Published in final edited form as:

J Steroid Biochem Mol Biol. 2016 June ; 160: 196–203. doi:10.1016/j.jsbmb.2015.11.019.

GABAergic regulation of the HPA and HPG axes and the impact of stress on reproductive function

Laverne Camille Melón¹ and Jamie Maguire¹

¹ Tufts University School of Medicine, Department of Neuroscience, Boston, MA 02111.

Abstract

The hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes are regulated by GABAergic signaling at the level of corticotropin-releasing hormone (CRH) and gonadotropin-releasing hormone (GnRH) neurons, respectively. Under basal conditions, activity of CRH and GnRH neurons are controlled in part by both phasic and tonic GABAergic inhibition, mediated by synaptic and extrasynaptic GABA_A receptors (GABA_ARs), respectively. For CRH neurons, this tonic GABAergic inhibition is mediated by extrasynaptic, δ subunit-containing GABA_ARs. Similarly, a THIP-sensitive tonic GABAergic current has been shown to regulate GnRH neurons, suggesting a role for δ subunit-containing GABA_ARs; however, this remains to be explicitly demonstrated. GABA_ARs incorporating the δ subunit confer neurosteroid sensitivity, suggesting a potential role for neurosteroid modulation in the regulation of the HPA and HPG axes. Thus, stress-derived neurosteroids may contribute to the impact of stress on reproductive function. Interestingly, excitatory actions of GABA have been demonstrated in both CRH neurons at the apex of control of the HPA axis and in GnRH neurons which mediate the HPG axis, adding to the complexity for the role of GABAergic signaling in the regulation of these systems. Here we review the effects that stress has on GnRH neurons and HPG axis function alongside evidence supporting GABA_ARs as a major interface between the stress and reproductive axes.

Keywords

Neurosteroids; stress; GABA_A receptors; KCC2; GnRH; estrous; puberty; LH; allopregnanolone; THDOC; tonic inhibition; extrasynaptic; Gabrd

I. INTRODUCTION

The high energy cost of mammalian reproduction and offspring care makes the timing of this important event sensitive to a variety of stressors. Indeed, in his initial report on the *general adaptation syndrome*, Hans Selye detailed significant reproductive deficits in rats that experienced a wide range of stressors, from cold exposure to excessive exercise. It is

All correspondence should be directed to: Jamie Maguire, Ph.D., Assistant Professor, Department of Neuroscience, Tufts University School of Medicine, 136 Harrison Ave., SC205, Boston, MA 02111, Tel: 617-636-3595 Fax: 617-636-2413.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

here that Selye hypothesizes that these effects-which included atrophy of the gonads and cessation of milk production in lactating rat dams, may involve some general stress-associated suppression of reproduction and suggests that activity of the stress axis must compete with and thus inhibit activity of the reproductive axis [1]. Since these observations, the specific mechanisms underlying the pathophysiological effects of stress on reproduction remain largely unknown. Given the significance of the gonadotropin releasing hormone (GnRH) system as the common central regulator of the hypothalamic-pituitary-gonadal (HPG) axis, it is hypothesized that the central effects of varied stressors converge on these neurons to disrupt their activity.

Stress, the body's response to the metabolic, immunological and psychological factors that threaten homeostasis, involves a coordinated cascade of events across the hypothalamic-pituitary-adrenal (HPA) axis. During the acute stress response, secretion of corticotropin releasing hormone (CRH) from parvocellular neurons of the hypothalamus evokes increased synthesis and release of adrenocorticotropin releasing hormone (ACTH) from the pituitary that acts in an endocrine fashion to induce release of mineralocorticoids and glucocorticoids from the adrenal cortex. These steroid hormones have a host of downstream effects that include cessation of this stress signal. Activation of this HPA axis also involves an increase in the synthesis and release of neuroactive derivative of steroid hormones, termed neurosteroids. This peripheral increase in neurosteroids contributes to the central increase of the 5 α reduced neurosteroids tetrahydrodeoxycorticosterone (THDOC) and allopregnanolone (ALLO). THDOC and ALLO are potent modulators of GABA_ARs, acting preferentially at extrasynaptic, δ subunit-containing GABA_ARs that mediate tonic inhibition [2, 3]. These receptors play a critical role in the regulation of the HPA axis [4] and have been implicated in the regulation of the HPG axis [5, 6]. Here we review the role for GABA_ARs, in particular neurosteroid modulation of δ subunit-containing GABA_ARs, in the regulation of the HPA and HPG axes. Further, we review the evidence that stress disrupts HPG function highlighting a potential role for neurosteroids and their interaction with GABA_A receptors. Given significant differences in the physiological characteristics of the HPG axis across species and sexes, this review focuses on findings from female rodents to build a cohesive model supporting GABA_A receptors as an interface of the stress and reproductive axes.

II. GABAergic regulation of the HPA axis

The physiological response to stress is mediated by the HPA axis, the activity of which is governed by CRH neurons in the paraventricular nucleus (PVN) of the hypothalamus. CRH neurons are at the apex of HPA axis control, initiating the release of CRH that signals the release of ACTH from the pituitary, which triggers the secretion of cortisol from the adrenal gland in humans (corticosterone in mice). CRH neurons integrate information from many different brain regions involving numerous neurotransmitter systems, but the activity of CRH neurons is ultimately regulated by GABAergic inhibition [for review see 7, 8].

The importance for GABAergic signaling in the regulation of the HPA axis is evident from the fact that nearly 50% of all synapses in the PVN are GABAergic [7, 9]. GABAergic inputs into the PVN arise from local interneurons surrounding the PVN (peri-PVN) as well as from the subparaventricular zone, the anterior hypothalamic area, dorsomedial

hypothalamic nucleus, the medial preoptic area, lateral hypothalamic area, and from multiple nuclei within the bed nucleus of the stria terminalis [BNST; 8, 10-12]. In addition, other brain regions exert control over the HPA axis through interneuron mediators [for review see 12].

CRH neurons are regulated by both phasic and tonic GABAergic inhibition, mediated by synaptic and extrasynaptic GABA_ARs, respectively [13]. Recently, CRH neurons have been shown to be regulated by δ subunit-containing GABA_ARs [4], which confer sensitivity to neurosteroid modulation [2, 14-17]. When exogenously administered to non-stressed mice the stress-derived neurosteroid, THDOC (10mM), decreases the activity of CRH neurons via actions on δ subunit-containing GABA_ARs [4]. These results were somewhat unexpected given that autoradiography and immunohistochemistry techniques failed to support a high expression of δ protein in the hypothalamus, especially when compared to regions like the thalamus and cerebellum, where δ is enriched [18-22]. However, combined electrophysiological and pharmacological techniques support tonic GABAergic inhibition in this area mediated by δ containing GABA_A receptors [4]. Indeed, reduced responsivity of CRH neurons to δ -targeting compounds has been demonstrated in the hypothalamus of global and CRH specific δ knockout mice [4, 22, 23]. Further, immunoreactivity protocols focused on signal enhancement reveals positive δ expression in the PVN [4, 23]. However, it is important to note that it remains somewhat controversial whether the tonic GABAergic current in parvocellular neurons in the PVN is mediated by GABA_AR δ -subunit containing receptors.

Interestingly, the GABAergic regulation of CRH neurons is fundamentally altered following acute stress. Acute restraint stress results in excitatory actions of GABA on CRH neurons due to a collapse in the chloride gradient and a shift in the reversal potential for GABA_A (E_{GABA}) [4, 28]. Following acute stress, there is dephosphorylation of KCC2 residue Ser940, which regulates surface expression and function of this transporter [24] and a downregulation of KCC2 in the PVN. KCC2 maintains low intracellular chloride levels in the adult brain, which is essential for the inhibitory actions of GABA [25-27]. Thus, following acute stress, the downregulation of KCC2 results in excitatory actions of GABA on CRH neurons and compromised GABAergic constraint on these neurons [4, 28]. Due to excitatory actions of GABA on CRH neurons, THDOC increases the activity of CRH neurons and increases the corticosterone levels following stress [4]. These excitatory effects of GABA following acute stress are specific for CRH neurons in the PVN since similar changes are not observed in other brain regions, such as the hippocampus [4]. Interestingly, following chronic stress, deficits in GABAergic inhibition are observed in extrahypothalamic regions and have been proposed to contribute to increased hippocampal excitability [29]. These findings demonstrate that the GABAergic regulation and neurosteroid modulation of the HPA axis are state-dependent. This topic has been reviewed in more detail elsewhere [8, 30, 31].

III. GABAergic regulation of the HPG axis

The HPG axis is controlled by GnRH neurons, which consist of just over 1000 neurons scattered across the hypothalamus. About 70% of the projections of these neurons terminate

in the median eminence where GnRH can be released into the hypophyseal portal blood system and act as the central regulator of the HPG axis [32, 33]. The pattern of activity of these GnRH neurons is integral for the increase in release of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), from the pituitary gland [34]. Thus, activity of GnRH neurons is coupled to release, which is tightly regulated by GABAergic signaling (Han, 2004; Moenter, 2005). Analyses of inhibitory inputs along GnRH neurons using vGAT expression reveal greater vGAT positive contacts along the GnRH initial dendrite than the soma [35], allowing possible multi-dendritic inhibitory signals from a single inhibitory afferent. Indeed, evidence supporting bundled dendrites coming into contact with a shared synaptic input has been demonstrated in anatomical findings from electron microscopy techniques [36]. These shared-synapses along the dendrites could have significant effects on release of the peptide, as dendrites of GnRH neurons terminating in the median eminence are able to initiate and propagate action potentials [37-39]. However, computational modeling suggests that significant effects of inhibitory inputs to GnRH neurons would involve inhibitory contacts nearer the soma rather than at distal dendrites or terminals at the level of the median eminence [40].

GnRH neurons have been shown to be regulated by both tonic and phasic GABAergic inhibition. These neurons exhibit a tonic GABAergic current sensitive to modulation by 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol (THIP), an agonist with high efficacy and selectivity for δ subunit-containing GABA_ARs [6, 15, 41-43]. This THIP-sensitive tonic current is indicative of involvement of extrasynaptic δ subunit-containing GABA_ARs in regulation of GnRH neurons and is further supported by δ subunit mRNA expression in these cells [6]. In addition to their high sensitivity to THIP, δ containing GABA_A receptors have high sensitivity to neurosteroids. ALLO (500nm-5uM) has been shown to alter the resting membrane potential of GnRH neurons [44] as well as augment the depolarizing response of GnRH neurons to GABA [45]. However, these concentrations of ALLO would potentiate the effect of GABA at most GABA_AR subtypes [46] and is thus not specific for δ subunit-containing GABA_ARs. Still, a recent report using a GnRH secreting immortalized mouse hypothalamic cell line found that a synthetic δ GABA_AR agonist (DS1) activated GnRH neurons [5]. This GABAergic control of GnRH neurons can influence reproductive function in several ways, including regulation of the estrous cycle and the initiation of puberty [47-49]. Despite the importance of GABAergic signaling in the control of GnRH neurons, it is interesting to note that the loss of the GABA_AR $\gamma 2$ subunit has very little effect on reproductive function [50]. However, the importance of extrasynaptic GABA_ARs in the regulation of reproductive function remains to be determined.

In female mice and rats, an increase in GnRH multiunit activity precedes the LH surge on the morning of proestrus. Distinct vesicle sorting of the gonadotropins underlies a delayed GnRH-mediated surge in plasma FSH [34]. Given the tight coupling of GnRH activity and LH release, plasma levels of the latter are often used as a surrogate for the former in many studies. Puberty, the period associated with maturation of the above-described reproductive system, may be said to also depend on a maturation of the secretory profile of GnRH, as the mature burst activity profile of GnRH neurons comes online at this time to complete this neuroendocrine circuit. For rodents, this maturation is characterized by a *gradual* increase in the frequency of GnRH neuron pulse, from every 90 minutes during the peripubertal period

to every 30 minutes after vaginal opening and complete pubertal maturation [51]. This switch to the pubertal/mature pulse profile of the GnRH system is thought to involve both downregulation of the inhibitory drive to these neurons and an increase in excitatory inputs [47].

It is important to note that there is some controversy regarding the direction of GABAergic signaling in GnRH neurons. Early pharmacology studies supported an inhibitory role for GABA in the regulation of the HPG axis, with a negative relationship between GABA activity and GnRH or LH activity. Indeed, the LH surge that precedes ovulation is accompanied by a significant decrease in GABA concentration surrounding GnRH cell bodies located in the preoptic area [52] and systemic administration of a GABA_A agonist inhibits the LH surge [53]. Still, depolarizing responses to GABA have been demonstrated for GnRH neurons in slices from adult female mice [54, 55] and from neuronal cultures of adult female rats [56]. Heterogeneous GABAergic responses have been demonstrated *in vitro* [54, 56-60] and *in vivo* [61]. Moreover, application of either a GABA_A antagonist or channel blocker has been shown to reduce firing rate of GnRH neurons in female mice [59, 61]. Another report found an increase in the firing rate of GnRH neurons after bath application of a GABA_A receptor antagonist [58], which could be due to disinhibition of glutamatergic afferents in the slice, as glutamate receptor antagonists were not included in the slice preparation [62]. Excitatory actions of GABA on GnRH neurons are supported by evidence that the reversal potential of GABA is more positive than the resting potential for these GnRH neurons [54, 60]. These studies support excitatory actions of GABA on GnRH neurons, but also highlight the heterogeneity of this effect. Diversity in the responses of GnRH neurons to GABA_A activation may be due to the fact that two-times as many GnRH neurons express NKCC1 mRNA than KCC2 mRNA, which supports high basal intracellular chloride in these neurons [54]. Although these putative NKCC1 transporters were shown to be expressed by fewer than one quarter of GnRH neurons, the NKCC1 blocker (bumetanide) significantly dampened the stimulatory response of GABA in GnRH neurons [56]. These data highlight the complex role of GABA in the regulation of GnRH neurons and the HPG axis under basal conditions. The relationship of GABAergic control of GnRH neurons becomes increasingly more complex when considering the impact of stress on the HPG axis.

IV. Impact of Stress on the HPG axis: role of GABA_ARs

Following Selye's original observations, additional reports confirmed a modulatory effect of HPA axis activity on the HPG axis. Of course, the direction of this stress effect may depend on the timing of the stressor exposure, the particular reproductive endpoint of interest, stressor type and the state of the hormonal milieu at the initiation of the stress.

Although one-third of GnRH neurons are thought to express CRH receptors [63], the diverse classes of stressors shown to suppress GnRH activity and associated indices of reproductive health and fertility are not all mediated by activity at CRH. For example, although lesions of the medial amygdala can dampen the restraint stress-induced increase in LH pulse interval, it does not affect similar LH deficiency seen after immunological or metabolic stressors [64]. Similarly, though evidence supporting glucocorticoid regulation of GnRH mRNA through inclusion of a negative glucocorticoid responsive element on the GnRH promoter have been

demonstrated from *in vitro* [65, 66] and *in vivo* [67] models, chronic corticosterone failed to disrupt LH pulse frequency or amplitude in ovariectomized rats [68]. These findings suggest that psychological and physiological stressors may differentially affect HPG function through distinct neural circuits.

A long history of research supports GABA's role in modulating GnRH physiology. Yet, only a handful of recent investigations implicate GABA_AR signaling in the effects of stress on this level of the reproductive axis. Thus far, only one study supports a direct relationship between GABA_AR activity in the mPOA and stress effects on GnRH activity (via increased LH interpulse interval). Using pharmacological manipulation of GABA_A and GABA_B receptors in the mPOA, this work showed a differential role of these GABA_AR subtypes in the effects of immunological versus restraint stress [69]. The effect of stress on GnRH neurons and associated reproductive events (puberty and estrous cycle) and evidence supporting role for GABAergic signaling in these effects are summarized in Table 1 and reviewed in detail below.

2.1. Stress, GABA_ARs, and puberty

Stress may be associated with *either* delayed or advanced pubertal onset for humans. In particular, children who have experienced early peri/postnatal stress show precocious pubertal development [70, 71], whereas those who have experienced juvenile or peripubertal stress, show a delay. This bidirectional effect of stress on pubertal onset may be said to depend on whether the stress occurs during the infantile period of GnRH neuronal activity or during the quiescent period of inactivity that precedes puberty for primates.

Juvenile rodents do not display a quiescent period of GnRH activity [57, 72]. This may be why, for rats and mice, both perinatal and peripubertal stressors and stress hormone exposure are most often associated with delayed pubertal onset. For example, female rats receiving continuous, intracerebroventricular (icv) administration of CRH beginning at P28, show a dose dependent delay in both vaginal opening and first estrous [73]. Similarly, early life stress, including neonatal stress hormone exposure, is negatively correlated with the delayed onset of both variables associated with sexual maturation in female rats [74] and mice [75, 76]. This delay may also be seen following metabolic stress, as forced fasting is a well-known model of pubertal delay in rats [77]. However, some potent stressors, like maternal separation, do fail to induce changes in pubertal onset [78, 79]. Furthermore, some stressors have been associated with precocious pubertal onset in rodents. Offspring of low-licking/grooming dams show both a hyperactive HPA axis and earlier onset of vaginal opening and estrus than the offspring of high-licking/grooming dams [80]. This early pubertal onset has also been reported following overexpression of CRH in the central nucleus of the amygdala [81]. Interestingly, although neonatal exposure to an immunological stressor is associated with a delay in both vaginal opening and first estrous, these effects are only seen when the immunological stress occurs before the second week of gestation [82]. Finally, metabolic stress may result in either pubertal delay [83, 84] or advanced puberty [85], depending upon whether the stress is associated with an energy surfeit or deficit [85, though see 86].

Fewer studies demonstrate the accompanying effects of these stressors on the pubertal maturation of GnRH activity in rodents. It may be inferred that the stressors associated with pubertal delay also defer the increased activity of GnRH neurons that precede sexual maturation and that stressors associated with precocious puberty do the opposite. Indeed, the GnRH interpulse interval in hypothalamic explants of female rats that experienced neonatal undernutrition with delayed pubertal onset is relatively prolonged, much more similar to the interpulse interval seen during the juvenile stage [87]. Furthermore, the early onset of puberty seen following the metabolic stress associated with high fat diet is accompanied by an early increase in LH pulse frequency [88]. Further efforts are required to define the relationship between stress, pubertal delay and GnRH neuronal activity, as well as the role of GABAergic signaling in mediating these effects.

Though activity of GABA_ARs is thought to play a role in the maturation of the HPG axis [89, 90], very few studies have investigated changes in the GABAergic control of GnRH neurons, or the HPG axis, following stress in the pre-pubertal system. One study on the HPG response to prepubertal (P15) and peripubertal (P30) exposure to an acute dose of bacterial endotoxin (LPS) found a reduction in hypothalamic GABA content and plasma LH only after peripubertal exposure to this metabolic stressor [91]. Chronic exposure to this metabolic stressor was also found to also cause a reduction in GABA content and plasma LH as well as a delay vaginal opening by 4 days [92]. Further research is required to fully appreciate the role of GABAergic signaling in mediating the effects of stress on GnRH neurons in the pubertal transition.

2.2. Stress, GABA_ARs, and mature HPG function

The mature HPG axis is also vulnerable to stress. However, the effects of stress on the estrous cycle, GnRH/LH activity, and fertility depend strongly on the type of stressor. Therefore, the effects of different types of stressors (metabolic, immunological, or psychogenic) on HPG function are reviewed separately below.

2.2.1 Metabolic stressors—Fasting in mice may disrupt regular estrous cycling by causing a delay in the transition to estrus, when the fasting is initiated during diestrus [93]. This metabolic stressor is associated with a dampened GnRH pulse profile [94]. Although the GnRH neurons of fasted mice still display action potentials in response to locally applied GABA (mM) and a reduction in the frequency of GABAergic sPSCs, these neurons also show an increase in the GABAergic postsynaptic response to leptin (50mM), including an increase in the rise time, amplitude and decay of sPSCs [94]. As current evidence suggests that leptin does not directly act on GnRH neurons through classic cascades associated with leptin receptor activation [95], it is possible that the enhanced GABAergic postsynaptic response to leptin following fasting still involves some change in the GABA_AR population on these GnRH neurons. Indeed, in the immature system, leptin has been shown to potentiate GABAergic-PSCs through an increase in the expression of functional GABAergic synapses as well as an induction of multiple signaling molecules thought to be involved in synaptic plasticity [96]. Thus, the effects of fasting-stress on GnRH activity may involve both changes in presynaptic inhibitory drive and alterations in the GABA_AR profile on

GnRH neurons. As such, mice with disrupted leptin signaling to GABAergic neurons show delayed puberty onset, reduced plasma LH and proestrus incidence.

Unlike fasting, metabolic stress associated with high fat intake results in an increase in GnRH secretion in mice [97]. This increased hypothalamic activity also augments plasma LH and disrupts regular cycling. Though female rats on a high fat diet also display an increase in irregular cycling, this is associated with a reduction in LH activity, much like fasting [98]. These findings highlight potential differences in the regulatory signals of GnRH activity across species. There is a gap in the literature regarding a role for GABA_ARs in the effects of high fat diet on GnRH activity. However, GABAergic signaling has been shown to be depressed in the dorsomedial hypothalamus of male rats on a high fat diet [99]. This hypothalamic nucleus is believed to be the location of the GnRH neurons integral to GnRH pulse generation, as their activity is most strongly associated with an exogenously induced LH surge [100] and deafferentation of this region from the rest of the brain disrupts ovulation [101]. High fat diet also reduces total GABA_A binding densities in the hypothalamus of male rats [102]. Further efforts are needed to determine whether the effects of high fat diet on the GABAergic system may be extended to females and whether they directly impact GnRH neurons.

2.2.2 Immunological stressors—The effects of some stressors (such as intermittent cold-stress) on GnRH activity are accompanied by changes in cytokine expression [103], which may have an impact on reproductive events. Indeed, administration of a bacterial endotoxin (LPS) that potently induces an inflammatory stress response, including activation of proinflammatory cytokines, consistently suppresses LH pulse frequency [104-106]. This effect may be recapitulated following administration of cytokines themselves [106-108]. Furthermore, the reduced LH pulse frequency may be accompanied by a tempering of the increased GnRH multiunit activity that usually precedes the LH surge [106]. There is also a reduction in the surge in GnRH transcription that occurs during the proestrus phase in rats [109]. Although immunological stressors have potent and fast inhibitory effects on GnRH activity, and the effects of chronic immunological stress on estrous cycling has been established in rats [110], lasting changes in the behavioral events associated with acute reduction in GnRH activity may depend on the state of the hormonal milieu at the time of the stressor. For example, an increase in estrous cycle irregularity following LPS administration was found to most consistently occur when LPS was administered during diestrus [111].

Activation of GABA_ARs on GnRH neurons by elements associated with these immunological stressors may underlie the fast inhibitory effect of these stressors on HPG activity. Indeed, infusion of a GABA_A receptor antagonist (bicuculline) into the mPOA blocks the effect of LPS on LH pulse frequency [105]. Furthermore, IL-1 β , a pro-inflammatory cytokine, has been shown to augment α_5 -mediated tonic current in the hippocampus [112]. As mRNA for this subunit was shown to be the most consistently expressed GABA_AR transcript in single GnRH positive neurons harvested for analysis from GFP-GnRH mice [113], similar effects may occur in GnRH neurons. Thus, the sensitivity of tonic inhibitory current to this integral inflammatory cytokine suggests that immunological stressors may modulate GnRH activity by potentiating the tonic current mediated by

extrasynaptic GABA_ARs exerting a hyperpolarizing effect on the resting membrane potential of GnRH neurons, suppressing the release of the peptide.

2.2.3 Psychogenic stressors—Restraint stress has been shown to block the surge in LH associated with the evening of proestrus in mice [114], block or dampen both the proestrus LH surge and ovulation in cycling rats [115] and prolong the time between LH pulses in estrogen-primed ovariectomized rats [105]. This delay in LH pulse is maintained even when the restraint is preceded by an intra-mPOA infusion of the GABA_AR antagonist bicuculline [105]. Although this suggests that GABA_ARs may not play a role in mediating the effects of this stressor on GnRH activity, it is possible that the bicuculline treatment is acting to alter the activity of afferents to GnRH neurons in this area in addition to blocking GABA_ARs on the GnRH neurons themselves. Indeed, like fasting stress, restraint stress may be associated with changes in presynaptic GABAergic drive to GnRH neurons, as this treatment is positively associated with activation of GAD67 cells in regions populated by GnRH neurons [116]. Though an increase in GAD transcription has been shown in the POA of chronically stressed male rats [117], this has not been confirmed for females. GAD immunoreactivity is enhanced for female rats that have received infusions of CRH into the BNST [118]. This procedure is meant to mimic the CRH activation that may accompany stressors like restraint, and has been shown to reduce the frequency (and increase the interpulse interval) of LH pulses [118]. Preliminary reports suggest that, in addition to presynaptic changes in GABAergic signaling to GnRH neurons, central increases in CRH may have postsynaptic effects on GABA_ARs. For example, CRH (50nM) was shown to decrease the frequency of GABAergic sIPSCs [119; conference abstract]. More efforts are needed to determine whether the effects of psychogenic stress in these rodent models affect GABA_AR signaling on GnRH neurons.

Restraint stress may also regulate activity of GABA_ARs on GnRH neurons by shifting the expression of NKCC1 and KCC2. As mentioned earlier, downregulation of KCC2 in CRH releasing parvocellular neurons of the hypothalamus play an integral role in the neuroendocrine and behavioral response to acute stress [4]. Similarly, downregulation of KCC2 in the PVN occurs following chronic social defeat stress [120]. This downregulation also occurs in the hippocampus following chronic restraint stress [29]. Therefore, reproductive effects associated with restraint stress may involve changes in KCC2 expression on GnRH neurons and a change in the postsynaptic response of GABA_ARs on these cells. The effects of stress on chloride homeostasis and the direction of GABAergic signaling and impact on HPG function remain to be determined.

There is also very little known about the contribution of specific GABA_AR subtypes in mediating the effects of stress on mature HPG function. Interestingly, loss of the GABA_AR γ 2 subunit, which mediates the phasic component of GABAergic signaling, has very little effect on reproductive function [50]. However, the role of these receptors in mediating the effects of stress on HPG function has not been explored. Similarly, to-date, no studies have investigated the role for extrasynaptic GABA_ARs, such as those containing the δ subunit, in mediating the effects of stress on the HPG axis. Nor has there been an investigation into the role of neurosteroid actions on GABA_ARs, in particular stress-derived neurosteroids, in the effects of stress on reproductive function.

V. Concluding Remarks

Stress negatively impacts reproductive function. However, much remains to be learned regarding the mechanisms whereby stress alters HPG function. Stress alters the activity of GnRH neurons and disrupts reproductive events, including the pubertal transition and the estrous cycle. GABA_ARs have been shown to regulate GnRH activity with recent reports supporting a role for δ subunit-containing GABA_AR-mediated tonic inhibition on these neurons. GABA_ARs incorporating the δ subunit display higher sensitivity to neurosteroids, including stress-derived neurosteroids. Thus, δ subunit-containing GABA_ARs may be perfectly poised to regulate crosstalk between the stress and reproductive axes. Future studies will explore the role of extrasynaptic GABA_ARs, tonic inhibition, and neurosteroid modulation in mediating the effects of stress on HPG function.

Acknowledgments

J.M. is supported by NIH-NINDS grant R01 NS073574 (J.M.). L.C.M is supported by NIH-NIGMS grant K12GM074869; an IRACDA training grant to Tufts University, Training in Education and Critical Research Skills (TEACRS).

References

1. Selye, H. The physiology and pathology of exposure to stress : a treatise based on the concepts of general-adaption-syndrome and the diseases of adaption. 1. ed.. Acta, Inc Med. Publ.; Montreal: 1950.
2. Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, et al. Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice. *Proc Natl Acad Sci U S A*. 1999; 96(22):12905–10. [PubMed: 10536021]
3. Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABAA receptors. *Proc Natl Acad Sci U S A*. 2003; 100(24):14439–44. [PubMed: 14623958]
4. Sarkar J, Wakefield S, MacKenzie G, Moss SJ, Maguire J. Neurosteroidogenesis is required for the physiological response to stress: role of neurosteroid-sensitive GABAA receptors. *The Journal of Neuroscience*. 2011; 31(50):18198–210. [PubMed: 22171026]
5. Sukhbaatar U, Mijiddorj T, Oride A, Kanasaki H. Stimulation of δ subunit-containing GABAA receptor by DS1 increases GnRH receptor expression but reduces GnRH mRNA expression in GnRH-producing GT1-7 cells. *Endocrine*. 2015; 49(1):222–30. [PubMed: 25355308]
6. Bhattarai JP, Park SA, Park JB, Lee SY, Herbison AE, Ryu PD, et al. Tonic extrasynaptic GABA(A) receptor currents control gonadotropin-releasing hormone neuron excitability in the mouse. *Endocrinology*. 2011; 152(4):1551–61. [PubMed: 21285326]
7. Decavel C, Van den Pol AN. GABA: a dominant neurotransmitter in the hypothalamus. *J Comp Neurol*. 1990; 302(4):1019–37. [PubMed: 2081813]
8. Herman JP, Mueller NK, Figueiredo H. Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. *Ann N Y Acad Sci*. 2004; 1018:35–45. [PubMed: 15240350]
9. Miklos IH, Kovacs KJ. GABAergic innervation of corticotropin-releasing hormone (CRH)-secreting parvocellular neurons and its plasticity as demonstrated by quantitative immunoelectron microscopy. *Neuroscience*. 2002; 113(3):581–92. [PubMed: 12150778]
10. Roland BL, Sawchenko PE. Local origins of some GABAergic projections to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *J Comp Neurol*. 1993; 332(1):123–43. [PubMed: 7685780]

11. Cullinan WE, Herman JP, Watson SJ. Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis. *J Comp Neurol.* 1993; 332(1):1–20. [PubMed: 7685778]
12. Cullinan WE, Ziegler DR, Herman JP. Functional role of local GABAergic influences on the HPA axis. *Brain Struct Funct.* 2008; 213(1-2):63–72. [PubMed: 18696110]
13. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci.* 2005; 6(3):215–29. [PubMed: 15738957]
14. Belelli D, Casula A, Ling A, Lambert JJ. The influence of subunit composition on the interaction of neurosteroids with GABA(A) receptors. *Neuropharmacology.* 2002; 43(4):651–61. [PubMed: 12367610]
15. Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA. Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA(A) receptors. *Br J Pharmacol.* 2002; 136(7):965–74. [PubMed: 12145096]
16. Spigelman I, Li Z, Liang J, Cagetti E, Samzadeh S, Mihalek RM, et al. Reduced inhibition and sensitivity to neurosteroids in hippocampus of mice lacking the GABA(A) receptor delta subunit. *J Neurophysiol.* 2003; 90(2):903–10. [PubMed: 12702713]
17. Wohlfarth KM, Bianchi MT, Macdonald RL. Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit. *J Neurosci.* 2002; 22(5):1541–9. [PubMed: 11880484]
18. Laurie DJ, Seeburg PH, Wisden W. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. *J Neurosci.* 1992; 12(3):1063–76. [PubMed: 1312132]
19. Wisden W, Laurie DJ, Monyer H, Seeburg PH. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci.* 1992; 12(3):1040–62. [PubMed: 1312131]
20. Fritschy JM, Mohler H. GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J Comp Neurol.* 1995; 359(1):154–94. [PubMed: 8557845]
21. Hortnagl H, Tasan RO, Wieselthaler A, Kirchmair E, Sieghart W, Sperk G. Patterns of mRNA and protein expression for 12 GABAA receptor subunits in the mouse brain. *Neuroscience.* 2013; 236:345–72. [PubMed: 23337532]
22. Gunn BG, Cunningham L, Cooper MA, Corteen NL, Seifi M, Swinny JD, et al. Dysfunctional astrocytic and synaptic regulation of hypothalamic glutamatergic transmission in a mouse model of early-life adversity: relevance to neurosteroids and programming of the stress response. *J Neurosci.* 2013; 33(50):19534–54. [PubMed: 24336719]
23. Lee V, Sarkar J, Maguire J. Loss of Gabrd in CRH neurons blunts the corticosterone response to stress and diminishes stress-related behaviors. *Psychoneuroendocrinology.* 2014; 41:75–88. [PubMed: 24495609]
24. Lee HH, Walker JA, Williams JR, Goodier RJ, Payne JA, Moss SJ. Direct protein kinase C-dependent phosphorylation regulates the cell surface stability and activity of the potassium chloride cotransporter KCC2. *J Biol Chem.* 2007; 282(41):29777–84. [PubMed: 17693402]
25. Payne JA, Rivera C, Voipio J, Kaila K. Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci.* 2003; 26(4):199–206. [PubMed: 12689771]
26. Rivera C, Voipio J, Kaila K. Two developmental switches in GABAergic signalling: the K⁺-Cl⁻ cotransporter KCC2 and carbonic anhydrase CAVII. *J Physiol.* 2005; 562(Pt 1):27–36. [PubMed: 15528236]
27. Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, et al. The K⁺/Cl⁻ co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature.* 1999; 397(6716):251–5. [PubMed: 9930699]
28. Hewitt SA, Wamsteeker JI, Kurz EU, Bains JS. Altered chloride homeostasis removes synaptic inhibitory constraint of the stress axis. *Nat Neurosci.* 2009; 12(4):438–43. [PubMed: 19252497]
29. MacKenzie G, Maguire J. Chronic stress shifts the GABA reversal potential in the hippocampus and increases seizure susceptibility. *Epilepsy research.* 2015; 109:13–27. [PubMed: 25524838]
30. Inoue W, Bains JS. Beyond inhibition: GABA synapses tune the neuroendocrine stress axis. *Bioessays.* 2014; 36(6):561–9. [PubMed: 24648386]

31. Mody I, Maguire J. The reciprocal regulation of stress hormones and GABA(A) receptors. *Front Cell Neurosci.* 2011; 6:4. [PubMed: 22319473]
32. Silverman AJ, Jhamandas J, Renaud LP. Localization of luteinizing hormone-releasing hormone (LHRH) neurons that project to the median eminence. *J Neurosci.* 1987; 7(8):2312–9. [PubMed: 3302127]
33. Merchenthaler I, Setalo G, Csontos C, Petrusz P, Flerko B, Negro-Vilar A. Combined retrograde tracing and immunocytochemical identification of luteinizing hormone-releasing hormone- and somatostatin-containing neurons projecting to the median eminence of the rat. *Endocrinology.* 1989; 125(6):2812–21. [PubMed: 2479526]
34. McArdle, CA.; Roberson, MS. Knobil and Neill's Physiology of Reproduction. Fourth Edition. Academic Press; San Diego: 2015. Chapter 10 - Gonadotropes and Gonadotropin-Releasing Hormone Signaling.; p. 335-97.
35. Cottrell EC, Campbell RE, Han S-K, Herbison AE. Postnatal remodeling of dendritic structure and spine density in gonadotropin-releasing hormone neurons. *Endocrinology.* 2006; 147(8):3652–61. [PubMed: 16644918]
36. Campbell RE, Gaidamaka G, Han S-K, Herbison AE. Dendro-dendritic bundling and shared synapses between gonadotropin-releasing hormone neurons. *Proceedings of the National Academy of Sciences.* 2009; 106(26):10835–40.
37. Roberts CB, Best JA, Suter KJ. Dendritic processing of excitatory synaptic input in hypothalamic gonadotropin releasing-hormone neurons. *Endocrinology.* 2006; 147(3):1545–55. [PubMed: 16373415]
38. Herde MK, Iremonger KJ, Constantin S, Herbison AE. GnRH neurons elaborate a long-range projection with shared axonal and dendritic functions. *The Journal of Neuroscience.* 2013; 33(31):12689–97. [PubMed: 23904605]
39. Iremonger KJ, Herbison AE. Initiation and propagation of action potentials in gonadotropin-releasing hormone neuron dendrites. *The Journal of Neuroscience.* 2012; 32(1):151–8. [PubMed: 22219278]
40. Chen X, Sneyd J. A Computational Model of the Dendron of the GnRH Neuron. *Bulletin of Mathematical Biology.* 2014;1–23.
41. Boehm SL 2nd, Homanics GE, Blednov YA, Harris RA. delta-Subunit containing GABAA receptor knockout mice are less sensitive to the actions of 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol. *Eur J Pharmacol.* 2006; 541(3):158–62. [PubMed: 16777089]
42. Meera P, Wallner M, Otis TS. Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic GABA(A) receptors. *J Neurophysiol.* 2011; 106(4):2057–64. [PubMed: 21795619]
43. Mortensen M, Ebert B, Wafford K, Smart TG. Distinct activities of GABA agonists at synaptic- and extrasynaptic-type GABAA receptors. *J Physiol.* 2010; 588(Pt 8):1251–68. [PubMed: 20176630]
44. Sullivan SD, Moenter SM. Neurosteroids alter gamma-aminobutyric acid postsynaptic currents in gonadotropin-releasing hormone neurons: a possible mechanism for direct steroidal control. *Endocrinology.* 2003; 144(10):4366–75. [PubMed: 12960018]
45. Sim JA, Skynner MJ, Herbison AE. Direct Regulation of Postnatal GnRH Neurons by the Progesterone Derivative Allopregnanolone in the Mouse. *Endocrinology.* 2001; 142(10):4448–53. [PubMed: 11564709]
46. Hosie AM, Clarke L, da Silva H, Smart TG. Conserved site for neurosteroid modulation of \GABAA\ receptors. *Neuropharmacology.* 2009; 56(1):149–54. [PubMed: 18762201]
47. Clarkson J, Herbison AE. Development of \GABA\ and glutamate signaling at the GnRH neuron in relation to puberty. *Molecular and Cellular Endocrinology.* 2006; 254-255(0):32–8. [PubMed: 16781054]
48. Mitsushima D, Tin Tin Win S, Kimura F. Sexual dimorphism in the GABAergic control of gonadotropin release in intact rats. *Neurosci Res.* 2003; 46(4):399–405. [PubMed: 12871761]
49. Maffucci JA, Gore AC. Chapter 2: hypothalamic neural systems controlling the female reproductive life cycle gonadotropin-releasing hormone, glutamate, and GABA. *Int Rev Cell Mol Biol.* 2009; 274:69–127. [PubMed: 19349036]

50. Lee K, Porteous R, Campbell RE, Lüscher B, Herbison AE. Knockdown of GABA(A) Receptor Signaling in GnRH Neurons Has Minimal Effects upon Fertility. *Endocrinology*. 2010; 151(9): 4428–36. [PubMed: 20573723]
51. Sisk CL, Richardson HN, Chappell PE, Levine JE. In Vivo Gonadotropin-Releasing Hormone Secretion in Female Rats during Peripubertal Development and on Proestrus 1. *Endocrinology*. 2001; 142(7):2929–36. [PubMed: 11416013]
52. Jarry H, Leonhardt S, Wuttke W. Gamma-aminobutyric acid neurons in the preoptic/anterior hypothalamic area synchronize the phasic activity of the gonadotropin-releasing hormone pulse generator in ovariectomized rats. *Neuroendocrinology*. 1991; 53(3):261–7. [PubMed: 2041587]
53. Herbison A, Chapman C, Dyer R. Role of medial preoptic GABA neurones in regulating luteinising hormone secretion in the ovariectomised rat. *Experimental brain research*. 1991; 87(2): 345–52. [PubMed: 1769388]
54. DeFazio RA, Heger S, Ojeda SR, Moenter SM. Activation of A-type gamma-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. *Molecular endocrinology*. 2002; 16(12):2872–91. [PubMed: 12456806]
55. Herbison AE, Moenter SM. Depolarising and hyperpolarising actions of GABA(A) receptor activation on gonadotrophin-releasing hormone neurones: towards an emerging consensus. *J Neuroendocrinol*. 2011; 23(7):557–69. [PubMed: 21518033]
56. Watanabe M, Sakuma Y, Kato M. GABAA receptors mediate excitation in adult rat GnRH neurons. *Biology of reproduction*. 2009; 81(2):327–32. [PubMed: 19357366]
57. Han S-K, Abraham IM, Herbison AE. Effect of GABA on GnRH neurons switches from depolarization to hyperpolarization at puberty in the female mouse. *Endocrinology*. 2002; 143(4): 1459–66. [PubMed: 11897704]
58. Han S-K, Todman MG, Herbison AE. Endogenous GABA release inhibits the firing of adult gonadotropin-releasing hormone neurons. *Endocrinology*. 2004; 145(2):495–9. [PubMed: 14617578]
59. Moenter SM, DeFazio RA. Endogenous gamma-aminobutyric acid can excite gonadotropin-releasing hormone neurons. *Endocrinology*. 2005; 146(12):5374–9. [PubMed: 16123153]
60. Yin C, Ishii H, Tanaka N, Sakuma Y, Kato M. Activation of A-type gamma-amino butyric acid receptors excites gonadotrophin-releasing hormone neurones isolated from adult rats. *Journal of Neuroendocrinology*. 2008; 20(5):566–75. [PubMed: 18363808]
61. Constantin S, Iremonger KJ, Herbison AE. In vivo recordings of GnRH neuron firing reveal heterogeneity and dependence upon GABAA receptor signaling. *J Neurosci*. 2013; 33(22):9394–401. [PubMed: 23719807]
62. Watanabe M, Fukuda A, Nabekura J. The role of GABA in the regulation of GnRH neurons. *Frontiers in Neuroscience*. 2014; 8:387. [PubMed: 25506316]
63. Jasoni CL, Todman MG, Han SK, Herbison AE. Expression of mRNAs encoding receptors that mediate stress signals in gonadotropin-releasing hormone neurons of the mouse. *Neuroendocrinology*. 2005; 82(5-6):320–8. [PubMed: 16721036]
64. Lin Y, Li X, Lupi M, Kinsey-Jones JS, Shao B, Lightman SL, et al. The role of the medial and central amygdala in stress-induced suppression of pulsatile LH secretion in female rats. *Endocrinology*. 2010; 152(2):545–55. [PubMed: 21159851]
65. Chandran UR, Attardi B, Friedman R, Dong KW, Roberts JL, DeFranco DB. Glucocorticoid receptor-mediated repression of gonadotropin-releasing hormone promoter activity in GT1 hypothalamic cell lines. *Endocrinology*. 1994; 134(3):1467–74. [PubMed: 8119188]
66. Chandran UR, Attardi B, Friedman R, Zheng Z, Roberts JL, DeFranco DB. Glucocorticoid repression of the mouse gonadotropin-releasing hormone gene is mediated by promoter elements that are recognized by heteromeric complexes containing glucocorticoid receptor. *J Biol Chem*. 1996; 271(34):20412–20. [PubMed: 8702778]
67. Gore AC, Attardi B, DeFranco DB. Glucocorticoid repression of the reproductive axis: effects on GnRH and gonadotropin subunit mRNA levels. *Mol Cell Endocrinol*. 2006; 256(1-2):40–8. [PubMed: 16839661]

68. Kinsey-Jones JS, Li XF, Luckman SM, O'Byrne KT. Effects of kisspeptin-10 on the electrophysiological manifestation of gonadotropin-releasing hormone pulse generator activity in the female rat. *Endocrinology*. 2008; 149(3):1004–8. [PubMed: 18063679]
69. Li X, Shao B, Lin C, O'Byrne K, Lin Y. Stress-induced inhibition of LH pulses in female rats: role of GABA in arcuate nucleus. *J Mol Endocrinol*. 2015
70. Ruttle PL, Shirtcliff EA, Armstrong JM, Klein MH, Essex MJ. Neuroendocrine coupling across adolescence and the longitudinal influence of early life stress. *Developmental psychobiology*. 2013
71. Saxbe DE, Negri S, Susman EJ, Trickett PK. Attenuated hypothalamic-pituitary-adrenal axis functioning predicts accelerated pubertal development in girls 1 year later. *Development and psychopathology*. 2014:1–10.
72. Jasoni CL, Romano N, Constantin S, Lee K, Herbison AE. Calcium dynamics in gonadotropin-releasing hormone neurons. *Frontiers in neuroendocrinology*. 2010; 31(3):259–69. [PubMed: 20594958]
73. Kinsey-Jones JS, Li XF, Knox AM, Lin YS, Milligan SR, Lightman SL, et al. Corticotrophin-releasing factor alters the timing of puberty in the female rat. *J Neuroendocrinol*. 2010; 22(2):102–9. [PubMed: 20002962]
74. Smith JT, Waddell BJ. Increased Fetal Glucocorticoid Exposure Delays Puberty Onset in Postnatal Life. *Endocrinology*. 2000; 141(7):2422–8. [PubMed: 10875242]
75. Harvey PW, Chevins PFD. Crowding during pregnancy delays puberty and alters estrous cycles of female offspring in mice. *Experientia*. 1987; 43(3):306–8. [PubMed: 3556526]
76. Politch JA, Herrenkohl LR. Effects of prenatal stress on reproduction in male and female mice. *Physiology and Behavior*. 1984; 32(1):95–9. [PubMed: 6718542]
77. Wade GN, Schneider JE. Metabolic fuels and reproduction in female mammals. *Neurosci Biobehav Rev*. 1992; 16(2):235–72. [PubMed: 1630733]
78. Lau C, Klinefelter G, Cameron AM. Reproductive Development and Functions in the Rat after Repeated Maternal Deprivation Stress. *Toxicological Sciences*. 1996; 30(2):298–301.
79. Rhees RW, Lephart ED, Eliason D. Effects of maternal separation during early postnatal development on male sexual behavior and female reproductive function. *Behavioural Brain Research*. 2001; 123(1):1–10. [PubMed: 11377724]
80. Cameron NM, Champagne FA, Parent C, Fish EW, Ozaki-Kuroda K, Meaney MJ. The programming of individual differences in defensive responses and reproductive strategies in the rat through variations in maternal care. *Neuroscience and Biobehavioral Reviews*. 2005; 29(4-5):843–65. [PubMed: 15893378]
81. Li XF, Hu MH, Li SY, Geach C, Hikima A, Rose S, et al. Overexpression of Corticotropin Releasing Factor in the Central Nucleus of the Amygdala Advances Puberty and Disrupts Reproductive Cycles in Female Rats. *Endocrinology*. 2014; 155(10):3934–44. [PubMed: 25051447]
82. Knox AMI, Li XF, Kinsey-Jones JS, Wilkinson ES, Wu XQ, Cheng YS, et al. Neonatal Lipopolysaccharide Exposure Delays Puberty and Alters Hypothalamic Kiss1 and Kiss1r mRNA Expression in the Female Rat. *Journal of Neuroendocrinology*. 2009; 21(8):683–9. [PubMed: 19500221]
83. Léonhardt M, Lesage J, Croix D, Dutriez-Casteloot I, Beauvillain JC, Dupouy JP. Effects of perinatal maternal food restriction on pituitary-gonadal axis and plasma leptin level in rat pup at birth and weaning and on timing of puberty. *Biology of Reproduction*. 2003; 68(2):390–400. [PubMed: 12533401]
84. Sánchez-Garrido MA, Castellano JM, Ruiz-Pino F, García-Galiano D, Manfredi-Lozano M, León S, et al. Metabolic Programming of Puberty: Sexually Dimorphic Responses to Early Nutritional Challenges. *Endocrinology*. 2013; 154(9):3387–400. [PubMed: 23751873]
85. Castellano JM, Bentsen AH, Sánchez-Garrido MA, Ruiz-Pino F, Romero M, García-Galiano D, et al. Early Metabolic Programming of Puberty Onset: Impact of Changes in Postnatal Feeding and Rearing Conditions on the Timing of Puberty and Development of the Hypothalamic Kisspeptin System. *Endocrinology*. 2011; 152(9):3396–408. [PubMed: 21712362]

86. Sloboda DM, Howie GJ, Pleasants A, Gluckman PD, Vickers MH. Pre- and Postnatal Nutritional Histories Influence Reproductive Maturation and Ovarian Function in the Rat. *PLoS ONE*. 2009; 4(8):e6744. [PubMed: 19707592]
87. Franssen D, Ioannou YS, Alvarez-real A, Gerard A, Mueller JK, Heger S, et al. Pubertal timing after neonatal diethylstilbestrol exposure in female rats: Neuroendocrine vs peripheral effects and additive role of prenatal food restriction. *Reproductive Toxicology*. 2014; 44(0):63–72. [PubMed: 24316331]
88. Li XF, Lin YS, Kinsey-Jones JS, O'Byrne KT. High-fat diet increases LH pulse frequency and kisspeptin-neurokinin B expression in puberty-advanced female rats. *Endocrinology*. 2012; 153(9): 4422–31. [PubMed: 22733968]
89. Feleder C, Ginzburg M, Wuttke W, Moguilevsky JA, Arias P. GABAergic activation inhibits the hypothalamic-pituitary-ovarian axis and sexual development in the immature female rat. Associated changes in hypothalamic glutamatergic and taurinergergic systems. *Brain Res Dev Brain Res*. 1999; 116(2):151–7. [PubMed: 10521559]
90. Roth C, Leonhardt S, Theiling K, Lakomek M, Jarry H, Wuttke W. Ontogeny of the GNRH-, glutaminase- and glutamate decarboxylase-gene expression in the hypothalamus of female rats. *Brain Res Dev Brain Res*. 1998; 110(1):105–14. [PubMed: 9733938]
91. Reynoso R, Ponzo O, Cardoso N, Szwarcfarb B, Carbone S, Moguilevsky J, et al. Effect of bacterial lipopolysaccharide on the reproductive axis of prepubertal and peripubertal female rats. Ontogenic changes in the immune-neuroendocrine interactions. *Neuroimmunomodulation*. 2008; 15(2):125–30. [PubMed: 18679051]
92. Cardoso N, Arias P, Szwarcfarb B, Ponzo O, Carbone S, Moguilevsky J, et al. Reproductive axis response to repeated lipopolysaccharide administration in peripubertal female rats. *J Physiol Biochem*. 2010; 66(3):237–44. [PubMed: 20652472]
93. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. *Nature*. 1996; 382(6588):250–2. [PubMed: 8717038]
94. Sullivan SD, DeFazio RA, Moenter SM. Metabolic regulation of fertility through presynaptic and postsynaptic signaling to gonadotropin-releasing hormone neurons. *The Journal of Neuroscience*. 2003; 23(24):8578–85. [PubMed: 13679427]
95. Quenell JH, Mulligan AC, Tups A, Liu X, Phipps SJ, Kemp CJ, et al. Leptin Indirectly Regulates Gonadotropin-Releasing Hormone Neuronal Function. *Endocrinology*. 2009; 150(6):2805–12. [PubMed: 19179437]
96. Guimond D, Diabira D, Porcher C, Bader F, Ferrand N, Zhu M, et al. Leptin potentiates GABAergic synaptic transmission in the developing rodent hippocampus. *Frontiers in Cellular Neuroscience*. 2014; 8:235. [PubMed: 25177272]
97. DiVall SA, Herrera D, Sklar B, Wu S, Wondisford F, Radovick S, et al. Insulin Receptor Signaling in the GnRH Neuron Plays a Role in the Abnormal GnRH Pulsatility of Obese Female Mice. *PLoS ONE*. 2015; 10(3):e0119995. [PubMed: 25780937]
98. Balasubramanian P, Jagannathan L, Mahaley RE, Subramanian M, Gilbreath ET, MohanKumar PS, et al. High Fat Diet Affects Reproductive Functions in Female Diet-Induced Obese and Dietary Resistant Rats. *Journal of Neuroendocrinology*. 2012; 24(5):748–55. [PubMed: 22192683]
99. Abreu AR, de Abreu AR, Santos LT, de Souza AA, da Silva LG Jr. DAC, et al. Blunted GABA-mediated inhibition within the dorsomedial hypothalamus potentiates the cardiovascular response to emotional stress in rats fed a high-fat diet. *Neuroscience*. 2014; 262(0):21–30. [PubMed: 24397951]
100. Funabashi T, Jinnai K, Kimura F. Bicuculline infusion advances the timing of Fos expression in LHRH neurons in the preoptic area of proestrous rats. *Neuroreport*. 1997; 8(3):771–4. [PubMed: 9106764]
101. Halász B, Pupp L. Hormone secretion of the anterior pituitary gland after physical interruption of all nervous pathways to the hypophysiotrophic area. *Endocrinology*. 1965; 77(3):553–62. [PubMed: 4284469]
102. Yu Y, Wu Y, Patch C, Wu Z, Szabo A, Li D, et al. DHA prevents altered 5-HT1A, 5-HT2A, CB1 and GABAA receptor binding densities in the brain of male rats fed a high-saturated-fat diet. *The Journal of Nutritional Biochemistry*. 2013; 24(7):1349–58. [PubMed: 23337348]

103. Tanebe, Nishijo; Muraguchi, Ono. Effects of Chronic Stress on Hypothalamic Interleukin-1 β , Interleukin-2, and Gonadotrophin-Releasing Hormone Gene Expression in Ovariectomized Rats. *Journal of Neuroendocrinology*. 2000; 12(1):13–21. [PubMed: 10692139]
104. Li XF, Bowe JE, Kinsey-Jones JS, Brain SD, Lightman SL, O'Byrne KT. Differential Role of Corticotrophin-Releasing Factor Receptor Types 1 and 2 in Stress-Induced Suppression of Pulsatile Luteinising Hormone Secretion in the Female Rat. *Journal of Neuroendocrinology*. 2006; 18(8):602–10. [PubMed: 16867181]
105. Lin YS, Li XF, Shao B, Hu MH, Goundry ALR, Jeyaram A, et al. The Role of GABAergic Signalling in Stress-Induced Suppression of Gonadotrophin-Releasing Hormone Pulse Generator Frequency in Female Rats. *Journal of Neuroendocrinology*. 2012; 24(3):477–88. [PubMed: 22172044]
106. Yoo M, Nishihara M, Takahashi M. Tumor necrosis factor- α mediates endotoxin induced suppression of gonadotropin-releasing hormone pulse generator activity in the rat. *Endocrine Journal*. 1997; 44:141–8. [PubMed: 9152627]
107. Ebisui O, Fukata J, Tominaga T, Murakami N, Kobayashi H, Segawa H, et al. Roles of interleukin-1 α and-1 β in endotoxin-induced suppression of plasma gonadotropin levels in rats. *Endocrinology*. 1992; 130(6):3307–13. [PubMed: 1597143]
108. Rettori V, Gimeno MF, Karara A, Gonzalez MC, McCann SM. Interleukin 1 α inhibits prostaglandin E2 release to suppress pulsatile release of luteinizing hormone but not follicle-stimulating hormone. *Proceedings of the National Academy of Sciences*. 1991; 88(7):2763–7.
109. Nappi RE, Rivest S. Effect of immune and metabolic challenges on the luteinizing hormone-releasing hormone neuronal system in cycling female rats: An evaluation at the transcriptional level. *Endocrinology*. 1997; 138(4):1374–84. [PubMed: 9075691]
110. Rivest S, Lee S, Attardi B, Rivier C. The chronic intracerebroventricular infusion of interleukin-1 β alters the activity of the hypothalamic-pituitary-gonadal axis of cycling rats. I. Effect on LHRH and gonadotropin biosynthesis and secretion. *Endocrinology*. 1993; 133(6):2424–30. [PubMed: 8243260]
111. Geary N, Asarian L, Sheahan J, Langhans W. Estradiol-mediated increases in the anorexia induced by intraperitoneal injection of bacterial lipopolysaccharide in female rats. *Physiology and Behavior*. 2004; 82(2-3):251–61. [PubMed: 15276786]
112. Wang D-S, Zurek AAA, Lecker I, Yu J, Abramian AAM, Avramescu S, et al. Memory Deficits Induced by Inflammation Are Regulated by α 5-Subunit-Containing GABAA Receptors. *Cell Reports*. 2012; 2(3):488–96. [PubMed: 22999935]
113. Sim JA, Skynner MJ, Pape J-R, Herbison AE. Late postnatal reorganization of GABAA receptor signalling in native GnRH neurons. *European Journal of Neuroscience*. 2000; 12(10):3497–504. [PubMed: 11029619]
114. Jeong K-H, Jacobson L, Widmaier EP, Majzoub JA. Normal Suppression of the Reproductive Axis Following Stress in Corticotropin-Releasing Hormone-Deficient Mice. *Endocrinology*. 1999; 140(4):1702–8. [PubMed: 10098506]
115. Roozendaal MM, Swarts HJ, Wiegant VM, Mattheij JA. Effect of restraint stress on the preovulatory luteinizing hormone profile and ovulation in the rat. *European journal of endocrinology*. 1995; 133(3):347–53. [PubMed: 7581953]
116. Mitchell J, Li X, Breen L, Thalabard J-C, O'Byrne K. The role of the locus coeruleus in corticotrophin-releasing hormone and stress-induced suppression of pulsatile luteinizing hormone secretion in the female rat. *Endocrinology*. 2005; 146(1):323–31. [PubMed: 15486230]
117. Bowers G, Cullinan WE, Herman JP. Region-Specific Regulation of Glutamic Acid Decarboxylase (GAD) mRNA Expression in Central Stress Circuits. *The Journal of Neuroscience*. 1998; 18(15):5938–47. [PubMed: 9671680]
118. Li XF, Lin YS, Kinsey-Jones JS, Milligan SR, Lightman SL, O'Byrne KT. The Role of the Bed Nucleus of the Stria Terminalis in Stress-Induced Inhibition of Pulsatile Luteinising Hormone Secretion in the Female Rat. *Journal of Neuroendocrinology*. 2011; 23(1):3–11. [PubMed: 21073554]
119. Chu Z, Moenter S. Corticotropin-releasing hormone inhibits gonadotropin-releasing hormone neuron activity. *Neuroscience Meeting Abstract*. 2009

120. Miller S, Maguire J. Deficits in KCC2 and activation of the HPA axis lead to depression-like behavior following social defeat. *Hormonal Studies*. 2014; 2(1):2.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HIGHLIGHTS

- Synaptic and extrasynaptic GABA_ARs play a role in the regulation of corticotropin releasing hormone (CRH) neurons and the stress axis
- Neurosteroid actions on GABA_ARs may modulate CRH neuron activity and stress axis function
- Gonadotropin releasing hormone (GnRH) neurons are regulated by a THIP-sensitive tonic GABAergic current, which likely contributes to the regulation of the reproductive axis
- Stress-derived neurosteroids via actions on GABA_ARs may mediate the effects of stress on the reproductive axis