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Gonadotropin-releasing hormone signaling in behavioral plasticity

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Sex and reproduction sculpt brain and behavior throughout life and evolution. In vertebrates, gonadotropin-releasing hormone (GnRH) is essential to these processes. Recent advances have uncovered novel regulatory mechanisms in GnRH signaling, such as the initiation of sexual maturation by kisspeptins. Yet despite our increasing molecular knowledge, we know very little about environmental influences on GnRH signaling and reproductive behavior. Alternative model systems have been crucial for understanding the plasticity of GnRH effects within an organismal context. For instance, GnRH signaling is under the control of seasonal cues in songbirds, whereas social signals regulate GnRH in cichlid fishes, with crucial consequences for reproduction and behavior. Analyzing cellular signaling cascades within an organismic context is essential for an integrative understanding of GnRH function.

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Introduction

Gonadotropin-releasing hormone (GnRH) is the master regulator of sexual maturation and reproduction in vertebrates [1]. The principal role of GnRH in controlling reproductive physiology and behavior through the synthesis and release of the gonadotropins FSH (follicle-stimulating hormone) and LH (luteinizing hormone) from the pituitary has long been established, and significant progress has been made in our understanding of the underlying signaling mechanisms. Although GnRHs play an important role in many different tissues, I here focus on their actions in the central nervous system, which are most pertinent to behavior. I discuss several exciting new insights, such as the role of the kisspeptin signaling pathway in the onset of puberty. I also emphasize how a comparative approach can add to our understanding. Studies in songbirds and cichlid fishes have highlighted the surprising plasticity exhibited by the GnRH system in adult animals in response to seasonal and social cues.

GnRH genes and GnRH receptor genes

GnRH is a decapeptide (see Box 1), the structure of which has been highly conserved throughout evolution, especially at the N- and C- termini, which are involved in receptor binding and activation [2,3]. *GnRH* genes encode a preprohormone precursor (see glossary) that needs to be cleaved and correctly folded before the peptide can become physiologically active [4]. Although peptide maturation has mostly been studied in cell lines, it is assumed that it is highly conserved across vertebrates. Similar to other neuropeptide systems [5•], a signal peptidase first removes the N-terminal sequence. The resulting prohormone is then further processed at specific sites by prohormone convertases in conjunction with specific regulators, such as proSAAS and 7B2. Next, the C-terminal basic residues are cleaved by carboxypeptidase E. Finally, a monooxygenase converts C-terminal Gly residues into a C-terminal amide. The C-terminus of the preprohormone might participate in this maturation process [2,4]. The GnRH-associated peptide (GAP), which originates from the C-terminus, possibly plays a role in GnRH processing; it has also been suggested that GAP is involved in prolactin regulation [4].

GnRH genes are present in all vertebrates, and their evolutionary origin is ancient, as GnRH homologs have been found in octopus [6] and the protochordate *Ciona* [7]. The ancestral gene was probably duplicated early in evolution, as all vertebrates examined to date have at least two GnRH genes. GnRH-2 is the best conserved of the GnRHs, although its function is just now being elucidated (see below). Gene duplication early during the teleost lineage has given rise to a third GnRH (GnRH-3) that is found only in this group [2].

The evolution of the GnRH receptors (GnRHRs) parallels that of their ligands, although which receptor is the cognate form for the different GnRHs is not always clear [2,8]. Interestingly, in humans, chimpanzees and a few other mammalian species (sheep, cow), the *GnRHR-2* gene has been disrupted by a frame shift mutation that resulted in a premature stop codon, thus a conventional receptor of this subtype might not be expressed in these species [8,9••]. However, GnRH-1 and GnRH-2 still have distinct functions because of their ligand-specific signaling through the type 1 receptor [8].

Glossary

Hypothalamo-hypophysial portal vasculature: A system of veins that originate at the median eminence (ventral boundary of the hypothalamus) and pass through the pituitary gland and into its anterior lobe, where they branch into a capillary bed and release stimulatory and inhibitory factors.

Life-history strategy: A suite of often plastic traits that increase survival and reproduction.

Photosensitivity and photorefractoriness: After a period of short days (i.e., winter), the HPG axis of many seasonally breeding vertebrates is photosensitive to increases in day length (during spring). GnRH-1 release and reproduction are then stimulated by light (photostimulation). The HPG axis becomes downregulated and the gonads regress towards the end of the breeding season, at which point the animals are insensitive (photorefractory) to increasing day length.

Preprohormone: Peptide hormone genes encode preprohormones, which contain an N-terminal signal sequence (required for transport), one or more copies of a peptide hormone, and other peptide sequences that might or might not have biological activities.

Prohormone: The preprohormone is processed by several enzymatic steps into a — still inactive — prohormone, which lacks the signal sequence. The prohormone is packaged into secretory vesicles with proteolytic enzymes that cleave the amino acid sequence to form the active hormone(s) and other fragments.

Box 1

For historical reasons, the nomenclature used for the different GnRHs and their receptors is very confusing. Peptide orthologs have often been named after the organism in which they were first found and the phylogenetic relationships of their cognate receptors have frequently been ignored. I follow here a rational nomenclature [2,3], according to which GnRH-1 is the hypothalamic peptide released into the pituitary, GnRH-2 the form found in the midbrain-tegmentum area of teleost fishes (although it is more widely distributed in many other organisms), and GnRH-3 the form found only in the terminal nerve of teleosts. Their cognate receptors are named accordingly, although there is much overlap in their binding affinities that is biologically relevant [2].

Function and regulation of GnRHs and their receptors

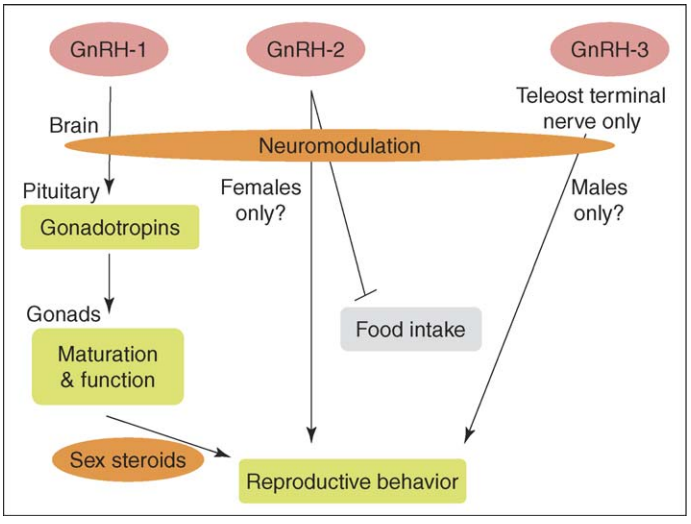
GnRHs are involved in many different physiological processes throughout the body [1]. Although many studies have investigated these actions in different tissues, including the peripheral nervous system, I focus here on the roles GnRHs play in the context of reproduction (Figure 1).

GnRH-1

GnRH was first studied for its fundamental role in sexual development and adult reproductive physiology [10]. GnRH neurons originate in the olfactory placode early in development and then migrate to their destinations. GnRH-1 neurons reside in the preoptic area (POA), and their projections in the median eminence release the peptide into the hypothalamo-hypophysial portal vasculature (see glossary), from which it ultimately reaches the pituitary gonadotropes (the cells that synthesize and release the gonadotropins). Fishes have no portal vasculature, hence their POA neurons project into the appropriate pituitary cell population directly.

GnRH-1 is released in a pulsatile manner, and gonadal steroid hormones provide important regulatory feedback on this process [1]. This temporal regularity arises from intrinsic properties of the GnRH-1 neurons, which include cAMP signaling and Ca^{2+} oscillations [11,12]. Interactions between GnRH-1 neurons and both glial and epithelial cells are important in the regulation of hormone release into the portal system. Epithelial cells of the median eminence use nitric oxide in this regulatory context, whereas astrocytes use members of

Figure 1



The three GnRH subtypes influence reproductive behavior through hormonal and neuromodulatory pathways. GnRH-1, which mainly controls gonadal maturation through gonadotropin release from the pituitary, probably also has neuromodulatory functions throughout the brain. Both GnRH-2 and GnRH-3 influence reproductive and probably other behaviors through neuromodulatory actions in the central nervous system.

the epidermal growth factor (EGF) family of growth factors among others [13]. A recent paper by Galbiati *et al.* [14] provides evidence that the transforming growth factor β (TGF-β) released from astrocytes regulates GnRH-1 neurons through the phosphorylation of Smad proteins in the target cells. All these interactions are temporally coordinated and result in plastic neuronal rearrangements throughout the reproductive cycle.

A surge of GnRH-1 signals the onset of mammalian puberty [1]. Significant progress has been made towards an understanding of the regulation of this process. de Roux *et al.* [15] and Seminara *et al.* [16] applied genetic mapping in several consanguineous families who had members afflicted by isolated hypogonadotropic hypogonadism (a deficiency in gonadotropin release from the pituitary that results in suppressed pubertal development and reproductive function). They found that the affected individuals were homozygous for a mutation in the GPR54 gene, which encodes a G protein-coupled receptor and was first described in the rat brain [17]. In addition, the kisspeptins (encoded by the metastasis suppressor gene *KiSS-1*) were determined as its natural ligands [18]. These discoveries have triggered numerous studies that established the KiSS-1–GPR54 pathway as crucial for the onset of both mammalian puberty and adult reproductive physiology through regulation of GnRH release [19].

Specifically, kisspeptin administration stimulates the rodent hypothalamic-pituitary-gonadal (HPG) axis [20]. In mice, GPR54 is co-localized in GnRH-1 neurons and administration of kisspeptins powerfully stimulates LH and FSH release through the secretion of GnRH [21••]. This effect is specifically and directly mediated by GPR54, as it does not occur in GPR54 knockout mice. Han *et al.* [22] performed electrophysiological recordings in mice and found that in adults, hypothalamic GnRH neurons respond with a long-lasting depolarization to low doses of kisspeptin, whereas in juveniles, only a small fraction of these neurons become activated at such a dose. Interestingly, this study also showed that ~90% of GnRH neurons express GPR54, independent of age. By contrast, KiSS-1 mRNA levels in the anteroventricular nucleus of the hypothalamus increased dramatically during the transition from juvenile to adult life stages. A corresponding pathway also exists in primates: hypothalamic *KiSS-1* and *GPR54* gene expression increase as rhesus monkeys transition from juvenile to mid-puberty, and kisspeptin injections elicit a potent GnRH-1 discharge [23].

Given the conservation of neuroendocrine pathways across vertebrates, one might ask whether the KiSS-1–GPR54 pathway is also important in the reproductive physiology of non-mammalian vertebrates. Parhar *et al.* [24] used single-cell quantitative real-time polymerase chain reaction (PCR) to demonstrate that GPR54 is expressed in all three GnRH neuron populations of a cichlid fish, at a significantly higher percentage in mature compared with that in immature animals. Although these results are intriguing, it is not yet clear how conserved the mechanisms really are.

Two recent papers have reinforced the important role of chemosensory modulation of the GnRH-1 system in the context of reproductive behavior [25,26]. They show that the GnRH-1 system communicates with a multitude of functionally diverse brain areas, thus confirming its central role as master regulator of reproduction. Interestingly, these authors also show that the effects on GnRH-1 by both pheromones and other odorants can be mediated by the main olfactory system alone, which came as a surprise for some. However, although the notion that pheromone processing is the exclusive domain of the vomeronasal organ was widely accepted [27], there has long been convincing evidence in the literature that both chemosensory systems can process pheromones in addition to other odorant signals [28].

GnRH-2

Much less is known about the function of GnRH-2, although it is the best conserved of the GnRHs [2]. It is found throughout the brain and expression patterns appear to be highly variable across species, suggesting a role as a neuromodulator. Although GnRH-2 does not appear to be a regulator of pituitary gonadotropins [29,30], new evidence suggests that it nevertheless influences reproductive behavior in female mammals. Specifically, administration of GnRH-2 heightens sexual behavior and inhibits short-term food intake in underfed female shrews, *Suncus murinus* (Figure 2). This effect is mediated by GnRHR-2 and independent of GNRHR-1 [31••].

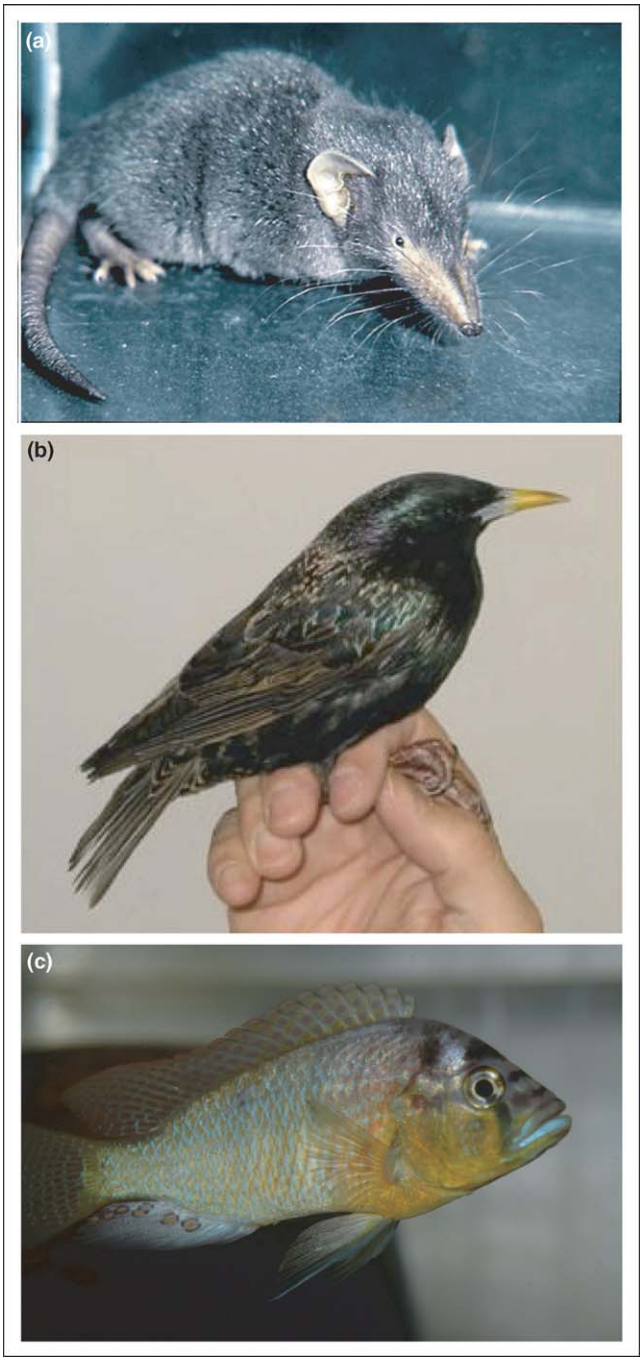
GnRH-3

GnRH-3, which is only present in teleosts, is perhaps the most mysterious of the three GnRHs. GnRH-3 neurons are found in the terminal nerve, with projections throughout the brain, which implies a neuromodulatory role [32]. Lesion experiments suggest that this peptide is important in the initiation of nest-building behavior in male dwarf gourami, *Colisa lalia* [33]. GnRH-3 neurons also contribute to a pathway projecting from the terminal nerve to the retina, which raises the possibility that this peptide could function as a neuromodulator there. Indeed, GnRH can modulate receptive field size of goldfish retinal ganglion cells [34].

GnRH receptors

GnRH receptors belong to the G protein-coupled receptor superfamily. Several up-to-date reviews discuss GnRH receptor structure, biochemistry and function [2,8] in addition to tissue-specific and hormonal regulation [35], which enables me to focus here on a few new insights into GnRH receptor regulation.

Figure 2



Comparative studies are crucial for an integrative understanding of GnRH function. In addition to established model systems such as mouse and rat, non-traditional models have yielded important insights into GnRH function in the context of behavioral plasticity. **(a)** Studies in musk shrew, *Suncus murinus*, provided insight into the role of GnRH-2 in reproductive behavior. **(b)** The European starling, *Sturnus vulgaris*, is an excellent model system to study how seasonal cues control reproductive physiology through their actions on GnRH-1. **(c)** Finally, the fascinating biology of the African cichlid fish *Astatotilapia burtoni* has been exploited to elucidate the social control of GnRH-1 plasticity in the context of aggression and territoriality. Photo credits: Emilie Rissman (a); Joseph Casto and Gregory Ball (b); Christian Landry and Hans Hofmann (c).

Roelle *et al.* [36] showed that two matrix metalloproteinases, MMP2 and MMP9, are crucial in GnRH receptor signaling through GnRH-dependent EGF receptor transactivation. Also, arrestin has been implicated in the C-terminus-dependent signaling of seven transmembrane domain receptors. Examining a *Xenopus* GnRHR, another recent paper [37] provides the first direct evidence that MMP2, MMP9 and arrestin are also involved in the signaling of GnRH receptors. In addition, this study shows that the mammalian GnRHR-1, which is exceptional in that it lacks a C-terminus, is not regulated by arrestin.

As mentioned above, the *GnRHR-2* gene of humans and a few other mammals carries a frame shift mutation that prevents the production of a conventional receptor [2]. However, this remnant is still transcriptionally active and encodes part of the full protein from the cytoplasmic end of transmembrane domain 5 all the way to the C-terminus of the full-length receptor. In an exciting paper, Pawson *et al.* [9••] have now been able to demonstrate that this GnRHR-2 remnant can inhibit the GnRHR-1-induced inositol phosphate signaling pathway, possibly by perturbing its normal processing. This effect hints at an interesting novel mechanism for the modulation of the GnRHR-1 signaling process.

Parhar and co-workers [38•] have adapted quantitative real-time PCR from microdissected single cells to assess the cellular and functional heterogeneity of the three GnRHs and their receptors in cichlid fishes. GnRHRs are expressed throughout the brain, although the patterns of expression are subtype-specific and suggestive of complex cross-talk among the different ligands. Another recent study [39•] extends these findings by showing that only GnRHR-2 is co-localized in all three GnRH neuron populations, suggesting that any autocrine feedback regulation of this receptor must occur through this subtype. Finally, there is an astonishing complexity of GnRHR expression in gonadotropic and non-gonadotropic cell populations of the cichlid pituitary that depended on reproductive status, with many cells expressing more than one receptor subtype simultaneously [40•].

Comparative analysis of the GnRH system

Insights from non-traditional model systems have highlighted the role the environment can play in regulating GnRH function. These studies are important for several reasons. First, they enable us to ask questions that are directly inspired by the life history (see glossary) of the animal, and thus compel us to integrate the reductionist studies within the larger and more complex biological context. Second, the comparative analysis enables us to make inferences about the evolution of the various GnRH pathways. For example, alignments of GnRH peptide sequences from different species helped to identify highly conserved regions that are important in receptor

binding and activation [2]. Third, the study of GnRH signaling systems in a broad range of vertebrate species enables us to identify those parts of the pathway that have diverged more, and thus might have been subject to diversifying selection, possibly related to differences in life history and behavior. Finally, comparative studies highlight how the challenges associated with naturalistic experiments can be overcome using elegantly designed tests. In the following, I present two examples that emphasize the power of the comparative approach and its implications for future research in standard model systems.

Seasonal plasticity of the GnRH system in the European starling

In many temperate-zone songbirds, reproductive physiology is regulated by day length throughout the seasons [41,42]. Thyroid hormones have been implicated in the control of this plastic process [42]. These annual changes are accompanied by corresponding changes in the song system and singing activity [41]. Hypothalamic GnRH levels and gonadal function are upregulated at the beginning of the breeding season, when the birds are photostimulated by increasing day length. Later in the season, with continuous exposure to long days, GnRH is downregulated and the gonads regress as the birds become photorefractory (see glossary). After experiencing a period of short days (i.e., winter), the animals become photosensitive. As first shown by Foster *et al.* [43] for the European starling, *Sturnus vulgaris* (Figure 2), hypothalamic GnRH is already upregulated at the end of winter in photosensitive birds (with their gonads still undeveloped), although its release is inhibited until stimulated by increasing day length.

Recent advances in our understanding of this process make us appreciate the complexity of GnRH signaling mechanisms within an organismal context. Because female reproductive performance increases with age, Sockman *et al.* [44••] hypothesized that prior breeding experience might 'prime' the HPG axis to respond faster and more vigorously in subsequent years. They found that experienced photosensitive female starlings indeed exhibit a higher concentration of circulating LH and more GnRH fibers in relation to the number of GnRH cells, a proxy for increased release of the hormone.

The excitatory transmitter glutamate is involved in the pulsatile release of hypothalamic GnRH through the activation of NMDA-subtype receptors, and treatment with exogenous NMDA causes increased secretion of LH [45]. Dawson [46] injected NMDA into starlings to assess the amount of GnRH-1 stored in hypothalamic neurons at four different reproductive stages. He found the greatest LH increase in photosensitive birds and a slight increase in photostimulated birds. Interestingly, the increase in LH levels in birds that had just experienced gonadal regression was significant when compared with that of fully photo refractory birds, who showed no response.

These studies demonstrate nicely that photoperiod can independently regulate the GnRH system at the level of both hormone synthesis and release in an experience-dependent manner. It is still unclear how these cues are encoded in the brain to regulate hypothalamic GnRH production and/or release. Interestingly, in photostimulated birds, gonadal maturation goes hand in hand with an increase in body weight [44••], which suggests that the reproductive, growth and food-intake systems interact, as has been proposed for mammalian puberty [47].

Social control of GnRH function and reproductive behavior in a cichlid fish

The diversity of life-history strategies makes teleost fishes ideal for the study of reproductive physiology and behavior. Fernald [48] and colleagues have established the Lake Tanganyikan cichlid *Astatotilapia* (formerly *Haplochromis*) *burtoni* as a model for the study of socially regulated physiology and behavior (Figure 2). Males of this species are either reproductively mature and territorial (T) or reproductively suppressed and non-territorial (NT). In response to social change, however, Ts and NTs often change into the other phenotype, and they can do so repeatedly throughout life. Because GnRH-1 is regulated as a consequence of social change [48], this system provides a unique opportunity to investigate GnRH-1 signaling from an organismal perspective. Two recent studies have significantly extended our understanding of how this regulation occurs.

Using electrophysiological recordings from a slice preparation, Greenwood and Fernald [49] determined that the electrical properties of POA GnRH-1 neurons depend on the social and/or reproductive status of the animal. Most of the differences found in basic electrical properties (e.g., membrane capacitance, input resistance) can be attributed to the previously described GnRH-1 cell size differences between Ts and NTs [48], whereas the activity patterns (partly suggestive of episodic firing) and spontaneous firing rates are independent of reproductive status. However, the action potential duration is increased in NTs, potentially limiting the maximum firing rate and thus GnRH release. These findings are reminiscent of the situation in mammals [12]. How then do social signals affect GnRH-1 signaling in these fish? One possible mechanism involves the activation of the immediate-early gene (IEG) *egr-1*. Using an elegant experimental paradigm, Burmeister *et al.* [50••] removed the dominant male from a stable hierarchy, which within minutes led to striking changes in body coloration and behavior of the subordinate male that were indicative of him assuming dominance. The authors found that this phenotypic transition was accompanied by the activation of *egr-1* in the anterior POA, an area that is densely populated by

GnRH-1 neurons. Importantly, established dominant males did not show this IEG activity, although they exhibited dominance behaviors similar to those of ascending males. Brain regions that express GnRH-2 and GnRH-3 were not activated. Because *egr-1* encodes a transcription factor thought to be important in neural plasticity, the gene might well be an upstream activator of GnRH-1 signaling and represent one of the key steps necessary to turn a social signal into physiological action. Although this has not been tested, it is tempting to speculate that *egr-1* induces KiSS-1 expression in these fish, which in turn could activate GnRH-1 neurons through GPR54 signaling [24].

Recent efforts have aimed to extend this promising molecular approach by assessing gene expression changes at a genomic scale [51•]. Renn *et al.* [52] have developed the resources, such as expressed-sequence tag (EST) databases and DNA microarrays, to examine neural gene expression patterns within and across cichlid species, and several interesting insights are beginning to emerge from these studies.

Conclusions

GnRH signaling is clearly a fundamental process in vertebrate physiology and evolution. Without it there is no gonadal maturation and no reproduction. But despite the tremendous progress in our understanding of the molecular and hormonal mechanisms, there are still many unanswered questions. Because I consider an integrative approach to the relationship among molecular, physiological and behavioral levels to be essential for a complete understanding, I will discuss future challenges from this perspective.

First, can we find evidence that *GnRH* genes and receptor genes (including their promoters) have been under diversifying selection in vertebrate species that differ in their life history? Clearly, the GnRH system is highly conserved across all vertebrates, underscoring its fundamental role in biology. However, there is considerable variation in both sequence space (outside the peptide-coding region) and regulatory mechanisms, which might be evidence of selection. A second challenge relates to the KiSS-1–GPR54 pathway. How conserved is this pathway across vertebrates and what do variations in some species tell us about its function? If kisspeptins throw the switch that induces puberty, what upstream factors activate them in the first place? Interestingly, increased growth and acquisition of body fat, along with sexual maturation, are hallmarks of puberty, and regulatory interactions among growth, food intake and reproductive systems are now being investigated [19,44••]. Uncovering these mechanisms might also shed more light on the role and plasticity of the KiSS-1–GPR54 pathway in adult reproductive function. Next, we need to know much more about the roles of GnRH-2 and, in fishes, GnRH-3. The recent studies reviewed here have opened up a whole new area of research that will continue to be productive. Finally, the studies in starlings and cichlids have demonstrated the astonishing plasticity of the adult GnRH system. How do seasonal and social factors affect GnRH signaling in mammals? Systematic studies demonstrating these effects in humans and rodents are scarce, yet they will provide crucial insights and might also prove important for medical considerations.

GnRHs are ancient peptides that still hold many secrets about their signaling mechanisms and regulatory responses. It will be exciting to see more and more of these secrets unveiled in an effort to understand behavioral and neural plasticity in vertebrate sex and reproduction.

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