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Sex differences in corticotropin releasing factor-evoked behavior and activated networks

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ABSTRACT

Hypersecretion of corticotropin releasing factor (CRF) is linked to the pathophysiology of major depression and post-traumatic stress disorder, disorders that are more common in women than men. Notably, preclinical studies have identified sex differences in CRF receptors that can increase neuronal sensitivity to CRF in female compared to male rodents. These cellular sex differences suggest that CRF may regulate brain circuits and behavior differently in males and females. To test this idea, we first evaluated whether there were sex differences in anxiety-related behaviors induced by the central infusion of CRF. High doses of CRF increased self-grooming more in female than in male rats, and the magnitude of this effect in females was greater when they were in the proestrous phase of their estrous cycle (higher ovarian hormones) compared to the diestrous phase (lower ovarian hormones), which suggests that ovarian hormones potentiate this anxiogenic effect of CRF. Brain regions associated with CRF-evoked self-grooming were identified by correlating a marker of neuronal activation, cFOS, with time spent grooming. In the infralimbic region, which is implicated in regulating anxiety, the correlation for CRF-induced neuronal activation and grooming was positive in proestrous females, but negative for males and diestrous females, indicating that ovarian hormones altered this relationship between neuronal activation and behavior. Because CRF regulates a number of regions that work together to coordinate different aspects of responding to stress, we then examined more broadly whether CRF-activated functional connectivity networks differed between males and cycling females. Interestingly, hormonal status altered correlations for CRF-induced neuronal activation between a variety of brain regions, but the most striking differences were found when comparing proestrous females to males, particularly when comparing neuronal activation between prefrontal cortical and other forebrain regions. These results suggest that ovarian hormones alter the way brain regions work together in response to CRF, which could drive different strategies for coping with stress in males versus females. These sex differences in stress responses could also help explain female vulnerability to psychiatric disorders characterized by CRF hypersecretion.

1. Introduction

Women are roughly twice as likely as men to suffer from stress-related psychiatric disorders, such as major depression and post-traumatic stress disorder (PTSD; Kessler et al., 2012). These disorders are considered stress-related because stress is associated with their onset and severity, and stress hormone levels are altered in patients with these disorders (Breslau, 2009; Holsboer, 2001). For example, a key mediator of the stress response, corticotropin releasing factor (CRF), is hypersecreted in patients with depression and PTSD (Bremner et al., 1997; Nemeroff et al., 1984). Given that disorders characterized by CRF dysregulation occur more frequently in women than in men, sex differences in the CRF system could contribute to the sex bias in disease prevalence (Bangasser and Valentino, 2014).

In a preclinical model, we previously identified sex differences in neuronal responses to CRF. Specifically, noradrenergic neurons in the locus coeruleus (LC)-arousal system were more sensitive to CRF in female than in male rats (Bangasser et al., 2010, 2013b; Curtis et al., 2006). This physiological sex difference was linked to sex differences in CRF_1 receptor coupling and signaling (Bangasser et al., 2010). Importantly, sex differences in CRF receptors are not limited

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to the LC. For example, CRF_1 receptor binding is higher in certain regions of the cortex and amygdala in adult female compared to adult male rats (Weathington and Cooke, 2012; Weathington et al., 2014). Additionally, sex differences in CRF receptor co-localization with GABAergic neurons in the dorsal raphe and delta opioid receptor-containing neurons in the hippocampus have been identified (Howerton et al., 2014; Williams et al., 2011). Collectively, these studies suggest widespread sex differences in CRF receptors at the cellular level. However, systems level sex differences in CRF-mediated behaviors and activated circuitry have been largely underexplored, because the majority of previous studies assessed systems level effects of CRF only in male rodents (but see, Toth et al., 2015, 2014).

In male rodents, central administration of CRF is known to evoke a number of stress-related behaviors, potentiating anxiety in novel environments and eliciting defensive responses (*e.g.*, Britton et al., 1982; Korte et al., 1994; Veldhuis and De Wied, 1984). Even in a familiar environment devoid of any anxiogenic stimuli, Howard et al. (2008) found that central administration of CRF evoked head shakes, burying, and self-grooming in male rats, behaviors that are thought to be defensive or reflect an anxiety-like state (De Boer et al., 1990; Handley and Singh, 1986; Homberg et al., 2002; Spruijt et al., 1992). At the anatomical level, CRF has been shown to increase neuronal activation in cortical, limbic, and hindbrain regions in males, but again, females were not included in these studies (Arnold et al., 1992; Imaki et al., 1993).

The present study was designed to test the hypotheses that there are sex differences in CRF-evoked behavior and activated brain circuits. To this end, we first utilized the CRF-evoked behavior procedure, which was previously established in males (Howard et al., 2008) and is known to elicit both defensive and anxiety-related behavior, to determine whether CRF would increase these behaviors more in female than in male rats. We also evaluated the estrous cycle of females to assess a role for circulating ovarian hormones in regulating CRF's behavioral effects. Putative brain regions linked to CRF-evoked behavior in males and cycling females were then assessed by correlating a marker of neuronal activation, cFOS, with behavior. Then, CRF-activated circuits were assessed more broadly by evaluating neuronal activation in a number of stress responsive brain regions and determining whether the relationships between neuronal activation in these brain regions differed by sex and cycle stage. This approach allowed us to assess the effect of sex and hormonal status on neuronal activation in stress-related functional connectivity networks.

2. Methods

2.1. Subjects, cytology, and stereotaxic surgery

Two sets of adult (>70 days old) male and female Sprague Dawley rats (Charles River Laboratories, Wilmington MA, USA) were used. The first set (male, n = 11; female, n = 10) was used to generate CRF dose-response curves for anxiety-related behavior. The second set (male, n = 23; female, n = 42) was used to test the effect of estrous cycle stage on CRF-evoked behavior and activated brain circuits. All rats were housed individually on a 12-h reverse light/dark cycle with dark onset at 9:00am and *ad libitum* water and food. Females were lavaged daily to assess estrous cycle stage. All studies were conducted in accordance with the Temple University Institutional Animal Use and Care Committee and the Institutional Animal Care and Use Committee.

Rats were implanted with a cannula aimed at the lateral ventricle (−1.1 mm A/P, −1.5 mm M/L, −4.4 mm D/V) as previously described (Bangasser et al., 2013a). Then they were allowed at least 7 days to recover before testing.

2.2. CRF dose-response curve for evoked behavior

We chose to utilize the CRF-evoked behavior task developed for male rats by Howard et al. (2008) to compare the effects of CRF in males and females. This task has some advantages. First, it evokes multiple types of stress-related behaviors (*e.g.*, defensive and anxiety-related), which can be independently assessed. Second, because it is performed in a familiar environment, baseline anxiety levels are similarly low in both males and females. Under red light, each rat in the dose-response study was habituated individually for 1 h to the experimental chamber (black plexiglass 61.0 cm × 30.5 cm × 21.6 cm, open top) that contained bedding (7.5 cm in depth) spread evenly across the floor. The following day, each rat was returned to the chamber for a 30 min acclimation session. Immediately after, the rat was infused *via* a microinfusion pump (Harvard Apparatus) at a rate of 1 μl/min with the artificial cerebrospinal fluid (aCSF) vehicle or one of three doses of ovine CRF (0.1 μg, 0.3 μg, and 3.0 μg in 3 μl of aCSF; American Peptides) as previously described (Bangasser et al., 2013a; Howard et al., 2008). Doses of CRF were chosen based on Howard et al. (2008) and were administered with a week-long washout period as described (Cole et al., 2016; Fig. 1A) and in a counterbalanced fashion using four different schedules that controlled for order and carryover effects (Fig. 1B).

Each rat was returned to the chamber where it was individually tested. After 10 min, their behavior was recorded for 1 h. CRF-evoked behaviors were scored by an experimenter blind to the condition using the behavioral scoring software Kinoscope (Kokras et al., 2015, https://sourceforge.net/projects/kinoscope). As in the Howard et al. (2008) study, head shakes were defined as a shaking motion originating in the head and extending through the entire body, burying—also referred to as defensive treading (Reynolds and Berridge, 2001)—was defined as repeated forward-and-backward movements of either one or both forepaws that moved bedding, and self-grooming, which we will refer to as grooming, was defined as paw strokes on the face or body, or licking of the forelimbs or body. Consistent with Howard et al. (2008) and the focus on stress-induced behaviors the other two prominent behaviors, locomotion and resting, were not scored.

2.3. Ovarian hormone effects on CRF-evoked behavior

In the first set of rats used for the dose-response curve study, the estrous cycle was tracked, but because the rat cycle is 4–5 days and testing occurred every 7 days, females were not tested in a particular cycle stage. In order to better gauge the contribution of circulating ovarian hormones, we used a second set of rats where CRF-evoked behaviors and activated brain circuits were evaluated in females in either the proestrous phase of their cycle (higher ovarian hormones) or the diestrous phase (lower ovarian hormones). Specifically, proestrous females, diestrous females, and males were infused with either aCSF or the 0.3 μg dose of CRF and tested on the evoked behavior procedure as detailed above (Fig. 1F). The rats were then sacrificed ~75 min after the session was complete and their tissue was processed for cFOS as detailed below.

2.4. Tissue collection and processing

Following behavioral testing, rats were deeply anesthetized and transcardially perfused. Brain tissue was sectioned (30 μm) on a cryostat as previously detailed (Bangasser et al., 2013a). Slices from the dose-response curve study were processed with cresyl violet to confirm placement. Sections from the rats in the subsequent study were processed for immunohistochemistry as previously described (Bangasser et al., 2013b). Briefly, every 4th section throughout the brain was quenched (0.75% H_2O_2, 20 min), blocked (phosphate