Estrogen and Extinction of Fear Memories: Implications for Posttraumatic Stress Disorder Treatment

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Abstract

Posttraumatic stress disorder (PTSD) is a psychiatric illness whose prevalence in women is more than twice the rate as men. Despite a burgeoning literature characterizing sex differences in PTSD incidence and its disproportionate burden on society, there is a dearth of literature describing biological mechanisms underlying these disparities. However, the recent identification of biomarkers of PTSD by translational neuroscientists offers a promising opportunity to explore sex interactions in PTSD phenotypes. A notable observation is that individuals with PTSD show deficits in their ability to inhibit conditioned fear responding after extinction training. Given that extinction procedures, via exposure-based cognitive behavioral therapy, make up one of the predominant modes of treatment in PTSD, there is a critical need for more research on sex interactions in this form of fear regulation. An emerging hypothesis is that fluctuating gonadal hormones, especially estrogen, in the menstrual cycle may play a critical role in fear extinction and, hence, PTSD vulnerability and symptom severity in women. The current review discusses how the study of putative activational effects of estrogen on fear extinction may be harnessed to advance the search for better treatments for PTSD in women. We conclude that estrogen treatment may be a putative pharmacological adjunct in extinction based therapies, and should be tracked in the menstrual cycle during the course of PTSD treatment.

Keywords

Extinction; Fear conditioning; Estrogen; PTSD; Anxiety; Sex differences

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Introduction

It is widely understood that women have a significantly higher occurrence of anxiety disorders than men (1, 2). Posttraumatic stress disorder (PTSD), a severe and debilitating anxiety disorder that may develop following a traumatic experience, is one of the most sex-polarized psychiatric illnesses, with women having more than twice the prevalence than men (3-6). Women are also more likely to experience chronic PTSD that persists for more than a year (4, 5, 7) and carry a greater burden of this illness on the healthcare infrastructure (1). While there is much debate about what factors contribute to these disparities –from sociocultural and environmental influences (8) to sexual dimorphisms in physiology – there is, surprisingly, very little known about the biological mechanisms that underlie sex differences in PTSD. The overreliance of male subjects in preclinical animal models and in clinical research has, thus far, limited our progress in understanding the nature of psychiatric illnesses that disproportionately affect women. Recently, however, a few studies have linked the cyclical release of ovarian hormones to PTSD vulnerability in women (9-11). These findings are particularly important given that anxiety disorders tend to emerge around puberty, when sex organs begin to release hormones that have activational effects on brain activity. The current review discusses how this recent work – along with an accumulating line of evidence suggesting activational effects of gonadal steroid hormones on female emotional regulation – may be harnessed to advance the search for better treatments for PTSD in women.

The role of fluctuating estrogen on anxiety-related behaviors

Sex differences in rates of anxiety disorders typically emerge after puberty, when circulating ovarian hormones increase (12, 13), implicating activational sex steroids (i.e., estrogens, progestogens, and androgens) in the expression of psychopathology (14). Among the sex steroids, estrogen has come forth as a leading candidate for the study of gonadal hormone influence on female brain and behavior. At sexual maturity, females experience cyclic fluctuations in estrogen secretions throughout their reproductive cycle, ending in rapid decline during menopause. Interestingly, women are more likely to report symptoms of depression and anxiety during pre-menstrual, post-partum and peri- and post-menopausal periods, when estrogen levels are relatively low (14-20).

Neuroimaging studies show greater activation of neural networks involved in fear excitation when women are scanned during the early follicular phase of their menstrual cycle (marked by low estrogen levels) relative to those scanned mid-cycle (high estrogen levels) (21, 22). Moreover, studies using rodent models of anxiety have shown that females in the pro-estrus phase of their cycle (marked by high estrogen levels) show less fear- and anxiety-related behaviors relative to females in the metestrus or diestrus phases (lower estrogen levels; (23-26). Together, these findings suggest that cyclical secretions of estrogen across the reproductive cycle may play a role in women's increased vulnerability to, and the severity of, PTSD symptoms after psychological trauma. However, very few studies have taken into account the estrous cycle, or studied phasic estrogen levels in laboratory-based models that explicitly probe for PTSD phenotypes. In fact, many studies intentionally exclude female subjects to eliminate this source of variability. However, a more inclusive research approach

is critical for understanding sex interactions in PTSD vulnerability. In the following sections, we discuss an effective approach for identifying biomarkers for PTSD, and describe research to date that have used this approach to examine an activational role of fluctuating estrogen on PTSD risk in women.

Use of fear conditioning models to study biomarkers for PTSD

Most individuals who experience trauma do not develop PTSD in its aftermath. Therefore, a major goal in psychiatric research is to identify what factors confer resilience or vulnerability to traumatic life events. The three primary symptom clusters that characterize PTSD, although differentially characterized by the Diagnostic and Statistical Manual for Mental Disorders (DSM) versions IV and 5, include: (1) persistent and intrusive recollections of the traumatic event, (2) hyperactivity and increased arousal, usually in response to trauma reminders, and (3) avoidance of sensory cues associated with the traumatic event (27); dominant features of the disorder that reflect memory-related mechanisms. Hence, many translational researchers have focused on the neural bases of fear-motivated learning and memory to better understand the biological underpinnings of this disorder. It is generally believed that traumatic memories form when individuals contemporaneously experience neutral stimuli with highly aversive stimuli in their environment. Many behavioral scientists attribute this to associative learning, a phenomenon that has been extensively modeled in animals including rodents and non-human primates (28-30).

In Pavlovian fear conditioning, a subject is exposed to a previously neutral conditioned stimulus (CS), which is often presented in the form of a discrete cue, such as an odor, light, or tone (cued fear conditioning), or a distinctive environment (context fear conditioning), that overlaps in time with an aversive unconditioned stimulus (US), such as a footshock or an aversive airblast. Consequently, subjects show a species-specific response, such as increased startle or freezing behavior, in the presence of the reinforced CS due to its prior association with the US. The CS may also elicit autonomic (e.g., changes in heart rate or blood pressure) and endocrine (e.g., hormone release) responses. Thus, memory is inferred from a quantifiable behavioral or physiological change observed in the presence versus the absence of the CS.

In the clinical presentation of PTSD, fear responses closely resemble those observed in fear conditioning models (i.e., increased arousal and hyper-reactivity in the presence of trauma-related cues). A major challenge in PTSD research has been to understand the neural mechanisms involved in the reduction of these conditioned fear responses, and finding tools in which one can inhibit or eliminate maladaptive behaviors associated with unwanted fear memories.

Fear extinction is one of the leading experimental models for measuring the ability to suppress a conditioned fear response. In this model, the amplitude and frequency of conditioned fear responses may be gradually reduced by repeated presentations of the previously CS in the absence of the aversive US. Our laboratory has consistently found that

people with PTSD show deficits in fear inhibition (31, 32) and extinction (33), suggesting that impaired inhibition of conditioned fear may be a biomarker of PTSD (34).

Although fear extinction models have been critical in shaping our understanding of the neurobiological basis of fear regulation, there are very few studies that examined sex differences or observed the female behavioral response using this paradigm. Given that extinction procedures comprise some of the evidence-based modes of treatment of PTSD and other trauma-, stressor-, and anxiety-related disorders (i.e., exposure-based cognitive behavioral therapy or CBT), there is a critical need for more research on sex interactions in fear conditioning and extinction processes.

Sex interactions in fear conditioning

In general, male rats outperform females in tests of conditioned fear acquisition. In contextual fear conditioning, male rats have shown enhanced acquisition (35, 36), which has been associated with increased long-term potentiation (LTP) in the hippocampus compared to females (35). This effect was not altered by castration of the adult male rat (37). However, ovariectomized (OVX) female rats showed enhanced fear *expression*, similar to males; an effect that normalized back to control levels with estrogen replacement (38). A similar pattern of results was found in tone-induced cued fear conditioning (35, 36, 39, 40). These findings suggest a role of estrogen in reducing the over-expression of fear following acquisition in females via synaptic plasticity, but indicate no such function of testosterone in males.

Conflicting findings have been reported when conditioned fear retention was measured and when the estrous cycle of females was taken into account. That is, female rats that were conditioned and tested during proestrus (when estrogen levels are at their peak) showed less contextual fear retention compared to both males and estrus females (when estrogen levels are low; (41). However, there was no sex difference in cued fear retention (41). Together, these findings implicate an important role of estrogen in hippocampal-dependent fear learning and memory.

Role of sex hormones in conditioned fear extinction

Fear extinction is believed to be a form of hippocampal-dependent learning in concert with brain areas including prefrontal cortical regions (42). Given previous evidence of estrogen involvement in other hippocampal-dependent fear conditioning tasks (35-37, 41), it is reasonable to hypothesize a role for estrogen in this model. Nevertheless, few studies have used fear extinction paradigms to examine sex and gonadal hormone interactions. In one of the first studies to implicate sex steroids in fear extinction processes, Rivas-Arancibia and Vazquez-Pereyra (43) found that both testosterone and estradiol treatment facilitated extinction of a single-trial passive avoidance task in male rats. Since steroid hormone treatments were given prior to avoidance training and then again before each of 10 weekly extinction trials, it is difficult to disentangle the mechanism of action of steroid hormones on fear acquisition versus extinction acquisition and/or retention (43). Also, the role of sex differences in extinction was not addressed as only male rats were examined.

Sex differences in fear extinction have since been reported in rats, whereby naturally cycling female rats exhibited impairments in extinction recall compared to males (39, 44). However, female estrous cycle phase was not an experimental variable in those studies. A formative study by Chang and colleagues (45) found significant sex and estrous phase differences in contextual fear extinction in rats. Specifically, they replicated previous findings that male rats exhibited enhanced context fear acquisition relative to naturally cycling females. However, when fear extinction rate was determined in relation to estrous stage, females in proestrus and estrus extinguished more rapidly than males and females in diestrus (45). In further support for a role of estrogen in extinction, they found that OVX females treated with estradiol extinguished more rapidly than both vehicle-treated and progesterone-treated OVX females. In addition, this facilitating effect of estradiol on extinction was replicated when OVX females were treated with a selective estrogen receptor (ER) β agonist, but not with a selective ERα agonist (45). These findings strongly implicate estrogen in fear extinction modulation due to ERβ activation.

In perhaps the most comprehensive set of studies examining sex and estrous cycle influences on fear extinction, Milad and colleagues have established an important role of estrogen in extinction mechanisms in female rats and women (see (46) for review). Importantly, they demonstrated that when cycle phase was not factored in their analyses, no differences in fear extinction were found between males and females. However, sex differences were evident when females were divided into low and high estradiol groups based on their phase in estrous or menstrual cycle (47-50). Specifically, females in the high estradiol group expressed extinction similar to males, and both groups expressed greater extinction retention than females in the low estradiol group (48, 49). A recent study that examined fear conditioning and extinction while collecting saliva hormonal levels also found that low estradiol, but not progesterone, levels were associated with higher fear responses during extinction (51). Moreover, this study found that women who had low levels of estrogen during the observation of violent film clips had more intrusive thoughts about these scenes in the following days; this finding is of high relevance to the re-experiencing symptoms of PTSD (51). It may appear contradictory that females, who should be protected by higher estrogen levels relative to men, show higher vulnerability to PTSD. However, taking into account the cyclic nature of estrogen in females and the more constant endogenous estrogen levels in males (albeit on average lower than those in females) these data suggest a phasic vulnerability in women. These findings underscore the importance of taking cycle phase into consideration when examining sex interactions in fear learning and memory. Given the additional observation that administration of selective ERβ but not ERα agonists to female rats in the metestrus phase (low estrogen) improved extinction recall (50) and that blockade of ERβ and ERα in proestrus females impaired extinction recall (48), there is convincing evidence to conclude that activational estrogen exposure is critical for fear extinction in females, and ERβ is a critical site of activation. These observations, tying fear extinction to estrogen activity at ERβ, hold clinical promise for the use of estrogen as a pharmacological adjunct in PTSD treatment. More clinical research is clearly needed to characterize estrogen function in women who are at risk for or have developed PTSD.

Estrogen and PTSD

It has been established that PTSD is a disorder of the fear memory circuitry and that individuals with PTSD show deficits in extinction. Neurobiological models of PTSD hold that the maladaptive fear responses observed in PTSD are due to heightened activity in the amygdala (52) in concert with impaired inhibitory control of the amygdala from prefrontal cortical regions (53). This fear regulatory network has been well-characterized as playing a critical role in extinction learning and memory (54, 55). Estrogen receptors, both ERα and ERβ, are densely expressed in the amygdala, hypothalamus, and hippocampal cortical regions (56-59), and express sexually dimorphic intracellular processing (60, 61), suggesting sex-specific estrogen modulation of emotion and cognition. However, the functional consequences of estrogen-induced plastic changes within the fear extinction network are not fully understood. Estrogen may influence extinction via genomic mechanisms by binding to classical nuclear receptors and initiating long-lasting transcription-related events. However, recent advances suggest that estrogen may also act at membrane receptors (which are similar or identical to classical receptors) to initiate rapid effects via signal transduction pathways (62, 63). The fact that acute estrogen administration induces rapid and reversible changes in synaptic plasticity within the extinction network (64, 65) and in extinction memory (48-50) suggests that non-genomic estrogen activity at membrane receptors may largely mediate these facilitatory effects. A number of candidate cellular and molecular markers have been linked to estrogen activity within the extinction network, such as increased NMDA- (66) and AMPA- (67) receptor activity, and increases in key synaptic proteins such as MAPK-dependent CREB phosphorylation (68, 69) and brain-derived neurotrophic factor (BDNF) expression (63, 70, 71). Nevertheless, a great deal of research is needed to delineate the precise mechanisms underlying estrogen modulation of fear extinction. By establishing a role of estrogen in this neural circuitry and in extinction function, there is a unique opportunity to pharmacologically target this behavioral phenotype in women with PTSD (72).

Functional magnetic resonance imaging studies (fMRI) have found sex differences in fear-associated neural activation patterns (46, 73, 74). Changes in brain activity in areas associated with fear regulation occur across the menstrual cycle in time with fluctuating gonadal hormones (21, 75). Women who are ovulating (high estrogen) show attenuated activity in the amygdala and related subcortical regions and increased activity in those regions during the early follicular phase of their menstrual cycle (low estrogen) (21, 22). Together, these findings establish a dynamic neuromodulatory role of estrogen on female brain function during emotion processing, and provide firm rationale for research examining the role of estrogen in PTSD vulnerability and symptom severity. In addition to the above neural activation, peripheral biomarkers are also influenced by menstrual cycle changes. For example, the acoustic startle reflex, which is mediated by amygdala activity, is enhanced during periods of lower estrogen (76, 77).

Current work from our group and others has employed a translational approach to characterize biomarkers of PTSD in a clinical population using fear potentiation of the acoustic startle response. By measuring conditioned fear in a traumatized sample, we are well suited to examine estrogen influences on PTSD risk in women. We previously found

that people with PTSD show impairments in their ability to inhibit conditioned fear responses in the presence of safety cues (78, 79), and also show deficits in fear extinction (79). In addition, women with PTSD exhibit higher expression of conditioned fear compared to men with PTSD (80). In the first study to examine estrogen interactions in these behavioral phenotypes in a clinical sample, we recently identified a potential role for estrogen in PTSD vulnerability and severity in women (9). Specifically, the mechanism underlying this role is still not well understood, but recent evidence suggests an interaction between ovarian hormone levels and genetic risk. For example, a single nucleotide polymorphism rs2267735 in the pituitary adenylate cyclase activating protein (PACAP) receptor gene, *ADCYAP1R1*, located within a canonical estrogen response element, is associated with increased PTSD symptoms (81), brain activation to fearful stimuli (82), and elevated startle responses (81). In addition, plasma levels of the PACAP peptide were positively associated with increased PTSD symptom expression (81). The PACAP—PAC1R pathway has been linked to numerous fear- and anxiety-related phenotypes with several animal models demonstrating a role of the PACAP pathway in controlling HPA-axis activation during stress responses, activation of adrenal medulla activation in the periphery, and corticosterone regulation ((83).

Notably, the sex differences in ADCYAP1R1 effects on startle responses were not observed in prepubertal children, emphasizing the relevance of activational effects of ovarian hormones (84). The interaction between PACAP receptor genomics and environmental influences ((85) remains under investigation and there have been some recent conflicting reports in the recent literature (86). Nevertheless, this type of gene by environment (G×E) analyses will better inform the tailoring of fear-and anxiety-disorder treatments that better account for genomic, hormonal, and environmental complexity and heterogeneity.

In a separate series of studies, we assessed fear conditioning and extinction in traumatized women with and without PTSD and assayed their serum estradiol levels. We then divided the sample into high versus low levels of estradiol at the time of testing (9). Our findings showed that high levels of estrogen was protective against the extinction deficit observed in women with PTSD and low estrogen, so that they did not differ from trauma controls. Figure 1 summarizes the effects of plasma estrogen levels on fear extinction in traumatized women within our psychophysiological paradigm and others in the literature. The optimal range of estrogen on in vivo fear learning and fear extinction in humans remains to be elucidated. Certainly, there are potentially adverse effects of estrogen levels that are too high that must be explored.

Clinical significance

The association between low estrogen and fear extinction has great clinical significance, as they suggest that elevating estrogen levels in some patients may prove beneficial in rescuing extinction deficits observed in PTSD. A recent proof-of-concept study found that administration of synthetic estrogen to normally cycling healthy volunteers increased extinction recall after fear extinction training (87). Due to the fact that clinical exposure therapy recruits neural learning mechanisms that mediate conditioned fear extinction, the adjuvant use of estrogen could facilitate therapeutic effects in fewer sessions. Such effects

have been found with other pharmacological agents that enhance learning, such as d-cycloserine (DCS; (88). Although the latter study by Rothbaum and colleagues (2014) did not demonstrate superior effects of DCS, as compared to placebo, in terms of clinical treatment response in general, the Rothbaum group found that DCS augmentation of extinction-based psychotherapy reduced acoustic startle reactivity more than did alprazolam or placebo. Thus, in similar clinical trial paradigms, it remains possible that estrogen could be used specifically in women with low circulating levels and high PTSD symptoms. Similar studies have found that estradiol administration significantly alleviated symptoms of depression in peri-menopausal women (89, 90). A recent naturalistic study compared sexual assault victims who either received or declined estrogen-based emergency contraceptives immediately after the assault and found that those who took estrogen had significantly lower PTSD symptoms 6 months later(91). The studies reviewed here support the use of estrogen as an individualized treatment strategy in women with PTSD.

Limitations/Future Directions

The facilitation of extinction learning through pharmacological and non-pharmacological manipulations has been the focus of translational research for several years. As discussed previously, interest in DCS was renewed within the pharmacological arena given its early clinical success ((92) whereas non-pharmacological efforts were driven by recent work in the area of reconsolidation updating as a means of enhancing fear extinction ((93-95). An important series of next steps will be further investigation of updating fear memories through reconsolidation in humans as well as determining the degree to which estrogen manipulations can enhance extinction learning and prevent the return of fear.

Follow-up studies will also address influences that have not been directly investigated in well-controlled studies. This includes, but is not limited to, a focus on other hormones and hormone-hormone interactions (e.g., testosterone and progesterone), assessment of hormonal metabolites (e.g.,allopregnanolone), and the within-subjects study of fear processing across phases of the menstrual cycle. Sex differences as indexed by estrogen levels can appear contradictory, given that women should, in general, have higher estrogen levels than men, yet are more vulnerable. A study by Milad and others (49) compared women with high and low estrogen to men and found that women with high estrogen had equally successful levels of extinction recall to men; Both groups differed from low estrogen women. It is likely that endogenous estrogen in men, combined with the cyclic nature of estrogen in women, accounts for these apparently counterintuitive findings.

Given that prolonged estrogen treatment has been previously linked to adverse health consequences, such as cancer, stroke, liver disease and lupus (96), its mental health benefits must be carefully balanced against other disease risks. Many factors must be taken into consideration when designing estrogen-based PTSD therapies, including dose and route of administration, as well as timing, and duration of estrogen exposure. In addition, individualized treatment strategies should be designed within the context of preexisting factors such as patients'; PTSD symptom severity, possible family history of disease and the existence of genetic polymorphisms associated with disease risk. Considering the enormous burden of mental health disease on society, there is an urgent need for more clinical research

examining the benefit potential of estrogen treatment while identifying those factors that predict health risks (97) keeping in mind the potential benefits of non-drug approaches, such as the systematic monitoring of menstrual cycle phase during therapy.

Conclusions

To summarize, translational studies of stress, trauma, and anxiety have often excluded female subjects due to the degree of variance introduced by fluctuating hormone levels. With a focus on both animal and human studies, this review not only highlights the importance of shifting focus to sex differences in laboratory investigations of psychiatric illness vulnerability and symptom severity, but suggests how such a focus may improve treatment outcome for specific traumatized populations. In this review, we focused on observed effects of estrogen; however, this research could be incorporated into an expanded optimization of treatment that includes assessment of genetic and environmental risk. There is strong evidence that estrogen levels are highly influential during and after the extinction of conditioned fear and, as such, clinical researchers and clinicians should capitalize on this potential avenue of therapeutic intervention.

Acknowledgments

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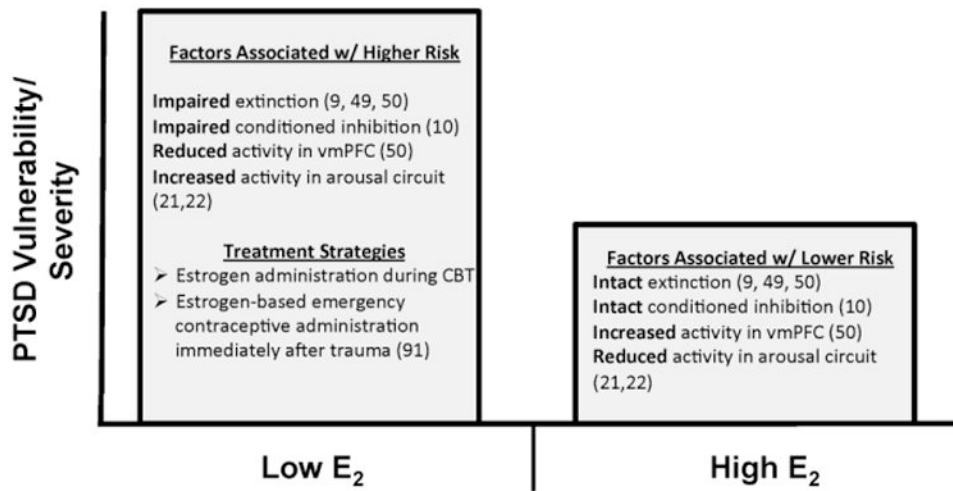
Figure 1.
Schematic summarizing risk factors related to estrogen levels in PTSD vulnerability and severity based on preclinical and clinical investigations (9-10; 45-51). Although not depicted in the figure, it is understood that these factors operate within the context of a larger gene by environment perspective and that the beneficial "dose" range of estrogen must be further explored.