Review

Kisspeptin Neurons and Estrogen–Estrogen Receptor α Signaling: Unraveling the Mystery of Steroid Feedback System Regulating Mammalian Reproduction

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Abstract: Estrogen produced by ovarian follicles plays a key role in the central mechanisms controlling reproduction via regulation of gonadotropin-releasing hormone (GnRH) release by its negative and positive feedback actions in female mammals. It has been well accepted that estrogen receptor α (ERα) mediates both estrogen feedback actions, but precise targets had remained as a mystery for decades. Ever since the discovery of kisspeptin neurons as afferent ERα-expressing neurons to govern GnRH neurons, the mechanisms mediating estrogen feedback are gradually being unraveled. The present article overviews the role of kisspeptin neurons in the arcuate nucleus (ARC), which are considered to drive pulsatile GnRH/gonadotropin release and folliculogenesis, in mediating the estrogen negative feedback action, and the role of kisspeptin neurons located in the anteroventral periventricular nucleus-periventricular nucleus (AVPV-PeN), which are thought to drive GnRH/luteinizing hormone (LH) surge and consequent ovulation, in mediating the estrogen positive feedback action. This implication has been confirmed by the studies showing that estrogen-bound ERα down- and up-regulates kisspeptin gene (Kiss1) expression in the ARC and AVPV-PeN kisspeptin neurons, respectively. The article also provides the molecular and epigenetic mechanisms regulating Kiss1 expression in kisspeptin neurons by estrogen. Further, afferent ERα-expressing neurons that may regulate kisspeptin release are discussed.

Keywords: estradiol; dynorphin A; follicle-stimulating hormone; follicular development; gonadotropin-releasing hormone; luteinizing hormone; Kiss1; neurokinin B; ovulation

1. Introduction

It has been well accepted that estrogen produced by the ovary plays an indispensable role in the female reproductive system via its feedback actions on gonadotropin-releasing hormone (GnRH) release in mammals. The central mechanisms of the estrogen feedback actions on GnRH release have been a mystery for decades. This is because no report has been available to show the expression of estrogen receptor α (ERα), a critical receptor isoform required for estrogen feedback actions, in the hypothalamic GnRH neurons. Intensive studies on the hypothalamic kisspeptin neurons, which express ERα, have been gradually unraveling the central mechanisms of the negative and positive feedback actions of estrogen on GnRH release. In this article, the physiological significance of the estrogen negative and positive feedback actions on the tonic pulsatile and surge modes of GnRH release—which control folliculogenesis and ovulation in female mammals, respectively—is outlined. Further, the molecular and epigenetic mechanisms mediating the regulation of kisspeptin gene (Kiss1) expression by estrogen–ERα signaling and afferent ERα-expressing neurons that may mediate estrogen-dependent modulation of kisspeptin release from the hypothalamic kisspeptin neurons are also discussed.
2. Feedback Actions of Estrogen on Pulsatile and Surge- Modes of Gonadotropin-Releasing Hormone (GnRH)/Gonadotropin Release

Mammalian reproduction is orchestrated by the interaction of hormones secreted by the hypothalamus–pituitary–gonadal (HPG) axis. Estrogen secreted from the ovary, downstream of the axis, feeds back to the higher hierarchy hypothalamus and pituitary to regulate GnRH/gonadotropin release. Estrogen production is stimulated by the tonic pulsatile release of gonadotropins, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the anterior pituitary gland, under the control of GnRH pulses. During the follicular development, circulating estrogen fine-tunes pulsatile release of GnRH to keep circulating levels of LH and FSH adequately. This estrogen action is referred to as “the negative feedback action of estrogen” on GnRH pulses. Under appropriate stimulation by LH and FSH, ovarian follicles develop into a large and mature state. Estrogen production and release gradually increase along with the follicular development, and consequent high levels of circulating estrogen derived from mature follicles (also known as Graafian follicles), in turn, induce a large release of hypothalamic GnRH and then pituitary LH (GnRH/LH surge). This is the so-called “positive feedback action of estrogen” on GnRH release, and the LH surge consequently evokes ovulation. Therefore, the circulating levels of estrogen serve as a messenger for transmitting the maturity status of ovarian follicles to the hypothalamus, which plays a pinnacle role in the hierarchical control of the HPG axis in female mammals.

The presence of hypothalamic GnRH was predicted by Harris and Jacobsohn in the early 1950s by showing that the function of the pituitary graft was restored only when the graft was placed under the median eminence in hypophysectomized rats [1]. This notion was further validated by McCann and colleagues in the early 1960s by showing the LH-releasing ability of hypothalamic extracts in rats [2,3]. The tonic pulsatile LH release, the preovulatory LH surge, and their regulation by estrogen feedback are then clearly demonstrated by Knobil and colleagues from the late 1960s to the early 1970s [4–8]. They demonstrated that tonic LH release was found in most periods of the menstrual cycle, and LH surge was found only in the midcycle before ovulation in humans and rhesus monkeys [4,5]. It was also demonstrated that ovariectomy increased plasma LH concentration, indicating the negative feedback action of some ovarian humoral factor(s) on tonic pulsatile LH release in rhesus monkeys [6]. Importantly, estrogen replacement at a physiological level (not a preovulatory level) suppressed tonic pulsatile LH release in ovariectomized (OVX) rhesus monkeys [8]. These findings suggest that estrogen secreted from the ovary is a major humoral factor that exerts its negative feedback action on the tonic pulsatile LH release. On the other hand, an administration of estrogen at a preovulatory dose induces a vast release of LH similar to the spontaneous preovulatory LH surge, suggesting that the high dose of estrogen exerts its positive feedback action on the LH surge-generating system [7,8].

In 1971, GnRH was isolated from the hypothalamus of pigs and sheep by two groups led by Schally and Guillemin [9,10]. In the early 1990s, Moenter et al. [11–13] suggested that GnRH dominantly controls LH release by showing that GnRH pulses and GnRH surge in the pituitary portal circulation were synchronized with LH pulses and LH surge in the peripheral circulation, respectively, in sheep. In addition, Pau et al. [14] showed simultaneous GnRH and LH surges in rhesus monkeys as well. To date, the pulsatile and surge modes of GnRH release have been hypothesized to be driven by the independent hypothalamic mechanisms, so-called “GnRH pulse and surge generators”, respectively [15–17]. It is plausible that estrogen regulates the activity of GnRH pulse and surge generators via the negative and positive feedback actions, respectively.

3. Indispensable Role of Estrogen Receptor α for Mammalian Reproduction

Accumulating evidence indicates that ERα is a critical estrogen receptor isoform responsible for both the negative and positive feedback actions of estrogen on GnRH/LH release. In fact, Esr1 (coding ERα) knockout mice [18,19] and rats [20] show hypersecretion of both LH and estrogen, indicating that ERα mainly mediates the estrogen negative
feedback action. Further, the Esr1 knockout mice and rats fail to show ovulation, albeit enlarged cystic follicles are found in Esr1 knockout mice and rats. This suggests that ERα is also responsible for the estrogen positive feedback action [18–20]. On the other hand, reproductive function of Esr2 (coding ERβ, known as another ER) knockout animal models were reportedly varied between animal models: Esr2 knockout mice showed normosecretion of LH and estrogen [19] and are subfertile with a small litter size [21,22]; Esr2 knockout rats are infertile with a lack of LH surge and ovulation [23]. In addition, previous studies demonstrated that selective antagonism of estrogen–ERα signaling, but not estrogen–ERβ signaling, eliminated the endogenous LH surge in rats [24]. Taken together, these findings suggest that ERα mainly mediates both estrogen feedback actions on GnRH/LH release.

In general, the ERα is known as a ligand-activated transcriptional factor that activates or represses the expression of target genes. The estrogen-bound ERα is reported to bind to the estrogen response element (ERE) in the target genes to control gene expression [25]. In addition, it is suggested that the estrogen-bound ERα interacts with other transcription factors, such as AP-1 and NF-κB, and the complex controls target gene expression via binding to non-ERE response elements through the transcriptional partner [26–28]. Intriguingly, a previous study demonstrated that ERα knock-in/knockout (KIKO) mice, in which a mutant ERα (E207A/G208A) lacks the binding ability for the ERE but is capable of interacting with other transcriptional partners [29,30], showed the negative, but not the positive, feedback action of estrogen on LH release [31]. These findings suggest that the negative feedback action of estrogen on GnRH pulses is likely mediated via some gene(s) controlled by the ERE-independent estrogen–ERα signaling and that the estrogen positive feedback action is likely mediated via some gene(s) controlled by the ERE-dependent estrogen–ERα signaling.

4. Possible Targets of the Negative and Positive Feedback Action of Estrogen in the Brain

Precise targets of the negative and positive feedback actions of estrogen on the GnRH pulse and surge generation have been a mystery for many years of the 20th century because no report has been available to show ERα expression in GnRH neurons [32]. Therefore, the most plausible explanations are that certain hypothalamic ERα-expressing cells serve as targets of the negative and positive feedback actions of estrogen on GnRH pulse and surge generation and that such ERα-expressing cells transmit the estrogen signals to GnRH neurons. A large number of ERα-expressing cells were found in the several hypothalamic nuclei—such as the anteroventral periventricular nucleus (AVPV), preoptic area (POA), arcuate nucleus (ARC), and ventromedial nucleus (VMH)—at both the mRNA and protein levels, as well as the paraventricular nucleus (PVN) and suprachiasmatic nucleus (SCN), where ERα expression was evident at the mRNA level in rats [33,34]. Similarly, ERα was mainly found in the POA, ARC, and VMH at both the mRNA and protein levels and in the PVN at the mRNA level in sheep [35,36]. These findings were well consistent with previous studies showing that radiolabeled estrogen was accumulated in the POA, ARC, and VMH in rats [37].

Previous studies suggest that the ARC is one of the most possible targets of negative feedback action of estrogen: Smith and Davidson [38] showed that estrogen implants in the mediobasal hypothalamus (MBH), including the ARC suppressed plasma LH levels in OVX rats in 1974; Akema et al. [39] showed that estrogen implants in the ARC suppressed LH pulses in OVX rats in 1983; furthermore, Nagatani et al. [40] showed that estrogen micro-implants in the ARC suppressed LH pulses in both fasted and re-fed OVX rats, while the estrogen implants in either the PVN or brainstem A2 region suppressed LH pulses in only fasted rats in 1994. These findings suggested that the negative feedback action of estrogen may be mediated by ERα-expressing neurons located in the ARC under the normal nutritional condition and by multiple ERα-expressing neurons located in the ARC, PVN, and brainstem A2 region under the malnutritional condition. The negative feedback actions of estrogen under the malnutritional condition are likely mediated by de novo
synthesized ERα in the PVN and brainstem A2 region because 48 h fasting increases the number of ERα-immunoreactive cells in the PVN and brainstem A2 region in O VX rats [41].

Previous studies suggest that ERα-expressing neurons in the AVPV and/or POA are the most possible targets of estrogen positive feedback action: Kawakami et al. [42] and Goodman [43] demonstrated in the late 1970s that estrogen implants into the AVPV or neighboring POA induced the LH surge in OVX rats; Wiegand et al. [44,45] showed in the late 1980s that an electrolytic lesion around the AVPV abolished the estrogen-induced LH surge in OVX rats; Petersen et al. [46,47] demonstrated in the late 1980s that implants of estrogen antagonists, such as LY-10074 or keoxifene, in the AVPV-POA region prevented estrogen-induced LH surge in OVX rats. These reports suggest that the ERα-expressing cells in the AVPV-POA region serve as targets of the estrogen positive feedback actions to induce GnRH/LH surge.

5. Kisspeptin Neurons as Targets of the Negative and Positive Feedback Actions of Estrogen

Intensive studies during the past 20 years demonstrate that ERα expression is evident in the hypothalamic kisspeptin neurons in rodents [48–51] and sheep [52] and that kisspeptin serves as a potent secretagogue of gonadotropin release in rodents [50, 53–58], ruminants [59, 60], and primates [61, 62]. To date, it is well accepted that ERα-expressing kisspeptin neurons mainly mediate the estrogen feedback on GnRH release in mammals, and the possible mechanism mediating the feedback effect is discussed in detail later in this article.

Kisspeptin was first discovered as an endogenous ligand for GPR54, an orphan Gq-coupled G-protein coupled receptor (GPCR), in humans in 2001 [63, 64]. In 2003, two independent research groups reported that inactivating mutations in the GPR54 gene caused hypogonadotropic hypogonadism in humans [65, 66]. These important findings shed light on the fact that kisspeptin–GPR54 signaling plays a pivotal role in the brain mechanism controlling GnRH/gonadotropin release and then puberty and fertility in mammals [65,66]. As expected, inactivating mutations in the KISS1 gene (coding kisspeptin) also caused hypogonadotropic hypogonadism in humans [67]. The infertile phenotype in humans carrying inactivating mutations of the KISS1 or GPR54 genes was recapitulated in Kiss1 or Gpr54 knockout rodent models [66, 68–72]. Importantly, GPR54 expression is evident in GnRH neurons in rodents [54, 68, 73–75], suggesting that kisspeptin directly stimulates GnRH release. Further, Kiss1 knockout rats show undetectable levels of LH and FSH even after ovariectomy, indicating failure of tonic pulsatile LH release [72]. In addition, the Kiss1 knockout rats also fail to show estrogen-induced LH surge. These findings suggest that kisspeptin–GPR54 signaling is indispensable for both GnRH pulse and surge generation and mediate feedback actions of estrogen on GnRH/LH release.

Histological studies in rodents revealed that cell bodies of kisspeptin neurons are mainly located in the anterior hypothalamic areas, such as the AVPV–periventricular nucleus continuum (AVPV-PeN), and in the posterior hypothalamic region—that is, the ARC [48–51, 76–78]. Importantly, ERα was found in both populations of hypothalamic kisspeptin neurons, and Kiss1 expression is controlled by estrogen in a brain region-specific fashion in rodents [48–51, 76]. More specifically, the ARC Kiss1 expression level was high at diestrus and was suppressed by estrogen treatment [48–51, 76], whereas the AVPV-PeN Kiss1 expression level was high at the afternoon of proestrus and was increased by estrogen treatment in rodents [48, 49, 51, 76]. These findings suggest that the ARC kisspeptin neurons are a target of the negative feedback action of estrogen and that the AVPV-PeN kisspeptin neurons are a target of the positive feedback action of estrogen. Figure 1 depicts the brain mechanism mediating the estrogen negative and positive feedback actions on GnRH/gonadotropin release to regulate follicular development and ovulation in rodents. As shown in the figure, it is most likely that the ARC kisspeptin neurons control GnRH/LH pulses via mediating the estrogen negative feedback action and that the AVPV-PeN kisspeptin neurons control GnRH/LH surge via mediating the estrogen positive feedback action.
Figure 1. Central mechanisms underlying the negative and positive feedback actions of estrogen on pulsatile and surge modes of gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) release in female rodents. Estrogen production along with follicular development is stimulated by GnRH/gonadotropin pulses. During the follicular development period, low levels of circulating estrogen fine-tune GnRH/H pulses via the negative feedback action of estrogen. The estrogen negative feedback action is considered to be mediated by estrogen receptor α (ERα)-expressing kisspeptin neurons located in the arcuate nucleus (ARC). Estrogen production and release gradually increase along with the follicular development, and consequent high levels of circulating estrogen derived from mature follicles, in turn, induce GnRH/LH surge and hence ovulation via the positive feedback action of estrogen. The estrogen positive feedback action is likely mediated by ERα-expressing kisspeptin neurons located in the anteroventral periventricular nucleus–periventricular nucleus continuum (AVPV-PeN).

5.1. The Molecular and Epigenetic Mechanism Mediating the Regulation of Arcuate Kiss1 Expression by Estrogen and the Role of arcuate Kisspeptin Neurons as the GnRH Pulse Generator in Mammals

To date, the ARC kisspeptin neurons have been considered to serve as a target of estrogen negative feedback action on GnRH pulse generation, and a similar population of kisspeptin neurons have been identified in the ARC in other species or infundibular nucleus in primates (equivalent to the ARC in others) of several mammalian species, including humans [79,80], macaque monkeys [80–83], sheep [52,84–86], goats [59,87,88], cattle [89], horse [90], pigs [91], and musk shrews [92]. Our studies and other previous studies demonstrated that estrogen treatment largely repressed ARC Kiss1 expression in rodents [48–51]. Similar to the rodent models, previous studies demonstrated estrogen-
dependent repression of \textit{KISS1} expression in the ARC kisspeptin neurons in sheep \cite{93,94} and the infundibular nucleus in primates including humans \cite{80}. These findings suggest that the estrogen negative feedback action on ARC kisspeptin neurons would be largely common among mammalian species.

According to the studies with rodent models, estrogen-dependent repression of \textit{Kiss1} expression in ARC kisspeptin neurons is likely mediated via the ERE-independent pathway because estrogen repressed the ARC \textit{Kiss1} expression even in ER\textsubscript{x} KIKO mice \cite{95–97}. In addition, our previous chromatin immunoprecipitation (ChIP) assay with antibodies against ER\textsubscript{x} and acetylated histone H3 revealed that estrogen-bound ER\textsubscript{x} induced histone H3 deacetylation of the \textit{Kiss1} promoter region in the ARC kisspeptin neurons by showing that estrogen treatment lowered acetylated histone H3 levels in the \textit{Kiss1} promoter region in mouse ARC tissue \cite{98}. These findings suggest that an estrogen-dependent inactivating modification of histone H3 of the \textit{Kiss1} promoter region resulted in the repression of \textit{Kiss1} expression. Furthermore, our in vivo reporter assay utilizing \textit{Kiss1}-GFP reporter mice suggested that the 5'-intergenic region of the \textit{Kiss1} gene is required for an induction of \textit{Kiss1} mRNA expression in the ARC of female mice \cite{99}. Indeed, reporter mice carrying the 5'-truncated \textit{Kiss1}-GFP transgene (RBRC09415 and RBRC09416) failed to display the GFP expression in ARC kisspeptin neurons even after ovariectomy. Importantly, the reporter mice displayed the GFP expression in the AVPV-PeN kisspeptin neurons in the presence of estrogen \cite{99}. Furthermore, other reporter mice carrying the full-length of \textit{Kiss1}-GFP transgene (RBRC09413) displayed the GFP expression in both ARC and AVPV-PeN kisspeptin neurons in OVX and estrogen-treated OVX conditions, respectively \cite{99}. Taken together, we speculate that the estrogen-bound ER\textsubscript{x} may cancel interaction, which is most likely chromatin loop formation, between the \textit{Kiss1} promoter and the 5'-intergenic regions of \textit{Kiss1} locus, resulting in the repression of \textit{Kiss1} expression in ARC kisspeptin neurons even after the ovariectomy.

Collectively, we envisage the molecular mechanism of estrogen negative feedback action on ARC \textit{Kiss1} expression as shown in Figure 2. Briefly, circulating estrogen most likely binds to ER\textsubscript{x} in the ARC kisspeptin neurons, and then the estrogen-bound ER\textsubscript{x} coupled with unknown transcriptional partner(s) may repress \textit{Kiss1} expression via a non-classic ERE-independent pathway in ARC kisspeptin neurons. The estrogen-bound ER\textsubscript{x} may induce histone H3 deacetylation at the \textit{Kiss1} promoter, and the estrogen-bound ER\textsubscript{x} and/or this inactivating histone modification may unwind chromatin loops between the \textit{Kiss1} promoter and the 5'-intergenic regions of \textit{Kiss1} locus, resulting in the repression of ARC \textit{Kiss1} expression in ARC kisspeptin neurons.

The vast majority of ARC kisspeptin neurons reportedly express neurokinin B (NKB) and dynorphin A (Dyn), thus the ARC kisspeptin neurons are also called KNDy neurons \cite{86,87,100–102}. Accumulating evidence suggests that the ARC KNDy neurons can serve as an intrinsic source of the GnRH pulse generator \cite{103–107}. The notion was recently confirmed by our study showing that rescuing \textit{Kiss1} expression only in ARC \textit{Tac3} (NKB gene)-expressing neurons recovered LH pulses and follicular development in global \textit{Kiss1} knockout rats \cite{108}. The multiple-unit activity (MUA) recording demonstrated that rhythmic increases in the MUA volley detected from the recording electrodes placed in close vicinity to ARC kisspeptin (KNDy) neurons were synchronized with LH pulses in goats \cite{59,87}. In addition, conditional ARC-specific \textit{Kiss1} knockout by using the Cre-loxP system severely or partially suppressed LH pulses in rats \cite{108} and mice \cite{109,110} according to the knockout rates in each individual. Further, the fiber photometry recording revealed that the mouse ARC kisspeptin neurons displayed rhythmic increases in intracellular Ca\textsuperscript{2+} levels that correspond to LH pulses \cite{111,112}. Thus, kisspeptin neurons may secrete kisspeptin in a pulsatile fashion and then induce GnRH/gonadotropin pulses. Indeed, Keen et al. \cite{113} and Kurian et al. \cite{114} showed pulsatile kisspeptin release that mostly corresponds to GnRH pulses at the median eminence in rhesus monkeys. Thus, the negative feedback action of estrogen directly acts on the intrinsic source of the GnRH pulse generator—namely, ARC kisspeptin neurons—and then suppresses GnRH/LH pulses.
In this context, the profound suppression of GnRH/LH pulses before the afternoon LH surge in the female rodents in the presence of a high dose of estrogen may be due to the abovementioned epigenetic repression of ARC Kiss1 expression and consequent deficiency of kisspeptin in ARC kisspeptin neurons. Indeed, chronic treatment of preovulatory levels of estrogen profoundly suppresses tonic LH release in the morning (before LH surge) in OVX rats, and the estrogen treatment largely decreased Kiss1 expression as well as kisspeptin-immunoreactivity in the ARC of female rats [51].

Figure 2. Putative molecular mechanism of the negative feedback action of estrogen on Kiss1 expression in the arcuate nucleus (ARC). Circulating estrogen seems to act on ARC kisspeptin neurons, in which estrogen-bound estrogen receptor α (ERα) coupled with an unknown transcriptional partner may repress Kiss1 expression via histone deacetylation and unwinding chromatin loops between the Kiss1 promoter and the 5′-intergenic regions of the Kiss1 locus. In the absence of estrogen, ARC Kiss1 expression may be up-regulated by histone acetylation and chromatin loop formation between the Kiss1 promoter and the 5′-intergenic regions of the Kiss1 locus.

In addition to the direct inhibiting action of estrogen on Kiss1 expression, estrogen may also inhibit the pulsatile activity of ARC kisspeptin neurons via other intra-kisspeptin neuronal mechanisms or some afferent ERα-expressing neurons to ARC kisspeptin neurons. The frequency of KNDy neuronal activity recorded by the MUA volley was increased and decreased by a central administration of NKB and Dyn, respectively, in goats [87,104]. A majority of KNDy neurons reportedly express both tachykinin NK3 receptor, a Gq-coupled GPCR for NKB, and kappa-opioid receptor (KOR), a Gi-coupled GPCR for Dyn in mice [102,115–117], rats [118,119], and sheep [120,121]. Considering the stimulatory or inhibitory signaling of NKB or Dyn, respectively, these findings suggest that the pulsatile activity of ARC kisspeptin (KNDy) neurons is controlled by NKB and Dyn in an autocrine/paracrine manner (please see review articles for details, [103–105]). Previous studies showed that estrogen decreased NKB gene (Tac2 in mice and Tac3/TAC3 in other mammals) expression in the ARC of mice [96,97,122] and sheep [123] and in the infundibular nucleus of rhesus monkeys [124]. In addition, estrogen decreased Dyn gene (Pdyn) expression in the ARC of mice [95,96] and rats [125]. These results suggest that estrogen may modulate kisspeptin release from the ARC KNDy neurons via changing stimulatory NKB and inhibitory Dyn inputs to the KNDy neurons.

Interestingly, the proestrous level of estrogen repressed ARC Kiss1 expression [50,51], whereas the diestrous level of estrogen, which exerted negative feedback action of LH pulses [78], failed to suppress ARC Kiss1 expression in female rats [50,51]. The dose of
estrogen required for the repression of ARC Kiss1 expression raises the possibility that certain afferent ERα-expressing neurons to ARC kisspeptin neurons may be involved in the negative feedback action of estrogen on kisspeptin release from the ARC kisspeptin neurons. This notion is supported by a previous study showing that estrogen effectively decreased plasma LH concentration even in kisspeptin neuron-specific ERα knockout mice, whose ARC Kiss1 expression was not repressed by estrogen treatment [126]. One of the candidates mediating the estrogen negative feedback action would be Dyn neurons located in the PVN. Our studies showed that estrogen increased the number of Pdyn-expressing cells in the PVN [118] and that nor-binaltorphimine (nor-BNI), a KOR antagonist, enhanced LH pulses in estrogen-treated OVX rats but not in OVX rats without estrogen replacement [127]. Further, our recent study showed that glucoprivation suppressed LH pulses and induced fos (coding c-Fos, a marker of neuronal activation) expression in PVN Dyn neurons, while central KOR antagonism blocked the glucoprivic suppression of LH pulses in estrogen-treated OVX rats [118]. These findings suggest that PVN Dyn neurons may partly mediate estrogen negative feedback action to suppress kisspeptin release via KOR expressed in ARC kisspeptin neurons and then suppress pulsatile GnRH/LH release.

5.2. The Role of Anteroventral Periventricular Nucleus-Periventricular Nucleus (AVPV-PeN)/Preoptic Area (POA) Kisspeptin Neurons as the GnRH/Luteinizing Hormone (LH) Surge Generator and the Molecular and Epigenetic Mechanism Mediating the Regulation of AVPV-PeN/POA Kiss1 Expression by Estrogen Positive Feedback Action

The AVPV-PeN kisspeptin neurons have been considered to serve as a target of estrogen positive feedback action on GnRH surge generation in rodents, as already mentioned in the article. To date, kisspeptin neurons were found in the POA in several mammalian species, including macaque monkeys [81,83], sheep [85], goats [88], cattle [89], and musk shrews [92], as well as in the PeN in pigs [91]. Previous studies demonstrated that estrogen treatment largely increased AVPV-PeN Kiss1 expression [48,49,51,128] and induced c-Fos expression in AVPV-PeN kisspeptin neurons in OVX rodent models [49,51]. Similarly, our and other previous studies demonstrated estrogen-induced Kiss1 and/or c-Fos expression in the POA/PeN kisspeptin neurons of macaque monkeys [81,83], sheep [85], goats [88], cattle [89], pigs [91], and musk shrews [92]. Thus, the POA/PeN kisspeptin neurons in those species are likely equivalent to AVPV-PeN kisspeptin neurons in rodents in terms of an estrogen positive feedback action site.

The notion that the AVPV-PeN kisspeptin neurons serve as an intrinsic source of the GnRH surge generator is more verified by the following studies on sex difference in LH surge generation in rodent models [129–132]. It is well-known that male rats failed to show LH surge even when they were treated with a preovulatory level of estrogen after castration in adulthood [133,134]. Concordantly, male rodents show only a few kisspeptin neurons in the AVPV-PeN even in the presence of estrogen, whereas females exhibit a cluster of AVPV-PeN kisspeptin neurons in the presence of estrogen [51,76,77]. Sex steroids originated from the perinatal testes are considered to cause defeminization of the AVPV-PeN kisspeptin neurons because neonatal castration allowed the estrogen-induced AVPV-PeN Kiss1 expression and LH surge in genetic male rats in adulthood to be shown [133,134]. In further support, neonatally androgenized/estrogenized female rats displayed the male-like pattern (few) of Kiss1 expression in the AVPV-PeN and failed to show LH surge in adulthood [76,133]. Thus, these findings suggest that the AVPV-PeN kisspeptin neurons serve as a target of the estrogen positive feedback action and are the intrinsic source of the GnRH surge generator in rodents.

Estrogen-induced Kiss1 expression in AVPV-PeN kisspeptin neurons is likely mediated via the ERE-dependent pathway because estrogen treatment failed to induce AVPV-PeN Kiss1 expression and LH surge generation in ERα KIKO mice [31,95]. In addition, our previous study using ChIP assay for ERα and acetylated histone H3 suggested that estrogen-bound ERα binds to the Kiss1 promoter and enhances histone H3 acetylation of the Kiss1 promoter region in the AVPV-PeN kisspeptin neurons because estrogen induces ERα binding and histone H3 acetylation at the Kiss1 promoter region in mouse AVPV-PeN
tissue [98]. The finding suggests that an estrogen-dependent activating modification of histone H3 of the Kiss1 promoter resulted in an induction of Kiss1 expression. Furthermore, chromatin conformation capture (3C) assay suggested that estrogen induces chromatin loop formation between the Kiss1 promoter and the 3′-intergenic regions of the Kiss1 locus in AVPV-PeN kisspeptin neurons in mice [98]. This result also suggests that the 3′-intergenic region of the Kiss1 locus serves as an enhancer for estrogen-induced Kiss1 expression in AVPV-PeN kisspeptin neurons. Indeed, our in vivo reporter assay utilizing Kiss1-GFP reporter mice suggested that the 3′-intergenic region of the Kiss1 gene is required for an induction of Kiss1 mRNA expression by estrogen in the AVPV-PeN of female mice [98]. More specifically, the reporter mice carrying the 3′-truncated Kiss1-GFP transgene (RBRC09417) failed to display estrogen-induced GFP expression in AVPV-PeN kisspeptin neurons, but displayed ovariectomy-induced GFP expression in ARC kisspeptin neurons. As described above, the reporter mice carrying the full-length of Kiss1-GFP transgene (RBRC09413) display GFP expression in the AVPV-PeN kisspeptin neurons of the OVX mice with estrogen treatment and the ARC kisspeptin neurons without estrogen treatment.

We envisage here the molecular mechanism responsible for the estrogen positive feedback action on AVPV-PeN Kiss1 expression in rodents, as shown in Figure 3. Briefly, at proestrus in rodents, circulating high levels of estrogen bind to ERα in the AVPV-PeN kisspeptin neurons, and the estrogen-bound ERα may bind to ERE in the Kiss1 promoter region and enhance histone H3 acetylation at the promoter region. The estrogen-ERα bindings and/or the activating histone modification may form chromatin loops between the Kiss1 promoter and the 3′-intergenic regions of Kiss1 locus, resulting in Kiss1 expression in AVPV-PeN kisspeptin neurons.

Figure 3. Putative molecular mechanism of the estrogen positive feedback action on Kiss1 expression in the anteroventral-periventricular nucleus-periventricular nucleus continuum (AVPV-PeN). Preovulatory levels of circulating estrogen seem to act on AVPV-PeN kisspeptin neurons, in which estrogen-bound estrogen receptor α (ERα) may increase Kiss1 expression via histone acetylation of Kiss1 promoter region and chromatin loop formation between the Kiss1 promoter and the 3′-intergenic regions of Kiss1 locus. In the absence of estrogen, AVPV-PeN Kiss1 expression may be down-regulated by histone deacetylation of the Kiss1 promoter region and unwinding chromatin loops between the Kiss1 promoter and the 3′-intergenic regions of the Kiss1 locus.

Interestingly, both the proestrous and diestrous levels of estrogen are capable of increasing AVPV-PeN Kiss1 expression in female rats [51], while only the proestrous level of estrogen evoked LH surge in female rats [51]. This fact raises the possibility that, in
addition to an increase in Kiss1 expression in AVPV-PeN kisspeptin neurons, certain afferent ERα-expressing neurons may be also involved in the positive feedback action of estrogen on kisspeptin release from the AVPV-PeN kisspeptin neurons. One of the candidates is brainstem noradrenergic neurons: previous studies showed that ERα expression was found in A2 noradrenergic neurons [135], where estrogen induced c-Fos expression [136], and that α1-adrenergic receptor antagonist attenuated afternoon LH surge in proestrous female rats [137]. Additionally, the SCN, where ERα mRNA expression was found in rats [33], might be also an estrogen positive feedback action site. It is well known that LH surge is timed by the circadian clock localized in the SCN and occurs in the afternoon of proestrus in rodents. A previous study suggested an involvement of SCN vasopressin neurons in the induction of afternoon LH surge because an administration of vasopressin V1 receptor antagonist attenuated afternoon LH surge in proestrous female rats [138]. Interestingly, an electrophysiological study showed that vasopressin treatment induced AVPV-PeN kisspeptin neuronal activity in estrogen-treated O VX mice but not in O VX mice [139], indicating that estrogen may enhance the sensitivity of AVPV-PeN kisspeptin neurons to vasopressin. Taken together, these findings suggest that A2 noradrenergic neurons and SCN vasopressin neurons may mediate estrogen positive feedback to induce kisspeptin release from AVPV-PeN kisspeptin neurons. Previous studies demonstrated that AVPV-PeN kisspeptin neurons largely project their axons to the GnRH cell bodies in the POA in mice [77,140] and that kisspeptin reportedly exerted a long-lasting excitation of GnRH neurons [141,142]. These findings suggest that kisspeptin secreted from the AVPV-PeN kisspeptin neurons may act on GnRH cell bodies to induce GnRH/LH surge.

6. Conclusions and Perspectives
Overall, the intensive studies on hypothalamic kisspeptin neurons in the past two decades have been gradually uncovering the cellular and molecular mechanisms of the negative and positive feedback actions of estrogen on GnRH pulse and surge generation in female mammals. Based on the findings currently available, we now postulate that the negative feedback action of estrogen, which fine-tunes GnRH pulses, is mainly mediated by the ARC kisspeptin neurons, in which estrogen directly represses Kiss1 expression. Further, estrogen may indirectly inhibit pulsatile kisspeptin release via likely afferent ERα-expressing neurons. Further studies are warranted to clarify the afferent inputs that convey estrogen signals to ARC kisspeptin neurons. In addition, we postulate that the positive feedback action of estrogen, which induces GnRH surge, is mainly mediated by the anterior population (the AVPV-PeN in rodents and the POA or PeN in other mammals) of hypothalamic kisspeptin neurons, in which estrogen directly induces Kiss1 expression. Furthermore, estrogen may indirectly stimulate surge-mode kisspeptin release via likely afferent ERα-expressing neurons, such as brainstem noradrenergic neurons and SCN vasopressin neurons. So far, only a few studies are available to show kisspeptin release, except for the studies in the rhesus monkeys [113,114], as described above. Further studies are needed to depict pulsatile and surge-modes of kisspeptin release and to clarify the mechanism of kisspeptin release controlled by the negative and positive feedback actions of estrogen.

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References

1. Harris, G.W.; Jacobsohn, D. Functional grafts of the anterior pituitary gland. Proc. R. Soc. Lond. B Biol. Sci. 1952, 139, 263–276. [PubMed]

2. McCann, S.M. A hypothalamic luteinizing-hormone-releasing factor. Am. J. Physiol. 1962, 202, 395–400. [CrossRef]

3. Ramirez, V.D.; McCann, S.M. A highly sensitive test for LH-releasing activity: The ovariectomized, estrogen progesterone-blocked rat. Endocrinology 1963, 73, 193–198. [CrossRef] [PubMed]

4. Neill, J.D.; Johansson, E.D.; Datta, J.K.; Knobil, E. Relationship between the plasma levels of luteinizing hormone and progesterone during the normal menstrual cycle. J. Clin. Endocrinol. Metab. 1967, 27, 1167–1173. [CrossRef] [PubMed]

5. Monroe, S.E.; Atkinson, L.E.; Knobil, E. Patterns of circulating luteinizing hormone and their relation to plasma progesterone levels during the menstrual cycle of the Rhesus monkey. Endocrinology 1970, 78, 453–455. [CrossRef]

6. Atkinson, L.E.; Bhattacharya, A.N.; Monroe, S.E.; Dierschke, D.J.; Knobil, E. Effects of gonadectomy on plasma LH concentration in the rhesus monkey. Endocrinology 1970, 78, 847–849. [CrossRef]

7. Yamaji, T.; Dierschke, D.K.; Weick, R.F.; Yamaji, T.; Knobil, E. Positive and negative feedback control by estrogen of luteinizing hormone secretion in the rhesus monkey. Endocrinology 1973, 92, 799–804. [CrossRef]

8. Matsuo, H.; Baba, Y.; Nair, R.M.; Arimura, A.; Schally, A.V. Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem. Biophys. Res. Commun. 1971, 43, 1334–1339. [CrossRef]

9. Yamaji, T.; Dierschke, D.K.; Hotchkiss, J.; Bhattacharya, A.N.; Surve, A.H.; Knobil, E. Estrogen induction of LH release in the female rat. Endocrinology 1971, 89, 1034–1041. [CrossRef]

10. Krege, J.H.; Hodgin, J.B.; Couse, J.F.; Enmark, E.; Warner, M.; Mahler, J.F.; Sar, M.; Korach, K.S.; Gustafsson, J.A.; Smithies, O. Generation and reproductive phenotypes of mice lacking estrogen receptor β. Proc. Natl. Acad. Sci. USA 1998, 95, 15677–15682. [CrossRef] [PubMed]

11. Moenter, S.M.; Brand, R.C.; Karsch, F.J. Dynamics of gonadotropin-releasing hormone (GnRH) secretion during the GnRH surge: Insights into the mechanism of GnRH surge induction. Endocrinology 1992, 130, 2978–2984. [CrossRef]

12. Amer, M.; Burgus, R.; Blackwell, R.; Vale, W.; Fellows, R.; Guillemin, R. Purification, amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. Biochem. Biophys. Res. Commun. 1971, 44, 205–210. [CrossRef]

13. Moenter, S.M.; Caraty, A.; Karsch, F.J. The estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 1990, 127, 1375–1384. [CrossRef]

14. Moenter, S.M.; Brand, R.M.; Midgley, A.R.; Karsch, F.J. Dynamics of gonadotropin-releasing hormone release during a pulse. Endocrinology 1992, 130, 503–510. [CrossRef] [PubMed]

15. Moenter, S.M.; Brand, R.C.; Karsch, F.J. Dynamics of gonadotropin-releasing hormone (GnRH) secretion during the GnRH surge: Insights into the mechanism of GnRH surge induction. Endocrinology 1992, 133, 1650–1656. [CrossRef] [PubMed]

16. Lincoln, D.W.; Fraser, H.M.; Lincoln, G.A.; Martin, G.B.; McNeill, A.S. Hypothalamic pulse generators. Recent Prog. Horm. Res. 1985, 41, 369–419. [PubMed]

17. Moenter, S.M.; Caraty, A.; Karsch, F.J. Estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 1990, 127, 1375–1384. [CrossRef]

18. Lincoln, D.W.; Fraser, H.M.; Lincoln, G.A.; Martin, G.B.; McNeill, A.S. Hypothalamic pulse generators. Recent Prog. Horm. Res. 1985, 41, 369–419. [PubMed]

19. Moenter, S.M.; Caraty, A.; Karsch, F.J. Estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 1990, 127, 1375–1384. [CrossRef]

20. Moenter, S.M.; Caraty, A.; Karsch, F.J. Estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 1990, 127, 1375–1384. [CrossRef]

21. Moenter, S.M.; Caraty, A.; Karsch, F.J. Estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 1990, 127, 1375–1384. [CrossRef]

22. Moenter, S.M.; Caraty, A.; Karsch, F.J. Estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 1990, 127, 1375–1384. [CrossRef]

23. Moenter, S.M.; Caraty, A.; Karsch, F.J. Estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 1990, 127, 1375–1384. [CrossRef] [PubMed]
51. Adachi, S.; Yamada, S.; Takatsu, Y.; Matsui, H.; Kinoshita, M.; Takase, K.; Sugihara, H.; Ohtaki, T.; Matsumoto, H.; Uenoyma, Y.; et al. Involvement of anteroventral periventricular metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats. *J. Reprod. Dev.* 2007, 53, 367–378. [CrossRef]

52. Franceschini, I.; Lomet, D.; Cateau, M.; Delsol, G.; Tillet, Y.; Caraty, A. Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha. *Neurosci. Lett.* 2006, 401, 223–230. [CrossRef] [PubMed]

53. Gottsch, M.L.; Cunningham, M.J.; Smith, J.T.; Popa, S.M.; Acohido, B.V.; Crowley, W.F.; Seminara, S.; Clifton, D.K.; Steiner, R.A. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology* 2004, 145, 4073–4077. [CrossRef] [PubMed]

54. Irwig, M.S.; Fraley, G.S.; Smith, J.T.; Acohido, B.V.; Popa, S.M.; Cunningham, M.J.; Gottsch, M.L.; Clifton, D.K.; Steiner, R.A. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KISS-1 mRNA in the male rat. *Neuroendocrinology* 2004, 80, 264–272. [CrossRef] [PubMed]

55. Matsui, H.; Takatsu, Y.; Kumanono, S.; Matsumoto, H.; Ohtaki, T. Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. *Biochem. Biophys. Res. Commun.* 2004, 320, 383–388. [CrossRef]

56. Pheng, V.; Uenoyma, Y.; Homma, T.; Inamoto, Y.; Takase, K.; Yoshizawa-Kumagaye, K.; Isaka, S.; Watanabe, T.X.; Ohkura, S.; Tomikawa, J.; et al. Potencies of centrally- or peripherally-injected full-length kisspeptin or its C-terminal decapeptide on LH release in intact male rats. *J. Reprod. Dev.* 2009, 55, 378–382. [CrossRef]

57. Navarro, V.M.; Castellano, J.M.; Fernandez-Fernandez, R.; Tovar, S.; Roa, J.; Mayen, A.; Nogueiras, R.; Vazquez, M.J.; Barreiro, M.L.; Magni, P.; et al. Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. *Endocrinology* 2005, 146, 156–163. [CrossRef] [PubMed]

58. Navarro, V.M.; Castellano, J.M.; Fernandez-Fernandez, R.; Tovar, S.; Roa, J.; Mayen, A.; Barreiro, M.L.; Casanueva, F.F.; Aguilar, E.; Dieguez, C.; et al. Effects of KiSS-1 peptide, the natural ligand of GPR54, on follicle-stimulating hormone secretion in the rat. *Endocrinology* 2005, 146, 1689–1697. [CrossRef]

59. Ohkura, S.; Takase, K.; Matsuyma, S.; Mogi, K.; Ichimaru, T.; Wakabayashi, Y.; Uenoyma, Y.; Mori, Y.; Steiner, R.A.; Tsukamura, H.; et al. Gonadotrophin-releasing hormone pulse generator activity in the hypothalamus of the goat. *J. Neuroendocrinol.* 2009, 21, 813–821. [CrossRef]

60. Naniwa, Y.; Nakatsukasa, K.; Setsuda, S.; Oishi, S.; Fuji, N.; Matsuda, F.; Uenoyma, Y.; Tsukamura, H.; Maeda, K.-I.; Ohkura, S.; et al. Effects of full-length kisspeptin administration on follicular development in Japanese Black beef cows. *J. Reprod. Dev.* 2010, 56, 588–594. [CrossRef]

61. Dhillo, W.S.; Chaudhri, O.B.; Patterson, M.; Thompson, E.L.; Murphy, K.G.; Badman, M.K.; McGowan, B.M.; Amber, V.; Patel, S.; Ghatei, M.A.; et al. Kisspeptin-54 stimulates the hypothalamo-pituitary gonadal axis in human males. *J. Clin. Endocrinol. Metab.* 2005, 90, 6609–6615. [CrossRef]

62. Shahab, M.; Mastronardi, C.; Seminara, S.B.; Crowley, W.F.; Ojeda, S.R.; Plant, T.M. Increased hypothalamic GPR54 signaling: A potential mechanism for initiation of puberty in primates. *Proc. Natl. Acad. Sci. USA* 2005, 102, 2129–2134. [CrossRef]

63. Ohtaki, T.; Shintani, Y.; Honda, S.; Matsuda, H.; Hori, A.; Kanemashi, K.; Terao, Y.; Kumanono, S.; Takatsu, Y.; Masuda, Y.; et al. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* 2001, 411, 613–617. [CrossRef]

64. Kotani, M.; Dethieux, M.; Vandenbogaerde, A.; Communi, D.; Vanderwinden, J.M.; Le Poul, E.; Brezillon, S.; Tyldesley, R.; Suarez-Huerta, N.; Vandeput, F.; et al. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J. Biol. Chem.* 2001, 276, 34631–34636. [CrossRef] [PubMed]

65. de Roux, N.; Genin, E.; Carel, J.C.; Matusuda, F.; Chauessain, J.L.; Milgrom, E. Hypogonadotropic hypogonadism due to loss of function of the KISS1-derived peptide receptor GPR54. *Proc. Natl. Acad. Sci. USA* 2003, 100, 10972–10976. [CrossRef]

66. Seminara, S.B.; Messager, S.; Chatzidaki, E.E.; Thresher, R.R.; Acienro, J.S., Jr.; Shagoury, J.K.; Bo-Abbas, Y.; Kuohung, W.; Schinof, K.M.; Hendrick, A.G.; et al. The GPR54 gene as a regulator of puberty. *N. Engl. J. Med.* 2003, 349, 1614–1627. [CrossRef]

67. Topaloglu, A.K.; Tello, J.A.; Kotan, L.D.; Ozbek, M.N.; Yilmaz, M.B.; Erdogan, S.; Gurbuz, E.; Temiz, F.; Millar, R.P.; Yuksel, B. Inactivating KISS1 mutation and hypogonadotropic hypogonadism. *N. Engl. J. Med.* 2012, 366, 629–635. [CrossRef]

68. Messenger, S.; Chatzidaki, E.E.; Ma, L.D.; Hendrick, A.G.; Zahn, D.; Dixon, J.; Thresher, R.R.; Malinge, I.; Lomet, D.; Carlson, M.B.; et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc. Natl. Acad. Sci. USA* 2005, 102, 1761–1766. [CrossRef] [PubMed]

69. d’Anglemont de Tassigny, X.; Fagg, L.A.; Dixon, J.P.; Day, K.; Leitch, H.G.; Hendrick, A.G.; Zahn, D.; Franceschini, I.; Caraty, A.; Carlson, M.B.; et al. Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. *Proc. Natl. Acad. Sci. USA* 2007, 104, 10714–10719. [CrossRef] [PubMed]

70. Lapatto, R.; Pallais, J.C.; Zhang, D.; Chan, Y.M.; Mahan, A.; Cerrato, F.; Le, W.W.; Hoffman, G.E.; Seminara, S.B. Kiss1-/- mice exhibit more variable hypogonadism than Gpr54-/- mice. *Endocrinology* 2007, 148, 4927–4936. [CrossRef]

71. Chan, Y.M.; Broder-Finger, S.; Song, K.M.; Seminara, S.B. Kisspeptin/Gpr54-independent gonadotrophin-releasing hormone activity in Kiss1 and Gpr54 mutant mice. *J. Neuroendocrinol.* 2009, 21, 1015–1023. [CrossRef] [PubMed]

72. Uenoyma, Y.; Nakamura, S.; Hayakawa, Y.; Iekami, K.; Watanabe, Y.; Deura, C.; Minabe, S.; Tomikawa, J.; Goto, T.; Ieda, N.; et al. Lack of pulse and surge modes and glutamatergic stimulation of LH release in Kiss1 knockout rats. *J. Neuroendocrinol.* 2015, 27, 187–197. [CrossRef] [PubMed]

73. Higo, S.; Honda, S.; Iijima, N.; Ozawa, H. Mapping of kisspeptin receptor mRNA in the whole rat brain and its co-localisation with oxytocin in the paraventricular nucleus. *J. Neuroendocrinol.* 2016, 28. [CrossRef] [PubMed]
74. Herbison, A.E.; de Tassigny, X.; Doran, J.; Colledge, W.H. Distribution and postnatal development of Gpr54 gene expression in mouse brain and gonadotropin-releasing hormone neurons. *Endocrinology* 2010, 151, 312–321. [CrossRef]

75. Han, S.K.; Gottsch, M.L.; Lee, K.J.; Popa, S.M.; Smith, J.T.; Jakowich, S.K.; Clifton, D.K.; Steiner, R.A.; Herbison, A.E. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J. Neurosci.* 2005, 25, 11349–11356. [CrossRef]

76. Kauffman, A.S.; Gottsch, M.L.; Roa, J.; Byquist, A.C.; Crown, A.; Clifton, D.K.; Hoffman, G.E.; Steiner, R.A.; Tena-Sempere, M. Sexual differentiation of Kiss gene expression in the brain of the rat. *Endocrinology* 2007, 148, 1774–1783. [CrossRef]

77. Clarkson, J.; Herbison, A.E. Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. *Endocrinology* 2006, 147, 5817–5825. [CrossRef]

78. Takase, K.; Uenoyma, Y.; Inoue, N.; Matsu, H.; Yamada, S.; Shimizu, M.; Homma, T.; Tomikawa, J.; Kanda, S.; Matsumoto, H.; et al. Possible role of oestrogen in pubertal increase of Kiss1/kisspeptin expression in discrete hypothalamic areas of female rats. *J. Neuroendocrinol.* 2009, 21, 527–537. [CrossRef]

79. Hrabovszky, E.; Takacs, S.; Gocz, B.; Skrapsits, K. New Perspectives for Anatomical and Molecular Studies of Kisspeptin Neurons in the Aging Human Brain. *Neuroendocrinology* 2019, 109, 230–241. [CrossRef]

80. Rometo, A.M.; Krajevski, S.J.; Voytko, M.L.; Rance, N.E. Hypertrophy and increased kisspeptin gene expression in the hypothalamic infundibular nucleus of postmenopausal women and ovariectomized monkeys. *J. Clin. Endocrinol. Metab.* 2007, 92, 2744–2750. [CrossRef]

81. Smith, J.T.; Shahab, M.; Pereira, A.; Pau, K.Y.; Clarke, I.J. Hypothalamic expression of KISS1 and gonadotropin inhibitory hormone genes during the menstrual cycle of a non-human primate. *Biol. Reprod.* 2010, 83, 568–577. [CrossRef]

82. Ramaswamy, S.; Guerrero, K.A.; Gibbs, R.B.; Plant, T.M. Structural interactions between kisspeptin and GnRH neurons in the mediobasal hypothalamus of the male rhesus monkey (Macaca mulatta) as revealed by double immunofluorescence and confocal microscopy. *Endocrinology* 2008, 149, 4387–4395. [CrossRef] [PubMed]

83. Watanabe, Y.; Uenoyma, Y.; Suzuki, J.; Takase, K.; Suetomi, Y.; Ohkura, S.; Inoue, N.; Maeda, K.-I.; Tsukamura, H. Oestrogen-induced activation of preoptic kisspeptin neurones may be involved in the luteinising hormone surge in male and female Japanese monkeys. *J. Neuroendocrinol.* 2014, 26, 909–917. [CrossRef] [PubMed]

84. Estrada, K.M.; Clay, C.M.; Pompolo, S.; Smith, J.T.; Clarke, I.J. Elevated KiSS-1 expression in the arcuate nucleus prior to the cyclic preovulatory gonadotrophin-releasing hormone/luteinising hormone surge in the ewe suggests a stimulatory role for kisspeptin in oestrogen-positive feedback. *J. Neuroendocrinol.* 2006, 18, 806–809. [CrossRef]

85. Smith, J.T.; Li, Q.; Pereira, A.; Clarke, I.J. Kisspeptin neurones in the ovine arcuate nucleus and preoptic area are involved in the preovulatory luteinizing hormone surge. *Endocrinology* 2009, 150, 5530–5538. [CrossRef] [PubMed]

86. Goodman, R.L.; Lehman, M.N.; Smith, J.T.; Coolen, L.M.; de Oliveira, C.V.; Jafarzadehshirazi, M.R.; Pereira, A.; Iqbal, J.; Caraty, A.; Ciofi, P.; et al. Kisspeptin neurones in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. *Endocrinology* 2007, 148, 5752–5760. [CrossRef]

87. Wakabayashi, Y.; Nakada, T.; Murata, K.; Ohkura, S.; Mogi, K.; Navarro, VM.; Clifton, D.K.; Mori, Y.; Tsukamura, H.; Maeda, K.-I.; et al. Neurokinin B and dynorphin A in kisspeptin neurones of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. *J. Neurosci.* 2010, 30, 3124–3132. [CrossRef] [PubMed]

88. Matsuda, F.; Nakatsukasa, K.; Suetomi, Y.; Naniwa, Y.; Ito, D.; Inoue, N.; Wakabayashi, Y.; Okamura, H.; Maeda, K.-I.; Uenoyma, Y.; et al. The LH surge-generating system is functional in male goats as in females: Involvement of kisspeptin neurones in the medial preoptic area. *J. Neuroendocrinol.* 2015, 27, 57–65. [CrossRef]

89. Hassaneen, A.A.A.; Naniwa, Y.; Suetomi, Y.; Matsuyama, S.; Kimura, K.; Ieda, N.; Inoue, N.; Uenoyma, Y.; Tsukamura, H.; Maeda, K.-I.; et al. Immunohistochemical characterization of the arcuate kisspeptin/neurokinin B/dynorphin (KNDy) and preoptic kisspeptin neuronal populations in the hypothalamus during the estrous cycle in heifers. *J. Reprod. Dev.* 2016, 62, 471–477. [CrossRef] [PubMed]

90. Decourt, C.; Tillet, Y.; Caraty, A.; Franceschini, I.; Briant, C. Kisspeptin immunoreactive neurones in the equine hypothalamic Interactions with GnRH neuronal system. *J. Chem. Neuroanat.* 2008, 36, 131–137. [CrossRef]

91. Tomikawa, J.; Homma, T.; Tajima, S.; Shibata, T.; Inamoto, Y.; Takase, K.; Inoue, N.; Ohkura, S.; Uenoyma, Y.; Maeda, K.-I.; et al. Molecular characterization and estrogen regulation of hypothalamic KISS1 gene in the pig. *Biol. Reprod.* 2010, 82, 313–319. [CrossRef]

92. Inoue, N.; Sasaegawa, K.; Ikai, K.; Sasaki, Y.; Tomikawa, J.; Oishi, S.; Fujii, N.; Ohmori, Y.; Yamamoto, N.; et al. Kisspeptin neurones mediate reflex ovulation in the musk shrew (Suncus murinus). *Proc. Natl. Acad. Sci. USA* 2011, 108, 17527–17532. [CrossRef] [PubMed]

93. Smith, J.T.; Clay, C.M.; Caraty, A., Clarke, I.J. KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. *Endocrinology* 2007, 148, 1150–1157. [CrossRef]

94. Smith, J.T.; Coolen, L.M.; Kriegsfeld, L.J.; Sari, I.P.; Jafarzadehshirazi, M.R.; Maltby, M.; Bateman, K.; Goodman, R.L.; Tilbrook, A.J.; Ubuka, T.; et al. Variation in kisspeptin and RFamide-related peptide (RFRP) expression and terminal connections to gonadotropin-releasing hormone neurones in the brain: A novel medium for seasonal breeding in the sheep. *Endocrinology* 2008, 149, 5770–5782. [CrossRef] [PubMed]
95. Gottsch, M.L.; Navarro, V.M.; Zhao, Z.; Glidewell-Kenney, C.; Weiss, J.; Jameson, J.L.; Clifton, D.K.; Levine, J.E.; Steiner, R.A. Regulation of Kiss1 and Dynorphin gene expression in the murine brain by classical and nonclassical estrogen receptor pathways. J. Neurosci. 2009, 29, 9390–9395. [CrossRef]

96. Yang, J.A.; Mamounis, K.J.; Yasrebi, A.; Roepke, T.A. Regulation of gene expression by 17β-estradiol in the arcuate nucleus of the mouse through ERε-dependent and ERε-independent mechanisms. Steroids 2016, 107, 128–138. [CrossRef] [PubMed]

97. Yang, J.A.; Stires, H.; Belden, W.J.; Roepke, T.A. The arcuate estrogen-regulated transcriptome: Estrogen response element-dependent and -independent signaling of ERs in female mice. Endocrinology 2017, 158, 612–626. [PubMed]

98. Tomikawa, J.; Uenoyama, Y.; Ozawa, M.; Fukanuma, T.; Takase, K.; Goto, T.; Abe, H.; Ieda, N.; Minabe, S.; Deura, C.; et al. Epigenetic regulation of Kiss1 gene expression mediating estrogen-positive feedback action in the mouse brain. Proc. Natl. Acad. Sci. USA 2012, 109, E1294–E1301. [CrossRef]

99. Goto, T.; Tomikawa, J.; Ikegami, K.; Minabe, S.; Abe, H.; Fukanuma, T.; Imamura, T.; Takase, K.; Sanbo, M.; Tomita, K.; et al. Identification of hypothalamic arcuate nucleus-specific enhancer region of Kiss1 gene in mice. Mol. Endocrinol. 2015, 29, 121–129. [CrossRef]

100. Lehman, M.N.; Coolen, L.M.; Goodman, R.L. Minireview: Kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: A central node in the control of gonadotropin-releasing hormone secretion. Endocrinology 2010, 151, 3479–3489. [CrossRef]

101. Murakawa, H.; Iwata, K.; Takeshita, T.; Ozawa, H. Immuno-electron microscopic observation of the subcellular localization of kisspeptin, neurokinin B and dynorphin A in KNDy neurons in the arcuate nucleus of the female rat. Neurosci. Lett. 2016, 612, 161–166. [CrossRef]

102. Navarro, V.M.; Gottsch, M.L.; Chavkin, C.; Okamura, H.; Clifton, D.K.; Steiner, R.A. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J. Neurosci. 2009, 29, 11859–11866. [CrossRef] [PubMed]

103. Maeda, K.; Ohkura, S.; Uenoyama, Y.; Wakabayashi, Y.; Oka, Y.; Tsukamura, H.; Okamura, H. Neurobiological mechanisms underlying GnRH pulse generation by the hypothalamus. Brain Res. 2010, 1364, 103–115. [CrossRef] [PubMed]

104. Okamura, H.; Tsukamura, H.; Ohkura, S.; Uenoyama, Y.; Wakabayashi, Y.; Maeda, K.-I. Kisspeptin and GnRH Pulse Generation. In Kisspeptin Signaling in Reproductive Biology; Kauffman, A.S., Smith, J.T., Eds.; Springer: New York, NY, USA, 2013; pp. 297–323.

105. Goodman, R.L.; Ohkura, S.; Okamura, H.; Coolen, L.M.; Lehman, M.N. KNDy hypothesis for generation of GnRH pulses: Evidence from sheep and goats. In The GnRH Neuron and Its Control; Herbison, A.E., Plant, T.M., Eds.; Wiley: Hoboken, NJ, USA, 2018; pp. 289–324.

106. Herbison, A.E. The gonadotropin-releasing hormone pulse generator. Endocrinology 2018, 159, 3723–3736. [CrossRef]

107. Uenoyama, Y.; Pheng, V.; Tsukamura, H.; Maeda, K.I. The roles of kisspeptin revisited: Inside and outside the hypothalamus. J. Reprod. Dev. 2016, 62, 537–545. [CrossRef] [PubMed]

108. Nagae, M.; Uenoyama, Y.; Okamoto, S.; Tsuchida, H.; Ikegami, K.; Goto, T.; Majarune, S.; Nakamura, S.; Sanbo, M.; Hirabayashi, M.; et al. Direct evidence that KNDy neurons maintain gonadotropin pulses and folliculogenesis as the GnRH pulse generator. Proc. Natl. Acad. Sci. USA 2021, 118, e2009156118. [CrossRef]

109. Ikegami, K.; Goto, T.; Nakamura, S.; Watanabe, Y.; Sugimoto, A.; Majarune, S.; Horihata, K.; Nagae, M.; Tomikawa, J.; Imamura, T.; et al. Conditional kisspeptin neuron-specific Kit1 knockout with newly generated Kiss1-floxed and Kiss1-Cre mice replicates a hypogonadal phenotype of global Kiss1−/− mice. J. Reprod. Dev. 2020, 66, 359–367. [CrossRef] [PubMed]

110. Minabe, S.; Nakamura, S.; Fukushima, E.; Sato, M.; Ikegami, K.; Goto, T.; Abe, H.; Ieda, N.; Minabe, S.; Deura, C.; et al. Inducible Kit1 knockdown in the hypothalamic arcuate nucleus suppresses pulsatile secretion of luteinizing hormone in male mice. J. Reprod. Dev. 2020, 66, 369–375. [CrossRef]

111. Clarkson, J.; Han, S.Y.; Piet, R.; McLennan, T.; Kane, G.M.; Ng, J.; Porteous, R.W.; Kim, J.S.; Colledge, W.H.; Iremonger, K.J.; et al. Definition of the hypothalamic GnRH pulse generator in mice. Proc. Natl. Acad. Sci. USA 2017, 114, E10216–E10223. [CrossRef]

112. Han, S.Y.; Kane, G.; Cheong, I.; Herbison, A.E. Characterization of GnRH Pulse Generator Activity in Male Mice Using GCaMP Fiber Photometry. Endocrinology 2019, 160, 557–567. [CrossRef] [PubMed]

113. Keen, K.L.; Wegner, F.H.; Bloom, S.R.; Ghatie, M.A.; Terasawa, E. An increase in kisspeptin-54 release occurs with the pubertal increase in luteinizing hormone-releasing hormone-1 release in the stalk-median eminence of female rhesus monkeys In Vivo. Endocrinology 2008, 149, 4151–4157. [CrossRef] [PubMed]

114. Kurian, J.R.; Keen, K.L.; Guerriero, K.A.; Terasawa, E. Tonic control of kisspeptin release in prepubertal male monkeys: Implications to the mechanism of puberty onset. Endocrinology 2012, 153, 3331–3336. [CrossRef]

115. Ikegami, K.; Minabe, S.; Ieda, N.; Goto, T.; Sugimoto, A.; Nakamura, S.; Inoue, N.; Oishi, S.; Maturana, A.D.; Sanbo, M.; et al. Evidence of involvement of neurone-glia/neurone-neurone communications via gap junctions in synchronised activity of KNDy neurones. J. Neuroendocrinol. 2017, 29, 2017. [CrossRef]

116. Navarro, V.M.; Gottsch, M.L.; Wu, M.; Garcia-Galiano, D.; Hobbs, S.J.; Bosch, M.A.; Pinilla, L.; Clifton, D.K.; Dearth, A.; Ronnekleiv, O.K.; et al. Regulation of NKB pathways and their roles in the control of Kiss1 neurons in the arcuate nucleus of the male mouse. Endocrinology 2011, 152, 4265–4275. [CrossRef]

117. Ruka, K.A.; Burger, L.L.; Moenter, S.M. Regulation of arcuate neurons coexpressing kisspeptin, neurokinin B, and dynorphin by modulators of neurokinin 3 and kappa-opioid receptors in adult male mice. Endocrinology 2013, 154, 2761–2771. [CrossRef]
Tsuchida, H.; Mostari, P.; Yamada, K.; Miyazaki, S.; Enomoto, Y.; Inoue, N.; Uenooyama, Y.; Tsukamura, H. Paraventricular dynorphin A neurons mediate LH pulse suppression induced by hindbrain glucoprivation in female rats. *Endocrinology* **2020**, *161*, bqaa161. [CrossRef] [PubMed]

Assadullahi; Ieda, N.; Kawai, N.; Ishii, H.; Ihara, K.; Inoue, N.; Uenooyama, Y.; Tsukamura, H. Co-expression of the calcitonin receptor gene in the hypothalamic kisspeptin neurons in female rats. *Reprod. Med. Biol.* **2018**, *17*, 164–172. [CrossRef] [PubMed]

Weems, P.W.; Witty, C.F.; Amstalden, M.; Coolen, L.M.; Goodman, R.L.; Lehman, M.N. Kappa opioid receptor is co-localized in GnRH and KNDy cells in the female ovine and rat brain. *Endocrinology* **2016**, *157*, 2367–2379. [CrossRef] [PubMed]

Amstalden, M.; Coolen, L.M.; Hemmerle, A.M.; Billings, H.J.; Connors, J.M.; Goodman, R.L.; Lehman, M.N. Neurokinin 3 receptor immunoreactivity in the septal region, preoptic area and hypothalamus of the female sheep: Colocalisation in neurokinin B cells of the arcuate nucleus but not in gonadotrophin-releasing hormone neurones. *J. Neuroendocrinol.* **2010**, *22*, 1–12. [CrossRef]

Dellovade, T.L.; Merchenthaler, I. Estrogen regulation of neurokinin B gene expression in the mouse arcuate nucleus is mediated by estrogen receptor α. *Endocrinology* **2004**, *145*, 736–742. [CrossRef]