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INVITED REVIEW

The electrophysiologic properties of gonadotropin-releasing hormone neurons

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Abstract

For about two decades, recordings of identified gonadotropin-releasing hormone (GnRH) neurons have provided a wealth of information on their properties. We describe areas of consensus and debate the intrinsic electrophysiologic properties of these cells, their response to fast synaptic and neuromodulatory input, Ca^{2+} imaging correlates of action potential firing, and signaling pathways regulating these aspects. How steroid feedback and development change these properties, functions of GnRH neuron subcompartments and local networks, as revealed by chemo- and optogenetic approaches, are also considered.

KEYWORDS

action potential, fertility, intrinsic properties, kisspeptin, luteinizing hormone, synaptic transmission

1 | INTRODUCTION

In the half century since GnRH was sequenced, studies using native GnRH decapeptide and antagonist analogs have demonstrated that the pattern of GnRH release is vital for normal physiology. The hypogonadal (*hpg*) mouse, a natural GnRH knockout, established this hormone as the critical final output from the central nervous system with respect to regulating the reproductive system through its effects upon the anterior pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone. Although there is a rich history of physiologic studies concerning the central control of reproduction, we focus here on the neurobiological mechanisms revealed by

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studies of identified GnRH neurons in brain slices, in nasal explants and in vivo, aiming to help explain how their intrinsic and synaptic properties change with reproductive state, as well as how these cells are integrated into steroid-responsive networks.

2 | THE BEFORE TIME

For the current and next generations of young scientists, for who fluorescence-identified living neurons are a norm, we briefly visit the time before green fluorescent protein (GFP) and other genetically-encoded marker genes. The first reported recordings of putative GnRH neurons were made in the laboratory of Martin Kelly in 1984.[1] A few statistics from that paper are worth noting. Guinea pig cells were recorded in the arcuate nucleus using electrodes containing procion-yellow, allowing post-hoc identification of recorded cells and, when combined with immunohistochemistry using an anti-GnRH antibody, identification of the recorded cell's phenotype. Of 102 cells recorded, just four were GnRH-immunopositive. It is important to note that the antibody used in these studies was reported the same year to non-specifically label neurons in the arcuate nucleus in the rat.[2] A later report, using a similar approach with a validated GnRH antibody, revealed that GnRH neurons were sensitive to estradiol and opioid receptor agonists.[3] Later efforts to identify recorded GnRH neurons post-hoc employed a reverse-transcriptase polymerase chain reaction on the cytosol from single neurons, collected after patch clamp recordings, to detect GnRH transcripts.[4] These labor-intensive methods of identification were supplanted by promoter transgenic approaches and have subsequently been abandoned.

3 | IDENTIFIED GnRH NEURONS

Near the turn of the century, promoter-driven genetic approaches made it possible to identify specific cell types in mammalian tissue. People investigating GnRH neurons were among the first to take advantage of this because of the difficulties of recording from this small, anatomically-diffuse population.[5] Early success was likely facilitated by the strength and cell-specificity of the GnRH promoter.[6] GnRH neurons have been identified in living tissue using β-galactosidase substrates,[7] Ca^{2+} indicators[8–13] or variations of GFP derived from *Aequorea victoria* in mice,[14–16] rats[17] and medaka fish.[18] GFP identification is the method that has been used most and will be the main source of studies covered in this review https://onlinelibrary.wiley.com/doi/10.1111/jne.13068. Because of space limitations, the reader is referred to the article on non-mammalian systems for discussion of the many substantial contributions to GnRH neuron electrophysiology from work on teleost fish.

4 | INTRINSIC PROPERTIES OF GnRH NEURONS

For an explanation of recording approaches, the reader is referred to previous reviews.[19–21] Initial studies of GnRH neuron properties suffered from a lack of consistent and rigorous methodology that had been established for patch clamp investigation; essentially no laboratory in the field escaped errors, ranging from use of inappropriate pipette solutions, to failure to report quality parameters allowing the reader to evaluate data integrity, as well as failure to explain how properties (e.g., action potential threshold, resting potential) were defined. These limitations resulted in variable data. We have chosen to focus our review on work performed after these initial growing pains.

The majority of GnRH neurons recorded in brain slices exhibit spontaneous action potential firing that is often arranged into short-term patterns called bursts, and an array of voltage-gated conductances typical of many neurons, including tetrodotoxin-sensitive Na^+,[22] multiple K^+,[10,23–25] Ca^{2+},[26,27] hyperpolarization-activated[28] and transient receptor potential (TRP).[29–31] These conductances enable action potential firing, regulate firing patterns and mediate membrane responses to inputs. From this rather standard list, we highlight four aspects that are important to GnRH neurons: action potential shape, firing patterns, currents regulating action potential initiation and Cl^- homeostasis.

4.1 | The GnRH neuron action potential

GnRH neurons are often spontaneously active from a basal potential between −75 and −60 mV in brain slices.[14,32] The GnRH neuron action potential is majestic (Figure 1A). In current clamp recordings with a pipette solution mimicking intracellular milieu and amplifier settings allowing more precise characterization of these rapid events (e.g., high-frequency (≥ 10 kHz) acquisition and filtering), the amplitude of the first action potential induced by current injection routinely achieves 90 mV from action potential threshold (defined as 1V/s).[23,33] Despite this amplitude, the spikes are over quickly, with full width at half maximum averaging under 0.8 ms. Spikes are followed by a pronounced afterhyperpolarization potential (AHP) of approximately 25 mV and show little evidence of frequency adaptation. Finally, a slow (peaking 1–2 s post threshold) afterdepolarizing potential (ADP) follows the AHP in many GnRH neurons and can contribute to ongoing firing.[34–36] These characteristics produce a spike profile that distinguishes these cells and is consistent among GnRH neurons (Figure 1). Where these action potentials are initiated is an interesting question. Simultaneous recordings of proximal dendrites and soma indicate that action potentials are initiated in dendrites in some GnRH neurons.[37,38] Immunoreactivity for ankyrin G, a protein often linked to the site of action potential initiation, revealed that it is typically located within 150 μm from the soma in GnRH neurons.[39] Consistent with action potential initiation in the dendrites, ankyrin was located in one of the dendrites of most (75%) GnRH neurons, as well as in the axon in a small proportion of these cells.[39] Future work should investigate whether the site of initiation is constant for a particular neuron, or whether it is regulated by the type of input being received, and/or can be in other regions such as the terminals.

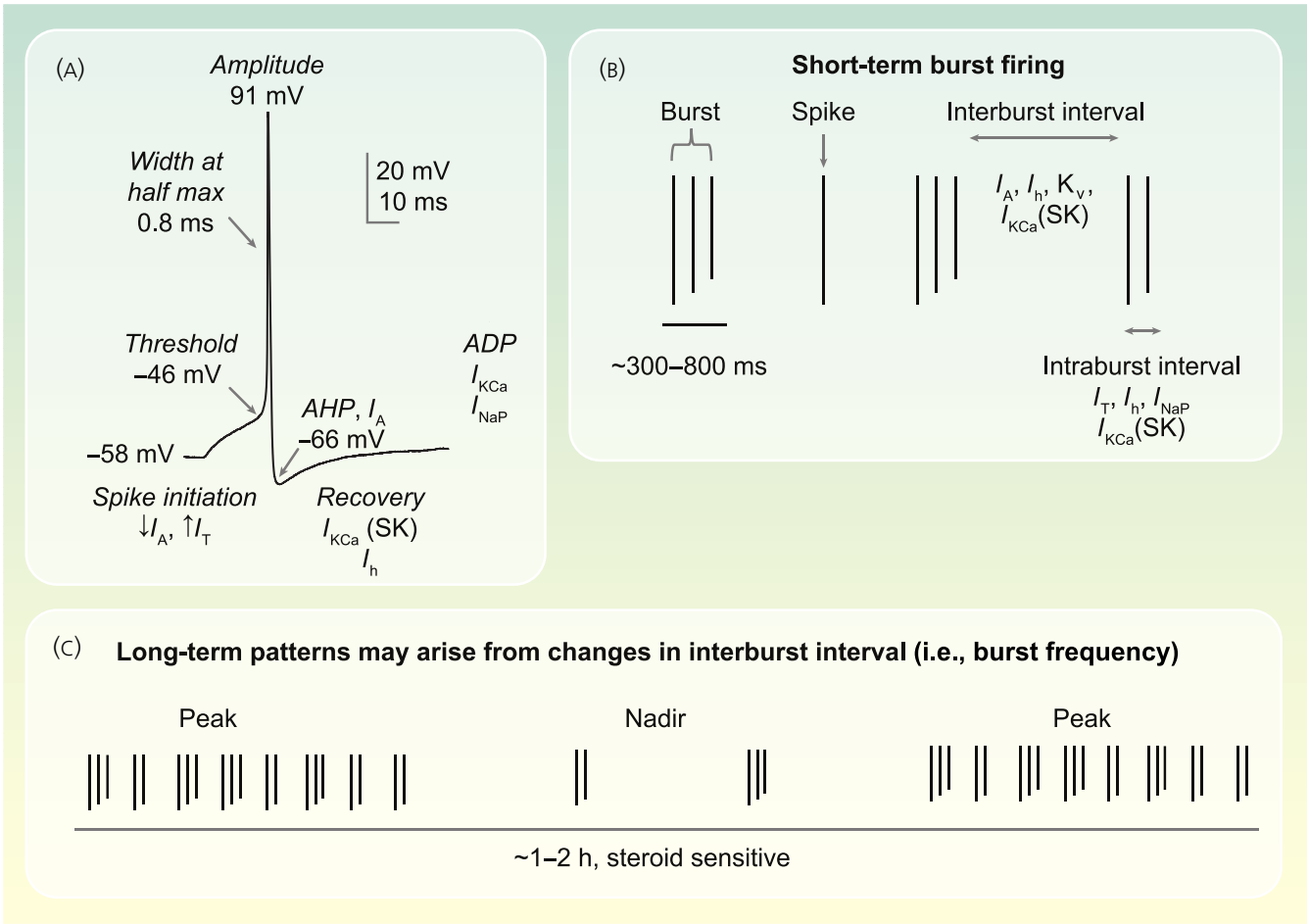

FIGURE 1 Action potential firing in gonadotropin-releasing hormone (GnRH) neurons. (A) GnRH neuron action potential waveform and its regulation by individual conductances. (B) Short-term burst firing and postulated underlying conductances. (C) Postulate for generation of long-term firing patterns from bursts. I_A, A-type K^+ current[58]; I_T, T-type Ca current[2+27]; I_{KCa}, Ca^{2+}-activated K^+ current; SK small conductance $I_{KCa;}$[10] I_{NaP}, persistent Na^+ current; I_h, hyperpolarization-activated current[28]; AHP, afterhyperpolarization potential

4.2 | GnRH neuron firing patterns

Spontaneous GnRH firing is not dependent upon fast synaptic transmission,[40] GnRH itself[41] or kisspeptin,[42] a major activator of GnRH neurons. An interesting feature of spontaneous GnRH neuron firing is that it exhibits short-term (burst firing) (Figure 1B) and long-term patterning (Figure 1C).[10,43–46] GnRH neuron bursts have a longer intraburst interval (approximately 150–400 ms) than neurons in the thalamus and cortex[47,48] or even magnocellular neuroendocrine cells,[49] and bursts are short, with typically two to eight spikes per burst.[50] This low spontaneous frequency is puzzling because GnRH neuron firing can be driven at much higher rates by current injection, suggesting that the ionic conductances of these cells are not in and of themselves limiting.[23,36] As reviewed previously,[20,21] GnRH neurons recorded in brain slices may be quiescent or spontaneously firing. Although most GnRH neurons exhibit irregular bursting, 1–2% of these cells exhibit parabolic bursting riding on marked slow (approximately 0.05 Hz) oscillations in membrane potential.[51] Burst firing is linked with peptide release in magnocellular neurons[52] and the higher frequency activity within bursts is likely important for GnRH release.[53] Long-term alterations between peaks and nadirs in firing rate have been observed in both sexes; the frequency of these peaks resembles that of LH release in vivo and is likewise modified by gonadal steroid feedback.[45,46] Short- and long-term patterns may be related. One investigation demonstrated that bursts within a GnRH neuron maintain consistent characteristics between peaks and nadirs, although the interburst interval increases during nadirs.[43] Further studies of this relationship and its modulation, the underlying ionic conductances and excitation–secretion coupling in GnRH neurons are required.

Interestingly, despite demonstration of synaptic connections, bundling and even cytoplasmic bridges among GnRH neurons,[54–56] there are no convincing data indicating that GnRH neurons in brain slices are coordinated with one another from dual patch clamp recordings[51,57] or calcium imaging.[9] It is important to emphasize that few attempts have been published despite the fascination of the field with pulsatile release and the presumption that this involves some sort of coordination among these cells. Such studies are technically difficult and the possible caveats of looking for coordination within a reduced slice preparation are many; for example, if connecting fibers

are severed during slice preparation, a false negative result will be obtained. This is an important area for future studies, which might take advantage of in vivo methods that can simultaneously monitor multiple cells.

4.3 | Ionic conductances of GnRH neurons

GnRH neurons express a typical set of voltage-gated ion channels that enables action potential firing. Channels that can be active at subthreshold membrane potentials help sculpt spike initiation. GnRH neurons have a very large fast-transient (A-type) K^+ current that fights depolarization,[24,58] and may contribute to both the narrow width of the spike and the large amplitude AHP. Ca^{2+}-activated K^+ currents in these cells appear to regulate intra and interburst intervals[10] and the amplitude of the ADP.[34] Opposing these outward currents are several smaller magnitude inward currents, specifically hyperpolarization-activated or I_h,[24,28] low-voltage-activated Ca^{2+} or I_T,[27] a Ca^{2+}-activated mixed cation or CAN,[59] and a tetrodotoxin-sensitive persistent Na^+ current or I_{NaP}.[34,59] Several of these latter conductances, along with Ca^{2+}-activated K^+ currents, have been implicated in both burst firing and pacemaker activity in other neurons. Both I_T and I_h contribute to rebound firing after the termination of a hyperpolarizing current injection and CAN and I_{NaP} augment the ADP in these cells. Interestingly, many of these are regulated by estradiol in a manner that would tend to increase GnRH neuron activity during positive feedback, and thus this may be important in the achievement of that state.

4.4 | Cl^- homeostasis

An interesting feature of GnRH neurons that was once controversial is their maintenance of higher intracellular Cl^- levels than is typical of most adult neurons. There is now consensus that the Cl^- reversal potential is depolarized relative to the action potential threshold in these cells.[60,61] As a result, activation of $GABA_A$ receptors, for which Cl^- is the main permeable ion, depolarizes these cells and can trigger action potential firing in preoptic GnRH neurons in rodents and terminal nerve GnRH neurons in teleost fish.[60–62] High Cl^- levels are likely attributable to continuing activity of the Cl^--accumulating cation cotransporter NKCC1 into adulthood, in contrast to most neurons in which the Cl^--depleting cotransporter KCC2 becomes dominant.[63] At the time of this discovery, GnRH neurons were among a small population of primarily sensory neurons for which excitatory GABA was identified, although this is now recognized in other hypothalamic cells[64,65] and throughout the adult brain.[66]

The induction of action potentials in response to GABA, widely recognized as the primary mode of fast inhibition in the brain, raises several interesting questions about GnRH neurons. First, is this a phenomenon limited to the perisomatic region where measurements have been made? Differential distribution of Cl^- cotransporters in subcompartments has been observed[67] and may lead to regional functional changes.[68] Although expression of NKCC1 is prominent, GnRH neurons do express KCC2, but subcellular localization requires further investigation.[61,69] Second, is regulating Cl^- homeostasis a possible mechanism for controlling GnRH activity? In this regard, the excitatory response to GABA appears to persist regardless of age, gonadal status or sex, even in mice in negative energy balance.[61,70] Reduced excitatory response to GABA was observed in prepubertal mice exposed to androgen in utero, a model used to generate a phenotype resembling polycystic ovary syndrome; this blunting was not attributable, however, to a change in the Cl^- reversal potential.[71] In the models studied, regulating intracellular Cl^- does not appear to be a major point of physiologic control. Third, is it GABA alone or does the initial depolarization initiated by GABA activate other inward currents that boost the depolarizing response[8,72]? Fourth, what inhibits GnRH neurons? Although no 'fast' synaptic inhibition is known, several neuromodulators, discussed below, may do so.

5 | FAST SYNAPTIC TRANSMISSION TO GnRH NEURONS

In addition to having a non-standard response to activation of the $GABA_A$ receptor, the frequency of spontaneous fast synaptic transmission to GnRH neurons is low compared to, for example, cortex,[73] and even other hypothalamic neurons regulating reproduction.[74] Fast synaptic transmission detectable at the cell soma is primarily GABAergic and glutamatergic; blocking AMPA, NMDA and $GABA_A$ receptors eliminates postsynaptic currents (PSCs) and no response is observed to local application of glycine, another Cl^--permeable ionotropic receptor.[70] Immunoreactivity for vGLUT2 and vGAT, markers of putative glutamatergic and GABAergic synaptic appositions, respectively, identified the proximal dendrite as the main input target.[75] Of note, knockout of subunits of either ionotropic GABA or glutamate receptors had little effect on reproduction.[76,77] This may indicate that fast synaptic transmission is not critically important, or that functional compensatory mechanisms were initiated by the knockout.

Curiously, the density of putative glutamatergic synapses detected with immunostaining methods is twice that of putative GABAergic inputs on GnRH neuron somata and proximal dendrites.[75] Functional measures of glutamatergic synaptic transmission directly to GnRH neurons made with patch clamp, however, indicate that it is infrequent. A low percentage of cells responding to glutamate receptor agonists, particularly NMDA receptor agonists, was noted in the first recordings of GFP-identified cells,[14] and confirmed in subsequent measurements of glutamatergic transmission[78,79] and of the percentage of cells responding to bath application of receptor agonists.[8,80] The spontaneous AMPA-mediated PSC frequency in mice is typically under 0.1 Hz. Suppression by estradiol negative feedback has been reported,[78,79] but should be interpreted with caution because small changes in transmission rate that may not be biologically important can result in large fold changes. Of note, higher rates of AMPA-mediated transmission to GnRH neurons occur in

rats, with an additional increase during estradiol positive feedback on proestrus, when Ca^{2+}-permeable AMPA receptors are inserted into the membrane.[81] Positive feedback is also associated with more spines, considered as a site for glutamatergic synaptic input, on murine GnRH neurons[82] and with changes in expression of genes encoding glutamate receptor subunits in these cells,[83] although, importantly, it is not known whether these anatomical and molecular changes are associated with functional differences.

Despite these latter observations, the low rates of glutamatergic transmission observed may call into question its relevance, but two points are worth making. First, the available data on spontaneous transmission is from somatic recordings. Input occurring at a distance from the soma, such as those impinging onto the very distal processes of GnRH neurons,[84] may have either decayed before detection by the somatic recording electrode or been eliminated by brain slice preparation. Second, GnRH neurons may have more glutamatergic input than is appreciated because increasing neurotransmission within a brain slice by blocking K^+ channels markedly increases glutamatergic transmission[79]; this may indicate low initial probability of release at these synapses under most conditions.

GABAergic transmission is more prevalent but still rarely exceeds 2 Hz, being more typically under 1 Hz. Several studies demonstrated that the frequency of GABAergic transmission to these cells correlates with what is expected for GnRH/LH output for that physiologic state. Progesterone negative feedback, estradiol negative feedback and negative energy balance reduce GnRH/LH pulse frequency and reduce the frequency and sometimes amplitude of GABAergic transmission to GnRH neurons.[70,85–87] By contrast, states that increase GnRH/LH release, such as mild androgen elevation and estradiol positive feedback, typically,[85–88] but not always,[89] correlate with increased GABAergic transmission and PSC amplitude. Consistent with these correlational studies, electrical stimulation of the anteroventral periventricular nucleus (AVPV) induces GABAergic and glutamatergic evoked PSCs in GnRH neurons[90]; GABA was predominant and required for AVPV stimulation to elicit the firing of most GnRH neurons. Optogenetic stimulation specifically of AVPV kisspeptin neurons, which use GABA as a cotransmitter, evokes $GABA_A$ receptor-dependent firing in GnRH neurons.[91] Finally, blocking $GABA_A$ receptors during in vivo recordings of GnRH neurons consistently reduced firing.[92] These findings consistently point to the action of GABA via the $GABA_A$ receptor as being excitatory to GnRH neurons. Activation of $GABA_B$ receptors, in contrast, inhibits GnRH neuron electrical activity,[90,93,94] indicating that GABA may bidirectionally control GnRH neuron firing depending on the receptor activated.

It should be noted that the rates and/or patterns of glutamate and GABA synaptic transmission to GnRH neurons in vivo may be substantially different because brain slice preparation removes many afferent soma. Changes in synaptic transmission detected in brain slices from animals in different physiologic or pathologic states nevertheless indicate functional synaptic plasticity and, as such, are meaningful. The identification of where fast synaptic inputs originate and the reproductive states and/or phases of a pulse cycle when these inputs are active represent interesting future directions.

6 | RECORDING GnRH NEURONS IN VIVO

The brain slice recordings used in the work discussed above and below allow the targeting of individual cells and mechanistic studies, but also have limitations. Perhaps most prominent is the reduced nature of these preparations, which maintains only local circuitry, removing distal input soma and even some proximal ones. Although this can be mitigated to some extent by using different slice orientations,[86,95] this is still far from the integrative in vivo situation in which the regulation of reproduction typically occurs. Recording from GnRH neurons in vivo avoids this limitation. This is challenging because the few GnRH neurons are scattered and deep relative to the surface of the brain. One paper has accomplished this by using a transpharyngeal approach to expose and record from GnRH neurons under anesthesia.[92] The only area in the mouse brain where GnRH-GFP neurons are sufficiently superficial to be targeted with a recording pipette is dorsal to the optic chiasm at the ventral-most surface of the anterior hypothalamic area, along the posterior cerebral artery. As a result of the nearby beating artery, it was only possible to achieve short duration targeted extracellular recordings. Despite these challenges, this heroic effort nonetheless provided important confirmatory evidence of several observations made in brain slices. Specifically, GnRH neuron firing is variable with spontaneous bursts, and responses to GABA, glutamate and kisspeptin-10 are all excitatory.

In sum, there are several aspects of GnRH neuron electrophysiology that are quite consistent among cells. These include basic properties such as a relatively high input resistance (e.g., compared to cortical and hippocampal pyramidal neurons), the observation of spontaneous firing activity, grouping of action potentials into bursts in most cells, action potential properties when recorded with physiologic chloride levels, a predominance of fast GABAergic over fast glutamatergic input in recordings made in the perisomatic region and a depolarizing/excitatory response to GABA. Burst properties can exhibit considerable heterogeneity among cells and the importance of this for neuroendocrine output is something that requires further investigation.

7 | NEUROMODULATION OF GnRH NEURON ELECTRICAL ACTIVITY

Subsequent to the first recordings of GnRH neurons, a large repertoire of neuromodulators altering their electrical activity has been compiled. Because of space limitations in the present review, we focus on the non-conventional neuromodulators that have emerged as important regulators of GnRH neuron activity, as well as on RF-amide neuropeptides. Detailed accounts of GnRH neuron regulation

by neurotransmitters and neuropeptides are available in previous excellent reviews.[96,97]

7.1 | Non-traditional neuromodulators

Studies of endocannabinoid, nitric oxide (NO) and prostaglandin E_2 regulation of GnRH neuron electrical activity are relatively recent. These neuromodulators are not stored in vesicles but rather produced on demand, acting directly or indirectly on GnRH neurons, or as retrograde transmitters synthesized by GnRH neurons to regulate synaptic transmission.

7.1.1 | Endocannabinoids

GnRH neuron membrane potential depolarization or activation of receptors stimulates synthesis and release of 2-arachidonoylglycerol (2-AG), which acts on presynaptic type-1 cannabinoid receptors to decrease GABA release.[98–101] Endocannabinoid (eCB)-dependent suppression of GABA release occurs in mice of both sexes and may occur constitutively, at least in brain slices.[98,99,101] GnRH neurons also synthesize anandamide, which may signal via TRP vanilloid channels to suppress constitutive eCB signaling from GnRH neurons.[30,31] Importantly, estradiol via estrogen receptor β (ERβ) rapidly promotes 2-AG synthesis, subsequent suppression of GABA release and decreased GnRH neuron firing in metestrous females,[100] suggesting a role of retrograde eCB suppression of GABA release as one mechanism of estradiol negative feedback.

7.1.2 | Nitric oxide

The gaseous neuromodulator NO may affect GnRH neuron electrical activity through multiple mechanisms with opposing effects. The "NO donor" L-arginine directly suppresses action potential firing in > 90% of GnRH neurons from both sexes via neuronal NO synthase (nNOS)-dependent NO synthesis, modulation of soluble guanylyl cyclase, subsequent suppression of a depolarizing plateau potential and activation of a K^+ current.[102] In addition to these direct inhibitory effects, NO produced in response to activation of specific receptors increases GABA and glutamate release, indirectly increasing GnRH neuron activity.[31,103,104] Of note, estradiol, acting at ERβ, rapidly increases neurotransmitter release and action potential firing in GnRH neurons by mobilizing this pathway in proestrous female mice, suggesting a role in estradiol positive feedback.[104] NO may also directly alter GnRH neuron excitability by accelerating recovery from prior exposure to kisspeptin-10, thereby enabling repeated responses to this neuropeptide (see below)[105] (Figure 2). Whether or not GnRH neurons synthesize NO is controversial. Neither NADP-diaphorase,[102] nor nNOS[106] is detectable in GnRH neurons, arguing against synthesis. By contrast, pharmacological experiments, and detection of *Nos1* mRNA and nNOS immunoreactivity at the ultrastructural level in GnRH neurons argue for synthesis,[31] opening the possibility that NO acts as a retrograde or neuromodulatory signal.

7.1.3 | Prostaglandin E_2

Prostaglandin E_2 (PGE_2) directly depolarizes the membrane potential and increases action potential firing of most GnRH neurons regardless of sex or estrous cycle stage. These effects are mediated via the prostaglandin EP2 receptor, adenylyl cyclase and protein kinase A to open a non-selective cation channel.[107] Ambient PGE_2 may help control GnRH neuron membrane potential and spontaneous firing because blocking its synthesis reduces activity in brain slices.[107] Evidence indicates that astrocytes are a primary source of PGE_2 release.[99,107,108]

These non-traditional neuromodulators directly and indirectly regulate GnRH neurons. Interestingly, there may be crosstalk among these pathways because PGE_2 is required for depolarization-induced, eCB-mediated, suppression of GABA transmission to GnRH neurons.[99] Moreover, additional neuropeptides and hormones may recruit these neuromodulators to produce their effects on GnRH neuron firing, as is the case of eCBs and NO.[31,101,103]

7.2 | RF-amide peptides

Exogenous kisspeptin-10 drives, with high potency (low nanomolar EC_{50}), prolonged membrane potential depolarizations, action potential firing and increased intracellular Ca^{2+} ($[Ca^{2+}]_i$) fluctuations in GnRH neurons, as well as GnRH secretion.[29,92,105,109–116] Kisspeptin receptor (Kiss1r) expression in GnRH neurons is both necessary and sufficient for kisspeptin-10 effects on GnRH neuron firing and LH secretion, as well as for puberty and overall fertility.[42,117] Kisspeptin-10 may also have indirect actions by increasing GABA and glutamate release on GnRH neurons, and by modulating NO-synthesizing neurons in the preoptic area.[106,110] Kisspeptin-10 actions in GnRH neurons are mediated through inhibition of multiple K^+ currents, including inward-rectifying, A-type and K^+ current mediating the slow AHP,[29,110,111,118–120] as well as via activation of a non-selective cationic current likely mediated by TRP canonical (TRPC) channels.[29,118] The role of $[Ca^{2+}]_i$ in GnRH neuron membrane responses to kisspeptin is somewhat unclear. Although kisspeptin-10 increases $[Ca^{2+}]_i$ in GnRH neuron somata, dendrites and terminals,[12,112,118] kisspeptin-10-induced excitation is resistant to Ca^{2+} buffering[111,121] and to depletion of Ca^{2+} stores[112,121] (Figure 2). Interestingly, kisspeptin-10-evoked increases in $[Ca^{2+}]_i$ are required for GnRH secretion at the median eminence (ME).[116,122] Activation of phospholipase C β (PLCβ) and c-Src tyrosine kinase are necessary for kisspeptin-10 effects.[29,118,121] PLCβ-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2), which inhibits TRPC channels in a subset of GnRH neurons, and

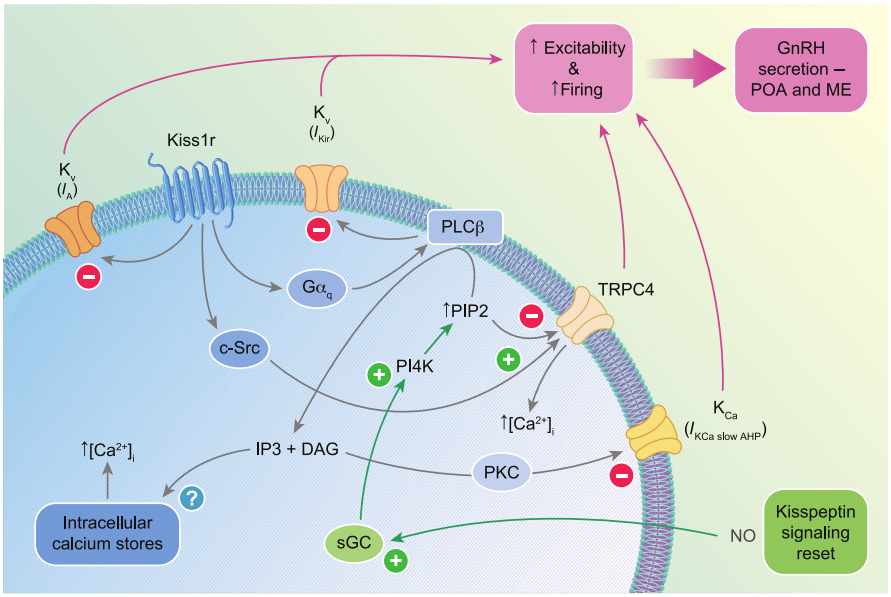

FIGURE 2 Kisspeptin-Kiss1r signaling in gonadotropin-releasing hormone (GnRH) neurons. Schematic illustration of the main intracellular pathways identified to date that contribute to kisspeptin-10-evoked stimulation of GnRH neurons. The main effects are to inhibit multiple K^+ conductances and to activate non-selective cation channels (TRPC4), resulting in membrane depolarization, further activation of voltage-gated currents and, eventually, action potential firing. For details and references, see text. AHP, afterhyperpolarization; c-Src, protein-tyrosine kinase Src; DAG, diacyl glycerol; I_A, A-type K^+ current; $I_{KCa\ slow\ AHP}$, Ca^{2+}-activated K^+ current mediating the slow AHP; I_{Kir}, inward rectifying K^+ current; IP3, inositol triphosphate; K_{Ca}, Ca^{2+}-activated K^+ channel; Kiss1r, kisspeptin receptor; K_v, voltage-gated K^+ channel; ME, median eminence; NO, nitric oxide; PI4K, phosphatidylinositol 4-kinase; PIP2 phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLCβ, phospholipase C β; POA, preoptic area; sGC, soluble guanylate cyclase; TRPC4, transient receptor potential channel 4

the ensuing PIP2 depletion may contribute to prolonged activation of these channels[121] (Figure 2).

The effects of exogenous kisspeptin-10 last many minutes and cannot be repeated at the GnRH neuron soma in brain slice recordings.[111,118] The prolonged nature of this response is likely the result of PIP2 depletion because inhibition of phosphatidylinositol 4-kinase (PI4K), which synthesizes PIP2, prolongs kisspeptin-10 excitation of GnRH neurons.[105,121] Of interest in this regard, NO increases PI4K activity[123] and thus PIP2 synthesis, thereby both shortening kisspeptin-10-mediated excitation and enabling subsequent kisspeptin-10 stimulations of GnRH neurons[105] (Figure 2). NO-mediated recovery from kisspeptin excitation may provide an "off" signal terminating kisspeptin-10-induced GnRH neuron activity/secretion and might facilitate periodic kisspeptin signaling to promote pulsatile GnRH secretion. Of note, because NO diffusion can potentially affect many GnRH neuronal elements, NO-modulation of kisspeptin signals might help coordinate GnRH secretion between many terminals.[105] It should be noted that endogenous kisspeptin actions may be repeatable as consecutive optogenetic stimulations of kisspeptin neurons at approximately 10-min intervals repeatedly evoke action potential firing in GnRH neurons[91] and LH secretion in vivo,[124] suggesting that "on–off" kisspeptin effects might require sustained activation of the kisspeptin receptor and associated signaling pathways. The impact of NO on kisspeptin signaling has only been assessed so far at the GnRH neuron somata[105] and it remains to be determined whether kisspeptin signaling in the ME is similarly regulated by NO. This is particularly important because GnRH neuron subcompartments may operate independently from one another in vivo[125] (see below). Lastly, the source of NO remains unknown. Bearing in mind the controversy around nNOS expression discussed above, GnRH neurons remain possible candidates. Alternatively, nNOS-expressing neurons are found in the preoptic area, some of which, interestingly, express Kiss1r, and the arcuate nucleus.[106]

RF amide-related peptide 3 (RFRP-3), the mammalian counterpart to avian gonadotropin-inhibiting hormone,[126] inhibits GnRH neuron $[Ca^{2+}]_i$ oscillations in nasal explants and decreases the firing rate of 50%–70% of prepubertal and adult male and female GnRH neurons via binding to neuropeptide FF receptor 1 and activation of K^+ currents.[127–129] This inhibition transiently opposes the prolonged actions of kisspeptin-10,[128,129] in keeping with the inhibitory effects of RFRP-3 on GnRH secretion in brain slices[122,130] and LH secretion in vivo,[131,132] making RFRP-3 a candidate "off" signal. It should be noted, however, that approximately 10% of GnRH neurons increase their firing in response to RFRP-3[127] and that central administration of RFRP-3 may stimulate LH secretion in males.[132]

8 | FUNCTIONAL INTERROGATION OF NATIVE CIRCUITS

Although the studies reviewed above have been very valuable, at least some of these results should be interpreted with caution. Bath

or local agonist and antagonist applications provide important information but have limited interpretative value as a result of off-target actions at all binding sites present within the preparation. Moreover, many neuromodulators are expressed in several brain areas and these neuronal populations may express multiple cotransmitters and/or be involved in completely different physiological functions. Inferring the functional impact of specific neuromodulatory populations thus requires a combination of agonist-induced changes in electrical activity/$[Ca^{2+}]_i$, neuronal tract-tracing and functional circuit interrogation with opto- and/or chemogenetics.

Applying these approaches to the control of GnRH neurons by kisspeptin revealed that kisspeptin neurons in the preotic area in females potently stimulate GnRH neuron firing and that a subset of preoptic kisspeptin neurons further excites GnRH neurons by co-releasing GABA.[91] These types of studies also revealed an unsuspected indirect mode of communication between arcuate kisspeptin neurons and GnRH neurons via projections to and stimulation of preoptic kisspeptin neurons; whether this pathway substantially contributes to GnRH and LH secretion in vivo is not known.[133] It is interesting to note that direct interrogation of the arcuate kisspeptin-to-GnRH pathway in brain slices still eludes researchers, probably because, in rodents, the former neurons project predominantly to the GnRH neuron distal processes near and in the ME,[134] a compartment almost inaccessible for conventional electrophysiology. Further studies aimed at determining direct inputs to the GnRH neurons, as well as their impact on the activity of these cells, are needed to gain deeper understanding of the regulation of GnRH release patterns.

9 | FUNCTION OF GnRH NEURON COMPARTMENTS

Another area that has progressed over the past 10–15 years has been the functional definition of GnRH neuron compartments. Most patch clamp recordings have been made of the soma, but dual recordings of soma and proximal processes with dendrite-like morphology revealed that these processes can initiate and actively propagate action potentials in some GnRH neurons.[37,38,135] In rodents, most GnRH neuron projections to the ME exhibit features such as the presence of spines, synaptic input, a larger diameter and ultrastructural profiles typically characteristic of dendrites.[84,135,136] Because of these attributes, which collectively suggest that these projections share dendritic and axonal properties, they have been referred to as "dendrons".[135] Near the ME, GnRH neuron processes often branch out into multiple axon-like profiles (i.e., smaller diameter) with putative secretory terminals in proximity to blood vessels.[84,135] Determining whether these unusual features of GnRH neuron projections exist beyond mice and rats will be an important future research direction.[137]

Understanding GnRH neuron subcompartments is particularly relevant because several studies suggest that functional differences may exist among GnRH somata and/or proximal processes in the preoptic area and GnRH neuronal elements near the ME. In vivo opto- and chemogenetic suppression of GnRH neuron activity in behaving mice revealed that distal GnRH neuron projections may operate independently of activity in their somata and proximal dendrites, and, furthermore, that these distal regions may be sufficient to control pulsatile, but not surge, LH secretion.[125] In brain slice studies aiming to investigate mechanisms, GnRH release from the perisomatic and terminal regions is differently regulated.[138] In agreement with this, distal projections near the ME are the overwhelming site of arcuate kisspeptin neuron inputs to GnRH neurons in rodents.[134] This region responds to local kisspeptin-10 application in an action potential-independent manner with increases in $[Ca^{2+}]_i$ and GnRH release.[12,13,138] Neurokinin B (NKB), dynorphin A and glutamate, co-expressed in arcuate kisspeptin neurons, do not alter $[Ca^{2+}]_i$ dynamics in the distal processes of GnRH neurons, although NKB application to the ME induces GnRH release in a kisspeptin-independent manner.[12,13,138] Differences in kisspeptin-10-induced signaling might also exist between GnRH somata and distal processes.[12,122] Such observations are consistent with those made in medial basal hypothalamic preparations from rodents and sheep, which contain arcuate kisspeptin but very few GnRH neuron somata. These preparations release GnRH in response to kisspeptin[114–116] and, in rodents, can release GnRH in a pattern reminiscent of pulsatile LH secretion in vivo.[139] This suggests that arcuate kisspeptin neurons and their projections to the distal processes of GnRH neurons might be sufficient to support pulsatile secretion. An even more reduced preparation of isolated rat ME exhibited episodic release,[140] perhaps indicating only the very distal processes of both GnRH and kisspeptin neurons are needed. Together, these observations suggest specialization of different regions of the GnRH neurons; understanding how various neuromodulators influence these functional compartments is a rich area for future studies.

10 | DEVELOPMENT OF GnRH NEURON PROPERTIES

10.1 | Early GnRH secretion

The ability to release GnRH in a pulsatile manner is a core function of these neurons. GnRH is detectable before these cells migrate from the olfactory placode and neurosecretion appears to arise early in their development.[130] Sexual differentiation of the brain requires a postnatal testosterone surge; this may rely upon GnRH secretion immediately after birth,[141] although it has been suggested that this may be GnRH independent.[142] In mice, connections from the putative pulse generator arcuate kisspeptin neuron and GnRH neurons are established before birth.[143,144] Consistent with this, fetal mediobasal hypothalamic tissue isolated from humans[145] and non-human primates[146] releases GnRH pulses, as do GnRH neurons derived from primate and rodent olfactory placodes.[147–150] Prenatal

preoptic tissue containing GnRH neurons restores LH pulses and/or gonadal function in models lacking endogenous GnRH, including lesioned adult female monkeys,[151] rats[152] and *hpg* male mice,[153,154] suggesting that prenatal tissue either has episodic release as an inherent function or can mature into this role.

Olfactory placode-derived GnRH neurons were an early model used to decipher the mechanisms underlying pulsatility and other aspects of GnRH physiology. An important advantage of this preparation is that whole GnRH neurons are present, unlike in most brain slice preparations, which cut processes. Ca^{2+} imaging was used to assess both GnRH neuron function and coordination. Bursts of action potentials occur concomitantly with $[Ca^{2+}]_i$ oscillations in placodal GnRH neurons and GnRH neurons in brain slices from adults.[10,12] Increases in $[Ca^{2+}]_i$ are often equated to action potential firing but, although often related,[10,12] this is not technically accurate. Fluctuations in $[Ca^{2+}]_i$ can reflect any of several phenomena, including changes in action potential-dependent or subthreshold Ca^{2+} entry, receptor-mediated Ca^{2+} influx and/or Ca^{2+} release from internal stores. An important advantage to Ca^{2+} imaging is the ability to monitor simultaneously several cells. In addition, Ca^{2+} imaging makes possible the recording of subcompartments, such as GnRH neuron terminal regions, which are not readily accessible with electrophysiology.

Ca^{2+} imaging of primate and mouse GnRH neurons in placode cultures revealed that GnRH neurons exhibit $[Ca^{2+}]_i$ oscillations at a higher frequency than is typical for LH release. These high-frequency oscillations are typically not coordinated among cells. Intriguingly, however, periodic coordination of these higher frequency oscillations occurred at a frequency similar to LH release; further work showed that this coordination is correlated with GnRH pulses.[150,155,156] These cultures are devoid of neuronal central nervous system inputs, suggesting that pulsatile GnRH secretion is an endogenous property. Supporting this postulate, the coordination of $[Ca^{2+}]_i$ oscillations in primate placodal cultures is followed by a delay in the resumption of $[Ca^{2+}]_i$ oscillations. However, there are several factors to consider before this postulate is accepted. First, increased frequency of the high-frequency oscillation can be driven by neuromodulators, such as GABA[156,157] and estradiol[158,159]; this increased frequency is associated with increased coordination of oscillations, raising the question of whether this is merely an increase in mathematical probability. Second, non-neuronal cells in placodal cultures are also coordinated with GnRH neuron $[Ca^{2+}]_i$ fluctuations and GnRH release.[160] Third, glial cells in placodal cultures express gap junctions and blocking these reduces coordination and GnRH release.[161] Consistent with this, the putative gliotransmitter ATP induces coordinated elevations in $[Ca^{2+}]_i$ in both GnRH neurons and non-neuronal cells.[162] Fourth, GnRH pulses in placodal cultures develop over time,[150] in parallel with the network surrounding GnRH neurons.[163] Although these mechanistic questions remain to be resolved, it is clear that GnRH neurons are equipped with the exocytotic machinery for peptidergic secretion and can sustain pulsatile secretion early on.

10.2 | Development of GnRH firing and signaling properties

The literature on the intrinsic properties of GnRH neurons before adulthood is sparse but the firing activity of GnRH neurons before weaning might shape their adult properties.[164] Placodal GnRH neurons display action potentials and are equipped with Na^+, K^+ (delayed rectifier and A-type) and Ca^{2+} (high- and low-voltage activated) conductances.[165,166] Recordings of GnRH neurons from prepubertal GnRH-GFP mice demonstrate adult-like bursting characteristics develop early and that the mean firing rate is developmentally regulated.[44,50] Placodal and adult GnRH neurons exhibit other consistent features, including excitation by activating $GABA_A$ receptors,[156,167] and less influence of glutamatergic signaling.[8,9] Early on, GnRH neurons also express G-protein-coupled receptors (GPCRs) and their coupling partners ($G_{i/o}$, $G_{q/11}$, G_s). $G_{i/o}$-coupled receptors provide a robust inhibition, directly via G-protein-gated inwardly rectifying K^+ channels.[94,168,169] $G_{q/11}$-coupled receptors provide a robust excitation, via PLC and downstream effectors.[29,112,118] By contrast, G_s-coupled receptors provide a mild excitation, via protein kinase A and downstream effectors.[11,120,170] Kisspeptin-10 potently elicits GnRH release from placodal cultures,[168] neonatal to juvenile[171] and adult preparations.[114] Importantly, the response of GnRH neurons to kisspeptin-10 increases during development.[109,130] The complexity of GnRH neuron cell signaling described above can be appreciated with the activation of $G_{q/11}$-coupled kisspeptin receptor in both placodal and adult GnRH neurons.[29,110,112,118–120] Many GPCRs identified in adult GnRH neurons[172] have been found in placodal GnRH neurons and are linked to a signaling pathway modulating the frequency of GnRH $[Ca^{2+}]_i$ oscillations.[162,168,173] The close signaling parallel between placodal and adult GnRH neuron highlights the precocity of GnRH neurons.

11 | CONCLUSIONS AND PERSPECTIVES

The above and continued characterization of GnRH neuron intrinsic properties is a prerequisite for understanding the physiologic and pathophysiologic regulation of their output, generating computational models and understanding the entire circuitry underlying GnRH release. Several critical and unanswered questions remain concerning the electrophysiologic properties of GnRH neurons, including how is coordination achieved, how does action potential firing relate to hormone release and is this a point of feedback regulation, does signaling and its functional outcomes differ depending on which domain of the GnRH neuron receives the input, and how do changes in ionic conductances in GnRH neurons contribute to long-term patterns of activity in these cells that correlates with hormone release? Pairing electrophysiologic approaches with opto- and chemogenetic interrogation of brain circuits in vivo is a powerful approach for determining the role of specific cell populations in GnRH secretion and will expand our understanding of the functional GnRH

network, generation of the secretory pattern and the regulation of these elements throughout postnatal development, via physiologic steroid feedback, internal and external cues, and under pathophysiologic conditions.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Kelly MJ, Ronnekleiv OK, Eskay RL. Identification of estrogen-responsive LHRH neurons in the guinea pig hypothalamus. *Brain Res Bull*. 1984;12:399-407.
2. Kozlowski GP, Dees WL. Immunocytochemistry for LHRH neurons in the arcuate nucleus area of the rat: fact or artifact? *J Histochem Cytochem*. 1984;32:83-91.
3. Lagrange AH, Ronnekleiv OK, Kelly MJ. Estradiol-17 beta and mu-opioid peptides rapidly hyperpolarize GnRH neurons: a cellular mechanism of negative feedback? *Endocrinology*. 1995;136(5):2341-2344. https://academic.oup.com/endo/article/136/5/2341/3037278?login=true
4. Sim JA, Skynner MJ, Pape JR, Herbison AE. Late postnatal reorganization of GABA(A) receptor signalling in native GnRH neurons. *Eur J Neurosci*. 2000;12:3497-3504.
5. Silverman AJ. The gonadotropin-releasing hormone (GnRH) neuronal systems: immunocytochemistry and in situ hybridization. In: Knobil E, eds. *The Physiology of Reproduction*, 1. 2nd edn. New York, NY: Raven Press, Ltd; 1994:1683-1709.
6. Mellon PL, Windle JJ, Goldsmith PC, Padula CA, Roberts JL, Weiner RI. Immortalization of hypothalamic GnRH neurons by genetically targeted tumorigenesis. *Neuron*. 1990;5:1-10.
7. Han SK, Abraham IM, Herbison AE. Effect of GABA on GnRH neurons switches from depolarization to hyperpolarization at puberty in the female mouse. *Endocrinology*. 2002;143:1459-1466.
8. Constantin S, Jasoni CL, Wadas B, Herbison AE. Gamma-aminobutyric acid and glutamate differentially regulate intracellular calcium concentrations in mouse gonadotropin-releasing hormone neurons. *Endocrinology*. 2010;151:262-270.
9. Jasoni CL, Todman MG, Strumia MM, Herbison AE. Cell type-specific expression of a genetically encoded calcium indicator reveals intrinsic calcium oscillations in adult gonadotropin-releasing hormone neurons. *J Neurosci*. 2007;27:860-867.
10. Lee K, Duan W, Sneyd J, Herbison AE. Two slow calcium-activated afterhyperpolarization currents control burst firing dynamics in gonadotropin-releasing hormone neurons. *J Neurosci*. 2010;30:6214-6224.
11. Piet R, Dunckley H, Lee K, Herbison AE. Vasoactive Intestinal peptide excites GnRH neurons in male and female mice. *Endocrinology*. 2016;157:3621-3630.
12. Iremonger KJ, Porteous R, Herbison AE. Spike and Neuropeptide-Dependent Mechanisms Control GnRH Neuron Nerve Terminal Ca(2+) over Diverse Time Scales. *J Neurosci*. 2017;37:3342-3351.
13. Liu X, Yeo SH, McQuillan HJ, et al. Highly redundant neuropeptide volume co-transmission underlying episodic activation of the GnRH neuron dendron. *Elife*. 2021;10:e62455.
14. Spergel DJ, Kruth U, Hanley DF, Sprengel R, Seeburg PH. GABA- and glutamate-activated channels in green fluorescent protein-tagged gonadotropin-releasing hormone neurons in transgenic mice. *J Neurosci*. 1999;19:2037-2050.
15. Suter KJ, Song WJ, Sampson TL, et al. Genetic targeting of green fluorescent protein to gonadotropin-releasing hormone neurons: characterization of whole-cell electrophysiological properties and morphology. *Endocrinology*. 2000;141:412-419.
16. Han SK, Todman MG, Herbison AE. Endogenous GABA release inhibits the firing of adult gonadotropin-releasing hormone neurons. *Endocrinology*. 2004;145:495-499.
17. Kato M, Ui-Tei K, Watanabe M, Sakuma Y. Characterization of voltage-gated calcium currents in gonadotropin-releasing hormone neurons tagged with green fluorescent protein in rats. *Endocrinology*. 2003;144:5118-5125.
18. Kanda S, Nishikawa K, Karigo T, et al. Regular pacemaker activity characterizes gonadotropin-releasing hormone 2 neurons recorded from green fluorescent protein-transgenic medaka. *Endocrinology*. 2010;151:695-701.
19. Hill CL, Stephens GJ. An Introduction to Patch Clamp Recording. *Methods Mol Biol*. 2020;2188:1-19. https://link.springer.com/protocol/10.1007%2F978-1-0716-0818-0_1
20. Moenter SM. Identified GnRH neuron electrophysiology: a decade of study. *Brain Res*. 2010;1364:10-24.
21. Piet R, Herbison AE. Electrophysiology of Rodent GnRH Neurons. In: Herbison AE, Plant TM, eds. *The GnRH Neuron and its Control*, 1st edn. Hoboken, NJ: Wiley Blackwell; 2018:177-202.
22. Wang Y, Kuehl-Kovarik MC. Flufenamic acid modulates multiple currents in gonadotropin-releasing hormone neurons. *Brain Res*. 2010;1353:94-105.
23. DeFazio RA, Moenter SM. Gonadotropin-Releasing Hormone (GnRH) Neuron Potassium Currents and Excitability in Both Sexes Exhibit Minimal Changes upon Removal of Negative Feedback. *eNeuro*. 2021;8:ENEURO.0126-0121.2021.
24. Zhang C, Bosch MA, Levine JE, Ronnekleiv OK, Kelly MJ. Gonadotropin-releasing hormone neurons express K(ATP) channels that are regulated by estrogen and responsive to glucose and metabolic inhibition. *J Neurosci*. 2007;27:10153-10164.
25. Liu X, Herbison AE. Small-conductance calcium-activated potassium channels control excitability and firing dynamics in

gonadotropin-releasing hormone (GnRH) neurons. *Endocrinology*. 2008;149:3598-3604.
26. Sun J, Chu Z, Moenter SM. Diurnal in vivo and rapid in vitro effects of estradiol on voltage-gated calcium channels in gonadotropin-releasing hormone neurons. *J Neurosci*. 2010;30:3912-3923.
27. Zhang C, Bosch MA, Rick EA, Kelly MJ, Ronnekleiv OK. 17Beta-estradiol regulation of T-type calcium channels in gonadotropin-releasing hormone neurons. *J Neurosci*. 2009;29:10552-10562.
28. Chu Z, Takagi H, Moenter SM. Hyperpolarization-activated currents in gonadotropin-releasing hormone (GnRH) neurons contribute to intrinsic excitability and are regulated by gonadal steroid feedback. *J Neurosci*. 2010;30:13373-13383.
29. Zhang C, Roepke TA, Kelly MJ, Ronnekleiv OK. Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-like cationic channels. *J Neurosci*. 2008;28:4423-4434.
30. Balint F, Csillag V, Vastagh C, Liposits Z, Farkas I. Insulin-like growth factor 1 (IGF-1) increases GABAergic neurotransmission to GnRH neurons via suppressing the retrograde tonic endocannabinoid signaling pathway in mice. *Neuroendocrinology*. 2021;111:1219-1230.
31. Farkas I, Vastagh C, Farkas E, et al. Glucagon-Like Peptide-1 Excites Firing and Increases GABAergic Miniature Postsynaptic Currents (mPSCs) in Gonadotropin-Releasing Hormone (GnRH) Neurons of the Male Mice via Activation of Nitric Oxide (NO) and Suppression of Endocannabinoid Signaling Pathways. *Front Cell Neurosci*. 2016;10:214.
32. Suter KJ, Wuarin JP, Smith BN, Dudek FE, Moenter SM. Whole-cell recordings from preoptic/hypothalamic slices reveal burst firing in gonadotropin-releasing hormone neurons identified with green fluorescent protein in transgenic mice. *Endocrinology*. 2000;141:3731-3736.
33. Adams C, Stroberg W, DeFazio RA, Schnell S, Moenter SM. Gonadotropin-Releasing Hormone (GnRH) Neuron Excitability Is Regulated by Estradiol Feedback and Kisspeptin. *J Neurosci*. 2018;38:1249-1263.
34. Chu Z, Moenter SM. Physiologic regulation of a tetrodotoxin-sensitive sodium influx that mediates a slow afterdepolarization potential in gonadotropin-releasing hormone neurons: possible implications for the central regulation of fertility. *J Neurosci*. 2006;26:11961-11973.
35. Roberts CB, O'Boyle MP, Suter KJ. Dendrites determine the contribution of after depolarization potentials (ADPs) to generation of repetitive action potentials in hypothalamic gonadotropin releasing-hormone (GnRH) neurons. *J Comput Neurosci*. 2009;26:39-53.
36. Kuehl-Kovarik MC, Partin KM, Handa RJ, Dudek FE. Spike-dependent depolarizing afterpotentials contribute to endogenous bursting in gonadotropin releasing hormone neurons. *Neuroscience*. 2005;134:295-300.
37. Roberts CB, Campbell RE, Herbison AE, Suter KJ. Dendritic action potential initiation in hypothalamic gonadotropin-releasing hormone neurons. *Endocrinology*. 2008;149:3355-3360.
38. Iremonger KJ, Herbison AE. Initiation and propagation of action potentials in gonadotropin-releasing hormone neuron dendrites. *J Neurosci*. 2012;32:151-158.
39. Herde MK, Herbison AE. Morphological Characterization of the Action Potential Initiation Segment in GnRH Neuron Dendrites and Axons of Male Mice. *Endocrinology*. 2015;156:4174-4186.
40. Nunemaker CS, DeFazio RA, Moenter SM. Estradiol-sensitive afferents modulate long-term episodic firing patterns of GnRH neurons. *Endocrinology*. 2002;143:2284-2292.
41. Gill JC, Wadas B, Chen P, et al. The gonadotropin-releasing hormone (GnRH) neuronal population is normal in size and distribution in GnRH-deficient and GnRH receptor-mutant hypogonadal mice. *Endocrinology*. 2008;149:4596-4604.
42. Kirilov M, Clarkson J, Liu X, et al. Dependence of fertility on kisspeptin-Gpr54 signaling at the GnRH neuron. *Nat Commun*. 2013;4:2492.
43. Nunemaker CS, Straume M, DeFazio RA, Moenter SM. Gonadotropin-releasing hormone neurons generate interacting rhythms in multiple time domains. *Endocrinology*. 2003;144:823-831.
44. Kuehl-Kovarik MC, Pouliot WA, Halterman GL, Handa RJ, Dudek FE, Partin KM. Episodic bursting activity and response to excitatory amino acids in acutely dissociated gonadotropin-releasing hormone neurons genetically targeted with green fluorescent protein. *J Neurosci*. 2002;22:2313-2322.
45. Pielecka J, Quaynor SD, Moenter SM. Androgens increase gonadotropin-releasing hormone neuron firing activity in females and interfere with progesterone negative feedback. *Endocrinology*. 2006;147:1474-1479.
46. Pielecka J, Moenter SM. Effect of steroid milieu on gonadotropin-releasing hormone-1 neuron firing pattern and luteinizing hormone levels in male mice. *Biol Reprod*. 2006;74(5):931-937. https://academic.oup.com/biolreprod/article/74/5/931/2667005
47. Llinas R, Jahnsen H. Electrophysiology of mammalian thalamic neurones in vitro. *Nature*. 1982;297:406-408. https://www.nature.com/articles/297406a0
48. Karvat G, Alyahyay M, Diester I. Spontaneous activity competes with externally evoked responses in sensory cortex. *Proc Natl Acad Sci USA*. 2021;118:e2023286118.
49. Lemos JR, Ortiz-Miranda SI, Cuadra AE, et al. Modulation/physiology of calcium channel sub-types in neurosecretory terminals. *Cell Calcium*. 2012;51:284-292.
50. Dulka EA, Moenter SM. Prepubertal development of gonadotropin-releasing hormone neuron activity is altered by sex, age, and prenatal androgen exposure. *Endocrinology*. 2017;158:3943-3953.
51. Chu Z, Tomaiuolo M, Bertram R, Moenter SM. Two types of burst firing in gonadotrophin-releasing hormone neurones. *J Neuroendocrinol*. 2012;24:1065-1077.
52. Cazalis M, Dayanithi G, Nordmann JJ. The role of patterned burst and interburst interval on the excitation-coupling mechanism in the isolated rat neural lobe. *J Physiol*. 1985;369:45-60.
53. Campos P, Herbison AE. Optogenetic activation of GnRH neurons reveals minimal requirements for pulsatile luteinizing hormone secretion. *Proc Natl Acad Sci U S A*. 2014;111:18387-18392.
54. Witkin JW, O'Sullivan H, Silverman AJ. Novel associations among gonadotropin-releasing hormone neurons. *Endocrinology*. 1995;136:4323-4330.
55. Witkin JW, Silverman AJ. Synaptology of luteinizing hormone-releasing hormone neurons in rat preoptic area. *Peptides*. 1985;6:263-271.
56. Campbell RE, Gaidamaka G, Han SK, Herbison AE. Dendro-dendritic bundling and shared synapses between gonadotropin-releasing hormone neurons. *Proc Natl Acad Sci U S A*. 2009;106:10835-10840.
57. Campbell RE, Ducret E, Porteous R, et al. Gap junctions between neuronal inputs but not gonadotropin-releasing hormone neurons control estrous cycles in the mouse. *Endocrinology*. 2011;152:2290-2301.
58. DeFazio RA, Moenter SM. Estradiol feedback alters potassium currents and firing properties of gonadotropin-releasing hormone neurons. *Mol Endocrinol*. 2002;16:2255-2265.
59. Wang Y, Garro M, Kuehl-Kovarik MC. Estradiol attenuates multiple tetrodotoxin-sensitive sodium currents in isolated gonadotropin-releasing hormone neurons. *Brain Res*. 2010;1345:137-145.
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. *J Neuroendocrinol*. 2008;20:566-575.

61. DeFazio RA, Heger S, Ojeda SR, Moenter SM. Activation of A-type gamma-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. *Mol Endocrinol*. 2002;16:2872-2891.
62. Nakane R, Oka Y. Excitatory action of GABA in the terminal nerve gonadotropin-releasing hormone neurons. *J Neurophysiol*. 2010;103:1375-1384.
63. 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;23:557-569.
64. Haam J, Popescu IR, Morton LA, et al. GABA is excitatory in adult vasopressinergic neuroendocrine cells. *J Neurosci*. 2012;32:572-582.
65. Kim YB, Colwell CS, Kim YI. Long-term ionic plasticity of GABAergic signalling in the hypothalamus. *J Neuroendocrinol*. 2019;31:e12753.
66. Marty A, Llano I. Excitatory effects of GABA in established brain networks. *Trends Neurosci*. 2005;28:284-289.
67. Kaila K, Price TJ, Payne JA, Puskarjov M, Voipio J. Cation-chloride cotransporters in neuronal development, plasticity and disease. *Nat Rev Neurosci*. 2014;15:637-654.
68. Khirug S, Yamada J, Afzalov R, Voipio J, Khiroug L, Kaila K. GABAergic depolarization of the axon initial segment in cortical principal neurons is caused by the Na-K-2Cl cotransporter NKCC1. *J Neurosci*. 2008;28:4635-4639.
69. Leupen SM, Tobet SA, Crowley WF Jr, Kaila K. Heterogeneous expression of the potassium-chloride cotransporter KCC2 in gonadotropin-releasing hormone neurons of the adult mouse. *Endocrinology*. 2003;144:3031-3036.
70. Sullivan SD, DeFazio RA, Moenter SM. Metabolic regulation of fertility through presynaptic and postsynaptic signaling to gonadotropin-releasing hormone neurons. *J Neurosci*. 2003;23:8578-8585.
71. Berg T, Silveira MA, Moenter SM. Prepubertal Development of GABAergic Transmission to Gonadotropin-Releasing Hormone (GnRH) Neurons and Postsynaptic Response Are Altered by Prenatal Androgenization. *J Neurosci*. 2018;38:2283-2293.
72. Hemond PJ, O'Boyle MP, Roberts CB, Delgado-Reyes A, Hemond Z, Suter KJ. Simulated GABA synaptic input and L-type calcium channels form functional microdomains in hypothalamic gonadotropin-releasing hormone neurons. *J Neurosci*. 2012;32:8756-8766.
73. Cummings DM, Andre VM, Uzgil BO, et al. Alterations in cortical excitation and inhibition in genetic mouse models of Huntington's disease. *J Neurosci*. 2009;29:10371-10386.
74. Wang L, Burger LL, Greenwald-Yarnell ML, Myers MG Jr, Moenter SM. Glutamatergic Transmission to Hypothalamic Kisspeptin Neurons Is Differentially Regulated by Estradiol through Estrogen Receptor alpha in Adult Female Mice. *J Neurosci*. 2018;38:1061-1072.
75. Yeo SH, Herde MK, Herbison AE. Morphological assessment of GABA and glutamate inputs to GnRH neurons in intact female mice using expansion microscopy. *J Neuroendocrinol*. 2021;33(9):e13021.
76. Shimshek DR, Bus T, Grinevich V, et al. Impaired reproductive behavior by lack of GluR-B containing AMPA receptors but not of NMDA receptors in hypothalamic and septal neurons. *Mol Endocrinol*. 2006;20:219-231.
77. Lee K, Porteous R, Campbell RE, Luscher B, Herbison AE. Knockdown of GABA(A) receptor signaling in GnRH neurons has minimal effects upon fertility. *Endocrinology*. 2010;151:4428-4436.
78. Christian CA, Pielecka-Fortuna J, Moenter SM. Estradiol suppresses glutamatergic transmission to gonadotropin-releasing hormone neurons in a model of negative feedback in mice. *Biol Reprod*. 2009;80:1128-1135.
79. Chen P, Moenter SM. GABAergic transmission to gonadotropin-releasing hormone (GnRH) neurons is regulated by GnRH in a concentration-dependent manner engaging multiple signaling pathways. *J Neurosci*. 2009;29:9809-9818.
80. Iremonger KJ, Constantin S, Liu X, Herbison AE. Glutamate regulation of GnRH neuron excitability. *Brain Res*. 2010;1364:35-43.
81. Tada H, Kuroki Y, Funabashi T, et al. Phasic synaptic incorporation of GluR2-lacking AMPA receptors at gonadotropin-releasing hormone neurons is involved in the generation of the luteinizing hormone surge in female rats. *Neuroscience*. 2013;248:664-669.
82. Chan H, Prescott M, Ong Z, Herde MK, Herbison AE, Campbell RE. Dendritic spine plasticity in gonadatropin-releasing hormone (GnRH) neurons activated at the time of the preovulatory surge. *Endocrinology*. 2011;152:4906-4914.
83. Vastagh C, Rodolosse A, Solymosi N, Liposits Z. Altered Expression of Genes Encoding Neurotransmitter Receptors in GnRH Neurons of Proestrous Mice. *Front Cell Neurosci*. 2016;10:230.
84. Moore AM, Prescott M, Czieselsky K, et al. Synaptic Innervation of the GnRH Neuron Distal Dendron in Female Mice. *Endocrinology*. 2018;159:3200-3208.
85. Adams C, Chen X, Moenter SM. Changes in GABAergic Transmission to and Intrinsic Excitability of Gonadotropin-Releasing Hormone (GnRH) Neurons during the Estrous Cycle in Mice. *eNeuro*. 2018;5:ENEURO.0171-0118.2018.
86. Christian CA, Moenter SM. Estradiol induces diurnal shifts in GABA transmission to gonadotropin-releasing hormone neurons to provide a neural signal for ovulation. *J Neurosci*. 2007;27:1913-1921.
87. Sullivan SD, Moenter SM. GABAergic integration of progesterone and androgen feedback to gonadotropin-releasing hormone neurons. *Biol Reprod*. 2005;72:33-41.
88. Sullivan SD, Moenter SM. Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurons: implications for a common fertility disorder. *Proc Natl Acad Sci U S A*. 2004;101:7129-7134.
89. Liu X, Porteous R, Herbison AE. Dynamics of GnRH Neuron Ionotropic GABA and glutamate synaptic receptors are unchanged during estrogen positive and negative feedback in female mice. *eNeuro*. 2017;4:ENEURO.0259-0217.2017.
90. Liu X, Porteous R, d'Anglemont de Tassigny X, et al. Frequency-dependent recruitment of fast amino acid and slow neuropeptide neurotransmitter release controls gonadotropin-releasing hormone neuron excitability. *J Neurosci*. 2011;31:2421-2430.
91. Piet R, Kalil B, McLennan T, Porteous R, Czieselsky K, Herbison AE. Dominant Neuropeptide Cotransmission in Kisspeptin-GABA Regulation of GnRH Neuron Firing Driving Ovulation. *J Neurosci*. 2018;38:6310-6322.
92. Constantin S, Iremonger KJ, Herbison AE. In vivo recordings of GnRH neuron firing reveal heterogeneity and dependence upon GABAA receptor signaling. *J Neurosci*. 2013;33:9394-9401.
93. Liu X, Herbison AE. Estrous cycle- and sex-dependent changes in pre- and postsynaptic GABAB control of GnRH neuron excitability. *Endocrinology*. 2011;152:4856-4864.
94. Zhang C, Bosch MA, Ronnekleiv OK, Kelly MJ. Gamma-aminobutyric acid B receptor mediated inhibition of gonadotropin-releasing hormone neurons is suppressed by kisspeptin-G protein-coupled receptor 54 signaling. *Endocrinology*. 2009;150:2388-2394.
95. Constantin S, Piet R, Iremonger K, et al. GnRH neuron firing and response to GABA in vitro depend on acute brain slice thickness and orientation. *Endocrinology*. 2012;153:3758-3769.
96. Spergel DJ. Modulation of Gonadotropin-Releasing Hormone Neuron Activity and Secretion in Mice by Non-peptide Neurotransmitters, Gasotransmitters, and Gliotransmitters. *Front Endocrinol*. 2019;10:329.

97. Spergel DJ. Neuropeptidergic modulation of GnRH neuronal activity and GnRH secretion controlling reproduction: insights from recent mouse studies. *Cell Tissue Res*. 2019;375:179-191.
98. Farkas I, Kallo I, Deli L, et al. Retrograde endocannabinoid signaling reduces GABAergic synaptic transmission to gonadotropin-releasing hormone neurons. *Endocrinology*. 2010;151(12):5818-5829. https://academic.oup.com/endo/article/151/12/5818/2456296
99. Glanowska KM, Moenter SM. Endocannabinoids and prostaglandins both contribute to GnRH neuron-GABAergic afferent local feedback circuits. *J Neurophysiol*. 2011;106:3073-3081.
100. Balint F, Liposits Z, Farkas I. Estrogen Receptor Beta and 2-arachidonoylglycerol Mediate the Suppressive Effects of Estradiol on Frequency of Postsynaptic Currents in Gonadotropin-Releasing Hormone Neurons of Metestrous Mice: An Acute Slice Electrophysiological Study. *Front Cell Neurosci*. 2016;10:77.
101. Farkas I, Vastagh C, Sarvari M, Liposits Z. Ghrelin decreases firing activity of gonadotropin-releasing hormone (GnRH) neurons in an estrous cycle and endocannabinoid signaling dependent manner. *PLoS One*. 2013;8:e78178.
102. Clasadonte J, Poulain P, Beauvillain JC, Prevot V. Activation of neuronal nitric oxide release inhibits spontaneous firing in adult gonadotropin-releasing hormone neurons: a possible local synchronizing signal. *Endocrinology*. 2008;149:587-596.
103. Csillag V, Vastagh C, Liposits Z, Farkas I. Secretin Regulates Excitatory GABAergic Neurotransmission to GnRH Neurons via Retrograde NO Signaling Pathway in Mice. *Front Cell Neurosci*. 2019;13:371.
104. Farkas I, Balint F, Farkas E, Vastagh C, Fekete C, Liposits Z. Estradiol Increases Glutamate and GABA Neurotransmission into GnRH Neurons via Retrograde NO-Signaling in Proestrous Mice during the Positive Estradiol Feedback Period. *eNeuro*. 2018;5:ENEURO.0057-0018.2018.
105. Constantin S, Reynolds D, Oh A, Pizano K, Wray S. Nitric oxide resets kisspeptin-excited GnRH neurons via PIP2 replenishment. *Proc Natl Acad Sci U S A*. 2021;118:e2012339118.
106. Hanchate NK, Parkash J, Bellefontaine N, et al. Kisspeptin-GPR54 signaling in mouse NO-synthesizing neurons participates in the hypothalamic control of ovulation. *J Neurosci*. 2012;32:932-945.
107. Clasadonte J, Poulain P, Hanchate NK, Corfas G, Ojeda SR, Prevot V. Prostaglandin E2 release from astrocytes triggers gonadotropin-releasing hormone (GnRH) neuron firing via EP2 receptor activation. *Proc Natl Acad Sci U S A*. 2011;108:16104-16109.
108. Vanacker C, Defazio RA, Sykes CM, Moenter SM. A role for glial fibrillary acidic protein (GFAP)-expressing cells in the regulation of gonadotropin-releasing hormone (GnRH) but not arcuate kisspeptin neuron output in male mice. *Elife*. 2021;10:e68205.
109. Han SK, Gottsch ML, Lee KJ, et al. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J Neurosci*. 2005;25:11349-11356.
110. Pielecka-Fortuna J, Chu Z, Moenter SM. Kisspeptin acts directly and indirectly to increase gonadotropin-releasing hormone neuron activity and its effects are modulated by estradiol. *Endocrinology*. 2008;149:1979-1986.
111. Dumalska I, Wu M, Morozova E, Liu R, van den Pol A, Alreja M. Excitatory effects of the puberty-initiating peptide kisspeptin and group I metabotropic glutamate receptor agonists differentiate two distinct subpopulations of gonadotropin-releasing hormone neurons. *J Neurosci*. 2008;28:8003-8013.
112. Constantin S, Caligioni CS, Stojilkovic S, Wray S. Kisspeptin-10 facilitates a plasma membrane-driven calcium oscillator in gonadotropin-releasing hormone-1 neurons. *Endocrinology*. 2009;150:1400-1412.
113. Glanowska KM, Venton BJ, Moenter SM. Fast scan cyclic voltammetry as a novel method for detection of real-time gonadotropin-releasing hormone release in mouse brain slices. *J Neurosci*. 2012;32:14664-14669.
114. d'Anglemont de Tassigny X, Fagg LA, Carlton MB, Colledge WH. Kisspeptin can stimulate gonadotropin-releasing hormone (GnRH) release by a direct action at GnRH nerve terminals. *Endocrinology*. 2008;149:3926-3932.
115. Smith JT, Li Q, Yap KS, et al. Kisspeptin is essential for the full preovulatory LH surge and stimulates GnRH release from the isolated ovine median eminence. *Endocrinology*. 2011;152:1001-1012.
116. Uenoyama Y, Inoue N, Pheng V, et al. Ultrastructural evidence of kisspeptin-gonadotrophin-releasing hormone (GnRH) interaction in the median eminence of female rats: implication of axo-axonal regulation of GnRH release. *J Neuroendocrinol*. 2011;23:863-870.
117. Novaira HJ, Sonko ML, Hoffman G, et al. Disrupted kisspeptin signaling in GnRH neurons leads to hypogonadotrophic hypogonadism. *Mol Endocrinol*. 2014;28:225-238.
118. Liu X, Lee K, Herbison AE. Kisspeptin excites gonadotropin-releasing hormone neurons through a phospholipase C/calcium-dependent pathway regulating multiple ion channels. *Endocrinology*. 2008;149(9):4605-4614. https://academic.oup.com/endo/article/149/9/4605/2455771
119. Pielecka-Fortuna J, DeFazio RA, Moenter SM. Voltage-gated potassium currents are targets of diurnal changes in estradiol feedback regulation and kisspeptin action on gonadotropin-releasing hormone neurons in mice. *Biol Reprod*. 2011;85:987-995.
120. Zhang C, Ronnekleiv OK, Kelly MJ. Kisspeptin inhibits a slow afterhyperpolarization current via protein kinase C and reduces spike frequency adaptation in GnRH neurons. *Am J Physiol Endocrinol Metab*. 2013;304:E1237-1244.
121. Zhang C, Bosch MA, Ronnekleiv OK, Kelly MJ. Kisspeptin activation of TRPC4 channels in female GnRH neurons requires PIP2 depletion and cSrc kinase activation. *Endocrinology*. 2013;154:2772-2783.
122. Glanowska KM, Moenter SM. Differential regulation of GnRH secretion in the preoptic area (POA) and the median eminence (ME) in male mice. *Endocrinology*. 2015;156:231-241.
123. Taoufiq Z, Eguchi K, Takahashi T. Rho-kinase accelerates synaptic vesicle endocytosis by linking cyclic GMP-dependent protein kinase activity to phosphatidylinositol-4,5-bisphosphate synthesis. *J Neurosci*. 2013;33:12099-12104.
124. Han SY, Cheong I, McLennan T, Herbison AE. Neural determinants of pulsatile luteinizing hormone secretion in male mice. *Endocrinology*. 2020;161:bqz045.
125. Wang L, Guo W, Shen X, et al. Different dendritic domains of the GnRH neuron underlie the pulse and surge modes of GnRH secretion in female mice. *Elife*. 2020;9:e53945.
126. Kriegsfeld LJ, Mei DF, Bentley GE, et al. Identification and characterization of a gonadotropin-inhibitory system in the brains of mammals. *Proc Natl Acad Sci U S A*. 2006;103:2410-2415.
127. Ducret E, Anderson GM, Herbison AE. RFamide-related peptide-3, a mammalian gonadotropin-inhibitory hormone ortholog, regulates gonadotropin-releasing hormone neuron firing in the mouse. *Endocrinology*. 2009;150:2799-2804.
128. Wu M, Dumalska I, Morozova E, van den Pol AN, Alreja M. Gonadotropin inhibitory hormone inhibits basal forebrain vGluT2-gonadotropin-releasing hormone neurons via a direct postsynaptic mechanism. *J Physiol*. 2009;587:1401-1411.
129. Constantin S, Pizano K, Matson K, Shan Y, Reynolds D, Wray S. An inhibitory circuit from brainstem to GnRH neurons in male mice: a new role for the RFRP receptor. *Endocrinology*. 2021;162:bqab030.
130. Glanowska KM, Burger LL, Moenter SM. Development of gonadotropin-releasing hormone secretion and pituitary response. *J Neurosci*. 2014;34:15060-15069.

131. Anderson GM, Relf HL, Rizwan MZ, Evans JJ. Central and peripheral effects of RFamide-related peptide-3 on luteinizing hormone and prolactin secretion in rats. *Endocrinology*. 2009;150:1834-1840.
132. Ancel C, Inglis MA, Anderson GM. Central RFRP-3 stimulates LH secretion in male mice and has cycle stage-dependent inhibitory effects in females. *Endocrinology*. 2017;158:2873-2883.
133. Qiu J, Nestor CC, Zhang C, et al. High-frequency stimulation-induced peptide release synchronizes arcuate kisspeptin neurons and excites GnRH neurons. *Elife*. 2016;5:e16246.
134. Yip SH, Boehm U, Herbison AE, Campbell RE. Conditional viral tract tracing delineates the projections of the distinct Kisspeptin neuron populations to gonadotropin-releasing hormone (GnRH) neurons in the mouse. *Endocrinology*. 2015;156:2582-2594.
135. Herde MK, Iremonger KJ, Constantin S, Herbison AE. GnRH neurons elaborate a long-range projection with shared axonal and dendritic functions. *J Neurosci*. 2013;33:12689-12697.
136. Yip SH, Campos P, Liu X, Porteous R, Herbison AE. Innervation of GnRH Neuron Distal Projections and Activation by Kisspeptin in a New GnRH-Cre Rat Model. *Endocrinology*. 2021;162.
137. Herbison AE. The dendron and episodic neuropeptide release. *J Neuroendocrinol*. 2021;33(11):e13024.
138. Gaskins GT, Glanowska KM, Moenter SM. Activation of neurokinin 3 receptors stimulates GnRH release in a location-dependent but kisspeptin-independent manner in adult mice. *Endocrinology*. 2013;154:3984-3989.
139. Purnelle G, Gerard A, Czajkowski V, Bourguignon JP. Pulsatile secretion of gonadotropin-releasing hormone by rat hypothalamic explants without cell bodies of GnRH neurons. *Neuroendocrinology*. 1997;66(5):305-312. https://www.karger.com/Article/Abstract/127253
140. Rasmussen DD. Episodic gonadotropin-releasing hormone release from the rat isolated median eminence in vitro. *Neuroendocrinology*. 1993;58:511-518.
141. Clarkson J, Busby ER, Kirilov M, Schutz G, Sherwood NM, Herbison AE. Sexual differentiation of the brain requires perinatal kisspeptin-GnRH neuron signaling. *J Neurosci*. 2014;34:15297-15305.
142. Poling MC, Kauffman AS. Sexually dimorphic testosterone secretion in prenatal and neonatal mice is independent of kisspeptin-Kiss1r and GnRH signaling. *Endocrinology*. 2012;153:782-793.
143. Kumar D, Periasamy V, Freese M, Voigt A, Boehm U. In utero development of Kisspeptin/GnRH neural circuitry in male mice. *Endocrinology*. 2015;156:3084-3090.
144. Kumar D, Freese M, Drexler D, Hermans-Borgmeyer I, Marquardt A, Boehm U. Murine arcuate nucleus kisspeptin neurons communicate with GnRH neurons in utero. *J Neurosci*. 2014;34:3756-3766.
145. Rasmussen DD, Gambacciani M, Swartz W, Tueros VS, Yen SS. Pulsatile gonadotropin-releasing hormone release from the human mediobasal hypothalamus in vitro: opiate receptor-mediated suppression. *Neuroendocrinology*. 1989;49:150-156.
146. Meyer DC, Pepe GJ. Gonadotropin-releasing hormone release from the mediobasal hypothalamus of fetal baboons. *Am J Primatol*. 1988;14:247-253.
147. Terasawa E, Keen KL, Mogi K, Claude P. Pulsatile release of luteinizing hormone-releasing hormone (LHRH) in cultured LHRH neurons derived from the embryonic olfactory placode of the rhesus monkey. *Endocrinology*. 1999;140:1432-1441.
148. Duittoz AH, Batailler M. Pulsatile GnRH secretion from primary cultures of sheep olfactory placode explants. *J Reprod Fertil*. 2000;120:391-396.
149. Funabashi T, Daikoku S, Shinohara K, Kimura F. Pulsatile gonadotropin-releasing hormone (GnRH) secretion is an inherent function of GnRH neurons, as revealed by the culture of medial olfactory placode obtained from embryonic rats. *Neuroendocrinology*. 2000;71:138-144.
150. Constantin S, Caraty A, Wray S, Duittoz AH. Development of gonadotropin-releasing hormone-1 secretion in mouse nasal explants. *Endocrinology*. 2009;150:3221-3227.
151. Saitoh Y, Luchansky LL, Claude P, Terasawa E. Transplantation of the fetal olfactory placode restores reproductive cycles in female rhesus monkeys (Mucaca mulatta) bearing lesions in the medial basal hypothalamus. *Endocrinology*. 1995;136:2760-2769.
152. Ohkura S, Tsukamura H, Maeda K. Effects of transplants of fetal mediobasal hypothalamus on luteinizing hormone pulses impaired by hypothalamic deafferentation in adult ovariectomized rats. *Neuroendocrinology*. 1992;55:422-426.
153. Kokoris GJ, Lam NY, Ferin M, Silverman AJ, Gibson MJ. Transplanted gonadotropin-releasing hormone neurons promote pulsatile luteinizing hormone secretion in congenitally hypogonadal (hpg) male mice. *Neuroendocrinology*. 1988;48:45-52.
154. Livne I, Gibson MJ, Silverman AJ. Brain grafts of migratory GnRH cells induce gonadal recovery in hypogonadal (hpg) mice. *Brain Res Dev Brain Res*. 1992;69:117-123.
155. Terasawa E, Schanhofer WK, Keen KL, Luchansky L. Intracellular Ca(2+) oscillations in luteinizing hormone-releasing hormone neurons derived from the embryonic olfactory placode of the rhesus monkey. *J Neurosci*. 1999;19:5898-5909.
156. Moore JP Jr, Shang E, Wray S. In situ GABAergic modulation of synchronous gonadotropin releasing hormone-1 neuronal activity. *J Neurosci*. 2002;22:8932-8941.
157. Funabashi T, Daikoku S, Suyama K, Mitsushima D, Sano A, Kimura F. Role of gamma-aminobutyric acid neurons in the release of gonadotropin-releasing hormone in cultured rat embryonic olfactory placodes. *Neuroendocrinology*. 2002;76:193-202.
158. Abe H, Keen KL, Terasawa E. Rapid action of estrogens on intracellular calcium oscillations in primate luteinizing hormone-releasing hormone-1 neurons. *Endocrinology*. 2008;149:1155-1162.
159. Noel SD, Keen KL, Baumann DI, Filardo EJ, Terasawa E. Involvement of G protein-coupled receptor 30 (GPR30) in rapid action of estrogen in primate LHRH neurons. *Mol Endocrinol*. 2009;23:349-359.
160. Richter TA, Keen KL, Terasawa E. Synchronization of Ca(2+) oscillations among primate LHRH neurons and nonneuronal cells in vitro. *J Neurophysiol*. 2002;88:1559-1567.
161. Pinet-Charvet C, Geller S, Desroziers E, et al. GnRH episodic secretion is altered by pharmacological blockade of gap junctions: possible involvement of glial cells. *Endocrinology*. 2015;157(1):304-322. https://academic.oup.com/endo/article/157/1/304/2251851
162. Terasawa E, Keen KL, Grendell RL, Golos TG. Possible role of 5'-adenosine triphosphate in synchronization of Ca2+ oscillations in primate luteinizing hormone-releasing hormone neurons. *Mol Endocrinol*. 2005;19:2736-2747.
163. Constantin S, Klenke U, Wray S. The calcium oscillator of GnRH-1 neurons is developmentally regulated. *Endocrinology*. 2010;151:3863-3873.
164. Dulka EA, DeFazio RA, Moenter SM. Chemogenetic Suppression of GnRH Neurons during Pubertal Development Can Alter Adult GnRH Neuron Firing Rate and Reproductive Parameters in Female Mice. *eNeuro*. 2020;7:ENEURO.0223-0220.2020.
165. Kusano K, Fueshko S, Gainer H, Wray S. Electrical and synaptic properties of embryonic luteinizing hormone-releasing hormone neurons in explant cultures. *Proc Natl Acad Sci U S A*. 1995;92:3918-3922.
166. Constantin S, Wray S. Gonadotropin-releasing hormone-1 neuronal activity is independent of cyclic nucleotide-gated channels. *Endocrinology*. 2008;149:279-290.
167. Romano N, Lee K, Abraham IM, Jasoni CL, Herbison AE. Nonclassical estrogen modulation of presynaptic GABA terminals modulates calcium dynamics in gonadotropin-releasing hormone neurons. *Endocrinology*. 2008;149(11):5335-5344. https://academic.oup.com/endo/article/149/11/5335/2455044

168. Constantin S, Wray S. Galanin activates G-protein gated inwardly rectifying potassium channels and suppresses kisspeptin-10 activation of GnRH neurons. *Endocrinology*. 2016;157:3197-3212.
169. Constantin S, Wray S. Nociceptin/Orphanin-FQ Inhibits Gonadotropin-Releasing Hormone Neurons via G-Protein-Gated Inwardly Rectifying Potassium Channels. *eNeuro*. 2018;5:ENEURO.0161-0118.2018.
170. Constantin S, Wray S. Gonadotropin-releasing hormone-1 neuronal activity is independent of hyperpolarization-activated cyclic nucleotide-modulated channels but is sensitive to protein kinase a-dependent phosphorylation. *Endocrinology*. 2008;149:3500-3511.
171. Castellano JM, Navarro VM, Fernandez-Fernandez R, et al. Ontogeny and mechanisms of action for the stimulatory effect of kisspeptin on gonadotropin-releasing hormone system of the rat. *Mol Cell Endocrinol*. 2006;257–258:75-83.
172. Todman MG, Han SK, Herbison AE. Profiling neurotransmitter receptor expression in mouse gonadotropin-releasing hormone neurons using green fluorescent protein-promoter transgenics and microarrays. *Neuroscience*. 2005;132:703-712.
173. Klenke U, Constantin S, Wray S. Neuropeptide Y directly inhibits neuronal activity in a subpopulation of gonadotropin-releasing hormone-1 neurons via Y1 receptors. *Endocrinology*. 2010;151:2736-2746.

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