Stress-related psychiatric disorders, such as anxiety and depression, occur twice as frequently in women than in men. Corticotropin-releasing factor (CRF) orchestrates the stress response and is dysregulated in these disorders. Thus, sex differences in CRF sensitivity could underlie their sex bias. We previously identified sex differences in CRF, receptor signaling and trafficking in the locus coeruleus that increase neuronal sensitivity to CRF in female compared to male rats. Although these CRF-induced physiological sex differences likely translate into sex differences in behavior, this possibility has not been systematically explored. The present study evaluated whether CRF induces greater anxiety-related behaviors in female than male rats. Circuits differentially activated by the central infusion of CRF in males and females also have been identified.

CRF-Evoked Behavior in Males and Females Across the Estrous Cycle

**Fig. 1.** Depiction of the CRF-evoked behavior procedure developed by Howard et al., (2008). Burying is a defensive coping strategy. Headshakes are related to high arousal. Grooming is an anxiety-related, displacement behavior associated with arousal reduction.

**Introduction**

Stress-related psychiatric disorders, such as anxiety and depression, occur twice as frequently in women than in men. Corticotropin-releasing factor (CRF) orchestrates the stress response and is dysregulated in these disorders. Thus, sex differences in CRF sensitivity could underlie their sex bias. We previously identified sex differences in CRF, receptor signaling and trafficking in the locus coeruleus that increase neuronal sensitivity to CRF in female compared to male rats. Although these CRF-induced physiological sex differences likely translate into sex differences in behavior, this possibility has not been systematically explored. The present study evaluated whether CRF induces greater anxiety-related behaviors in female than male rats. Circuits differentially activated by the central infusion of CRF in males and females also have been identified.

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**Pattern 1: Sex Difference in CRF-Induced cFOS**

**Locus Coeruleus**

- **aCSF**
- **CRF Male**
- **CRF Diestrus**
- **CRF Proestrus**

**Fig. 4.** Photomicrographs depict cFOS-positive neurons in the locus coeruleus in CRF-treated rats. CRF significantly increased cFOS-positive neurons in females. Asterisks indicate (p<0.01).

**Pattern 2: CRF-induced cFOS in Diestrus Females**

- **LDTg**
- **PAG**
- **vmDR**

**Fig. 5.** Photomicrographs depict cFOS-positive neurons in the lateral dorsal tegmental nucleus (LDTg), dorsomedial periaqueductal gray (PAG), and ventral medial dorsal raphe (vmDR). CRF significantly increased cFOS-positive neurons in the diestrus group. Asterisks indicate (p<0.05).

**Pattern 3: CRF-induced cFOS in Males and Proestrus Females**

- **Basal Nucleus**
- **Nucleus Accumbens**

**Fig. 6.** Photomicrographs depict cFOS-positive cells in the basal nucleus and nucleus accumbens in CRF-treated rats. CRF significantly increased cFOS in males and proestrus females. Asterisks indicate (p<0.05).

**Correlations Between cFOS and Grooming**

**PVN**

**AcbC**

**IL**

**Fig. 7.** The nature of the relationship between CRF-induced brain activation and grooming differed across hormonal cycle for the paraventricular nucleus (PVN), nucleus accumbens core (AcbC), and infralimbic cortex (IL). Fisher’s z transformation revealed significant differences between the correlations for males and proestrus females in all regions, as well as between diestrus and proestrus females in the IL.

**Conclusions**

- CRF evoked more grooming in proestrus female rats compared to diestrus female rats and male rats. Because proestrus is the estrous cycle phase when levels of progesterone and estrogen are higher, this result suggests that one or both of these hormones potentiate the effect of CRF on grooming.
- Interestingly, CRF activated different circuits in males and females. Differences in regional activation were further observed across the female estrous cycle.
- For the PVN, AcbC, and IL, the nature of the relationship between CRF-induced brain activation and grooming was dependent on hormonal status.
- The differential effect of CRF on various circuits in males and females could be an important mechanism by which sex differences in stress responses, anxiety, and stress-related disorders are established.

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