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Sex Differences in the Co-expression of Estrogen Receptor Alpha with Corticotropin Releasing Factor

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Abstract

Women are far more likely to develop anxiety and depression than men. It is believed that the dysregulation of the HPA axis by the binding of corticotropin -releasing factor (CRF) to the corticotropin-releasing factor receptor 1 (CRFR1) contributes to the likelihood of these stress-related disorders. Estrogens acting through Estrogen receptor alpha (ERa) have been shown to increase anxiety production upon activating the HPA axis. In this current study, we explored whether CRF-expressing neurons in various regions of the brain express ERa. The levels of ERa were counted in the bed nucleus of the stria terminalis (BST), the medial preoptic nucleus (MPN), and the medial preoptic area (MPOA). We expect there to be differences in the expression of ERα between males and females within all 3 regions. ERα is suspected to act as a pathway for the CRF neurons to pass through which would amplify the stress response in females. If females have certain CRF-activated cells expressed disproportionately to males in different brain regions, this will provide further evidence that there are sex differences present in the mice. In the future, this would support the possibility of sex differences between men and women in terms of stress-related mood disorders considering CRF has been shown to have a relationship with stress responses in the brain.

Keywords: *HPA axis, Corticotropin-releasing Factor, Estrogen, ERa, Stress*

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Introduction

Women are twice as likely to develop stress-related mood disorders such as anxiety or depression. Overall, there is an overwhelming percentage of people who will develop these disorders in their lifetime. These developments have been linked to the Hypothalamic-Pituitary-Adrenal axis. Basically, when stress is incurred, this axis is activated. The HPA axis functions as a negative feedback loop to maintain a response to external stress. When the HPA axis is dysregulated, we see the onset of stress disorders. HPA axis performs negative feedback. The hypothalamus releases corticotropin-releasing hormone and when that eventually leads to enough cortisol being produced, a signal will be sent to stop the production of CRH. This stimulates the anterior pituitary to release adrenocorticotropic hormone. Finally, leading to the release of CORT by the adrenal cortex which specifically refers to corticosterone in mice and cortisol in humans. Cortisol is known as the stress hormone. It works to increase our heart rate and blood pressure. The short-term increase in cortisol is necessary for our recovery from stress so that we don't incur fatigue or damaged adrenal glands from too much stress. However, dysregulation of the HPA axis does lead to less negative feedback. The continuous output of cortisol, which again is stimulated by stress, can eventually have a negative impact on the HPA axis, meaning that the body becomes less resilient to stress.

The corticotropin-releasing factor is a regulator of the HPA axis. It also activates one of its receptors, CRFR1, and this binding can regulate stress-associated behaviors. Estrogen Receptor Alpha is a transcription factor that regulates the transcription of certain target genes. ERα is typically found in the nucleus. ERα has been seen to produce anxiogenic effects in rodents. This implies that when ERα binds within mice, it can induce the behaviors that are indicative of anxiety. The binding to ERa has also been shown to increase the HPA axis response which would cause increased levels of corticotropin-releasing hormone. This would

ultimately result in greater corticosterone production. Therefore, there is a possibility that the colocalization of ERα and CRF can increase the stress response seen in mice.

There are many different overlapping functions within the brain. For this study, there are three main brain regions that can allow for the evaluation of stress and anxiety levels in mice. The medial pre-optic area is located within the hypothalamus, and it is known for its prolonged responses to anxiety-producing stressors (Zhang et al., 2021). We also studied the medial preoptic nucleus that regulates the HPA responses maintain homeostasis (Williamson et al., 2010). Finally, inside of the amygdala there is the bed nucleus of the stria terminalis and that is known for controlling these anxiety-like behaviors in mice (Zhang et al., 2021).

In this study, we gathered cells from the brain regions chosen within the mice. After this, we performed microscopy. We also did cell counting through ImageJ software for quantification of the genes activated in the region of interest. Then, I performed fluorescent immunohistochemistry for a final visualization of the cells.

Methods and Materials

Brain Slicing

We crossed a CRF/Cre mouse with an Ai9TDTom mouse for the mice used in this experiment. This crossing was important because the TDTomato allows the CRF to present to us as red for better identification and stain the ERα cells green for visualization. We collected the brains from the rodents for sectioning. These brains were stored in the freezer. We use an Optimal cutting temperature compound to create a platform for the embedding of the brain. As the cryostat froze slightly, we gently patted the brain dry with a KimWipe and cut off the cerebellum of the brain to make a steady flat bottom for the brain to stick to the plate. Next, we set the brain onto the platform created by the OCT compound and ensured that the brain was

symmetrically placed. Then, we began covering the brain with an even coat of OCT compound and freezing it in short increments until the entire brain was covered in the OCT compound. Now we placed the platform with the brain attached in the object holder. From this point, we were able to create even brain slices and gather them with a PBS-covered paintbrush.

Fluorescent Immunohistochemistry

We begin by preparing the brain sections onto a tissue slide. Then we perform blocking to prevent nonspecific antibodies from binding. After blocking, we add a primary antibody for 30-60 minutes. Now we can wash the slides 3 times for 5 mins each. Next, we add a secondary antibody for 30-60 minutes and wash again. Then, we add the substrate and wash again before we counterstain. The cell is then prepared for mounting of the tissue onto the slides. Finally, we can perform imaging of the tissue.

Microscopy and Cell Counting

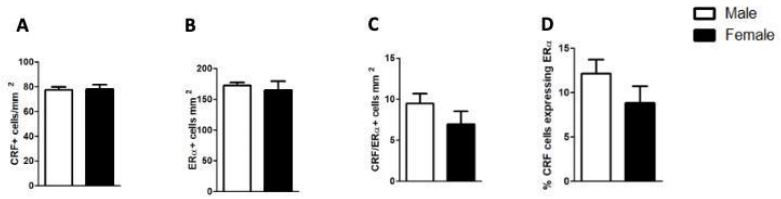
Cells were visualized underneath a microscope. Then the color differentiation allows us to see CRF and ERα cells. We counted these cells for quantification for which cells are seen more often in each of the sexes.

Results

The goal of this research was to discover any possibly sex differences in the stress-related responses of the mice. This would indicate that it is possible a correlation between the onset of stress and anxiety responses with the expression of ERa, CRF, and the co-expression of both.

Figure 1

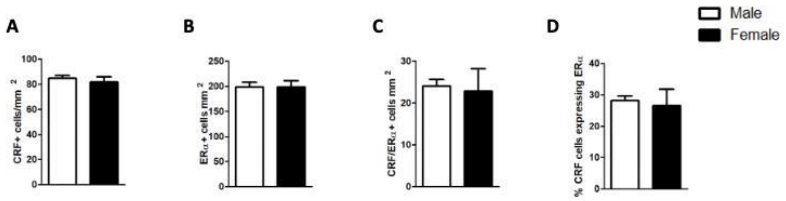
BSTd Region Cell Analysis



Note. There was a small appearance of a difference in ERa and CRF cell counts between females and males and a greater percentage and count of CRF cells expressing ERa in males than there was in females. However, this data recorded was statistically insignificant.

Figure 2

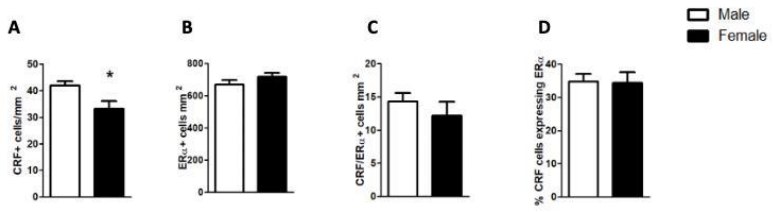
BSTv Region Cell Analysis



Note. There was no significant difference in the number of CRF and ERa seen between females and males. There is also no significance to the difference in colocalization seen in the sexes.

Figure 3

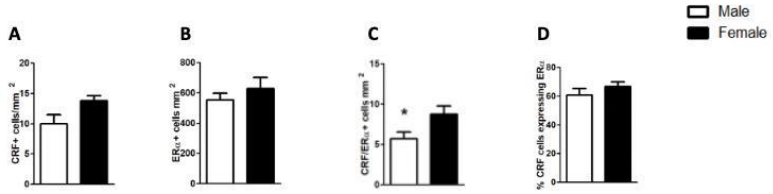
MPN Region Cell Analysis



Note. **A:** There was statistical significance to the decreased number of CRF cells seen in the MPN regions ($p < 0.05$). There was much less CRF seen in females than in males. **B-D:** There was no significant difference in the ERa cells between the sexes in this region.

Figure 4

MPOA Region Cell Analysis



Note. **A-B:** There was no significance to this data. **C:** There has been less colocalization of CRF with ERa cells in males than in females. There was statistical significance to this data ($p < .05$). **D:** This data is statistically insignificant.

Discussion

In this study, we divulged 3 main brain regions. Estrogen uses these brain regions to impact stress responses. The BST region has an abundance of Estrogen receptors, so it is logical to investigate this region to see if there are a sufficient number of CRF neurons expressing ERa (Zuloaga et al., 2020). The BST brain region is a crucial region for the regulation of both anxiety and depression as well as the possible sex differences within these stress-related mood disorders. The anteroventral region of the BST has been shown to have high levels of estrogen receptors that can lead to the sex difference in stress response. The CRF in the dorsolateral region of the BST works with stress response regulation. The BST brain region has been implicated as the route that gonadal hormones use to create neuroendocrine stress responses. Considering its location within the hypothalamus, the BST is needed to regulate the HPA axis during chronic stress. The MPN region also has an ample amount of ERa. This area also expresses the beta variation of the estrogen receptor while playing a certain role in the rapid estrogen activity throughout the brain (Ábrahám, 2004). The MPN region functions to regulate the HPA axis to maintain homeostasis. The MPOA region has been shown to have CRF acting as a mediator of the anxiogenic effects of ERa (Dagnault et al., 1997). In the MPOA region, CRF neurons and ERa work together within the brain for other functions (i.e anorectic impacts). The nuclei of the MPOA region operating within the hypothalamus work to control the HPA axis function. By regulating the HPA axis, these regions modulate stress responses.

In both sexes, there is coexpression of ERa in CRF neurons. Our study has demonstrated there is a higher amount of colocalization between ERa and CRF cells within the MPOA region for females than males. This would insinuate that in this region estrogen acts on these CRF cells

to impact the stress responses differently between the female and male mice. There was also a higher level of ERa reported in the female mice than in the male mice in this region.

The increased colocalization level within females implies that there could possibly be a higher responsiveness for females regarding carrying out the functions of the ERa in the cell. ERa causes anxiogenic effects with the rodents; therefore, there would be an increased anxiogenic stress response for the females who have an ERa and CRF colocalization. This is due to the inhibition of negative feedback within the HPA axis causing excess stress hormones to be released. Once we properly understand the sex differences in the co-expression of estrogen receptor alpha and corticotropin-releasing factor in mice, we can translationally observe what the difference in estrogen expression means for a possible dissimilarity in stress response between men and women.

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