OPEN

Citation: Transl Psychiatry (2014) 4, e422; doi:10.1038/tp.2014.67

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REVIEW

Mechanisms of estradiol in fear circuitry: implications for sex differences in psychopathology

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Over the past two decades, substantial knowledge has been attained about the mechanisms underlying the acquisition and subsequent extinction of conditioned fear. Knowledge gained on the biological basis of Pavlovian conditioning has led to the general acceptance that fear extinction may be a useful model in understanding the underlying mechanisms in the pathophysiology of anxiety disorders and may also be a good model for current therapies treating these disorders. Lacking in the current knowledge is how men and women may or may not differ in the biology of fear and its extinction. It is also unclear how the neural correlates of fear extinction may mediate sex differences in the etiology, maintenance, and prevalence of psychiatric disorders. In this review, we begin by highlighting the epidemiological differences in incidence rate. We then discuss how estradiol (E2), a primary gonadal hormone, may modulate the mechanisms of fear extinction and mediate some of the sex differences observed in psychiatric disorders.

Translational Psychiatry (2014) **4,** e422; doi:10.1038/tp.2014.67; published online 5 August 2014

We form associations between emotional events and co-occurring cues that can guide future behavioral outcomes. This is the basis of classical conditioning, a paradigm used to study mechanisms of associative learning and memory. In the past few decades, conditioned fear and its extinction have been the focus of extensive research efforts, in part, due to the clinical relevance of fear to the etiology and pathophysiology of many psychiatric disorders. Key nodes of brain regions involved in conditioned fear and fear extinction learning have been identified in rodents and humans.[1] The majority of the rodent studies have been conducted in males and those conducted in humans, for the most part, disregard the role of sex differences in this form of learning (Figure 1). Below, we begin by outlining why this is an issue that deserves attention from a clinical perspective; a point previously alluded to by others.[2] We review evidence for the relevance of fear extinction in studying anxiety disorders and then discuss the mechanisms by which estrogens might interact with the function of the fear extinction network. We conclude with a discussion of how natural variations, or exogenous manipulations, of estrogens throughout a woman's lifespan may translate to heightened vulnerability to psychopathology.

SEX DIFFERENCES ACROSS PSYCHIATRIC DISORDERS

Epidemiological studies highlight significant differences between men and women in the incidence of psychiatric disorders (Figure 2). There is a higher incidence in men for autism, attention deficit hyperactivity disorder, schizophrenia and Parkinson's disease. Conversely, women are more susceptible to depression, anxiety and posttraumatic stress disorder (PTSD). In addition to differences in incidence, many psychiatric disorders are characterized by marked sex differences in progression and severity. Women are twice as likely to be diagnosed with PTSD;[3–6] have longer symptom duration,[7] higher symptom severity and functional impairment,[8] and have worse quality of life.[9] Women with obsessive compulsive disorder are more likely to have more contamination/cleaning obsessions[10] and their symptoms begin or worsen at menarche and postpartum.[11] Women comprise 60% of individuals with generalized anxiety disorder and are more likely to develop comorbid psychiatric disorders and have worse prognosis and impairment.[12,13] In addition to increased incidence of panic disorder in women, studies also suggest that panic attacks occur more frequently in women relative to men.[14,15] Data indicate that women are at higher risk of developing anxiety disorders during reproductive life events such as menarche, menstruation, pregnancy, parturition and menopause.[16,17] All together, these epidemiological data suggest that gonadal hormones may have a role in the onset of psychiatric disorders in women.

FEAR EXTINCTION AS A MODEL FOR ANXIETY-RELATED DISORDERS

The inability to appropriately inhibit fear is a central underlying feature of anxiety disorders, with individuals avoiding fear-provoking situations or employing maladaptive safety behaviors. PTSD, for example, is marked by uncontrollable recurring memories of a traumatic life event with sufferers unable to extinguish their fear to stimuli related to the event. Conditioned fear paradigms elicit the symptomatic behaviors that mimic those observed in anxiety disorders and fear extinction protocols directly assess the core dysfunction, thus providing a means to investigate the underlying neural pathophysiology. The neural mechanisms underlying fear extinction have been extensively studied in rodents and have been reviewed elsewhere.[28–31] Briefly, the brain circuitry underlying extinction memory consolidation

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Received 12 March 2014; revised 2 June 2014; accepted 23 June 2014

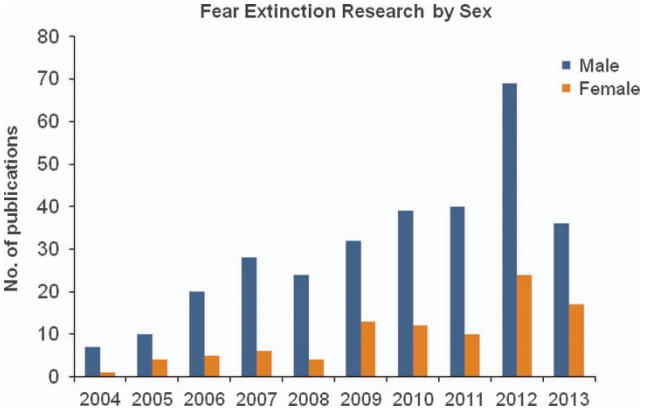

Figure 1. Studies published within the past decade that focus on fear extinction research. To highlight the disparity in research focused on women and female animals, we used keywords 'fear extinction' and 'male' or 'female'.

Disorder	Women/Men Lifetime Incidence Ratio
PTSD	2.08[a]
Panic Disorder	1.96[b]
Generalized Anxiety	1.88[b]
Major Depressive Disorder	1.75[b]
OCD*	1.6[c]
Alzheimer's Disease	1.30[d]
Bipolar Disorder	~1.0[e]
Schizophrenia	0.71[f]
Parkinson's Disease	0.53[g]
Drug Use Disorder	0.51[h]
Alcohol Dependence	0.46[b]
ADHD	0.42[i]

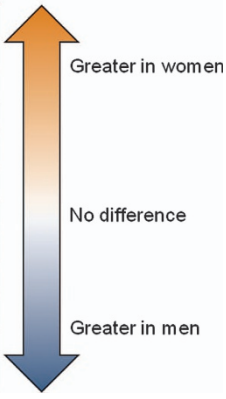

Figure 2. Sex differences in the lifetime incidence of psychiatric disorders vary from higher incidence in women, to no differences, to higher in men. Women/men lifetime incidence ratio was obtained directly from the publications referenced within the table or were calculated from the percentages of lifetime incidence published in the referenced studies. Superscripted letters next to each ratio reflects the citation from which we obtained such data: a, ref. 18; b, ref. 19; c, ref. 20; d, ref. 21; e, 22; f, ref. 23; g, ref. 24; h, ref. 25; i, ref. 26. *Of note, a sex bias for OCD is under debate and may depend on age; one study reports greater incidence among boys than girls. ADHD, attention deficit hyperactivity disorder; OCD, obsessive compulsive disorder; PTSD, posttraumatic stress disorder.[27]

involves an integrated network of the amygdala, hippocampus and the ventromedial prefrontal cortex (vmPFC).[1] Fear expression can be modulated by interactions within different nodes of this circuit. Input from the prelimbic cortex of the PFC to the basolateral amygdala increases activity in the central nucleus of the amygdala for increased fear output.[32] Input from the infralimbic cortex (IL) to the inhibitory intercalated neurons of the amygdala suppresses activity within the central amygdala to reduce fear responses.[33] Based on the context in which extinction learning took place, the hippocampus can either allow or suppress the expression of the fear memory by activating the IL.[34]

The brain regions involved in fear conditioning and extinction in humans parallel those described in rodents.[1] It has been suggested that the dorsal anterior cingulate and the vmPFC may be the functional homologs to the rat prelimbic cortex and IL, respectively. Along with the amygdala, increased activation of the vmPFC and dorsal anterior cingulate has been reported during fear conditioning and extinction in humans.[35] Specifically, increased vmPFC activation to the extinguished cue during extinction recall positively correlated with the magnitude of extinction recall.[36–38] In addition, context-specific hippocampal activation supports the role of this structure in modulating the network based on contextual information.[37] Neuroimaging studies with PTSD patients show deficits in this network, including dorsal anterior cingulate hyperactivity and vmPFC hypoactivity correlating with impaired extinction.[39] However, not all neuroimaging studies report results congruent with the fear extinction model. For example, although the model predicts blunted vmPFC activity in PTSD patients, several studies have reported hyperactivation or no differences within this brain region between PTSD and trauma-exposed or healthy individuals during symptom provocation.[40–42] In addition, the fear extinction model does not capture all features of anxiety such as anticipatory symptoms nor does it accurately model disorders like obsessive compulsive disorder. Despite these limitations, this model is a useful tool for studying the neural mechanisms and vulnerability for anxiety, as well as evaluating treatment efficacy.

All of the above studies have not examined sex differences in the activation of the different nodes of the fear extinction network nor have they examined how sex hormones such as estrogen might manipulate their responsivity. Before discussing how estradiol may influence fear extinction memory, we first provide a brief overview of the different types of estrogens and their receptors and briefly describe the localization of estrogen receptor expression.

TYPES OF ESTROGENS

Estrogens are the primary female sex hormones and are produced by the ovaries and adrenal gland. The four primary steroidal estrogens are estrone (E1), estradiol (E2), estriol (E3) and estetrol (E4). E2 is the most potent in nonpregnant females whereas E1 is predominant during menopause and E3 and E4 are greatest during pregnancy.[43] Estrogen synthesis also occurs in males. Although the adrenal glands and testes produce low levels of estrogens, males rely on the conversion of testosterone, by the enzyme aromatase, into estrogen for physiological functioning.[44–47] In both sexes, high levels of aromatase localize in the hypothalamus, amygdala, hippocampus, midbrain and cortex, thus denoting sites of estrogen synthesis.[43] As 17β E2 is the most potent circulating estrogen in males and nonpregnant females, we focus our discussion on this type of estrogen.

ESTRADIOL RECEPTORS

E2 acts primarily through estrogen receptor subtypes alpha (ERα) and beta (ERβ). ERα is functionally related with reproductive behavior[48] whereas ERβ is associated with nonreproductive behaviors such as learning and memory[49] and anxiety-related behaviors.[50] These receptors are expressed throughout the brain and may localize in the nucleus, cytoplasm and cell membrane.[43] The ERs have similar distribution in male and female brains but may differ in relative expression.[51,52] ERα and ERβ expression patterns generally overlap, though ERα dominates hypothalamic subregions[53] whereas ERβ is more abundant in the hippocampus[52] and cerebral cortex.[54] The ERs show distinct expression within the amygdala subregions, however, ERα is the predominate receptor.[51,55] Regarding the vmPFC, both ERα and ERβ have been detected in the rat IL and prelimbic cortex.[56–58] A summary of relative receptor distribution within the fear extinction network is illustrated in Figure 3.

E2 receptors exhibit sensitivity to estradiol fluctuations, with expression and cellular localization varying across the phases of the rat estrous cycle in the hippocampus[57] and hypothalamus.[60] In the cornu ammonis 1 (CA1) subregion of the hippocampus, for

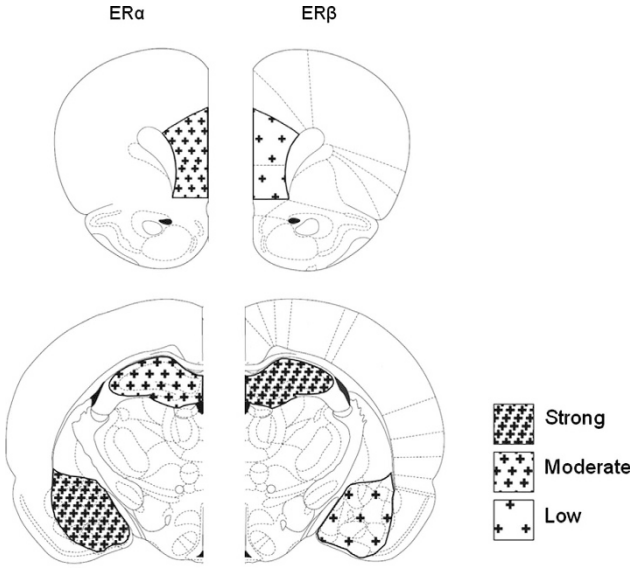

Figure 3. Relative estrogen receptor distribution within the rat fear extinction network. Estrogen receptor alpha (ERα; left) is expressed moderately in the ventromedial prefrontal cortex (vmPFC) and hippocampus and strongly in the amygdala. Estrogen receptor beta (ERβ; right) is weakly expressed in the vmPFC and amygdala and strongly in the hippocampus. These relative distributions are compiled from studies employing immunoreactivity and *in situ* hybridization methodologies.[51,52,55,56–58] Atlas images are adapted from Paxinos and Watson.[59]

instance, ERα expression correlates with serum estradiol levels and shows greatest expression during estrus, whereas ERβ is upregulated during both estrus and metestrus phases.[57] In addition, ERα localizes in the CA1 cytoplasm and translocates to the nucleus during diestrus, whereas ERβ localizes in the nucleus throughout the estrous cycle.[57] Halting E2 production through ovariectomy causes both ER downregulation and desensitization,[61] suggesting that circulating E2 preserves ER density. These data suggest that ovariectomy may drastically alter E2 signaling. Therefore, careful consideration should be made as to the translational validity of studies utilizing ovariectomized females.

ESTRADIOL MODULATES FEAR EXTINCTION

Rodents

Almost all of the data on the neural mechanisms underlying the fear extinction network in rodents have come from studies conducted in males.[62] Relatively few studies have examined female rats, and there are few published studies that examine the role of gonadal hormones specifically in fear extinction. However, there is evidence indicating that estradiol modulates extinction processes. For example, studies have reported that estradiol facilitates extinction in passive avoidance task[63] and conditioned taste aversion tasks.[64] In our laboratory, we have demonstrated that the natural fluctuations in estradiol can influence recall of fear extinction memory. Specifically, female rats that extinguished during the low-E2 metestrus phase of the estrous cycle exhibit poor extinction recall, whereas females that underwent extinction training during the high-E2 proestrus phase displayed improved extinction recall.[65] Moreover, systemic E2 administration before extinction training in metestrus rats significantly improved extinction recall, whereas blocking E2 receptors in female rats impaired extinction memory consolidation.[65,66] Together, these data suggest that the consolidation of fear extinction memory is dependent on the female's naturally fluctuating levels of E2 throughout the estrous cycle.

Humans

Neuroimaging studies have shown that measures of fear and arousal are associated with changes in hormonal levels across the menstrual cycle and correlate with changes in the functional reactivity of the amygdala and hippocampus.[67,68] In the Go-No-Go task, a measure of emotional response inhibition, women exhibited increased dorsal lateral PFC reactivity during the high-E2 luteal phase relative to the lower E2 follicular phase. Moreover, this reactivity was positively correlated to positive stimuli and negatively to negative stimuli,[69] suggesting that estradiol may facilitate the functional activation of the PFC with specificity for valence. We have recently shown that women with high estradiol exhibit significantly enhanced fear extinction recall relative to women with low estradiol levels; the increased extinction capacity in women with high estradiol was associated with increased vmPFC, hippocampus and amygdala function during extinction recall.[66] Consistent with our findings, women in low estradiol states showed impaired fear inhibition in a fear-potentiated startle task relative to women with elevated estradiol levels.[70]

Performance in fear-related tasks may be indicative of risk for psychopathology, namely PTSD. Sex differences persist among individuals with PTSD;[71] women exhibit greater acquisition of the conditioned fear response than men[72] and have greater difficulty extinguishing fear responses. Low estradiol levels appear to be associated with impaired fear extinction and may be a vulnerability factor for developing PTSD.[73]

MOLECULAR MECHANISMS OF FEAR EXTINCTION

Numerous studies have described the molecular and cellular cascades that are necessary for the acquisition, consolidation and retrieval of fear extinction memory. In these studies, activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway appears necessary for the consolidation of fear extinction memory.[74–77] Specifically, intra-amygdalar infusions of an MAPK inhibitor before extinction training significantly impair extinction recall of conditioned fear-potentiated startle, whereas hippocampal infusions do not.[76] Moreover, extinction learning increases phosphorylated MAPK/ERK in the basolateral amygdala.[74] In addition, it has been demonstrated that consolidation of fear extinction is dependent on MAPK/ERK signaling and protein synthesis in the medial PFC.[75,78] Enhancing extinction learning with pretraining administration of D-serine, an NMDAR agonist, correlated with ERK phosphorylation in the hippocampus during extinction training and in the basolateral amygdala during recall.[79] This finding of enhanced ERK phosphorylation in the amygdala after extinction recall may reflect the feedback mechanism from the basolateral amygdala to IL in suppressing fear expression.

Another molecular pathway that has been shown to be critical for fear extinction learning is the phosphoinositide 3-kinase (PI3K) cascade. Successful fear extinction is associated with Akt phosphorylation in the CA1[80] and dephosphorylation in the amygdala.[81] Infusing a PI3K inhibitor in the IL following extinction training resulted in impaired extinction consolidation in male rats.[82] The MAPK/ERK and PI3K signaling pathways converge in the activation of cAMP response element-binding protein (CREB), resulting in transcription of brain-derived neurotrophic factor (BDNF), and may be a critical component of this model (Figure 4). BDNF is a neurotrophin that critically supports long-term potentiation (LTP), spinogenesis and dendritic plasticity, mechanisms that underlie learning and memory. Binding to receptor TrkB activates MAPK/ERK and PI3K pathways.[83] BDNF and TrkB are expressed abundantly in the brain, including the PFC,

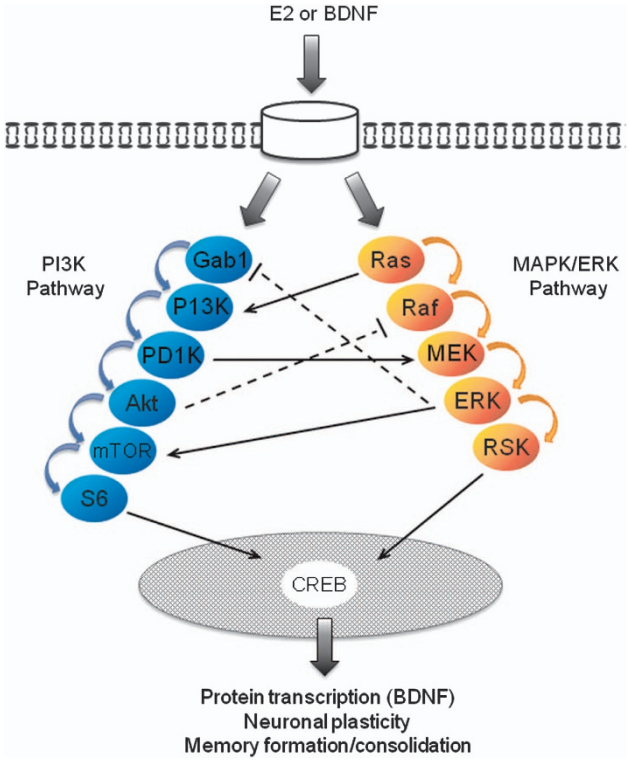

Figure 4. Schematic illustration of two molecular pathways implicated in fear extinction that are induced by estradiol (E2) or brain-derived neurotrophic factor (BDNF). In this diagram, PI3K (left) and MAPK/ERK (right) protein cascades may be activated by E2 or BDNF-bound membrane receptors. Both pathways phosphorylate CREB resulting in protein transcription, neuronal plasticity and memory formation and consolidation. Several examples of intra-pathway crosstalk are illustrated with facilitative activation represented with solid arrows and inhibitory actions by dashed lines. CREB, cAMP response element-binding protein; Gab1, GRB2-associated-binding protein 1; MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; MTOR, mammalian target of rapamycin; PDK1, pyruvate dehydrogenase lipoamide kinase isozyme 1; PI3K, phosphoinositide 3-kinase; RSK, ribosomal s6 kinase.

hippocampus and amygdala. As these regions are involved in fear circuitry, it is unsurprising that BDNF modulates fear extinction.[84,85] BDNF lowers the threshold for LTP induction, facilitates extinction consolidation in the amygdala and supports cue-dependent extinction in the hippocampus.[86]

In summary, MAPK/ERK, PI3K and BDNF are several molecular markers that appear to be critical for the consolidation of fear extinction. Most of the reviewed data were obtained from males. As previously noted, estradiol enhanced the consolidation of extinction memory in female rodents and in women. Could this effect of estradiol in females be mediated via modulation of these pathways during fear extinction?

CELLULAR PATHWAYS ACTIVATED BY ESTRADIOL

To date, the literature points to at least three different (but potentially convergent) cellular pathways through which estradiol appears to influence gene expression and learning-induced plasticity. Most of these data have been gathered from studies focusing on the hippocampus and have recently been reviewed in detail.[87–89] Below, we provide a brief overview of these pathways.

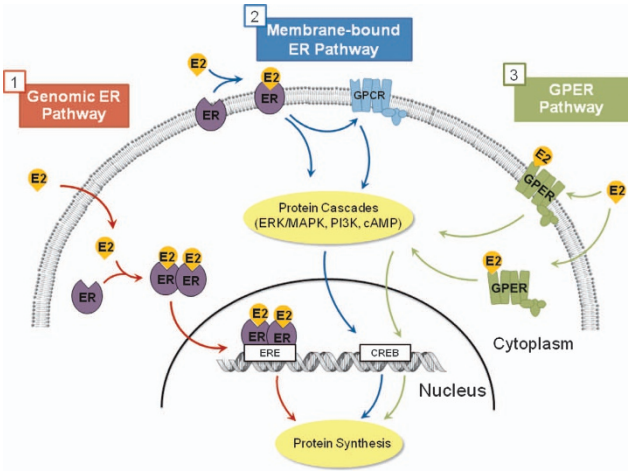

Figure 5. Schematic illustration of the different estrogen signaling pathways. Genomic ER pathway (1): estradiol mediates gene transcription by activating E2 receptors (ERs) located in the cytoplasm and nucleus, which bind to the estrogen-response element of gene promoters and induce gene transcription (hours to days). Membrane-bound ER pathway (2): membrane ERs activate intracellular cascades and neighboring GPCRs (such as metabotropic glutamate receptors), promoting CREB-modulated protein transcription. GPER pathway (3): localized in either the cell membrane or cytoplasm, E2-activated GPER initiates intracellular protein signaling resulting in CREB activation and gene transcription. Both membrane-bound ER and GPER pathways exert effects within seconds or minutes of activation. CREB, cAMP response element-binding protein; GPCR, G protein-coupled receptor; GPER, G protein-coupled estrogen receptor.

Genomic pathway

ERs located in the cytoplasm and nucleus serve as ligand-activated transcription factors. The binding of E2 to its cytoplasmic receptor forms a steroid receptor complex that dimerizes, enters the nucleus and binds to the estrogen-response elements of target gene promoters to regulate transcription (Figure 5.1). It has been suggested that this genomic pathway mediates the long-term effects of E2 exposure, with gene products detected within 12–24 h.[90] Activated nuclear ERs may also regulate gene expression indirectly by binding to transcription factors such as AP-1 and Sp1.[91] These independent mechanisms enable selective estrogen receptor modulators such as tamoxifen to act as both an antagonist (via the estrogen-response element-dependent mechanism) and agonist (through AP-1 binding),[92] data highlighting the complexity of the role that ERs and their ligands may have on synaptic plasticity.

Membrane-bound pathway

ERs may also be trafficked to the cell membrane, providing rapid (seconds to minutes) and transient signaling by modulating intracellular signaling cascades, including PI3K, MAPK/ERK, cyclic adenosine monophosphate (cAMP) and other protein kinases. Membrane-bound ERs may also activate other G protein-coupled receptors, notably metabotropic glutamate receptors (mGluRs; Figure 5.2).[93,94] Through ER–mGluR coupling, estradiol modulates intracellular calcium levels, CREB phosphorylation, and modulates L-type calcium channel currents;[88] cellular events that support synaptic plasticity and learning. ERα-mGluR coupling in CA1 post-synaptic neurons has been shown to mediate inhibitor post-synaptic potential suppression.[95] ER–mGluR coupling in the hippocampus mediates MAPK and CREB phosphorylation, an effect that has only been found in female neurons.[88] These findings, in addition to sex differences in local E2 synthesis,[96] suggests that the membrane-bound ER pathway mediates a

mechanistic sex difference in hippocampal-dependent learning and memory.

GPER pathway

GPER (formerly known as GPR30) is a recently discovered G protein-coupled receptor found to localize in the cell membrane, nucleus and endoplasmic reticulum.[97] GPER is strongly expressed in the hippocampus, cortex and limbic system[98,99] and modulates anxiety-related behavior in rodents.[100,101] As with the membrane-bound ER pathway, the GPER pathway may facilitate the rapid non-genomic effects of estradiol by activating intracellular cascades (Figure 5.3).

INFLUENCE OF ESTRADIOL ON PLASTICITY

Estradiol induces neuronal plasticity underlying cognitive function. Acute estradiol treatment promotes hippocampal neurogenesis in the female rat,[102,103] which has been linked to hippocampal-dependent learning and memory.[104,105] In female rats, E2 rapidly increases synaptic and spine density in the CA1 to enhance LTP.[106] The ER subtypes have been reported to drive opposing synaptic events. For example, ERα agonists facilitate long-term depression in the CA1, whereas ERβ agonists suppress it.[96] Estradiol has also been shown to induce dendritic remodeling in the PFC and hippocampus.[107] For instance, spine density in hippocampal pyramidal cells fluctuates with rat estrous cycle.[108,109] ERβ is thought to mediate spinogenesis in the cortex, whereas spine formation in the hippocampus has been attributed to ERα.[54,110] Investigation of the mechanisms driving dendritic spinogenesis in CA1 pyramidal cells suggest that E2 binds to membrane ERα, activating an intracellular cascade involving the MAPK/ERK pathway.[96]

Lastly, estradiol may promote spinogenesis through interactions with BDNF. BDNF transcription fluctuates across the estrous cycle, correlating with changes in hippocampal excitability.[111] Ovariectomized rats exhibit reduced BDNF expression in the hippocampus and cortex; expression that is restored with E2 treatment and correlates with enhanced recognition memory.[112,113] E2 activates BDNF transcription through both genomic and non-genomic ER pathways resulting in increases in dendritic spine density, which may be supportive of memory enhancement.[111] Altogether, there is a strong positive correlation between elevated E2, BDNF levels, spine density and enhanced memory.

THE INTERSECTION OF FEAR EXTINCTION, ESTRADIOL AND MOLECULAR SIGNALING

Our overview of estradiol signaling highlights a diverse and complex system involving multiple types of receptors and signaling pathways to produce region-specific functional and behavioral effects in the female brain. Merging this information with current knowledge of the fear extinction circuitry may further our understanding of how E2 could contribute to some of the inherent sex differences we observe in fear extinction learning, specifically as they relate to molecular signaling in the vmPFC and amygdala. We propose that elevated estradiol levels during extinction training may acutely induce MAPK/ERK signaling in the IL by (1) membrane ERβ—G protein-coupled receptor coupling resulting in ERK phosphorylation, LTP and spine remodeling and (2) enhancing BDNF transcription to promote dendritic spine growth, with both mechanisms of plasticity enhancing memory formation and consolidation. Estradiol may utilize both MAPK/ERK and PI3K pathways to phosphorylate CREB, which in turn prompts transcription of proteins involved in synaptic plasticity. This molecular process will strengthen the newly formed synaptic connections between the IL and intercalated amygdalar cells responsible for suppressing fear responses. Interestingly, there is recent evidence to suggest that there is crosstalk between the MAPK/ERK and PI3K cascades,[114] which may amplify the functional effects of membrane ER transmission. According to our hypotheses, impaired fear extinction that accompanies low-E2 states may be attributed to a reduction in activation of membrane-bound ERβ, consequently resulting in less CREB phosphorylation, and a lack of LTP and dendritic spine growth. In addition, the synergistic effects provided by BDNF are absent, as estradiol is not present to initiate protein transcription.

Given its strong expression in the hippocampus, ERβ may also support extinction learning through actions within this region. It has been suggested that ERβ may serve as a negative regulator of ERα transcription and that cognitive memory depends on the relative interactions between E2 and the ER subtypes.[115] In addition, infusing BDNF into the IL enhances extinction memory whereas increased BDNF levels in the ventral hippocampus is associated with increased neuronal firing within the IL.[116] Therefore, it is possible that during fear extinction hippocampal ERβ suppresses the anxiogenic effects associated with ERα by inhibiting its transcription as well as enhancing extinction memory through BDNF modulation.

Our predictions are not conclusive and will need further examination, as they apply knowledge of estrogen-mediated signaling to a fear extinction network built on male animal studies. The overwhelming prevalence of fear-related disorders in women suggests that there may be intrinsic sex differences in fear circuitry. Investigating the neural mechanisms underlying fear extinction in female rats with respect to gonadal hormone levels will aid in identifying these differences.

FEAR EXTINCTION AND OTHER GONADAL HORMONES

In addition to E2, there are several other sex hormones that fluctuate and differ in concentration between males and females. Progesterone is one of these key hormones that may be interacting with or contributing to the effects of E2 on extinction memory. In fact, we have observed facilitative effects of progesterone administration on extinction recall in female rodents, an effect that is comparable with that attained with E2 administration.[65] This effect, however, was not observed in women.[117] Although these discrepant findings may be due to differences in species, it is more likely that progesterone may have its effects on the fear network through its metabolites. This is consistent with other findings demonstrating the protective effects of its metabolite allopregnanolone.[118,119] More recent imaging studies have, in fact, shown that allopregnanolone is associated with reduced amygdala responsivity to aversive stimuli, further supporting the anxiolytic role of this hormone.[120,121] These studies highlight the need to further examine the role of this hormone on the mechanisms associated with emotional memory formation.

Another important sex hormone is testosterone. Testosterone and its metabolites have been linked to reduced anxiety behaviors and enhanced cognition in male rodents.[122–124] As noted earlier in this review, testosterone is aromatized to estradiol in the brain via the enzyme aromatase. Fadrazole, an aromatase inhibitor, prevents estrogen synthesis. In our laboratory, we have demonstrated that administration of fadrazole before extinction training impairs fear extinction recall in male rats.[125] In humans, low doses of testosterone administration appears to be associated with reduced anxiety.[126] In a recent study, we have shown that extinction learning and extinction memory recall is best in men with an elevated testosterone to cortisol ratio,[127] further implicating this hormone in fear extinction. Future studies are needed to examine the influence of testosterone on the mechanisms mediating fear extinction and its interactions with estrogens and other sex hormones.

POTENTIAL CONTRIBUTION OF ESTRADIOL TO VULNERABILITY FOR MOOD AND ANXIETY DISORDERS

The experimental evidence reviewed thus far clearly indicates that endogenous fluctuations as well as exogenous manipulations of E2 influence emotional memory consolidation. Specifically, low levels of E2 appear to be associated with reduced memory consolidation whereas elevated E2 is associated with enhanced memory consolidation. Drastic hormonal fluctuations occur throughout the woman's lifespan and appear to coincide with vulnerability for mood disturbances. Risk for depression and anxiety increases at the onset of puberty,[128] and mood disturbances such as premenstrual dysphoric disorder are associated with hormonal changes during menstruation.[129] The sharp drop in estradiol production at menopause coincides with cognitive deficits[130] and increased risk for depression.[131] During pregnancy, a period of extremely high hormone levels, women exhibit a blunted stress response[132] and have a lower risk for mood disorders than nonpregnant women.[133] However, the dramatic decrease in hormone levels following pregnancy accompanies a significant risk for postpartum depression.[134] These data suggest that fluctuations of E2 and other sex hormones may potentially place women at risk for developing mood and anxiety disorders. In support of this possibility, there are several studies indicating that estradiol therapy improves anxiety and depressive symptoms in postnatal depression,[135,136] recurrent postpartum affective disorder,[137] and menopause.[138–140]

In addition to natural fluctuations of E2, hormonal contraception induces an overall reduction in circulating E2. Hormonal contraceptives (HCs) are used by a large percent of women and inhibit ovarian production of estradiol and progesterone. HC treatment has been associated with altered functional connectivity in regions important for cognitive and emotional processing.[141] We recently conducted a translational investigation on the impact of HCs on fear extinction in healthy women and female rats.[142] In our study, HC-using women demonstrated significantly impaired extinction recall compared with naturally cycling women. This impairment was also found in HC-treated rats and correlated with reduced serum estradiol levels. Extinction impairment was rescued in rats through administration of ER agonists before extinction learning or by halting HC treatment after fear learning, both correlating to restored serum estradiol levels. In addition, a single dose of estradiol to low-estrogen naturally cycling women significantly enhanced extinction recall.[142] It is not clear if the use of contraceptives may also increase vulnerability to psychopathology. However, a recent study compared the development of PTSD symptoms in HC-using women who did or did not take emergency contraception following sexual assault. Women who took Ogestrel, a combination estradiol and progesterone emergency contraceptive, reported less severe PTSD symptoms 6 months later compared with women who took Plan B (a progesterone-only drug) or declined contraceptive treatment.[143] One possible explanation for this finding is that the dose of E2 immediately following the traumatic event partially rescued HC-induced vulnerability and conferred resilience against long-term PTSD symptoms.

Although our model for vulnerability implies a negative influence of HC use, it should be noted that HCs have differing effects on mood and cognition depending on task and type of hormone. Combined estradiol and progestin HCs has been associated with enhanced verbal memory[144] and overall cognitive functioning.[145] However, the progestins used in HCs have been suggested to have a masculinizing effect in certain tasks. Both men and HC users differ from naturally cycling women in expressing enhanced recall for gist as opposed to story details in an emotional memory task.[146,147] In a cognitive task involving number processing, HC users performed similarly to women in the low hormone follicular phase but showed neural activation similar to men.[148] Examining the hormones used in combined HCs, one study correlated deficits in mental rotation and verbal fluency to androgenic testosterone-derived progestins.[149] Few studies have examined the influence on HCs on mood; however, epidemiological data suggest that the combined HC is protective against mood disorders whereas progestin-only contraceptives may have a deleterious influence.[150]

FUTURE DIRECTIONS

The reviewed data indicate that low E2 levels in females may be associated with deficits in fear extinction recall and may potentially be related to vulnerability to anxiety, fear and mood disorders. In males, low levels of estradiol do not appear to impair extinction recall. This may be due to the effects of testosterone, which has been reported to have anxiolytic properties.[151] It is also probable that estradiol engages male and female brains differently. As such, we cannot preclude the possible roles that other hormones, or their interactions with E2, may have in this phenomenon. It is also important to note that while low levels of estradiol are disadvantageous to extinction memory consolidation, it is likely that fluctuations rather than absolute levels of estradiol may be the critical factor for elevated risk of anxiety.

We have reviewed evidence that estradiol may influence the molecular and cellular machinery involved in fear extinction, a behavioral process that models the psychopathology of PTSD and anxiety disorders. Together, these data highlight the association between the dynamic estrogen states that occur across the female lifespan and increased vulnerability to anxiety-related disorders. It is imperative that future studies investigate fluctuations in levels of E2 to determine their possible associations with, and contributions to, vulnerability to mood and anxiety disorders in women. There are many questions that remain to be answered in this field that are related to where, how and when E2 modifies neural function to elicit its effects on extinction memory recall (Figure 6). Future research aimed at localizing and identifying cellular and molecular mechanisms by which estrogen modulates fear extinction and anxiety can better inform us of treatment targets and improve the efficacy of clinical applications.

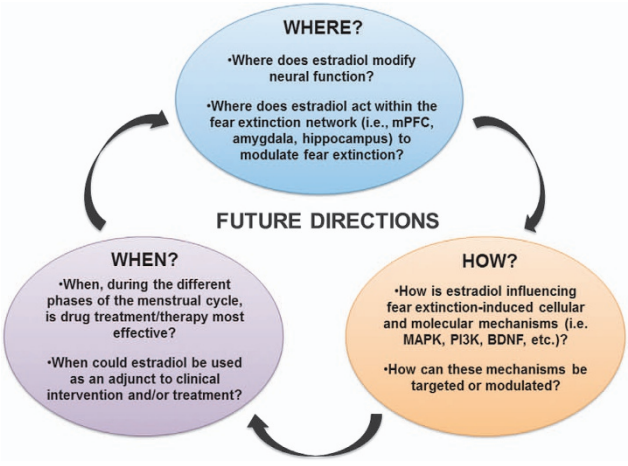

Figure 6. Future directions for exploring the role of estradiol in fear extinction and psychopathology. An apparent correlation between fluctuating estradiol states and vulnerability for fear and anxiety disorders necessitates further research into where, how and when estradiol modulates the fear extinction network. Investigating these questions may provide new options for targeted, and thus more effective, treatment and therapy in the clinic. BDNF, brain-derived neurotrophic factor; MAPK, mitogen-activated protein kinase; mPFC, medial prefrontal cortex; PI3K, phosphoinositide 3-kinase.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank members of the Behavioral Neuroscience Program in the Department of Psychiatry at Massachusetts General Hospital for helpful comments on this manuscript. MRM is supported by 1R01MH097880-001.

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