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Emerging insights into Hypothalamic-pituitary-gonadal (HPG) axis regulation and interaction with stress signaling

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Abstract

Reproduction and fertility are regulated via hormones of the hypothalamic-pituitary-gonadal (HPG) axis. Control of this reproductive axis occurs at all levels, including the brain and pituitary, and allows for promotion or inhibition of gonadal sex steroid secretion and function. In addition to guiding proper gonadal development and function, gonadal sex steroids also act in negative and positive feedback loops to regulate reproductive circuitry in the brain, including kisspeptin neurons, thereby modulating overall HPG axis status. Additional regulation is also provided by sex steroids made within the brain, including neuroprogestins. Furthermore, as reproduction and survival need to be coordinated and balanced, the HPG axis is able to modulate—and be modulated by—stress hormone signaling, including cortiscosterone, from the hypothalamic-pituitary-adrenal (HPA) axis. This review covers recent data related to the neural, hormonal, and stress regulation of the HPG axis and emerging interactions between the HPG and HPA axes, focusing on actions at the level of the brain and pituitary.

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1. Introduction

Reproduction is necessary for the continuation of species, and reproductive success is dependent on many neuropeptide and hormonal systems working in concert to regulate gonadal function and sexual behavior. Reproduction is governed by the hypothalamic-pituitary-gonadal (HPG) axis. The hypothalamic control of reproduction is coordinated through the release of gonadotropin-releasing hormone (GnRH). GnRH, typically secreted in pulses, stimulates secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the anterior pituitary gonadotropes. These hormones function at the gonads to stimulate the production of gametes and promote gonadal release of sex steroids [i.e., testosterone (T), estradiol (primarily 17β-estradiol, E2), and progesterone (P4)]. Along with guiding reproductive function in the peripheral tissues, these gonadal steroids can also feedback and modulate upstream HPG components. Each level of the HPG axis is tightly regulated but can be modulated to influence reproductive status. This review will focus on neural and hormonal factors controlling and modulating HPG axis activity, as well as how the HPG axis can influence and be influenced by stress signaling.

2. The Neuroendocrine Reproductive Axis and its Upstream Control by Kisspeptin and RFRP-3

Within the brain, an interconnected network of neurons regulates the pulsatile release of GnRH; however, many of the key mechanisms and factors involved in the regulation of GnRH release, including modulation of GnRH pulsatile and surge modes of secretion, remain poorly-defined. Within the past decade, the neuropeptides kisspeptin and RFRP-3 have been shown to have potent stimulatory or inhibitory actions on GnRH secretion in mammals, thereby modulating reproductive status.

The *Kiss1* gene encodes the neuropeptide kisspeptin, which potently stimulates GnRH secretion[1]. Early experiments determined that exogenous kisspeptin treatment of rodents and other species, including humans, robustly increases circulating LH and FSH levels (reviewed in [1,2]). Subsequent studies from numerous species have now provided a wealth of information supporting the model that hypothalamic-derived kisspeptin directly activates GnRH neurons via kisspeptin receptor (Kiss1R) to stimulate the reproductive axis (reviewed in [1,2]). In a wide range of mammals, including rodents, sheep, and primates, *Kiss1* mRNA or kisspeptin protein has been detected primarily in two discrete regions of the hypothalamus, the preoptic area [POA; which in rodents includes the morphological continuum comprising the anteroventral periventricular nucleus and neighboring periventricular nucleus (AVPV/PeN)] and, more caudally, the arcuate nucleus (ARC; analogous to the primate infundibular nucleus) (reviewed in [3,4]).

In adulthood, the secretion of GnRH is regulated by positive and negative feedback actions of gonadal sex steroids. Importantly, GnRH neurons do not express estrogen receptor α (ERα) or the androgen receptor (AR), the receptors that mediate sex steroid feedback, indicating that other sex steroid-sensitive circuits "upstream" of GnRH neurons receive and relay sex steroid feedback signals to GnRH cells[3]. Recent evidence suggests that hypothalamic kisspeptin neurons, which express ERα and AR, are these upstream sex steroid-sensitive neurons. Kisspeptin neurons in the ARC are likely involved in promoting pulsatile GnRH and LH secretion and responding to sex steroid hormone negative feedback[1,2]. In contrast, kisspeptin neurons in the AVPV/PeN are thought to be involved in mediating E2-positive feedback which triggers the preovulatory LH surge in females[5]. Supporting this hypothesis, AVPV/PeN *Kiss1* gene expression is greater in females than males (males do not show an LH surge), *Kiss1* neurons in the AVPV/PeN express ERα and show increased neuronal activation exclusively during the LH surge, and *Kiss1* knockout (KO) mice and Kiss1R KO mice do not exhibit an LH surge even with exogenous E2 treatment[3,5].

In contrast to kisspeptin, another neuropeptide, RFamide-related peptide-3 (RFRP-3), has potent inhibitory actions on LH secretion in many mammalian species[6–8]. RFRP-3 is encoded by the *Rfrp* gene and is the mammalian orthologue of avian gonadotropin-inhibiting hormone (GnIH). RFRP-3 neurons are found exclusively in the dorsal-medial nucleus of the hypothalamus (DMN) of rodents[7,8] and may be involved in influencing the onset of puberty, the onset of cyclicity in seasonally reproductive mammals, and the suppression of GnRH-induced release of gonadotropins[9,10]. RFRP-3 has been functionally shown to inhibit the electrical firing of some GnRH and ARC kisspeptin neurons[11–13] suggesting that RFRP-3 may inhibit the reproductive axis, in part, by signaling to kisspeptin and/or GnRH populations. Interestingly, both RFRP-3 and kisspeptin neurons are influenced by stress hormone regulation, as will be discussed further below.

3. GnRH Regulation of Pituitary Gonadotropin Production

The gonadotrope is a target for many different endocrine inputs; yet, none of these is more fundamental than GnRH. The unique phenotype of gonadotropes is defined by expression of the GnRH receptor (GnRHR) on the plasma membrane and the synthesis and secretion of LH and FSH. It is well established that GnRH induces the release of stored LH secretory granules into the systemic circulation[14,15]. Increased GnRH pulse frequency and amplitude favor LH secretion, while a slower frequency favors FSH secretion[16,17]. This elegant mechanism is particularly important for female reproduction, where increases in GnRH pulsatility are required for the LH surge and, thus, ovulation.

GnRH binding to its cognate membrane receptor leads to an increase in GnRHR numbers on the plasma membrane and increased expression of the different gonadotropin subunits. The GnRHR is a member of the rhodopsin like family of seven transmembrane domain G protein coupled receptors (GPCR)[18]. Hormone binding the GnRHR activates Gαq/11 which initiates multiple phospholipase activities leading to formation of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol[19,20]. Receptor activation also increases intracellular

calcium concentrations through release of intracellular stores and opening of voltage gated L-type calcium channels, which underlie activation of protein kinase C (PKC) isoforms[21].

Transcription of the LHβ gene is highly sensitive to stimulation by GnRH. The proximal 140 base pairs (bp) of the LHβ gene promoter is highly conserved across species, but the GnRH responsive promoter regions vary between species. The proximal 140bp consist of tandem copies of DNA binding motifs for the early growth response protein 1 (Egr-1) and steroidogenic factor 1 (SF-1) transcription factors[22,23]. Sandwiched between the tandem Egr and gonadotrope specific element (GSE) motifs, is the binding site for Pitx1, a paired-like homeodomain transcription factor[24]. Data from transgenic mice suggest that the GSE and the Pitx1 elements are required for GnRH induced LHβ gene promoter activity[23,25]. Additionally, GnRH stimulation results in an increase in Egr-1 mRNA and protein, which is tightly linked to an increase in LHβ synthesis[26].

GnRH also induces epigenetic mechanisms to regulate LHβ gene expression. Lim et al. showed that the gene encoding LHβ, *Lhb*, is occupied by histone deacetylases (HDACs) in αT3-1 cells resulting in transcriptional silencing[27]. In this cell line, GnRH stimulated the export of class IIa HDACs resulting in increased acetylation of gonadotropin genes, while in LβT2 cells, another gonadotrope derived cell line, GnRH treatment enhanced the association of the histone acetyltransferase p300 with the *Lhb* gene[28]. Chromatin organizing the β-subunit gene promoters also contains low levels of trimethylated histone H3 lysine 4 (H3K4me3). GnRH stimulation increases this modification resulting in chromatin decondensation by menin and the menin-mixed-lineage leukemia (MLL) 1/2 methyl transferase complex[29]. Work by Khan *et al.* shows that GnRH also induces the citrullination of histone H3 arginine residues 2, 8, and 17 to stimulate expression of the *Lhb* gene[30]. Thus, GnRH stimulation initiates both epigenetic and transcriptional mechanisms to increase LHβ gene expression in gonadotrope cells.

4. Estrogen Modulation of Reproductive Hypothalamic Circuits

Estrogens, primarily E2, feedback to the brain to control the pulsatile release of GnRH into the primary portal plexus through bimodal interactions with the hypothalamic neurocircuitry that modulate GnRH neurons. These neuronal circuits include kisspeptin neurons, both in the AVPV and the ARC nuclei, vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) neurons of the suprachiasmatic nucleus (SCN), RFRP-3 neurons of the DMN, corticotropin-releasing hormone (CRH) neurons of the paraventricular nucleus (PVN), and the melanocortin (proopiomelanocortin (POMC) or neuropeptide Y and agouti-related peptide (NPY/AgRP)) neurons of the ARC[31–33]. These hypothalamic circuits collectively express the range of ER and employ both nuclear-initiated estrogen signaling (through estrogen response element (ERE)-dependent and ERE-independent pathways) and membrane-initiated estrogen signaling (through palmitoylated nuclear ER (α/β) and E2-responsive GPCR)[34].

Nuclear-initiated ERα and ERβ signaling controls gene expression primarily through direct binding to DNA via an ERE. After ligand-dependent receptor activation, ER disassociates from molecular chaperones, dimerizes, and binds to coactivators or corepressors to initiate

or suppress transcription[34]. Alternatively, ERα and ERβ can modulate transcription through protein-protein interactions with other transcription factors interacting with DNA in non-ERE promoter sites.[35] Membrane-associated ERα and ERβ can also activate signaling cascades to modulate cell physiology and control gene expression including phosphatidylinositol 3′-kinase (PI3K), phospholipase C (PLC), MAPK and protein kinase pathways (PKA, PKC, etc.)[36] . A second putative, Gq-coupled, E2-responsive GPCR (Gq-mER) controls PLC-mediated signaling in a wide variety of hypothalamic neurosecretory neurons[36,37].

ERα has been identified as the key ER mediating the negative and positive feedback of E2. ERα KO mice are infertile and do not have a normal estrous cycle or the proestrus surge of LH, while ERβ KO mice are subfertile with regular estrous cycles[38]. E2 also modulates GABAergic tone of GABA neurons in the POA, and specific deletion of ERα in hypothalamic GABA neurons produces infertile female mice due to a failure to generate an LH surge and ovulation[39]. However, GnRH neurons from both sexes, and from many species, express ERβ but not ERα[31]. Indeed, membrane-initiated signaling via ERβ activates a PLC-mediated signaling cascade leading to retrograde endocannabinoid signaling to reduce the frequency of presynaptic GABA release and spontaneous postsynaptic currents[40]. E2 at high physiological concentrations enhance GnRH firing frequency through modulation of the after-hyperpolarization potential via a membrane-mediated ERβ-PKA pathway in mice[41]. Furthermore, activation of GPER by its selective ligand STX in mouse GnRH neurons controlled the expression and activity of cation channels involved in burst firing and membrane hyperpolarization[42,43], indicating the E2 has multiple pathways and receptors to control GnRH neuronal excitability, gene expression, and peptide release. ER expression across the various cell types of the hypothalamic-pituitary circuit are summarized in table 1.

As previously discussed, ARC-derived kisspeptin is the primary “gatekeeper” of GnRH pulsatility. Both populations of kisspeptin neurons express ERα (with low ERβ expression) but respond differentially to E2 through ERE-dependent and ERE-independent mechanisms[44,45]. In females, ARC kisspeptin expression is suppressed through ERα-mediated, ERE-independent mechanisms or de-repressed through a hormone response element[44,46]. Neurokinin B and dynorphin, co-expressed with kisspeptin (KNDy neurons) in the ARC nucleus and vital components of the GnRH pulse generator, are also downregulated by E2 but through ERα-mediated, ERE-dependent mechanisms[44,45]. In AVPV/PeN kisspeptin neurons, *Kiss1* mRNA expression and kisspeptin synthesis are stimulated by E2 through ERα-mediated, ERE-dependent mechanisms[45]. This E2-induced expression of AVPV/PeN kisspeptin occurs with an increase in kisspeptin neuronal activation and peptide release onto GnRH neurons to drive the pre-ovulatory surge of GnRH and LH[3,5].

In rodents, neurons of the SCN, the body’s central circadian clock, play a prominent role in the promotion and timing of the LH surge initiated at the beginning of the dark cycle. These SCN neurons synthesize either AVP and project to AVPV/PeN kisspeptin and RFRP-3 neurons or synthesize VIP and project to GnRH and RFRP-3 neurons, thereby controlling the timing of the LH surge[47]. Neither AVP nor VIP neurons of the SCN express detectable ERα or ERβ protein in the mouse[48]. However, E2 restores AVP-induced activation of

kisspeptin neurons in the AVPV/PeN in ovariectomized (OVX) female mice[49,50], and E2 perfusion can rapidly depolarize SCN neurons by increasing the frequency of excitatory postsynaptic currents leading to an increase in firing frequency[51]. Elevated E2 levels preceding the surge of GnRH and LH activate SCN neurons to accelerate the downstream release of kisspeptin and simultaneously decrease the inhibition of GnRH secretion provided by RFRP-3[32,52].

E2 also impacts the inhibitory hypothalamic inputs, including DMN RFRP-3 neurons. E2 reduces RFRP-3 expression in the DMN of mice, although only ~20% of RFRP-3 neurons express detectable levels of ERα protein and no detectable ERβ[53]. In the hamster, E2 reduces RFRP-3 neuronal activation, perhaps to promote downstream GnRH surge secretion, with ~40% of RFRP-3 neurons in the female hamster expressing ERα[7]. The low expression of ERα and ERβ in RFRP-3 neurons suggests that membrane-initiated estrogen signaling may be key to the regulation of these neurons, as has recently been characterized in ARC NPY neurons[54]. However, this has yet to be studied in RFRP-3 neurons.

Several other populations of hypothalamic neurons also can excite or inhibit GnRH neurons and mediate E2's influence on the pulsatile and surge release of LH. One such population is CRH neurons of the PVN, which are the master control neurons of the stress response. CRH intracerebroventricular (ICV) administration suppresses both LH pulsatility and the LH surge, and this suppression is enhanced by E2[55], potentially through CRH innervation of the locus coeruleus[56]. However, recent evidence indicates that CRH directly modulates GnRH excitability in a dose-dependent and receptor-specific manner under the influence of E2[57]. CRH neurons are also activated by membrane-initiated E2 signaling through the Gq-mER to suppress the M-current and augment excitatory postsynaptic current amplitude[58]. Another population of neurons modulated by E2 is the melanocortin neurons of the ARC nucleus that express POMC or NPY and AgRP. The two neuropeptides produced by POMC neurons, α-MSH and β-endorphin, differentially control GnRH excitability and release. α-MSH activation of the melanocortin 4 (MC4) receptor increases GnRH action potential firing while β-endorphin signaling through μ-opioid receptors inhibit GnRH excitability[33,59]. Furthermore, NPY can excite and inhibit GnRH neurons through the Y1 and Y4 receptors, respectively, and AgRP, an endogenous agonist to MC4 receptors, can also excite and inhibit GnRH neurons[33,60]. Both POMC and NPY neurons express ERα protein, although at strikingly different levels[54,61]. Furthermore, both neurons express the Gq-mER, which disinhibits GABA and μ-opioid tone in POMC neurons and augments GABAergic tone in NPY neurons[54]. The distinct E2-induced responses in the melanocortin neurons potentially amplify E2's role in the negative feedback on the HPG axis.

5. Neuroprogesterone Control of the Reproductive Axis

Emerging evidence indicates that steroid hormones synthesized in the brain, termed "neurosteroids," have impact on the reproductive axis. In some brain areas, locally derived pregnenalone is converted to P4 via 3β-hydroxysteroid dehydrogenase (3β-HSD) which is then metabolized to allopregnenalone (ALLO), the main active derivate, by 5α-reductase and 3α-HSD[62]. Both 5α-reductase and 3α-HSD are expressed in the cortex, hippocampus, and amygdala[62], supporting ALLO's potential ability to modulate behavior.

Neurosteroids, particularly P4 and ALLO, have been associated with mood and anxiety disorders[63]. While P4 may exert behavioral effects through the classical nuclear P4 receptor (PR) mechanism[64], most of P4's effect may correspond to P4 conversion to ALLO[63]. ALLO interacts with the $GABA_A$ receptors as an allosteric modulator[63,65] and enhances GABA-mediated inhibition at $GABA_A$ receptors, and, at high doses, ALLO acts as a $GABA_A$ receptor agonist. Additionally in the last decade, a new pathway of ALLO action through membrane P4 receptors (mPR α and β) was discovered allowing for rapid responses[65]. ALLO and its stereoisomer, pregnanolone, can also be sulphated allowing them to modulate glutamatergic neurotransmission through NMDA receptors[63]. It is under debate whether sulphated neurosteroids reach neurophysiological levels to exert endogenous modulatory effects on glutamatergic neurotransmission[63]. The mechanisms of actions of ALLO need more clarification due to the differences found between receptors and areas of action.

ALLO interaction with $GABA_A$ receptors contributes to ALLO's anxiolytic, neuroprotective, neuromodulatory, and anti-gonadotropic properties[66–68]. In serotonergic neurons, ALLO, not P4, potentiated $GABA_A$ receptor response to agonists[69]. Serotonin signalling disruption is associated with mood disorders thereby suggesting an association between ALLO and serotonergic signalling. This effect of ALLO on serotonin signalling may be pertinent for disorders linked to reproductive hormone fluctuations such as premenstrual dysphoric disorder and postpartum depression[69].

ALLO fluctuations during the estrous cycle have a strong impact on reproductive function[70,71]. ALLO levels decrease in the hypothalamus during proestrus, when ovulation occurs, which supports a role for ALLO in modulating the HPG axis to control ovulation[70]. One ICV administered dose of ALLO on the morning of proestrus has an inhibitory effect on both ovulation rate and sexual receptivity[71,72]. When ALLO ICV injection was coupled with a $GABA_A$ receptor antagonist, sexual receptivity was recovered, suggesting that some ALLO action involves $GABA_A$ signalling[71,72]. Furthermore, Giuliani et al. observed increased GnRH release from hypothalamic slices following treatment with ALLO which was absent when slices were also treated with an NMDA receptor antagonist. This suggests that ALLO may also modulate NMDA receptor activity to alter HPG axis activity[73].

ALLO signalling can also influence development of the ovary. Rats treated with a single ICV injection of ALLO on the morning of proestrus showed ovarian disorders, such as follicular atresia, ovarian cysts, and delayed luteolysis, an apoptotic process of the corpus luteum[71]. ALLO treatment also caused alterations in both follicular and luteal dynamics during the estrous cycle[74].

6. Stress and Reproduction: Bidirectional Interactions

Stress signaling is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Upon exposure to a stressor, the PVN is activated to release CRH which then stimulates release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH, in turn, stimulates release of glucocorticoids (CORT; corticosterone in rodents, cortisol in humans) from the adrenals which then provide negative feedback back to the brain in a classic homeostatic feedback

loop to fine-tune HPA axis signaling. The HPG and the HPA axes are tightly linked to balance reproduction and survival.

6a. Influence of Stress on Reproductive Status and Brain Circuitry

Stress, including psychological stress, can negatively impact reproduction in many mammalian species, including humans. In females, stress can disrupt ovarian cyclicity as well as upstream gonadotropin synthesis and secretion[75,76]. For example, restraint stress, a commonly-used model of psychosocial stress, reduces LH and FSH in rodents and other species[77,78]. In multiple species, psychosocial stress exposure or treatment with exogenous CORT inhibited the pulsatile release of LH[79–81] which is assumed to reflect inhibition of upstream GnRH pulsatile secretion. To date, little is known about underlying mechanisms for stress suppression of GnRH and downstream LH secretion, especially at the level of the brain. Given the importance of ARC kisspeptin neurons in directing pulsatile GnRH secretion, AVPV/PeN kisspeptin neurons in generating the LH surge, and RFRP-3 neurons in inhibiting GnRH and LH output, it is possible that stress alters downstream GnRH and LH secretion via upstream actions on kisspeptin and/or RFRP-3 neurons.

The E2-induced surge mode of GnRH and LH secretion (i.e., positive feedback), which governs ovulation in females, is vulnerable to the effects of stress. Isolation-restraint stress can block the LH surge in rats, and in sheep and monkeys, the LH surge can be delayed or blocked by stress[82,83]. Despite this evidence, the fundamental mechanisms that result in disrupted surge LH secretion are not well understood. In a recent collaboration between the Breen and Kauffman labs, exogenous CORT treatment of gonad-intact female mice arrested cyclicity in diestrus[84]. A separate cohort of OVX female mice were treated with an LH surge-inducing E2 implant, as well as a CORT or cholesterol (control) pellet, and assessed two days later for LH levels on the evening of the anticipated LH surge. All cholesterol-treated females showed a clear LH surge, whereas LH levels were undetectable in CORT-treated females[84]. Brain analyses revealed that CORT robustly suppressed the percentage of AVPV/PeN *Kiss1* cells co-expressing *cfos*, as well as reduced the number of AVPV/PeN *Kiss1*-expressing cells, compared to expression in control brains[84]. Collectively, these findings in female mice support the hypothesis that physiological stress-levels of CORT disrupt ovarian cyclicity, in part, through disruption of positive feedback mechanisms at hypothalamic kisspeptin circuits which are known to be necessary for LH surge generation.

The effects of stress on LH pulsatility in mice, whose small size posed a decades-long technical challenge for assaying LH in repeated blood samples, was recently tested for the first time when a new LH assay and blood sampling methodology was invented. OVX female mice were either not stressed (controls) or exposed to 90 min of restraint stress, during which time serial blood samples were collected every 5 minutes to measure pulsatile LH levels. LH pulse frequency and interpulse intervals were significantly and rapidly decreased in stressed female mice compared to controls, with no effects on LH pulse amplitude[85]. Next, to determine the effects of acute restraint stress on reproductive neuropeptides known to regulate GnRH neurons, OVX female mice were separated into control (no stress) or 45, 90, or 180-min restraint stressed groups. Acute restraint stress had no effect on *Kiss1* mRNA levels, though there was a significant decrease in *Kiss1* neuronal

activation at all time points, suggesting a decrease in stimulatory kisspeptin input to GnRH neurons[85]. *Rfrp* neuronal activation in female mice was significantly increased after 45 min of restraint stress but not following longer stress durations, suggesting that increased RFRP-3 signaling, known to inhibit GnRH and LH, may occur rapidly following acute stress[85]. Furthermore, *Rfrp* mRNA levels in female mice were elevated after 180 min of restraint stress, perhaps as a compensatory response to replenish RFRP-3 stores that were reduced when these neurons were more active during initial stress exposure[85].

Collectively, the data described in this section suggest that stress-induced suppression of the reproductive axis in female mice may be due, at least in part, to regulation at levels upstream of GnRH neurons, either directly or indirectly, through ARC kisspeptin and/or DMN RFRP-3 neurons. Supporting the mouse data, in rats, 5 hours of restraint stress increased hypothalamic *Rfrp* expression and decreased mean LH secretion via a pathway dependent on elevated CORT[77]. The specific underlying neural, hormonal, and cellular mechanisms by which stress alters RFRP-3 and kisspeptin neurons remain to be determined.

6b. Influence of Reproductive Hormones on the HPA axis

Anxiety and mood disorders are associated with HPA axis dysregulation and show large gender disparities, with women being more likely to experience these disorders[86]. This gender disparity in prevalence supports a role for gonadal steroids, E2, P4, and T, in modulating the HPA axis. Through these gonadal steroids, the HPG axis can exert a modulatory effect over the HPA axis (for review see [63,86]).

Numerous studies find that basal CORT levels are higher in females than males and that following restraint stress, glucocorticoid release was higher in females[86], suggesting that E2 increases HPA axis reactivity. This is further supported by observations that during the estrus phase of the estrous cycle, when E2 levels are lowest, CORT response to stress is reduced compared to during proestrus when E2 and P4 levels are elevated[87]. Additionally, OVX females showed reduced levels of CORT release following restraint stress[86], and gonadectomized (GDX) males treated with E2[88] or estradiol benozoate (EB)[89] showed elevated CORT release following acute restraint stress compared to control animals. While these data suggest that E2 increases HPA axis reactivity, the effects of E2 were not always consistent[86].

This inconsistency of E2's effect on the HPA axis relates to E2 activity at ERα and ERβ. Signaling through these 2 receptors yield opposing effects on HPA axis reactivity, with ERα increasing and ERβ inhibiting HPA axis reactivity (*vide infra*). Both ER receptors are found throughout the brain and with overlapping expression in numerous areas important in HPA axis regulation[86]. Male GDX rats treated with an ERα agonist showed increased HPA axis reactivity following acute stress[88]. When rats were treated with EB or an ERα agonist near the PVN, glucocorticoid receptor (GR)-mediated suppression of CORT release following acute restraint stress was inhibited, and this inhibition was not seen when animals were treated with an ERβ agonist. These results suggest that the GR-induced negative feedback of the HPA axis was impaired by ERα on GABAergic neurons in the peri-PVN area[90]. ERα is also expressed in other GABAergic neurons that project into the PVN, suggesting that ERα

may reduce inhibitory input into the PVN, thereby reducing negative feedback from the glucocorticoids[90].

Rats[88,89] and mice[91] treated with an ERβ selective agonist showed decreased stress-induced ACTH and CORT release compared to vehicle-treated animals. This reduction in HPA axis reactivity was also seen when ERβ in the PVN were selectively activated[88]. Within the PVN, ERβ is expressed in a large population of oxytocin neurons, in approximately half of CRH neurons, and a small population of AVP neurons[92]. Oxytocin has been shown to reduce HPA axis reactivity, and E2, via ERβ signaling, increases oxytocin mRNA levels (for review see [93]). Additionally, ERβ has been shown to bind to the promoter of all three neuropeptides[86]. The mechanism through which ERβ reduces HPA axis reactivity has yet to be determined but could be through modulation of these PVN neuropeptides.

Progesterone and ALLO have also been implicated in HPA axis modulation and in human psychopathology (for review see [63]). Stress increases brain and plasma P4 and ALLO concentrations, and increased concentrations may allow for termination of HPA axis signaling[63]. The effects of P4 on HPA axis reactivity are thought to be primarily due to conversion of P4 to ALLO rather than through intracellular PR signaling[63]. As previously discussed, ALLO binds to $GABA_A$ receptors to promote inhibition[63]; however, ALLO administration may also reduce HPA axis reactivity through reduction of CRH and AVP gene transcription[63].

While E2 increases HPA axis reactivity, T has been found to reduce HPA axis reactivity[86]. T binds to AR, which is expressed in hypothalamic reproductive areas, such as the ARC, VMN and POA nuclei[86]. GDX male rats showed elevated CORT release following stressor exposure compared to gonad-intact males[94]. This elevated CORT release was reduced with administration of T or DHT, a T metabolite[94]. Additionally, treatment with DHT reduced CORT release following restraint stress[88,89]. T treatment reduced CORT release in response to stressors when administered into brain areas that synapse onto the PVN[86] as well as when DHT was administered just above the PVN[88]. However, the effect of DHT within the PVN is not solely through AR signaling, as AR antagonist treatment does not completely abolish the effect of T on reducing HPA axis reactivity[88]. DHT can be metabolized to 3β-diol which is an ERβ specific ligand thereby contributing to DHT's inhibition of HPA axis reactivity[88].

6c. Betamethasone Effects on the HPG Axis in Development and Adulthood

During intrauterine development, the hormonal systems that regulate the HPG and HPA axes plays a crucial role in the growth and development of fetal tissues[95]. In the third trimester of pregnancy, an increase in fetal glucocorticoid concentration occurs[95]. This increase provides a number of modifications in several tissues and organs essential for fetal survival, including lung maturation and pulmonary surfactant production[96]. With preterm births, these changes have yet to occur as endogenous concentrations of glucocorticoids are still low. Therefore, antenatal therapy using glucocorticoids, especially betamethasone, is critical to promote lung maturation in these scenarios[97].

In rodents, a critical window of study is between GD 12 to GD 19[98–100]. During this period of intrauterine growth, important changes in fetal development are occurring, such as the

gonadogenesis, the establishment of the HPG axis and the morphogenesis of reproductive ducts and glands (epididymis, vas deferens, seminal vesicle and prostate)[98–100]. Thus, glucocorticoid exposure in this critical window of development becomes important, as any disturbance can lead to irreversible damage on the reproductive tract[95,101]. The increase in glucocorticoid concentration with antenatal therapy directly interferes in the control of the HPG axis, leading to a decrease in GnRH release and, consequently, a decrease in FSH and LH[102,103]. In addition, the increase in glucocorticoid concentrations interfere with serum T concentration since it inhibits T biosynthesis leading to decreased rates of T secretion from Leydig cells[104,105].

The secretion of T, especially in the final periods of fetal development, is fundamental for the processes of masculinization and defeminization of the hypothalamus. Pereira et al. observed that intrauterine exposure to hydrocortisone during this critical window of fetal development interfered with these two processes[98], and Pereira & Piffer observed an incomplete masculinization of the hypothalamus followed by damage of seminal vesicle contractility in adult rats exposed *in utero* to hydrocortisone[99]. Piffer et al. observed changes in serum T concentrations, sperm production and sexual preference of adult males following intrauterine betamethasone exposure[100]. These findings demonstrate the impact of glucocorticoid exposure on the masculinization process of the hypothalamus. Borges et al. reinforced these findings and also demonstrated that the impact of fetal programming promoted by betamethasone exposure was seen at birth, as low pup weight, at puberty, as reduced serum T levels and delayed puberty onset, and at adulthood, as direct effects on gonadal morphology and function, sperm quality and fertility[106–108]. Indeed, *in utero* betamethasone exposure confirmed its effects acting mainly on the HPG axis, since the same pattern of hormonal alteration was observed in both, male and female pups, as well as in the following two generations[106–109].

Pedrana et al. also observed that *in utero* betamethasone exposure directly affected the development of sheep testes, decreasing the size of the testicular cords and interstitial space, suggesting an impact on Leydig cells, since they express GR[110]. Reduction of the seminiferous tubules diameter as well as reduced T production were also observed by Borges et al[107,108]. Moreover, the intrauterine exposure to betamethasone promoted drastic alterations in the seminiferous tubule morphology, compromising its cytoarchitecture and spermatogenesis[108].

Overall, treatment with glucocorticoids during a critical window of development promotes alterations to the reproductive system, for both, male and female offspring, which continued into adulthood. Additional studies should be conducted to clarify, based on the exposure periods, how the reproductive organs can be reprogrammed throughout development, especially considering the critical periods of organogenesis and the establishment of the HPG axis.

7. Conclusions

Altogether, we can see that the HPG axis is an elegantly-regulated endocrine system that allows for successful reproduction and species propagation. Many components, including

neural and hormonal factors, contribute to the proper regulation of the HPG axis and provide many interesting avenues for future research. The complex interactions between these hormonal and neuronal systems are summarized in Figure 1. As reproduction and survival are necessary for life, it follows that the two systems promoting these aspects, the HPG and HPA axes respectively, would be interlinked. Through further study of the HPG axis and the components regulating it, we can better understand factors contributing to reproductive maladies and health disorders.

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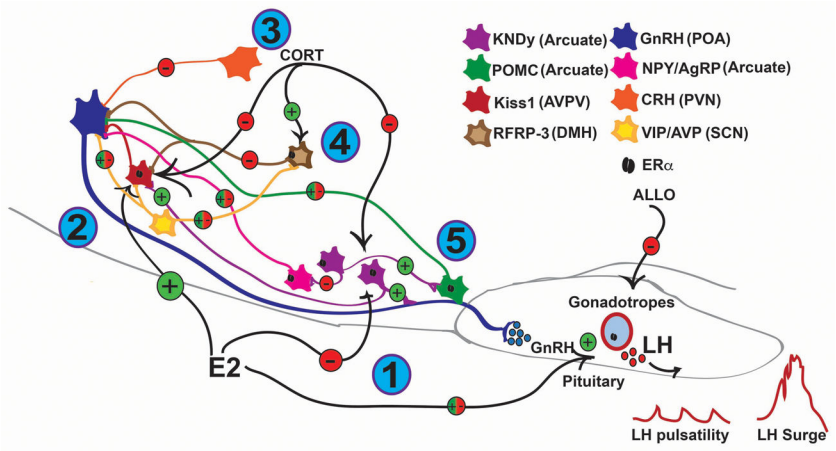

Figure 1.

Table 1

Estrogen receptor expression in the hypothalamic-pituitary circuits

Cell Type	ERα	ERβ	GPER1	Gq-mER
GnRH	−	+	+	+
Kiss1	+	+	+	ND
KNDy	+	+	+	ND
POMC	+	−	ND	+
NPY/AgRP	+	+	ND	+
CRH	−	+	+	+
RFRP-3	+	−	ND	ND
SCN - AVP	−	−	ND	ND
SCN - VIP	−	−	ND	ND
Gonadotropes	+	+	+	ND

+: expression; -: no expression; ND: no data