Ion channels and information processing in GnRH neuron dendrites

Rachael Norberg,[1,2] Rebecca Campbell[3,4] and Kelly J. Suter[1,2,*]

[1]Department of Biology; University of Texas San Antonio; San Antonio, TX USA; [2]Neurosciences Institute; University of Texas San Antonio; San Antonio, TX USA; [3]Department of Physiology; University of Otago; Dunedin, New Zealand; [4]Centre for Neuroendocrinology; University of Otago; Dunedin, New Zealand

Recent findings indicate that a majority of action potentials originate from dendrites of GnRH neurons. This localization of the dendrite as the principle site of action potential initiation has sparked considerable interest in the nature of ionic channels throughout GnRH neurons. This paper will review the ionic conductances described within GnRH neurons and their implications for physiological output, such as sensitivity to steroids and diurnal state. To date, a majority of information regarding ionic conductances in GnRH neurons pertains to somata and the first 50–100 μm of dendrite length. Thus, unraveling the tapestry created by the nature and distribution of dendritic conductances in GnRH neurons lies at the forefront of understanding the control of reproductive hormone secretion.

Gonadotropin releasing-hormone (GnRH) is a small neuropeptide that drives pituitary release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), peripheral hormones critical in regulating gonadal function and fertility. The cell bodies of the GnRH-containing neurons are distributed diffusely throughout the rostral hypothalamus. Their axons project to the median eminence where they release the GnRH peptide into the hypophysiotropic portal system. In the portal capillaries, GnRH travels to the anterior pituitary gland to stimulate release of gonadotropins into the systemic circulation. GnRH release is not continuous, but rather is released in episodic pulses, approximately once/hour.[1] It is well-established that the intermittent manner of GnRH release is essential for sexual reproduction and that changes in the frequency and amplitude of GnRH pulses govern transitions between reproductive states.[2]

The neuronal substrate that results in pulsatile GnRH release, and therefore comprises the "GnRH pulse generator," is unknown. The intermittent stimulus for GnRH release may arise from input to the GnRH neurons and reflect synaptic interactions between GnRH neurons and a secondary network.[3]

Alternatively, pulsatile GnRH release may be a consequence of spontaneous activity of the GnRH neurons themselves, and therefore, is the result of properties that are intrinsic to this population of hypothalamic cells. These two hypotheses are not mutually exclusive; the GnRH pulse generator is likely derived from a combination of intrinsic properties and synaptic interactions.

With the development of transgenic rodent models in which the GnRH neurons express green fluorescent protein[4-6] (GFP), it has become increasingly possible to study GnRH neurons using a variety of electrophysiological approaches.[7] Thus, there is now a rapidly expanding literature focusing on the activity of GnRH neurons, their ionic conductances, and their physiological modulation by a variety of internal and external factors. Our purpose is to review what is known about the conductances described in both somatic and dendritic compartments of GnRH neurons, and their role within the mammalian reproductive axis.

This review will consider GnRH neurons as a single population. However, GnRH neurons display a physiological diversity that suggests they may not be one singular population of neurons. Before the broad-spread use of GnRH-GFP rodent models, Sim et al. (2000)[8] provided a notable glimpse into the diversity that exists within the total population of GnRH neurons. Using post hoc identification of GnRH neurons, they demonstrated that sub-populations of GnRH neurons could be identified based on key electrophysiological properties such as repetitive firing and rectification. Since these behaviors reflect the expression of specific ion conductances, their study indicated that individual GnRH neurons likely differed in the type and densities of specific ion channels. Thus, while this review discusses GnRH neurons as a single population with the same, or largely the same ionic channels, it should be noted that even from the earliest junctures it has been clear that this is unlikely.

Ionic Channels in GnRH Neurons

Table 1 describes some of the many ionic conductances that have been defined in GnRH neurons. The voltage-gated ion channels in GnRH neurons include sodium channels, potassium channels, and calcium channels. The combined impact of these channels largely determines the intrinsic properties of GnRH neurons.

*Correspondence to: Kelly J. Suter; Email: Kelly.suter@utsa.edu

Table 1. Conductances and estrogen sensitivity

Current	E Sensitive	Diurnal	Citation
I_h	No	No	Sim et al., 2001;[8] Zhang et al., 2008;[18] Chu et al., 2010[64]
Sodium			
TTX-sensitive	Yes	No	Spergel et al., 1999;[4] Suter et al., 2000;[5] Kuehl-Kovarik et al., 2005;[11] Chu and Moenter, 2006;[12] Liu and Herbison, 2008;[13] Roberts et al., 2009[14]
Kisspeptin -induced inward	No	No	Zhang et al., 2008[18]
Potassium			
I_A	Yes	Yes	DeFazio and Moenter, 2002;[20] Xu et al., 2008;[21] Pielecka-Fortuna et al., 2011[26]
I_K	Yes	Yes	Herbison, 1998;[25] Pielecka-Fortuna et al., 2011[26]
I_M	No	No	Xu et al., 2008[21]
S_K	Yes	No	Bosch et al., 2003;[24] Kelly et al., 2002;[22] Kelly et al., 2003;[23] Kato et al., 2006;[29] Liu and Herbison, 2008;[13] Lee et al., 2010[30]
B_K	No	No	Hiraizumi et al., 2008[31]
KATP	Yes	No	Zhang et al., 2007[27]
Calcium			
L-type	Yes	Yes	Nunemaker et al., 2003;[33] Sun et al., 2010
N-type	Yes	Yes	Nunemaker et al., 2003;[33] Sun et al., 2010
P-type	No	No	Nunemaker et al., 2003[33]
Q-type	No	No	Nunemaker et al., 2003[33]
R-type	Yes	Yes	Nunemaker et al., 2003;[33] Sun et al., 2010
T-type	Yes	No	Kato et al., 2003;[34] Nunemaker et al., 2003;[33] Zhang et al., 2009[36]
Chloride			
$GABA_A$	No	No	Weyler et al., 1999; Herbison and Moenter, 2011[38]

Summary table of the ionic conductances described for GnRH neurons. Intrinsic GnRH conductances, estrogen sensitivity, and diurnal effects are listed per conductance type. Citations that describe conductances found in GnRH neurons are provided.

The relatively high input resistance of GnRH neurons suggests that at resting membrane potentials, most ionic conductances are inactive. However, even the earliest studies noted marked excursions and oscillations in resting membrane potential (Suter 2006) even in the absence of synaptic inputs (Keuhl 2009). Thus, ionic conductances in GnRH neurons likely underlie and modulate this critical electrophysiological behavior.

Sodium channels. There is general agreement that GnRH neurons express the classic TTX-sensitive voltage-gated sodium channels. Blocking these channels eliminates spontaneous action potentials in GnRH neurons as well as action potentials in response to current injection pulses. Thus, similar to other neurons, TTX-sensitive voltage-gated sodium channels underlie the rapid depolarization phase of the action potential in GnRH neurons.[4,6] It seems a substantial fraction of voltage-gated sodium channels (~93%) are inactive at resting membrane potentials. Spike threshold in GnRH neurons has been reported to range from –49 to –32 mV with the sodium current activating at about –40 mV.[4] Our own studies agree with the more depolarized range of spike threshold (~35 mV) which is consistent with the finding that the upstroke of the action potential is generated almost exclusively by voltage gated sodium channels.[4]

A TTX-sensitive sodium channel also contributes to slow after depolarizing potentials (so-called ADPs) in GnRH neurons. In other neurosecretory cells, ADPs have been implicated in repetitive firing, the mode of neuronal activity that facilitates hormone release.[10] Both cultured GnRH somata and GnRH neurons in hypothalamic slices express ADPs.[11-14] In female mice, ADPs recorded in GnRH neurons are modulated by estradiol, a key component of the positive and negative feedback system within the reproductive axis. High levels of estradiol, associated with positive feedback, increase the amplitude of ADPs and low levels of estradiol, associated with negative feedback, either have no effect[15] or decrease ADP amplitude.[16] The augmentation of ADP amplitude in response to estradiol has been suggested to increase the activity of GnRH neurons during times of maximum hormone release; for example, during the preovulatory surge of release in females.[12] The latter finding is consistent with

an additional study from the same laboratory indicating that low levels of estrogen decrease the conductance of both a persistent sodium current as well as the classical voltage-gated sodium channel.[17] Similar studies have not been performed in males but estradiol is likely the negative feedback signal. Therefore, GnRH neurons from males may respond in a similar manner. The neuropeptide kisspeptin opens TRPC-like cation channels through which sodium influx makes a large contribution to kisspeptin's depolarizing effect on GnRH neurons.[18] Kisspeptin, a potent stimulator of GnRH release has been implicated in pubertal development, regulation of the female estrous cycle, and also as the effector in multiple signals that regulate reproduction.[19]

Potassium channels. Similar to other neurons, multiple potassium conductances are expressed in GnRH neurons. Most of these potassium conductances in neurons are activated by subthreshold levels of membrane depolarization and can be distinguished by differing kinetic properties with respect to voltage and time. In GnRH neurons, the slowly inactivating potassium current (I_K) and the fast inactivating A-type potassium current (I_A) are both expressed.[20] However, I_A activates at more hyperpolarized voltages (~–50 mV) than I_K (~–40 mV). I_A inactivates rather quickly (~100 ms) and impacts the inter-action potential interval and therefore, the frequency of action potential firing. I_K, on the other hand, can remain activated for up to 5 sec, and generally acts to repolarize the membrane potential to remove the inactivation of sodium channels and enable repetitive firing. The so-called M-type potassium current (I_M) is also expressed in GnRH neurons. M-type potassium currents are non-inactivating, sub-threshold currents that inhibit cell excitability. The presence of these currents on GnRH neurons may provide a mechanism in which GnRH autoregulates GnRH neurons via a self-feedback loop.[21]

Potassium channels in GnRH neurons create a rich substrate for modulation of activity. Kelly et al. (2002 and 2003)[22,23] and Bosch et al. 2002[2] provided some of the earliest evidence for the regulation of reproductive hormone secretion via estrogen actions on potassium channels. Kelly et al.[22,23] observed an estrogen-mediated reduction of inwardly rectifying potassium currents activated by μ-opioids in POMC neurons. Moreover, neurons throughout the preoptic area and the arcuate nuclei were hyperpolarized by steroids with a particularly marked response in GnRH neurons. In recent studies, estradiol has been shown to alter both I_K and I_A, two voltage-gated potassium conductances that are largely responsible for the overall excitability and discharge activity in GnRH neurons.[20] Since the effects of estradiol are relatively rapid, it most likely operates through a mechanism other than genomic signaling.[25] Estradiol shifts (in the depolarizing direction) the voltage of both the half activation and half inactivation of I_A in female mice. In addition, action potential latency was reduced in animals treated with estradiol following ovariectomy when compared with females without estrogen replacement following ovariectomy.[20] These effects of estradiol appear to be modulated by time of day, a finding that has the potential to explain how estrogen shifts between the positive and negative modes of feedback regulation on GnRH neurons.[26] The GnRH peptide itself also has been reported to activate I_M to
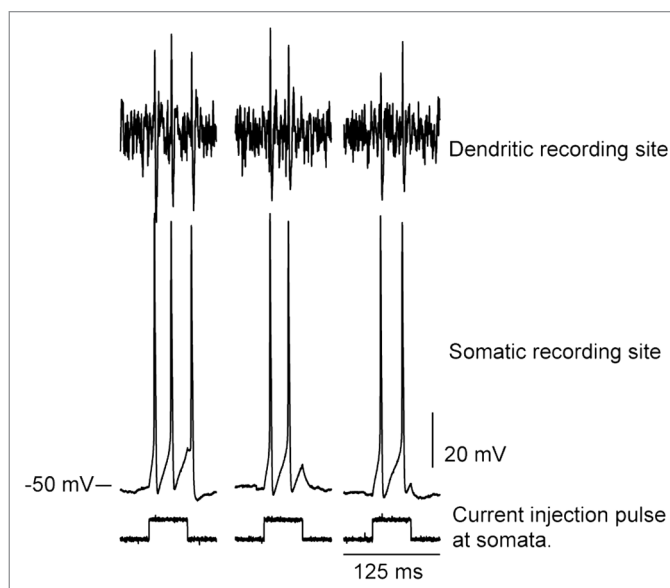

Figure 1. Back propagation of high frequency action potentials. Dual-recording at GnRH neuron soma and dendrites illustrates faithful back propagation of somatically generated high frequency action potentials. Expression of TTX-sensitive voltage-gated sodium channels and fast potassium conductances results in the active properties of GNRH neuron dendrites. Used with permission from the Endocrine Society, from reference 47.

hyperpolarize GnRH neurons and thus form a potential auto-regulatory mechanism of negative feedback to GnRH neurons.[21]

Perhaps the most interesting potassium current in GnRH neurons is the ATP-sensitive K_{ATP}. This inwardly rectifying conductance is expressed in about 50% of GnRH neurons from both male and female mice, which approximates the proportion of GnRH neurons that are also glucose sensitive. The current carried by K_{ATP} channels (when input resistance is about 1.0 Ω) ranges from about 35 pA in males and varies in females based on estradiol levels (~27 pA in estrogen treated females vs. ~14 pA in oil treated females).[27] In a variety of species, the release of GnRH is influenced by metabolic status.[28] Thus, it is possible that the strong hyperpolarization imposed by K_{ATP} (~–8.0 mV) in either GnRH somata or dendrites is partly responsible for the link between nutritional status and fertility.

Repetitive action potentials result in the accumulation of calcium in neurons. The elevated levels of intracellular calcium activate calcium-dependent potassium currents. One of these currents has a relatively small conductance. Thus, it is called a small conductance potassium channel or S_K. A second calcium-sensitive potassium conductance exists in most neurons. Because it has a larger conductance than S_K channels, it is called the large-conductance calcium activated potassium channel or B_K (for big potassium conductance). There is evidence for both S_K and B_K conductance in GnRH neurons. The S_K conductance is reported to be present in all GnRH neurons derived from mice[13] and rats.[29] Tonic activity of S_K currents in GnRH neurons accounts for a relatively large hyperpolarization (~7 mV). Thus, multiple parameters shift in the presence of apamin, a specific blocker
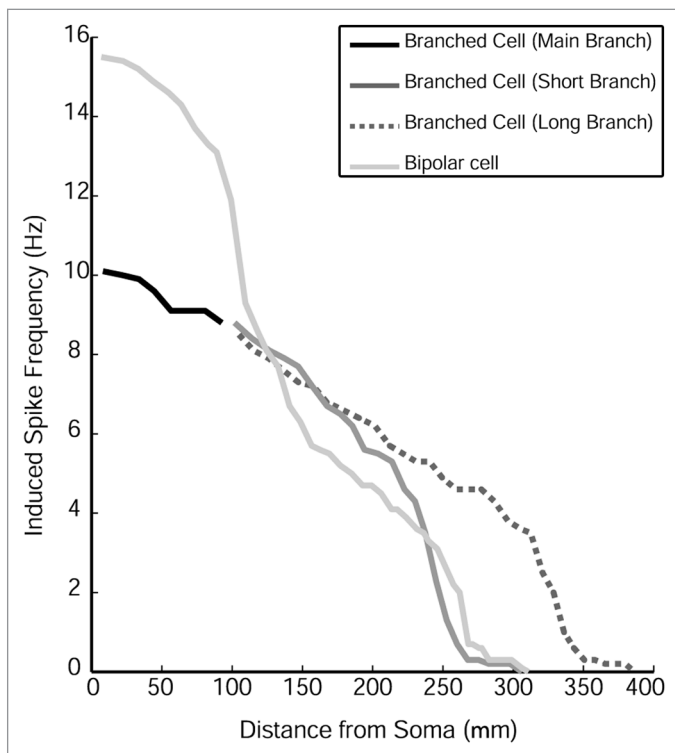
Figure 2. Spike frequency and dendritic location. The application of simulated AMPA conductances results in robust action potentials until about 150 μm of dendrite length. Beyond this most proximal portion of the GnRH dendrite, the passive filtering of synaptic input prevents generation of somatic action potentials. Used with permission from the Endocrine Society, from reference 60.

of S_K channels. For example, the action potential frequency increases. However, the changes in the underlying pattern of action potentials account for the higher frequency of discharge. More repetitive (as opposed to basal) action potentials are exhibited and the duration of episodes of repetitive action potentials is longer.[13] Broad calcium transients have also been found to control slow calcium-dependent potassium conductances, including an apamin-sensitive conductance, which regulates activity within and between bursts of activity.[30] Like S_K, the B_K conductance has also been reported to control activity patterns in GnRH neurons from rats.[31]

Calcium. Voltage-gated calcium channels can be broadly characterized as high voltage-gated channels (HVGCCs) and low voltage-activated channels (LVA). HVGCC are activated by strong depolarization and further distinguished as L-, N-, R- and Q/P subtypes. Each of these channels requires depolarization to activate them but they differ in terms of the required magnitude and/or duration of depolarization for activation. They also differ in the duration of the active conductance. Low voltage activated calcium channels are T-type conductances. Voltage-gated calcium channels can also be distinguished based on their region of expression through the body and by their physiological function.[32]

Multiple calcium conductances are present in GnRH neurons. GnRH somata in mice express high voltage activated calcium

currents, with the L-type calcium channel dominating over N-, P, Q- and R-type. Low voltage-activated calcium channels have not been detected in mouse GnRH neurons.[33] In GnRH somata derived from rats, HVGCC have been detected, and a small component of a T-type conductance is reported to emerge around the time of puberty.[34] However, in mice, Spergel[35] did not report a developmental change in GnRH neuron calcium currents. Nonetheless, the observation of an increase in T-type currents in rats is interesting in that puberty requires a shift in activity of GnRH neurons to support increased GnRH release. Consistent with the findings of Kato et al.,[34] T-type calcium conductances are frequently present in cells with rhythmic bursting or pacemaker activity.[32] The potential role of T-type calcium channels during times of elevated GnRH secretion is underscored by the recent findings of Zhang et al. (2009),[36] indicating that T-type channels are positively modulated by estrogen and may be particularly important in the large release of GnRH that results in ovulation.

Chloride channels: A ligand-gated ion channel. Until recently, chloride ions were assumed to be passively distributed across neuronal membranes. Thus, the emergence of chloride channels in neurons has attracted intense interest.[37] In GnRH neurons, the GABA$_A$ receptor is the major chloride ion channel identified thus far. As both receptor and channel, GABA$_A$ is involved in synaptic transmission to GnRH neurons to modulate their firing patterns. However, whether its activation results in excitation or inhibition of GnRH neurons remains a topic of controversy and discussion.[38] In most adult neurons, GABA$_A$ receptor activation causes hyperpolarization through chloride entry into the cell. This hyperpolarization is dependent upon low intracellular chloride concentration driving Cl$^-$ movement into the cell down its concentration gradient. If intracellular chloride concentration is elevated, the reversal potential for chloride will be depolarized and chloride ions will move out of the cell causing depolarization of the membrane. It is now well established that GABA$_A$ activation is depolarizing and excitatory in most immature neurons, and switches to become hyperpolarizing and inhibitory in most mature neurons. However, there is evidence that Cl$^-$ conductance may remain predominantly depolarizing in peripubertal[39] and even adult GnRH neurons.[40] Despite the interest surrounding the distribution of chloride in GnRH neurons, GnRH neuron-specific knockout of GABA$_A$ channels does not ultimately disrupt normal fertility.[41]

Ion Channels and Information Processing in GnRH Neuron Dendrites

It is clear that the dendrites in many, if not most, populations of neurons express active ionic conductances that endow them with active properties. The expression of active conductances in dendrites compensates for the capacitance-induced distortion of the temporal aspects of synaptic potentials.[42] These active conductances can underlie plateau potential generation and create bistability leading to repetitive action potentials. Finally, the effectiveness of distal synaptic inputs often relies on active conductances in the intervening segments of the dendrite to boost
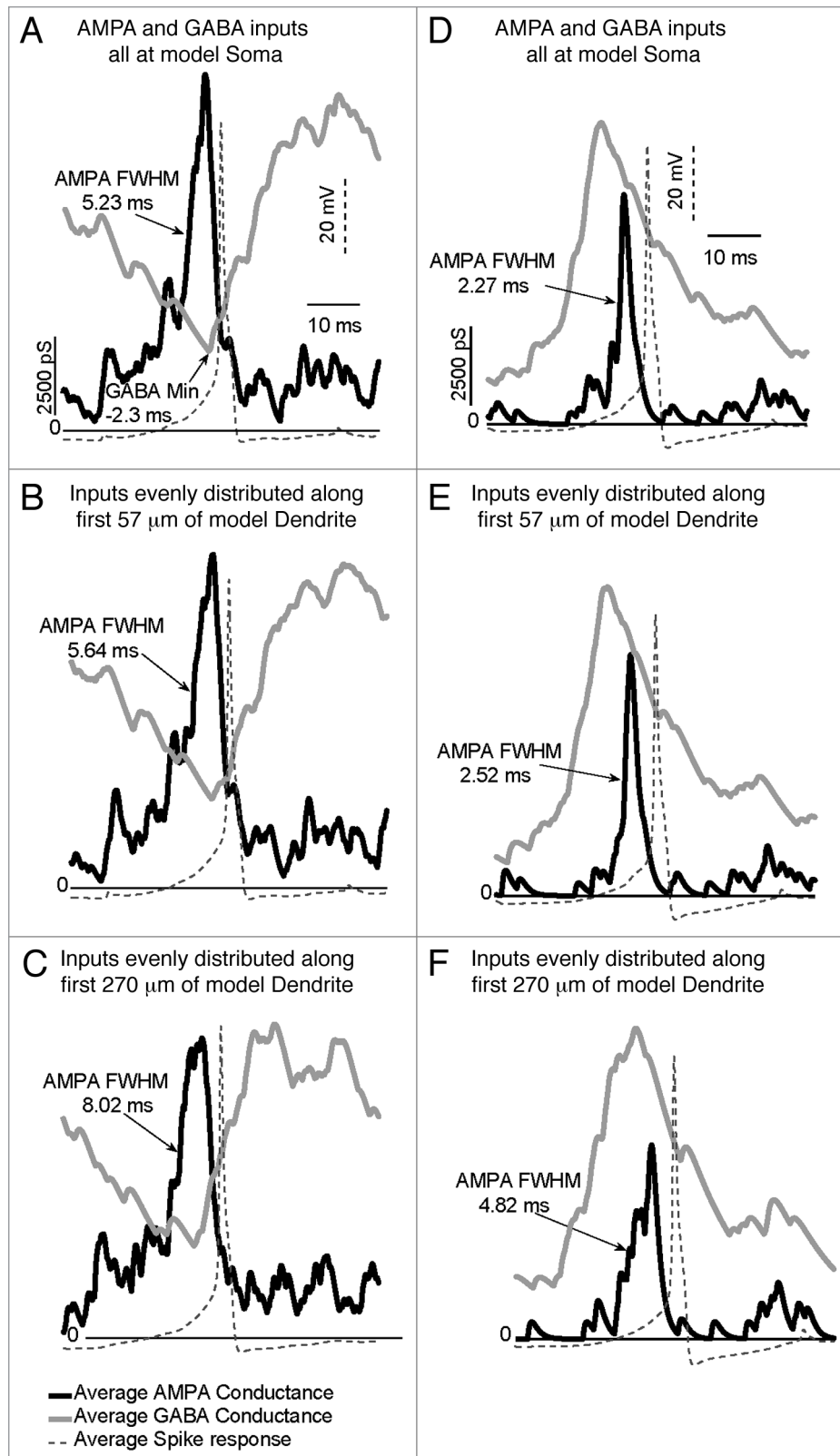
Figure 3. Model schematic of GnRH input and firing. Distributed inputs on model GnRH dendrites widen the temporal window for integration of AMPA-type inputs with GABA-A type inputs in GnRH somata panels (**A and D**), proximal dendrite panels (**B and E**) and in distal dendrite panels (**C**) and (**F**). Panels **A–C** show the integration of AMPA and GABA inputs when GABA is excitatory. Panels **D–F** show integration of these inputs when GABA is inhibitory. The dashed wave form in all panels indicates aligned action potentials in response to the respective simulations. Distributing inputs along dendrites increases the temporal window for integration by introducing propagation delays. Used with permission from reference 61.
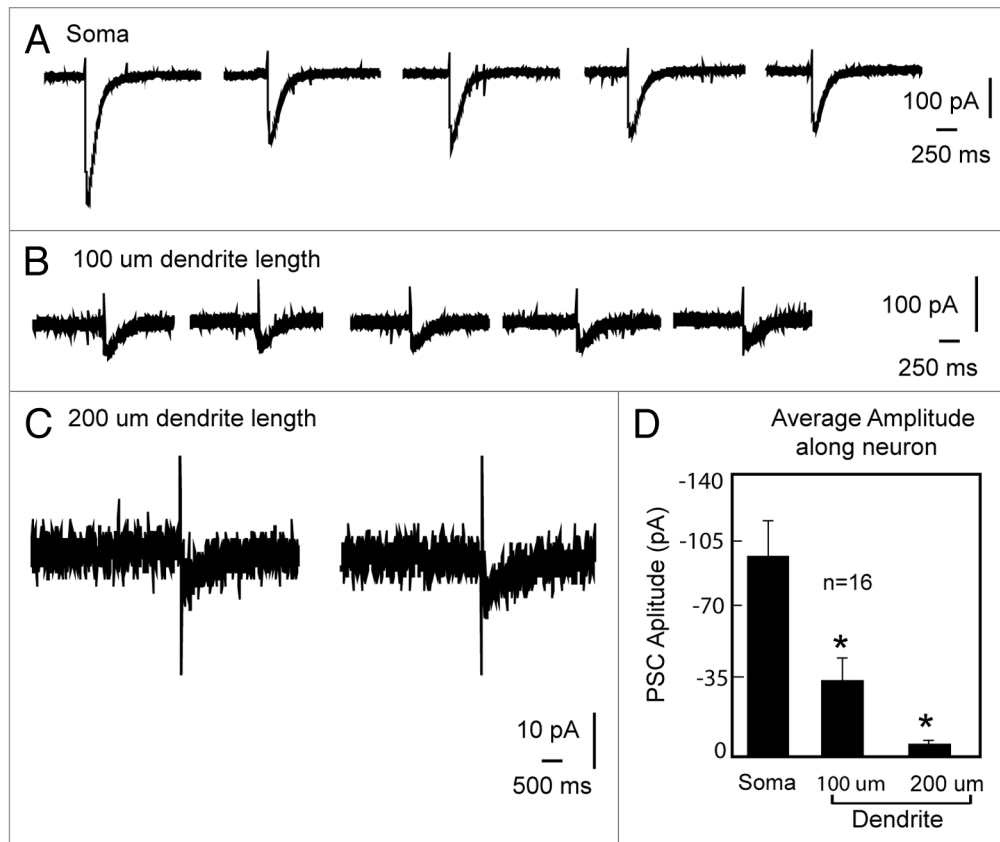
Figure 4. Attenuation of GABAergic PSPs and fendritic location. The GABAergic post synaptic current evoked by uncaging GABA decreases along the GnRH dendrite. Multiple trials at soma, proximal and distal dendritic locations are shown. Used with permission from reference 63.

signals that would, in the absence of active properties, decay before reaching somata.[43] Therefore, the expression of active conductances in dendrites not only defines, in part, neuronal excitability; these conductances often fundamentally alter how neurons integrate information.[44-47]

The full complement, density and distribution of ion channels with their respective conductances in dendrites of any population of neurons is not completely known, but the following parameters described here are generally characteristic within a population of neurons. Conductances can vary from uniform along the entire length of a dendrite, or as gradients that either increase or decrease along the dendrite length (see ref. 48 for detailed discussion on the issue of distribution of active conductances within dendrites). The most common active conductances in dendrites are the voltage-gated sodium channels, voltage-dependent potassium channels, hyperpolarization-activated cation ion current (I_h), voltage-dependent calcium channels and the NMDA receptor channels. In some dendrites, I_h reduces the time course of subthreshold events that would otherwise be distorted by the increased capacitive load of larger diameter segments of dendrites.[49] The opening of the I_h channel reduces membrane resistance. This slows the time course of events by offsetting the increased capacitance. Multiple forms of potassium conductances are also expressed in dendrites, most notably fast potassium channels (A-type) or slower D-type potassium channels. Many dendrites also express voltage-gated calcium channels of several

possible varieties (L, N, P/Q, R and T). Depending on the population of neurons, there can either be preferential expression of the high or low-voltage activated calcium channels in dendrites, or expression of the entire complement of voltage-activated calcium channels. The high voltage activated channels appear to be important for amplifying synaptic input, inducing synaptic plasticity, generating dendritic spikes and underlying calcium-dependent neurotransmitter release from dendrites.[32] The T-type calcium channels in dendrites are implicated in burst firing patterns. Perhaps the most important and most commonly expressed active conductance in dendrites is the voltage-gated sodium channel. This voltage-gated sodium channel is the same tetrodotoxin (TTX)-sensitive voltage-gated sodium channel that underlies both the generation of the action potential at the axon hillock and the propagation of action potential in axons. These voltage gated sodium channels serve the same function in dendrites.

Most of the electrophysiological studies in GnRH neurons to date have relied on recordings from isolated GnRH somata or somata of GnRH neurons in acute brain slice preparations. Thus, many of the conductances noted above may actually exist in GnRH neuron dendrites but, due to the location of recordings, cannot be definitively attributed to any more than the proximal portions of dendrites. The persistence of ADPs detected in isolated GnRH somata indicates that they are most likely generated in the proximal regions of the GnRH neuron. This could be somata or within the first 30 μm of the dendrite that is
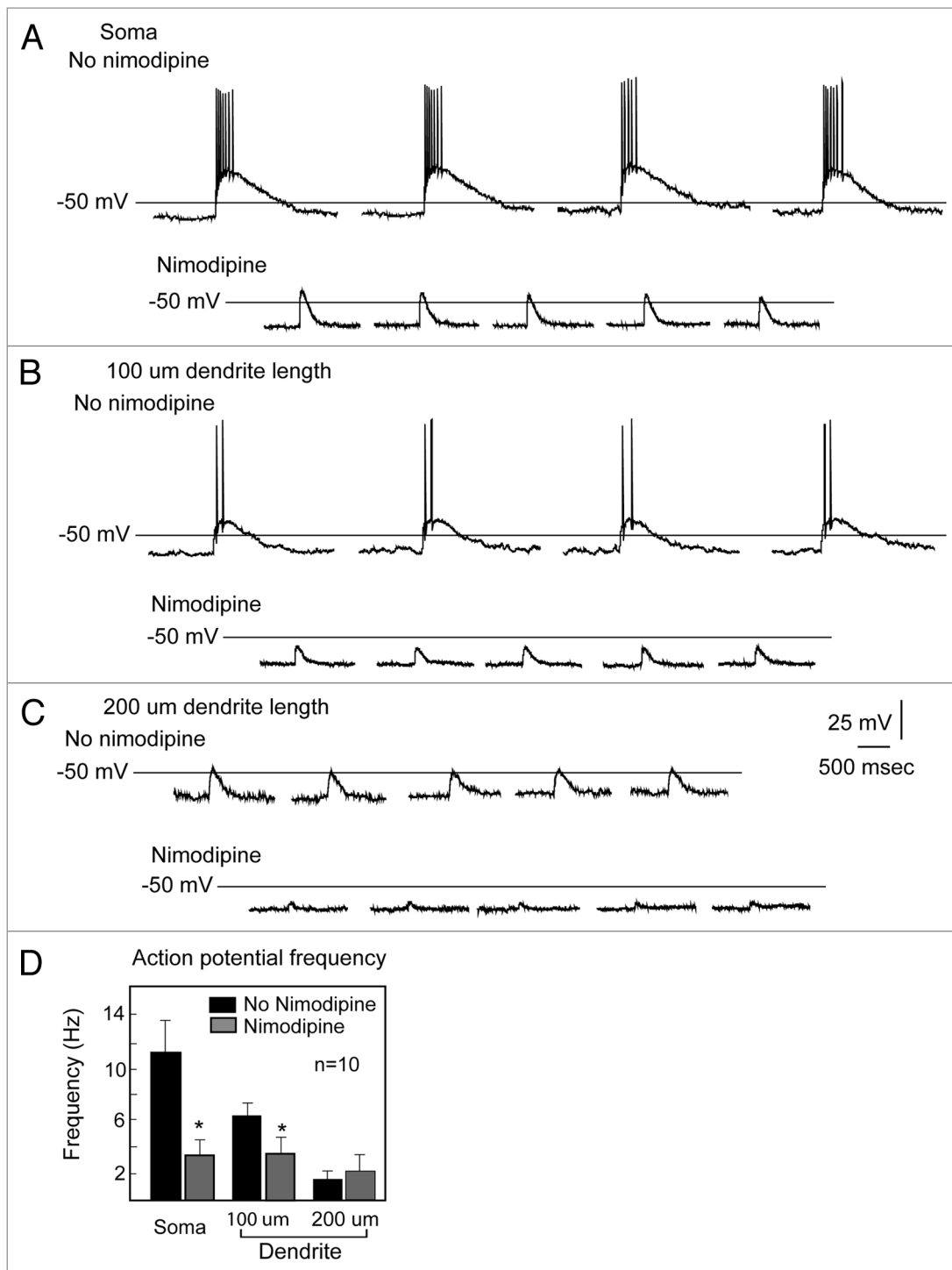
Figure 5. L-type calcium channels and GABA excitation. Blocking L-type calcium channels in GnRH neurons eliminates the response to excitatory GABA. Thus, interaction with an intrinsic conductance long the length of the GnRH dendrite is required for excitatory GABA to generate action potentials. Used with permission from reference 63.

usually present in cultured somata.[9] Therefore the TTX-sensitive sodium-based current that has been implicated in the generation of ADPs may reside in the proximal dendrite.

Until recently, very little was understood about the GnRH neuron dendrite beyond the most proximal portions. It is now clear that the dendrites of GnRH neurons are morphologically and functionally more complex than was previously appreciated. Structurally, dendrites of GnRH neurons are now recognized as being exceptionally long processes that[50,51] bundle with one another[52] and exhibit extensive branching in areas outside the blood-brain barrier.[53] Based on work from our laboratory[54] and those of the Herbison group,[55] dendrites are also the major
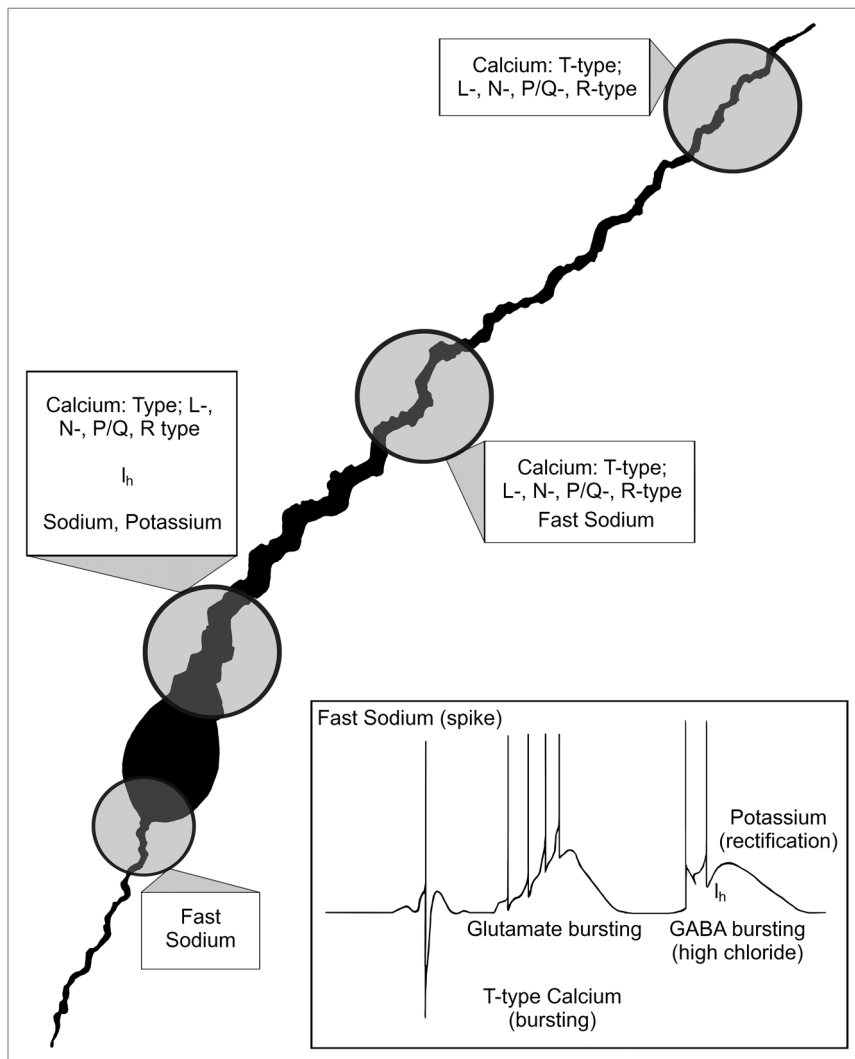
Figure 6. Morphology and corresponding physiology of GnRH neurons. Illustration summarizing the morphology and physiology of GnRH neurons. Known conductances are shown within their respective regions of the GnRH neuron. Inset summarizes the conductances observed in physiological recordings.

potassium channels in appropriate densities. In the absence of a rapid repolarization of dendritic membrane potential, repetitive action potentials could not be generated due to inactivation of the voltage-gated sodium channels. In contrast to the strong back-propagation of action potentials into GnRH neuron dendrites, CA1 pyramidal neurons exhibit a marked attenuation of high frequency action potentials during back-propagation into their dendrites.[57] The observation of back-propagation failure during high frequency discharges presumably reflects persistent inactivation of voltage-gated sodium channels due to lack of the membrane repolarization generally provided by voltage-gated potassium channels. In the case of pyramidal neurons, the density of I_A current channels decreases as the dendrite extends from the soma.[57] Thus, the failure of high-fidelity back-propagation of somatically-generated action potentials is specifically due to weak dendritic expression of potassium channels which underlie membrane repolarization.[58,59] In contrast, the robust back-propagation of somatically induced action potentials suggests the presence of sufficient densities of potassium conductances, mostly likely the fast-activating I_A in GnRH dendrites. Moreover, if GnRH dendrites have enriched I_A expression, the modulations of estradiol on I_A channels (see above) may well be exerted on GnRH dendrites and somata.

The I_h current is a nonspecific cation current that utilizes sodium and potassium ions as current carriers. It is activated by hyperpolarization and inactivated by depolarization. It can serve as an important component of pacemaking in some neurons with primarily *fast* oscillations on the millisecond time scale (like cortical neurons).[60] At resting membrane potentials, I_h can reduce/offset local elevations in membrane resistance (R_m) due to its opening and closing. These local changes in R_m would have substantial implications for activity in the thin distal dendrites of GnRH neurons. Moreover, because GnRH neurons have been reported in some cases to exhibit periodic activity,[4,9,61-63] one might reasonably assume that I_h would be a dominant current. In the early study of Sim et al. (2000)[7] only about 5% of GnRH neurons exhibited the inward rectification consistent with an I_h current. In contrast, later studies[18,35,64] suggested that as many as half of GnRH neurons express I_h. An evaluation of the expression of I_h in GnRH dendrites may reconcile these differences.

Interaction of Ionic Channels and Synaptic Inputs

The GnRH neurons express a host of post-synaptic receptors that allow them to integrate multiple signals about internal

site of action potential generation in GnRH neurons in brain slices. Both slow basal firing[54] and repetitive action potentials[46,55] primarily originate in GnRH dendrites. Thus, the fast sodium conductance is present in GnRH dendrites. Moreover, somatically-induced action potentials faithfully back-propagate in GnRH dendrites.[54] As shown in **Figure 1**, action potentials occur in both somata and dendrites following applied current injection pulses at somata. However, this signal shows consistent back-propagation into dendrites whereby each induced action potential in somata also results in a corresponding action potential in dendrites. The physiological significance of back-propagation of action potentials into dendrites may include activation of voltage-gated calcium channels or removing the voltage-dependent magnesium block on NMDA receptors.[56]

In any regard, the ability of the GnRH neuron dendrite to back-propagate relatively high-frequency action potentials supports the notion that these dendrites also express voltage-gated

homeostasis and the external environment.[57] In response to these signals, activation of intrinsic conductances can shape the overall response of the GnRH cell. One of the most intriguing issues is how dendrites might process multiple and sometimes conflicting signals. In other neurons with elaborate dendritic arbors, individual branches within the same neuron function as the smallest unit of dendritic integration.[58,59] Because segments of branches have unique ionic channel expression, even the same synaptic signal can be processed quite differently depending on the location of the synapse on the dendrite and the filtering properties of that dendritic segment. Segments of the exceedingly long GnRH dendrite have different passive filtering properties due to their morphology.[13,60] However, the majority of GnRH neuron dendrites examined to date express only modest branching; those that project into the oranum vasculosum of the lamina terminalis (OVLT) being an exception.[53] Thus, some of the tactics of most neurons would not be well-suited for the long, relatively unbranched dendrite of GnRH neurons.

The most common of experimental approaches used in other neurons to characterize interactions between incoming synaptic signals and intrinsic conductances (i.e., whole-cell recordings) are largely intractable in the very thin GnRH neuron dendrites. To provide insight into dendritic integration in GnRH dendrites, multi-compartmental models of GnRH neurons have been useful.[47,60,61]

Glutamate and GABA are key neurotransmitters throughout the brain and the neuroendocrine hypothalamus is no exception. Each bind to specific ligand-gated channels in GnRH neurons. As described above, GABA causes the opening of the $GABA_A$ chloride channel in the GnRH neurons. Glutamate can bind to the ionotropic glutamate receptors, NMDA and AMPA, which are non-specific cation channels. Multiple studies in a variety of species have provided compelling evidence that glutamate provides a key excitatory drive to GnRH neurons.[62] However, GABA can be excitatory or inhibitory in GnRH neurons.[37] The latter circumstance adds an additional layer of complexity for processing in GnRH neurons. Both glutamate and GABA have linear current-voltage relationships, thus making them quite amenable for computational modeling approaches.

Using compartmental models of GnRH neurons, one can examine the filtering properties of GnRH dendrites[60] (**Fig. 2**). Part of the power of this approach is that one can distribute synaptic inputs along the simulated dendrite. When simulated synaptic inputs are applied along the length of the model GnRH neurons' dendrite, the induced action potential frequency is reduced as synapses are placed in increasingly distal positions. This occurs in the dendrite shaft (in black line in **Fig. 2**) of a GnRH neuron with a branched dendrite (dark gray and dark, dashed gray lines) and along the dendrite of the classic bipolar GnRH neuron with a non-branching dendrite (light gray trace; **Fig. 2**). Since these model neurons do not contain simulated voltage-gated sodium channels in their dendrites, the response represents the impact of passive filtering by the plasma membrane.

Using the above approach, one can also determine the impact of GABA in both the excitatory and inhibitory mode in the same GnRH neuron (albeit a simulated one) using a measure called

spike-triggered averaging on conductance. This analysis allows one to directly relate action potentials to a particular temporal pattern and/or spatial arrangements of simulated synaptic inputs.[61] As shown in **Figure 3**, distributing inputs along the model dendrite alters temporal aspects of synaptic integration of glutamate and GABA. Independent of whether GABA is in the inhibitory mode (**Fig. 3A–C**) or in the excitatory mode (**Fig. 3D–F**), action potentials are most effectively generated at all locations when the GABA conductance is at a nadir or declining relative to the peak AMPA conductance. As simulated synaptic inputs are distributed through increasing dendrite lengths (**Fig. 3B–F**), propagation delays (from site of simulated input to somata) are introduced. This can be assessed by measuring a conductance's full width at half maximum (FWHM). Distribution of inputs along the dendrite more strongly impacts on AMPA inputs than GABA input due to the longer time course of GABA conductances. Thus, the effect of distributing inputs is to increase the time domain over which glutamatergic and GABAergic inputs can be integrated.

To study the issue of synaptic integration from the perspective of endogenous receptor activation, we have turned to photolysis of caged compounds, often termed "uncaging."[64] At the core of uncaging, is a molecule that contains a neurotransmitter "encased" in a photo-labile moiety. When the caged moiety is briefly exposed to light, its conformation changes and the functional portion of the caged compound is either released from the cage or at least exposed. It can then interact as a ligand with its receptors. Responses to a particular uncaged moiety indicate the presence of its receptor and how the neuron responds when that receptor is activated. Thus, we use can use photolysis of caged compounds to probe GnRH dendrites for their processing capabilities.

Using uncaging along the length of the GnRH dendrite, we have identified a functional microdomain composed of GABAergic receptors and L-type calcium channels.[63] Previously, Constantin et al. (2010)[65] demonstrated entry of calcium through L-type calcium channels when $GABA_A$ type receptors were activated. We used the release of caged GABA to activate $GABA_A$ receptors in GnRH somata and dendrites (**Fig. 4**). We rendered GABA excitatory by using high chloride in the pipette solution such that the reversal potential for chloride was –36.5 mV.[37] Consistent with earlier observations using anatomical approaches,[66] there is a decreasing response to GABA as the GnRH dendrite extends. Moreover, blocking L-type calcium channels eliminates the response to uncaging GABA (**Fig. 5**). Note that the post-synaptic potential in response to uncaging GABA in the soma approximates that at 200 μm of dendrite length. The increase in the GABA induced post-synaptic potential between 100 and 200 μm of dendrite length reflects a higher density of the L-type calcium channels in the distal portions of the dendrite. Thus, for their action on GnRH neurons, GABAergic inputs must engage L-type calcium channels, and the density of the intrinsic L-type calcium channels creates areas of variable sensitivity.

One of the hallmarks of the GnRH neuron population is the extensive range of internal and external cues that influence their

activity. The active properties and unique morphology of GnRH neuron dendrites render them a multi-functional substrate on which internal and external cues can exert their signaling. Thus, understanding the physiology, morphology and behavior of GnRH neuron dendrites has moved to the forefront of the GnRH field (**Fig. 6**).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Goodman RL, Parfitt DB, Evans NP, Dahl GE, Karsch FJ. Endogenous opioid peptides control the amplitude and shape of gonadotropin-releasing hormone pulses in the ewe. Endocrinology 1995; 136:2412-20; PMID:7750462; http://dx.doi.org/10.1210/en.136.6.2412

2. Herbison A. Physiology of the GnRH neuronal network. In: Neill JD, ed. Knobil and Neill's Physiology of Reproduction. San Deigo, CA: Academic Press, 2006:1415-82

3. 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; 92:3918-22; PMID:7537379; http://dx.doi.org/10.1073/pnas.92.9.3918

4. Spergel DJ, Krüth 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; 19:2037-50; PMID:10066257

5. Suter KJ, Song WJ, Sampson TL, Wuarin JP, Saunders JT, Dudek FE, et al. Genetic targeting of green fluorescent protein to gonadotropin-releasing hormone neurons: characterization of whole-cell electrophysiological properties and morphology. Endocrinology 2000; 141:412-9; PMID:10614664; http://dx.doi.org/10.1210/en.141.1.412

6. 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; 141:3731-6; PMID:11014229; http://dx.doi.org/10.1210/en.141.10.3731

7. Moenter SM. Identified GnRH neuron electrophysiology: a decade of study. Brain Res 2010; 1364:10-24; PMID:20920482; http://dx.doi.org/10.1016/j.brainres.2010.09.066

8. Sim JA, Skynner MJ, Herbison AE. Heterogeneity in the basic membrane properties of postnatal gonadotropin-releasing hormone neurons in the mouse. J Neurosci 2001; 21:1067-75; PMID:11157093

9. 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; 22:2313-22; PMID:11896170

10. Dutton A, Dyball RE. Phasic firing enhances vasopressin release from the rat neurohypophysis. J Physiol 1979; 290:433-40; PMID:469785

11. 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; PMID:15961246; http://dx.doi.org/10.1016/j.neuroscience.2005.03.047

12. 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; 26:11961-73; PMID:17108170; http://dx.doi.org/10.1523/JNEUROSCI.3171-06.2006

13. Liu X, Herbison AE. Small-conductance calcium-activated potassium channels control excitability and firing dynamics in gonadotropin-releasing hormone (GnRH) neurons. Endocrinology 2008; 149:3598-604; PMID:18372332; http://dx.doi.org/10.1210/en.2007-1631

14. 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; PMID:18461432; http://dx.doi.org/10.1007/s10827-008-0095-5

15. Chu Z, Andrade J, Shupnik MA, Moenter SM. Differential regulation of gonadotropin-releasing hormone neuron activity and membrane properties by acutely applied estradiol: dependence on dose and estrogen receptor subtype. J Neurosci 2009; 29:5616-27; PMID:19403828; http://dx.doi.org/10.1523/JNEUROSCI.0352-09.2009

16. Wang Y, Kuehl-Kovarik MC. Estradiol directly attenuates sodium currents and depolarizing afterpotentials in isolated gonadotropin-releasing hormone neurons. Brain Res 2012; 1436:81-91; PMID:22209345; http://dx.doi.org/10.1016/j.brainres.2011.12.013

17. Wang Y, Garro M, Kuehl-Kovarik MC. Estradiol attenuates multiple tetrodotoxin-sensitive sodium currents in isolated gonadotropin-releasing hormone neurons. Brain Res 2010; 1345:137-45; PMID:20580637; http://dx.doi.org/10.1016/j.brainres.2010.05.031

18. Zhang C, Roepke TA, Kelly MJ, Rønnekleiv OK. Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-like cationic channels. J Neurosci 2008; 28:4423-34; PMID:18434521; http://dx.doi.org/10.1523/JNEUROSCI.5352-07.2008

19. Oakley AE, Clifton DK, Steiner RA. Kisspeptin signaling in the brain. Endocr Rev 2009; 30:713-43; PMID:19770291; http://dx.doi.org/10.1210/er.2009-0005

20. DeFazio RA, Moenter SM. Estradiol feedback alters potassium currents and firing properties of gonadotropin-releasing hormone neurons. Mol Endocrinol 2002; 16:2255-65; PMID:12351691; http://dx.doi.org/10.1210/me.2002-0155

21. Xu C, Roepke TA, Zhang C, Rønnekleiv OK, Kelly MJ. Gonadotropin-releasing hormone (GnRH) activates the m-current in GnRH neurons: an autoregulatory negative feedback mechanism? Endocrinology 2008; 149:2459-66; PMID:18218690; http://dx.doi.org/10.1210/en.2007-1178

22. Kelly MJ, Rønnekleiv OK, Ibrahim N, Lagrange AH, Wagner EJ. Estrogen modulation of K(+) channel activity in hypothalamic neurons involved in the control of the reproductive axis. Steroids 2002; 67:447-56; PMID:11960620; http://dx.doi.org/10.1016/S0039-128X(01)00181-7

23. Kelly MJ, Qiu J, Rønnekleiv OK. Estrogen modulation of G-protein-coupled receptor activation of potassium channels in the central nervous system. Ann N Y Acad Sci 2003; 1007:6-16; PMID:14993035; http://dx.doi.org/10.1196/annals.1286.001

24. Bosch MA, Kelly MJ, Rønnekleiv OK. Distribution, neuronal colocalization, and 17beta-E2 modulation of small conductance calcium-activated K(+) channel (SK3) mRNA in the guinea pig brain. Endocrinology 2002; 143:1097-107; PMID:11861537; http://dx.doi.org/10.1210/en.143.3.1097

25. Herbison AE. Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons. Endocr Rev 1998; 19:302-30; PMID:9626556; http://dx.doi.org/10.1210/er.19.3.302

26. 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; 85:987-95; PMID:21778142; http://dx.doi.org/10.1095/biolreprod.111.093492

27. Zhang C, Bosch MA, Levine JE, Rønnekleiv 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; 27:10153-64; PMID:17881521; http://dx.doi.org/10.1523/JNEUROSCI.1657-07.2007

28. Wade GN, Jones JE. Neuroendocrinology of nutritional infertility. Am J Physiol Regul Integr Comp Physiol 2004; 287:R1277-96; PMID:15528398; http://dx.doi.org/10.1152/ajpregu.00475.2004

29. Kato M, Tanaka N, Usui S, Sakuma Y. The SK channel blocker apamin inhibits slow afterhyperpolarization currents in rat gonadotropin-releasing hormone neurones. J Physiol 2006; 574:431-42; PMID:16627563; http://dx.doi.org/10.1113/jphysiol.2006.110155

30. 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; 30:6214-24; PMID:20445047; http://dx.doi.org/10.1523/JNEUROSCI.6156-09.2010

31. Hiraizumi Y, Nishimura I, Ishii H, Tanaka N, Takeshita T, Sakuma Y, et al. Rat GnRH neurons exhibit large conductance voltage- and Ca2+-Activated K+ (BK) currents and express BK channel mRNAs. J Physiol Sci 2008; 58:21-9; PMID:18177544; http://dx.doi.org/10.2170/physiolsci.RP013207

32. Catterall WA. Voltage-gated calcium channels. Cold Spring Harb Perspect Biol 2011; 3:a003947; PMID:21746798; http://dx.doi.org/10.1101/cshperspect.a003947

33. Nunemaker CS, DeFazio RA, Moenter SM. Calcium current subtypes in GnRH neurons. Biol Reprod 2003; 69:1914-22; PMID:12904316; http://dx.doi.org/10.1095/biolreprod.103.019265

34. 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; 144:5118-25; PMID:12960038; http://dx.doi.org/10.1210/en.2003-0213

35. Spergel DJ. Calcium and small-conductance calcium-activated potassium channels in gonadotropin-releasing hormone neurons before, during, and after puberty. Endocrinology 2007; 148:2383-90; PMID:17289846; http://dx.doi.org/10.1210/en.2006-1693

36. Zhang C, Bosch MA, Rick EA, Kelly MJ, Rønnekleiv OK. 17Beta-estradiol regulation of T-type calcium channels in gonadotropin-releasing hormone neurons. J Neurosci 2009; 29:10552-62; PMID:19710308; http://dx.doi.org/10.1523/JNEUROSCI.2962-09.2009

37. Jentsch TJ, Stein V, Weinreich F, Zdebik AA. Molecular structure and physiological function of chloride channels. Physiol Rev 2002; 82:503-68; PMID:11917096

38. 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; 23:557-69; PMID:21518033; http://dx.doi.org/10.1111/j.1365-2826.2011.02145.x

39. 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; 143:1459-66; PMID:11897704; http://dx.doi.org/10.1210/en.143.4.1459

40. DeFazio RA, Heger S, Ojeda SR, Moenter SM. Activation of A-type gamma-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. Mol Endocrinol 2002; 16:2872-91; PMID:12456806; http://dx.doi.org/10.1210/me.2002-0163

41. Lee K, Porteous R, Campbell RE, Lüscher B, Herbison AE. Knockdown of GABA(A) receptor signaling in GnRH neurons has minimal effects upon fertility. Endocrinology 2010; 151:4428-36; PMID:20573723; http://dx.doi.org/10.1210/en.2010-0314

42. Cook EP, Johnston D. Voltage-dependent properties of dendrites that eliminate location-dependent variability of synaptic input. J Neurophysiol 1999; 81:535-43; PMID:10036257

43. Schiller J, Major G, Koester HJ, Schiller Y. NMDA spikes in basal dendrites of cortical pyramidal neurons. Nature 2000; 404:285-9; PMID:10749211; http://dx.doi.org/10.1038/35005094

44. Williams SR, Stuart GJ. Voltage- and site-dependent control of the somatic impact of dendritic IPSPs. J Neurosci 2003; 23:7358-67; PMID:12917370

45. Häusser M, Mel B. Dendrites: bug or feature? Curr Opin Neurobiol 2003; 13:372-83; PMID:12850223; http://dx.doi.org/10.1016/S0959-4388(03)00075-8

46. Spruston N, Kath WL. Dendritic arithmetic. Nat Neurosci 2004; 7:567-9; PMID:15162161; http://dx.doi.org/10.1038/nn0604-567

47. Gulledge AT, Kampa BM, Stuart GJ. Synaptic integration in dendritic trees. J Neurobiol 2005; 64:75-90; PMID:15884003; http://dx.doi.org/10.1002/neu.20144

48. Migliore M, Shepherd GM. Emerging rules for the distributions of active dendritic conductances. Nat Rev Neurosci 2002; 3:362-70; PMID:11988775; http://dx.doi.org/10.1038/nrn810

49. Lüthi A, McCormick DA. H-current: properties of a neuronal and network pacemaker. Neuron 1998; 21:9-12; PMID:9697847; http://dx.doi.org/10.1016/S0896-6273(00)80509-7

50. Campbell RE, Han SK, Herbison AE. Biocytin filling of adult gonadotropin-releasing hormone neurons in situ reveals extensive, spiny, dendritic processes. Endocrinology 2005; 146:1163-9; PMID:15564319; http://dx.doi.org/10.1210/en.2004-1369

51. Roberts CB, Best JA, Suter KJ. Dendritic processing of excitatory synaptic input in hypothalamic gonadotropin releasing-hormone neurons. Endocrinology 2006; 147:3415; PMID:16373415; http://dx.doi.org/10.1210/en.2005-1350

52. 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; 106:10835-40; PMID:19541658; http://dx.doi.org/10.1073/pnas.0903463106

53. Herde MK, Geist K, Campbell RE, Herbison AE. Gonadotropin-releasing hormone neurons extend complex highly branched dendritic trees outside the blood-brain barrier. Endocrinology 2011; 152:3832-41; PMID:21791557; http://dx.doi.org/10.1210/en.2011-1228

54. Roberts CB, Campbell RE, Herbison AE, Suter KJ. Dendritic action potential initiation in hypothalamic gonadotropin-releasing hormone neurons. Endocrinology 2008; 149:3355-60; PMID:18403488; http://dx.doi.org/10.1210/en.2008-0152

55. Iremonger KJ, Herbison AE. Initiation and propagation of action potentials in gonadotropin-releasing hormone neuron dendrites. J Neurosci 2012; 32:151-8; PMID:22219278; http://dx.doi.org/10.1523/JNEUROSCI.3739-11.2012

56. Häusser M, Spruston N, Stuart GJ. Diversity and dynamics of dendritic signaling. Science 2000; 290:739-44; PMID:11052929; http://dx.doi.org/10.1126/science.290.5492.739

57. Hoffman DA, Magee JC, Colbert CM, Johnston DK. K+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. Nature 1997; 387:869-75; PMID:9202119; http://dx.doi.org/10.1038/42571

58. Colbert CM, Magee JC, Hoffman DA, Johnston D. Slow recovery from inactivation of Na+ channels underlies the activity-dependent attenuation of dendritic action potentials in hippocampal CA1 pyramidal neurons. J Neurosci 1997; 17:6512-21; PMID:9254663

59. Jung HY, Mickus T, Spruston N. Prolonged sodium channel inactivation contributes to dendritic action potential attenuation in hippocampal pyramidal neurons. J Neurosci 1997; 17:6639-46; PMID:9254676

60. Dickson CT, Magistretti J, Shalinsky MH, Fransén E, Hasselmo ME, Alonso A. Properties and role of I(h) in the pacing of subthreshold oscillations in entorhinal cortex layer II neurons. J Neurophysiol 2000; 83:2562-79; PMID:10805658

61. Nunemaker CS, DeFazio RA, Moenter SM. Estradiol-sensitive afferents modulate long-term episodic firing patterns of GnRH neurons. Endocrinology 2002; 143:2284-92; PMID:12021193; http://dx.doi.org/10.1210/en.143.6.2284

62. Gay VL, Hemond PJ, Schmidt D, O'Boyle MP, Hemond Z, Best J, et al. Hormone secretion in transgenic rats and electrophysiological activity in their gonadotropin releasing-hormone neurons. Am J Physiol Endocrinol Metab 2012; 303:E243-52; PMID:22621869; http://dx.doi.org/10.1152/ajpendo.00157.2012

63. Kelly MJ, Wagner EJ. GnRH neurons and episodic bursting activity. Trends Endocrinol Metab 2002; 13:409-10; PMID:12431832; http://dx.doi.org/10.1016/S1043-2760(02)00698-7

64. 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; 30:13373-83; PMID:20926664; http://dx.doi.org/10.1523/JNEUROSCI.1687-10.2010

65. 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; 151:262-70; PMID:19864483; http://dx.doi.org/10.1210/en.2009-0817

66. Cottrell EC, Campbell RE, Han SK, Herbison AE. Postnatal remodeling of dendritic structure and spine density in gonadotropin-releasing hormone neurons. Endocrinology 2006; 147:3652-61; PMID:16644918; http://dx.doi.org/10.1210/en.2006-0296