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Hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axes: sex differences in regulation of stress responsivity

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Abstract

Gonadal hormones play a key role in the establishment, activation, and regulation of the hypothalamic–pituitary–adrenal (HPA) axis. By influencing the response and sensitivity to releasing factors, neurotransmitters, and hormones, gonadal steroids help orchestrate the gain of the HPA axis to fine-tune the levels of stress hormones in the general circulation. From early life to adulthood, gonadal steroids can differentially affect the HPA axis, resulting in sex differences in the responsivity of this axis. The HPA axis influences many physiological functions making an organism's response to changes in the environment appropriate for its reproductive status. Although the acute HPA response to stressors is a beneficial response, constant activation of this circuitry by chronic or traumatic stressful episodes may lead to a dysregulation of the HPA axis and cause pathology. Compared to males, female mice and rats show a more robust HPA axis response, as a result of circulating estradiol levels which elevate stress hormone levels during non-threatening situations, and during and after stressors. Fluctuating levels of gonadal steroids in females across the estrous cycle are a major factor contributing to sex differences in the robustness of HPA activity in females compared to males. Moreover, gonadal steroids may also contribute to epigenetic and organizational influences on the HPA axis even before puberty. Correspondingly, crosstalk between the hypothalamic–pituitary–gonadal (HPG) and HPA axes could lead to abnormalities of stress responses. In humans, a dysregulated stress response is one of the most common symptoms seen across many neuropsychiatric disorders, and as a result, such interactions may exacerbate peripheral pathologies. In this review, we discuss the HPA and HPG axes and review how gonadal steroids interact with the HPA axis to regulate the stress circuitry during all stages in life.

Keywords

Hypothalamic–pituitary–adrenal axis; hypothalamic–pituitary–gonadal axis; gonadal steroids; estrogen; progesterone; testosterone; sex differences; stress circuitry

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Introduction

Stress is the natural response of living organisms to environmental perturbations. Appropriate reactivity to an acute stressor and timely cessation of the stress response are critical for an organism's survival. Although in our society stress has a negative connotation, this is somewhat misleading since the acute activation of stress responses makes an individual more adaptable and allows one to cope with events that jeopardize homeostasis, whether real or perceived, by increasing awareness to our surroundings, and enhancing analgesia, cognition, and euphoria (Charmandari, Tsigos, & Chrousos, 2005). Chronic and traumatic stressors, however, have the opposite effect, causing detrimental changes in the brain and other organs, and have been linked to the development of pathology including neuropsychiatric disorders, such as post-traumatic stress disorder, depression, chronic anxiety (Smith & Vale, 2006), and cardiometabolic diseases (Levine, Levine, & Levine, 2014). Moreover, the mechanisms to cope with stressors differ between males and females (Bale & Epperson, 2015). The basis for these differences is unknown, in part because much of the early work in this field was performed on male rodents, perhaps due to the challenges associated with carrying out experiments that account for fluctuating gonadal hormones in females. Across the estrous cycle of rodents, the changes in progesterone and estrogen levels have been shown to have a direct impact on the way that females respond to stress, with differential responses dependent upon the stage of the cycle (Viau & Meaney, 1991). Thus, the most appropriate study of the neuroendocrine stress response requires that fluctuations in gonadal steroid hormones be taken into account.

Gonadal steroids are largely responsible for the differential response to physical or psychological threats between sexes. Likewise, elevated stress hormones can negatively regulate the reproductive neuroendocrine axis and consequently, the levels of circulating gonadal hormones (Rivier & Rivest, 1991). Biological and physiological differences have been identified between males and females, especially in the way that they react to stress. Exploring these differences using animal models may help explain why psychiatric disorders, like anxiety and major depressive disorder, are more prevalent among females (Bekker & van Mens-Verhulst, 2007). In this review, we will define the HPA axis, identify the differences in its function between sexes, and discuss how gonadal hormones interact with the HPA axis. Finally, we will discuss potential mechanisms by which gonadal hormones may influence the development of the HPA axis.

The HPA axis – overview

The hypothalamic–pituitary–adrenal (HPA) axis is a neuroendocrine axis that utilizes three primary structures, allowing it to respond appropriately to stressful life-events. These include the paraventricular nucleus of the hypothalamus (PVN), the anterior pituitary gland, and the adrenal gland (Figure 1). At its core, the PVN computes and integrates neuronal and humoral inputs to activate a specialized group of cells that synthesize and secrete corticotropin-releasing hormone (CRH) into the hypophyseal portal vasculature. CRH is a 41-amino acid peptide that, at the level of the anterior pituitary gland, stimulates the synthesis and release of adrenocorticotrophic hormone (ACTH) into the blood stream. In turn ACTH, upon reaching the adrenal cortex, signals the synthesis of glucocorticoids (Rivier &

Vale, 1985; Ulrich-Lai & Herman, 2009). Typical of all steroid hormones, glucocorticoids are immediately released following their synthesis in adrenal cortical cells. The primary glucocorticoids are cortisol in humans and corticosterone in mice and rats. These steroid hormones bind mineralocorticoid (MR) and glucocorticoid (GR) receptors with different affinities to promote an appropriate response to environmental perturbations, whether perceived or psychological (de Kloet, Joels, & Holsboer, 2005; Reul & de Kloet, 1985). The HPA axis is ultimately inhibited by the same hormones acting in different brain regions. This negative feedback process reestablishes the baseline homeostatic state. These responses are known to be adaptive and have effects that influence future HPA axis responses (McEwen & Gianaros, 2010). A dysregulation of these carefully orchestrated interactions can result in pathologies such as immunodeficiency, memory impairment, obesity, and cardiometabolic disorders (Levine, Levine, & Levine, 2014; Munck, Guyre, & Holbrook, 1984; Sapolsky, Romero, & Munck, 2000; Smith & Vale, 2006).

The paraventricular nucleus of the hypothalamus (PVN)

The PVN houses the key neurons controlling the level of activation of the HPA axis, regulation of metabolism, growth, and immune functions, as well as pre-autonomic control of gastrointestinal, cardiovascular and renal functions (Ferguson, Latchford, & Samson, 2008). Close to a century ago, Gurdjian, using the rat model, classified these neurons into two broad groups based on their size (Gurdjian, 1927). The medial parvocellular region has neurons with small nuclei, whereas the lateral magnocellular region houses neurons with large, dense nuclei. Since then, the cyto- and chemo-architecture of the PVN has been studied extensively in the rat. In contrast, the PVN of other species, like the mouse and human, reportedly have noteworthy differences in the chemical and cytological distribution (Biag et al., 2012). Nonetheless, most of the information available regarding the morphology and chemical composition of the PVN is predominantly based on the rat model.

The PVN is composed of two main cellular types classified by their fiber distribution: neuroendocrine and pre-autonomic. Neuroendocrine cells residing in the PVN project to the median eminence or to the posterior pituitary gland. At the median eminence, their chemical content is released into the hypothalamo-hypophyseal portal veins, a series of veins that connect two venous capillary beds, thereby allowing for fast transport and exchange of hormones between the hypothalamus and the anterior pituitary gland (Ferguson, Latchford, & Samson, 2008; Swanson & Sawchenko, 1983). Once these releasing factors reach the anterior pituitary, they trigger the release of hormones into the general circulation. CRH, thyrotropin releasing hormone (TRH), oxytocin (OT), dopamine, somatostatin, and vasopressin (AVP) expressing neurons are among those that project to the median eminence (Ferguson, Latchford, & Samson, 2008). Additionally, OT and AVP magnocellular neurons project to the posterior pituitary and secrete directly into the general circulation (Sawchenko, Imaki, & Vale, 1992). PVN pre-autonomic neurons, on the other hand, directly project to preganglionic autonomic neurons of the brainstem and spinal cord for the regulation of cardiovascular, thermoregulatory, and gastrointestinal functions, to name a few (Geerling, Shin, Chimenti, & Loewy, 2010). In addition to the median eminence and autonomic nuclei projecting neurons, the PVN contains neurons that project to the forebrain and other

hypothalamic nuclei to mediate freezing and other behavioral responses (Fuzesi, Daviu, Wamstecker Cusulin, Bonin, & Bains, 2016; Knobloch et al., 2012).

The rat neuroendocrine neurons of the PVN can be divided into magno- and parvocellular divisions based on each neuron's size and projections (Swanson & Kuypers, 1980). OT and AVP neurons are numerous in the magnocellular division, and project to the posterior pituitary gland to release their respective hormones. However, PVN neurons that directly release neuropeptides like CRH, AVP, and TRH into the hypothalamo-hypophyseal portal vasculature are part of the neurosecretory parvocellular division (Cunningham & Sawchenko, 1991; Swanson & Sawchenko, 1980; Wotjak et al., 1996). Of importance for HPA axis regulation, these neuropeptides are carried by the hypothalamo-hypophyseal portal veins to the anterior pituitary, where they activate corticotrophs that release ACTH into the blood stream (Smith & Vale, 2006).

Sex differences in the PVN composition—Interestingly, as far as is known, in rodents and humans, the morphology of the male and female PVN is quite similar, with some exceptions. For instance, women reportedly have larger PVN AVP neurons than men (Ishunina & Swaab, 1999). Otherwise, the lack of sex differences in the morphology of the PVN suggests that the chemical composition or wiring of the PVN is what makes the activation of the HPA axis more robust in females than in males. For example, following exposure to a stressor, the release of CRH from PVN neurons triggers a larger ACTH response in female than in male rats (Le Mevel, Abitbol, Beraud, & Maniey, 1979). Additionally, female rats have a much stronger release of AVP following stress (Williams, Carter, & Lightman, 1985), indicating a robust sexual dimorphism in mounting the HPA response to environmental threats at the PVN level. More on these sex differences will be covered in later sections.

The pituitary gland

The pituitary gland, through its secretion of protein hormones, plays a critical role in the maintenance of homeostasis during and after stress, as well as during other physiological processes, like growth and metabolism (Perez-Castro, Renner, Haedo, Stalla, & Arzt, 2012). This structure serves as the main humoral communication gateway from the brain to peripheral organs. Strategically located at the base of the hypothalamus and enclosed by the bony *sella turcica*, it interacts with the hypothalamus via compounds that are delivered to it through the hypothalamo-hypophyseal portal veins (Melmed, 2011). The pituitary is divided into two lobes, the anterior lobe (or adenohypophysis) and the posterior lobe (or neurohypophysis). This division is based upon their relative anatomical positions, function, and developmental precursors. The posterior pituitary receives axonal inputs from magnocellular OT and AVP neurons residing in the supraoptic nucleus and PVN (Perez-Castro, Renner, Haedo, Stalla, & Arzt, 2012), where they release their secretory product into the general circulation. In contrast, corticotrophs are the key anterior pituitary cell type involved in HPA axis regulation and the production of ACTH. These cells contain receptors that bind CRH to activate the synthesis of ACTH in response to humoral signals from the hypothalamus. Activation of the CRH receptor type 1 (CRH-R1) (Bale & Vale, 2004) initiates ACTH secretion from the corticotrophs.

There are two main types of the CRH receptor in mammals, although the CRH-R1 is the primary receptor responsible for driving secretion of ACTH from corticotrophs. Both CRH-R1 and CRH-R2 are 7-transmembrane G-protein coupled receptors, which act to increase cAMP activity through stimulation of adenylate cyclase. The release of ACTH is the result of an increase in intracellular calcium driven by the cAMP-protein kinase A pathway (Majzoub, 2006). Corticotrophs in the rat pituitary also express both estrogen receptor subtypes (α and β), but only a pituitary-specific truncated estrogen receptor product (TERP) mRNA transcript changes levels (4-to 7-fold increase) following estradiol treatment (Mitchner, Garlick, & Ben-Jonathan, 1998). Whether these receptors are co-expressed with CRH receptor-containing corticotrophs to regulate the release of ACTH at this level remains unknown. The importance of these receptors in the regulation of the HPA axis has been highlighted recently, especially with its upstream modulatory targets to the PVN, which have been observed to work through CRH receptors. For instance, in a recent article, Henckens et al. (2016) demonstrated that the posterior part of the bed nucleus of the stria terminalis (BnST) modulates the HPA axis (Henckens et al., 2016). They showed this by specifically activating CRHR2-expressing neurons using an optogenetic approach, which resulted in a blunted neuroendocrine response to stress, as well as decreased anxiety and PTSD-like related behaviors. Furthermore, Dabrowska et al. (2011) have shown that not only does the BnST interact with the PVN, but also vice versa, presumably through an OT and CRHR2 mechanism (Dabrowska et al., 2011). They showed that OT and CRHR2 fibers present in the BnST originated in the PVN. These data suggest a potential reciprocal modulation between these two nuclei.

The *Crh* gene and promoter activity have been shown to be regulated by estrogens (Lalmansingh & Uht, 2008; Miller, Suzuki, Miller, Handa, & Uht, 2004). Estradiol treatment increases *Crh* gene expression and basal levels of ACTH in ovariectomized rats (Ochedalski, Subburaju, Wynn, & Aguilera, 2007) and CRH neurons in the rat PVN express estrogen receptor β , but not estrogen receptor α (Laflamme, Nappi, Drolet, Labrie, & Rivest, 1998; Mitra et al., 2003). The estradiol-dependent regulation of CRH neurons in the PVN is thought to be regulated by the estrogen receptor β (Lund, Hinds, & Handa, 2006). *In vitro* studies have shown that the estrogen receptor β , but not α , has a strong stimulatory action on the *Crh* promoter (Miller, Suzuki, Miller, Handa, & Uht, 2004). These studies suggest that estradiol-dependent effects in the regulation of the HPA axis may be driven by their direct action through the estrogen receptor β in CRH neurons of the PVN. While the size of the pituitary gland has been reported to differ between sexes in humans (MacMaster et al., 2007), and its response to hormones in mice (Sanchez-Cardenas et al., 2010), little is known about the sex differences at this level contributing to the differential response to stressors between males and females.

CRH binding proteins and sex differences in the pituitary gland—CRH-binding protein (CRH-BP) is a secreted protein that binds CRH with similar or greater affinity than the CRH receptor. It is secreted by anterior pituitary corticotropes, and subsequently binds free CRH (Figure 1), thus preventing it from binding to its receptor and thereby blocking the actions of CRH on anterior pituitary corticotrophs (Cortright, Nicoletti, & Seasholtz, 1995;

Seasholtz, Burrows, Karolyi, & Camper, 2001). These data suggest a local inhibitory role at the pituitary level.

In female rats, there is a more robust ACTH response to stress as compared to males (Handa et al., 1994; Young, 1996). In a recent report, Stinnett et al. (2015), indicated higher *Crh-BP* mRNA transcripts in pituitary from female mice versus male mice, even after restraint stress (Stinnett, Westphal, & Seasholtz, 2015). Further, the increase in *Crh-BP* mRNA levels observed following restraint stress is blunted by adrenalectomy, or depletion of endogenous glucocorticoids, indicating that glucocorticoids play a fundamental role in the regulation of CRH-BP (McClennen, Cortright, & Seasholtz, 1998). The level of CRH-BP is also higher in the murine pituitary and the cells expressing *Crh-BP* mRNA may also be involved in the regulation of reproductive hormones, such as prolactin and luteinizing hormone β (Speert, McClennen, & Seasholtz, 2002). In fact, on proestrus, 80% of the CRH-BP of the murine female pituitary is present in prolactin-expressing cells, suggesting that high estrogen levels drive the expression of CRH-BP and restrict the amounts of bioactive CRH that can activate corticotrophs. However, this goes against studies showing enhanced secretion of ACTH on proestrus (Viau & Meaney, 1991). Thus, these data may indicate that there are other upstream factors involved. In support of this, the estrogen receptors α and β have been reported to regulate the CRH-BP promoter *in vitro*, and estradiol has been shown to promote the induction of endogenous CRH-BP (van de Stolpe et al., 2004). Thus, it is possible that CRH-BP could be mediating the levels of CRH sensed by the pituitary gland through an ER-dependent mechanism, bringing CRH sensitivity back to homeostatic levels after stress. However, the actions of CRH-BP on the efficacy of CRH at the level of the pituitary seems to be context specific, or during periods like pregnancy, when exposure to CRH is chronic.

The adrenal gland

The adrenal glands have strategic access to the vascular system allowing them to regulate basal and reactive hormone levels (Rosol, Yarrington, Latendresse, & Capen, 2001). The adrenal cortex is composed of three distinct concentric zones: the zona glomerulosa, zona fasciculata, and zona reticularis from outside to inside. In addition to the cortical zones, the adrenal medulla is also involved in the regulation of homeostasis, as it secretes epinephrine and norepinephrine following autonomic nervous system activation (Mitani, 2014). However, the main adrenal region responsible for glucocorticoid secretion is the zona fasciculata. Cells in the zona fasciculata express the melanocortin receptor-2 (MC2R) (Figure 1). ACTH in the general circulation reaches the adrenal gland to activate MC2R, thereby triggering the synthesis of CORT in the zona fasciculata of the adrenal cortex (Rivier & Vale, 1985; Vale, Spiess, Rivier, & Rivier, 1981).

There are five melanocortin receptor subtypes (MC1R-MC5R), all of which have different expression patterns and specific functions (Yang, 2011). These receptors are part of the G-protein coupled receptor (GPCR) superfamily. MC2R expression is predominantly restricted to the adrenal cortex, pituitary, and adipose tissue (Beuschlein, Fassnacht, Klink, Allolio, & Reincke, 2001; Boston & Cone, 1996; Forti, Dias, & Armelin, 2006). Upon ACTH binding and activating MC2R, genes involved in steroidogenesis are activated by the canonical intracellular molecular pathway involving adenylyl cyclase, cAMP, and protein kinase A

(Lefkowitz, Roth, Pricer, & Pastan, 1970; Lehoux, Fleury, & Ducharme, 1998) to allow the synthesis and secretion of adrenal glucocorticoids. The metabolic pathway that gives rise to adrenal steroid hormones has been reviewed in Oyola, Malysz, Mani, and Handa (2016).

Sex differences in adrenal function—The adrenal release of CORT has been shown to differ between the sexes, particularly following stress. CORT increases following a stressor are higher and remain elevated for longer in female rats (Figueiredo, Dolgas, & Herman, 2002). Both estrogen and testosterone have been shown to modulate the levels of CORT, and the degree to which activation of the HPA axis is driven is also influenced by both of these hormones, suggesting an interaction between the HPA and the HPG axes (see below). However, gonadal steroids impact HPA axis reactivity differentially. Gonadectomy of male rats elevates, while androgen replacement blunts the CORT and ACTH response to stress (Handa et al., 1994). In contrast, ovariectomy reduces, while estradiol treatment increases, the gain of the HPA axis (Lund, Munson, Haldy, & Handa, 2004; Viau & Meaney, 1991). The reciprocal relationship between the HPA axis and the regulation of the HPG axis will be further discussed in a later section.

Corticosteroid binding proteins—CORT signaling can be regulated at many levels. The main control of CORT bioavailability is the plasma corticosteroid binding globulin (CBG), which binds CORT in the blood to render it inactive (de Kloet, Joels, & Holsboer, 2005). CBG, or transcortin, is synthesized mainly by hepatocytes and binds CORT with high affinity to transport CORT in blood. This interaction regulates bioavailability during high CORT secretion (Meyer, Nenke, Rankin, Lewis, & Torpy, 2016). Of note, CBG also binds other hormones, including androgens and progesterone, which in humans are thought to displace cortisol from CBG (Dunn, Nisula, & Rodbard, 1981). Moreover, CBG is higher in female than in male rats (Gala & Westphal, 1965), perhaps normalizing the free biological component of circulating CORT to levels observe in males. However, the levels of stress-induced biologically active CORT may be elevated in male rats in part because the CBG is negatively regulated by stress (Tannenbaum et al., 1997), though, it would be interesting to know if the same is true in female rats. Finally, estradiol treatment has been shown to positively regulate CBG in male rats (Gala & Westphal, 1966; Young & Altemus, 2004), but perhaps not over a short time course that is insufficient to increase HPA responses to stress (Lund et al., 2004), indicating that estrogens and androgens may both play a role in the regulation of the HPA axis in part, through modulation of CBG levels.

Negative feedback and corticosteroid receptors

An important step in HPA axis control is its ability to keep its own secretion in check, thereby allowing return to baseline levels. This occurs through inhibition of the entire axis by adrenal glucocorticoids. There are at least two time-domains known to be involved in negative feedback: a time-delayed feedback and a fast acting, non-genomic feedback (Keller-Wood & Dallman, 1984). The more studied of these mechanisms is delayed feedback, which involves corticosteroid receptors and transcriptional regulation in multiple brain regions (Figure 1). Two types of receptors have been described for corticosteroids, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). While GRs are ubiquitously expressed throughout the brain, they are highly expressed in the hippocampus

(De Kloet, Vreugdenhil, Oitzl, & Joels, 1998) and hypophysiotropic neurons of the PVN (Kovacs, Foldes, & Sawchenko, 2000; Sawchenko, 1987), two brain regions key to negative feedback. However, because GRs are present throughout the brain, the involvement of many different brain region in negative feedback cannot be overlooked.

CORT can affect glucocorticoid-sensitive brain regions by binding GRs and MRs to directly regulate gene transcription (Reul & de Kloet, 1985). Mineralocorticoid receptors have a much higher affinity ($K_d = 0.5$ nM) for CORT than do GRs ($K_d = 5.0$ nM) (De Kloet, Veldhuis, Wagenaars, & Bergink, 1984; De Kloet, Vreugdenhil, Oitzl, & Joels, 1998), and consequently, the MRs are mostly occupied when CORT levels are low, such as during the morning trough of CORT in rodents (Bradbury et al., 1991). On the other hand, it is thought that GRs likely become occupied following rises in CORT that can occur after activation of the HPA axis (Reul, van den Bosch, & de Kloet, 1987) and/or at peak concentrations achieved during the diurnal cycle.

Malfunction in the negative feedback loop of the HPA axis results in abnormal CORT levels and can give rise to HPA axis dysregulation. In a series of early studies, PVN infusion of glucocorticoids resulted in decreased activation of the HPA axis, suggesting that this brain nucleus is central to this adaptive process (Kovacs & Makara, 1988; Sawchenko, 1987).

Sex difference in and sites of negative feedback—CRH and AVP neurons of the PVN express GR (Uht, McKelvy, Harrison, & Bohn, 1988) and as a result, corticosterone can act directly on CRH and AVP neurons of the PVN as well as in anterior pituitary corticotropes to regulate synthesis of these key secretagogues. CORT can also act indirectly in extra-PVN regions expressing MR and GR, such as the hippocampus, to inhibit the secretion of CORT (Akana, Cascio, Du, Levin, & Dallman, 1986; Dallman et al., 1987; Kovacs, Foldes, & Sawchenko, 2000; Sawchenko, 1987) through a trans-synaptic mechanism (Herman et al., 2016). Additional evidence for CORT acting directly on PVN neurons comes from studies where targeted deletion of GR in the PVN increased CRH expression and CORT and ACTH levels in male mice (Jeanneteau et al., 2012). Moreover, Solomon et al. (2015) reported that in a transgenic mouse model, where reductions in the levels of GR were targeted to the PVN, there were corresponding increases in ACTH and CORT levels after acute but not chronic stress, thus supporting the hypothesis that PVN GR directly mediates negative feedback. The same reduction in GR in the PVN increased the levels of the diurnal CORT nadir, thus implicating GR in the maintenance of baseline levels of HPA axis control as well. Further, there were suppressed responses to acute and chronic stress-induced ACTH in the transgenic female mice (Solomon et al., 2015), indicating that GR negative feedback occurred predominantly at the hypothalamus. Previous studies by this group showed that forebrain GR does not play as large a role in regulating the HPA of female mice compared to males, since the PVN selective loss of GR caused a striking increase in HPA reactivity and depression-like behaviors in males but not females (Solomon et al., 2012).

Estrogens have also been shown to regulate negative feedback at multiple sites. Weiser and Handa (2009) showed that treatment with estradiol could inhibit the negative feedback effects of the synthetic glucocorticoid, dexamethasone, likely through effects on or near the

PVN (Weiser & Handa, 2009). In this work, direct implant of wax pellets containing E2 or the ER α agonist (PPT) proximal to the PVN increased stress-induced and diurnal peak CORT. The opposite was found when pellets with DPN, the ER β agonist, were placed in the same region. The Peri-PVN region contains, mostly, GABAergic neurons that project into the PVN. Thus, E might be working through activation of ER α in the peri-PVN to increase the HPA axis reactivity, and reduce GR through activation of ER β . Peripheral administration of the estrogen receptor β agonist, R-DPN, prevented the increased HPA axis reactivity and increased anxiety-like behaviors in female rats in response to the implantation of a wax pellet containing a GR agonist, adjacent to the central nucleus of the amygdala (Weiser, Foradori, & Handa, 2010). Together, such data suggest a possible mechanism for estradiol actions in inhibiting HPA axis reactivity through estrogen receptor β . These results suggest a strong modulation of GR on the HPA axis, marked by a striking sex difference in the negative feedback regulation as a result of estradiol interactions with estrogen receptors.

Gonadal hormone fluctuations and the HPA axis

Fluctuations in gonadal hormones have been shown to modulate the way that males and females react to stress, although the exact mechanism for this has yet to be completely resolved. Changes in gonadal steroid levels are driven by the HPG axis and result in physiological and behavioral changes across the estrous cycle in females. The estrous cycle of the female rodent is characterized by cyclic fluctuations in estradiol and progesterone throughout the reproductive lifespan. For female rodents, there are four stages: proestrus, estrus, metestrus, and diestrus, which can be differentiated by changes in circulating hormone patterns (Levine, 2015). Estradiol begins to rise in the evening of diestrus and peaks near midday on proestrus. This stage is followed by an increase in progesterone in the afternoon of proestrus. These hormonal changes correlate with higher stress hormone levels in female rats noted on proestrus when compared to rats that are in estrus, a time when estradiol and progesterone levels are reduced (Viau & Meaney, 1991). These internal changes are further reflected behaviorally by increased anxiety-like behavioral responses to stressors during proestrus, as compared to estrus (Lovick, 2012). This relationship challenges the experimental approach of using intact females in the study of the stress circuitry and indicates that estrous cyclicity should be monitored in order to allow interpretation of results from females.

In males, the response of the HPA axis to stressors changes dramatically during pubertal development. Testosterone rises shortly before puberty, and subsequently, its levels remain relatively constant. Interestingly, although pre-pubertal and adult male rats exhibit similar CORT and ACTH peak levels following acute stress, pre-pubertal animals take 30–45 min longer to return to baseline levels (Romeo et al., 2006; Romeo, Lee, & McEwen, 2004). These results suggest that changes in the transition from puberty to adulthood have a marked effect on the male HPA axis response to stress. However, whether these changes are driven by the rise in testosterone observed during puberty is not entirely clear. Subsequently, Evuarherhe et al. (2009) tested this hypothesis, and concluded that the rise in testosterone during the pubertal period is indeed key for the proper HPA axis response during adulthood. Thus, gonadal steroids are not only critical for the correct programming of the HPA axis

during the perinatal period, but also play a role in establishing the HPA axis response to stress at puberty.

Gonadal hormones and neuropeptidergic regulation of the HPA

Studying the neuropeptidergic expression profile of hypothalamic neurons is key to the understanding of HPA function. PVN neuropeptide neurons are the drivers of the HPA axis, yet, estrogen receptors are expressed in different proportions in CRH, AVP, and OT neurons populating the PVN (Hrabovszky et al., 2004; Laflamme, Nappi, Drolet, Labrie, & Rivest, 1998; Suzuki & Handa, 2004). Such results suggest their involvement in the estrogen-mediated regulation of the HPA axis.

CRH regulation by estrogen and androgen receptors

CRH neurons play a primary role in the integration of limbic and sensory inputs, and through their varied projections can regulate behavior (Fuzesi, Daviu, Wamsteeker Cusulin, Bonin, & Bains, 2016), autonomic, and neuroendocrine responses (Bale & Vale, 2004). As a mediator of estradiol actions, ER β is expressed at different levels in various subdivisions of PVN CRH neurons in the rat, ranging from 5% co-localization in the medial PVN to 60–80% co-localization in the caudolateral division (Laflamme, Nappi, Drolet, Labrie, & Rivest, 1998). These types of data suggest a potential interaction between these two molecules, where nuclear receptors may act to directly regulate gene transcription (Kushner et al., 2000; Lefstin & Yamamoto, 1998) such as CRH (Miller, Suzuki, Miller, Handa, & Uht, 2004). Examination of the regulation of CRH by estradiol was first described by Vamvakopoulos & Chrousos (1993), who demonstrated that the *Crh* promoter contains several ERE half sites, suggesting the involvement of ERE-mediated effects of estrogen receptor. In support of this, they showed that estradiol treatment increased human *Crh* promoter activity by almost twofold *in vitro* in cells co-transfected with the ER α and a *Crh* promoter–reporter gene construct.

Later *in vitro* studies by Miller et al. (2004) used the *Crh* proximal promoter in transformed rat hypothalamic neurons to show that ER β can bind the *Crh* promoter to regulate transcription (Miller, Suzuki, Miller, Handa, & Uht, 2004). In addition, the *Crh* promoter was differentially regulated by splice variants of ER β , consistent with the demonstration that a variant that lacks the full DNA binding domain (ER β 1 delta3) could signal through non-classical mechanisms by protein: protein interactions involving an SP1 element rather than an ERE (Price et al., 2001). Of importance, regulation by ER α was weak (Miller, Suzuki, Miller, Handa, & Uht, 2004). Similarly, *Crh* promoter activity can be augmented by estradiol or the ER β agonist, DPN, but not the ER α agonist, PPT (Ogura, Kageyama, Hanada, Kasckow, & Suda, 2008). Moreover, the ER β agonist, 3 β -diol, an important metabolite of the non-aromatizable androgen, DHT, can also increase *Crh* promoter activity in CHO-K1 cell lines overexpressing ER β (Huang, Zhu, Fischer, & Zhou, 2008). Together, these studies suggest that estrogens are able to modulate *Crh* gene expression by acting through ER β directly. The fact that only a relatively low percentage of ER β cells co-localize with CRH neurons in the PVN, yet treatment with an ER β agonist is able to decrease the activation of the stress response, is still a conundrum. However, there are a few ways in which these

effects could be explained. First, the anxiolytic properties of ER β might be mediated by extra-PVN regions, which ultimately interact with neurosecretory cell in the PVN. Second, it is possible that the techniques utilized in previous works do not reflect the actual co-localization numbers due to technical limitations (e.g. IHC and microscopy limitations). Third, there are various splice variants of ER β , which may not be necessarily recognized by the antibodies used for the co-localization studies.

Steroid hormone control of arginine vasopressin and regulation of the HPA

The neuropeptide, AVP, has been shown to be involved in the regulation of the stress response and circadian rhythms, as well as osmoregulation, paternal behaviors, and aggression (Sladek, 2010). Expression of this potent neuromodulator has been found in brain regions including the BST, medial amygdala (MeA), supraoptic nucleus (SON), suprachiasmatic nucleus, and PVN (Dumais & Veenema, 2016; Whitnall, Mezey, & Gainer, 1985). AVP can be co-expressed with CRH in the rat PVN, and can be co-secreted with CRH where it acts at the pituitary to enhance the secretagogue activity of CRH (Rivier, Rivier, Mormede, & Vale, 1984; Rivier & Vale, 1983, 1985). Hypothalamic AVP neurons have been shown to be involved in regulation of the HPA axis. In a study using adrenalectomized rats implanted with pellets delivering physiological CORT levels, *Avp* transcription and mRNA levels were significantly elevated (Herman, 1995). Restraint stress was shown to cause a similar increase in AVP activity, suggesting a tight relationship between stress, circulating glucocorticoids, and PVN AVP neurons. In particular, AVP has been shown to play a prominent role mediating the glucocorticoid negative feedback upon the HPA axis (Kovacs & Makara, 1988).

Data from our laboratory and others indicate that the estrogen and androgen regulation of AVP expression could be mediated by direct actions of ER β . For instance, it was shown that the *Avp* promoter could be differentially regulated by ER α and ER β *in vitro* through an upstream ERE. The differential regulatory effects of ER α and ER β on AVP could be due to the constitutive activity of ER β on the *Avp* promoter (Pak, Chung, Hinds, & Handa, 2007; Shapiro, Xu, & Dorsa, 2000). Similarly, the effects of DHT could also be mediated by ER β or AR. *In vivo* studies using pre- and post-pubertal gonadectomized female rats showed that the DHT metabolite, 5 α androstane 3 β -17 β -diol (3 β -diol), increased the number of *Avp* mRNA-expressing cells in the BST, although this increment was not as marked as in animals treated with estradiol (Pak, Chung, Hinds, & Handa, 2009). In summary, although the gonadal steroid regulation of AVP is evident, the mechanism by which this is occurring requires further investigation. However, it is plausible that this mechanism involves several intracellular players, such as AR, ER β , and ER α , acting in parallel, but further regulated by ligand-specific actions.

Gonadal steroid effects on oxytocin and the HPA axis

The neuropeptide hormone, oxytocin, is primarily produced by neurons residing in the PVN and SON (Onaka, 2004). Oxytocin is widely known for its actions on parturition, lactation, and maternal behaviors (Leng, Meddle, & Douglas, 2008). OT is synthesized at two main sites in the hypothalamus: the PVN and the supraoptic nucleus (SON). OT neurons in the PVN can also regulate the HPA axis since PVN OT is released into the hypothalamo-

hypophyseal portal vasculature, enhancing the CRH-mediated release of ACTH via binding the V1b type vasopressin receptor (Schlosser, Almeida, Patchev, Yassouridis, & Elands, 1994). Of note, the OT-ergic regulation of the HPA axis has been shown by several laboratories. Although OT can act at the pituitary to enhance CRH action, when delivered to the PVN, OT decreased HPA axis gain, as shown by decreased CORT and ACTH levels (Neumann, Krömer, Toschi, & Ebner, 2000; Windle et al., 2004; Windle, Shanks, Lightman, & Ingram, 1997). Furthermore, global deletion of OT in female mice results in increased stress-induced CORT secretion and *Crh* mRNA expression in the PVN (Mantella, Vollmer, Rinaman, Li, & Amico, 2004; Nomura et al., 2003), suggesting that OT may regulate HPA axis gain at the PVN level as well as the pituitary.

Gonadal steroids can act in a synergistic manner to decrease HPA axis gain and anxiety-related behaviors (Kudwa, McGivern, & Handa, 2014; McCarthy, McDonald, Brooks, & Goldman, 1996), and this may be mediated through modulation of OT neuron function. OT neurons in the PVN express androgen receptors (Zhou, Blaustein, & De Vries, 1994). However, 85% of OT neurons in the PVN also express ER β in the rat (Hrabovszky et al., 2004; Suzuki & Handa, 2004) and in mice (Oyola, Thomson, Handa, & Handa, 2017). Interestingly, the ER β -driven reduction in stress-induced CORT and ACTH levels, and PVN neuronal activation is very similar to that observed following OT treatment (Ochedalski, Subburaju, Wynn, & Aguilera, 2007; Windle et al., 2006, 2004).

Another twist on the potential mechanism for the HPA down-regulation by testosterone is through its conversion to 3 β -diol, a weak androgen, which has been shown to bind ER β [reviewed in Handa, Weiser, and Zuloaga (2009b)]. Supporting this possibility, studies using a rat hypothalamic cell line that constitutively expresses OT, reported that levels of OT mRNA were significantly increased following 3 β -diol treatment (Sharma, Handa, & Uht, 2012), and this was accompanied by an increased interaction between the *Ot* promoter, cAMP response element-binding protein, and the transcriptional regulatory protein, steroid receptor co-activator-1. These findings suggest that 3 β -diol aids in the molecular machinery assembly involving cAMP response element and the steroid receptor coactivator-1 to drive the expression of the *Ot* gene. Consistent with these findings, 3 β -diol also increases *Ot* mRNA expression in the midcaudal, but not the rostral portion, of the rat PVN (Hiroi et al., 2013). This study also reported an increase in *Ot* mRNA in hypothalamic N38 cells following 3 β -diol treatment, indicating its effectiveness in regulating OT *in vivo* and *in vitro*. These effects of ER β agonists were tracked to an ERE half-site in the *Ot* proximal promoter and thereby verify some molecular mechanisms for *Ot* gene expression regulation by ER β . Moreover, the role of OT in mediating the actions of ER β on the HPA axis can be demonstrated by studies showing that an OT antagonist can partially block the inhibitory effects of an ER β agonist on HPA axis function (Kudwa, McGivern, & Handa, 2014). These fundamental observations help dissect the molecular mechanisms underlying the actions of ER on OT regulation and may aid our understanding of the complexities of HPA axis regulation and further begin to point out potential therapeutic targets for the control of stress-related pathologies in the future.

The hypothalamic–pituitary–gonadal axis

Reproduction is driven and directed by another neuroendocrine axis, the hypothalamo–pituitary–gonadal (HPG) axis. Like all neuroendocrine axes, the HPG axis consists of interactions between the hypothalamus and the anterior pituitary gland, and in the case of the HPG axis, this drives the growth and maturation of germ cells, and the synthesis of gonadal steroids in both, male and female gonads. At its apex, the decapeptide, gonadotropin releasing hormone (GnRH), is widely accepted as the hypothalamic peptide controlling reproduction. It stimulates the secretion of the anterior pituitary hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), that ultimately control the production of gonadal steroids and gametogenesis (Figure 2).

Gonadotropin releasing hormone (GnRH)

GnRH, independently discovered by Andrew Schally and Roger Guillemin and coworkers over 45 years ago (Burgus et al., 1972; Schally, Nair, Redding, & Arimura, 1971), is synthesized and secreted from a subset of neurons in the rostral forebrain of rodents (Jennes & Conn, 1994). Hypothalamic GnRH neurons are specialized neurosecretory cells that release GnRH into the hypothalamo-hypophyseal portal vasculature in a regular pulsatile fashion. GnRH acts on the pituitary to stimulate the secretion of the gonadotropins, LH and FSH, which are also secreted in lock step with the pulsatile release of GnRH from the hypothalamus.

GnRH neurons have a very curious lifespan, arising in the nasal placode in early development and then migrating into the brain along the vomeronasal nerve to eventually take up their adult location in the hypothalamus (Schwanzel-Fukuda & Pfaff, 1989; Tobet & Schwarting, 2006; Wray, Nieburgs, & Elkabes, 1989). In the rodent, GnRH neurons are located in a swath from the medial septum to the medial pre-optic area, whereas in the human and non-human primate they are found further caudal in the medial basal hypothalamus (Rance, Young, & McMullen, 1994; Witkin, Paden, & Silverman, 1982). From there, GnRH neurons extend axons to the hypophyseal portal vasculature. A comprehensive review of the development of GnRH neurons can be found in Wray (2010).

In the rodent, hypophysiotropic GnRH neurons project to the median eminence where they release GnRH in a regular pulsatile manner (Bakker, Rubin, & Baum, 1999; Herbison, 2016; Jimenez-Linan, Rubin, & King, 1997; Silverman, Kokoris, & Gibson, 1988). The organization of these pulses is critical for puberty and reproduction (Herbison, 2016). The activity of GnRH is regulated by neurotransmitters, steroid hormones, and growth factors (Watanabe, Fukuda, & Nabekura, 2014). GnRH pulses are required for reproductive functions in both sexes, and the importance of these pulses in driving reproduction has been described in primates and humans (Tsutsumi & Webster, 2009). In women with hypogonadotropic hypogonadism, regular ovulatory cycles can be reinstated by the timed pulsatile infusion of GnRH but not by similar levels of GnRH delivered in a constant fashion (Tsutsumi & Webster, 2009). Moreover, studies have demonstrated that slow release of GnRH can selectively drive the synthesis of FSH- β in the anterior pituitary gland, whereas fast pulses of GnRH selectively drive LH- β synthesis (Burger, Dalkin, Aylor, Haisenleder, &

Marshall, 2002; Haisenleder, Dalkin, Ortolano, Marshall, & Shupnik, 1991; Kaiser, Jakubowiak, Steinberger, & Chin, 1997).

GnRH action at the anterior pituitary

Mediating the effects of GnRH on anterior pituitary gonadotrophs is the GnRH receptor, a seven transmembrane receptor that is intracellularly coupled to G α q. Stimulation of GnRH receptors on gonadotrophs results in elevations in intracellular calcium, which drives the release of LH and FSH by exocytosis into the general circulation to eventually reach the gonads (Duran-Pasten & Fiordelasio, 2013). Curiously, GnRH receptors have also been described in numerous tissues throughout the body, including the brain (Cheung & Wong, 2008; Wilson et al., 2006), but their functional significance has not been completely determined.

LH and FSH action on steroidogenesis: males

LH and FSH release is critical for the maintenance of spermatogenesis and steroidogenesis in males. LH receptors are present on Leydig cells in the testis, whereas FSH receptors are expressed predominantly by Sertoli cells. LH stimulates the synthesis of testosterone by Leydig cells (Ramaswamy & Weinbauer, 2014), whereas FSH stimulates sperm cell maturation and supports Sertoli cells. Ultimately, the secretion of testosterone is involved in controlling the HPG axis through its negative feedback action on hypothalamic GnRH neurons and anterior pituitary gonadotrophs. This relationship has been demonstrated numerous times by studies using castrated male rats, and other species, where castration results in increased levels of gonadotropins and testosterone replacement drives secretion back to baseline (Damassa, Kobashigawa, Smith, & Davidson, 1976; Tilbrook & Clarke, 1995).

LH and FSH action on steroidogenesis: females

In females, LH and FSH are essential for the production of estrogens and progesterone. In the ovary, LH controls steroidogenesis by acting upon thecal and granulosa cells. During the follicular phase of the human menstrual cycle, LH acts upon thecal cells to increase synthesis of enzymes involved in the production of the androgen, androstenedione. Androstenedione is released from thecal cells and taken up by adjacent granulosa cells, where it is converted to estrone by the actions of aromatase and then to estradiol by the actions of 17 β -HSD-1. In granulosa cells, these two enzymes are regulated by FSH binding to the FSH receptor. The net result of this coordinated action of LH and FSH on thecal and granulosa cells of the ovary gives rise to increases in circulating estradiol and supports the two cell – two gonadotropin hypothesis (Stocco, 2001). While estrogens are made by the conversion of androstenedione into estradiol in ovarian granulosa cells, these cells lack the enzymes required to directly metabolize progesterone into the androgenic precursors of estrogens (Doshi & Agarwal, 2013) and must depend on the-cal cells to provide estrogenic precursors. At mid-cycle, elevated LH levels not only are responsible for ovulation, but also drive the luteinization of the follicle and the development of the corpus luteum. Increases in LH receptor in granulosa cells further stimulate enzymes required for the production of progesterone, which can be now be secreted in large amounts (Kaiser, 2011).

Steroid feedback loop

Under basal conditions, estradiol acts through a negative feedback loop to restrict the secretion of GnRH (Figure 2). This long-loop negative feedback is similar to that of many neuroendocrine axes, where the end product, in this case, estradiol or testosterone, acts upon hypothalamic “motorneurons” that drive the neuroendocrine axis. This system helps maintain relatively constant levels of HPG drive and gonadal hormone secretion.

In rodents, estradiol is also known to have a stimulatory effect on GnRH secretion, and this positive feedback action is essential for ovulation in the female (Evans, Dahl, Mauger, & Karsch, 1995; Levine & Ramirez, 1980; Sarkar & Fink, 1980). As a result of follicle growth and the rising titers of estradiol that culminate late on proestrus morning, a surge of LH is initiated in the afternoon, which drives ovulation. This positive feedback response is a sexually differentiated phenomenon, where male rodents are incapable of responding to elevated estradiol with a surge of LH (Corbier, 1985; Gogan, Beattie, Hery, Laplante, & Kordon, 1980; Olster & Blaustein, 1991). Evidence that positive feedback is sexually differentiated by the neonatal actions of testosterone comes from studies showing that neonatal castration of male rats allows them to respond to estradiol in adulthood with an LH surge (Handa, Corbier, Shryne, Schoonmaker, & Gorski, 1985). Conversely, estrogen or testosterone treatment of neonatal females defeminizes the brain such that they can no longer respond to estradiol in adulthood with a surge of LH (Handa, Corbier, Shryne, Schoonmaker, & Gorski, 1985). Thus, sensitivity of the post-pubertal female brain to respond to elevations in estradiol with a surge of LH is organized by the absence of estradiol derived from testosterone in neonatal females.

The site of negative and positive feedback on the HPG axis is still being explored. Although it would be easy to hypothesize that estradiol acts directly on GnRH neurons to limit their activity, this does not appear to be the case. Early studies failed to detect the presence of estrogen receptor in GnRH neurons, giving rise to the hypothesis that estrogen negative feedback was indirect, perhaps through estrogen receptors that were found in neighboring GABAergic neurons (Shivers, Harlan, Morrell, & Pfaff, 1983). Some studies using immunohistochemistry in rat brain have indeed shown the presence of ER β in GnRH neuron (Butler, Sjoberg, & Coen, 1999; Hrabovszky et al., 2007; Hrabovszky et al., 2001; Kallo, Butler, Barkovics-Kallo, Goubillon, & Coen, 2001; Navarro et al., 2003; Roy, Angelini, & Belsham, 1999). However, a conundrum arises in that ER β has not been detected in GnRH neurons of mice, yet they still respond to estradiol (Handa, Ogawa, Wang, & Herbison, 2012).

GnRH neurons do not seem to express ER α either, suggesting that another upstream player is involved in the regulation of GnRH (Herbison & Theodosis, 1992). A relatively recent player implicated in the regulation of GnRH was introduced with the discovery of the Rfamide, kisspeptin (Lee et al., 1996). Kisspeptin is a hypothalamic peptide encoded by the *KISS 1* gene (Kotani et al., 2001) and it has been found to be a potent stimulus for GnRH secretion (Herbison, 2016). Kisspeptin neurons have been shown to express ER α (Adachi et al., 2007; Clarkson, d'Anglemon de Tassigny, Moreno, Colledge, & Herbison, 2008; Franceschini et al., 2006) and the kisspeptin receptor GPR54 is expressed in GnRH neurons (Clements et al., 2001; Kotani et al., 2001; Muir et al., 2001; Ohtaki et al., 2001).

Kisspeptin neurons have been found in two main brain areas: the arcuate nucleus and the anteroventral periventricular nucleus (Clarkson, d'Anglemont de Tassigny, Colledge, Caraty, & Herbison, 2009; Clarkson & Herbison, 2006). These two populations of kisspeptin neurons are responsible for vastly different responses in GnRH neurons. Moreover, Clarkson and Herbison (2006) detected a female bias in the levels of kisspeptin expression in the anteroventral and pre-optic periventricular nucleus of the mouse hypothalamus (Clarkson & Herbison, 2006). Rising titers of estradiol can activate kisspeptin neurons in the AVPV causing a concomitant activation of GnRH neurons, which apparently drives the pre-ovulatory surge of GnRH from the hypothalamus. In contrast, activation of kisspeptin neurons in the arcuate nucleus has been shown to directly influence the regulation of pulsatile GnRH release (Han et al., 2005; Millar et al., 2010). The first observations indicating that kisspeptin neurons might be involved in the negative feedback regulation by gonadal steroids were seen in gonadectomized rats, where removal of the gonads significantly increased *KiSS 1* mRNA levels, and this effect was reversed by replacement with gonadal steroids (Navarro et al., 2004).

In addition to kisspeptin, mRNA levels of neurokinin B are also upregulated by gonadectomy (Sandoval-Guzman, Stalcup, Krajewski, Voytko, & Rance, 2004). Both, neurokinin B and kisspeptin, have now been shown to have stimulatory effects on the release of GnRH and are both co-localized in some neurons of the arcuate nucleus. A third neuropeptide dynorphin, has also been found in arcuate nucleus kisspeptin neurons but has an inhibitory effect on GnRH secretion (Plant, 2015). Thus, it appears to be the interaction of these three players that sculpt the pulsatile patterns of GnRH secretion. While kisspeptin and neurokinin B can increase the frequency of GnRH pulses, secretion of dynorphin will reduce pulse frequency. These data suggest a complexity whereby estradiol can control both positive and negative feedback action. Arcuate nucleus kisspeptin, neurokinin B, and dynorphin co-expressing neurons initiate pulsatile hormone patterns in the absence of estradiol and are inhibited at low levels of estradiol to account for negative feedback, yet AVPV localized kisspeptin neurons are activated by rising titers of estradiol and initiate the pre-ovulatory surge of LH (Wang, DeFazio, & Moenter, 2016).

Steroid metabolites and the control of the HPA axis

Peripheral steroid hormones are able to cross the brain-blood barrier, due to their lipophilic nature, and can be converted to biologically active metabolites within the brain through actions of several enzymes such as aromatase, 3 β -HSD, and 5 α -reductase (Lephart, Lund, & Horvath, 2001). These metabolites are thought to regulate cell function by interacting with classical steroid hormone receptors. For example, the effects of testosterone on the brain are often controlled by the intra-cellular conversion of testosterone to estradiol by the aromatase enzyme or to DHT by the 5 α -reductase enzyme (Arevalo, Azcoitia, & Garcia-Segura, 2015). Inhibition of 5 α -reductase using finasteride abolishes the inhibitory effects of testosterone on the HPA axis in male rats (Handa, Kudwa, Donner, McGivern, & Brown, 2013). 5 α -Reductase also converts progesterone and glucocorticoids into their respective metabolites in the brain (Celotti, Negri-Cesi, & Poletti, 1997; Lephart, 1996; Lephart, Lund, & Horvath, 2001). The non-aromatizable androgen, DHT, may also be further metabolized to the estrogen-like androgen, 3 β -diol, via 3 β -HSD and then metabolized by the cytochrome P450

enzyme CYP7B1 (Handa, Weiser, & Zuloaga, 2009a). 3 β -diol acts by binding and activating estrogen receptor β , rather than an androgen receptor (Weihua et al., 2001). As mentioned in previous sections, these metabolites have regulatory effects on the HPA axis (Figure 3) potentially through the regulation of the *Ot* or *Avp* promoter (Hiroi et al., 2013; Pak, Chung, Hinds, & Handa, 2007).

Interestingly, the mRNA transcripts levels of aromatase, 5 α -reductase, and CYP7B1 are highly present in the PVN, indicating their potential role in the local metabolism of steroids and further regulation of the HPA axis (Lund, Hinds, & Handa, 2006). The presence of these enzymes in stress-regulated brain regions, and their regulatory potential, raises questions regarding the classical view of estrogen modulation of the HPA axis. For example, testosterone in males may work through estrogen receptors, rather than androgen receptors, by its aromatization to estradiol. Moreover, testosterone can be further converted into 3 β -diol and thereby act through ER β to reduce the gain of the HPA axis (Figure 3). Correspondingly, if testosterone is converted into estradiol, both ERs could be activated due to the indiscriminative binding properties of estradiol to both ERs. Since ERs can have opposing effects on HPA activity (ER β decreases, while ER α increases HPA axis gain), then the gain of the HPA axis in males could be dependent upon the enzymes levels that are present. Moreover, additional regulation could be provided by two other factors. First, the quantity of ERs present in a particular brain region, and, second, the ratio of ER α vs. ER β at a particular time point. Physiological fluctuations in the ER α : ER β ratio could dictate how the HPA axis would respond to a stressor. However, this possibility has not been explored in relation to the HPA axis.

Some of the enzymes involved in the local metabolism of estrogens and androgens in the brain are sexually dimorphic. One of the most widely studied of these is aromatase, whose expression levels are much higher in the male stria terminalis, paraventricular pre-optic area, ventromedial nucleus, pre-optic nucleus, and anterior nucleus of the hypothalamus (Roselli, 1991). Also, aromatization of testosterone into estrogen is known to trigger neurite growth and neuronal plasticity in sexually dimorphic brain regions, including the sexually dimorphic nucleus of the rat pre-optic area (Davis, Popper, & Gorski, 1996; Davis, Shryne, & Gorski, 1996). Brain-metabolized estradiol also has a strong influence on stress, reproduction, learning, and memory, as well as sexual behaviors (Blomqvist, 2000; Fink, Sumner, McQueen, Wilson, & Rosie, 1998).

As discussed here, internal cues are critical for the regulation of the HPG axis. However, external signals, like stress, are well known to affect this axis also. Thus, whether through peripheral hormones acting centrally, or metabolism to other steroids through the major metabolic pathways, a mutual relationship exists between the HPA and HPG axes for allostatic regulation and species survival.

Gonadal steroid receptors as regulators of gene transcription

Upon reaching their target cells, steroid hormones readily diffuse through the cell membrane and interact with cytoplasmic or nuclear receptors. The cascade of signaling events triggered by gonadal steroids is regulated by the binding of the hormone to its cognate receptor in a

tissue- and region-specific manner. Binding of the steroid hormones, such as corticosterone, to the unliganded steroid receptor induces a conformational change and disassociation from chaperone proteins such as heat shock proteins (HSP90). This modification leads to dimerization of the steroid hormone receptor complex, followed by translocation of the steroid hormone receptor to the target DNA. The steroid hormone receptor complex is accessible to DNA regulatory regions, which contain specific hormone response elements. These hormone response elements have at their core, a palindromic sequence of nucleotides that can bind the hormone receptor dimer. Coactivators or co-regulatory proteins, such as p160 and p300 are also recruited to uncoil the inactive DNA from histone proteins, exposing regulatory regions and transcriptional initiation sites of target genes. Transcription of target genes is initiated through activation of the pre-initiation complex (Beato, 1989; Beato & Klug, 2000; Tyagi et al., 2000). In addition to this classic hormone response element dependent-regulation of gene transcription, steroid receptors can also interact with other transcription factor proteins, such as Fos and Jun, that work through other DNA elements such as the AP-1 response element, to regulate gene transcription [for GR and ER actions review see Weikum, Knuesel, Ortlund, & Yamamoto (2017) and Paech et al. (1997) respectively].

Finally, steroid hormones can affect cell function through their interaction with membrane-bound proteins, such as GPR30, also known as GPER [for review, see Prossnitz & Barton (2011)]. Steroid hormone actions through these membrane-bound steroid receptors have rapid effects and involve the alteration in membrane potential and second messenger pathways including extracellular regulate kinase, phospholipase C, Ca^{2+} , mitogen-activated protein, nitric oxide protein kinase A, and protein kinase B. Alternatively, evidence also exists for steroid receptors being localized in caveolae to modulate neurotransmitter receptors, such as mGlu receptors (Meitzen & Mermelstein, 2011). For review of the rapid effects of glucocorticoids see Jiang, Liu, & Tasker (2014).

Sexual differentiation of the rodent brain

The study of sex differences in the brain has unveiled similarities and dissimilarities in the mechanism of neuronal development between sexes (Amateau & McCarthy, 2004; McCarthy & Arnold, 2011; Schwarz, Liang, Thompson, & McCarthy, 2008). Steroid hormones have significant effects on the developing brain and are known to program adult functions required for sex-specific reproductive behaviors. Many behavioral sex differences observed in the adult are the consequences of sex-specific exposure to steroid hormones during critical windows of development. In rodents, male-typical reproductive behaviors, such as mounting, and female-typical behaviors, such as lordosis, are some behaviors organized by gonadal steroid hormones. In the rodent, the absence of testosterone during a critical perinatal period results in the expression of the default program of the brain, which is female-like (McCarthy & Arnold, 2011), whereas perinatal treatment of females with testosterone will defeminize the brain and prevent the expression of female reproductive behaviors in adulthood.

The origin of sexual differences in the brain starts even before gonadal hormones are detected in the plasma (Arnold, 1996; Wade & Arnold, 1996). These changes are thought to

begin when genes on the sex chromosomes (X and Y) determine the type of internal and external morphology to be developed, and hence the level of hormones to be present in the fetus. Questions regarding the effects of X and Y gene imbalance have been explored over the past decade, and the data suggests that X and Y gene dose discrepancies account for several sex-biased phenotypes in the brain and periphery (Arnold, 2004; Arnold et al., 2016; Burgoyne et al., 1995). The study of direct sex chromosomes effects in brain sexual differentiation, however, is challenging, mainly because chromosomal manipulation could lead to confounding changes in gonadal steroid levels. Development of mouse models that bypass this issue has been used to further advance our understanding of how sex chromosomes work to influence development of the brain and behavior. For a detailed review on the direct influence of sex chromosomes on brain sexual differentiation see Arnold (2017) and Burgoyne and Arnold (2016).

Sexual differentiation of the HPA axis

The sexually dimorphic behavioral and physiological responses to stress are evident from pre-pubertal stages through adulthood. For instance, basal and stress-induced CORT have been shown to be greater in female than in male rodents (Critchlow, Liebelt, Bar-Sela, Mountcastle, & Lipscomb, 1963; Handa, Burgess, Kerr, & O'Keefe, 1994; Kitay, 1961). Furthermore, these sex-dependent patterns are also observed in the activation of PVN neurons controlling HPA axis reactivity following stress (Larkin, Binks, Li, & Selvage, 2010; Seale, Wood, Atkinson, Harbuz, & Lightman, 2004; Viau, Bingham, Davis, Lee, & Wong, 2005). These responses are thought to be delineated in part by gonadal steroids acting during pre- and perinatal stages as well as at puberty (McCormick & Mathews, 2007). For example, removal of endogenous gonadal hormones by castration of male rats during the peri-natal stage causes a female pattern of ACTH and CORT response to stress during adulthood (McCormick, Furey, Child, Sawyer, & Donohue, 1998).

In line with classical mechanisms for sexual differentiation of the rodent brain, the effects of neonatal gonadectomy on male HPA axis function can be reversed by testosterone treatment during the perinatal period (Bingham & Viau, 2008). Moreover, sex differences in the pulsatile pattern of CORT secretion have been identified by the Lightman group, where females have a higher amplitude and frequency of CORT pulses than males (Lightman & Conway-Campbell, 2010). Interestingly, the CORT pulsatile pattern of adult female rats can be masculinized by treatment with testosterone 24 h after birth, resulting in CORT pulse patterns in adulthood that are similar to those observed in males (Seale, Wood, Atkinson, Harbuz, & Lightman, 2005). On the other hand, aromatization blockade in males immediately after birth results in an augmented CORT and ACTH response to stress in adulthood, similar to that of females (Bingham, Wang, Innala, & Viau, 2012), and akin to that observed following perinatal castration. Thus, these studies further demonstrate the importance of testosterone and its metabolites in the sexual differentiation of the HPA axis.

Summary and conclusion

To increase survival and reproductive success, the HPA and the HPG axes work together to fine-tune each other thereby integrating environmental, psychological, reproductive, and

genetic factors. The HPG and HPA axes both exhibit striking sex differences, which are organized and activated by dynamic changes in sex and stress hormones across the lifespan. It can be speculated that, if applicable to humans, the developmental programs determining sex differences in the HPA axis may influence vulnerability to develop neuropathological disorders triggered by stress in adulthood. To maintain homeostasis, the neuroendocrine system continuously monitors the levels of gonadal steroids using estrogen and androgen receptors found in the hypothalamus. Hypothalamic neurons expressing gonadal steroid receptors are of great importance for the proper regulation of the HPA and HPG axes, and thus, the composition of these cells requires further investigation. Dysregulation of either or both of these axes can result in compromised responses to stressful life events. The fluctuations in estradiol and progesterone present across the estrous cycle of adult rodents are arguably responsible for most activational effects in the female brain, thereby regulating the behavioral and physiological repertoire to stress seen during adulthood. In sum, the HPG and HPA axes work in a parallel, and in a carefully choreographed fashion, to successfully maintain species survival and perhaps minimize allostatic load. Although steady advancements have been achieved in the last few years, there are still questions remaining to explore. First, although much has been revealed as to how individual steroid hormones can regulate neuropeptide regulators of the HPA axis from a molecular perspective, the interactions between steroid hormone receptors on specific neuropeptide promoters has not been described and would allow us to better understand the molecular perspective by which specific hormones interact to have distinct and sex specific effects across cell types. Second, elucidating the co-regulatory proteins involved in steroidal regulation of the neuropeptide genes would shed light on specific cell types and intracellular messenger pathways, or brain-specific effects.

Further investigation of how the neonatal hormone milieu influences adult HPA axis function may allow us to understand epigenetic programming of gene expression associated with the HPA axis. It is now known that hormones have specific actions in given brain regions. Thus, the localization, expression levels, and activity of steroid hormone receptors may further help explain why certain hormones and their metabolites have sex-specific effects. On the same note, understanding steroid hormone metabolism and subsequent pre-receptor modulation of the HPA axis is still underway.

Finally, throughout this article we focused on the HPA axis from the perspective of the hypothalamus and its downstream targets. Much less is known about the upstream regulators of the HPA axis. For instance, how do decisional making processing centers, like the prefrontal cortex, interact, either directly or indirectly, with upstream targets of the PVN to regulate the activation of the HPA and HPG axes, and how do extra-hypothalamic regions, such as the BnST help to further coordinate the neuroendocrine stress responses and its response to gonadal steroid hormones? Future research probing these areas will provide new and exciting mechanisms and avenues for exploration of sex differences and steroidal modulation of stress responses.

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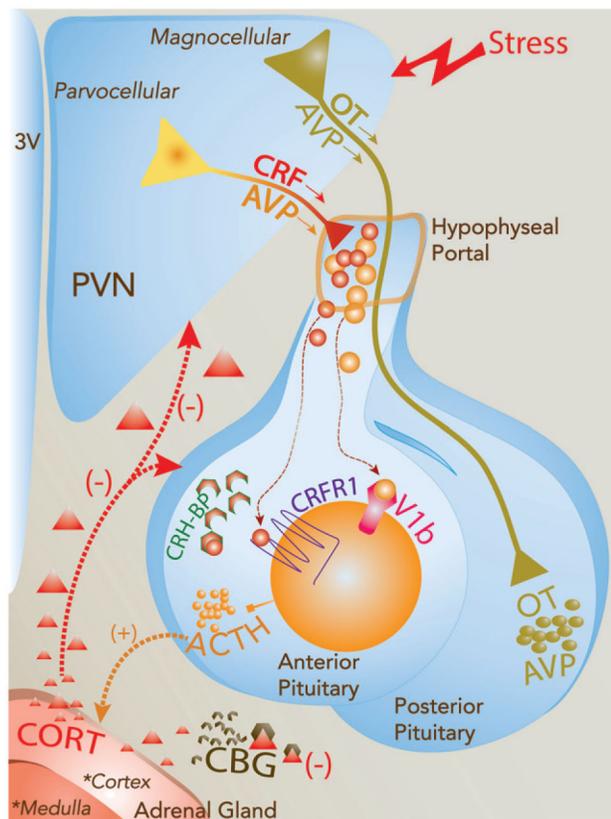


Figure 1. Schematic diagram of the HPA axis and factors controlling the neuro-endocrine response to stress. Stress signals are relayed to the paraventricular nucleus of the hypothalamus (PVN) where oxytocin (OT), vasopressin (AVP), and corticotropin-releasing hormone (CRH) neurons reside. These neurons are recruited to trigger the HPA axis response. First, a subset of hypophysiotropic PVN neurons releases CRH and AVP into the hypophysial portal vasculature. These neuropeptides are transported to the anterior pituitary to act on corticotropes through their binding to CRHR1 and V1b receptors thereby stimulating the synthesis and release of adrenocorticotropic hormone (ACTH) into the general circulation. CRH activity on corticotropes is also regulated by the CRH-binding protein, which impedes its interaction with CRHR1. OT and AVP also control CRH actions at the pituitary level. In the adrenal cortex, the binding of circulating ACTH to the melanocortin type 2 receptor (MC2R) stimulates the synthesis and secretion of corticosterone (CORT) into the general circulation. Circulating CORT can then modulate behavioral and physiological responses to stress. CORT is also capable of inhibiting HPA axis activation (negative feedback) by acting in brain regions including the PVN, pituitary, hippocampus, and other limbic areas. The activity of circulating CORT is in part regulated by the CORT binding globulin (CBG). In contrast, axons of OT and AVP in the magnocellular division of the PVN project to the posterior pituitary, where they release OT and AVP to the general circulation to regulate the physiological response to stressors, osmoregulation, and reproductive function.

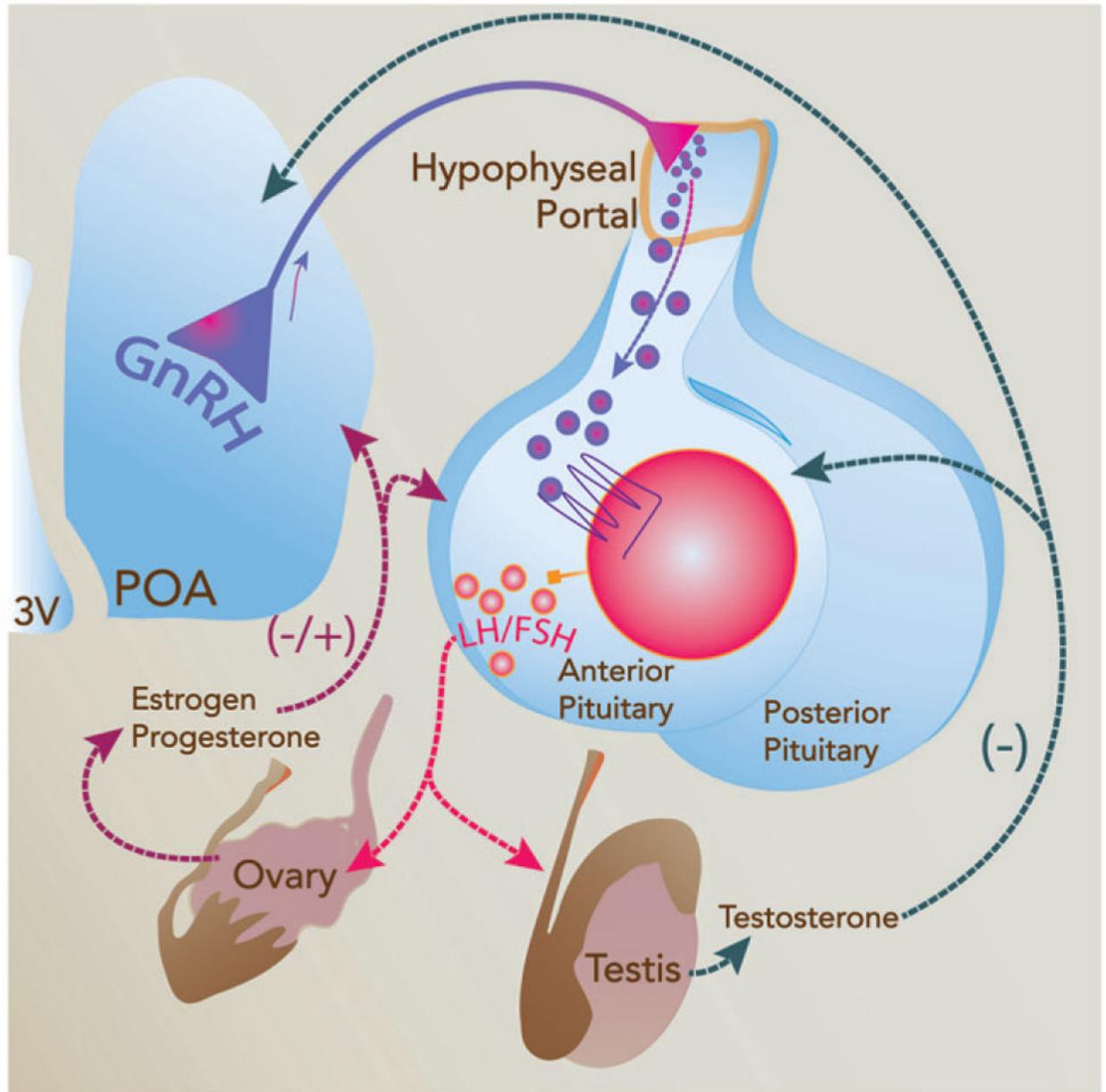


Figure 2.

Schematic diagram showing the hypothalamic–pituitary–gonadal (HPG) axis and factors involved in its regulation. Hypophysiotropic neurons in the rodent hypothalamus secrete gonadotropin-releasing hormone (GnRH) into the hypothalamo-hypophyseal portal vasculature at the median eminence, where it is carried to the anterior pituitary. Once in the anterior pituitary, GnRH stimulates gonadotroph cells to synthesize and secrete luteinizing hormone (LH) and follicular stimulating hormone (FSH). LH and FSH act on the ovary and testis to regulate steroidogenesis and gametogenesis. Once released, steroid hormones signal through the anterior pituitary and steroid-sensitive hypothalamic neurons to feedback upon

the HPG axis to regulate its activity and to other brain areas thereby controlling reproductive behaviors and functions, including HPA axis activity.

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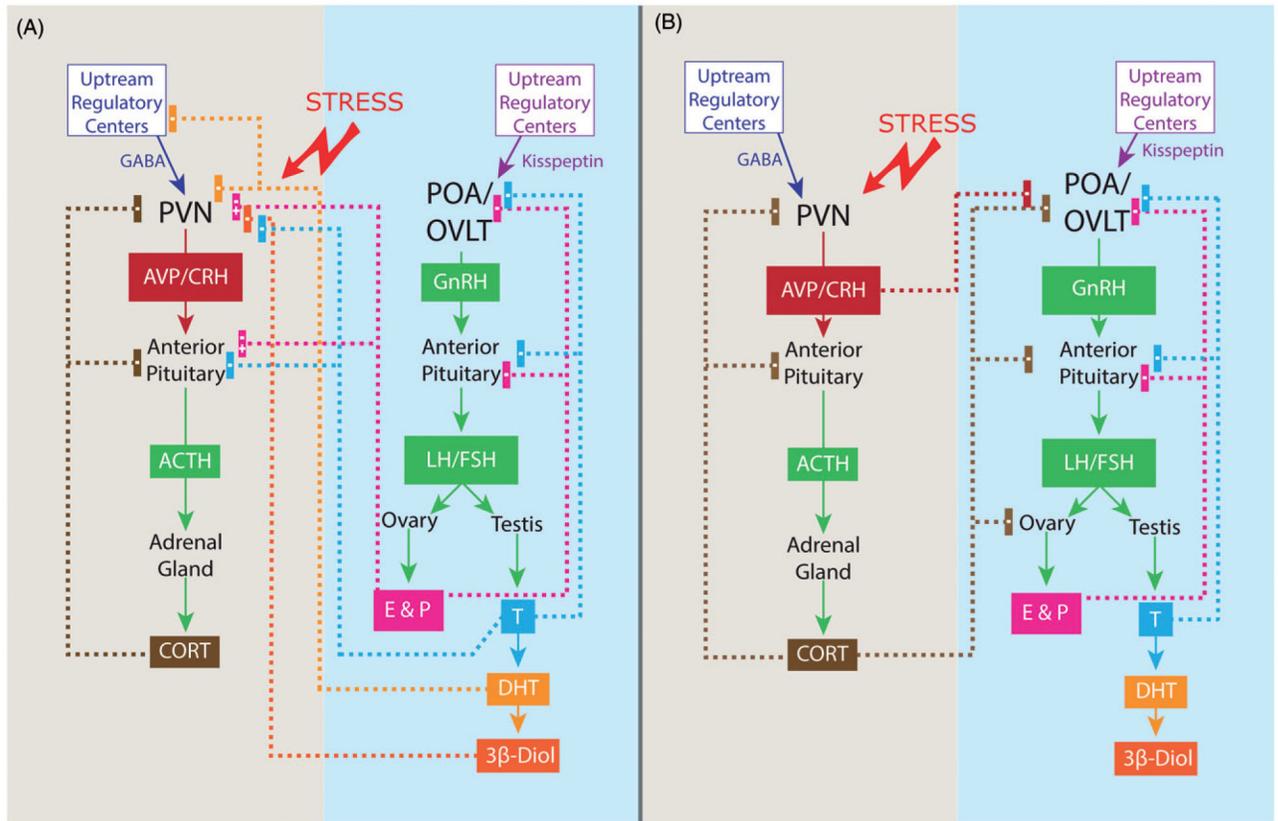


Figure 3.

Schematic diagram demonstrating the reciprocal interaction between the HPA and HPG axes. Panel A: Hormones of the HPG axis are involved in the regulation of the HPA axis at different levels, as delineated by dotted lines. Both axes are influenced by upstream regulatory centers. In the case of the HPA axis, most of these centers release GABA either directly into the PVN or in its immediate periphery. In the case of the HPG axis, upstream centers release kisspeptin to regulate GnRH activity. Panel B: Hormones from the HPA axis are involved in the regulation of the HPG axis at different levels, as delineated by dotted lines. Abbreviations: PVN: paraventricular nucleus of the hypothalamus; AVP: arginine vasopressin; CRH: corticotropin releasing hormone; ACTH: adrenocorticotrophic hormone; CORT: corticosterone; POA: pre-optic area; OVLT: organum vasculosum lamina terminalis; GnRH: gonadotropin releasing hormone; LH: luteinizing hormone; FSH: follicle stimulating hormone; E: estrogen; P: progesterone; T: testosterone; DHT: dihydrotestosterone.