

Classical Estrogen Receptor α Signaling Mediates Negative and Positive Feedback on Gonadotropin-Releasing Hormone Neuron Firing

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During the female reproductive cycle, the neuroendocrine action of estradiol switches from negative feedback to positive feedback to initiate the preovulatory GnRH and subsequent LH surges. Estrogen receptor- α (ER α) is required for both estradiol negative and positive feedback regulation of LH. ER α may signal through estrogen response elements (EREs) in DNA and/or via ERE-independent pathways. Previously, a knock-in mutant allele (ER α ^{-/AA}) that selectively restores ERE-independent signaling onto the ER α ^{-/-} background was shown to confer partial negative but not positive estradiol feedback on serum LH. The current study investigated the roles of the ERE-dependent and ERE-independent ER α pathways for estradiol feedback at the level of GnRH neuron firing activity. The above ER α genetic models were crossed with GnRH-green fluorescent protein mice to enable identification of GnRH neurons in brain slices. Targeted

extracellular recordings were used to monitor GnRH neuron firing activity using an ovariectomized, estradiol-treated mouse model that exhibits diurnal switches between negative and positive feedback. In wild-type mice, GnRH neuron firing decreased in response to estradiol during negative feedback and increased during positive feedback. In contrast, both positive and negative responses to estradiol were absent in GnRH neurons from ER α ^{-/-} and ER α ^{-/AA} mice. ERE-dependent signaling is thus required to increase GnRH neuron firing to generate a GnRH/LH surge. Furthermore, ERE-dependent and -independent ER α signaling pathways both appear necessary to mediate estradiol negative feedback on serum LH levels, suggesting central and pituitary estradiol feedback may use different combinations of ER α signaling pathways. (*Endocrinology* 149: 5328–5334, 2008)

GnRH NEURONS FORM the final common pathway in the neuroendocrine regulation of reproduction. GnRH stimulates the secretion of the pituitary gonadotropins LH and FSH. During most of the female reproductive cycle, ovarian estradiol exerts negative feedback to reduce gonadotropin release (1, 2). In the late follicular phase, in response to sustained high levels of estradiol from preovulatory follicles, the action of estradiol switches from negative to positive feedback, resulting in a surge release of GnRH, that is likely due to increased GnRH neuron firing activity (3). The GnRH surge triggers a surge of LH secretion to initiate ovulation (4–7).

The α -isoform of the estrogen receptor- α (ER α) appears to be critical for estradiol feedback. ER α , but not ER β , knockout mice have elevated LH, indicating a lack of estradiol negative feedback, and also lack the positive feedback LH surge response to estradiol (2, 8). A neuron-specific ER α knockout mouse also lacks estradiol positive feedback (9), sug-

gesting estradiol action is at least in part via a neural mechanism.

ER α exerts its cellular effects by interacting with multiple signaling pathways and transcription factors (10). In the classical genomic pathway, ER α translocates into the nucleus and binds and recruits cofactors to estrogen response element (ERE) regulatory sites in DNA to alter gene transcription (11, 12). Alternatively, ER α may signal through ERE-independent genomic pathways via protein-protein interactions to alter transcription of genes at non-ERE DNA sites (13–16). Estradiol effects may also be triggered via membrane-initiated signaling cascades (17–20). Physiologically, these pathways are not mutually exclusive and may converge to modulate specific genes or cellular responses (17).

Previously, a mutant receptor (E07A/G208A; AA) with disrupted DNA binding but intact ERE-independent activity (13) was used to develop a nonclassical ER knock-in (NERKI) mouse model (16). The AA mutant ER α allele was bred onto an ER α null (ER α KO) background (ER α ^{-/-}) to generate a mouse model with isolated ERE-independent ER α signaling (ER α ^{-/AA}). This model was used to characterize the distinct *in vivo* roles for ER α pathways in mediating estradiol effects in bone (21, 22), uterus (23), the male neuroendocrine axis, and reproductive behavior (24). Additionally, in the female, ERE-independent ER α signaling was found to be capable of conveying partial estradiol negative feedback on serum LH, whereas positive feedback required ERE-dependent ER α signaling (25).

The purpose of the current study was to examine whether

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Abbreviations: ER α , Estrogen receptor- α ; ERE, estrogen response element; ER α KO, ER α null; GABA, γ -aminobutyric acid; GFP, green fluorescent protein; NERKI, nonclassical ER knock-in; OVX, ovariectomized; WT, wild-type.

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ERE-dependent and/or ERE-independent ER α pathways are needed for estradiol feedback at the level of GnRH neuron firing activity. GnRH-green fluorescent protein (GFP) transgenic mice (26) were crossed with the NERKI and ER α KO mouse models to allow single-unit recordings of GnRH neurons in living brain slices. We used an estradiol treatment regimen in which wild-type (WT) mice exhibit daily switches between estradiol negative feedback, with low LH levels and low GnRH neuron activity, and estradiol positive feedback, with high LH levels and high GnRH neuron activity (3). Because the ER $\alpha^{-/AA}$ and ER $\alpha^{-/-}$ genotypes do not exhibit normal estrous cycles (8, 9, 16, 25), it was necessary to use a surge-induction protocol. Ovariectomy and treatment with a constant physiological level of estradiol causes WT mice to exhibit daily switches between low GnRH neuron activity with low LH levels and high GnRH neuron activity with high LH levels (3). The present study indicates that ERE-dependent signaling is required for both negative and positive feedback regulation of GnRH neuron firing but that both ERE-dependent and ERE-independent signaling are needed for full suppression of serum LH.

Materials and Methods

Animals

All procedures were approved by the Animal Care and Use Committees of Northwestern University and the University of Virginia. Mice were maintained on a 14-h light, 10-h dark photoperiod (Virginia: lights off at 1630 h EST) with food (Northwestern: Harlan Teklad 7912, Indianapolis, IN; Virginia: Harlan 2916) and water available *ad libitum*.

Introduction of the GnRH-GFP transgene into NERKI and ER α KO mouse models

NERKI mice were on a 129SvJ background (16) and ER α KO mice on a C57BL/6 background (27). To produce congenic strains, the NERKI allele was previously outcrossed 14 generations to the ER α KO. GnRH-GFP mice (26) were transferred from the University of Virginia to Northwestern University, where they were outcrossed to NERKI and ER α KO colonies. It was assumed that the GnRH transgene was present on one chromosome. Thus, male progeny from this union were considered obligate heterozygotes for the GnRH-GFP transgene. Those heterozygous for NERKI (ER $\alpha^{+/AA}$) or ER α KO (ER $\alpha^{+/-}$) alleles were backcrossed to GnRH-GFP founder, and thus considered homozygous, female mice. Genetic test crosses were performed on male heterozygous NERKI (ER $\alpha^{+/AA}$) and ER α KO (ER $\alpha^{+/-}$) progeny by outcrossing them to WT C57BL/6J female mice (000664; Jackson Laboratory, Bar Harbor, ME). Male mice that transmitted the GnRH transgene to 100% of at least 20 pups were selected as founders for new NERKI and ER α KO colonies that stably express the GnRH-GFP transgene. To prepare additional female breeding stock, ER α KO GnRH-GFP founder males were outcrossed to the original GnRH-GFP founder female mice. Resultant female progeny heterozygous for the ER α KO allele (ER $\alpha^{+/-}$) were bred to either NERKI (ER $\alpha^{+/AA}$) or ER α KO (ER $\alpha^{+/-}$) GnRH-GFP founder males identified by genetic test cross. This produced NERKI/ER α KO (ER $\alpha^{-/AA}$), homozygous ER α KO (ER $\alpha^{-/-}$), and WT littermate control (ER $\alpha^{+/+}$) mice expressing the GnRH-GFP transgene. Mice were transferred to the University of Virginia between 6 and 8 wk of age.

Animal surgery and treatment to induce estradiol negative and positive feedback

After a 1-month quarantine period, mice were ovariectomized (OVX) and either simultaneously implanted with an estradiol capsule (OVX+E: WT $n = 15$; ER $\alpha^{-/AA}$ $n = 16$; ER $\alpha^{-/-}$ $n = 17$ mice) or not treated further (OVX: WT $n = 6$; ER $\alpha^{-/AA}$ $n = 5$; ER $\alpha^{-/-}$ $n = 5$ mice). OVX+E treatment in this manner generates daily LH surges that peak around the time of lights off, with LH levels showing a significant increase beginning at

1500 h (3). For electrophysiology experiments, mice were euthanized at either 0900–1030 h (negative feedback groups) or 1430–1500 h (positive feedback groups). Uterine weight is known to increase in response to estradiol and was measured at the time of brain slice preparation to confirm endocrine status. The average weight of uteri from WT mice was significantly increased in response to estradiol treatment (OVX 31.2 \pm 1.8 mg, OVX+E WT 117.1 \pm 0.1 mg; $P < 0.0001$). In contrast, uteri from ER $\alpha^{-/-}$ and ER $\alpha^{-/AA}$ mice were hypoplastic and did not respond to estradiol with increased weight ($P > 0.6$), confirming previous results (23). For LH level measurements, trunk blood was collected either at the time of brain slice preparation or after CO $_2$ anesthesia. Serum was separated and stored at -20°C until assay. Serum LH concentration was determined using a two-site mouse LH sandwich RIA at the Center for Research in Reproduction Ligand and Assay Core at the University of Virginia (28, 29). All samples were run in the same assay with an intraassay coefficient of variation of 3.9%, and level of detection was 0.04 ng/ml; all samples read within the reportable range.

Brain slice preparation

Reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless noted. Two to four days after surgery, sagittal brain slices were prepared using slight modifications (3, 30) of previous descriptions (31). For recording, slices were placed in a recording chamber and continuously superfused with oxygenated normal saline maintained at 30–32°C with an in-line heating unit (Warner Instruments, Hamden, CT). Estradiol treatment was solely *in vivo*, and estradiol was not present in any recording solutions.

Recordings

Targeted single-unit extracellular recordings (loose-patch) were used in this study because this configuration allows recording with minimal impact on the behavior of the recorded cell (31). GFP-GnRH neurons in the preoptic area and ventral hypothalamus were identified by brief illumination at 470 nm. Recording pipettes (1–3 M Ω) were filled with normal HEPES-buffered solution (31). Seal resistances (5–50 M Ω) were monitored at least every 30 min during recording. Recordings were made in voltage-clamp mode with the pipette holding potential at 0 mV, and signals were filtered at 10 kHz. After a recording stabilization period of several minutes after electrode placement, cells were recorded for 30–60 min. If no firing was observed after 60 min, 15 mM KCl was added to the bath solution to depolarize cells and induce firing. If no firing occurred with KCl treatment, the data were discarded because it was not possible to confirm recording integrity or cell health. Experiments were performed using an EPC 8 amplifier (HEKA Electronics, Lambrecht/Pfalz, Germany) with the Pulse Control XOP (Instrutech, Port Washington, NY) running in Igor Pro software (Wavemetrics, Lake Oswego, OR) on a G4 Macintosh computer. Signals were digitized at 16-bit resolution through an ITC-18 acquisition interface (Instrutech). Action currents (events), the membrane currents associated with action potential firing, were detected using Event Tracker software in Igor Pro. For simplicity, we use the terms firing rate or firing activity to refer to currents recorded extracellularly.

Recordings were performed between 1030 and 1400 h (negative feedback groups) or 1600 and 1930 h (positive feedback groups). No more than three cells per animal and only one cell per slice were recorded. Within-animal variance was of the same magnitude as between-animal variance, and thus cells are considered as independent observations. Similarly, values from d 2–4 after surgery were grouped together because variance among days was similar to variance within values for each day.

Data analysis

Each time the recording trace crossed the threshold for event detection, 10 msec worth of data centered on the threshold crossing were recorded and stored to a digital file. Events were detected offline using custom programs in Igor Pro (31) and binned at 1-min intervals. Binned data were transferred to Excel (Microsoft, Redmond, WA) and Prism4 (GraphPad, San Diego, CA) for further analysis. Data were analyzed for mean firing rate, percentage of time spent in quiescence, and longest duration of quiescent periods. Mean firing rate (in Hertz) was calculated by dividing the total number of events over the duration of recording

in seconds. Quiescent time was defined as 1-min bins containing no more than one event. The percentage of bins that were quiescent (a measure of overall cell activity) and the longest duration of consecutive quiescent bins (a measure of firing pattern) were calculated for each cell. Data were log transformed and group means compared using two-way ANOVA followed by Bonferroni multiple-comparisons tests for effects of estradiol treatment and genotype. Data are presented as means \pm SEM. Statistical significance was set at $P < 0.05$.

Results

The daily surge estradiol replacement paradigm confirms LH data

Previous experiments in which OVX WT, ER $\alpha^{-/AA}$, and ER $\alpha^{-/-}$ mice were treated with an estradiol capsule for 1 wk, followed by a subsequent estradiol benzoate injection showed that ERE-independent ER α signaling partially restores negative but not positive feedback on LH levels (25). In the current study, a 2- to 4-d constant estradiol treatment regimen (3) was used to examine diurnal shifts between negative and positive feedback on LH levels in WT, ER $\alpha^{-/AA}$, and ER $\alpha^{-/-}$ mice [two-way ANOVA, $F_{(4,38)} = 12.4$; $P < 0.0001$]. During negative feedback (measured between 0900 and 1030 h), LH levels in OVX+E WT mice showed a decrease in comparison with OVX animals ($P < 0.001$) (Fig. 1). Estradiol treatment significantly lowered LH in ER $\alpha^{-/AA}$ mice ($P < 0.01$), although LH levels in OVX+E ER $\alpha^{-/AA}$ animals were still significantly higher than in WT mice ($P < 0.001$). This result agrees with a previous report (25) indicating the ERE-independent ER α signaling pathway can convey partial estradiol negative feedback but that complete suppression of LH requires ERE-dependent ER α signaling as well. As previously observed (25, 32), estradiol had no effect on LH levels in ER $\alpha^{-/-}$ mice, indicating ER α is required to mediate estradiol negative feedback.

During positive feedback (measured between 1600 and 1630 h), LH levels in WT OVX+E mice increased 32-fold ($P < 0.001$) compared with levels during negative feedback

(Fig. 1). The LH increase was absent in ER $\alpha^{-/AA}$ and ER $\alpha^{-/-}$ mice, confirming a requirement for ERE-dependent signaling to convey estradiol positive feedback on serum LH (25). As the daily surge estradiol treatment paradigm recapitulated both the negative and positive feedback serum LH results previously reported for this genetic model, it was used for all experiments described here.

GnRH neuron firing is not altered by ligand-independent ER α effects

To investigate whether these changes in LH levels are associated with alterations in GnRH neuron firing activity, targeted single-unit recordings were used to monitor the firing of GnRH neurons from WT, ER $\alpha^{-/AA}$, and ER $\alpha^{-/-}$ mice [two-way ANOVA: mean firing rate $F_{(4,80)} = 8.75$, $P < 0.0001$; percentage of time in quiescence $F_{(4,80)} = 5.69$, $P = 0.0004$; duration of quiescent time $F_{(4,80)} = 4.36$, $P = 0.0031$]. GnRH neurons from WT OVX mice not treated with estradiol show no diurnal changes in GnRH neuron firing activity (3). To examine whether genotype alone in the absence of gonadal steroids altered the firing of GnRH neurons, recordings of firing activity of OVX cells were collected during the time of negative feedback (WT $n = 9$ cells from six mice; ER $\alpha^{-/AA}$ $n = 9$ cells from five mice; ER $\alpha^{-/-}$ $n = 11$ cells from five mice) (Fig. 2). There was no effect of genotype on GnRH neurons from OVX mice on mean firing rate (Fig. 3, $P > 0.9$), percentage of time in quiescence (Fig. 4A, $P > 0.6$), or duration of quiescent time (Fig. 4B, $P > 0.9$). This suggests neither the long-term absence of ER α nor ligand-independent ER α signaling alters GnRH neuron firing activity in this model (33, 34). Consistent with this interpretation, elevated serum LH was reported in a ligand-independent ER knock-in mouse, indicating ligand-independent functions of this receptor are not sufficient to convey negative feedback (35).

Estradiol negative feedback effects on GnRH neuron firing activity require ERE-dependent ER α signaling

LH levels in OVX+E and OVX mice during negative feedback confirmed that the isolated ERE-independent signaling pathway was able to mediate a significant, although incomplete, decrease in pituitary output (25) (Fig. 1). Estradiol-induced decreases in LH levels in WT mice were previously shown to correlate with decreased GnRH neuron activity (3), an observation repeated in the present study. In cells from WT mice ($n = 10$ cells from six mice), estradiol decreased mean firing rate (Fig. 3, $P < 0.001$) and increased measures of quiescence (Fig. 4, $P < 0.05$). In contrast, cells from ER $\alpha^{-/AA}$ and ER $\alpha^{-/-}$ mice showed no response to estradiol treatment in any of the firing parameters examined during the time of negative feedback (ER $\alpha^{-/AA}$ $n = 9$ cells from four mice; ER $\alpha^{-/-}$ $n = 10$ cells from five mice) (Figs. 3 and 4). Consistent with this, the firing of both ER $\alpha^{-/AA}$ and ER $\alpha^{-/-}$ OVX+E cells were greater than WT cells ($P < 0.05$, all parameters) and not different from each other. Thus, although the ERE-independent pathway is sufficient to mediate some degree of estradiol negative feedback on LH levels, ERE-dependent signaling is required for negative effects on GnRH neuron firing.

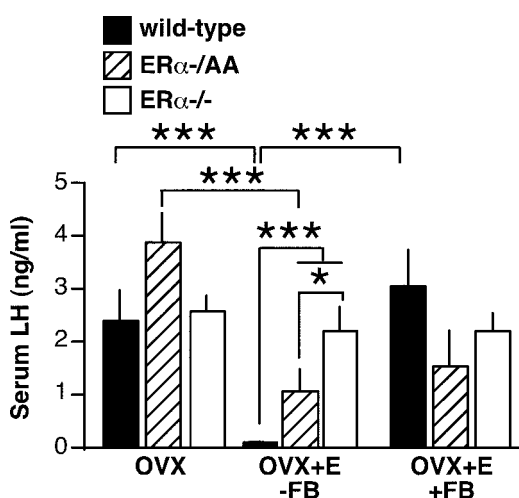


FIG. 1. ERE-independent ER α signaling partially restores negative feedback but not positive feedback on serum LH levels. Bars represent serum LH concentrations (mean \pm SEM). Serum samples were collected during the times of negative (-FB) or positive (+FB) feedback in WT (black), ER $\alpha^{-/AA}$ (hatched), and ER $\alpha^{-/-}$ (white). OVX $n = 4$ –6 mice per group; OVX+E -FB $n = 6$ –7 mice per group; OVX+E +FB $n = 4$ mice per group. *, $P < 0.05$; ***, $P < 0.001$.

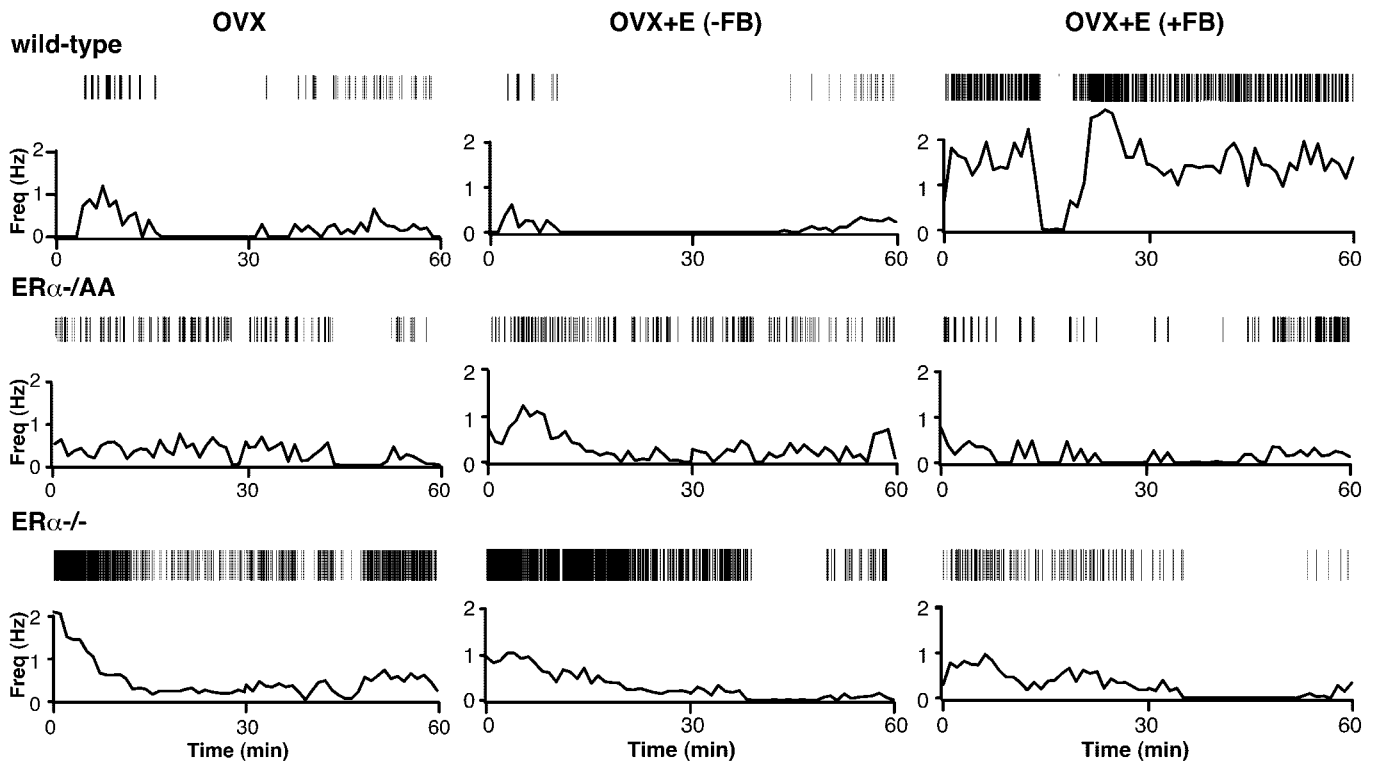


FIG. 2. Representative recordings of cells from OVX (left column), OVX+E negative feedback (–FB) (middle column) and positive feedback (+FB) (right column) WT (top row), ER $\alpha^{-/-AA}$ (middle row), and ER $\alpha^{-/-}$ (bottom row) mice. Firing rate is displayed at 1-min intervals. Vertical lines at the top of each panel illustrate timing of individual action currents detected. Recordings were chosen as representative on the basis of mean firing rate.

Estradiol positive feedback effects on GnRH neuron firing activity require ERE-dependent ER α signaling

The lack of an estradiol-induced LH surge in both ER $\alpha^{-/-AA}$ and ER $\alpha^{-/-}$ mice (2, 8, 9, 25) (Fig. 1) may be due to a failure of estradiol to increase GnRH neuron activity, GnRH release, and/or pituitary response. To examine the first possibility, firing activity of cells from OVX+E animals was recorded during the time of positive feedback (WT n = 10 cells from five mice; ER $\alpha^{-/-AA}$ n = 9 cells from six mice; ER $\alpha^{-/-}$ n = 12 cells from six mice) (Fig. 2). As reported (3), the mean firing rate of WT OVX+E cells increased (Fig. 3, $P <$

0.001), and the percentage and duration of quiescence decreased (Fig. 4, $P <$ 0.001) during positive compared with negative feedback. In contrast, there was no change in any measured parameter of GnRH neuron activity from OVX+E ER $\alpha^{-/-AA}$ or ER $\alpha^{-/-}$ mice (Figs. 3 and 4). Moreover, the mean firing rates of OVX+E ER $\alpha^{-/-AA}$ and ER $\alpha^{-/-}$ OVX+E GnRH neurons were lower than WT neurons during positive feedback (Fig. 3, $P <$ 0.01). Compared with WT, ER $\alpha^{-/-AA}$ cells showed increased percentage of time in quiescence (Fig. 4A, $P <$ 0.01) but no difference in the duration of quiescence (Fig. 4B). ER $\alpha^{-/-}$ cells showed no differences in quiescence compared with either WT or ER $\alpha^{-/-AA}$ cells (Fig. 4). Thus, consistent with the lack of LH surges, the overall level of firing activity is significantly lower in these genotypes. These data indicate genomic ERE-dependent ER α signaling is required for estradiol to increase GnRH neuron firing during positive feedback.

Discussion

ER α signaling is crucial for estradiol negative and positive feedback regulation of LH. ER α can signal via ERE-dependent or -independent genomic, as well as ERE-independent nongenomic mechanisms (37). Previously, and as confirmed in Fig. 1, the ERE-independent ER α signaling pathway was shown to convey partial estradiol negative feedback on serum LH levels, whereas the ERE-dependent ER α signaling pathway was required for estradiol positive feedback regulation of LH (25). Here, the relative roles of these ER α signaling pathways in mediating estradiol effects on GnRH

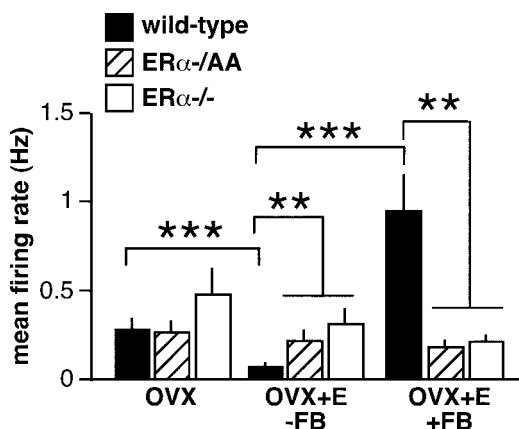


FIG. 3. Summary of effects of genotype and estradiol treatment on mean firing rate of GnRH neurons (mean \pm SEM for each group). n = 9–12 cells per group. *, $P <$ 0.05; **, $P <$ 0.01; ***, $P <$ 0.001.

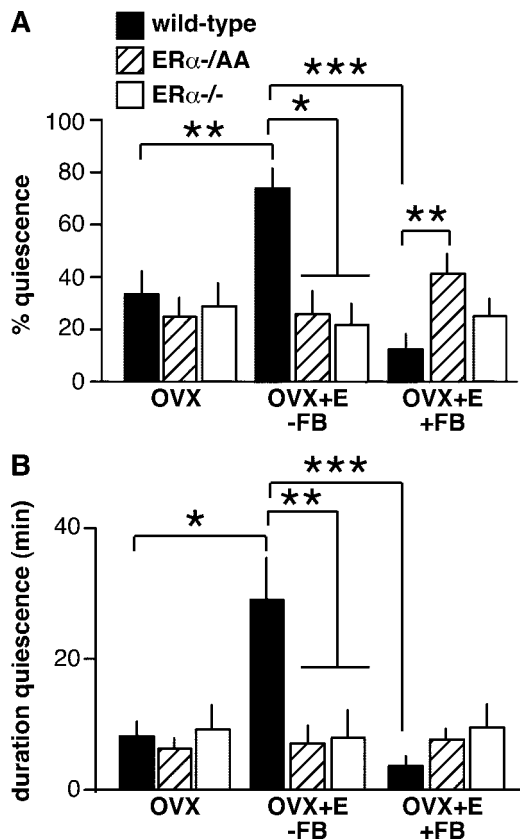


FIG. 4. Summary of effects of genotype and estradiol treatment on percentage of time spent in quiescence (A) and longest duration of quiescent time (B) of GnRH neurons (mean \pm SEM for each group). $n = 9$ –12 cells per group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

neuron firing activity were directly examined. In mice with isolated ERE-independent ER α signaling, no estradiol-induced changes in GnRH neuron activity were observed. These data suggest ERE-dependent ER α signaling is required for both positive and negative estradiol feedback effects on GnRH neuron activity in this animal model. Together with the LH data, these results suggest that negative feedback on serum LH levels is differentially mediated by both ERE-dependent and ERE-independent signaling. ERE-dependent ER α signaling appears to suppress LH with a concomitant reduction in GnRH neuron activity. In contrast, ERE-independent ER α signaling partially suppresses serum LH via a mechanism not reflected by decreased GnRH neuron firing.

The ERE-independent pathway was able to convey significant but incomplete suppression of serum LH but not GnRH neuron firing activity. There are several explanations for this dissociation. First, ERE-independent signaling may act at the pituitary to decrease responsiveness to GnRH. In this regard, LH release is rapidly lost after injection of estradiol benzoate in OVX ewes; this loss occurs before the loss of GnRH pulses, consistent with differential action of estradiol on the release of these two hormones (38). In male mice, steroid milieu producing reduced serum LH release but increased GnRH neuron activity have been reported, again suggesting differential regulation (39). In a previous report, female mice with isolated ERE-independent ER α signaling did not exhibit a decreased pituitary LH response to exogenous GnRH (25). That study, however, used a single supraphysiological dose of GnRH, which may not have revealed decreased pituitary sensitivity to endogenous or pulsatile GnRH stimulation. Second, although neural activity has been associated with hormone release (40, 41), ERE-independent signaling may alter the amount of GnRH released per action potential. Estradiol negative feedback can reduce GnRH pulse amplitude (38, 42, 43), which may combine with negative actions on pituitary gonadotropes to reduce gonadotropin release (38, 44–47). Third, the ER α ^{-/-AA} genotype could alter the coordination of GnRH neurons that is likely needed to produce pulses of hormone that reach the pituitary. Finally, neuroanatomical changes such as alterations in glial ensheathment of axon terminals at the median eminence (48) may play a role in decreasing GnRH release in the absence of a decrease in GnRH neuron activity. Thus, altered GnRH release may not have been reflected by GnRH neuron firing and the present methodology. It is important to point out, however, that GnRH neuron activity did accurately parallel LH release in both the WT and ER α ^{-/-} conditions.

The lack of normal cycles in the genetic models altering estradiol signaling mandated use of a GnRH/LH surge induction protocol to gather these data. We chose a model in which removal of the ovaries and replacement with a constant physiological estradiol level provided via an implant produces daily switches between negative and positive feedback (3). Daily surges have been demonstrated in several rodent species (3, 49, 50) and are postulated to reveal that the central signal for ovulation can occur on a daily basis if estradiol levels are sufficient. Consistent with this, the rise in estradiol production by the developing follicles to levels sufficient to induce a GnRH/LH surge appears to be an important factor contributing to the difference in length of the follicular phase in mammals. This daily surge model has now been used to examine several parameters associated with surge induction including GnRH activity, neurotransmission, and neuromodulation (3, 51, 52). Although no differences have been observed in any of these parameters within the day range used (daily surge d 2–4), it is important to point out that we cannot exclude the possibility that these daily LH surges differ in mechanism from one another. It is also possible that the mechanisms of negative and/or positive feedback in this model differ from those during the natural cycle.

The location of ER α needed for feedback responses of GnRH neurons is under debate. Although ER α has been shown to be expressed in immortalized GnRH neuronal cell lines (53, 54), it has not yet been detected in native adult GnRH neurons (55). Estradiol effects may be conveyed indirectly to GnRH neurons through transsynaptic contacts with ER α -expressing neurons, astrocytes, or other glial or endothelial cells (2, 56). The anteroventral periventricular area, in particular, appears to be a source of ER α -expressing neural afferents that is critical for GnRH regulation (9, 57–59). Direct action of estradiol on GnRH neurons may also be possible via ER β (60, 61), which is expressed in all genotypes used in the current study. The lack of an effect of estradiol on GnRH neuron firing in the ER α ^{-/-} indicates a requirement for ER α signaling but does not exclude a potential

modulatory role for ER β (62), for example via rapid effects of estradiol on GnRH neurons (63–66).

A role for synaptic inputs in transmitting estradiol feedback is supported by recent work indicating estradiol feedback on GnRH neuron firing activity may be mediated through changes in γ -aminobutyric acid (GABA) transmission. Although controversial (67, 68), there is considerable evidence that GABA_A receptor activation can excite GnRH neurons (69–71). Furthermore, estradiol decreases GABA transmission to GnRH neurons during negative feedback but increases it during positive feedback (51). In addition, neuromodulators such as kisspeptin (72), vasoactive intestinal polypeptide (52, 73, 74), gonadotropin-inhibitory hormone (75), vasopressin (76, 77), catecholamines (78), and neuropeptide Y (36) have been implicated in negative feedback and/or surge generation. Thus, a likely primary role for ERE-dependent signaling is to drive changes in neurotransmission via fast synaptic transmission and/or neuromodulatory factors that appear critical to negative and positive feedback regulation of GnRH neurons.

In summary, the present studies demonstrate estradiol positive and negative feedback on GnRH neuron firing activity require signaling via ER α , and specifically via the ERE-dependent genomic pathway. Furthermore, estradiol negative feedback is distinctly conveyed through ERE-dependent and -independent ER α signaling pathways and dissociable at the levels of GnRH neuron firing activity and serum LH. Future studies will be necessary to elucidate the sites of estrogen action that lead to LH suppression and to identify the molecular targets of ERE-dependent and ERE-independent pathways in the hypothalamus and pituitary.

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