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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 stressful events. 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 the number of cFOS-positive cells between regions and then identifying differences within each sex in correlations for aCSF-treated and CRF-treated groups. 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 together 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.

1. Introduction

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 post-traumatic 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 CRF_1 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

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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 that were in the proestrous phase (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-responsive regions. This analysis allows us to begin to gauge if and where ovarian hormones play a role in regulating the effect of CRF.

2. Methods

2.1. 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.

2.2. Stereotaxic surgery

Rats were anesthetized and then maintained on a 2% isofluorane-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.

2.3. Microinfusions, tissue and plasma collection, and tissue processing

Rats were infused using a microinfusion pump (Harvard Apparatus) at a rate of 1 μl/min with artificial cerebrospinal fluid (aCSF, 3 μl) or ovine CRF (American Peptides) 0.3 μg in 3 μl 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 3000 × *g* at 4 °C. Plasma was separated and frozen for later analysis (see hormone assays). Tissue was stored until sectioned (30 μm) 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).

2.4. 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.

2.5. 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 0.2-50 ng/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 10–3000 pg/ml range. This 17B–estradiol antibody cross-reacts 100% with estradiol-17β, 20% with estrone, 1.51% with estriol, and 0.68% with 17α-estradiol. The intra-assay variability was 4.7% and the inter-assay variability was 9.1%.

2.6. 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 R 2017a, MathWorks). This analysis was used to determine community structure

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