Gonadotropin-Releasing Hormone Containing Neurons and Olfactory Fibers During Development: From Lamprey to Mammals

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ABSTRACT: Gonadotropin releasing-hormone (GnRH) regulates the hypothalmo–pituitary–gonadal axis in all vertebrates. The vast majority of GnRH neurons are thought to be derived from progenitor cells in medial olfactory placodes. Several antibodies and lectins that recognize cell surface carbohydrates have been useful for delineating the migratory pathway from the olfactory placodes and vomeronasal organ, through the nasal compartment, and across the cribriform plate into the brain. In rats, α-galactosyl-linked glycoconjugates (immunoreactive with the CC2 monoclonal antibody) are expressed on fibers along the GnRH migration pathway and approximately 10% of the GnRH neuronal population. In lamprey, the α-galactosyl binding lectin, Grifonia simplicifolia-I (GS-1), identifies cells and fibers of the developing olfactory system. In contrast to the CC2 immunoreactive GnRH neurons in rats, the GS-1 does not label a subpopulation of presumptive GnRH neurons in lamprey. Results from these and other experiments suggest that GnRH neurons in developing lamprey do not originate within the olfactory placode, but rather within proliferative zones of the diencephalon. However, the overlap of olfactory- and GnRH-containing fibers from prolarval stages to metamorphosis, suggest that olfactory stimuli may play a major role in the regulation of GnRH secretion in lamprey throughout life. By contrast, olfactory fibers are directly relevant to the migration of GnRH neurons from the olfactory placodes in mammalian species. Primary interactions between olfactory fibers and GnRH neurons are likely transient in mammals, and so in later life olfactory modulation of GnRH secretion is likely to be indirect. © 1997 Elsevier Science Inc.

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INTRODUCTION

Gonadotropin releasing-hormone (GnRH or luteinizing hormone-releasing hormone; LHRH) regulates the hypothalmo–pituitary–gonadal axis in all vertebrates that have been studied [57]. The primary structures of several forms of GnRH have been identified in various species of vertebrate, and there are still several forms that have yet to have their sequences determined. Although specific amino acids in the middle of the GnRH decapetide sequence have differed from lamprey to mammals [58], essential molecular features at the ends of the peptide, and its length, have been conserved [56]. Several different genes containing the coding sequence for different GnRH forms have been identified within individual species [70], and usually the different forms are found in different cell populations [39]. In most vertebrates, neurons containing forms of GnRH that are important for the hypothalamo–pituitary axis are derived from outside the central nervous system. Data from a number of vertebrate species suggest that these critical neurons migrate from birth sites in epithelium of medial olfactory placode, across the nasal septum, and cribriform plate to the forebrain (mice [50,71], chicken [1,35,42], and amphibians [34,44]). An alternative to olfactory placode origin has been suggested [2] however, this still involves migration along the same route from the nasal compartment into the forebrain. By contrast, neurons containing some alternate forms of GnRH, which may not be used to regulate the hypothalamic–pituitary axis, may be derived from progenitor cells within the central nervous system (e.g., [42,44]). In this review we will examine how recent data in lampreys may alter this perception.

We will examine the relationship of the olfactory system to GnRH neurons from the perspective that all GnRH neurons are in search of target destinations within the central nervous system. In this perspective, peripheral ‘‘ganglia’’ of GnRH neurons are groups of cells that are in search of the central nervous system when found during development, or have failed to find central nervous system destinations when found in adulthood (ignoring bullfrog sympathetic ganglia [22]). We will examine the olfactory system from the perspective that projections from nasal epithelial cells into the brain share some fundamental characteristics of origination, and specific differences in their final targets (Fig. 1). In this perspective, the fiber pathways derived from the peripheral olfactory system have three targets: (1) the olfactory bulbs, (2) the accessory olfactory bulbs, and (3) scattered sites in the rostral forebrain. A number of investigators since the turn of the century have referred to these three projections in different animals as the olfactory, vomeronasal, and terminal nerves, respectively. Cells that can give rise to such fibers can be neuroepithelial cells in the main or vomeronasal olfactory epithelium, cells that have migrated out along fibers from those sources, or in some cases, cells in the trigeminal ganglia [15]. The discovery of GnRH neurons and their fibers in the peripheral olfactory system has significantly contrib-
Lampreys spawn once, and then die. If caught in streams of undetermined pretreatment, Gauchers also have found high levels of alpha-galactose containing carbohydrates that recognize cell surface carbohydrates have been useful for delineating the migratory pathway from the olfactory placodes/vomeronasal organ (VNO), through the nasal compartment, and across the cribiform plate into the brain. In rats, α-galactosyl–linked glycoconjugates (immunoactive with the CC2 monoclonal antibody [55]) are expressed on fibers along the GnRH migration pathway [63]. In addition, the only CC2 immunoreactive cells in the brain were approximately 10% of the GnRH neuronal population, suggesting that this glycoconjugate marker further identified the olfactory origins of GnRH neurons. Others also have found high levels of alpha-galactose containing glycoconjugates associated with the olfactory system [3,16,21,32, 53–55]. In additional experiments in rats, we found that the lectin and travel dorsally and caudally. At E16, a bundle of fibers oriented toward the developing accessory olfactory bulb is distinct from a caudal group that remains defasciculated. During the next few days, the projection to the accessory olfactory bulb forms a large, tightly bundled fascicle, whereas the number of caudally directed fibers become increasingly difficult to detect. Labeling studies were also conducted by placing DiI in the medial forebrain at E16. Cells and axon bundles were labeled along the trajectories of vomeronasal nerves all the way back to the vomeronasal epithelium. These studies demonstrate the existence of a projection that diverges from the main vomeronasal nerve, and extends along the medial surface of the olfactory bulb and rostral forebrain [72]. One difference between rats and mice is that the caudal projection into the forebrain of the mouse makes a significant ventral turn and grows deeper into the basal forebrain than in the rat, perhaps suggesting their exposure to differential regulation during development. As numerous investigators have found over the years (see [12]), the projections arising from the vomeronasal organ often differ subtly from species to species. The variety has contributed to differing interpretations of what constitutes the “terminal nerve” vs. what might constitute an “extrabulbar olfactory projection” [49] vs. what might simply constitute a component of the vomeronasal system [72].

Several antibodies and lectins that recognize cell surface carbohydrates have been useful for delineating the migratory pathway from the olfactory placodes and vomeronasal organ, through the nasal compartment, and across the cribiform plate into the brain. In rats, α-galactosyl–linked glycoconjugates (immunoactive with the CC2 monoclonal antibody [55]) are expressed on fibers along the GnRH migration pathway [63]. In addition, the only CC2 immunoreactive cells in the brain were approximately 10% of the GnRH neuronal population, suggesting that this glycoconjugate marker further identified the olfactory origins of GnRH neurons. Others also have found high levels of alpha-galactose containing glycoconjugates associated with the olfactory system [3,16,21,32, 53–55]. In additional experiments in rats, we found that the lectin

![Diagram of Mouse and Lamprey Brain](https://example.com/diagram)

**FIG. 1.** Schematic diagrams represent parasagittal or horizontal views of developing mouse and lamprey brains. In the majority of vertebrate species (e.g., mouse) GnRH-containing cells (small black rectangles) originate in the olfactory placodes/vomeronasal organ (VNO), migrate across the cribiform plate (CP) into the brain, and then move caudally toward the preoptic area and hypothalamus (H). In lamprey, GnRH-containing cells arise within the preoptic area/rostral hypothalamus and migrate only a short distance radially away from the ventricle. In the mouse, fibers from diverse sources such as the olfactory epithelium (OE), epithelium of the vomeronasal organ, cells lying along the fibers extending from these first two sources, and fibers from cells in the trigeminal ganglia collect at the CP before entering the brain and traveling toward the olfactory bulb (OB) or basal hypothalamus. In lamprey, olfactory fibers emerge from the single midline-olfactory epithelium and cross into the brain. Such fibers may serve as migratory guides for GnRH neurons in most species (e.g., the mouse), and perhaps guides for GnRH fibers in others (e.g., lamprey). T = telencephalon, V = ventricle, P = pituitary.
Dolichos biflorus (DBA), which recognizes a subset of α-galactosyl-linked glycoconjugates, and Ulex europus-1 (UEA-1), which recognizes a subset of fucosyl-linked glycoconjugates, both recognize selective projections of olfactory derived fibers across the cribriform plate (Fig. 2A and C), with little reactivity in the forebrain per se. They differ in that DBA recognizes a strikingly larger population of more rostral neuroepithelial cells than UEA-1. The monoclonal antibody HNK-1, which recognizes glycoconjugates bearing a sulfated glucuronic acid epitope, also recognizes selective projections of olfactory derived fibers across the cribriform plate (Fig. 2B), but is also significantly more prevalent within the central nervous system [41].

In lamprey, the α-galactosyl-binding lectin, Grifonia simplicifolia-1 (GS-1-B4 isoform), similarly identifies cells and fibers of the developing olfactory system [65]. Projections from the olfactory epithelium to the developing brain are seen in the lamprey from prolarval ages through metamorphosis. Fibers stretch from the olfactory epithelia and primordial olfactory bulbs towards the base of the diencephalon (Fig. 2D) above the adenohypophysis. Olfactory fibers follow a medial pathway that remains lateral to a cell dense periventricular zone. Occasional GS-1-B4 reactive cells are noted along the fiber pathway, but they never penetrate the cell dense periventricular zone. As development proceeds, the GS-1-B4 reactive olfactory epithelium expands, and the projection...
through the telencephalic lobes to the hypothalamus increases. A portion of the caudal projection may originate from cells in the olfactory epithelium and from a small group of cells in the rostral olfactory bulb. A separate reactive fiber bundle is directed toward the hypothalamus from the region of the glomeruli. Using the lectin DBA a different pattern of reactivity is seen in the OE. Isolated olfactory epithelial cells are DBA reactive, but reactive fibers do not exit the OE for the central nervous system. In contrast to the CC2 immunoreactive GnRH neurons in rats, GS-1-B4 reactive cells do not appear to indicate a subpopulation of presumptive GnRH neurons in lamprey.

Several studies have implicated N-CAM as an important glycoconjugate adhesion molecule during development of the olfactory system. In vivo, GnRH neurons are strongly associated with fibers immunopositive for the polysialylated form of N-CAM (PSA-N-CAM) in rodents [31,72] and chickens [33]. Data from explant experiments have shown a strong tendency of GnRH neurons to migrate along fibers delineated by immunoreactive NCAM [9,62]. In vivo lesion experiments in chickens have further suggested the importance of PSA-N-CAM containing fibers for GnRH neuron migration in the nasal compartment. Following selective lesions only those GnRH neurons that could reach alternate PSA-NCAM fibers were found to continue their migration, albeit along aberrant routes that did not reach the forebrain [37]. Microinjection of N-CAM antibodies into the olfactory placode in mice reduced the total number of GnRH neurons throughout the migratory route [52]. Recent studies were performed to investigate the role of polysialic acid in the migration of GnRH neurons [73]. Mouse embryos on day 12 of gestation (E12) were treated with endoneuraminidase (endo-N), an enzyme that specifically removes PSA from NCAM. After 3 days in the presence of endo-N, the positions of GnRH neurons were determined. Results suggest that acute removal of PSA during this 3-day period inhibits the migration of approximately 40% of GnRH neurons.

The distribution of PSA in the lamprey does not appear to match the olfactory distribution of PSA in mammals. The monoclonal antibody 5A5, which recognizes PSA, reveals numerous immunoreactive cells throughout the central nervous system of larval lamprey (Fig. 3A). In contrast to mammals, the cells of the lamprey OE are not 5A5 immunoreactive. Although several studies have examined the distribution of NCAM in different vertebrates, virtually all studies of PSA have been conducted in mammals. Nevertheless, data in lamprey suggests that GnRH neurons and PSA may be related across diverse vertebrate species. In particular, immunopositive profiles are seen in the direct vicinity of GnRH neurons during larval development (Fig. 3B and C).

A variety of adhesion molecules are thought to play significant roles in development of the nervous system in addition to NCAM. Other examples include various forms of tenascin [24] and laminin [7]. In cultures of embryonic olfactory neurons, laminin was a poor substrate for cell adhesion but enhanced cell migration [6]. Additional laminin isoforms are expressed in the developing olfactory system, and that adhesion can be mediated by an endogenous lectin, L-14 [28]. L-14 is capable of promoting olfactory axon fasciculation by crosslinking adjacent axons and can promote axonal adhesion to the extracellular matrix. TAG-1 is a glycosylphosphatidylinositol (GPI)-linked cell surface glycoprotein [17] that also has promise in aiding an analysis of the developing olfactory system and GnRH neurons in rodents. The TAG-1 adhesion molecule is expressed preferentially on olfactory-derived fibers that reach toward the hypothalamus, and its expression is restricted in time during the period of GnRH neuron migration [72]. In E12 mice, small subsets of GnRH neurons coexpress TAG-1 (K. Yoshida and G.A. Schwarting, unpublished observations), which raises the possibility that homophilic TAG-1/TAG-1 interactions might contribute to adhesion of GnRH cells to TAG-1" axons.

**IDENTIFIED CELLS MIGRATING THROUGH THE OLFACTORY SYSTEM**

Much data concerning the migration of cells from the olfactory system towards the brain also is derived from histochemical studies examining particular molecules at successive ages (e.g., [8,32,45,46,68]). Subsets of cells that migrate upon the pathway also maintain selective carbohydrates on their cell surfaces. Evidence suggests that both neurons and glial cells migrate from origins in the olfactory epithelial zones towards the cribiform plate. Such cells have been identified based on their morphology by electron microscopy [29], or the presence of specific antigens detected by either immunocytochemistry [8,46,68] or lectin histochemistry [32,45]. Immunocytochemical and lectin histochemical studies further suggest the potential involvement of specific cell surface carbohydrates in the migration of these additional cells through the nasal compartment. The same glycoconjugate directed reagents that identify fibers also identify individual cells that can be seen along those fibers (Fig. 2). In sections double-labeled for UEA-1 and GnRH, the two cell types are often located in close proximity. Interactions between these cells during the migration process have not been examined.

We have recently utilized antisera directed against y-aminobutyrlic acid (GABA) to identify the neurotransmitter phenotype of cells moving through the nasal compartment of rats, mice, and humans [66]. Previously, the peptides molluscan cardioexcitatory tetrapeptide (FRMFamide) in fish (e.g.,[69]), and somatostatin [36] and neuropeptide Y [18] in chickens had been identified in cells migrating through the olfactory system. However, these peptides have not been observed in the olfactory migration routes of mammalian species. The dipeptide carnosine has been found in migrating cells in mammals, however, its role in neurotransmission is unknown [61]. Recently, a homodimeric glycoprotein was discovered in the pituitary of adult lampreys, and the olfactory system of larval lampreys, and named nasohypophysial factor (NHF [59]). In larval lampreys, the distribution of immunoreactive NHF is similar to GS-1 reactive cells and fibers from the olfactory epithelium to the hypothalamus. Interactions among the different migrating cell populations may provide important determinants of the migratory process in the nasal compartment and at the cribiform plate. GABA, in particular, may play important neurotrophic roles [25], and/or significantly influence neuronal migration [5] during development. At a minimum, these markers help identify the phenotypes of cells that migrate through the olfactory system and perhaps into the brain.

The analysis of potential GABA-GnRH interactions was recently extended to pirlarval lamprey [27]. Sections from animals processed for GABA immunocytochemistry were compared with those processed separately for GnRH immunocytochemistry. Although technical considerations precluded direct double-label experiments, GnRH neurons were found in regions close to cells that contained immunoreactive GABA. Immunoreactive GABA was present early in lamprey brain development (within 10 days of fertilization), prior to the appearance of immunoreactive GnRH (between 20 and 30 days after fertilization). The distribution of GABA immunoreactive cells was significantly more widespread than that of GnRH, however, there always appeared to be a gap in the GABA cell distribution where GnRH neurons would be expected. Thus, the overwhelming impression was that GABA neurons surrounded GnRH neurons. There was no indication that either populations of GABA or GnRH neurons originated in the olfactory placode. The proximity of GABA to GnRH neurons...
lends support to the idea that GABA may influence the development of GnRH neurons in the lamprey.

OLFACTORY PATHWAYS AND GNRH CELLS

In species for which two or more forms of GnRH have been identified, the different GnRH forms are usually found in different cell populations (e.g., [10,26,30], reviewed [39]). However, evidence suggests that in one of the oldest extant vertebrates, the agnathan lamprey, at least two different forms of GnRH in sea lamprey are found in the same cells [64,65]. The population of GnRH immunoreactive cells in lamprey is confined to a densely packed tight arc within the rostral preoptic area. Both lamprey GnRH-I and lamprey GnRH-III are found within this same cell population from the earliest points in development, including metamorphosis [23,64]. While in some species (e.g., a teleost: *Catostomus commersonii*) cells containing different immunoreactive forms of GnRH can be located close to one another [48], in lamprey GnRH neurons are packed side by side with virtually no room for other cell types. Interestingly, in humans where some of the first data for multiple forms of GnRH in a single species was described, immunocytochemical evidence suggested that an alternative form (then tentatively identified as a lamprey GnRH-I form) is present in cells within the same regions as those containing the mammalian form [60]. In humans, different immunoreactive forms of GnRH may coexist in the same cells, or be found in nearby cells.

In the vertebrates that have been analyzed, the form of GnRH not thought to regulate the hypothalamic–pituitary–gonadal axis usually resides in more caudal sites. For example, in axolotls, the caudal cell population (containing chicken GnRH-II) is not derived from the olfactory placode [44]. Again, lampreys provide an exception, because neurons containing two lamprey forms of GnRH may be derived from cells within the brain and not the olfactory placode [65]. On the other hand, a second population of cells containing only lamprey GnRH-III was found transiently during larval lamprey development [64].

A combination of several experiments utilizing different approaches led us to suggest that in lamprey GnRH neurons are born within the central nervous system [64,65]. Thus, neurons containing immunoreactive GnRH appear first within the brain within weeks of fertilization and the numbers increase slowly over the course of the first year, with all added neurons appearing in the same preoptic/hypothalamic location. Cells in this region can incorporate the mitotic indicator bromodeoxyuridine (BrdU) dur-
ing the larval period when GnRH neuron number is rising slowly. These neuronal birthdating studies in larval lampreys also indicated that cells born in the region where GnRH neurons appear often move radially away from the ventricles. Birthdating studies in prolarval lampreys indicated that a majority of cells born in the olfactory epithelium from 10 to 30 days after fertilization are not postmitotic because olfactory epithelial labeling at these ages dilutes out significantly within 10–30 days.

All fibers containing immunoreactive GnRH are located caudal to the olfactory bulbs at prolarval, larval, and metamorphic stages and only appear to have originated from sources in the preoptic area. At all points in development, olfactory fibers (as indicated by GS-1 reactivity) coursed lateral to the locations of GnRH cells. The boundary between the cell dense periventricular zone and the lateral fiber region appeared to preclude direct contacts of GS-1-B4 reactive olfactory fibers and cells with the more medial perikarya containing immunoreactive lamprey GnRH. GnRH neurons themselves never appeared GS-1 reactive. As fibers containing immunoreactive lamprey GnRH exited the cell dense medial zone to stream caudally toward the neurohypophysis, they did mingle with GS-1-B4 reactive olfactory fibers travelling the same path.

Unlike other vertebrates, in lampreys both forms of GnRH act as neurohormones that stimulate the pituitary–gonadal axis [13]. Thus, the function and distribution of GnRH differs between lamprey and other vertebrates. A central nervous system origin of GnRH cells in the lamprey would be consistent with the hypothesis that a central nervous system origin is a phylogenetically older pattern. An extension of this hypothesis is that as new forms of GnRH evolved that more specifically modulate pituitary function (n pituitary directed function). Newer forms of GnRH may have been disturbed in vitro provide support for the suggestion that GnRH neurons utilize olfactory system fibers for guidance over a portion of their migration in the brain.

In the lamprey, the opportunity exists to expose the olfactory system in vivo to selective agents by adding them to the lamprey’s normal aqueous environment. As reviewed above, there appears to be extensive opportunity for olfactory fiber interactions with the developing GnRH system. An advantage of using prolarval lamprey is that they do not use nutrients from the environment, as they still feed from their embryonic yolk proteins. In preliminary experiments we utilized prolarvae 20 days after fertilization, before the first detectable GnRH neurons appear. Lamprey were placed into 0.02 M solutions containing α-methyl-galactoside, melibiose, or nothing extra. Lamprey were raised in this environment for 16–22 days and then immersed in fixative prior to processing for GnRH immunocytochemistry. When the number of neurons per brain containing immunoreactive lamprey GnRH-III in 36- and 42-day old lamprey were counted, control animals had 17.8 ± 3.2 (n = 8), whereas α-methyl-galactoside–exposed animals had almost twice as many 30.7 ± 3.4 (n = 9), and those treated with the alternate carbohydrate, melibiose, were not different from control (20.9 ± 4.3; n = 9). These preliminary data are consistent with the hypothesis that environmental exposure to specific carbohydrates may influence the development of the GnRH system in lamprey. Exogenous carbohydrate exposure may influence the olfactory system either by binding to receptors on the surface of olfactory neuroepithelia or by interfering (e.g., competitive inhibition) with endogenous interactions between α-galactose–linked glycoconjugates and the carbohydrate binding proteins with which they normally interact. Experiments such as these can help determine factors that influence the development of GnRH neurons.

**CONCLUSIONS**

In mammals the existence of a transient pathway that extends from olfactory neuroepithelial cells to regions caudal to the olfactory bulbs has been shown based on Dil labeling, lectin histochemistry, and immunocytochemistry for identified molecules. Our studies in lamprey show that the olfactory fibers projecting caudally, beyond the primordial glomerular layer, are evident from very early in development. Thus, where primary interactions between olfactory fibers and GnRH neurons are likely transient in mammals, olfactory modulation of GnRH secretion in adulthood is likely to be indirect. In lamprey, where primary interactions between olfactory fibers and GnRH projections are likely lifelong, olfactory modulation of GnRH secretion in adulthood may be more direct.
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