amaral, 2005; Rubinov and Sporns, 2011; Tanimizu et al., 2017). Both the within-module degree z-scores and participation coefficients were determined based on the positive correlations (Guimera and Nunes Amaral, 2005; Tanimizu et al., 2017). To determine how CRF treatment altered the correlations between males and females, Fisher r-to-z transformations were performed followed by Fisher's z-tests as previously described (Maras et al., 2014; Wiersielis et al., 2016).

Pearson product moment correlations were also performed on estradiol levels and cFOS counts, as well as on progesterone levels and cFOS counts for each brain region within the stress network in females. Again, differences in these correlations between aCSF-treated and CRF-treated females were assessed using Fisher's z-tests performed on r-to-z transformed data. Values that exceeded 2 SDs above or below the group mean were considered outliers and dropped (Wiersielis, et al. 2016).

Results

Correlation matrices and community structure analysis

Representative photomicrographs of cFOS staining are presented in Supplementary Materials (SM) Figure 1 and average cFOS cell counts for each condition are presented in Supplementary Materials (SM) Table 1. These cell counts were then correlated for each treatment and sex. For aCSF-treated males, we found 9 significant correlations for neuronal activation between brain regions, only one of which was in the negative direction (Table 1, Fig. 1a). CRF treatment increased the number of significant correlations for neuronal activation to 16, and 13 of these correlations were positive (Table 1, Fig. 1b). In female rats, a different pattern of network activation was found. Females treated with aCSF had 26 correlations for neuronal activation between brain regions, and 23 of these were positive (Table 2, Fig. 1c). Similarly, in the CRF-treated females, many correlations for neuronal activation were found. There were 27 in total and 23 of them were positive (Table 2, Fig. 1d). The full analysis is in the Supplemental Materials (SM Tables 2–4)

Next, the community structure of each network was determined. Three modules were identified for males treated with aCSF ($Q = .23$) and for males treated with CRF ($Q = .33$). Females in the aCSF and CRF conditions also had three modules ($Q = 0.19$, $Q = 0.26$, respectively). Notably, the structures that comprised these modules differed by sex and treatment. Modules are denoted by colors on Figure 1 (a–d) and structures that share the same color belong to the same module. For each condition, the within-module degree z-scores and participation coefficients for regions were plotted (Fig. 1e–h). Regions with a

high within-module degree z-score and low participation coefficient, such as the basal nucleus of Meynert (BN) and anterodorsal nucleus bed nucleus of the stria terminalis (adBNST) for CRF-treated males, have a high degree of connectivity within their module, but low connectivity with other modules, suggesting that they selectively participate within their module. In contrast, regions that score highly on both measures, such as the infralimbic (IL) cortex in CRF-treated females, connect frequently within and between modules and may mediate interactions with different parts of the network (Tanimizu et al., 2017). Notably, the structures with the highest within-module degree z-scores differed between sex and treatment condition.

Evaluating how CRF treatment altered functional connectivity for each sex

CRF-activated networks were then compared to aCSF-activated networks within sex. To assess whether patterns of neuronal activation differed between aCSF-treated and CRF-treated males, Fisher's z-tests were used, and they identified 7 significant differences between correlations for neuronal activation (Fig. 2a,c). Correlations for neuronal activation between the DR, medial prefrontal cortex (mPFC), and amygdala were most altered by CRF treatment, suggesting that circuits connected to these regions would drive responses to CRF in males.

To compare whether these significant correlations differed between aCSF-treated and CRF-treated females, we again performed the Fisher's z-test. Seven of these correlations significantly differed between the treatment conditions in females (Fig. 2b,d). Despite the fact that the female stress network was more correlated in both the aCSF and CRF conditions than the network of males, CRF treatment changed a similar number of correlations in both sexes. Yet, unlike in males, the regions in females that had the greatest changes in correlations for neuronal activation following CRF treatment were the BNST and periaqueductal gray (PAG), suggesting that circuits connected to these regions drive stress responses in females. The full analysis is in the Supplemental Materials (SM Tables 6–7).

Ovarian hormone analysis

To determine whether circulating ovarian hormone levels were related to neuronal activation in females, plasma levels of estradiol and progesterone were correlated with levels of neuronal activation in the regions within the network. Estradiol was not correlated with cFOS counts in the aCSF treatment group for the adBNST [$r(9) = -.459$, $p = .159$] (Fig. 3a), basolateral amygdala (BLA) [$r(14) = .051$, $p = .852$] (Fig. 3b), and central nucleus of the amygdala (CeA) [$r(14) = -.185$, $p = .494$] (Fig. 3c), regions that are considered to be part of the extended amygdala (Alheid, 2003; De Olmos et al., 2004). However, following CRF treatment, there were significant negative correlations between estradiol levels and neuronal activation for these regions as follows: adBNST, [$r(13) = -.525$, $p = .044$] (Fig. 3a), BLA [$r(18) = -.534$, $p = .015$] (Fig. 3b), and CeA [$r(18) = -.558$, $p = .011$] (Fig. 3c). In contrast, estradiol levels were positively correlated with dmPAG at baseline [$r(15) = .560$, $p = .030$], but this correlation was no longer significant following CRF treatment [$r(13) = .126$, $p = .655$] (Fig. 3d). Progesterone levels were significantly correlated with neuronal activation in only one brain region, the medial septum (MS), and the correlation was significant in aCSF-treated [$r(10) = -.777$, $p = .003$], but not CRF-treated females [$r(13) = -.148$, $p = .599$] (Fig.

3e). Fisher's z-tests revealed a significant difference between these MS correlations [$z(13) = -2.155, p = .031$]. Significant differences in the correlations were not found for any other relationship between neuronal activation and ovarian hormones. The full analysis is contained in the Supplemental Materials (SM Tables 4–5, 7).

Discussion

CRF orchestrates stress responses, in part, by activating neurons in a number of brain regions. Previous work has largely focused on the activation of individual areas (Bangasser et al., 2010; Howerton et al., 2014), but multiple regions must work in concert to coordinate complex responses to stress. Here we assessed how CRF altered the functional connectivity of stress-related brain areas in males and females. We found that neuronal activation between these regions was more highly correlated in female than in male rats at baseline and following CRF treatment. In both sexes, CRF treatment altered a similar number of correlations for neuronal activation. However, the brain regions affected largely differed between males and females, suggesting that CRF can alter stress-networks to bias males and females towards different strategies for responding to stress. Ovarian hormones can regulate neuronal activation and contribute to many sex differences in the brain (Figueiredo et al., 2002; Hoffman et al., 1993; Nappi et al., 1997). To examine the role of circulating ovarian hormones in females, neuronal activation in stress-related brain regions of aCSF-treated and CRF-treated rats were correlated with plasma levels of estradiol and progesterone. Of note were the negative correlations between estradiol levels and neuronal activation in the regions within the extended amygdala in the CRF, but not the aCSF, treatment group. Given that the extended amygdala is implicated in mediating fear and anxiety, this result suggests that circulating estrogens and CRF interact to modulate these processes.

CRF-activated functional connectivity networks in males and females

Male rats in the aCSF condition had the fewest significant correlations between regions in the network. Thus, in an unstressed state, these regions are not very synchronized in males, which is not surprising due to their primary involvement in responding to stress. CRF treatment in males increased the number of correlations for neuronal activation, suggesting that this stress peptide enhances coordination between these stress responsive circuits. Neuronal activation in the mPFC, BNST, paraventricular nucleus (PVN), and laterodorsal tegmental nucleus (LDTg) was highly correlated with other regions in the stress network following CRF treatment, suggesting that these areas are critical nodes responsible for driving responses to stress in males. Interestingly, in both the aCSF and CRF treatment groups, the PAG appeared to be an important node, however, the regions to which neuronal activation in the PAG was correlated differed between the conditions.

In contrast to males, neural activation between brain regions was highly correlated in the aCSF-treated group in females. This result indicates that even in an unstressed state, these stress-related regions are working together in females. A recent human imaging study found that women have higher resting-state functional connectivity than men between the amygdala and cortical regions (Kogler et al., 2016), which is consistent with our results in rats. Other human imaging studies also provide evidence of greater overall connectivity in

women compared to men (Gong et al., 2009; Tomasi and Volkow, 2012). Collectively, these studies indicate that baseline sex differences in functional connectivity exist across species. These differences may impact sex differences in behavior in an unstressed state.

CRF-treated females also had many significant correlations for neuronal activation. Interestingly, in both the aCSF and CRF treatment groups, the mPFC, nucleus accumbens (NAc), amygdala, and DR appeared to be important nodes coordinating their activity with neuronal activation in many other brain regions. That said, the brain regions that were correlated with the nodes, in some cases, differed following CRF treatment. There were also additional nodes in the CRF-treated group, including the septum, hippocampus, and BNST. Taken together, the pattern in females suggests that many brain regions are working together in both the unstressed and CRF-induced stress state, and some brain regions are further engaged by CRF administration. This pattern contrasts with males, where correlations were low in the aCSF treated group but greatly increased by CRF treatment.

We performed a community structure analysis on the stress network for each sex and treatment condition. Although the brain regions analyzed were chosen because they respond to stress and contain CRF receptors, this analysis further segregated these structures into communities or modules, which are thought to represent specialized functional subnetworks (Rubinov and Sporns, 2010). Interestingly, the structures that comprised the modules and those with high within-module and between-module connectivity largely differed by sex and treatment condition. This result suggests that these brain regions work together differently in males and females under aCSF- and CRF-treatment, an effect that could drive different behaviors.

We next wanted to determine how correlations for neuronal activation changed following CRF administration for each sex. The number of correlations for neuronal activation that were significantly altered by CRF treatment was similar in both males and females. However, the circuits that were affected most by CRF differed quite a bit by sex. CRF treatment in males altered correlations particularly with the mPFC, amygdala, and DR. Given that these regions are involved in cognition, fear, and mood, respectively, this suggests that CRF alters how regions that mediate these processes work together in males. In contrast, CRF treatment in females altered correlations primarily with the PAG and BNST, suggesting differences in defensive behavior as well as anxiety. Thus, the way that CRF alters the stress-network differs in each sex, suggesting that different strategies for responding to stress would be engaged in males vs. females following CRF release.

Although most prior studies investigating CRF have only used male subjects (Arnold et al., 1992; Imaki et al., 1993), when females are included, CRF-mediated responses have been shown to differ between males and females in a way that is fairly consistent with the network analysis. For example, CRF altered functional connectivity with the BNST predominantly in females. This finding is consistent with previous studies suggesting that females are more susceptible than males to light-enhanced startle, an anxiety-related behavior mediated by CRF and the BNST (Toufexis et al., 2005; Walker et al., 2008; Walker et al., 2003). As noted, CRF in the LC increases neuronal responses in females (Bangasser et al., 2010; Curtis et al., 2006), while CRF in the DR increases anxiety-related behavior in

males (Howerton et al., 2014). Surprisingly, in females, correlations with the LC were not significantly changed by CRF treatment, as might be expected based on previous results. One limitation of the present study is that cFOS was only assessed at one time point following CRF administration, and perhaps this time course did not best reflect the rapid effects of CRF on the LC. In contrast, the network analysis does support the prominence of the DR in males. Moreover, the analysis provides additional information indicating that it is likely that the DR is working with the mPFC and LC (regions where the correlations for neuronal activation with the DR differ following CRF treatment) to mediate anxiety in males. An advantage of the analysis performed here is that it provides a framework for extending studies from a focus on one brain region to a focus on circuits. Here we investigated circuits that were specifically activated by CRF because of the known sex differences within CRF pathways and CRF's relevance to psychiatric disorders (Bangasser et al., 2010; Bremner et al., 1997; Howerton et al., 2014; Waters et al., 2015). However, it is expected that stressor exposure, which modulates many neurotransmitters and neuropeptides, would have additional effects on this circuitry, some of which would differ based on sex. Importantly, this circuitry analysis can help guide the design of future studies aimed at understanding the networks by which stress differentially influences behavior in males and females.

Ovarian hormones and neuronal activation

Our previous study assessed the effects of estrous cycle stage on CRF-activated circuits and found that circuits activated in female rats in the proestrous phase of their cycle (higher levels of ovarian hormones) differed largely from those activated by CRF in females in the diestrous stage (lower levels of ovarian hormones) and males (Wiersielis et al., 2016). Many of these differences involved correlations with structures comprising extended amygdala, which includes the amygdala and the BNST (Alheid, 2003; De Olmos et al., 2004). These prior results suggested that ovarian hormones can influence CRF activated networks (Wiersielis, et al. 2016). However, a limitation to tracking cycle stage is that the individual contribution of estradiol and progesterone cannot be determined. In the present study, we addressed this limitation by directly assaying levels of plasma estradiol and progesterone and correlating them with neuronal activation in female aCSF-treated and CRF-treated rats. An interesting pattern emerged with the adBNST, CeA, and BLA. Estradiol was not correlated with neuronal activation in the aCSF control group, but estradiol was negatively correlated with neural activation in these regions in the CRF-treated females. This result indicates that estradiol and CRF work together to alter neuronal activation. Moreover, these findings suggest that it is the estradiol levels in proestrus (i.e., rather than progesterone levels) that account for our previously observed effects of estrous cycle stage on CRF-activated extended amygdala circuitry (Wiersielis, et al. 2016).

Although estradiol can induce genomic changes via nuclear receptors, the time course in this study suggests a more rapid effect of estradiol likely linked to estrogen receptor signaling. Rapid effects are possible because G-protein-coupled estrogen receptors are present in these regions (Hazell et al., 2009; Tian et al., 2013). It is important to note that estradiol alone did not significantly alter neuronal activation in these regions, but rather worked synergistically with CRF. CRF activates its receptors, which are G-protein-coupled receptors, to induce

signaling. Thus, it is possible that estradiol-induced and CRF-induced signaling converge on intracellular processes to reduce neuronal activation. Additionally, there is evidence that CRF receptors can form heterodimers with the membrane estrogen receptor, G-protein-coupled receptor 30, an effect that could also alter signaling (Akama et al., 2013).

Estradiol can regulate anxiety. In humans, estrogen replacement can reduce anxiety (Gleason et al., 2015; Misra et al., 2013, but see, Demetrio et al., 2011), and on the contrary, anxiety symptoms increase during phases of the menstrual cycle when estradiol levels decline (Cameron et al., 1988). Similarly in rodents, treatment of ovariectomized females with estradiol often reduces anxiety-like behavior (Kalandakanond-Thongsong et al., 2012; Tian et al., 2013, but see, Mora et al., 1996). In naturally cycling female rats, anxiety-related behavior is reduced in the proestrous phase of the cycle, when estradiol levels are highest (Mora et al., 1996). Additionally, estradiol facilitates extinction of fear memories and this effect is associated with decreased cFOS expression in the amygdala (Zeidan et al., 2011). The results here expand on this literature by indicating that one mechanism by which estradiol can regulate fear and anxiety is via interactions with CRF in the extended amygdala.

Unlike the extended amygdala, neuronal activation in the dmPAG and MS were positively correlated with estradiol and progesterone, respectively, in the aCSF condition. However, these correlations disappeared following CRF treatment. The dmPAG is associated with defensive behaviors (Carvalho-Netto et al., 2007; Keay and Bandler, 2001), while the MS is associated with mnemonic processing (Everitt and Robbins, 1997). Thus, these findings indicate that in the unstressed state, higher levels of ovarian hormones promote defensive and mnemonic processes. Yet CRF overcomes the ability of these ovarian hormones to regulate these regions, suggesting that during a stressful experience females would engage in a similar suite of defensive and cognitive processes, regardless of circulating ovarian hormone levels.

Conclusion

The present results suggest that stress via CRF preferentially activates different circuits in males and females. This preliminary analysis can form the basis of future studies to specifically test whether these functional network changes result in sex differences in stress response strategies, some of which may promote vulnerability to pathology in a sex-specific manner. As the field of neuroscience considers how sex and gender influence disease, it will be important to expand this work to human neuroimaging studies that assess stress. There are few neuroimaging examples of functional connectivity analysis performed during stressful conditions where gender differences are a focus (but see, Mather et al., 2010). Yet, identifying the precise circuitry by which men and women respond to stress and any differences therein could guide future sex-specific interventions to promote stress resilience.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Excess corticotropin releasing factor (CRF) is linked to disorders common in women
- Sex differences in responses to CRF could contribute to this disparity
- In rats, we found that CRF activates different circuits in males and females
- In some cases, ovarian hormones are related to neuronal activation
- Sex differences in CRF-activated circuits could bias pathology in males vs. females

Figure 1.

Networks for aCSF-treated and CRF-treated male and female rats. Schematics depict the network of brain regions analyzed for males treated with aCSF (**A**), males treated with CRF (**B**), females treated with aCSF (**C**), and females treated with CRF (**D**). The arrows illustrate anatomical connections and thick black arrows indicate significant correlations. The community structure of each network was determined and the modules are denoted by colors. Within-module degree z-scores and participation coefficients were determined for each region and plotted for all conditions: male aCSF (**E**), male CRF (**F**), female aCSF (**G**), and female CRF (**H**). ACC - anterior cingulate cortex; ad - anterodorsal; BLA - basolateral amygdala; BN - basal nucleus of Meynert; BNST - bed nucleus of the stria terminalis; CeA - central nucleus of the amygdala; dm – dorsomedial; DR - dorsal raphe; HPC - hippocampus; IL – infralimbic; LC - locus coeruleus; LDTg - laterodorsal tegmental nucleus; l - lateral; lw – lateral wing; m - medial; mPFC - medial prefrontal cortex; NAc - nucleus accumbens; o - oval nucleus; PAG - periaqueductal gray; PL - prelimbic; PVN - paraventricular nucleus of the hypothalamus; vm - ventromedial.

Figure 2.

CRF-induced changes in functional connectivity for male and female rats. Tables show correlations and Fisher's z-tests for areas where correlations for neuronal activation differed between aCSF and CRF treatment in males (A) and females (B). Schematics depict differences between the aCSF and CRF networks for males (C) and females (D). Red arrows indicate correlations that significantly differed between aCSF and CRF conditions. ACC - anterior cingulate cortex; ad - anterodorsal; BLA - basolateral amygdala; BN - basal nucleus of Meynert; BNST - bed nucleus of the stria terminalis; CeA - central nucleus of the amygdala; dm - dorsomedial; DR - dorsal raphe; HPC - hippocampus; IL - infralimbic; LC - locus coeruleus; LDTg - laterodorsal tegmental nucleus; l - lateral; lw - lateral wing; m - medial; mPFC - medial prefrontal cortex; NAc - nucleus accumbens; o - oval nucleus; PAG - periaqueductal gray; PL - prelimbic; PVN - paraventricular nucleus of the hypothalamus; vm - ventromedial.

Figure 3.

Correlations between ovarian hormones and cFOS counts for aCSF-treated (left column) and CRF-treated (right column) females. Significant correlations between estradiol levels and cFOS counts were found only in the CRF-treated females for the adBNST (A), CeA (B), and BLA (C). In the dmPAG, estradiol levels and cFOS counts were significantly correlated for aCSF-treated females, but not the CRF-treated females (D). Progesterone levels were significantly correlated with cFOS counts in the aCSF, but not CRF treatment group for the MS (E). adBNST – anterodorsal nucleus bed nucleus of the stria terminalis; BLA –

basolateral nucleus of the amygdala; CeA - central nucleus of the amygdala; dmPAG – dorsomedial periaqueductal gray; MS – medial septum

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Table 1

Significant correlations of cFOS counts between brain regions for aCSF-treated and CRF-treated male rats.

	Correlations	
	aCSF	CRF
IL vs. PL	$r = 0.84$ $p = \mathbf{0.019^*}$	$r = 0.94$ $p < \mathbf{0.001^*}$
NAc_Shell vs. PL	$r = .097$ $p = 0.877$	$r = 0.84$ $p = \mathbf{0.005^*}$
NAc_Shell vs. IL	$r = 0.24$ $p = 0.694$	$r = 0.85$ $p = \mathbf{0.003^*}$
adBNST vs IL	$r = 0.62$ $p = 0.268$	$r = 0.85$ $p = \mathbf{0.031^*}$
adBNST vs. NAc_Shell	$r = 0.74$ $p = 0.095$	$r = 0.78$ $p = \mathbf{0.038^*}$
BN & LS	$r = -0.07$ $p = 0.873$	$r = 0.64$ $p = \mathbf{0.046^*}$
PVN vs. NAc_Shell	$r = 0.3$ $p = 0.569$	$r = 0.68$ $p = \mathbf{0.032^*}$
adBNST vs. PVN	$r = 0.7$ $p = 0.123$	$r = 0.81$ $p = \mathbf{0.026^*}$
dmPAG vs. PVN	$r = -0.72$ $p = 0.279$	$r = 0.64$ $p = \mathbf{0.046^*}$
dmPAG vs. CeA	$r = -0.66$ $p = 0.224$	$r = -0.70$ $p = \mathbf{0.035^*}$
lwDR vs. NAc_Shell	$r = -0.51$ $p = 0.242$	$r = -0.7$ $p = \mathbf{0.038^*}$
LDTg vs. IL	$r = -0.31$ $p = 0.612$	$r = 0.72$ $p = \mathbf{0.030^*}$
LDTg vs. adBNST	$r = -0.18$ $p = 0.729$	$r = 0.83$ $p = \mathbf{0.021^*}$
LDTG vs. PVN	$r = 0.54$ $p = 0.348$	$r = 0.84$ $p = \mathbf{0.002^*}$
LDTG vs. dmPAG	$r = 0.43$ $p = 0.338$	$r = 0.69$ $p = \mathbf{0.030^*}$
dmPAG vs. MS	$r = 0.90$ $p = \mathbf{0.037^*}$	$r = -0.05$ $p = 0.900$
IPAG vs. NAc_Shell	$r = -0.78$ $p = \mathbf{0.035^*}$	$r = -0.23$ $p = 0.550$
IPAG vs. dmPAG	$r = 0.79$ $p = \mathbf{0.035^*}$	$r = 0.26$ $p = 0.537$
lwDR vs. IPAG	$r = 0.71$ $p = \mathbf{0.031^*}$	$r = -0.15$ $p = 0.726$
LC vs. CeA	$r = 0.89$ $p = \mathbf{0.045^*}$	$r = -0.07$ $p = 0.846$
LC vs. vmDR	$r = 0.81$ $p = 0.095$	$r = -0.73$ $p = \mathbf{0.027^*}$
CeA vs. adBNST	$r = 0.89$ $p = \mathbf{0.008^*}$	$r = -0.19$ $p = 0.683$
BLA vs. IL	$r = 0.98$ $p = \mathbf{0.001^*}$	$r = 0.42$ $p = 0.223$
lwDR vs. PL	$r = 0.77$	$r = -0.61$

	Correlations	
	aCSF	CRF
	p = 0.045*	p = 0.060

Table 2

Significant correlations of cFOS counts between brain regions for aCSF-treated and CRF-treated female rats.

	Correlations	
	aCSF	CRF
IL vs. PL	$r = 0.88$ $p < \mathbf{0.001}^*$	$r = -0.72$ $p = \mathbf{0.024}^*$
NAc_Shell vs. IL	$r = 0.5$ $p = 0.035^*$	$r = 0.74$ $p = 0.006^*$
adBNST vs. PVN	$r = -0.55$ $p = 0.064$	$r = -0.69$ $p = \mathbf{0.009}^*$
LDTg vs. IL	$r = 0.65$ $p = 0.005$	$r = 0.68$ $p = \mathbf{0.031}^*$
LDTG vs. dmPAG	$r = 0.7$ $p = \mathbf{0.004}^*$	$r = 0.50$ $p = \mathbf{0.048}^*$
Cea vs. AdBNST	$r = -0.06$ $p = 0.851$	$r = 0.52$ $p = \mathbf{0.040}^*$
NAc_CORE vs. NAc_Shell	$r = 0.74$ $p < \mathbf{0.001}^*$	$r = 0.74$ $p < \mathbf{0.001}^*$
NAc_CORE vs. LS	$r = 0.63$ $p = \mathbf{0.006}^*$	$r = 0.04$ $p = 0.885$
oBNST vs. ACC	$r = -0.91$ $p = \mathbf{0.033}^*$	$r = -0.35$ $p = 0.326$
PVN vs. PL	$r = 0.50$ $p = \mathbf{0.048}^*$	$r = 0.40$ $p = 0.194$
CeA vs. NAc_Core	$r = 0.602$ $p = \mathbf{0.011}^*$	$r = 0.29$ $p = 0.246$
CeA vs. LS	$r = 0.57$ $p = \mathbf{0.018}^*$	$r = 0.21$ $p = 0.414$
BLA vs. CeA	$r = 0.53$ $p = \mathbf{0.023}^*$	$r = 0.40$ $p = 0.071$
HPC vs. ACC	$r = 0.74$ $p = \mathbf{0.010}^*$	$r = 0.21$ $p = 0.554$
dmPAG vs. NAc_Shell	$r = 0.55$ $p = \mathbf{0.035}^*$	$r = 0.43$ $p = 0.142$
vmDR vs. IPAG	$r = 0.56$ $p = \mathbf{0.048}^*$	$r = -0.60$ $p = \mathbf{0.023}^*$
lwDR & IL	$r = 0.6$ $p = \mathbf{0.015}^*$	$r = 0.63$ $p = 0.052$
lwDR & NAc_Core	$r = 0.66$ $p = \mathbf{0.006}^*$	$r = 0.55$ $p = \mathbf{0.034}^*$
lwDR vs. BLA	$r = 0.55$ $p = \mathbf{0.035}^*$	$r = 0.19$ $p = 0.465$
lwDR vs. dmPAG	$r = 0.68$ $p = \mathbf{0.008}^*$	$r = 0.1$ $p = 0.749$
LDTg vs. PL	$r = 0.57$ $p = \mathbf{.021}^*$	$r = 0.39$ $p = 0.304$
LDTg vs. NAc_Core	$r = 0.5$ $p = \mathbf{0.044}^*$	$r = 0.15$ $p = 0.599$
LDTg vs. vmDR	$r = 0.6$ $p = \mathbf{0.025}^*$	$r = 0.53$ $p = \mathbf{0.043}^*$
LDTg vs. lwDR	$r = 0.55$	$r = -0.06$

	Correlations	
	aCSF	CRF
	p = 0.034*	p = 0.839
LC vs. vmDR	r = 0.8 p < 0.001*	r = 0.5 p = 0.084
NAc_Shell vs. ACC	r = 0.21 p = 0.531	r = 0.6 p = 0.024*
NAc_Core vs. ACC	r = 0.33 p = 0.323	r = 0.62 p = 0.018*
NAc_Core vs. IL	r = 0.46 p = 0.058	r = 0.66 p = 0.019*
MS & LS	r = 0.38 p = 0.122	r = 0.62 p = 0.014*
BN & IL	r = 0.28 p = 0.265	r = 0.67 p = 0.012*
BN & NAc_Core	r = 0.16 p = 0.547	r = 0.52 p = 0.031*
BN & oBNST	r = -0.22 p = 0.683	r = 0.63 p = 0.021*
BLA vs. ACC	r = 0.30 p = 0.370	r = 0.68 p = 0.004*
BLA vs. NAc_Shell	r = 0.35 p = 0.172	r = 0.65 p = 0.004*
BLA vs. NAc_Core	r = 0.44 p = 0.079	r = 0.67 p = 0.002*
BLA vs. MS	r = -0.13 p = 0.625	r = 0.54 p = 0.031*
BLA vs. adBNST	r = 0.19 p = 0.544	r = 0.5 p = 0.049*
HPC vs. CeA	r = -0.07 p = 0.811	r = 0.66 p = 0.019*
vmDR vs. LS	r = 0.13 p = 0.637	r = -0.52 p = 0.048*
lwDR vs. CeA	r = 0.07 p = 0.809	r = 0.56 p = 0.020*
LC & LDTg	r = 0.36 p = 0.151	r = 0.58 p = 0.025*
BLA vs. IL	r = 0.86 p < 0.001*	r = 0.27 p = 0.38
BLA vs. PL	r = 0.76 p < 0.001*	r = 0.085 p = 0.793
HPC vs. adBNST	r = -0.66 p = 0.023*	r = 0.65 p = 0.032*
dmPAG vs. NAc_Core	r = 0.68 p = 0.005*	r = -0.05 p = 0.873
MS vs. adBNST	r = -0.11 p = 0.735	r = 0.73 p = 0.010*
adBNST vs. ACC	r = -0.66 p = 0.073*	r = 0.38 p = 0.226