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Sex differences and chronic stress effects on the neural circuitry underlying fear conditioning and extinction

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HIGHLIGHTS

- Expression and rates of stress-dependent psychopathology are sex-dependent.
- Remodeling of fear circuits may contribute to sex differences in psychopathology.
- Sex and stress alter medial prefrontal cortex dendritic morphology.
- There are sex-dependent stress effects on fear conditioning and extinction.
- Dendritic remodeling may underlie sex and stress effects on emotional learning.

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ABSTRACT

There are sex differences in the rates of many stress-sensitive psychological disorders such as posttraumatic stress disorder (PTSD). As medial prefrontal cortex and amygdala are implicated in many of these disorders, understanding differential stress effects in these regions may shed light on the mechanisms underlying sex-dependent expression of disorders like depression and anxiety. Prefrontal cortex and amygdala are key regions in the neural circuitry underlying fear conditioning and extinction, which thus has emerged as a useful model of stress influences on the neural circuitry underlying regulation of emotional behavior. This review outlines the current literature on sex differences and stress effects on dendritic morphology within medial prefrontal cortex and basolateral amygdala. Such structural differences and/or alterations can have important effects on fear conditioning and extinction, behaviors that are mediated by the basolateral amygdala and prefrontal cortex, respectively. Given the importance of extinction-based exposure therapy as a treatment for anxiety disorders such as PTSD, understanding the neural mechanisms by which stress differentially influences fear learning and extinction in males and females is an important goal for developing sex-appropriate interventions for stress-related disorders.

1. Chronic stress, psychopathology, and corticolimbic structure and function

Women are more susceptible than men to stress-related mental illness and twice as likely to experience depression [1,2]. There is also a greater incidence of most types of anxiety disorders, such as social anxiety, phobias, and posttraumatic stress disorder (PTSD), among women compared to men [3]. However, after women experience menopause, a stage of life marked by a pronounced decline in ovarian hormones, this sex difference diminishes [4,5]. In women, depression is also more likely to occur during periods of hormonal fluctuation, such as prior to menses, immediately after pregnancy, and during and after menopause [6,7]. Thus, there may be a role for the cyclic release of ovarian hormones in exacerbating the high incidence of stress-sensitive psychological disorders in women.

Chronic stress is linked to cognitive and emotional dysfunctions. For instance, stressful life events play a role in precipitating episodes of major depression, and can trigger posttraumatic stress disorder [8,9]. Chronic stress also has detrimental effects on many behaviors. For instance, several studies have demonstrated stress-induced deficits on a variety of cognitive tasks, including fear conditioning and retrieval of extinction, attentional set-shifting, spatial learning and recognition, and working memory [reviewed in 10–13]. However, it is not well understood how stress acts on the brain to contribute to the development of psychopathology.

Several stress-related disorders, including anxiety [14], depression [15], PTSD [16], and schizophrenia [17], have been associated with changes in the volume of prefrontal cortex and amygdala, implicating these regions as important targets for investigating stress effects in the brain. In fact, both prefrontal cortex and amygdala contain

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corticosteroid receptors and are involved in the regulation of hypothalamic–pituitary–adrenal axis activity [18–20]. Further, there is interconnectivity between prefrontal cortex and amygdala [21], allowing for prefrontal inhibition of amygdala activity [22–25]. Connections between these two regions are critical modulators of a useful model of the regulation of emotional behavior, fear conditioning and extinction.

Fear conditioning and extinction provide an excellent model system for examining how stress-induced changes in corticolimbic morphology are related to stress-induced changes in neural function and behavior, as the neural circuitry underlying this behavior is well characterized, and involves both medial prefrontal cortex and basolateral amygdala. During fear conditioning, an animal is placed in an operant chamber and acquires a learned fear response to a neutral stimulus, such as a tone, that is paired with an aversive unconditioned stimulus, such as a shock. Repeated pairings of the tone with the shock result in a conditioned fear response to the conditioned stimulus (CS) tone. In a rodent model, a common measure of the conditioned fear response (CR) is the animal's freezing during the tone, which is defined as absence of all movements except that due to breathing. Time away from the operant chamber (typically ranging from 1 to 24 h) is necessary for consolidation of the fear memory. The animal is then placed back in the operant chamber for subsequent presentations of the CS in the absence of the unconditioned stimulus. Multiple presentations of the CS will result in extinction of the fear response—the animal learns that the tone no longer predicts the shock, and no longer freezes in response to presentation of the tone [12,26]. Memory for extinction of conditioned fear can be measured by presentation of the CS on subsequent days. High levels of freezing during the CS indicate poor retrieval of the extinction memory.

Variations in the ability to consolidate and/or retrieve the extinction memory could contribute to disorders such as PTSD [27,28], which makes stress effects on retrieval of extinction an especially important topic of study. Patients with PTSD have impairments in both the ability to extinguish an aversive conditioned response [29,30] and later retrieval of the extinction memory [27]. These extinction deficits could be responsible for the persistence of traumatic memories in the absence of the trauma-inducing stimulus, a hallmark of PTSD [28,31].

Patients with PTSD have reduced ventral medial prefrontal cortex activity and increased activity in the amygdala [32–34], and alterations in prefrontal and amygdalar activity are associated with extinction deficits in these patients [27]. Indeed, connections between medial prefrontal cortex and amygdala are important for fear conditioning and extinction. Basolateral amygdala is a key site of convergence for unconditioned and conditioned stimuli, a critical requirement for the neuroplasticity necessary for learning of fear conditioning [35], and the acquisition of fear conditioning is mediated by amygdala [36,37]. Basolateral amygdala is specifically involved in mediating the initial acquisition of extinction, as either temporary inactivation [38] or blockade of glutamatergic transmission [39,40] in basolateral amygdala prevented or attenuated extinction.

Medial prefrontal cortex contributes to both acquisition and retrieval of extinction of conditioned fear [41]. Male rats with infralimbic cortex lesions showed normal acquisition of fear conditioning and initial extinction, but deficits in the ability to retrieve extinction memory [42]. Likewise, stimulation [43] or pharmacological activation [44] of infralimbic cortex facilitated extinction retrieval, while blockade of infralimbic cortex activity impaired extinction retrieval [45]. Further, neurons in infralimbic cortex showed an increase in firing in response to the CS during extinction retrieval [46]. This evidence suggests that infralimbic cortex is necessary for inhibiting fear responses during extinction [47].

Prelimbic cortex, on the other hand, seems to be involved in the expression of conditioned fear [47]. Temporary inactivation of prelimbic cortex during extinction disrupted conditioned fear expression [48], while stimulation of prelimbic cortex during extinction increased fear expression and slowed extinction of the fear memory [49]. Neural activity in prelimbic cortex is associated with freezing during extinction [50]. Thus, there is a regional specificity in the involvement of medial prefrontal cortex in the modulation of fear conditioning and extinction: prelimbic cortex facilitates fear expression while infralimbic cortex is involved in fear extinction.

The different output targets of infralimbic cortex and prelimbic cortex are the key to the differential effects of each region on fear expression. Although a small percentage of infralimbic neurons innervate basolateral amygdala [51], infralimbic cortex neurons also heavily innervate the intercalated cells of the amygdala and the lateral division of the central nucleus of the amygdala [21,52]. These regions contain GABAergic neurons that inhibit output neurons of the medial division of central amygdala to inhibit fear [53]. Lesion of intercalated cells impaired extinction [22], while activation of intercalated cells facilitated extinction [54]. On the other hand, prelimbic cortex targets basolateral amygdala. Basolateral amygdala connects via excitatory projections to output neurons of central amygdala [24,55], and these neurons trigger midbrain and hypothalamic structures, resulting in the expression of fear [56]. Further, basolateral amygdala provides projections to both prelimbic cortex and infralimbic cortex [57,58]. The presence of these reciprocal connections highlights the importance of investigating multiple structures within the circuit.

Not only is this corticolimbic circuit linked to psychopathology, it also is involved in the regulation of stress response [20], and plays a role in emotion regulation [59]. Its involvement in fear conditioning and extinction is well documented, and thus provides a neural substrate to address questions of stress effects on behavior. However, despite the sex differences in the rates and expression of stress-related psychological disorders, most of the research on the neurobiological mechanisms underlying stress effects on emotional behavior focuses on males. Thus, research into the mechanisms underlying potential stress-induced plasticity of corticolimbic structures in females may provide the groundwork necessary to develop sex-specific treatment for stress-related psychopathology. In this review, we will focus on sex differences and potential differential effects of chronic stress on morphology of basolateral amygdala and medial prefrontal cortex and fear conditioning and extinction in rodents.

2. Chronic stress effects on neuronal morphology

As in primates, prefrontal cortex in rodents can be subdivided into several major subregions. Medial prefrontal cortex includes anterior cingulate, prelimbic, and infralimbic cortex. This region is functionally homologous to the primate dorsolateral and ventromedial prefrontal cortices, and plays a role in autonomic and HPA axis regulation, emotion regulation [e.g., prelimbic cortex plays a role in expression of conditioned fear, while infralimbic cortex plays a role in retrieval of extinction, see 60 and above], and working memory. Orbitofrontal cortex, which includes the medial, ventral, and lateral orbitofrontal subregions, is functionally homologous to primate orbitofrontal cortex and appears to play a role in modulating behavioral responses based on changing incentive values of reward-related stimuli [13].

Medial prefrontal cortex is involved in cognitive tasks that are influenced by chronic stress [as reviewed in 13], is a target for stress hormones like corticosterone [61], and helps regulate HPA axis activity [62]. In male rats, chronic restraint stress produced retraction of apical dendrites of pyramidal neurons in male prelimbic cortex [63–70], an effect that was mimicked with chronic corticosterone administration [71–73]. A similar pattern of stress-induced retraction was seen for apical dendritic branches of neurons within the infralimbic region of medial prefrontal cortex [74,75]. Finally, even shorter, milder episodes of stress were sufficient to produce dendritic atrophy. Ten minutes of stress for 10 days [76] or 3 weeks of vehicle injection alone [71] reduced dendritic arborization within medial prefrontal cortex, again with retraction occurring only in distal portions of the apical arbor.

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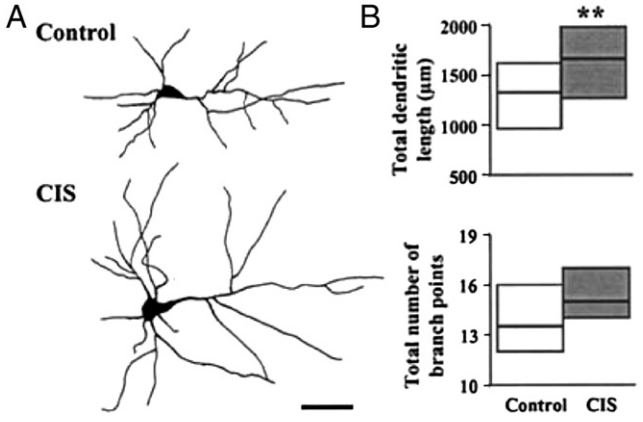

Fig. 1. Chronic immobilization stress (CIS) resulted in dendritic hypertrophy in basolateral amygdala pyramidal neurons in male rats. A. Reconstructions of representative neurons from unstressed and stressed male rats. Scale bar, 50 μm. B. Medians and inter-quartile ranges for total dendritic length (top) and total number of branch points (bottom) for neurons from unstressed and stressed male rats. *$p < 0.05$, **$p < 0.01$. Modified from [83].

Medial prefrontal cortex appears to be particularly sensitive to even acute stress, as a single episode of forced swimming produced reductions in infralimbic apical dendritic branching in mice [74].

The amygdala is made up of separate subnuclei with disparate functions, including reproduction, aggression, and learning [as reviewed in 77]. Many studies have shown sex differences in medial amygdala [78–81], yet less is known about sex differences and stress effects in basolateral amygdala, a critical structure in the fear and extinction circuit (see above). The amygdala is rich in corticosteroid receptors [82] and is involved in the regulation of glucocorticoid activity [62], and thus is a likely target for stress. Indeed, like neurons within medial prefrontal cortex, basolateral amygdala neurons are affected by chronic stress. Long-term (10–21 days) immobilization stress increased dendritic length of pyramidal cells within the basolateral amygdala in male rats [83,84] (see Fig. 1). There is stressor sensitivity within the amygdala, as chronic immobilization stress but not chronic unpredictable stress induced dendritic remodeling in basolateral amygdala [85]. However, unlike recovery effects observed in medial prefrontal cortex [67], 21 days of stress-free recovery did not rescue the stress-induced hypertrophy of the amygdala [86].

2.1. Chronic stress effects on behavior

Given that there is a relationship between dendritic structure, dendritic spines and synaptic input, and neural firing rates [87], stress-induced structural alterations may have important effects on neural function and behavior. Because chronic stress in males produces profound changes in dendritic morphology of medial prefrontal cortex and basolateral amygdala, it is not surprising that stress has important effects on fear conditioning and extinction. Several studies have shown that in males, the acquisition of either contextual or cued fear conditioning can be enhanced with chronic restraint stress, with stressed males freezing more than unstressed males [88–91]. Facilitated acquisition of conditioning is also seen in male rats with glucocorticoid exposure similar to that of chronic stress levels [92]. Such evidence suggests that chronic stress-induced changes in amygdala could be responsible for stress-induced changes in behavior; however, little research has been done to address this question.

Other studies have shown chronic stress effects on extinction in males. For instance, one week of chronic restraint stress impaired retrieval of extinction of conditioned fear [89,93,94]. Subsequent studies have shown that one week of unpredictable mild stress produced similarly specific changes in extinction retrieval [95], as does a longer-term stressor [6 h of daily restraint stress for 3 weeks; 96]. In mice, one episode of swim stress occurring 24 h before fear conditioning attenuated the rate of extinction, though the authors could not differentiate between extinction acquisition and extinction retrieval [74]. Further, stress-induced changes seen in medial prefrontal cortex were responsible for the stress-induced behavioral impairment in retrieval, as removal of infralimbic cortex before the chronic stressor occluded the stress-induced impairment of extinction retrieval [89]. Finally, in male rats, stress-induced alterations in fear conditioning and extinction were associated with stress-induced changes in physiology, as the stress-induced deficit in extinction retrieval was accompanied by alterations in activity of medial prefrontal cortex neurons [94]. In prelimbic cortex, stress prevented the tone-evoked inhibition of activity seen in unstressed rats. In infralimbic cortex, neurons in unstressed rats exhibited increased firing rate in response to the CS, whereas stressed rats showed inhibition of infralimbic cortex firing during the tone. Thus, the stress-induced dendritic changes seen in medial prefrontal cortex are consistent with the stress-induced impairment of a prefrontally mediated behavior, extinction retrieval, as well as alterations in neural activity in medial prefrontal cortex.

Differential dendritic remodeling patterns in medial prefrontal cortex and basolateral amygdala of male rats in response to chronic stress may contribute to the stress-induced impairment versus facilitation of medial prefrontal cortex- and amygdala-mediated behaviors. Dendritic retraction seen in medial prefrontal cortex may reflect less excitatory input and hypoactivity of the structure, resulting in impairment of extinction retrieval. On the other hand, dendritic proliferation seen in basolateral amygdala may reflect more excitatory input and hyperactivity of the structure, resulting in facilitation of the acquisition of fear conditioning. Whether or not this is the case remains to be determined, as one study found that stress-induced dendritic retraction within area CA3 of the hippocampus was associated with increased neuronal excitability [97]. Though work from our lab is consistent with the hypothesis that the stress-induced changes seen in male prefrontal cortex may be a neural mechanism for the stress-induced impairment in extinction retrieval [89], a direct demonstration that stress-induced dendritic changes in prefrontal cortex and basolateral amygdala are responsible for alterations in fear conditioning and extinction still awaits further testing. In addition, because medial prefrontal cortex and basolateral amygdala are interconnected, altering the structure of one region could alter function of the other region, thus influencing behaviors mediated by both. For example, if activity in infralimbic cortex is abnormally suppressed by chronic stress exposure, there may be an accompanying facilitation of fear expression from excessive activation in prelimbic cortex and amygdala. Thus, the balance of activity among regions in the circuit could be especially important, and disruption of that balance by chronic stress may have detrimental effects on behavior.

3. Sex differences in dendritic morphology

In rats, medial prefrontal cortex is sexually dimorphic. For instance, intact, unstressed females had smaller and less complex apical dendritic arbors in prelimbic cortex pyramidal neurons than intact, unstressed males [69,98,99] (see Fig. 2). Given that medial prefrontal cortex contains estrogen and progesterone receptors [100], this sexual dimorphism could be mediated at least in part by gonadal hormones. In female rats, ovarian hormones fluctuate over a 4 to 5 day cycle characterized by elevated levels of estrogens and progesterone in proestrus compared to lower levels of ovarian hormones in estrus, metestrus, and diestrus [101].

Evidence from other limbic brain regions suggests that sexual dimorphism in dendritic structure is mediated by gonadal hormones. For instance, in the hippocampus, changes in circulating estrogens across the cycle and manipulation of sex steroids have profound effects on dendritic morphology and spine density [reviewed in 102]. Estrogen modulates spine density in CA1 of the hippocampus, with a decrease in spine density in ovariectomized females compared to ovariectomized females with estradiol replacement [103–105]. Again, within CA1 of

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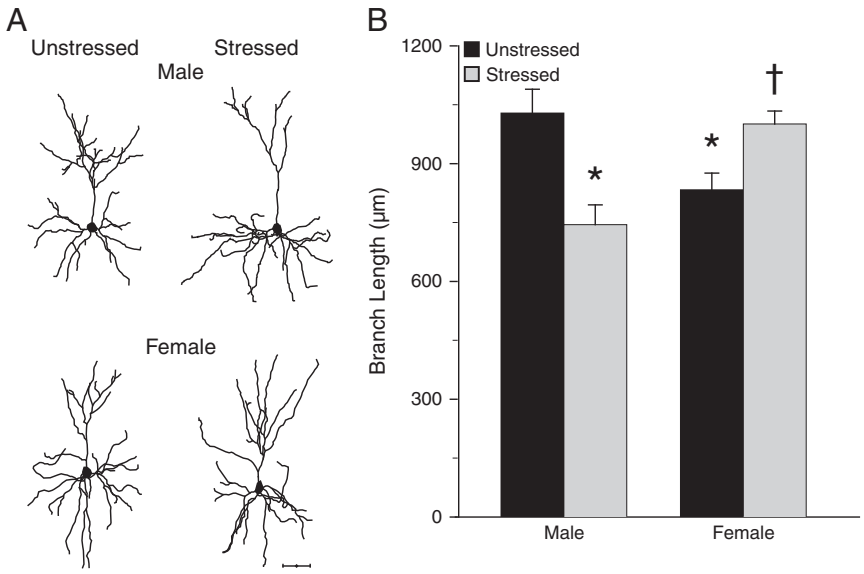

Fig. 2. Chronic restraint stress produced apical dendritic retraction in pyramidal neurons in prelimbic cortex of males, and apical dendritic hypertrophy in females. A. Reconstructions of representative neurons from unstressed and stressed male and female rats. Scale bar, 50 μm. B. Mean (±SEM) length of apical dendrites for male and female unstressed versus stressed rats. *$p < 0.05$ relative to unstressed males, †$p < 0.05$ relative to unstressed females. Modified from [69].

the hippocampus, intact females showed the largest spine densities while in proestrus and the smallest spine densities while in late estrus [106–108]. In comparison, little is known about the effect of sex steroids on dendritic morphology within medial prefrontal cortex. There was no effect of ovariectomy on dendritic morphology within prelimbic cortex [69] or in infralimbic cortex [109]; thus, exogenous manipulation of estradiol alone does not appear to have an effect on medial prefrontal cortex dendritic morphology. Nonetheless, because fluctuations in estradiol and progesterone can influence dendritic morphology, it is likely that potential remodeling of dendritic arbor in medial prefrontal cortex across the estrous cycle could occur, and should be assessed.

The amygdala has a high concentration of androgen and estrogen receptors [110,111], suggesting that sex steroids may modulate the morphology or function of amygdalar neurons. Consistent with this hypothesis, estradiol treatment decreased the amplitude of excitatory postsynaptic potentials in basolateral amygdala neurons in vitro [112] and impaired performance on basolateral amygdala-dependent behavioral tasks such as the conditioned place preference task [113]. Though sex differences or estrous phase effects on dendritic morphology within basolateral amygdala have not been examined, sex differences in spine densities in basolateral amygdala have been reported. Male rats had greater spine density on pyramidal neurons than females [114]. Although this study failed to find differences in spine density across estrous phases, the authors only compared proestrous and estrous rats, leaving open the possibility of differences across proestrous and diestrous phases. Further, no study has explored the effect of experimental manipulations of gonadal hormones on basolateral amygdala dendritic morphology.

3.1. Sex differences in chronic stress effects on neuronal morphology and behavior

The relationship between sex and stress steroids in rodents is complex. There are sex differences in basal and stress-induced glucocorticoid levels, as males have lower levels of glucocorticoids than females and release less corticosterone during stress than females [115–118]. Further, corticosterone levels vary over the estrous cycle. Female rats in proestrus have higher basal and stress-induced plasma corticosterone levels compared to rats in other phases of the estrous cycle [115,116,118–121], and these fluctuations in estradiol and changes in glucocorticoid levels alter stress sensitivity in female rodents [119]. Given these sex- and gonadal-hormone-dependent differences in glucocorticoid levels, it is likely that stress differentially influences corticolimbic dendritic morphology in males and females. While several studies have demonstrated such sex differences in stress effects on hippocampal morphology [reviewed in 10], little is known about potential sex-dependent stress effects in amygdala and prefrontal cortex.

Whereas chronic stress produced dendritic retraction in male medial prefrontal cortex, female rats showed stress-induced dendritic proliferation in prelimbic cortex neurons (see Fig. 2), and this effect was estradiol-dependent [69]. The stress-induced hypertrophy of apical dendrites was eliminated with removal of gonadal hormones by ovariectomy and rescued with ovariectomy plus estradiol treatment. Within infralimbic cortex of ovariectomized females implanted with estradiol, chronic stress-induced proliferation has been reported in a sub-population of neurons that project to basolateral amygdala [109]. This effect was absent in ovariectomized females without estradiol replacement, again suggesting a role for estradiol in the stress-induced dendritic proliferation in medial prefrontal cortex. Thus, there are sex differences in the morphology of pyramidal neurons in prelimbic and infralimbic cortices, and stress differentially affects structure of medial prefrontal cortex in male and female rats. However, it is unknown how chronic stress affects basolateral amygdala dendritic morphology in females, nor is it known how fluctuations in circulating hormones over the estrous cycle may interact with chronic stress to affect dendritic morphology within either medial prefrontal cortex or basolateral amygdala.

Sex differences in medial prefrontal cortex and basolateral amygdala morphology may contribute to sex differences in medial prefrontal cortex and amygdala-dependent behaviors. There are sex differences in prefrontal- and amygdala-mediated cognitive and emotional behaviors, for instance working memory [122–124] and anxiety [125,126]. These sex differences may be mediated in part by gonadal hormones. Estrogen administration improved prefrontal cortex-dependent cognitive tasks (e.g., delayed nonmatching-to-sample recognition memory task) in primates [127], while removal of endogenous estrogens via ovariectomy impaired prefrontal cortex-dependent cognitive tasks (e.g. object recognition memory task) in rodents [128]. The effect of ovarian hormones may be task-specific; for example, estradiol administration impaired latent inhibition [129] and delayed spatial alternation [130], yet improved working memory [131]. Estrogens can also alter fear learning and fear inhibition [132], contributing to sex differences in fear expression. The

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first study of sex differences in fear conditioning showed that males and females differed in contextual but not cued fear conditioning, with males displaying more freezing to context than intact females [133]. Subsequent studies investigated how activational effects of ovarian hormones contribute to this sex difference. Ovariectomized females froze to the context CS at levels similar to those of males and more than those of intact females, and the ovariectomy-induced effect could be reversed with estradiol replacement [134], suggesting that removal of endogenous estrogens increased females' freezing to the context CS. Thus, the activational effect of estrogens may contribute to the sexually dimorphic nature of acquisition of contextual fear conditioning. Contextual and cued fear conditioning involve different but overlapping neural circuits. Contextual fear conditioning is hippocampally-dependent [135–137], while cued conditioning is not. Thus, sex differences in the hippocampus [e.g. 106,138] could be responsible for the sex difference in contextual conditioning.

Consistent with Maren and colleagues' earlier report, Milad and colleagues [139] showed that during cued conditioning, when behavioral responses from females are collapsed across estrous cycle phase, there was no sex difference between males and females during any phase of fear conditioning or extinction. However, when estrous cycle phase was considered, females that were in diestrus during initial extinction showed levels of freezing 24 h later during extinction retrieval that were similar to that of males. In contrast, females that were in proestrus during extinction displayed levels of freezing 24 h later during extinction retrieval that were lower than those of diestrous females or males. Further, it seems that having high versus low ovarian hormone levels may be important only during extinction [139]. Females in proestrus during conditioning or extinction retrieval showed no differences in freezing from females in diestrus during conditioning or extinction retrieval. These data suggest that circulating levels of ovarian hormones over a three-day testing protocol can have important effects on behavior. Further, peak levels of circulating estrogens during initial extinction may be especially important for retrieval of the extinction memory 24 h later.

However, there are conflicting findings. A study from Baran and colleagues found that, while they did not differ from males in acquisition of conditioned fear, intact females did not extinguish the conditioned fear [96]. One possible explanation for the differential findings is that the studies used somewhat different fear conditioning and extinction paradigms. Examples of variation in experimental paradigm included behavioral testing in light versus dark cycle, number of tone-shock pairings, intensity of shock, time period for extinction consolidation (1 h vs. 24 h), and whether or not rats were trained to bar press for food pellets during conditioning and extinction as a means of ensuring a baseline level of activity against which to measure freezing during massed extinction sessions [see 42]. Further, Baran and colleagues [96] examined females without regard to estrous phase, which could also contribute to the conflicting results. Alternatively, the difference between these studies could be due to handling effects. Baran and colleagues subsequently demonstrated that, unlike in their previous study, intact females that had been handled daily prior to fear conditioning acquired the fear response and subsequently were able to acquire extinction, but failed to retrieve the previous extinction session on the next day [140]. This pattern of results is reminiscent of that seen in Milad and colleagues' [139] study, in which daily handling (vaginal lavage for characterization of estrous phase) occurred.

Changes in levels of circulating estrogens may mediate the effects of estrous phase on extinction retrieval. In nonovariectomized females, either immediate post-extinction injection of estradiol or estrogen receptor β activation via injection of the estrogen receptor β agonist diarylpropionitrile facilitated extinction retrieval [141]. Moreover, extinction retrieval testing following estradiol administration led to decreased c-Fos expression in basolateral amygdala and increased c-Fos expression in infralimbic cortex [141]. Thus, high levels of estradiol are associated with improvements in the retrieval of extinguished fear by modulating activity within infralimbic cortex and basolateral amygdala, likely through estrogen receptor β activation. However, there are some limitations to administering estradiol in intact, non-ovariectomized animals. Though all rats underwent extinction and estradiol or drug injection while in metestrus (when estrogens are low), administration of estradiol can alter subsequent release of ovarian hormones through feedback mechanisms [reviewed in 142].

Because females exhibit contrasting chronic stress-induced dendritic remodeling in medial prefrontal cortex compared to males, it is likely that stressed females also show a different behavioral pattern in fear learning and extinction. To date, only one study has addressed this issue. Baran and colleagues [96] demonstrated that, in contrast to males, chronically stressed female rats showed impaired acquisition of fear conditioning, but were not impaired in retrieval of fear extinction. However, handling again appears to influence this effect. A subsequent study by this group found that chronically stressed females that had been handled demonstrated facilitated acquisition of conditioning and more freezing to the tone during extinction than unstressed, handled control females, with no effect of estradiol treatment [143].

The paucity of evidence and inconsistencies in the existing literature currently prohibit development of a complete model to explain how stress-induced alterations in corticolimbic morphology might influence fear conditioning and extinction in females. However, it is tantalizing to speculate that the dendritic proliferation in prelimbic pyramidal neurons after stress in females [69] provides for hyperexcitability of prelimbic neurons. The resultant facilitation of expression of conditioned fear could account for Hoffman and colleagues' [143] finding of facilitated acquisition of conditioning in handled, stressed females. This hypothesis could be tested with studies examining the activity of neurons in medial prefrontal cortex during fear conditioning and extinction in stressed and unstressed females.

Thus, at this point the fledgling literature on both sex differences and differential stress effects in prefrontal–amygdalar circuitry and fear conditioning and extinction has yielded inconsistent results, and further studies are required to fully characterize the nature of the sex differences and to clarify the role of gonadal hormones. An important first step is to determine whether or not chronic stress differentially influences dendritic morphology in basolateral amygdala in males versus females. Moreover, while gonadal hormonal status in adulthood has been implicated as being permissive of stress effects in medial prefrontal cortex in females, a similar examination of the potential influence of gonadal hormones on stress effects in males is absent. Further, a major unresolved question that must be addressed is the potential role of gonadal hormones acting early in development to organize sex differences in structure and function in prefrontal–amygdalar circuitry and the behaviors they mediate. For example, the sex differences in response to stress could reflect differences in circulating gonadal hormones (type, normal fluctuations) at adulthood or the consequences of the actions of those gonadal hormones during development, resulting in structural and/or functional differences that provide the substrate for the differential stress effects seen in adult males and females.

4. Summary and conclusions

The current literature describes sex differences in dendritic morphology of pyramidal neurons in medial prefrontal cortex and spine density in basolateral amygdala, as well as sex differences in behavioral responses during fear conditioning and extinction. While chronic stress effects on these variables are well-documented in males, the data on females is sparse. Nonetheless, these data show that there are sex differences in dendritic morphology within the prelimbic and infralimbic regions of medial prefrontal cortex. Further, sex-dependent stress effects on dendritic morphology in medial prefrontal cortex have been documented. Overall, existing behavioral studies suggest that a) there are sex differences in fear conditioning and extinction, which may be

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estrogen-dependent; and b) stress effects on fear conditioning and extinction vary in males and females. However, methodological differences and limitations across published studies make it difficult to determine the exact nature of these differences and their dependence on estrogens. For instance, the literature on stress-induced changes in performance during fear conditioning and extinction in females often does not take into account gonadal hormonal status, or uses daily injections of estradiol (an additional stressor and possibly disruptive of the estrous cycle) to mimic natural variations in estradiol. No previous study examining fear conditioning and extinction has manipulated ovarian hormones via ovariectomy and estradiol replacement. In addition to the differences in using intact females versus females with gonadal hormone manipulation (with differences across studies in use of daily injections to mimic estrous cycle, dose and type of hormone used, duration of hormone treatment), differences in fear conditioning paradigms across previous studies further limit our ability to compare results across studies, and therefore potential differences across groups. Finally, there are differences across studies in handling (daily lavage or injection versus no handling) that may affect behavioral responses during fear conditioning and extinction. Nonetheless, collectively these studies provide our first evidence that stress may alter fear conditioning and extinction in a sex-dependent manner. It is now important to begin to understand the interaction between chronic stress, sex, and ovarian hormones as a first step in elucidating the mechanisms underlying both basic sex differences in this model of emotion regulation, and differential effects of stress in the model system. Given sex differences in rates of anxiety disorders, and deficits in extinction and dysfunction of prefrontal cortex and amygdala that characterize disorders such as PTSD, uncovering these mechanisms is an essential first step in developing sex-specific treatment strategies for stress-dependent psychopathology.

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