Published in final edited form as:

Curr Psychiatry Rep. ; 20(6): 40. doi:10.1007/s11920-018-0906-y.

Startling Differences: Using the Acoustic Startle Response to Study Sex Differences and Neurosteroids in Affective Disorders

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Abstract

Purpose of Review—Neuroactive steroid hormones, such as estradiol and progesterone, likely play a role in the pathophysiology of female-specific psychiatric disorders such as premenstrual dysphoric disorder (PMDD) and postpartum depression, and may contribute to the marked sex differences observed in the incidence and presentation of affective disorders. However, few tools are available to study the precise contributions of these neuroactive steroids (NSs). In this review, we propose that the acoustic startle response (ASR), an objective measure of an organism's response to an emotional context or stressor, is sensitive to neuroactive steroids (NSs). As such, the ASR represents a unique translational tool that may help to elucidate the contribution of NSs to sex differences in psychiatric disorders.

Recent findings—Findings suggest that anxiety potentiated startle (APS) and prepulse inhibition of startle (PPI) are the most robust ASR paradigms for assessing contribution of NSs to affective disorders, while affective startle response modulation (ASRM) appears less diagnostic of sex or menstrual cycle (MC) effects. However, few studies have appropriately used ASR to test *a priori* hypotheses about sex or MC differences.

Summary—We recommend that ASR studies account for sex as a biological variable (SABV) and hormonal status to further knowledge of NS contribution to affective disorders.

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Conflict of Interest

Carla Golden, Sara Kornfield, and Christian Grillon, each declare no potential conflicts of interest. Liisa Hantsoo and C. Neill Epperson are section editors for *Current Psychiatry Reports*.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Keywords

sex; startle; affect; psychophysiology; electromyography; translational

INTRODUCTION

Affective disorders, including anxiety and depressive disorders, are neuropsychiatric conditions that are characterized by altered physiologic arousal and reactivity to emotional stimuli. These disorders show strong sex biases; major depressive disorder (MDD) and a number of anxiety disorders are significantly more prevalent in women than men, and risk increases at points of hormonal fluctuation, such as perinatally and perimenopausally [1–3]. However, little work has been done to assess sex differences in physiologic arousal regulation in the affective disorders, which may provide insights to the neurocircuitry involved in affective disorder pathophysiology. The hypothalamus and amygdala, for instance, are heavily embedded with gonadal hormone receptors, which may contribute to sex differences in vulnerability to affective disorders. Thus, techniques that enable study of these neuroactive steroid (NS)-sensitive regions across hormonal states (i.e. sex, menstrual cycle, the perinatal and perimenopausal phases) are an important tool for dissecting the physiologic basis of sex differences. The acoustic startle response (ASR) is one such technique, and sex differences in ASR may reflect differential activity of sexually variable brain regions involved in stress response or affective disorder pathophysiology. Relevant to the clinician, clarifying the role of NSs in affective disorders may lead to more precise treatments for anxiety or depressive disorders, or female-specific disorders such as perinatal depression or premenstrual dysphoric disorder (PMDD). ASR may also be useful as a biological predictor of response to existing treatments such as SSRIs. In this review, we detail how ASR may be used to study sex, menstrual cycle (MC), and perinatal differences in arousal regulation, and how this may provide insight on the contribution of NSs to affective and female-specific disorders.

Physiologic arousal is the somatic reflection of an organism's affective state. Arousal includes the autonomic response to a stressor or emotional stimulus, which can be objectively measured in humans using non-invasive laboratory techniques. While psychophysiological measures such as heart rate (HR) and skin conductance (SC) are used to assess arousal [4,5], ASR has unique characteristics, particularly sensitivity to neuroactive steroids (NSs) that make it well-suited to assessing influence of gonadal hormones in mood, anxiety, and stress-related disorders.

Acoustic Startle Response: Basics & the Psychoneuroendocrine Context

The startle reflex refers to the automatic defensive muscle contraction in response to a sudden stimulus such as a loud noise, and occurs in vertebrates ranging from zebrafish to humans. In fact, the startle response is highly conserved across species, with similar circuitry and pharmacology, and is therefore highly amenable to translational research. The most commonly used startle stimulus in clinical research settings is a burst of white noise administered via earphones, usually 90 – 120 decibels, lasting up to 50 milliseconds. The eyeblink response occurs about 20–50 milliseconds following the noise burst, and ASR is

measured via electromyography (EMG) of the orbicularis oculi muscle. A strong or pronounced blink is reflected in a higher amplitude peak in the EMG readout [6]. ASR can be measured at baseline, or in response to external stimuli, such as emotionally valenced images or threat of shock. The most frequently used ASR techniques are affective startle response modulation (ASRM), prepulse inhibition (PPI), and anxiety- and fear-potentiated startle (APS, FPS)).

Research suggests ASR amplitude is influenced by hormonal status, e.g. sex or menstrual cycle. The NSs, such as estradiol, progesterone, testosterone and their metabolites, are secreted by the gonads and are principally known for their role in reproductive physiology (e.g. regulating the female menstrual cycle). Notably, NSs are also synthesized and exert effects in the central nervous system (CNS); the primary circuit underlying ASR is regulated by input from NS-sensitive brain regions including the amygdala and the bed nucleus of the stria terminalis (BNST) [7,8]. The BNST modulates anxious anticipation, and is sensitive to the progesterone metabolite allopregnanolone (ALLO), a powerful γ -aminobutyric acid (GABA)-A receptor (R) modulator [9].

ACOUSTIC STARTLE PARADIGMS

1. Affective Startle Response Modulation (ASRM)

Human ASR magnitude is acutely influenced by affective state [10]. Generally, presentation of a pleasant stimulus reduces ASR magnitude, while an unpleasant stimulus increases ASR magnitude compared with neutral stimuli. ASRM experiments typically utilize the International Affective Picture System (IAPS) [11], images standardized for valence and arousal.

Sex and menstrual cycle may influence ASRM in healthy adults—Women tend to exhibit larger ASR to unpleasant stimuli than neutral or pleasant images, while men's ASR does not vary by affective stimulus condition [12–14]. Some studies failed to show differences in ASRM between women and men [15–17], or found sex differences only at certain points in the menstrual cycle. Women around ovulation and in the late luteal phase had larger ASR than men across affective conditions, but not in other phases of the menstrual cycle [18]. Ovulation and the late luteal phase are both characterized by fluctuations in estradiol, but without hormone measurements, the expected luteal phase increase in estradiol and progesterone cannot be confirmed. An earlier study found that menstrual cycle phase, confirmed via progesterone level, had no influence on ASRM [19].

While a true ASRM protocol in rodents is difficult given the nature of the affective stimuli, baseline ASR in rodents provides clues about hormonal status in moderating ASR. In rats and mice, baseline ASR is higher in males than in females [20,21], but may be due to strain [22], body weight or anxiety behavior [21,23]. ASR is sensitive to hormonal manipulation, i.e. gonadectomy and hormone replacement, in male rodents but not females [24–26], and ASR does not vary across the estrus cycle in female rodents [20,21]. While there is no effect of estrus cycle or exogenous hormones on ASR in female rodents, the sex difference suggests gonadal hormones in general may still have an effect.

Affective modulation of startle is altered in Major Depressive Disorder—

Lifetime history of depression is roughly twice that in women compared to men [27], and female MDD risk increases at points of hormonal fluctuation such as perinatally and perimenopausally, suggestive of involvement of NSs. However, ASR studies in depressed samples have generally not examined sex or menstrual cycle differences. Moderately depressed individuals exhibit the typical pattern of attenuated ASR to pleasant imagery and enhanced ASR to unpleasant imagery [28], but those with severe MDD have a generally blunted ASRM [29–32]. While sex was not a significant contributor [30–32], these studies may not have been powered to detect an actual sex difference. Of note, these studies did not assess menstrual cycle phase, and it is possible that potential sex differences were obscured by menstrual cycle effects.

Indeed, most ASRM studies have not been powered to address questions of sex or have not assessed hormonal impact [33]. A well-designed study to assess sex differences in ASRM in depression would include a priori hypotheses, a sufficient sample size to examine sex differences, and control for hormonal factors such as menstrual cycle or use of hormonal contraceptives in female participants. While ASR differences across cycle phase were apparent in healthy female controls [18], they were not apparent in women with MDD in the samples studied. On the other hand, preclinical studies suggest dysregulated ASR in females with a depressive phenotype. Female mice housed in isolation to precipitate a depression-like phenotype developed a dampened ASR [34], although influence of estrous cycle was not assessed.

Affective modulation of startle is altered in anxiety disorders—Nearly all the anxiety disorders have a higher lifetime prevalence in women than in men [35] and many women with these diagnoses experience premenstrual exacerbation of symptoms [36], again suggesting a role for NSs [2]. While ASRM is enhanced in response to threat-related or worry-inducing cues in individuals with social anxiety disorder, panic disorder, specific phobia, and GAD, compared with controls [37–40], none of these studies assessed sex differences or menstrual cycle effects.

Affective modulation of startle may be dysregulated in PMDD—PMDD is a disorder characterized by mood symptoms in the luteal phase of the menstrual cycle, likely due to aberrant CNS sensitivity to hormonal fluctuations. Women with PMDD show evidence of dysregulated ASR, potentially a trait phenomenon reflecting physiologic hypersensitivity that is unmasked by hormonal fluctuations [41]. Baseline ASR increased from the follicular to the luteal phase in participants with PMDD, but remained consistent across the menstrual cycle in controls [19]. During the follicular phase, when women with PMDD were asymptomatic, their baseline ASR was similar to that of the controls. Women with PMDD also had enhanced ASR in anticipation of positive and negative images, compared with controls, during the luteal phase [42]. This group previously found that women with PMDD had higher baseline ASR across the entire menstrual cycle, compared with healthy controls [41]. While Epperson and colleagues' study [19] assigned cycle phase via hormone level, Kask and colleagues [41] based phase on symptom severity, which may not have coincided with hormone levels. Hormone levels may be more critical to ASR than

symptomatology, which may explain the difference between Epperson's and Kask's findings. Indeed, in a rat progesterone withdrawal (PWD) model of premenstrual anxiety, females, but not males, exhibited greater ASR following PWD [43]. While ASR magnitude doubled in female rats that had undergone PWD, in controls that had not undergone PWD the ASR magnitude was lower in females compared to males. The females that had undergone PWD also experienced a 2–3 fold increase in GABA-A-R alpha-4 subunits in the amygdala, while PWD males did not. Again, this points to a role for gonadal hormones in arousal regulation, particularly in females, via structures such as the amygdala.

Pregnancy influences affective modulation of startle—Pregnancy is characterized by dramatic increases in NSs including estradiol, progesterone, and ALLO. While ASRM in pregnant women was similar to that of healthy non-pregnant women, at four to six weeks postpartum, higher progesterone levels correlated with a greater difference between positive and negative anticipation stimuli, underscoring ASR's sensitivity to gonadal hormones [44]. Preliminary findings from our laboratory in a within-subjects study suggest that ASR to pleasant and unpleasant conditions is dampened in pregnancy compared with postpartum [45]. The marked fluctuations in gonadal hormones that occur during and following pregnancy may be a useful model for hormonal influence on psychophysiology and arousal regulation. In rodent models, the hormonal fluctuations occurring with pregnancy, parturition and lactation clearly influence ASR. Baseline ASR was greater in female rats at postpartum day 14 than at postpartum day 2 or diestrus females, when progesterone levels are higher [46]. However, ASR at higher decibel levels was significantly attenuated in lactating female rats compared to normally cycling female rats [47]. In addition to progesterone and estradiol, levels of corticotropin releasing factor (CRF) increase by up to 10,000 times during pregnancy [48]. CRF, a primary hormone in the HPA axis, has a key role in stress response.

In rodents, CRF enhances ASR, and in turn, progesterone reduces CRF-enhanced startle, likely via its metabolite ALLO [8]. ALLO appears to impact ASR to contextual cues via the BNST [8]. Similarly in humans, Grillon and colleagues have established that cortisol administration accentuates anxiety-potentiated startle (APS) but not fear-potentiated startle (FPS), which will be discussed in a separate section [49].

2. Anxiety-Potentiated & Fear-Potentiated Startle

Fear-potentiated startle (FPS) refers to the phasic potentiation of ASR to a predictable threat, while anxiety-potentiated startle (APS) refers to sustained potentiation of ASR to an unpredictable threat. FPS is mediated, in large, by the amygdala [50], while APS is mediated by the BNST [51–53]. FPS and APS have been operationalized in humans via Grillon's Neutral, Predictable, Unpredictable (NPU) threat of shock paradigm [54]. Participants view cues on a computer screen that indicate the likelihood of receiving a shock. The cues indicate "safe" conditions when the participant will not receive a shock, or "threat" conditions during which the participant can receive either a predictable or unpredictable shock. The predictable shock condition corresponds to acute fear, while the unpredictable shock condition corresponds to anxious anticipation.

Women have greater ASR response to sustained anxiety than men, but not phasic fear—Women showed greater eyeblink magnitude during the anxious anticipation of a shock (APS) than men, but there were no sex differences in FPS [55]. ASR was similarly heightened among women during anticipation of an uncomfortable esophageal balloon distention, but not in men, with no effect of affective images [56]. This is a somewhat different design from the usual APS / FPS paradigm, but similarly suggests that women have heightened ASR when anticipating aversive stimuli. In contrast to Grillon's findings, there were no sex differences in ASR during unpredictable threat periods (APS) in healthy controls [57]. This study included users and non-users of hormonal contraceptives, and did not control for menstrual cycle phase, which may explain why results differed from that of Grillon. A recent study indicated no changes in FPS across the menstrual cycle in healthy women with regular menstrual cycles, and that free cycling women trended toward larger FPS than hormonal contraceptive users [58]. Unfortunately, this study did not assess APS, and its FPS paradigm was quite different from Grillon's NPU standard. Preliminary data from our laboratory similarly suggests that APS and FPS do not vary across the menstrual cycle in healthy controls [59], however, additional work is needed in a larger sample. To summarize, women appear to have increased ASR when anticipating aversive stimuli, while men do not. The potential impact of menstrual cycle on APS needs to be studied more precisely. There is mixed evidence for whether FPS differs between men and women, but data suggest that FPS is uniform across the menstrual cycle.

Similar to humans, female rodents have larger ASR in periods of sustained anxiety compared to males [60], but no sex differences in FPS [61]. CRF-enhanced startle (similar to APS) is sensitive to progesterone and its metabolite, ALLO; ALLO attenuates CRF-enhanced startle, but not FPS, in female rats [8]. Other rodent studies similarly suggest that APS is sensitive to NSs, but FPS is not. Intact female, pregnant female and castrated male rats had greater APS than gonadally-intact males, and pregnant female rats had greater APS than cycling and lactating females [61]. Testosterone dampened APS in male rats but not females, but testosterone did not affect FPS in male rats. Further, estradiol plus dihydrotestosterone also decreased APS in castrated males, but not in ovariectomized females, potentially mediated by the HPA axis and/or BNST.

There is little research on sex differences in APS or FPS in depressive disorders—High-risk children of probands with MDD had heightened FPS [62]. Among the probands' grandchildren, high-risk girls had elevated ASR, but boys did not. Similarly, the daughters of parents with an anxiety disorder had elevated baseline ASR, while sons had higher FPS but normal baseline ASR [63]. This may represent vulnerability factors for affective disorders, particularly increased vulnerability to anxiety and mood disorders in women. However, studies in adults with depressive disorders have not often reported on sex differences in APS or FPS. Psychiatric inpatients with MDD had increased APS compared to controls, but the authors did not find significant differences by sex [64]. Inpatients with MDD and a history of suicide attempts had greater FPS magnitude than those without a history of suicide attempt, but APS was not affected by suicide attempt history [65]. The authors did not report on sex differences or menstrual cycle effects. Individuals with MDD alone or with comorbid PTSD did not exhibit increased FPS [66]. However, sex differences

were not reported. Similarly, individuals with panic disorder, with or without comorbid MDD, had increased ASR to predictable and unpredictable threats; those with MDD alone did not exhibit this heightened ASR [67]. Sex was controlled in analyses, but no sex differences were reported specifically. Taken together, these results suggest that APS is increased in MDD, but may differ in those with a history of suicide attempts. Also, studies of APS and FPS among women with PMDD are needed. Given the evidence of suboptimal sensitivity to ALLO among women with PMDD, we hypothesize that women with PMDD would have altered APS compared with controls, given APS's roots in the highly GABAergic and ALLO-sensitive BNST.

APS and FPS are differentially altered in specific anxiety disorders—A number of studies have assessed APS or FPS in individuals with panic disorder, specific phobias, or GAD. As described above, individuals with panic disorder had increased ASR to predictable and unpredictable threats, essentially indicating that those with panic disorder have heightened APS and FPS over healthy controls [67]. Indeed, studies have found increased FPS in individuals with panic disorder compared with controls [68,69], although sex and MC differences were not examined. The latter sample included a wide age range (18 – 51 years), and the enhanced startle was particularly evident in the younger participants (aged 40 years and below), potentially reflecting changes in ASR with age or menopausal status. However, in a follow-up study, APS was greater in those with panic disorder than controls, and FPS did not differ between groups [70]. Individuals with panic disorder had greater startle in a verbal threat of shock condition than controls while those with comorbid depression did not; sex differences were not examined [71]. The mixed findings in panic disorder may due to sex or age differences, or comorbidities.

Individuals with GAD appear to have similar APS and FPS as controls, with no sex differences [72,73]. Individuals with social anxiety disorder or a specific phobia had increased FPS than patients with panic disorder or PTSD, or controls [74]. In another study, their startle potentiation to unpredictable threat, but not predictable threat, was greater than that of controls or individuals with MDD or GAD [73]. In these analyses, sex was included as a covariate, with no significant influence. Participants with social anxiety disorder undergoing a virtual reality social-evaluative threat condition had potentiated startle compared with controls [75], but the authors did not report whether there were significant sex differences.

Although PTSD is no longer classified as an anxiety disorder, the fact that an exaggerated startle response is among its hallmark symptoms makes it relevant to discussion in this review. Many of the PTSD studies utilizing ASR have focused on fear conditioning and extinction [76], which is a different paradigm than the APS/FPS studies discussed in this review. Often, studies on ASR in PTSD use male only samples of war veterans. War veterans with PTSD had greater FPS than those without PTSD in a male sample [77], similar to a mixed sex sample of non-veterans [78]. Other work suggests individuals with PTSD have similar FPS as controls, but heightened contextual anxiety, with no sex differences [72].

To summarize, there is evidence for increased FPS relative to controls in social anxiety disorder, specific phobia, PTSD, and potentially panic disorder. There is evidence of increased APS in those with panic disorder. GAD does not appear to influence FPS or APS. Sex differences and menstrual cycle differences in FPS and APS have not been carefully studied in these populations. Of clinical relevance, APS may be useful as a biological marker of treatment response to SSRIs. In work by Grillon and colleagues, acute treatment of healthy controls with the SSRI citalopram, which is believed to reduce brain levels of serotonin, increased APS, while a lengthier two-week treatment with citalopram, that increases brain levels of serotonin, reduced APS [79]. Given this, one could hypothesize that anxious patients with high APS might be more responsive to SSRI treatment

3. Pre-Pulse Inhibition

PPI varies by sex and by menstrual cycle phase—Prepulse inhibition (PPI) of the startle response is a measure of sensorimotor gating, an organism's ability to inhibit sensory input. Thus, when a relatively weak and brief "pre-pulse" tone is administered 30–500 milliseconds prior to the startle tone, blink response to the startle probe decreases [80]. PPI is an important measure because it shows clear sex and menstrual cycle differences, and variance in particular psychiatric disorders. Women typically have lower PPI than men, i.e. women have less capacity to inhibit startle [81–83]. While one study found no sex differences in PPI among healthy controls, this study only assessed women in the follicular phase of the menstrual cycle, and included postmenopausal women, which may have masked differences [84]. Indeed, lower PPI in women is detectable particularly in the luteal phase of the menstrual cycle when both estradiol and progesterone are elevated [85,86], and this sex difference disappears in the hypogonadal state of menopause [83,87,88]. As healthy women move through the menstrual cycle from the follicular to the luteal phase, their PPI levels decrease [86], indicating less inhibition of startle as they approach menses. This suggests estradiol has a dampening impact on PPI. However, in another study that examined PPI from follicular through the luteal phase when both estradiol and progesterone would be increasing, only increase in progesterone level was correlated with the decrease in PPI. Further, acute administration of oral 17β -estradiol to premenopausal women did not affect PPI [89]. While these human menstrual cycle and the animal studies described below indicate the importance of estradiol levels in modulating PPI, that higher levels of follicular to luteal phase increases in progesterone is associated with greater dampening of the PPI emphasizes the importance of progesterone and/or its metabolites in ASR regulation. Perhaps the impact of estradiol on sensorimotor gating is mediated through the hormones' modulation of dopaminergic and serotonergic function [90] while ALLO modulation of GABAA receptors in regions including the amygdala and BNST mediates the effects of progesterone on PPI [91,92]. Because of the potential sex difference, it is recommended that PPI studies match participants by sex or stratify by sex in analyses [93], and researchers should also consider menstrual cycle effects.

Similar to humans, female rats have lower PPI than male rats [20], although this may vary by strain [21,22]. The observed sex difference in PPI among rodents is likely due, in large, to the modulatory effects of gonadal steroids. Similar to humans, PPI was significantly reduced in female rats in proestrus (high estrogen and progesterone) compared to estrus (low

estrogen and progesterone), and PPI during estrus was lower than PPI in males [94]. Again, the reduced PPI in proestrus is likely due to estrogen's modulation of the dopaminergic and serotonergic systems, which play a role in regulating PPI. Hormone manipulation also provides insight into hormonal modulation of PPI. In rats, ovariectomy did not impact PPI [95], but treatment of ovariectomized animals with 17 β -estradiol increased PPI relative to ovariectomized rats, as did treatment with testosterone to a lesser degree [24,95]. Given the dampening effect of estradiol on PPI in previous studies, the authors posit other NSs in addition to estradiol modulate PPI. Dihydrotestosterone, which cannot be converted by aromatase to estrogen, did not impact PPI in these ovariectomized females. Thus, the authors conclude that testosterone's ability to enhance PPI is via its conversion to estrogen, thereby implicating brain regions rich in aromatase.

PPI may exhibit sex differences in affective disorders—The few studies of PPI in individuals with MDD suggest that PPI is not impaired in this condition [96–98], although this may differ in depressed men versus depressed women. Tryptophan (TRP) depletion, which is used experimentally to reduce central serotonin levels and is associated with relapse of depression symptoms among vulnerable groups [99], reduced baseline ASR in a sample of healthy female controls but did not affect PPI [100]. The authors confirmed reductions in TRP via plasma levels, but did not assess menstrual cycle. Conversely, in a small male-only sample, TRP depletion dampened PPI [101]. In a large study of individuals with MDD and age and sex-matched controls, men with MDD had PPI deficits, while women with MDD did not [93], consistent with the TRP depletion findings. Further, in males, MDD severity was correlated with PPI, such that greater depressive severity was associated with lower PPI. As suggested above, there is evidence that gonadal hormones may regulate stress neurocircuitry particularly in women with MDD, which may explain this sex difference [102]. A similar relationship has been observed in other mood disorders - men with bipolar disorder had lower PPI than control males, while women with bipolar disorder had significantly greater PPI than female controls [103]. In this study, women were measured at a point in the menstrual cycle corresponding to low circulating estradiol and progesterone. Together, these results suggest that among individuals with MDD, men may exhibit lower PPI, while women do not.

There is little research on sex differences in PPI in anxiety and stress disorders—There is less research on PPI in anxiety disorders. Individuals with panic disorder had lower PPI than controls, but sex differences were not assessed [104]. Further, most of the participants in this study were taking SSRIs, which could influence results. Results are mixed regarding altered PPI in PTSD. A number of studies found no differences in PPI between controls and individuals with PTSD [105], but sex differences were not examined, and some samples included only males [106] or only females [107]. One study found that individuals with PTSD had reduced PPI compared to healthy controls [108] and/or trauma-exposed controls [109], however, sex differences were not reported. While women with PTSD showed lower PPI than trauma-exposed female controls, there was no influence of menstrual cycle phase [110].

Women with PMDD exhibit PPI deficits—Women with PMDD exhibit a reduction in PPI in the late luteal phase compared to controls, but not in the follicular phase [41]. Interestingly, women without PMDD but who were experiencing adverse mood reactions to oral contraceptives (OCs) also exhibited deficits in PPI compared to women taking OCs without adverse mood reactions [111]. That non-PMDD women who were sensitive to the exogenous steroids included in their OC formulations also had low PPI suggests that this may be due in part to abnormal response to NSs, and is consistent with the conceptualization of PMDD as a disorder of NS sensitivity.

PPI is lowered in pregnancy, and in postpartum depression—Among pregnant women in the third trimester of pregnancy, when both estradiol and progesterone levels are greatly elevated, PPI was attenuated compared with women who were more than 48 hours postpartum [112]. However, baseline ASR remained intact, with no differences between the pregnant or postpartum groups. By 48 hours postpartum, progesterone levels have dropped precipitously, suggesting that the attenuated PPI during pregnancy may have been due to high levels of gonadal and placental hormones. However, it is possible that this effect persists in women with postpartum depression. Women with postpartum depression had lower PPI than control women at six to twelve weeks postpartum [113]. Postpartum rats had significantly reduced PPI compared to diestrus controls [46], again suggesting NS influence on ASR.

Post-menopause, PPI negatively correlates with neurosteroid levels—Prior to menopause, women generally have lower PPI than men [88], particularly in the luteal phase [85,86]. Post-menopause, women were similar in PPI to men, but had greater pre-pulse facilitation (PPF) than men [88]. Within a sample of women, postmenopausal women had greater PPI than normally cycling premenopausal women [114]. Among the postmenopausal women, there were no differences in PPI based on hormone replacement therapy (HRT) use. However, postmenopausal women with serum estradiol levels in the same range as cycling women had a lower PPI than postmenopausal women with low estradiol, suggesting that PPI remains sensitive to estradiol levels during reproductive senescence. Differences based on progesterone or ALLO levels was not reported, so the results may have been due not only to estradiol but potentially progesterone and its metabolites.

CONCLUSIONS

In this review, we have summarized the evidence for sex and menstrual cycle impacts on ASR and ASR modulation. ASR is a useful tool for a number of reasons. Perhaps most importantly, ASR provides an objective measure of an organism's response to a particular emotional context, perceptual context, or stressor. This characteristic is valuable when studying affective disorders, which are characterized by maladaptive responses to emotional stimuli or stressors. Clinically, this may be reflected in increased irritability or greater sensitivity to threat-related stimuli. Critical to this review, ASR provides a unique means for studying sex differences in pathophysiology, as particular ASR paradigms are sensitive to NSs [8,18,19,21,42,47,61,85,95,112]. Because ASR reflects differential activity of sexually divergent brain regions involved in the pathophysiology of affective disorders, ASR studies have important implications for identifying pathophysiological mechanisms and for

developing more tailored treatments, particularly for disorders such as PMDD or perinatal depression.

Regarding the literature in which investigators actively modulated the individual's appetitive state through exposure to emotionally or contextually-valenced stimuli, there is mixed evidence for sex differences or menstrual cycle effects on baseline ASR or ASRM. Some studies have suggested greater ASRM among women to specific emotional stimuli, particularly in the periovulatory or late luteal phases of the menstrual cycle, but other studies report no difference in sex or menstrual cycle status. Studies that are powered to assess sex differences are needed, and should also account for potential impact of menstrual cycle and menopausal status. Overall, studies that are powered to assess sex differences in general and with respect to modulation by the specific valence of emotional stimuli in humans are lacking. Findings from the rodent literature support greater baseline ASR in males compared to females, but few studies indicate that ASR is sensitive to fluctuations in gonadal steroid across the estrus cycle. Hence, we suggest that at the very least sex is considered as a biological variable (SABV) in both human and rodent studies in which baseline ASR and affective modulation of the ASR are being investigated. Menstrual cycle and estrus phase measurements in healthy rodent and human populations may be less critical with these ASR paradigms than others reviewed herein.

When studying adults with MDD, there is no evidence to date that affective modulation of the startle response magnitude varies within this population by sex or menstrual cycle phase. It is possible that reproductive subtypes of depression, such as PMDD or postpartum depression, differ from classical major depression in involvement of NSs in pathophysiology [115], which is important to consider in clinical case conceptualization and treatment planning. However, few ASR studies have been powered to control for SABV or to consider hormonal status in clinical samples. There are sex differences in other biomarkers, such as inflammatory molecules and urine metabolites, among individuals with MDD [116]. Preclinical studies would suggest a modulating effect of sex, although even here, estrus cycle effects have not been fully explored. Rodent models suggest that baseline ASR is dampened in females who underwent a laboratory stressor to induce a depressive phenotype [34]. Similarly, among women with PMDD, there sufficient data to indicate that menstrual cycle phase should be confirmed with serum or plasma hormone levels in all studies examining baseline and affective modulation of ASR. As pregnant and lactating rats had greater ASR than virgin female rats, reproductive status of subjects should be considered in both clinical and preclinical models. In anxiety disorders, there is evidence for increased ASR to threat-related stimuli in social anxiety disorder, panic disorder, and specific phobias, but sex differences and menstrual cycle differences have not been examined. Based upon the literature to date, we suggest that SABV should be considered in both preclinical and clinical studies in which startle paradigms are being used to study major affective disorders.

In contrast, there is already substantial preclinical and sufficient clinical evidence that NSs (progesterone, estradiol, ALLO, testosterone) modulate APS. Therefore, consideration of SABV and measurement of gonadal steroid levels should be considered as the gold standard for all studies utilizing psychophysiology paradigms including contextual threat. In general women appear to have greater APS in anticipation of aversive stimuli compared to men,

though the role of gonadal steroids in humans is less clear and often not examined using peripheral hormonal measures. There is little evidence to date that FPS differs between men and women, but data suggest that FPS is uniform across the menstrual and estrus cycle and testosterone administration does not appear to modulate FPS in rodent males. Regarding impact of psychiatric status, individuals with MDD had increased APS, but not FPS. Additional studies are needed to assess sex differences in APS and FPS in depressed individuals, and menstrual cycle effect on APS and FPS in PMDD. In contrast to MDD, there is consistent evidence for increased FPS in individuals with PTSD and anxiety disorders including social anxiety disorder, specific phobia, and panic disorder, and for unaltered APS and FPS in individuals with GAD. Again, sex differences and menstrual cycle differences in FPS and APS have not been carefully studied in these disorders.

Regarding the various startle paradigms, the most robust evidence for sex and menstrual cycle phase effects is with the PPI paradigm. Healthy women consistently have lower PPI than men, which is particularly detectable in the luteal phase of the menstrual cycle. This is consistent with rodent literature, which demonstrates sex and estrus cycle impact on PPI. Evidence suggests that PPI is not impaired in MDD, although this may vary by sex, as men with MDD had PPI deficits, while women with MDD did not. In contrast, women with PMDD exhibit lower PPI compared to healthy normally cycling women, as do healthy women in the third trimester of pregnancy compared to postpartum women, also suggesting hormonal modulation. There is no clear evidence on PPI alterations in anxiety disorders, particularly regarding sex or menstrual cycle differences.

In sum, evidence for sex or menstrual cycle differences in ASR is strongest for APS and PPI. APS may be particularly useful for studying potential sex differences in PTSD and anxiety disorders as well as hormonal contributions to the underlying pathophysiology of PMDD.. APS also has promise as a potential biomarker for SSRI treatment response, particularly in anxiety disorders [79]. PPI has been utilized extensively to study sex differences in schizophrenia, particularly via translational research, providing better understanding of its pathophysiology and treatment [82]. We believe ASR may be similarly useful for providing translational, mechanistic insights on sex differences in affective disorders, which may lead to more precise and effective treatments. Finally, the current state of the preclinical and clinical literature supports the recommendation that SABV be considered and peripheral gonadal steroids measured in all studies using the ASR, APS and PPI as assessments of the underlying psychophysiology of healthy and psychiatrically impacted populations. That each of these can be assessed to some degree in both rodents as well as humans highlights the translational possibilities of these measures in our efforts to understand the mechanisms underlying sex differences in neuropsychiatric conditions.

Acknowledgments

The editors would like to thank Dr. Bradley Gaynes for taking the time to review this article.

The authors are supported by the Intramural Research Program of the National Institute of Mental Health (K23MH107831 Hantsoo; F31MH115656 Golden; MH002798 Grillon; K23MH102360 Kornfield; P50 MH099910 Epperson & Hantsoo), Brain & Behavior Research Foundation NARSAD Young Investigator Award (Hantsoo), Office of Research on Women's Health (P50 MH099919 Epperson & Hantsoo; K12HD085848

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