



NEUROPSYCHOPHARMACOLOGY REVIEWS

Sex differences in the hypothalamic–pituitary–adrenal axis' response to stress: an important role for gonadal hormones

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The hypothalamic–pituitary–adrenal (HPA) axis, a neuroendocrine network that controls hormonal responses to internal and external challenges in an organism's environment, exhibits strikingly sex-biased activity. In adult female rodents, acute HPA function following a stressor is markedly greater than it is in males, and this difference has largely been attributed to modulation by the gonadal hormones testosterone and estradiol. These gonadal hormones are produced by the hypothalamic–pituitary–gonadal (HPG) axis and have been shown to determine sex differences in adult HPA function after acute stress via their activational and organizational effects. Although these actions of gonadal hormones are well supported, the possibility that sex chromosomes similarly influence HPA activity is unexplored. Moreover, questions remain regarding sex differences in the activity of the HPA axis following chronic stress and the underlying contributions of gonadal hormones and sex chromosomes. The present review examines what is currently known about sex differences in the neuroendocrine response to stress, as well as outstanding questions regarding this sex bias. Although it primarily focuses on the rodent literature, a brief discussion of sex differences in the human HPA axis is also included.

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INTRODUCTION

The ability of all mammals to cope with any environmental or homeostatic challenge (i.e., stressor), or with perceptual threats to homeostasis, relies upon activation of a neuroendocrine signaling cascade called the hypothalamic–pituitary–adrenal (HPA) axis. The HPA axis is activated in response to real or perceived stressors and culminates in the production and secretion of glucocorticoids by the adrenal glands. These act upon virtually all tissues to facilitate a body-wide stress response. When acutely elevated by stressors, glucocorticoids induce physiological and behavioral changes that are beneficial and indispensable for survival [1, 2]. However, persistent rises in glucocorticoids due to chronic stress or disease states are detrimental and increase risk for stress-related pathology [2–4]. Strikingly, women are at twice the risk of men for developing many of these diseases, likely due to sex differences in the function and regulation of the HPA axis [5]. Thus, understanding the nature and causes of such sex differences in the HPA axis following stress has important implications for understanding sex-biased risk for disease.

Much of what is known about sex differences in the stress-induced activity of the HPA axis and their underlying mechanisms comes from studies done in rodents. Such studies have demonstrated that sex differences in the HPA axis can arise from the influence of gonadal hormones during adulthood or during key developmental periods [6–9]. Additionally, they support the possibility that sex biases result from sex chromosomal effects, although supporting evidence is currently limited [10]. The present review accordingly focuses on advances revealed from rodent studies in our understanding of sex differences in the stress-induced activity of the HPA axis in adulthood. We primarily outline what is known about how gonadal hormones and sex

chromosomes modulate HPA axis activity following acute stress, and then focus on sex-biased HPA axis activity post-chronic stress, which is far less well understood. We conclude with a brief discussion of sex differences in the human HPA axis with the caveat that they are less pronounced than in rodents and are largely dependent on the stress modality.

OVERVIEW OF THE HPA AXIS

Activation of the HPA axis

Activation of the neuroendocrine response to stressors occurs at the level of the hypothalamic paraventricular nucleus (PVN), which receives varying inputs depending on the nature of the stressor. Some stressors involve an immediate threat to physiological homeostasis and require the rapid relay of peripheral signals to PVN neurons via direct serotonergic or catecholaminergic projections from brainstem nuclei [9, 11]. Other stressors, alternatively, have psychological components that can activate neuroendocrine responses in the absence of a direct threat to survival [12]. These responses involve anticipation of a homeostatic challenge that requires interpretation by higher brain structures in order to assign significance to an external event based on instinctual fears and/or prior experiences with homeostatic challenges [12]. Because these stressors likely involve preparing for a threat to survival rather than immediately coping with the threat itself, there is time for information to first be processed in limbic forebrain regions before it reaches the PVN [12].

The amygdala is a limbic structure that plays a notable role in activating the neuroendocrine response to various stressors [13]. It is divided into subnuclei, including the central (CeA), medial (MeA), and basolateral amygdaloid (BLA) nuclei, that all have this

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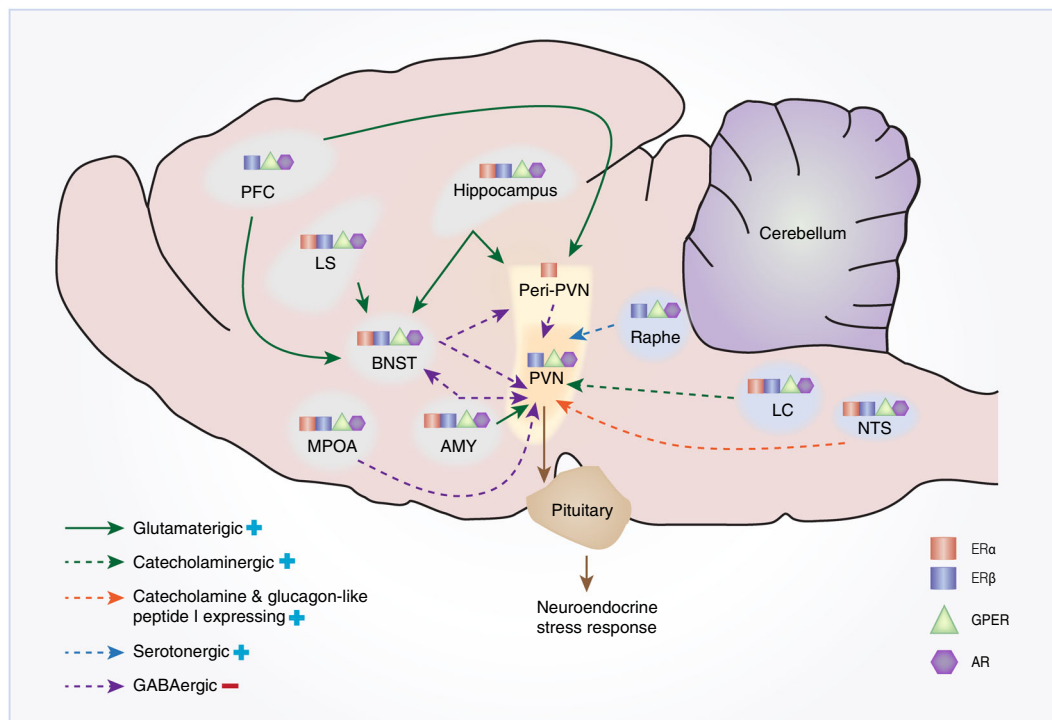


Fig. 1 Stress-related neuronal inputs to the PVN are candidates for gonadal hormone influence on the HPA activity. A diagrammatic representation of the major limbic and brainstem structures that send projections to the PVN to either enhance or inhibit the activity of the HPA axis in response to stressors is shown. Projections either directly contact PVN neurosecretory neurons or are indirect in nature and first synapse in the peri-PVN or limbic relay nuclei, such as the BNST. Excitatory and inhibitory projections are indicated by blue plus and red minus signs, respectively. Because their limbic and brainstem origins express androgen and/or estrogen receptors, these projections are potential targets for gonadal hormone modulation of HPA activity. PVN paraventricular nucleus, PFC prefrontal cortex, LS lateral septum, BNST bed nucleus of the stria terminalis, MPOA medial preoptic area, AMY amygdala, LC locus coeruleus, NTS nucleus of the solitary tract, Raphe represents both dorsal and medial raphe nuclei

excitatory effect [13]. However, each subnucleus is preferentially responsive to specific types of stressors (see ref. [13] for review) and may have sex-dependent function [14–17]. Notably, the amygdala sends few direct projections to the PVN [12, 18]. Thus, it largely modulates PVN neuronal activity by first innervating limbic or brainstem relay centers [19]. For instance, both the MeA and CeA send GABAergic projections to discrete regions of the bed nucleus of the stria terminalis (BNST) that ultimately function to decrease its GABAergic input to the PVN [12].

Ultimately, regardless of the nature of the stressor and corresponding neural circuitry, all pathways converge on PVN neurons that initiate the HPA axis and eventual hormonal response to stress [11] (Fig. 1).

The HPA axis is activated by a distinct population of hypophysiotropic neurons in the medial parvocellular division of the PVN [12, 20]. These neurons synthesize a cocktail of neuropeptides, including corticotropin-releasing hormone (CRH), vasopressin (AVP), and oxytocin (OT), which are carried through the hypothalamo-hypophyseal portal vasculature where they can stimulate production of adrenocorticotropin (ACTH) by corticotrophs in the anterior pituitary gland [21–23]. The subsequent increase in the release of ACTH into the systemic circulation can then act in the adrenal cortex to induce the synthesis and release of glucocorticoids (i.e., cortisol in humans, and corticosterone (CORT) in rats and mice). Glucocorticoids act on virtually all tissues to mobilize energy stores, enhance cognition, and increase cardiovascular activity, while suppressing immune, reproductive, and digestive functions [2]. In the short term, stress-related elevations in glucocorticoids permit the beneficial physiological and behavioral changes necessary for acute stress responses. Unfortunately, prolonged elevations in glucocorticoids induced by

chronic stress can have detrimental health consequences. Thus, numerous mechanisms exist to enable the tight regulatory control of the HPA axis [2–4].

Inhibition of the HPA axis

Glucocorticoid production by the HPA axis is partially maintained within homeostatic limits via neural inhibitory pathways (Fig. 1). Local brain areas, including the BNST, numerous hypothalamic nuclei, and the region immediately surrounding the PVN (i.e., peri-PVN), project to and directly inhibit PVN neuronal activity [9, 11]. These relay centers contain dense populations of GABAergic neurons. Thus, glutamatergic projections from limbic structures, such as the prefrontal cortex and hippocampus, can inhibit PVN neuronal activation by increasing tone of the inhibitory relay center [12, 24]. Notably, the involvement of limbic structures in constraining the activity of the HPA axis is largely restricted to stressors with a psychological component [11]. The neural inhibition of HPA activity induced by physical stress is far less well understood, but may involve inhibition of excitatory glucagon-like peptide 1-expressing neurons [25, 26]. Nevertheless, inhibitory neural pathways cannot act alone to inhibit the HPA axis and synthesis of glucocorticoids.

The actions of such inhibitory neural pathways are complemented by a glucocorticoid-mediated negative feedback system [11]. In this system, glucocorticoids alter the gain of key HPA regulatory regions, including limbic structures (i.e., hippocampus and prefrontal cortex), the PVN, and the anterior pituitary, to ultimately reduce adrenal glucocorticoid production [26]. Glucocorticoids act through either the mineralocorticoid receptor (MR) or the glucocorticoid receptor (GR) to exert these effects [27]. However, the differential expression and affinity for CORT of the

MR and GR give them functionally distinct feedback roles. The MR is mostly found in limbic structures and has a high affinity for CORT [28, 29]. Consequently, MRs are thought to be occupied when CORT is at basal levels and are important for maintaining low basal glucocorticoid secretion [27, 28, 30]. GR, alternatively, is expressed in limbic areas, the PVN, and the anterior pituitary, and it has relatively lower affinity for CORT [27, 28]. It is selectively occupied by stress-induced elevations in CORT levels and, therefore, plays a significant role in reducing HPA axis activity following a stressor [28, 30]. Accordingly, GR mediates the majority of glucocorticoid negative feedback processes [31]. In support of this, GRs regulate two well-established phases of glucocorticoid feedback on the HPA axis: fast and delayed. Fast feedback involves membrane-bound GRs that induce endocannabinoid suppression of the HPA axis [32, 33], whereas delayed feedback involves a classical nuclear receptor-mediated mechanism [34, 35]. In this classical mechanism, unoccupied GR is found in the cell cytoplasm and translocates to the cell nucleus upon ligand binding, where it can directly influence gene transcription by binding glucocorticoid response elements and/or by interacting with other transcriptional regulators [36]. It ultimately adjusts HPA axis activity by altering expression of HPA-related genes, such as *Crh* and *Avp* [37, 38]. A thorough discussion of the mechanisms of GR action can be found in ref. [39].

SEX DIFFERENCES IN HPA AXIS ACTIVITY

In rodents, prominent sex differences exist in the HPA axis response to stress. Females typically have a more robust neuroendocrine response to acute stress, as evidenced by their increased CORT and ACTH levels compared to those of males following exposure to a number of stressors with different modalities [40–44]. Sex differences at each level of the HPA axis, as well as in regulatory limbic structures, may underlie this sex-biased HPA output. In the PVN, neuronal activation following numerous stressors, as measured by the expression of immediate early genes such as *c-Fos*, is greater in females [6, 40, 45]. Additionally, expression of HPA-related genes is sexually dimorphic. Female rats have been shown to have greater expression of AVP and CRH mRNA in the PVN and greater expression of the ACTH precursor, proopiomelanocortin (POMC) mRNA, in the anterior pituitary following acute stressors than males [40, 42, 43, 46].

In addition to their greater hormonal response to stress, female rats have been shown to have a delayed return to baseline ACTH and CORT levels after acute stress, indicating sex differences in the negative feedback regulation of the HPA axis [40–43]. Sex differences are present in neural pathways that may explain this sex-biased inhibition. In limbic structures known to activate inhibitory inputs to the HPA axis, including the frontal cortex, cingulate cortex, piriform cortex, and hippocampus, neuronal activation following acute restraint stress is reduced in females compared to males [47].

Sex differences also exist in glucocorticoid feedback mechanisms that may similarly contribute to the less robust negative feedback on the HPA axis in females. Glucocorticoid binding is lower in the hypothalamus of female versus male rats, suggesting that females have fewer hypothalamic corticosteroid receptors [48]. In the pituitary, female rats also have a reduced density of MRs and GRs compared to males [49]. Furthermore, regulation of corticosteroid receptors in response to acute stressors varies between males and females in structures regulating HPA activity, including the hippocampus, hypothalamus, and pituitary [50, 51]. For example, acute stressors upregulate GR and MR mRNA in the hypothalamus of male, but not female rats [51].

GR knockout studies have further highlighted sex differences in the feedback regulation of the HPA axis by glucocorticoids. In mice with GR selectively depleted in forebrain regions, including

the hippocampus, medial prefrontal cortex, and basolateral amygdala, sex differences have been identified in the necessity of GR in these regions for HPA axis regulation [52]. The loss of forebrain GR results in HPA axis dysregulation in males but not females, suggesting that forebrain GR is largely inconsequential for normal HPA regulation in females [52]. The same group also examined the effects of selectively deleting GR in the PVN in male versus female mice [53]. Interestingly, males show increases in ACTH and CORT responses to acute stress above those of wild-type animals, but this response is absent in females [53]. Accordingly, PVN GR may be necessary for feedback inhibition of stress-induced ACTH and CORT in males, whereas GR in other, unexamined areas may be more important in females.

The availability of corticosteroids in brain regions where they can modulate HPA axis function may also influence their sex-dependent actions. Following their release from the adrenal gland, most corticosteroids are bound by corticosteroid-binding globulin (CBG), a glycoprotein produced by the liver [4]. The role of CBG is to protect corticosteroids from degradation as they are transported in the plasma to their target tissues [4]. Upon reaching the target, CBG releases corticosteroids by a number of mechanisms (see ref. [54] for review), as corticosteroids can only bind their intracellular receptors if they are not bound by CBG [55]. Thus, CBGs modulate the amount of plasma corticosteroids available to act on their targets, and it is important to make the distinction between total plasma CORT levels and bioavailable “free” CORT when considering sex differences in the activity of the HPA axis [54]. One study examining sex differences in basal CBG and free CORT levels found that females have approximately twice the binding activity of males, but only slightly higher (not statistically significant) levels of free CORT [56]. Consequently, the higher basal levels of total CORT in females may be partly buffered by their higher levels of CBG [56, 57]. It is also possible that the increased CBG levels contribute to the enhanced basal and stress-induced activity of the HPA axis observed in females, because CBG makes CORT less available for the negative feedback mechanisms that shut down the HPA axis. In males, CBG is negatively regulated by acute stress, which makes CORT more available for negative feedback [58]. Whether or not this is true for females as well remains to be determined. Thus, higher CBG levels may make show increases vulnerable to elevated HPA axis activity following acute stress but further research is necessary.

ACTIVATIONAL EFFECTS OF GONADAL HORMONES

Adult sex differences in the neuroendocrine response to acute stress are due in part to interactions between the HPA axis and a parallel neuroendocrine network that controls reproduction, the hypothalamic–pituitary–gonadal (HPG) axis [59]. The HPG axis culminates in the production of testosterone and synthesis of estrogens from aromatizable androgens in the testis and ovary, respectively. Both testosterone and estradiol can in turn modulate the HPA axis in adulthood (i.e., have activational effects) and contribute to its sex-dependent function, as will be explored in this section. Notably, gonadal steroids also play a role in altering HPA axis function in aging rodents, although this topic fall beyond the scope of this review [60].

Gonadal hormone receptors and their localization

Estrogens and androgens influence the activity of the HPA axis in adulthood by acting through estrogen receptors (ERs) and androgen receptors (ARs), respectively [9]. The androgens, testosterone and its metabolite, dihydrotestosterone (DHT), both bind the androgen receptor, whereas the potent endogenous estrogen, estradiol, can activate either of two major ER subtypes: estrogen receptor alpha (ER α) or beta (ER β). AR, ER α , and ER β all belong to the same family of receptors, nuclear receptor subfamily 3, which also includes GR, MR, and the progesterone receptor (PR)

[61]. All receptors in this subfamily function classically as ligand-activated transcription factors [62]. They reside as multiprotein complexes in the nucleus or cytoplasm until ligand binding triggers their shuttling to chromatin DNA, where they can influence transcription [62]. To alter transcription, these receptors (1) bind directly to hormone response elements on DNA and/or (2) interact with other transcriptional regulators [62–64]. As is the case for other members of nuclear receptor subfamily 3, ERs and ARs can also be found as membrane receptors that have faster (non-classical) influences on neuronal function and/or transcriptional activity by modulating second messenger pathways and ion channels [65, 66]. Moreover, a membrane estrogen receptor, G-protein-coupled estrogen receptor (GPER), has been identified that has high affinity for estradiol and can also mediate its rapid effects [67]. Thus, through classical and non-classical roles, gonadal hormone receptors enable gonadal hormones to influence HPA axis activity in a rapid and delayed manner.

The widespread expression of gonadal steroid hormone receptors in key parts of the neural circuitry controlling the HPA axis enables gonadal steroids to modify the neuroendocrine response to stress in concert with changes in reproductive function (Fig. 1). AR, ER α , and ER β expression accordingly has been found in cortex, hippocampus, MeA, BNST, and numerous hypothalamic areas, including the medial preoptic area (MPOA) [68]. GPER is also expressed in the hippocampus and hypothalamic areas [67]. In neuroendocrine PVN neurons that direct the activity of the HPA axis; however, ER β s and GPERs are robustly expressed, whereas ARs are limited, suggesting that gonadal steroids modulate HPA activity by distinct mechanisms of action [41, 69]. Estradiol can directly alter HPA function by acting in neuroendocrine PVN neurons or indirectly by acting through upstream brain regions that project to the PVN; androgens, alternatively, have been shown to have predominantly indirect effects [41]. The MPOA and BNST, for example, are particularly important mediators of androgens' actions, as they have abundant AR expression and project to the PVN and peri-PVN [9, 70, 71].

The estrous cycle and the HPA axis

Sex differences in the activity of the HPA axis may largely be influenced by HPG-mediated fluctuations in the levels of estradiol that occur across a 4-day estrous cycle in female rodents. Early investigations revealed that, as estradiol concentrations increase throughout the estrous cycle in female rats, so do the basal and stress-induced activity of the HPA axis. Accordingly, female rodents in diestrus (low estradiol) are similar to males, in that they exhibit low resting glucocorticoid secretion and a relatively quick on–off response to stressors [72, 73]. Females in proestrus (high estradiol, high progesterone) and estrus (recent exposure to peak estradiol), alternatively, have elevated basal and stress-induced ACTH and CORT [72–74]. Notably, elevations in HPA output are greatest on proestrous morning, when estradiol levels are peaking, but elevations in progesterone have not yet occurred. Progesterone appears to reduce estradiol's effects on HPA output. Accordingly, in OVX'd female rats replaced with physiological levels of estradiol with or without progesterone to mimic the estrous cycle, estradiol treatment enhances HPA activity more than estradiol and progesterone treatments combined [72]. Thus, the extent of estradiol's ability to increase HPA output on proestrus depends on whether there is a high or low background level of progesterone [72, 75]. This is well aligned with the findings of rodent studies demonstrating that progesterone treatment alone suppresses HPA axis stress reactivity [76, 77].

Female rats in proestrus and estrus also have a delayed return to baseline glucocorticoid secretion following stress [72, 73]. This may be due to less robust mechanisms of glucocorticoid negative feedback on the HPA axis and/or lesser input from limbic structures that are known to inhibit the HPA axis [47, 73]. Indeed, neuronal activation in cortical and hippocampal areas is lower in

proestrous and estrous females than diestrous females [47]. Overall, studies of HPA axis function across the reproductive cycle have highlighted an important role for estradiol in controlling the neuroendocrine response to stress.

Estradiol and the HPA axis

In rodents, estradiol often enhances the activity of the HPA axis. Numerous studies correspondingly report that the removal of most endogenous estrogens by ovariectomy decreases stress-induced ACTH and CORT levels, while estradiol treatment has the opposite effects [6, 72, 78]. However, there are exceptions to this pattern, as some studies demonstrate that estradiol treatment can inhibit the HPA axis [79, 80]. Such exceptions will be discussed shortly. Nonetheless, support for the excitatory effects of estradiol has been found at all levels of the HPA axis. In the PVN, estradiol has been shown to increase stress-induced neuronal activation [45], as well as *Crh* and *Avp* gene expression [6, 81]. Additionally, estradiol increases POMC mRNA [6] and ACTH content [82] in the pituitary; and it increases sensitivity to ACTH in the adrenal gland [78]. Lastly, estradiol disrupts GR-mediated negative feedback on the HPA axis, as it reduces the repression of PVN *crh* by glucocorticoids [83] and interferes with GR expression and binding in the pituitary and hippocampus [84]. Estradiol also upregulates CBG levels [85], but this is not necessarily responsible for estradiol effects on the acute neuroendocrine stress response, since estradiol upregulates the HPA axis even in the absence of CBG changes [86].

Other studies show that estradiol inhibits neuroendocrine responses to stress or has no effect [42, 79, 80]. Accordingly, under certain physiological conditions, estradiol has been shown to decrease PVN neuronal activation [78] and *Crh* gene expression [79, 87]. Reports of such apparently opposing effects of estradiol on HPA activity may be due to varying experimental conditions, such as the dose or duration of estradiol treatment. Interestingly, a recent study measuring the effects of estradiol on stress-related, anxiety-like behaviors showed that the response to estradiol is opposite if females are ovariectomized and fed a standard chow versus a phytoestrogen-free diet [88]. HPA axis activity was not measured in this study, unfortunately, but its findings suggest that food composition can greatly affect the way that animals respond to estradiol.

Additionally, it is likely that estradiol will have contrasting actions based on whether ER α or ER β -mediated signaling is invoked [9]. Whereas selective activation of ER α with propylpyrazoletriol (PPT) increases glucocorticoid secretion following stressors, selective activation of ER β with diarylpropionitrile (DPN) decreases it in ovariectomized female rats [9, 89–91]. ER α and ER β also modulate HPA activity via distinct mechanisms of action. ER β can directly modify PVN action and downstream HPA output, as it is co-expressed by PVN neurons that express CRH, AVP, and OT [90, 92, 93]. Notably, ER β co-expression with these neuropeptides varies based on the species and neuropeptide examined. For example, ER β is found in 2/3 of AVP neurons in the rat PVN, whereas it is expressed in <20% of these neurons in the mouse PVN [92, 94]. A total of 60–80% of OT neurons, alternatively, co-express ER β in both rats and mice [92, 94]. In contrast to the presumably direct actions of ER β , ER α may influence HPA activity more indirectly. Although some ER α expression has recently been reported in mouse PVN neurons, very limited expression has been found in the rat PVN [92, 94]. More substantial expression of ER α has been found in brain regions that project to the PVN, such as the peri-PVN, BNST, and hippocampus [89, 93], supporting its indirect effects on HPA activity. Accordingly, studies indicate that ER α can inhibit glucocorticoid negative feedback on the HPA axis by disinhibiting the inhibitory signal from the peri-PVN to the PVN [89].

Moreover, estradiol may decrease excitatory inputs to the PVN by binding GPERs. The PVN receives serotonergic projections from the median and dorsal raphe nuclei of the brainstem that

generally induce activation of the HPA axis (Fig. 1) [95], and estradiol has been shown to partially decrease this effect by desensitizing serotonin receptor signaling in the PVN [96]. Studies in ovariectomized rats have correspondingly shown that treatment with a serotonin receptor agonist increases plasma ACTH levels and that estradiol administration limits this effect [97]. Interestingly, GPER, but not ER β , has been shown to be necessary for the desensitizing effects of estradiol on serotonin signaling in the PVN [97, 98].

Androgens and the HPA axis

Androgens generally inhibit the activity of the HPA axis. Thus, removal of most endogenous androgens by gonadectomy increases stress-induced ACTH and CORT secretion, whereas testosterone treatment has the opposite effects [6, 46, 99]. Notably, the reduction of testosterone to the more potent androgen, DHT, is necessary for its suppression of glucocorticoid secretion after stress [100]. Accordingly, inhibition of the enzyme that converts testosterone to DHT, 5 α -reductase, with central infusion of finasteride increases stress-enhanced glucocorticoid secretion in male rats [100]. This effect is reversed by treatment with DHT, but not testosterone, indicating that testosterone's inhibition of HPA activity depends on 5 α -reductase activity within the central nervous system in males [100]. Moreover, estradiol does not potentiate the effects of DHT on the HPA axis in adulthood as may occur during development [101] and reproductive behaviors [102]. DHT placed near the PVN in adult, gonadectomized male rats reduces, whereas estradiol and ER α -selective agonists increase the ACTH and CORT responses to acute stress [90].

As illustrated for estrogens, the overall effect of androgens on the neuroendocrine stress response is partially mediated by androgen effects on the hypothalamus. In the PVN, androgens decrease neuronal activation, as well as elevations in CRH and AVP mRNA brought on by varying types of stressors [6, 103, 104]. These effects of androgens on PVN function and neuropeptide expression are likely mediated by ARs in limbic structures upstream of the PVN, because neuroendocrine PVN neurons do not express ARs [69, 105]. The MPOA and BNST, alternatively, are rich in AR expression and send AR immunoreactivity expressing projections to the PVN [70, 71]. Thus, they are favorably positioned to facilitate regulation of the HPA axis by androgens. Accordingly, androgen treatment administered directly to the MPOA decreases neuronal activation and *Avp* gene expression in the PVN, as well as glucocorticoid secretion following acute stress [70, 106]. Stereotaxic placement of DHT in the BNST, on the other hand, increases PVN AVP mRNA and stress-induced neuronal activation [107]. While further examination is necessary to determine if this unexpected role of androgens in the BNST depends on the type or duration of stress exposure, it supports the idea that androgens can act on brain regions upstream of the PVN to modulate the function of the HPA axis [107].

Androgens also act downstream of the PVN to influence HPA axis activity. At the level of the pituitary, POMC mRNA is downregulated by androgens [6]. Androgens also decrease pituitary ACTH content [82] and adrenal CORT content [108]. Moreover, androgens enhance GR-mediated negative feedback on the HPA axis. This is indirectly supported by evidence that testosterone increases GR binding in the MPOA, an area that mediates some of the inhibitory effects of glucocorticoids on the HPA axis [106]. It is also supported by evidence that testosterone decreases plasma CBG levels, which makes CORT more accessible to its receptors that facilitate glucocorticoid negative feedback [99].

Importantly, most studies examining androgen effects on the HPA axis employ DHT, which has high affinity for ARs, but cannot be aromatized to estradiol [105]. However, DHT can be metabolized to 5 α -androstane-3 β , 17 β -diol (3 β -diol), which has

been shown to bind and activate ER β [105]. Like DHT and ER β agonists, 3 β -diol inhibits the HPA axis response to stress [90, 105]. Accordingly, administration of either DHT or 3 β -diol just above the PVN decreases ACTH and CORT responses to an acute stressor [90]. It has been hypothesized that 3 β -diol and DHT, via its metabolism to 3 β -diol, act through ER β s in PVN neurons to exert these effects, as rat PVN neuroendocrine neurons do not express ARs [105]. In support of this hypothesis, an ER but not AR antagonist blocks the local actions of DHT and 3 β -diol in the PVN [90]. Additionally, 3 β -diol has been shown to directly regulate the OT and AVP promoters through binding to ER β [109, 110]. Together, these findings suggest that the inhibitory effects of DHT on HPA axis activity may be partially mediated by 3 β -diol signaling through ER β . They also emphasize the importance of further considering the roles ER β may play in facilitating androgen regulation of the HPA axis.

ORGANIZATIONAL EFFECTS OF GONADAL HORMONES

Perinatal gonadal hormones and the HPA axis

Sex differences in HPA function appear to also be organized by exposure to gonadal steroids during key developmental periods that program lasting differences in the HPA axis (i.e., organizational effects) [8] (Fig. 2). During perinatal development, male rats and mice encounter two surges in testosterone release that can presumably masculinize/defeminize the brain prior to puberty. One occurs late in gestation (G18 in the rat) and the other occurs shortly after birth [111, 112]. Accordingly, if neonatal testosterone is removed by gonadectomy, or if ARs are antagonized with flutamide, during the prenatal or postnatal periods in male rats, increased basal and stress-induced concentrations of CORT are found in adulthood [7, 113–115]. Male rats treated perinatally with flutamide also have altered patterns of basal CORT secretion as adults [7]. Normally, both male and female adult rodents secrete basal glucocorticoids in a pulsatile fashion throughout the day; but females have higher frequency and amplitude pulses than do males [6]. Perinatal AR antagonism in males increases their frequency and amplitude of CORT pulses in adulthood such that they resemble those of females. Notably, the effects of perinatal gonadectomy on basal and stress-enhanced glucocorticoid secretion in male rats are reversed by a single neonatal dose or neonatal doses of testosterone, but not prolonged testosterone treatment in adulthood [113, 114]. In females, a single neonatal dose of testosterone also dampens their HPA activity as adults [116]. Together, these findings indicate that neonatal testosterone acts through AR to produce lasting masculinization of glucocorticoid secretion by the HPA axis.

In rodents, the conversion of testosterone to estradiol by the aromatase enzyme, and estradiol's subsequent actions in organizing brain function, set the stage for functional sex differences in the HPA axis in adulthood (Fig. 2). Support for such estradiol-dependent sexual differentiation of the HPA axis comes from the identification of high levels of estrogen receptors [117] and aromatase enzymes [118] in the prenatal and neonatal rat brain. Additionally, treatment with an aromatase inhibitor or estradiol during the perinatal period increases and decreases basal and stress-induced HPA output in adult male rats, respectively [7, 114, 119]. Similarly, chronic estradiol treatment (estrogenization) of female neonates results in an evening corticosterone rise in adulthood that resembles that of male rather than diestrous female rats [120]. Thus, both early androgen and estrogen actions through ARs or ERs can establish sex differences in glucocorticoid secretion in adulthood, which are supported by sexually dimorphic activity at all levels of the HPA axis.

Current evidence supports a role for neonatal exposure to gonadal hormones in altering PVN neuroendocrine function during adulthood. Gonadectomy of male rats as neonates increases their basal and restraint-induced *c-Fos* expression (a

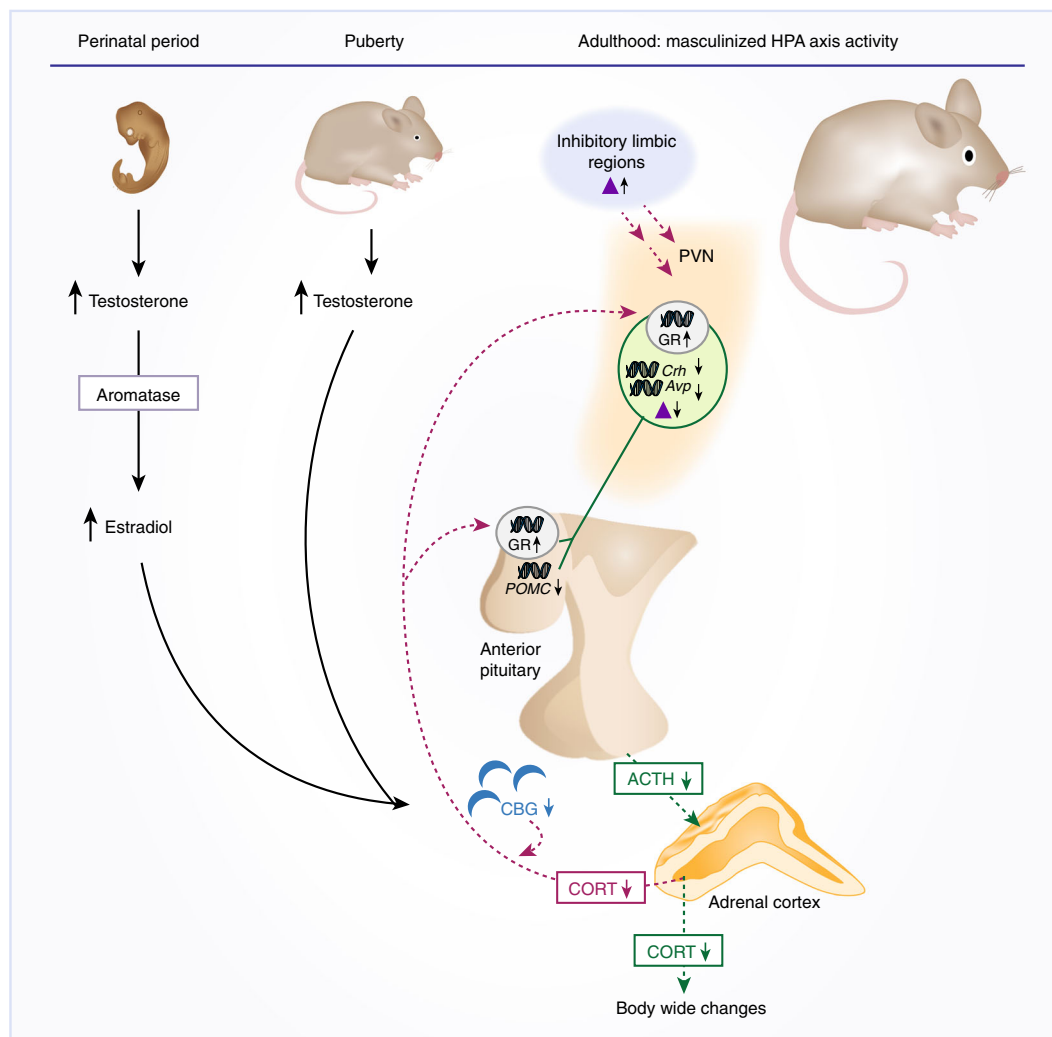


Fig. 2 Organizational actions of gonadal hormones program lasting changes in the adult male HPA axis response to stress. Testosterone surges that occur during the perinatal period and puberty play important roles in masculinizing the HPA axis response to stress in adult male rodents. During the perinatal period, testosterone, largely via its conversion to estradiol by the aromatase enzyme, is involved in establishing a blueprint for a male typical pattern of HPA axis activity in adulthood. Pubertal testosterone then acts on this blueprint to complete the development of the masculinized HPA axis. Thus, in adulthood, males have decreased HPA axis responses to acute stressors characterized by decreased PVN neuronal activation and gene expression, decreased pituitary expression of POMC, and decreased ACTH and CORT responses to acute stressors. Adult males also have enhanced negative feedback resulting from their relatively reduced CBG levels, their increased PVN and pituitary GR gene expression, and their increased neuronal activation in limbic regions that inhibit the HPA axis. Organizational effects of testosterone, therefore, are important contributors to sex differences in the adult HPA axis in which females have relatively enhanced activity. Triangles indicate neuronal activation

measure of neuronal activation) in the medial parvocellular part of the PVN in adulthood [113]. These effects are reversed by neonatal, but not adult, treatment with testosterone, suggesting that neonatal testosterone contributes to male typical stress-induced PVN neuronal activation [113]. Furthermore, adult male rats treated with an aromatase inhibitor (1,4,6-androstatriene-3,17-dione (ATD)) as neonates have increased restraint-stimulated c-Fos mRNA in the PVN [119]. Collectively, these findings suggest that neonatal testosterone treatment, at least partially via its conversion to estradiol, contributes to male typical PVN neuronal activation following stress.

Early exposure to gonadal hormones also alters the expression of genes associated with HPA activation in adulthood. A single injection of testosterone in neonatal females is sufficient to decrease their stress-generated levels of PVN CRH, PVN AVP, and anterior pituitary POMC mRNAs as adults [116]. Additionally, estradiol treatment of female neonatal rats reduces basal PVN *Crh* gene expression in adulthood such that it resembles that of males

rather than that of control diestrous females [120]. Such estrogenized females also do not exhibit increases in PVN CRH mRNA following adult ovariectomy and estradiol replacement as do control females [120]. In males, on the other hand, perinatal exposure to either an AR antagonist (flutamide) or ATD markedly increases PVN CRH and anterior pituitary POMC mRNA following stress in adulthood [7]. Consequently, both testosterone and its aromatization to estradiol are important for the development of masculinized HPA gene expression, which is reduced in adult males compared to females post stress.

Limbic regulation of the HPA axis is also subject to organizational actions of gonadal steroids. Neonatal treatment with an aromatase inhibitor, ATD, increases neuronal activation in limbic brain regions known to regulate the HPA axis in adult males, such as the PFC, lateral septum, and MeA [119]. Moreover, neonatal gonadectomy modifies expression of AR in the MeA and BNST, two areas that are important for regulating PVN neuronal activation and glucocorticoid secretion in response to stress

[113]. Both stressed and unstressed adult male rats that were gonadectomized as neonates have decreased numbers of AR-expressing cells in both brain regions, and this effect is reversed by neonatal testosterone treatment [113]. Consequently, early testosterone exposure and aromatization enable the normal development of limbic structures that regulate the HPA axis in adult males.

Gonadal hormones similarly influence glucocorticoid negative feedback on the HPA axis, which is generally enhanced in males relative to females in adulthood. Accordingly, GR expression is often greater in males than females in structures important for driving the HPA axis, such as the hippocampus, PVN, and pituitary [48, 50, 51]. However, this sex difference in adulthood likely depends on the estrous stage of the females examined, as one study found that adult diestrous females have greater hippocampal and PVN GR expression than males [120]. Nonetheless, early gonadal hormone exposure has been shown to modulate GR expression and contribute to its sex-biased expression in adulthood. One study found that neonatal estradiol treatment decreases adult levels of hippocampal GR mRNA such that they are indistinguishable from those in adult males, suggesting that early estradiol exposure has “defeminizing” effects [120]. Furthermore, PVN GR mRNA is decreased, or “feminized”, in adult male rats treated neonatally with either an AR antagonist or an aromatase inhibitor, whereas it is increased (defeminized) in adult females treated neonatally with testosterone or estradiol [7, 116, 120]. In the anterior pituitary, adult males also have decreased GR binding if they were neonatally gonadectomized [114]. Lastly, perinatal gonadectomy elevates CBG levels in adult males [114]. Collectively, these findings suggest that the increased HPA negative feedback observed in adult males may be due, at least in part, to androgen-mediated effects via ARs and ERs on the epigenetic control of corticosteroid receptor expression and ligand availability.

Studies indicating sex differences in the necessity of forebrain and PVN GRs for HPA axis suppression may further support an organizational hypothesis of gonadal hormone action. These studies revealed that the loss of forebrain and PVN GRs results in dysregulation of HPA axis activity in males but is largely inconsequential in females [52, 53]. The use of randomly cycling females in these studies leaves open the possibility that activational effects of gonadal hormones influence GR involvement in HPA regulation. However, the substantial differences in HPA function between control females and those containing selective GR depletion—all of which were randomly cycling—would suggest that the effects of GR knockdown are not modified by estrous cycle stage. Rather, organizational effects of gonadal hormones may be at play and suggest that females use a different mechanism or neurocircuitry to inhibit HPA axis function.

Pubertal gonadal hormones and the HPA axis

Rises in gonadal steroids that occur at puberty provide another signal that can drive permanent changes in brain function. Although limited, some evidence indicates that a second critical period for the organizational actions of gonadal hormones on the HPA axis may occur near puberty [121]. The increases in gonadal steroids during this period drive numerous neural and behavioral changes to produce adult-like characteristics [121]. These include changes in neuroendocrine function, such as the stress-induced activity of the HPA axis. Accordingly, rats of both sexes have greater glucocorticoid responses to acute stressors before puberty than they do in adulthood [122, 123]. A thorough review of such age-dependent changes in the HPA axis can be found in ref. [124].

Notably, neuroendocrine sex differences that begin to develop in the perinatal period are completed during puberty [121]. As is the case for testosterone exposure during the perinatal period, exposure during puberty is important for the masculinization of neuroendocrine responses to stress in adulthood [125]. Adult

female mice given testosterone during puberty have decreased CORT levels following acute restraint stress in adulthood, which are similar to those observed in males [125]. Moreover, pubertal testosterone exposure is important for organizing the adult male typical sensitivity of the HPA axis to androgen regulation [126]. Unlike males gonadectomized in adulthood, adult male rats gonadectomized before puberty do not respond to testosterone administration with decreases in basal or stress-induced PVN gene expression or glucocorticoid secretion [126]. Thus, puberty is potentially a critical organizational period during which rising levels of androgens play a vital role in sculpting the correct development of a male typical, androgen-sensitive adult HPA phenotype.

Pubertal rises in estradiol may also play a role, although less pronounced, in the organization of the adult HPA axis. Prior to puberty, estradiol decreases the activity of the HPA axis in female rats, whereas in adulthood it increases HPA activity [127]. Interestingly, this change in the HPA response to estrogens does not appear to depend on ovarian steroids [127]. In adult rats, estradiol treatment increases basal and stress-induced glucocorticoid secretion regardless of whether they were ovariectomized before or after puberty [127]. Thus, pubertal estrogen exposure is not essential for establishing the stimulatory effects of estradiol on the HPA axis as in adult females.

Adolescence and the HPA axis

Adolescence marks a transitional period between weaning and adulthood characterized by significant brain development, including changes in the HPA axis [128]. Although adolescence includes puberty and the associated effects of gonadal steroids on the HPA that occur during this period (discussed above), it can be more specifically divided into three stages: (1) prepubescence/early adolescence, (2) mid-adolescence, which includes pubertal onset, and (3) late adolescence during which puberty terminates [128]. Sex differences in HPA activity have been reported to emerge throughout adolescence. For example, adrenal volume increases in the late adolescence period more so in females than in males [129]. Sex biases also exist in the process whereby GRs translocate from the cytosol to the nucleus to influence gene transcription. One study demonstrated that GR translocation increases following acute stress in female, but not male, adolescent rats [130]. However, when adolescent rats have a history of chronic stress exposure, co-chaperones that inhibit GR translocation are upregulated and glucocorticoid negative feedback is impaired following an acute stressor only in females [130]. Additionally, gonadal hormones have been shown to play a role in regulating HPA function during adolescence, which can have lasting consequences. One study demonstrated that exposure to androgens irreversibly masculinizes CBG concentrations in adulthood. Accordingly, adult males have increased levels of CBG (resembling those of females) if gonadectomized during early adolescence [131]. A thorough review of sex differences in the HPA axis during adolescence and the influence of gonadal hormones can be found in refs. [8] and [132].

REMAINING QUESTIONS REGARDING SEX DIFFERENCES IN THE HPA AXIS

Do sex chromosomes affect the HPA axis?

Although activational and organizational actions of gonadal hormones are essential mediators of sex differences in neural and behavioral phenotypes, more recent evidence suggests that sex chromosomes can have direct effects in non-gonadal tissues that act alongside or even independent of gonadal hormones secretions [10]. Such chromosome effects partially involve expression of male- and female-specific genes by the Y and X chromosomes, respectively (see ref. [133] for review). For example, expression of the gene *Sry* by the Y chromosome in males not only

causes differentiation of the testis, but can also act in the brain to alter its function [133, 134]. Thus, differences intrinsic to XX and XY cells may be key mediators of sex differences in diverse aspects of physiology and behavior, including the neuroendocrine response to stress [133].

Unfortunately, studies examining the effects of XX versus XY on sex differences in the adult HPA axis are lacking. Nevertheless, rodent models now exist that would greatly aid in such investigations. The four core genotypes model has been used most frequently, and involves the uncoupling of gonadal (ovaries versus testes) and chromosomal (XX versus XY) sex. Subjects accordingly include: (1) XX gonadal females, (2) XY gonadal females, (3) XX gonadal males, and (4) XY gonadal males (see ref. [135] for review). Thus, the four core genotypes allow for testing of the organizational/activational effects of hormones resulting from gonadal sex independent of the intrinsic differences in XX versus XY cells [10]. This model has previously been used to examine sex differences in the morphology of AVP fibers in the lateral septum, a region known to modulate activity of the HPA axis. Results indicate that sexually dimorphic septal AVP not only arises due to organizational/activational effects of gonadal steroids, but also due to chromosomal effects [136, 137]. Thus, it is highly likely that such sex chromosomal effects alter other aspects of HPA axis regulation, but this remains to be determined.

Are there sex differences in HPA axis following chronic stress?

Acute activation of the HPA axis by stress is considered an adaptive response to the increased metabolic demand required to deal with the stressor. Yet, in the face of chronic stress, this same response can be considered maladaptive and lead to greater risk for disease states [4]. A particularly well-established association exists between hyperactivity of the HPA axis and the presentation of affective disorders, such as depression and anxiety [5, 138]. Such disorders are of notable interest since they exhibit a striking sex bias in prevalence. Women are at least twice as likely as men to present with these conditions, and this bias is likely driven (at least in part) by sex differences in the physiological response to chronic stress [5]. Thus, some studies have begun to examine sex differences in the activity of the HPA axis under chronic stress conditions in the interest of understanding why women have increased vulnerability to affective disorders.

Models frequently used to examine HPA dysregulation that may predispose or trigger stress-related diseases are the repeated (homotypic) stress and the chronic mild stress (CMS)/chronic variable stress (CVS) models. The repeated stress model involves presentation of the same stimulus continually until a reduction in the physiological response, or “habituation”, to that stressor occurs [139]. The CMS/CVS model, alternatively, prevents habituation of the stress responses by exposing animals to randomly presented stressors over a prolonged period [140]. Both models are thought to be etiologically relevant for human disease risk and have been used to examine sex differences in the HPA axis response to chronic stress as discussed below.

Sex differences following repeated stress

After a number of exposures to the same stressor, sex differences are present in the basal activity of the HPA axis in which female rats have higher plasma CORT and CBG levels than males [141, 142]. Additionally, sex differences may be found in the habituation of HPA activity in repeated stress scenarios. In both sexes, this habituation is characterized by decreased ACTH and CORT responses to a stressor, as well as reduced stress-induced neuronal activation in the medial parvocellular part of the PVN, as the number of exposures increases [143]. However, the degree to which the habituation of HPA activity occurs in male and female rodents may vary. Some studies report similar habituation between the sexes. Accordingly, CORT responses to restraint or loud noise stressors incrementally decrease by the same amount

in male and female rats [144]. The number of activated, c-Fos-expressing neurons that co-express AVP following restraint stress also similarly decreases in males and female rats [143]. Other studies, alternatively, suggest that habituation in a repeated restraint stress paradigm is reduced or slower in female versus male rats [142, 145, 146]. Notably, this sex difference has also been shown to be accompanied by a decrease in plasma CBG levels only in female rats [142].

Morphological changes may also accompany chronic stressors and show sex biases. Whereas repeatedly restrained males exhibit apical dendritic atrophy of CA3c pyramidal neurons in the hippocampus, females show a decrease in basal dendritic branching [142]. Ultimately, whether or not habituation is sex-biased likely depends on the type of stressor and/or experimental paradigm. Nonetheless, findings to date largely support the possibility that females have enhanced HPA axis responses during repeated stress, which may contribute to their increased susceptibility to stress-related diseases.

Some studies have also examined sex differences in facilitation, a process that occurs when repeatedly stressed subjects have an equal or greater response to a novel stressor than control subjects [147]. When exposed to a novel acute restraint stress, chronically cold-stressed male mice exhibit similar ACTH and CORT responses to control males [148]. Cold-stressed females, on the other hand, have lower ACTH responses than, but similar CORT responses to, unstressed control females following a novel stressor [148]. These findings suggest that both sexes show facilitation, but that it may be more prominent in males. Conversely, another study found no sex differences in the HPA axis response to a novel environment following repeated exposure to either audiogenic or restraint stress [144]. Together, these studies suggest that sex differences in facilitation are stressor dependent.

The influence of gonadal hormones on HPA responses to repeated stressors has also been investigated. During repeated restraint stress, estradiol has been shown to limit habituation. Thus, in OVX female rats treated with estradiol, the decrease in plasma CORT responses following repeated stress exposure is lesser than it is in vehicle-treated controls [81]. In the same estradiol-treated OVX females, PVN CRF and AVP mRNA levels are also enhanced by chronic stress, and the habituation of AVP mRNA is impaired [81]. These findings suggest that, like in acute stress studies, estradiol increases HPA responses to repeated stress in female rats. Conversely, estradiol has been shown to dampen the effects of repeated footshock stress on activity in the PVN [149]. Accordingly, in OVX female rats given estradiol, c-Fos expression is lesser in the PVN following repeated stress than in vehicle-treated controls [149]. The differential effects of estradiol on the repeated stress-induced activity of the HPA axis may be due to varying experimental parameters, such as the type of stressor or the treatment duration. However, it is also possible that estradiol has varying effects depending on whether ER α or ER β -mediated mechanisms are involved, as discussed above for acute stress situations. In support of this possibility, the study in ref. [149] demonstrated that estradiol treatment increases the number of ER β -expressing cells in the PVN observed during chronic footshock stress, supporting ER β -mediated mechanisms of PVN neuronal inhibition. Regardless of the direction of influence, the studies presented here demonstrate an important role for estradiol in determining how females adapt to chronic stress, which may influence sex differences in stress-related pathology.

The effect of gonadal hormones on chronic stress-induced HPA activity has also been investigated in male subjects. In male rats exposed to repeated restraint stress, gonadectomy enhances the habituation of the plasma CORT response to the restraint. However, habituation of the neuronal c-Fos response to repeated restraint stress in the PVN, MeA, and locus coeruleus are not affected by gonadectomy [148]. Therefore, androgens may minimally decrease

HPA activity during repeated stress, but further study is necessary to fully determine this. Another study demonstrated that testosterone has organizational rather than activational effects that influence the habituation of the HPA axis during repeated psychogenic stress in male rats [101]. Accordingly, males treated with an androgen receptor antagonist (flutamide) or an aromatase inhibitor (ATD) during the perinatal period have a reduced decline in ACTH and CORT responses to repeated stress in adulthood. When adult males are gonadectomized, on the other hand, no changes in the habituation of the CORT response to stress are evident [101]. Thus, both the activation of androgen receptors and the conversion of testosterone to estradiol during the perinatal period—but not androgen exposure during adulthood—are essential for males to exhibit habituation during adulthood [101].

Sex differences following chronic mild or variable stress

Studies using the CMS and CVS models in male rodents have revealed that chronic stress generally enhances HPA axis activity, as indicated by increased basal CORT secretion, increased PVN CRH and AVP mRNA, and decreased GR expression in inhibitory brain regions [150, 151]. Although some studies have begun to compare HPA activity following CMS/ CVS in male and female rodents, these are relatively few in number.

HPA axis dysregulation has been shown to be greater following CMS and CVS in female versus male rats, as females have larger elevations in basal CORT secretion than males post CMS and CVS [141, 152, 153]. Yet, chronically stressed males do exhibit adrenal hypertrophy and have elevated CORT levels when faced with a novel stressor, whereas chronically stressed females respond similarly to control, non-CMS females [141, 153]. This suggests that in males, but not females, a chronic stress history results in a potentiated HPA axis response to a novel stressor. Thus, females may be more vulnerable than males to chronic stress in the first place, but they utilize different or better coping skills when faced with a novel stressor. Whether these results indicate that one sex is better or worse off cannot be determined without more information. Females may adapt better in a CMS scenario, which may be important for stress resilience. However, if an acute response to a novel stressor following CMS is beneficial, females may be relatively impaired.

Sex differences in PVN neuronal activity following CMS have also been examined. In CMS-exposed rats, c-Fos expression is increased in the PVN of socially housed males, but not socially housed females [154]. Yet, both males and females have increased PVN c-Fos expression following CMS when singly housed [154]. These findings suggest that social support for females enables them to better cope with stress and is beneficial, whereas social housing in males is not beneficial and may even exacerbate their vulnerability to stress [154]. They support the possibility that social environment modifies the activity of the HPA axis [155]. Additionally, sex differences in PVN gene expression changes have been observed following CMS [156]. Constitutive PVN *Crh* gene expression is increased in males, but not females, post CMS which may be due to the females' increased basal PVN CRH mRNA levels relative to those of males [156]. Accordingly, males may require greater increases in CRH from their lower basal levels to meet the demands of CMS. It is also possible that females have greater increases in *Avp* or *Ot* gene expression to make up for their lower CRH levels post CMS; or females could have CBG and/or MR levels that result in decreased glucocorticoid negative feedback. Although these possibilities require further investigation, the findings of this study and that showing sex differences in PVN neuronal activation support the possibility that HPA axis activity post CMS is increased in males and socially isolated, but not group housed, females.

Future directions for chronic stress studies

Further examination of the HPA axis response to chronic stress in both sexes is necessary to better assess sex differences. Although

studies have begun to examine sex differences in PVN gene expression and neuronal activation following repeated stress and CMS, questions remain regarding sex differences at other levels of HPA axis regulation. For instance, are there sex differences in the limbic and brainstem structures that control the activity of the HPA axis following chronic stress? One study suggests that female, but not male rats, have decreased serotonergic activity in the hippocampus after CMS [153]. This difference may underlie the increased vulnerability of females to CMS, but it remains unclear how other regulatory brain regions may be affected to further contribute to this sensitivity. Similarly, following repeated stress, sex-biased changes in the morphology of hippocampal neurons have been observed; however, investigation of changes in other regulatory brain regions is lacking [142]. Additionally, whether or not sex differences exist in *Ot* and *Avp* gene expression in the PVN and/or in POMC expression in the anterior pituitary remains to be better determined using both chronic stress models. Sex differences in adrenal sensitivity to ACTH and glucocorticoid feedback regulation are similarly under-investigated. One study did examine the role of the PVN GR in the regulation of chronic stress reactivity, but found that selective deletion of GR in the PVN has no effect on the CORT response to CMS in either male or female mice [53]. This finding suggests that glucocorticoid control of the HPA axis after CMS likely does not involve the PVN and raises questions about whether other limbic and brainstem regions and/or the anterior pituitary serve as compensatory targets for glucocorticoid negative feedback in a sex-dependent manner. In a repeated stress model, alternatively, females show greater numbers of c-Fos and GR-positive PVN cells than males, suggesting that females may be more sensitive to glucocorticoid negative feedback in this case, but further investigation is necessary [143]. Furthermore, in both chronic stress models, sex differences in the mechanisms used by the GR and MR to negatively regulate the activity of the HPA axis have not been examined. Sex differences in receptor activation, assembly, or transactivation, for instance, may be present. Ultimately, it will be important to investigate sex differences at all levels of HPA axis regulation in response to repeated and variable chronic stressors moving forward. Such investigations will provide a more complete picture of the neurobiological mechanisms that coordinate to produce sex differences in vulnerability and resilience to chronic stress.

Moreover, few, if any, studies have examined the influence of gonadal hormones on sex-dependent HPA activity following CMS/ CVS. The corresponding studies following repeated stress are greater in number, but still limited. This leaves questions regarding whether changes at each level of HPA axis regulation are influenced by the organizational and/or activational actions of gonadal hormones as has been demonstrated in acute stress studies. There are also especially large gaps in our understanding of how gonadal steroids may come to program lasting sex differences in the HPA axis response to stress in females [157]. Although further investigation is necessary, the rodent literature certainly supports the possibility that gonadal hormones alter the chronic stress-induced activity of the HPA axis in both sexes. For one, chronic stress is well associated with a dysregulation of the HPG axis, which involves inhibition of estrogen and testosterone secretion, as well as desynchronization of estrous cycling in females [153] (see refs. [54] and [158] for review). Thus, it is likely that chronic stress disrupts the HPG axis to contribute to the dysregulation of the HPA axis classically found following chronic stress [159]. Additionally, endogenous gonadal hormone levels may have a profound influence on HPA activity when altered by chronic stress exposure during critical periods. Early life stress, for instance, can disrupt key organizing actions of gonadal hormones that masculinize the HPA axis and produce lasting effects on rodents' vulnerability to chronic stress in the future [60]. Similarly, the activational action of gonadal hormones can be disturbed by chronic stress in adulthood [60]. Ultimately, understanding how

gonadal hormones modulate the HPA axis response to chronic stress has important implications for understanding the pathology of affective disorders, which are associated with major changes in gonadal hormone levels (see ref. [160] for review).

SEX DIFFERENCES IN THE HPA AXIS AND DISEASE IN HUMANS

Understanding sex differences in the human HPA axis has been a topic of much interest, as dysregulation of the HPA axis (hypo- or hyper-reactivity) is a hallmark of many stress-related diseases, which are known to differentially present in men versus women [5]. In humans, as in rodents, sex differences exist in the HPA axis response to stress and are largely age- and modality-dependent, although findings are not always consistent between the species. Before puberty, male and female rodents exhibit limited differences in their physiological stress response, as do boys and girls [161, 162]. However, during adolescence and into adulthood, male rodents begin to show a blunted HPA axis response to stress relative to females and human findings are much less consistent [5, 163]. While some studies suggest that adult women have enhanced HPA responses to acute stressors compared to males, others indicate greater responses in males or no significant difference [5, 162, 164–166]. These contradicting findings could be due to a number of factors, with the type of stressor being important, as well as the age, overall health, and menstrual cycle stage of the female participants [5, 164, 166]. For a more thorough review of sex differences in the human HPA axis, please see refs. [5] and [162].

Organizational/activational effects of gonadal hormones also influence sex differences in the human HPA axis as they do in rodents. Studies examining females in varying stages of the menstrual cycle, compared to males, have accordingly found that sex differences in the salivary cortisol response to psychosocial stress are present when women are in the low-estrogen state of the follicular phase but not the luteal phase [167]. This suggests a role for estrogen and progesterone in regulating stress responsiveness in women across the menstrual cycle. Moreover, support for a role of gonadal hormones comes from the sex-biased prevalence of stress-related diseases, such as major depressive disorder, which develops after puberty [60]. During early gestation, exposure to maternal and/or environmental stress produces more adverse outcomes in male than female offspring [60, 168]. Accordingly, neurodevelopmental disorders, such as autism spectrum disorder and attention-deficit hyperactivity disorder, are more common in boys than girls [169, 170]. This discrepancy potentially reflects a failure of neonatal testosterone to prepare the brain in early life for adult levels of testosterone, which complete the masculinization of the brain during puberty (see ref. [60] for review). During adolescence and throughout adulthood, however, females have increased risk for affective disorders, such as depression and anxiety, especially if they have experienced early life adversity [5, 171]. Such findings are also consistent with the greater risk for comorbidity of major depressive disorder with cardiometabolic diseases in women, which may be related to sex-biased HPA activity as well as sex-biased activity in the autonomic nervous system in which females have enhanced parasympathetic tone [172, 173]. Collectively, these clinical findings indicate that the timing of developmental perturbations can have long-term sex-biased outcomes; and they support a role for adult levels of female gonadal hormones in increasing risk for stress-related diseases (see ref. [60] for review). Ultimately, prior to starting any therapy for stress-related diseases, clinicians should be aware of sex differences in HPA axis activity and their underlying sources.

CONCLUSIONS

Striking sex differences exist in the neuroendocrine response to acute stress and its underlying circuitry in rodents. Generally,

females exhibit enhanced glucocorticoid secretion in response to various acute stressors, which is supported by evidence of sex differences at all levels of HPA axis regulation [9]. Abundant evidence also supports organizational and activational roles for gonadal hormones in driving sex differences in acute HPA axis activity [8]. However, further study is necessary to rule out the possibility of sex chromosome effects that act with or without gonadal steroids to modulate HPA activity. Moreover, sex differences in the neuroendocrine stress response after chronic stress remain largely under-investigated. Chronic rather than acute stress results in the dysregulation of the HPA axis that may increase risk for stress-related diseases in humans [4]. Thus, investigations of sex differences in chronic stress-induced HPA dysregulation and the underlying influences of gonadal hormones and sex chromosomes may have important therapeutic consequences in the future.

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