

Translational studies of estradiol and progesterone in fear and PTSD

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ABSTRACT

Translational models of fear have greatly informed our understanding of PTSD and its underlying fear circuitry. One of the most replicated findings in the field is the two-fold higher PTSD incidence in females compared to males. While sociocultural factors play a role, the most robust biological influencers to date are gonadal hormones, such as estradiol and progesterone, which fluctuate across the menstrual cycle. Among studies that account for these hormones, most do so in isolation or collect both and only report one. Variation in study findings suggests that the ratio between these two hormones (the P/E ratio) may be an important and missing variable to further understand gonadal hormone influences on fear. Here we review cross-species examinations of fear and PTSD, within the contexts of estradiol and progesterone as well as P/E ratios that were calculated based on extant literature. We then provide recommendations for best practices in assay methods and reporting to improve research on the P/E ratio in fear and PTSD. Ultimately, greater understanding of this important variable will advance efforts to characterize gonadal hormone influences on fear learning processes in humans and animals.

Estudios traslacionales sobre el estradiol y la progesterona en miedo y en el TEPT

Los modelos traslacionales del miedo han enriquecido nuestro conocimiento sobre el TEPT y el circuito del miedo subyacente. Uno de los hallazgos más replicados en el campo es la incidencia dos veces mayor de TEPT en mujeres en comparación a los hombres. Aunque los factores socioculturales juegan un rol, las influencias biológicas más robustas a la fecha son las hormonas gonadales, tales como el estradiol y la progesterona, que fluctúan durante el ciclo menstrual. Entre los estudios que dan cuenta de estas hormonas, la mayoría las estudian de forma aislada u obtienen ambas y solo reportan una. La variación en los hallazgos de los estudios sugiere que la razón entre estas dos hormonas (la razón P/E) puede ser una variable poco estudiada e importante para mejorar la comprensión de la influencia de las hormonas gonadales en el miedo. En este trabajo hacemos una revisión de hallazgos trans-especie sobre el miedo y el TEPT, dentro del contexto del estradiol y la progesterona así como de la razón P/E que fue calculada basándose en la literatura existente. Posteriormente proveemos recomendaciones para mejorar las prácticas en los métodos de ensayo y reportes para mejorar la investigación sobre la razón P/E en miedo y TEPT. En última instancia, mayor comprensión de esta importante variable hará que progresen los esfuerzos para caracterizar la influencia de las hormonas gonadales en procesos de aprendizaje del miedo en humanos y animales.

雌二醇和孕酮在恐惧和PTSD中的转化研究

恐惧的转换模型极大地帮助了我们对PTSD及其潜在恐惧回路的理解。该领域中最具可重复性的发现之一是女性的PTSD发病率是男性的两倍。尽管社会文化因素起一定作用，但迄今为止，最有力的生物学影响因素是性腺激素，例如在月经周期中会波动的雌二醇和孕酮。在研究这些激素的研究中，大多数都是单独进行或收集两者而仅报告一种。研究结果的变化表明，这两种激素之间的比率（P/E比率）可能是重要的变量，该变量的缺失导致我们无法进一步了解性腺激素对恐惧的影响。在此我们回顾了对恐惧和PTSD的跨物种研究中关于雌二醇和孕酮的讨论，并根据现有文献计算P/E比率。然后，我们为测定和报告的最佳实践提供建议，以改善对恐惧和PTSD中对P/E比率的研究。最终，对这一重要变量的更多了解将推动人们努力研究性腺激素对人类和动物恐惧学习过程的影响。

ARTICLE HISTORY

Received 4 November 2019
Revised 23 December 2019
Accepted 8 January 2020

KEYWORDS

Fear; PTSD; estradiol; progesterone; translational; sex differences

PALABRAS CLAVE

miedo; TEPT; estradiol; progesterona; traslacional; diferencias de sexo

关键词

恐惧; 创伤后应激障碍; 雌二醇; 黄体酮; 转化; 性别差异

HIGHLIGHTS

- The prevalence of PTSD in females is twice that of males, and gonadal hormones such as estradiol and progesterone appear to play a role.
- The ratio between estradiol and progesterone (the P/E ratio) may be an important and missing variable to understand hormone influences on fear and PTSD.
- Cross-species examinations of fear and PTSD within the contexts of estradiol and progesterone are reviewed, as well as P/E ratios.
- Recommendations for best practices in assay methods and reporting to are provided.

1. Introduction

The strong conservation of fear circuitry across species has provided significant insights into how humans develop posttraumatic stress disorder

(PTSD) following trauma exposure. This provides excellent translational opportunities to understand factors that place individuals at greater risk of developing PTSD (e.g. heightened sympathetic arousal)

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and insight into potential therapies (e.g. exposure therapy based on Pavlovian conditioning principles of extinction learning). From these models, we can begin to better understand why a PTSD diagnosis is twice as likely in females as compared to males (Kessler et al., 2005; Kilpatrick et al., 2013; Tolin & Foa, 2006). While sociocultural factors certainly play a role, focused biological studies have implicated gonadal hormones, such as estradiol and progesterone, as robust PTSD modulators. Within the context of estradiol and progesterone, we will review findings from both human and animal studies and discuss how these have informed our understanding of normative and pathological fear. This approach is beneficial as pathological fear is a debilitating psychological diagnosis criterion for PTSD. We will also discuss an important gap that exists in the current literature; that is, how the ratio between estradiol and progesterone, as opposed to absolute levels of each, may help the field better understand the relationship between these gonadal hormones and PTSD risk and fear-related symptoms. It is possible that absolute levels of estradiol and progesterone may be less meaningful biological indicators than the relationship between them, which has implications for future sex differences research in fear and PTSD. We will end with recommended best practices for incorporating this ratio into sex differences research in human and animal studies.

2. Cross-species examination of fear and PTSD

During a traumatic event, a neutral stimulus, present in the environment is often paired with the original unconditioned stimulus (US) and subsequently trigger the fear response on its own (having become a conditioned stimulus; CS). Thus, individuals with PTSD tend to demonstrate poor fear inhibition, that is, a strong fear response in the absence of an environmental threat. Fear conditioning paradigms are useful translational tools for understanding these processes. They model fear acquisition by repeatedly pairing an aversive US (e.g. air blast, electric shock) with a neutral stimulus and measuring indices of fear responsivity (e.g. eyeblink startle response or skin conductance). After repeated pairings, fear learning takes place and the neutral stimulus alone is sufficient to elicit a fear response, thus becoming a conditioned stimulus (CS+). Subsequent extinction paradigms measure the fear response after repeated CS+ only presentations, with decreases in fear as the individual learns that the CS+ is no longer a danger cue. Following extinction training, extinction retention which represents the strength of the extinction memory, can then be tested, usually 24 hours following extinction training.

Similarly, rodent fear conditioning studies start with an acquisition stage where a once neutral cue, CS+, is repeatedly paired with a US, such that associative learning takes place and can be measured by conditioned responses (CRs – commonly freezing behaviour (measured as percent freeze time)). Like human studies, repeated presentation of the CS+, without the US, results in fear extinction. Fear extinction is not the erasure of the old memory but, rather, represents a new memory that has formed. The consolidation and recall of this (new) extinction memory can also be tested and usually occurs 24 hours following extinction training. Collectively, the similarity of conditioning models and the conservation of many elements of fear neurobiology make rodent models extremely useful for examining normative and pathological changes in fear-related behaviours (Lebron-Milad & Milad, 2012). The translational utility of these fear models is particularly useful for investigating the role of sex in fear and fear-related PTSD symptoms, which is critical given the elevated risk for PTSD among females.

3. The importance of studying sex in fear and PTSD

Sex refers to the binary, biological distinction between males and females that is based on a person's genetics and reproductive organs, while *gender* is a non-binary term that encompasses the socially constructed definition of man and woman, giving rise to the concept of masculinity and femininity. For the purpose of this paper we will focus specifically on biological *sex* differences in fear and PTSD. One of the most established findings in the literature is that following puberty, PTSD is twice as prevalent in females as compared to males (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995; Olf, Langeland, Draijer, & Gersons, 2007). Psychosocial risk factors for trauma exposure are strongly correlated with sex. For males, trauma is most commonly related to non-assaultive trauma, whereas females are more likely to develop PTSD following interpersonal trauma (Breslau, 2002; Breslau & Anthony, 2007; Kessler et al., 1995). When both sexes experience the same type of trauma, females are still more likely to develop PTSD and report more chronic symptoms as compared to males (Breslau, 2002; Tolin & Foa, 2006). Moreover, females are more likely to appraise traumatic events as stressful and report greater loss of personal control and lack of available coping mechanisms (Eisler & Skidmore, 1987; Timmer, Veroff, & Colten, 1985).

Female rodents provide a useful model for examining sex differences in fear-based PTSD symptoms, given currently available gonadal hormone tools, as well as the conservation of fear circuitry across

humans and rodents. As it relates to gonadal hormone tools, both naturally cycling and ovariectomized female mice can be used to assess the role of these hormones in fear processes. Naturally cycling methods involve accounting for estrous cycle stage most often through vaginal cytology assessment. Ovariectomy involves the surgical removal of the ovaries, typically followed by a synthetic hormone replacement of estradiol and/or progesterone. Given the language reliance of PTSD diagnosis, PTSD itself cannot be modelled in mice. However, highly conserved physiological symptoms in response to threat, can be used to model normative and pathological fear using Pavlovian fear conditioning paradigms.

4. Estrogen and progesterone in the human menstrual cycle

The human menstrual cycle is 28-days long and is comprised of two primary phases: follicular and luteal (see Figure 1). The follicular phase encompasses days 1–14 and includes menstruation on days 1–7 and ovulation beginning around day 14, while the luteal phase encompasses days 15–28. In the early follicular phase, both estrogen and progesterone levels are low, and estrogen levels begin to rise in the mid-follicular phase while progesterone remains relatively low. By the late follicular phase, estrogen levels begin to peak and progesterone rises. Estrogen continues to peak in the early luteal phase as ovulation ends, followed by a decrease that is followed by a second, smaller peak before dropping at the late-luteal phase. At this time, progesterone levels continue to rise and they peak at

the mid-luteal phase before dropping at the late-luteal phase.

5. Estrogen and progesterone in the rodent estrous cycle

Like the human menstrual cycle, the rodent estrous cycle is also characterized by fluctuating levels of estradiol and progesterone (see Figure 2). The estrous cycle typically lasts four to six days, and is separated into four distinct phases: Pro-Estrus, Estrus, Metestrus, and Diestrus. Pro-Estrus is characterized by high circulating levels of estradiol and progesterone. During the Estrus phase, both progesterone and estrogen levels fall and remain low during late estrus and as well as early and late Metestrus. Whilst estradiol levels remain low during Diestrus, there is less consensus regarding the rise of progesterone levels during this phase. Most studies show that circulating progesterone levels are high in Diestrus, but it is not certain if these levels remain high or fall before rising again during Pro-Estrus.

6. Human research on estradiol and progesterone in fear and PTSD

6.1. Human research on estradiol in fear and PTSD

Findings regarding estradiol levels and fear learning processes in humans are mixed. For example, one study found that pre-pulse inhibition (PPI) was reduced among healthy females in the luteal phase, compared to those in the follicular phase. PPI is

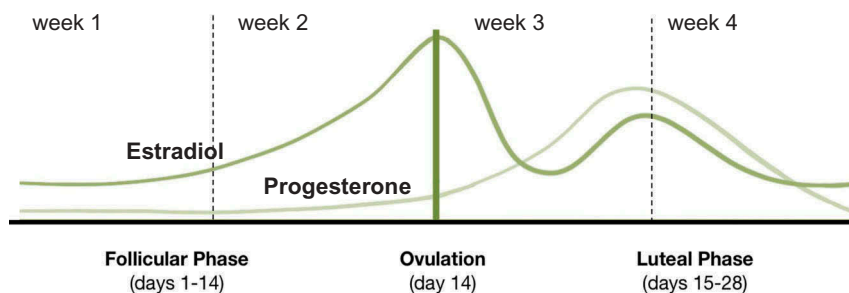


Figure 1. Human menstrual cycle.

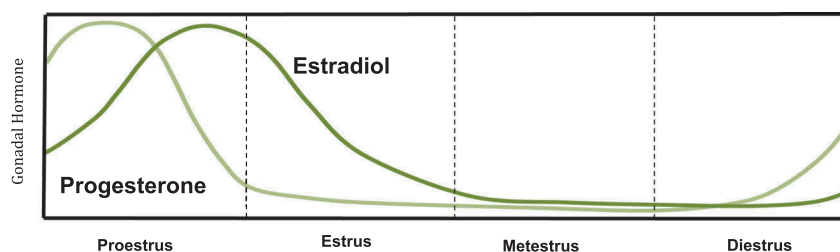


Figure 2. Rodent estrous cycle.

a measure of sensorimotor filtering which facilitates startle response inhibition, indexed by the eye blink startle response. As such, reduced PPI reflects sensorimotor filtering deficits which may be a contributory mechanism to recurrent intrusive memory symptoms related to past trauma. Therefore, this finding suggests that higher estradiol is associated with worse inhibition, or sensorimotor gating (Jovanovic et al., 2004). Similarly, another study found that healthy females in the late-follicular phase demonstrated worse extinction memory than those in the early follicular phase (measured by skin conductance response; SCR) (Milad et al., 2006). For this study, menstrual phase (see Figure 1) was determined by self-reported cycle day, however the authors measured serum estradiol levels in two follow-up studies, where they found that females with high circulating estradiol demonstrated better extinction recall compared to those with low estradiol (measured by SCR) (Milad et al., 2010; Zeidan et al., 2011). In both studies, differences were not observed in fear acquisition or extinction, suggesting that low circulating estradiol levels may specifically diminish extinction consolidation or expression. Similar findings were reported in another study, such that mid-cycle females demonstrated better extinction recall (as measured by SCR) than early-cycle females, again supporting the moderating role of estradiol on extinction retention processes (Antov & Stockhorst, 2014). Only one study with healthy females have found differences in extinction learning, showing that those with low estradiol levels demonstrated larger SCRs than those with high estradiol levels (Wegerer, Kerschbaum, Blechert, & Wilhelm, 2014). Overall, this work suggests that among healthy females, high levels of estradiol during extinction learning enhance fear learning and regulation, as well as extinction memory consolidation.

Findings related to the influence of estradiol on fear and PTSD have also been variable. Two studies tested the moderating effect of estradiol on fear-potentiated startle (FPS; eye blink startle response) during fear conditioning and extinction (Glover et al., 2012, 2013). While PPI is specifically a measure of inhibition/sensorimotor gating obtained using eye blink startle, FPS is a measure of the eye blink startle response to a CS+ or CS-. FPS accounts for baseline startle by subtracting a person's baseline startle from that of their startle to the CS+ or CS- (i.e. it is an index of how much the startle response is potentiated relative to baseline). Among females with PTSD, those with low circulating estradiol demonstrated worse fear inhibition and extinction than those with high estradiol. In contrast, a different study found no effect of menstrual phase on PPI (early follicular vs. mid-luteal) among females with PTSD (Pineles et al.,

2016a). However, the same sample also completed fear conditioning and females in the mid-luteal phase demonstrated deficits in extinction retention compared to those in the early follicular phase (measured by SCR) (Pineles et al., 2016b). Given that SCR is an index of sympathetic arousal and FPS is an index of a brainstem-mediated reflex, this suggests that specific fear-related pathways could be differentially influenced by estradiol levels.

6.2. Human research on progesterone in fear and PTSD

Considering the cyclical fluctuation of both estradiol and progesterone across phases of the menstrual cycle, some studies have also focused on the role of progesterone in fear. Findings among healthy females suggest that progesterone does not influence fear acquisition, extinction, or extinction recall (Graham & Milad, 2013; Milad et al., 2010; Zeidan et al., 2011). However, researchers who reported these findings noted that they divided females into high and low estradiol groups and found that females in the high estradiol group also had high levels of progesterone, and *vice versa*. Thus, there was insufficient evidence to suggest a lack of progestogenic effects on fear memory in these studies. On the other hand, studies using emotional film viewing as a proxy for traumatic stress among healthy females have reported inconsistent findings regarding the influence of progesterone on fear memory. While one study found an association between higher progesterone levels and more film-related intrusive memories, which can be regarded as conditioned emotional reactions to trauma reminders (Ferree, Kamat, & Cahill, 2011), three other studies found null results for the effect of progesterone on these types of intrusions (Cheung, Chervonsky, Felmingham, & Bryant, 2013; Soni, Curran, & Kamboj, 2013; Wegerer et al., 2014). Notably, all four of these studies assayed hormone levels through saliva, which limits comparison to studies that assayed via plasma (an issue that will be discussed below in terms of best practices/recommendations). Given the variability in methodologies and findings as well as a dearth of progesterone-specific human studies, more evidence is needed to develop a consistent picture of progesterone's role in fear learning processes among healthy individuals.

A series of studies on a sample of trauma-exposed females have provided data on progesterone levels during specific menstrual phases and its associations with various aspects of fear learning (Nillni et al., 2015; Pineles et al., 2016a, 2016b, 2018). Using a PPI paradigm, one study found that greater progesterone levels (assayed from plasma) were significantly associated with increased PPI among trauma-exposed females in

the early follicular phase (Pineles et al., 2016a). Conversely, a prior study in healthy females reported greater PPI among those in the follicular phase compared to those in the luteal phase (Jovanovic et al., 2004). Since gonadal hormone levels were not measured in that study, it is difficult to determine whether PPI was higher in the follicular phase because estradiol was high (late follicular) or low (early follicular), since progesterone remains low throughout the phase. Taken together, these studies could suggest that in the early follicular phase when estradiol is low, higher progesterone may confer better sensorimotor gating, but future research with confirmed hormone levels will be needed to accurately answer this question. Regardless, these findings appear to suggest that progesterone may promote improved sensorimotor gating among trauma-exposed females.

In the same sample of trauma-exposed females, the effects of progesterone on extinction retention were different for those with and without PTSD (Pineles et al., 2016b). Among those without PTSD, low levels of progesterone were associated with worse extinction retention. However, among those with PTSD, high levels of progesterone were associated with worse extinction retention (Pineles et al., 2016b). Importantly, this study tested females in either the early follicular or mid-luteal phase of the menstrual cycle – stages of the menstrual cycle where estradiol and progesterone levels are low and high, respectively. Given the stages investigated, it is difficult to assess the individual effects of each hormone; however, these studies did assess the ratio between estradiol and progesterone, which will be reviewed below. Nonetheless, the group differences suggest that the effects of progesterone may vary depending on PTSD status. To further investigate these findings, a subsequent study of the same sample examined ALLO synthesis (allopregnanolone and pregnanolone, metabolites of progesterone) as a potential mechanism given its anxiolytic properties. They found that the PTSD group evidenced a deficit in ALLO synthesis, and this was not moderated by menstrual phase (Pineles et al., 2018). This may suggest that deficient ALLO synthesis, and not high progesterone per se, explain the findings related to poor extinction retention in PTSD.

Although this review has focused on endogenous sources of gonadal hormones, work by Ferree and colleagues further argues that exogenous gonadal hormones modulate the consolidation of fear memory (Ferree, Wheeler, & Cahill, 2012). In their study, the authors examined the effect of emergency hormone contraceptives on the development of PTSD symptoms following sexual assault. Examination of symptom clusters demonstrated that synthetic estradiol and progesterone were associated with significantly fewer intrusive symptoms, suggesting that their

combined administration may confer better psychological outcomes. This supports the notion that the relationship between these two influential hormones may be critical in fear and PTSD.

7. Animal research on estradiol and progesterone in fear

7.1. Animal research on estradiol in fear

In rodent studies, effects of gonadal hormones are examined under three models: 1) endogenous sources of gonadal hormones are removed via ovariectomy (OVX) and 2) replaced with synthetic hormone treatment (OVX+E2), or 3) endogenous gonadal hormones are assessed by monitoring estrous cycle phase. To date, most studies focus on the role of estradiol in extinction recall. As it relates to fear conditioning and extinction learning, the collective data are often difficult to interpret. For example, Jasnow, Schulkin, and Pfaff (2006) showed that high dose treatment of estradiol-benzoate (EB), a synthetic estradiol, increased freezing during fear conditioning, while Gupta, Sen, Diepenhorst, Rudick, and Maren (2001) demonstrated no effect on freezing at this stage (Gupta et al., 2001). While fear conditioning was performed similarly in both studies, Gupta and colleagues included an additional four-day handling period prior to experimentation, potentially (but not necessarily) helping to explain similar threat responsiveness observed in both groups during experimentation. Moreover, this study also showed that OVX, compared to sham-operated, female rodents demonstrated impaired extinction of fear, suggesting that endogenous gonadal hormones presence and cyclic levels are necessary for enhanced extinction learning (Gupta et al., 2001).

These results are supported by a separate investigation using systemic hormone contraceptive treatment, which prevents natural gonadal hormone fluctuations. The group demonstrated that chronic treatment for four days prior to conditioning led to impaired extinction learning (Graham & Milad, 2013). Yet again in opposition to this, another study demonstrated that EB treated mice showed increased fear behaviour (as measured by freezing) during extinction training and reported no differences in extinction recall (Jasnow et al., 2006). Though not a direct measurement of estradiol, one study examined the effect of estradiol synthesis inhibition (via an aromatase inhibitor) in male rodents and found that it significantly impaired extinction recall (Graham & Milad, 2014). Given the limited number of studies, additional experiments are needed to fully determine whether exogenous or endogenous estradiol influences fear learning during the conditioning and extinction phases.

Most naturally cycling and OVX+ E2 studies report that high circulating estradiol levels improve extinction recall (Graham & Scott, 2018; Lebron-Milad & Milad, 2012; Matsumoto, Kasai, & Tomihara, 2018). However, there are a few studies with conflicting results, likely due to methodological and, by extension, data interpretation differences. For example, there are studies reporting either the opposite (decreased recall) or no effect (Garcia, Walker, & Zoellner, 2018). For studies showing enhancing effects, dose-dependent differences have been reported, as well as differences between acute and chronic estradiol treatment (Graham & Milad, 2013; Matsumoto et al., 2018; Milad, Igoe, Lebron-Milad, & Novales, 2009). These results suggest that the estradiol effect on improving extinction recall is not as simple as suggested by earlier studies, and, as we will further argue, estradiol and progesterone interactions may have unexplored modulatory effects on extinction processes.

7.2. Animal research on progesterone in fear

As compared to estradiol research, there has been limited focus on the role of progesterone in fear memory processes. Here, we argue that progesterone levels do influence fear processes, though most available data focus only on extinction recall. We draw this conclusion based on: 1) research suggesting that low levels of estradiol and progesterone leads to decreased extinction and extinction recall and 2) the systemic use of progesterone receptor (PR) agonists and antagonists resulting in enhancement of extinction consolidation (Graham & Daher, 2016; Graham & Milad, 2013; Gupta et al., 2001). Studies that inhibit fluctuating hormone levels (for example, through ovariectomy) have shown that sham-operated females extinguish fear more rapidly than ovariectomized females (Gupta et al., 2001). These data support the idea that fluctuating progesterone (much like estradiol) is potentially necessary for enhancing extinction recall. Additionally, a study examining antagonism of PR or estradiol receptors (ER), as compared to vehicle, showed that only systemic antagonism of PR increased freezing during recall testing (Milad et al., 2009) – further showing that progestogenic effects are important for facilitating extinction consolidation or recall. Collectively, these data suggest that progestogenic effects on fear memory processes are modulated via progesterone receptors as well as the progesterone metabolite, allopregnanolone, which mediates inhibitory effects via the GABA_A receptor (Lebron-Milad & Milad, 2012). Regarding fear conditioning, progesterone-based studies are few and generally report no effects. Though progesterone is often ignored in these studies, collectively, the evidence discussed suggests that the progesterone system must, in part, be

responsible for modulating fear extinction and its consolidation, whether in isolation or in conjunction with estradiol.

While not fully understood, it is worth noting the potential role of testosterone in fear and PTSD as indicated by three possible mechanisms. First, there appears to be an interactive relationship between the hypothalamic-pituitary-adrenal (HPA; secretes cortisol) and hypothalamic-pituitary-gonadal (HPG; secretes testosterone) axes, such that these two systems may both facilitate (Francis, 1981; Kreuz, Rose, & Jennings, 1972) and inhibit (Sutton, Coleman, Casey, & Lazarus, 1973) one another, which may differentially affect fear and PTSD. Second, testosterone may affect fear and PTSD through its indirect effect on estradiol, such that testosterone is converted to estradiol through the aromatase enzyme (see Maeng and Milad for a review (Maeng & Milad, 2015)). A third explanation is that testosterone's activation of androgen receptors reduces fear memory (Ramzan, Azam, Monks, & Zovkic, 2018). Human studies have demonstrated that blunted testosterone and cortisol reactivity have been associated with worse symptom severity (Josephs, Cobb, Lancaster, Lee, & Telch, 2017), and extinction learning may be improved among males with higher levels of testosterone (Pace-Schott et al., 2013). Thus, there may be an important role of testosterone in fear and PTSD but additional research is necessary in order to elucidate what that role is.

8. The progesterone-estradiol ratio

Given the findings regarding estradiol and progesterone and considering their fluctuation during the human and rodent menstrual/estrous cycles, both hormones should be assessed when examining their role in fear processes. One way of doing so is to examine the ratio between estradiol and progesterone and determine whether it may be a more accurate and influential biomarker of fear. Testing the statistical interaction between these hormones is another way of examining their relationship with the advantages of 1) increased power and 2) decreased Type I error but the disadvantages of 1) information loss and 2) lack of quantitative measurement. Although using the ratio may have the disadvantages of 1) increased Type I error risk in the case of multiple testing and 2) decreased power, it has the advantages of 1) providing a true quantitative measure that accounts for the actual P/E relationship in each individual, 2) no information loss due to its continuous nature, and 3) it has become a standard across disciplines, which facilitates comparison among studies. We will first review a selection of non-PTSD human studies where levels of both estradiol and progesterone were reported, including the progesterone-to-

estradiol (P/E) ratios that we calculated based on these levels. Thereafter, we will discuss the P/E ratio from the few PTSD studies that reported estradiol and progesterone levels and, lastly, examine ratios obtained from any animal studies with reports of these gonadal hormone levels. Estradiol was always reported in pg/mL, and progesterone as pg/mL or ng/mL. Human and rodent ratios were calculated as either: 1) progesterone divided by estradiol (if progesterone reported in pg/mL), or 2) progesterone *1000, divided by estradiol (if progesterone reported in ng/mL). See Tables 1 and 2 for all calculated P/E ratios in humans and animals, as well as a visual depiction of the cyclical nature of each hormone across the menstrual and estrous cycle, respectively (Figures 1 and 2).

8.1. P/E ratio in healthy females

Among healthy females, the within-studies P/E ratios largely demonstrate a higher ratio during the luteal, as compared to the follicular, phase (Table 1). Between-studies P/E are, however, more variable, potentially partly due to differences in biological samples used for analysis, such as plasma versus saliva. For example, ratios in the early follicular phase range from 7.65 to 27.03, and those in the mid luteal phase range from 17.14 to 90.60 (Jasnow et al., 2006; Lebron-Milad & Milad, 2012)⁰. While a slight majority of studies focused on these phases, others reported estradiol and progesterone for less-studied phases, such as mid-follicular and late luteal (Soni et al., 2013). Thus, comparisons among those studies are not yet possible. The paradigms included negative imagery/film (Andreano & Cahill, 2010; Cheung et al., 2013; Soni et al., 2013), general mental imagery (Wassell, Rogers, Felmingam, Bryant, & Pearson, 2015), emotional memory (Ertman, Andreano, & Cahill, 2011; Nielsen et al., 2013), sympathoexcitation (Carter, Fu, Minson, & Joyner, 2013), and fear conditioning/extinction (Milad et al., 2010).

Table 2. Progesterone to Estrogen (P/E) ratios in Studies involving Female Rodents.

Proestrus	Estrus	Metestrus	Diestrus
75 ¹	218 ¹	400 ¹	100 ¹
167 ²	15 ²	140 ²	365 ²
273 ³	344 ³	118 ³	437 ³
63 ⁴	425 ⁴	400 ⁴	314 ⁴
625 ⁵	500 ⁵	750 ⁵	1250 ⁵
155 ⁶	105 ⁶	80 ⁶	350 ⁶

Note. All plasma assays. Studies: 1–3 performed in mice; 4–6 performed in rats.

Estradiol reported in pg/mL, progesterone as pg/mL or ng/mL.

Ratios were calculated as either: 1) progesterone divided by estradiol (if progesterone reported in pg/mL), or 2) progesterone *1000, divided by estradiol (if progesterone reported in ng/mL).

¹Nelson et al., 1981; ²Wood et al., 2007; ³Annie et al., 2019; ⁴Faccio et al., 2013; ⁵Bowlby et al., 2016; ⁶Nilsson et al., 2015.

Several studies directly examined the P/E ratio. During negative picture viewing, one study did not find a significant association between the P/E ratio and amygdala reactivity, though they did find that absolute estradiol levels were associated with decreased reactivity (Andreano & Cahill, 2010). In contrast, a different study did not find an association between intrusive memories (after watching a distressing film) and estradiol or progesterone levels, but the P/E ratio was significantly negatively associated with intrusion frequency (Soni et al., 2013). This suggests that the combination of low estradiol and high progesterone may confer less risk in terms of symptoms akin to re-experiencing in PTSD. In a study examining sympathoexcitation (where peripheral nerves are directly stimulated with an electrode), the P/E ratio was significantly negatively associated with changes in muscle sympathetic nerve activity (a measure of blood pressure control), suggesting that sympathetic activity is more pronounced when estradiol is low, and progesterone is high (Carter et al., 2013) (this study appeared to calculate its P/E ratio as estradiol divided by progesterone, which likely explains this finding). During fear conditioning, extinction, and extinction recall, no differences were found based on the P/E ratio (using a median split) (Milad et al., 2010). Unlike other studies, the authors did not report hormone levels by phase but instead

Table 1. Progesterone to Estrogen (P/E) ratios in Studies involving Female Humans.

Early follicular	Mid follicular	Late follicular	Follicular	Early luteal	Mid luteal	Late luteal	Luteal
Healthy females							
7.65 ^a (saliva)	8.39 ^c (saliva)	22.88 ^d (saliva)	17.36 ^e (saliva)	20.61 ^c (saliva)	17.14 ^a (saliva)	34.16 ^c (saliva)	22.53 ^e (saliva)
27.03 ^b (plasma)			36.84 ^f (saliva)		51.20 ^d (saliva)		43.75 ^f (saliva)
					90.60 ^b (plasma)		
Trauma-exposed females							
					167.20 ^g (plasma)		
22.05 ^g (plasma)							

Estradiol reported in pg/mL, progesterone as pg/mL or ng/mL.

Ratios were calculated as either: 1) progesterone divided by estradiol (if progesterone reported in pg/mL), or 2) progesterone *1000, divided by estradiol (if progesterone reported in ng/mL).

^aAndreano and Cahill (2010); ^bCarter et al. (2013); ^cSoni et al. (2013); ^dWassell et al. (2015); ^eErtman et al. (2011); ^fNielsen, Ahmed, and Cahill (2013); ^gPineles et al. (2016a, 2016b, 2018) (same sample; average ratio).

examined high versus low estradiol. The ratios we calculated are therefore not phase-specific, but based on gonadal hormone levels, and they were nearly identical in both the low and high estradiol groups. estradiol and progesterone levels for the low estradiol group were 57.50 and 1.60, respectively; estradiol and progesterone for the high estradiol group were 256.70 and 7.20, respectively. These data suggest that phase-specific analysis of P/E ratio may be necessary to determine the advantages and/or limitations of its utility.

8.2. P/E ratio in trauma-exposed females

Only one independent study reported both estradiol and progesterone levels among trauma-exposed females (with and without PTSD) (Pineles et al., 2016a, 2016b, 2018). Given that the hormone levels reported in the individual manuscripts were obtained from the same sample, we first calculated the individual P/E ratios and then averaged them. The average early follicular ratio (22.05) is similar to that of healthy females in a prior study (27.03) (Carter et al., 2013), both of which used plasma assay, but higher than that of another study (7.65) (Andreano & Cahill, 2010), which measured hormone levels using saliva. In contrast to healthy females, the average mid-luteal ratio that we calculated for the trauma-exposed sample was 167.20, which is notably higher than the ratios obtained in healthy females using both saliva and plasma (Andreano & Cahill, 2010; Carter et al., 2013; Wassell et al., 2015).

When looking at individual hormone levels across studies, there is wide variability. For example, when assayed via saliva, estradiol in healthy mid-luteal females ranged from 3.97 to 8.46 pg/mL and progesterone ranged from 145 to 203.26 pg/mL (Andreano & Cahill, 2010; Wassell et al., 2015). When assayed via plasma, estradiol in healthy mid-luteal females was 117 pg/mL and progesterone was 10.6 ng/mL, while they were 75.34 pg/mL and 12.57 ng/mL, respectively in mid-luteal trauma-exposed females. This variability in individual hormone levels is necessary to capture as it directly impacts the P/E ratio (e.g. significantly higher progesterone levels in one sample will inflate the overall ratio). Further, the method by which phase is determined will impact the accuracy of such estimates (e.g. calculating based on day count versus actual hormone levels). Improved characterization of individual variability, phase determination, and assay methods are essential to disentangling their effects from those of trauma and PTSD.

In the context of fear conditioning, trauma-exposed females without PTSD demonstrated worse extinction retention when both estradiol and progesterone were low (Pineles et al., 2016b). However, those with PTSD demonstrated worse extinction

retention when progesterone was high and estradiol was low (i.e. a high P/E ratio) (Pineles et al., 2016b). As discussed above, a potential mechanistic explanation for this discrepancy is that individuals with PTSD exhibit deficits in ALLO synthesis (i.e. high progesterone alone may not be problematic) (Pineles et al., 2018).

8.3. P/E ratio in female mice

In female rodents, only one group has investigated the combined effect of estradiol and progesterone in fear memory and found that progesterone treatment, 6 hours after estradiol treatment (with OVX females), enhanced fear extinction memory recall (Graham & Daher, 2016). This same effect was not seen when progesterone was administered 24 hours post-E2 treatment, or when estradiol or progesterone was administered alone (though OVX+ E2 showed lower freezing than vehicle and E + P 24H) (Graham & Daher, 2016). This study is promising in its suggestion that, in combination, both estradiol and progesterone can have an enhancing effect on extinction memory. However, as we shift to a more translational approach to the understanding of fear memory, we suggest that it is the ratio between estradiol and progesterone that will prove most applicable going forward.

There does exist some data reporting absolute hormone levels of estradiol and progesterone, from which the P/E ratio can be calculated. In the study discussed above, we calculated the P/E ratio to be 50 (based on the doses administered), though this value may not be precise due to the delay of progesterone administration. The researchers did report that the concentrations were chosen to mimic pro-estrus (Graham & Daher, 2016). Other reports of hormone levels, though not with the intent of studying fear processing, have also provided us with data for estradiol and progesterone levels individually, and we were then able to calculate the ratio for each phase. Though only examining rodents of the same age range and subfamilies, between study results were still highly variable. Moreover, within study ratio trends from six rodent studies (see Table 2) are not as clear as those gleaned from human studies. Within studies, there is a trend of higher P/E ratios during metestrus or diestrus, which is not surprising given that concomitant higher and lower levels of progesterone and estrogen, respectively, are associated with these phases. On the other hand, a phase-specific association with lower ratios is less obvious which may be due to inaccuracies related to the difficulty of capturing the shorter pro-estrus (higher estrogen) phase of the estrous cycle.

Additional intra- and inter-rodent subfamily differences are present. Pro-Estrus and estrus rat studies

reported higher hormonal levels compared to mouse studies. Moreover, Nelson and colleagues also reported their own calculated ratios and found that older female mice had overall lower ratios than those of younger mice (Nelson, Felicio, Osterburg, & Finch, 1981). This data suggests that a standardized approach for examining the P/E ratio in rodents must be taken into consideration. Based on the available data, we found no determinable difference between the ratios when different assays were used. There was also no categorizable difference between studies using the same or different assays to measure estrogen and progesterone. With such limited data available, it is difficult to draw any further conclusions that could explain the variability.

The P/E ratio provides an opportunity for standardizing the study of gonadal hormones in fear processes, as well as potentially clarifying the data reported when estrogen and progesterone are studied in isolation. However, understanding and rectifying sources of inter- and intra- study variability will be needed to realize the full utility of the P/E ratio in rodent fear studies. Only then, can we assess the translational utility of the ratio in human and rodent studies of normative and pathological fear.

9. Recommendations for future research

Other than providing a potential solution to understanding how gonadal hormones play a role in fear in both rodents and humans, a shift of focus to a P/E ratio could offer several benefits to capture the relative hormone levels and thus the contribution of both hormones to modulating fear behaviours. We will now shift to understanding how the methodological inconsistencies between studies of both estradiol and progesterone complicate interpretation of the results and increase variability both within and across studies, and then outline specific benefits and downfalls to techniques used and how they could be adapted for studying estradiol and progesterone as a ratio. We will then explain how the P/E ratio could decrease this variability in studies going forward, as well as offer our own suggestions for the most translationally useful methodologies when studying the relationship between gonadal hormones and fear processes.

9.1. Recommendations for future human research on the P/E ratio

Perhaps one of the simplest recommendations for human research is to report levels of both estradiol and progesterone (and their respective units) so that their ratio may be calculated, and to do so in both females and males. While the ratio itself may be helpful to report, it is only informative if researchers

know the individual hormone levels as well. For example, a P/E ratio of 15 would be obtained for an individual with 45 pg/mL progesterone and 3 pg/mL estradiol, as well as an individual with 150 pg/mL progesterone and 10 pg/mL estradiol. Further, reporting these levels by phase would allow for more specific comparisons and may account for some of the variability that would otherwise be found. While it would be helpful to at least report values for the follicular and luteal phases, a more nuanced approach would be to report sub-phases, such as early/mid/late follicular and luteal. The strongest of these would be to report sub-phase levels of estradiol and progesterone levels within the same subjects. Although this is much less feasible given study constraints, it would allow for the most robust analyses that account for both within- and between-subject variability in the P/E ratio across the menstrual cycle. Moreover, an important consideration in PTSD research is the timing of trauma. While this varies significantly, any efforts to estimate and report estradiol and progesterone levels (or the P/E ratio) at the time of trauma would provide invaluable data on how the P/E ratio affects fear consolidation versus later fear inhibition deficits and PTSD.

A related recommendation involves collection methodology. One of the greatest challenges to interpreting different study findings and making replications or comparisons is variable assay methods. For example, some studies assay plasma whilst others use saliva. Additionally, there are differences in time of day that the assay is obtained. Where feasible, we recommend using plasma given that it is most accurate. Obtaining plasma in the morning would further reduce variability given that estradiol and progesterone do fluctuate throughout the day. If this is not possible due to study constraints, a 'time of collection' variable could be created and subsequently controlled for in analyses. Additionally, collecting estradiol and progesterone levels at lesser-studied sub-phases (e.g. mid follicular, late luteal) would allow researchers to better understand how the P/E ratio fluctuates throughout the entire menstrual phase among trauma-exposed females, and how different P/E ratios affect fear learning processes and PTSD. A related point regarding collection methodology is to consider the index of fear used (e.g. eye blink startle, skin conductance), which varies between studies. Use of one fear index versus another may be hypotheses-driven or based on logistics, but little is known about how these indices are differentially sensitive to variation in sex hormone levels. Thus, in addition to using the same hormone assay methods when possible, it is important for researchers to consider the fear index used when comparing study findings to prior literature and attempting replication.

An important consideration in human research on the P/E ratio, and PTSD research in particular, is the influence of stress on progesterone and estradiol levels. In addition to cortisol, the adrenal glands secrete progesterone as part of the stress response and higher progesterone levels have been associated with higher levels of available cortisol (Deis, Leguizamon, & Jahn, 1989; Herrera, Nielsen, & Mather, 2016; Roca et al., 2003; Wirth, Meier, Fredrickson, & Schultheiss, 2007). Thus, during high progesterone phases of the menstrual cycle, the contribution of stress may further increase progesterone levels and inflate the P/E ratio. Further, rodent studies suggest that stress may alter the expression of estrogen receptors (Blume et al., 2017; Isgor, Cecchi, Kabbaj, Akil, & Watson, 2003). Consideration of general stress may include a self-report measure, such as the Perceived Stress Scale (Cohen, Kamarck, & Mermelstein, 1983). However, researchers using experimental paradigms that increase stress (e.g. fear conditioning) are encouraged to measure progesterone and estradiol levels before and after such manipulations where possible, as well as circulating cortisol levels to provide convergent validity.

9.2. Recommendations for future animal research on the P/E ratio

Both naturally cycling and OVX females have been used to study estradiol and/or progesterone in isolation. While naturally cycling provides a more translationally relevant model, the variability in assessment of stage, as well as an inability to isolate a specific hormone often render this method less effective. However, the use of OVX females with hormone replacement risks inconsistencies that results from the number of variables that must be accounted for (for example, timing, concentration, as well as method for hormone replacement) (Graham & Milad, 2014; Matsumoto et al., 2018; Milad et al., 2006). Additionally, much of the translational relevance is lost when natural hormone fluctuations are removed.

Transitioning to a P/E ratio would overcome many of these methodological issues. Naturally cycling methods could easily be transitioned from a language describing high estradiol and progesterone levels, to one that bases different phases on the ratio between the two gonadal hormones. With an estradiol and progesterone ratio, OVX + hormone replacement could likely maintain the translational validity that is not preserved when assessing these hormones in isolation, and variability across studies could be greatly reduced if standard ratios (along with more uniform methods of administration and timing) were implemented. However, the greatest challenges are the lack of data on individual hormones levels, P/E ratios, as well as methodological differences across human and rodent studies of fear. It is now

important to shift to a more holistic approach that incorporates all potential gonadal hormone contributors to fear memory. This entails not only the addition of progesterone to many of these studies, but the understanding that there is likely an interaction between progesterone and estradiol that act in concert to modulate many aspects of fear.

10. Conclusions

We hope that the above review has highlighted the utility of translational models of fear and PTSD, and how these models can enrich our understanding of gonadal hormone contributions to sex differences. While methodological discrepancies exist and data are currently scarce, the P/E ratio is a promising avenue for future study given that both estradiol and progesterone impact fear learning processes. Their mutual fluctuation and relative ratio are potentially a key variable to provide insight into fear processes and PTSD among females. More consistent methodology and reporting will greatly advance current efforts to understand how the P/E ratio influences fear learning processes among humans and animals.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work is supported by NIH (T32MH016259, R21MH112956, P50MH115874, R01MH094757 and R01MH106595), The Helen Hay Whitney Foundation, and the Frazier Foundation Grant for Mood and Anxiety Research.

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