Estrogen-Dopamine Interactions in Female Rats During Extinction Learning
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Abstract
Women are twice as likely as men to develop Post Traumatic Stress Disorder (PTSD), but the neurobiological factors underlying this discrepancy are mostly unknown. In preclinical studies using fear conditioning and extinction paradigms, female rats with low estrogen levels exhibit impaired extinction retrieval. We have shown previously that estrogen can modulate dopaminergic transmission to rescue extinction retrieval impairments, suggesting that estrogen-dopamine interactions may be important during extinction learning. However, the physiological effects of estrogen on dopamine (DA) transmission during fear extinction are unknown. Intact female Long-Evans rats underwent a 2-day fear conditioning and extinction learning paradigm. Rats were then grouped according to estrous phase during extinction learning (day 2). All rats showed comparable freezing levels during fear conditioning and extinction regardless of estrous phase. We performed immunohistochemistry for c-fos expression in prelimbic and infralimbic regions of medial prefrontal cortex during extinction learning, and found that low-estradiol rats had increased activation of intralaminar neurons compared to high-estradiol rats. We also used immunohistochemistry for c-fos and tyrosine hydroxylase (TH) to quantify activation of DA neurons in the ventral tegmental area (VTA). Despite equal numbers of total TH + cells in all groups, high-estradiol rats had a greater percentage of c-fos labeled TH + cells in the VTA than low-estradiol rats.

Introduction
Women are twice as likely as men to develop Post Traumatic Stress Disorder (PTSD), but the neurobiological factors underlying this discrepancy are mostly unknown. In the rat brain, the prelimbic region (PL) of the medial prefrontal cortex (mPFC) excites the amygdala to express conditioned fear, while the infralimbic region (IL) inhibits fear responses during extinction and enhances retrieval of the extinction memory. Data from our lab and others demonstrate that animals in the low estrogen phases of the estrous cycle (Estrus, metestrus, diestrus) during extinction learning exhibit impaired extinction retrieval compared to animals in the high estrogen phase (PRO; proestrus). However, it is unknown how estrous phase may affect neural activation following extinction learning.

We have shown previously that circulating estrogen may interact with dopamine to alter freezing in females. D1 agonist treatment 30 min before extinction learning facilitates extinction consolidation in low-estrogen rats. However, the physiological effects of estrogen on dopamine transmission during fear extinction are unknown.

Methods

Figure 1. Effects of pre-extinction D1 agonist administration on extinction retrieval in EMD and proestrous female rats. SKF38393 induced impaired extinction retrieval in proestrus animals, and enhanced extinction retrieval in EMD animals. **p<0.006 compared with PRO-SKF, ***p<0.002 compared with EMD-VEH, and ****p<0.001 compared with same treatment/opposite estrogen state. From Rey et al., Neuropsychopharmacology 39, 1282-1289 (2014).

Figure 2. On day one, rats underwent seven tone-shock pairings and acquired the conditioned fear. On day 2, rats received twenty tone-alone extinction trials. Ninety minutes after the last tone rats were sacrificed and brains were extracted for subsequent immunostaining for markers of neural activation and dopamine signaling.

Figure 3. Above: As expected, there were no significant differences in percent freezing to the tone between EMD and PRO rats during fear conditioning or extinction.

Figure 4. Right: Representative photomicrograph of PL c-fos immunohistochemistry in proestrus (PRO) rats.

Figure 5. Left: Number of c-fos+ cells counted in PL (left) and IL (right) regions of mPFC in EMD and PRO rats. In PL, there was a significant increase in the number of c-fos+ cells in PRO rats compared to EMD rats. In IL, there were no significant differences in the number of c-fos+ cells in EMD compared to PRO rats. ***, p=0.001 EMD vs. PRO.

Does circulating estrogen alter activation of TH-positive VTA neurons during extinction learning?

Figure 7. Left: Shaded areas represent sampled sections of the VTA. Right: representative photomicrograph of VTA immunohistochemistry.

Figure 8. The number of TH+ neurons does not vary between EMD and PRO rats in either the anterior region of the VTA (Left). There is a greater percentage of c-fos+ TH+ neurons of anterior VTA in PRO compared to EMD rats. (Right).

Figure 9. Model of estrogen-dopamine interactions on the inverted-U curve. In the baseline configuration, PRO rats exhibit optimal D1 signaling and PFC performance. After D1 simulation, EMD rats improve and PRO rats exhibit impairment.

Conclusions
• Circulating estrogen shifted the balance of mPFC activity during extinction
  • PRO rats had greater PL activation during extinction learning than EMD rats
  • Is recruitment of PL necessary for enhanced consolidation?
• Anatomically specific effect of high circulating estrogen on VTA activation during extinction learning
  • Increased c-fos expression in TH+ neurons of anterior VTA of PRO vs EMD rats
• Role for estrogen-dopamine interactions as a modulator of fear suppression in females