Characterizing the Role of Estradiol in Neural Systems Underlying Social Stress Susceptibility in Female Rats

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Introduction

Repeated exposure to social stress is a common risk factor for development of anxiety disorders. Interestingly, women are twice as likely as men to suffer from anxiety [1]. However, the mechanism underlying this increased stress susceptibility in females is unclear. It is known that the increased susceptibility in females is confined between the onset of puberty and the end of menopause, suggesting that ovarian hormones may contribute to this disproportionate risk of stress-related disorders. Previously, we have shown that witness stress (WS) produces anxiety-like behaviors selectively in intact, cycling female rats [2]. Ovariectomized (OVX) females are largely resistant to witness stress. Moreover, intact females exposed to WS exhibit a distinct increase in corticotropin releasing factor (CRF) expression in the central nucleus of the amygdala (CeA). Thus, this study aimed to understand the specific 17-β estradiol-induced behavioral responses and neural regulation that may contribute to the heightened susceptibility to social stress among females. To do this, we observed the CeA and the hippocampus (hipp), two stress sensitive brain regions that express CRF and have estrogen receptors.

Methods

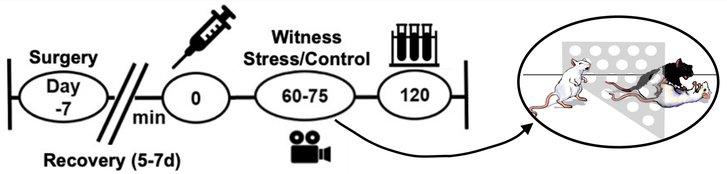

Figure 1. Study Timeline and Witness Stress Paradigm Diagram

Surgery: Sprague-Dawley rats underwent sham or ovariectomy (OVX) surgery

Drug/Vehicle Treatment: Subcutaneous injection of 17-β estradiol (17-βE, 10µg/rat) or sesame oil vehicle (veh, 0µg/rat)

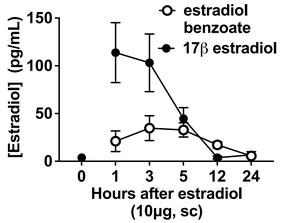

Figure 2. In the present investigation, 10µg of 17-βE was selected as we have confirmed that it mimics the natural peak of estradiol release in a normally cycling rat. 10ug of estradiol benzoate was less effective at mimicking this peak.

Witness Stress (WS): Female rats were subjected to witnessing an aggressive social-defeat encounter between male rats for 15 minutes; controls were handled by humans for 15 seconds.

Outcome Measures: Witness stress-evoked anxiety-like behaviors (burying, rearing, and freezing), corticosterone (CORT) levels, and CRF expression in stress sensitive brain regions were quantified in response to witness stress or control.

Statistical Analyses: 2-way ANOVAs (stress x hormone) or One-way ANOVA was conducted for behaviors quantified only in witnesses (Fig. 5).

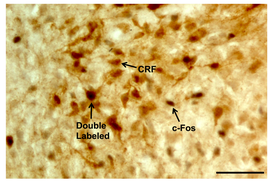

Figure 3. Immunohistochemical double labeling of CeA shows neurons expressing both corticotropin releasing factor (CRF;brown staining) and c-Fos (purple-black staining); scale bar = 1000µm.

Pro-Estrus Estrogen Levels Regulate WS Evoked Burying

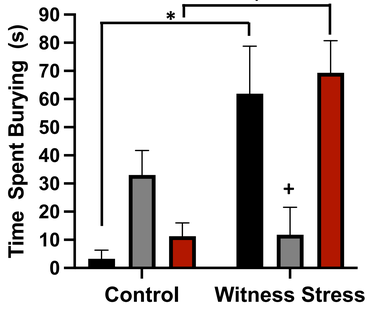

Figure 4. Witness stress (WS)-induced anxiety-like behavior as indicated by greater burying duration among witnesses vs controls. Witness stress-evoked burying that emerged in intact females was blunted by OVX and reinstated by 17-βE (*p<0.05, +p =0.055 vs sham + veh and OVX + 17-βE).

Pro-Estrus Estrogen Levels Do Not Regulate WS Evoked Freezing or Rearing

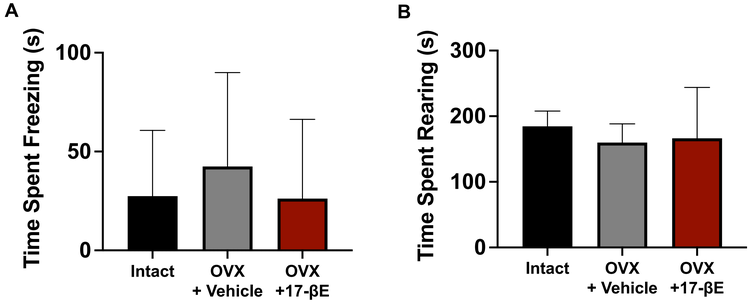

Figure 5. Witness stress-induced anxiety-like freezing (A) and rearing (B) behaviors were not regulated by estrogen (p>0.05).

Pro-Estrus Estrogen Levels Increase WS Induced Corticosterone Levels

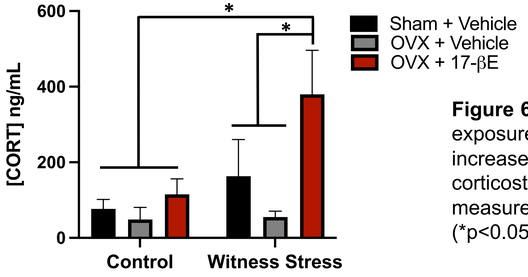

Figure 6. Sixty-mins after a single exposure to WS, 17-βE (10µg) increased the stress-induced corticosterone response as measured in plasma samples (*p<0.05 vs all groups).

Acknowledgements

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Pro-Estrus Estrogen Levels Promote CRF Neuronal Activation in CeA

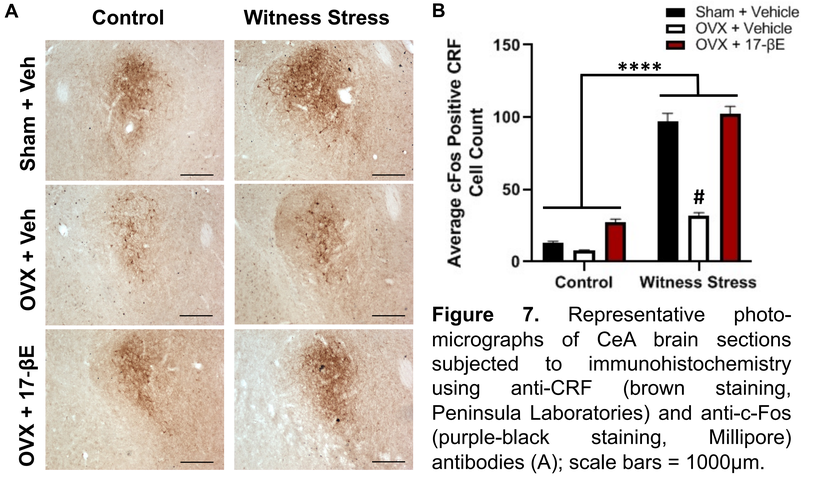

Figure 7. Representative photo-micrographs of CeA brain sections subjected to immunohistochemistry using anti-CRF (brown staining, Peninsula Laboratories) and anti-c-Fos (purple-black staining, Millipore) antibodies (A); scale bars = 1000µm.

c-Fos positive CRF expression in the CeA was robustly enhanced by 17-βE during WS, but not control (B) (****p<0.0001, #p=<0.0001 vs sham + veh and OVX + 17-βE).

Pro-Estrus Estrogen Levels Do Not Promote CRF Neuronal Activation in Hipp

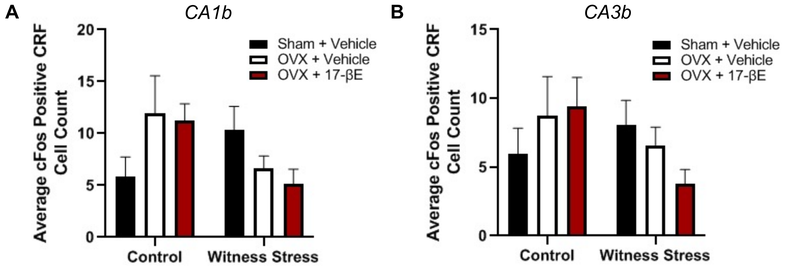

Figure 8. In the CA1 (A) and CA3 (B) subfields of the hippocampus, there was no effect of WS or 17-βE on c-Fos expression in CRF positive neurons (p>0.05).

Conclusions

- 17-βE is important in facilitating the anxiety-like responses to WS and the CeA acts as a region-specific regulator of these responses.
- While estradiol was shown to regulate CRF in the CeA, this is not universal throughout the brain as was shown in the hippocampus.
- By identifying neural systems activated in stress sensitive versus resilient conditions, this study will lay the foundation for identifying pharmacological targets to increase stress resilience.

References

1- Kessler, R.C., et. al (1993). *Journal of Affective Disorders.* 29(2-3):85-96.
2 - Finnell, J.E., et. al (2018). *Biological Psychiatry*.