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### Publication Date

2019-12-01

### DOI

10.1016/j.mce.2019.110579

Peer reviewed



# HHS Public Access

Author manuscript

*Mol Cell Endocrinol.* Author manuscript; available in PMC 2020 December 01.

Published in final edited form as:

*Mol Cell Endocrinol.* 2019 December 01; 498: 110579. doi:10.1016/j.mce.2019.110579.

## Neural and Endocrine Mechanisms Underlying Stress-Induced Suppression of Pulsatile LH Secretion

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### Abstract

Stress is well-known to inhibit a variety of reproductive processes, including the suppression of episodic Gonadotropin releasing hormone (GnRH) secretion, typically measured via downstream luteinizing hormone (LH) secretion. Since pulsatile secretion of GnRH and LH are necessary for proper reproductive function in both males and females, and stress is common for both human and animals, understanding the fundamental mechanisms by which stress impairs LH pulses is of critical importance. Activation of the hypothalamic-pituitary-adrenal axis, and its corresponding endocrine factors, is a key feature of the stress response, so dissecting the role of stress hormones, including corticotrophin releasing hormone (CRH) and corticosterone, in the inhibition of LH secretion has been one key research focus. However, some evidence suggests that stress hormone alone are not sufficient for the full inhibition of LH caused by stress, implicating the additional involvement of other hormonal or neural signaling pathways in this process (including inputs from the brainstem, amygdala, parabrachial nucleus, and dorsomedial nucleus). Moreover, different stress types, such as metabolic stress (hypoglycemia), immune stress, and psychosocial stress, appear to suppress LH secretion via partially unique neural and endocrine pathways. The mechanisms underlying the suppression of LH pulses in these models offer interesting comparisons and contrasts, including the specific roles of amygdaloid nuclei and CRH receptor types. This review focuses on the most recent and emerging insights into endocrine and neural mechanisms responsible for the suppression of pulsatile LH secretion in mammals, and offers insights in important gaps in knowledge and future directions.

### Keywords

Luteinizing Hormone; Gonadotropin; Reproduction; Stress; GnRH; Kisspeptin; Kiss1; RFRP3; Rfrp

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**Disclosure Statement:** The authors have nothing to disclose.

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## INTRODUCTION

Stress is defined as a real or perceived threat to homeostasis; without an adequate and appropriate physiological response to stressful stimuli, detrimental health or even death will occur (1). An important feature of the body's stress response is a core set of physiological processes that is initiated regardless of which stressful stimulus is applied. Among these is the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the suppression of the hypothalamic-pituitary-gonadal (HPG) axis (2). This makes physiologic sense as reproduction is energetically costly and inhibition of reproduction during stressful periods is beneficial for conserving energy. Indeed, there is clear evidence that stress impacts numerous tissues and processes necessary for reproduction [stress effects on the gonads and reproductive tract have been reviewed recently (3–5)]; here, we will focus on discussing recent and emerging insights into the neural and endocrine mechanisms by which stress impairs pulsatile luteinizing hormone (LH) secretion. Although follicle stimulating hormone (FSH) is a clinically and physiologically important gonadotropin, discussion of this hormone is beyond the scope of this review as its secretory patterns have been recently reviewed recently (6–8), including in another article in this Special Issue.

LH is secreted from anterior pituitary gonadotrope cells and acts to govern sex steroid synthesis and secretion, as well as ovulation, by the gonads (ovaries and testes). The pattern of LH secretion is driven primarily by upstream secretion of gonadotropin releasing hormone (GnRH) from the brain. GnRH itself is typically secreted in pulses, occurring approximately every 20 min to 120 min, depending on the species and hormonal milieu. There is now general agreement that the guiding neural mechanism controlling GnRH pulses—the GnRH pulse generator—is formed by an afferent population of neurons in the arcuate nucleus of the hypothalamus (ARC) that express the neuropeptides kisspeptin, neurokinin B, and dynorphin (KNDy cells; ARC<sup>Kiss1</sup> cells) (9, 10). Kisspeptin acts on GnRH cells (11) or GnRH axon terminals in rodents (12) to stimulate GnRH secretion. Each pulse of GnRH stimulates a corresponding pulse of LH from gonadotrope cells of the anterior pituitary gland (13). Thus, suppressed gonadotropin secretion following stress could be the result of impairment of either ARC<sup>Kiss1</sup> cells, GnRH cells, or gonadotropes (or some combination of these sites).

Broadly speaking, the majority of the published work in this field addresses one of three main questions: 1) Where in the body (primarily, the brain) is the proximate stressor detected? 2) How is this stress signal transmitted in the brain (i.e. what are the neural substrates and which signaling molecules are involved)? 3) What is the site of impaired gonadotropin secretion (i.e. ARC<sup>Kiss1</sup> cells, GnRH cells, or gonadotropes)? Conceptually, these questions represent three phases of the neural process by which stress impairs gonadotropin secretion.

## ELEMENTS OF THE HPA AXIS

During the response to stress, the HPA axis is robustly and rapidly activated, resulting in elevated secretion of corticotropin-releasing hormone (CRH) in the brain, adrenocorticotropin-releasing hormone (ACTH) from the pituitary, and glucocorticoids

(corticosterone in rodents, cortisol in larger mammals) from the adrenals (2). These stress hormones represent common mediators by which diverse types of stress might suppress LH. This section will highlight evidence supporting the sufficiency of HPA axis signaling molecules in the suppression of LH secretion in response to stress.

The neuropeptide CRH is produced in several brain regions, including hypophysiotropic neurons in the paraventricular nucleus (PVN) (14, 15). Early reports that intracerebroventricular (ICV) injection of CRH suppressed LH secretion in rats (16) and monkeys (17–19), and that CRH receptor antagonists prevented stress-induced suppression of LH (20–22), supported the hypothesis that CRH is necessary and sufficient for suppression of gonadotropin secretion during stress. However, follow-up studies reported highly variable responses to CRH, including either no effect (23, 24) or even stimulatory actions (23, 25). Many factors may account for these diverse effects, such as dose, route of administration, CRH preparation, species, and gonad (and hence, sex steroid) status of the experimental model. The inconsistent effects of CRH on LH secretion (inhibitory *vs.* stimulatory *vs.* no effect) are in contrast to the robust suppression of LH that is observed during numerous models of stress, which supports the hypothesis that additional signaling molecules are involved in the suppression of LH during stress. It should be noted that there are occasional reports of increased LH secretion during stress (26–29); these events are largely associated with surge-type LH secretion rather than pulsatile LH secretion. An additional complication is that the CRH antagonists used in these early studies (e.g.,  $\alpha$ -helical CRH and [D-Phe<sup>12</sup>, Nle<sup>21,38</sup>]hCRF-[21–41]) act on both CRHR1 (high-affinity receptor for CRH) and CRHR2 (low affinity receptor for CRH; displaying 70% homology to CRHR1) (30, 31). This makes interpretation of the discrepant results complex and challenging. More recently, a specific CRHR1 antagonist partially reversed the inhibitory effect of acute psychosocial stress, but not immune or metabolic stress, on LH in female rats (32), which indicates that CRH acting via CRHR1 may be necessary for the response to some acute stress types, but not others.

Arginine vasopressin (AVP) is co-secreted with CRH during stress (33) and acts synergistically with CRH to induce ACTH secretion, though the relative importance of these two neuropeptides varies by species (34). ICV administration of AVP suppressed LH secretion in rats (35) and OVX rhesus monkeys (20, 36), but stimulated LH secretion in gonad-intact baboons (37). Intriguingly, Knobil's group reported that AVP infusion caused a slight increase in frequency of ARC multi-unit activity (MUA) volleys (neuron electrical activity), despite lower mean LH levels in peripheral blood. Similarly, bath application of AVP increased intracellular ARC<sup>Kiss1</sup> calcium concentrations in female mice (38). Thus, in females there may be a stimulatory effect of AVP on ARC<sup>Kiss1</sup> cells that can occur with lower LH concentrations, due to factors such as gonadal status or responsiveness of the GnRH cell or gonadotrope to highfrequency ARC<sup>Kiss1</sup> stimulation. An important caveat to these studies is that AVP is synthesized in multiple brain regions to modulate numerous physiological processes; thus, exogenous AVP may target multiple cell-types and brain regions. Indeed, AVP synapses onto GnRH cells have also been described (39), though function and regional source of these connections is unknown. It should be noted that AVP treatment of dispersed pituitary cells from OVX rats did not alter *in vitro* LH release (40).

Peripheral administration of ACTH did not decrease LH secretion in OVX monkeys (41). Moreover, scant expression of MC2R, the receptor for ACTH, outside of the adrenal gland (42) has likely further dampened enthusiasm for investigating a role for this signaling molecule in stress-induced suppression of gonadotropin secretion.

The inhibitory effects of glucocorticoids on reproductive function have been extensively studied in numerous species, and several key differences have been reported. In castrated male monkeys, chronic treatment with hydrocortisone acetate (rapidly converted to cortisol) suppressed pulsatile LH secretion, but did not alter the LH response to exogenous GnRH (43). Similarly, in OVX pigs, chronic hydrocortisone acetate suppressed LH pulse frequency, but not in animals with pituitary stalk transections given exogenous GnRH (44), which implies a hypothalamic site of action because LH pulse frequency is modulated by neural (rather than pituitary) mechanisms. However, glucocorticoid-treated pigs have reduced LH response to GnRH challenge, indicating a pituitary effect as well (45). These data are consistent with a gonadal steroid-independent suppression of GnRH secretion by glucocorticoids, with minimal effect directly on pituitary function, at least in these species. In contrast, OVX sheep given sustained cortisol treatment displayed suppressed LH pulse amplitude, but not frequency, via a reduction in pituitary sensitivity to GnRH, indicating a pituitary effect in this species (46–49). Conversely, in the presence of gonadal steroids, GnRH and LH pulse frequency are suppressed by sustained cortisol treatment (50, 51). This estradiol-dependent suppression of GnRH and LH pulse frequency in sheep is similar to the recent report in female mice that showed an inhibitory effect of chronic corticosterone on LH pulse frequency in OVX mice only when they were also treated with estradiol (52). Intriguingly, in both sheep and mice, the majority of ARCKiss1 neurons contain glucocorticoid receptor (GR) (52, 53), which raises the possibility of direct action in these cells. In contrast, in OVX and estradiol replaced rats, a similar chronic corticosterone treatment did not alter either LH pulse amplitude or pulse frequency (54). Perhaps relatedly, it was reported that virtually no ARCKiss1 (<3%) cells contain GR in rats (55).

## REACTIVE STRESS

Reactive stress is induced by stimuli that present an actual physical challenge to homeostasis; thus, the body is ‘reacting’ to internal stimuli. The most studied are immune/inflammatory and metabolic stress, but also include hemorrhage, osmotic stress and visceral or somatic pain. Immune stress is often modeled by injection of lipopolysaccharide (LPS), a non-replicative component of the gram negative cell wall that induces a nearly-full immune response. Models of metabolic stress include: 1) insulin-induced hypoglycemia (IIH), in which a bolus insulin injection causes rapid peripheral glucose uptake, resulting in hypoglycemia; or 2) injection of 2-deoxyglucose, which models glucoprivation by inhibiting glucose-6-phosphate isomerase, acutely interfering with ATP production.

### How and where is the proximate stressor detected?

Technically, all cells respond to changes in glucose concentrations (i.e. metabolically slowing as energy is depleted). However, there are several populations of neurons that display acute changes in firing rate in response to physiological changes in blood glucose

concentrations. One important region is the area postrema of the brainstem (56). Lesion of the area postrema completely prevents suppression of LH pulses caused by IHH in rats (57), which supports the hypothesis that low blood glucose is primarily detected within this specialized hindbrain region.

Detection of immune stress is comparatively more diffuse. In experimental conditions, LPS activates the innate immune system via toll-like receptor 4, which is expressed throughout the body including several brain regions (58). Toll-like receptor 4 activation causes secretion of proinflammatory cytokines, including interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ). Blockade of either of these cytokine receptors partially reversed the inhibition of LH pulses by LPS treatment (59, 60), suggesting that these cytokines contribute to the inhibitory effect of LPS on LH secretion. Receptors for IL-1 $\beta$  and TNF $\alpha$  are expressed in numerous tissues and cell-types, including the area postrema (61, 62). Like other circumventricular areas, the area postrema has a rarefied blood-brain barrier, and therefore is prime for sensing circulating cytokines. Lesion of the area postrema blunted the ACTH rise following intravenous administration of IL-1 $\beta$  (63), which raises the possibility that other stress-sensitive endocrine systems, such as the network controlling LH, are also modulated by the detection of circulating cytokines via actions within the area postrema. Whether vagal afferents contribute to the suppression of LH following peripheral administration of inflammatory cytokines (64), as they do for induction of ACTH secretion, is unknown.

#### **What are the neural substrates and which signaling molecules are involved?**

The majority of the neural projections from the area postrema are to medullary and pontine nuclei (65), including dense innervation of the nucleus of the solitary tract (NTS), which includes the A2 population of norepinephrine neurons (A2<sup>NE</sup>) (66). Activation of A2<sup>NE</sup> neurons has been demonstrated in response to a variety of stressors, including metabolic (67) and immune stress (68), and A2<sup>NE</sup> neurons project broadly throughout the hypothalamus, including to the PVN (69). Interestingly, NE microinjection into the PVN suppressed pulsatile LH secretion in rats (70). Ablation of NE cells that project to the PVN prevented the disruption of estrous cycles caused by glucoprivation in rats (71), supporting the possibility that a brainstem NE neuron to PVN circuit is necessary for suppression of LH during metabolic stress, and possibly other stress types. It is important to note that this neuron ablation technique lesioned other NE cell populations, in addition to the brainstem A2<sup>NE</sup> neurons, and reduced NE fiber density in other hypothalamic regions, complicating final interpretation.

Several lines of evidence support the hypothesis that CRHR signaling contributes the suppression of LH. The suppression of LH caused by NE injection into the PVN was reversed by central administration of a non-selective CRH receptor antagonist in rats (70), which is consistent with the finding that a different non-selective CRH receptor antagonist prevented the suppression of LH during IHH in rhesus monkeys (72). Importantly, pharmacological blockade of CRHR2, but not CRHR1, prevented the suppression of pulsatile LH in estradiol-treated rats during either metabolic or immune stress (32). Together, these data support the hypothesis that NE release in the PVN (presumably from A2 neurons) suppresses LH secretion via downstream CRHR2 signaling. In support of this

idea, central administration of urocortin 2 (UCN2; a potent and specific agonist of CRHR2) suppressed LH pulse frequency in rats (73). Thus, CRHR2 activation is both necessary and sufficient for the suppression of LH during two types of reactive stress. ARC<sup>Kiss1</sup> cells receive direct synaptic innervation from several neuron types in the PVN, but not from CRH neurons in the PVN (74). Ultimately, a multi-synaptic brainstem to ARC<sup>Kiss1</sup> pathway is supported by a recent wheat germ agglutinin tracing study (75). However, the precise cellular intermediates are not completely resolved.

Another signaling molecule of interest is calcitonin gene related peptide (CGRP), which is produced in neurons of the parabrachial nucleus. These neurons are innervated by A2<sup>NE</sup> cells (76) and activated during a variety of stress types (77). Furthermore, central administration of CGRP suppressed LH pulses (78). Interestingly, a CGRP receptor antagonist reversed the inhibitory effect of IIH on LH pulses (78), indicating a critical role for this signaling molecule in metabolic stress-induced suppression of LH. The neural sites of action for CGRP is an outstanding question.

Several lines of evidence support the hypothesis that cells the dorsomedial nucleus (DMN) and amygdala may also be important for the response to immune stress. First, RFRP-3, a peptide made in the DMN, suppressed LH concentrations in rodents (79, 80) and sheep (81). Second, LPS induced c-Fos expression (a marker of neuronal activation) in the DMN in several species (82), and in sheep, the RFRP-3 cells were activated by LPS (83). Similarly, in rats, *Rfrp* mRNA abundance was increased in micropunches of the DMN following high dose LPS treatment (84). Though *Rfrp* expression, cell number, or cell activity was not reduced by 12 hr of fasting (79), it remains possible that only more extreme stress regulates these cells because a high, but not a low dose of LPS altered *Rfrp* mRNA levels (84). Thus, future work will be needed to determine if RFRP-3 cells are also regulated by metabolic stress, and if this neuropeptide (or the cells that produce it) is necessary for the suppression of LH during reactive stress types. Since RFRP-3 stimulates corticosterone secretion and CRH neuron activation (85), it is possible that RFRP-3 is upstream of HPA axis activation. Indeed, mechanisms for RFRP-3 cell activation are a significant outstanding question. Immune stress also induced c-Fos in the amygdala, though the phenotype of these cells is unknown (86). Excitotoxic lesion of the central amygdala partially reversed the inhibitory effects of LPS, but not of IIH or restraint stress (87), which implicates the central amygdala in the suppression of LH during immune stress and further demonstrates stressor-specific activation of different neural pathways.

### What is the site of impaired LH secretion?

Metabolic stress suppressed LH pulse frequency in numerous species (72, 88–90) and inhibited MUA volleys in the MBH of monkeys (72), which is consistent with actions on ARC<sup>Kiss1</sup> cells. *Kiss1* mRNA in ARC punches was reduced following IIH (54), though the rapid cessation of LH pulses observed during metabolic stress (often within 20 min), is unlikely to be mediated by an inhibition of peptide synthesis, which occurs on the order of hours vs. minutes. The possibility that metabolic stress impairs GnRH cell function, independent from ARC<sup>Kiss1</sup>, is supported by the finding that a CRHR2 agonist suppressed GnRH cell firing in brain slice preparation (91), though this effect is unlikely to be mediated

directly on GnRH neurons because few GnRH cells contain CRH receptors (92). Metabolic stress does not appear to alter pituitary function as LH responses to exogenous GnRH were similar during euglycemic control periods and IHH in rats (93) and monkeys (94). Moreover, in rats with area postrema lesions, pulsatile LH secretion patterns during IHH and non-stress conditions are identical (57), which supports the hypothesis that low blood sugar (or high insulin) does not directly impair ARC<sup>Kiss1</sup> cells, GnRH neurons, or gonadotropes, but rather activates neural afferents to indirectly suppress ARC<sup>Kiss1</sup> (or possibly GnRH) neurons.

Immune stress presents an interesting contrast to metabolic stress in that LPS suppressed both hypothalamic and pituitary function. An inhibitory action of LPS in the hypothalamus is supported by a reduction in the frequency of MUA volleys in goats (95) and a suppression in the frequency of GnRH pulses in sheep (96). Additionally, LPS reduced *Kiss1* mRNA in micropunches of the ARC in rats (54). An inhibitory action at the pituitary level was demonstrated in sheep, whereby lower doses of LPS suppressed LH secretion without altering GnRH pulses (97). This inhibition of pituitary function in response to LPS was shown to occur independent of the action of cortisol (49), but rather via a prostaglandin-dependent pathway (97). In contrast to sheep, rats treated with LPS exhibit low LH without a reduction in the response to exogenous GnRH (98), indicating possible species differences in the role of altered pituitary function in response to stress-induced mediators.

## PSYCHOSOCIAL STRESS

### How and where is the proximate stressor detected?

Psychosocial stress is psychogenic in nature and characterized by the perception of a threat to homeostasis, which is experience dependent. Acute psychosocial stress is often modeled with physical restraint, typically including isolation from cage or pen mates and/or exposure to predator smells or sounds (e.g. fox urine or dog barking). Thus, sensory inputs (particularly olfactory inputs in rodents) and cognitive processes likely activate limbic brain regions resulting in the neuroendocrine responses to psychosocial stress.

### What are the neural substrates and which signaling molecules are involved?

Assessment of c-Fos distribution following restraint stress has implicated several brain regions, including the DMN and amygdala in the response to psychosocial stress (99). A study in gonad-intact male rats reported an increase in *Rfrp* mRNA (expressed in the DMN) following restraint stress (100). In OVX females, restraint induced a 20% increase in Rfrp cell number 3 hr after restraint stress, but not at earlier time points (101). In contrast, no change in *Rfrp* cell number was observed in castrated males over the same observation period (102). Interestingly and perhaps more importantly, acute increases in *Rfrp* cell activation, as assessed by *cfos* co-labeling, were observed after 45 min of restraint stress in both gonadectomized male and female mice (101, 102). These findings support the hypothesis that psychosocial stress increases RFRP-3 signaling, which is inhibitory on the reproductive axis. Indeed, a functional role for RFRP-3 in the suppression of LH is supported by the observation that knock-down of *Rfrp* in the DMN restored fertility in female rats exposed to repeated restraint stress (103). *Rfrp* is likely regulated by an acute rise in glucocorticoids, as these cells contain glucocorticoid receptor, and adrenalectomy



with low dose corticosterone replacement prevented the upregulation of *Rfrp* in gonad-intact male rats (100). However, in contrast to the rodent studies, restraint stress did not alter RFRP-3 cell number or activation in OVX sheep, though this stress paradigm produced only a modest suppression of LH secretion in that study (104).

The amygdala is part of the limbic system and is well known for its role in fear and emotion processing. This brain region is therefore an interesting potential target for stress regulation of reproductive processes. While the central amygdala may regulate immune stress as previously described, lesions of the medial amygdala partially prevented restraint stress-induced suppression of LH in female rats (87). Since lesion of the medial amygdala also prevented the induction of c-Fos expression in the PVN (105), it is possible that neuropeptides produced in the PVN, such as CRH, are important for the inhibitory effect of restraint stress on LH. It also suggests that the medial amygdala may be upstream of the PVN in the psychosocial stress neural circuitry.

Specific blockade of CRHR1 partially prevented the suppression of LH during restraint stress in rats (32). However, CRH knock-out mice have normal suppression of LH during restraint stress (106). This disparity could reflect compensatory changes in whole-body knock-out animals during development, actions of the other CRHR1 agonist urocortin 1, or species differences. Similar to the physical stressors (immune and metabolic), CRHR2 blockade also partially prevented restraint stress-induced suppression of LH (73). Thus, it appears activation of CRHR2 represents a commonality between psychosocial and physical stressors. It will be interesting to determine whether the neural source of endogenous CRHR2 ligands (i.e. the population of cells that produce the peptides) are also common between stress types.

### What is site of impaired LH secretion?

Restraint stress suppressed LH pulse frequency in several species, including mice (101), rats (107), and sheep (108), which implies a hypothalamic site of action as discussed above. Strong evidence supports a mediatory role of ARC<sup>Kiss1</sup> cells in the suppression of LH pulse frequency in response to restraint stress in female mice. In OVX mice, restraint elicited a slowing of pulse frequency demonstrated by a lengthening of interpulse interval from 20 min to 58 min during the 90 min stress period (Figure 1). Associated with this reduction in pulse frequency was a 50% reduction in the percentage of *Kiss1* cells that contained *cfos* at 45, 90 and 180 minutes of restraint stress, thus demonstrating suppression of ARC<sup>Kiss1</sup> cell activation (101). In contrast, castrated male mice had a more modest suppression (~30%) in the percentage of ARC<sup>Kiss1</sup> cells containing *cfos* at 3hr of restraint stress, but not at earlier times (45 or 90 min) (102). These observations provide correlative evidence that restraint stress impairs LH via inhibition of ARC<sup>Kiss1</sup> cells. Whether the possible sex difference in *Kiss1* cell suppression following restraint stress reflects alternate mechanisms operating in the sexes or is a consequence of the technique used to assess cell activation (*cfos* induction) is unknown. Consistent with an action upon the ARC<sup>Kiss1</sup> cell population, ARC *Kiss1* mRNA abundance was suppressed by 1hr of restraint stress in estradiol-treated OVX rats (note: neural tissue was collected 5 hr after release from restraint, 6 hr after initiation of the restraint) (54). However, in castrate male and OVX female mice, no change in the number of

*Kiss1* cells was observed during 3 hr of continuous restraint (101, 102). Several differences exist in these experimental paradigms, including timing of tissue collection, steroid status, and species, as well as mRNA quantification technique.

In addition to the effects of acute psychosocial stress on reproductive brain circuits, evidence in some species support the hypothesis that pituitary function is also affected. For example, OVX ewes treated with gonadal steroids displayed a reduction in LH pulse amplitude during restraint and isolation stress (108). Transport stress reduced LH pulse frequency in intact ewes during the mid-follicular phase (109), and suppressed the LH response to GnRH injections (110). In OVX sheep, an acute layered psychosocial stressor model (isolation, restraint, blindfolding and barking dog sounds), suppressed both GnRH pulse amplitude and LH pulse amplitude (111). This suppression of just the latter is reversed by the GR antagonist RU486 (112, 113) suggesting an action of cortisol on the gonadotrope cell. In contrast to these inhibitory effects on LH pulse amplitude in sheep, in castrated male mice pulse amplitude during restraint stress was increased (102), which may reflect the release of a larger stored pool of LH during each pulse. Considering that acute effects of glucocorticoids on LH in rodents remains unclear, analysis of LH secretion in a GnRH-clamp experiment during restraint stress would be necessary to determine whether there is any suppression of pituitary function in rodents during this stress type.

## CONCLUSIONS

The inhibitory actions of many of the elements of the HPA axis on pulsatile LH secretion have been studied extensively and appear to contribute to some, but not all, of the inhibitory effects of stress. It is becoming increasingly evident that different types of stress suppress LH secretion through distinct mechanisms, which is summarized in Figure 2. CRH/CRHR1 signaling presents in interesting example of a mechanism contributing to some (psychosocial) but not other (immune and metabolic) stress types. The specific role of CRH and the ligands for CRHR2 in the suppression of LH during stress remain interesting and important questions. During reactive stress types, a brainstem to hypothalamus circuit ultimately resulting in suppression of ARC<sup>Kiss1</sup> cells is activated; the role of the peptides RFRP-3 and CGRP, as well as contributions from the amygdala remain interesting additions to this neurocircuit. For psychosocial stress it is apparent that the inputs from the amygdala, RFRP-3, as well as CRHR1 and CRHR2 ligands, all contribute to the suppression of ARC<sup>Kiss1</sup> cells. Thus, numerous signaling molecules and brain regions contribute to the suppression of LH during stress (Figure 2); an important challenge moving forward will be to integrate the actions of these diverse molecules and form ordered pathways for their effects on reproduction.

## Acknowledgments

**Grant Support:** The authors are supported by NSF grant IOS-1457226 and NIH grants R01 HD090161, R01 HD086100, P50 HD012303, T32 HD007203 and F32 HD096811.

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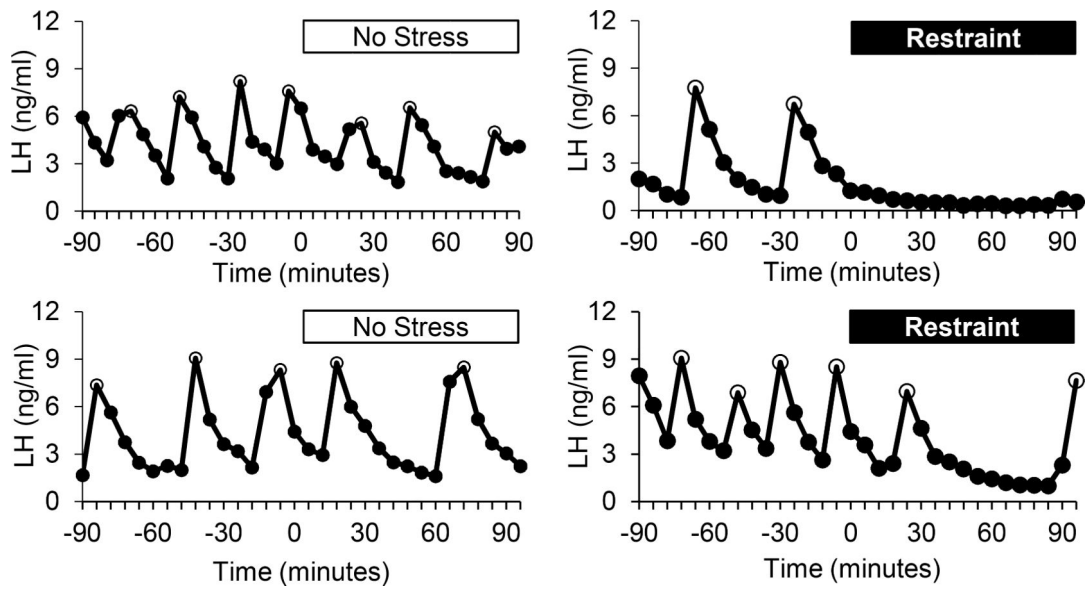
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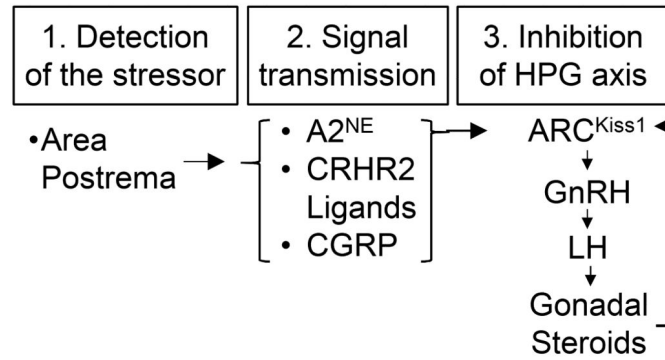


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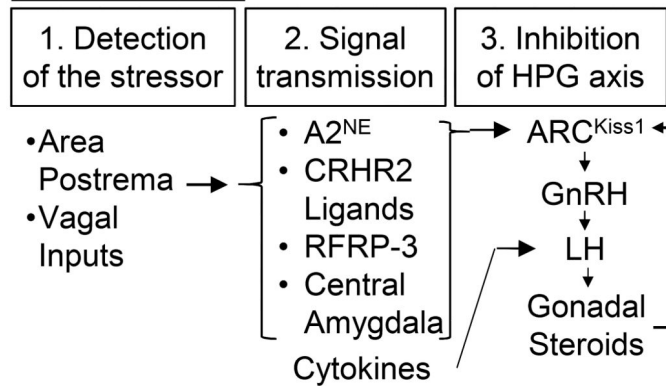


**Figure 1.** Representative LH pulse patterns in OVX mice exposed to no stress (left) or restraint stress for 90 minutes (right). Open circles represent LH pulses. [Note, these are unpublished LH pulse patterns from a published study. (101)]

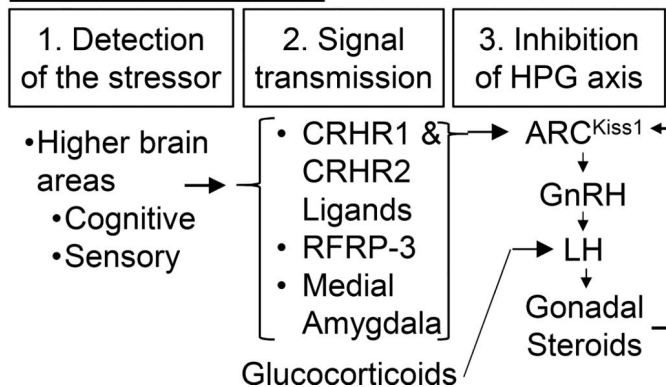
**Metabolic stress:**



**Immune stress:**



**Psychosocial stress:**



**Figure 2:** Schematic for neural pathways by which reactive stress (metabolic and immune) and psychosocial stress impair reproductive hormone secretion in mammals.