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Minireview: Recent Progress in Gonadotropin-Releasing Hormone Neuronal Migration

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Neurons that synthesize GnRH are critical brain regulators of the reproductive axis, yet they originate outside the brain and must migrate over long distances and varied environments to get to their appropriate positions during development. Many studies, past and present, are providing clues for the types of molecules encountered and movements expected along the migratory route. Recent studies provide real-time views of the behavior of GnRH neurons in the context of *in vitro* preparations that model those *in vivo*. Live images provide direct evidence of the changing behavior of GnRH neurons in their different environments, showing that GnRH neurons move with greater frequency and with more alterations in direction after they enter the brain. The heterogeneity of molecular phenotypes for GnRH neurons likely ensures that multiple external factors will be found that regulate the migration of different portions of the GnRH neuronal population at different steps along the route. Molecules distributed in gradients both in the peripheral olfactory system and basal forebrain may be particularly influential in directing the appropriate movement of GnRH neurons along their arduous migration. Molecules that mediate the adhesion of GnRH neurons to changing surfaces may also play critical roles. It is likely that the multiple external factors converge on selective signal transduction pathways to engage the mechanical mechanisms needed to modulate GnRH neuronal movement and ultimately migration. (*Endocrinology* 147: 1159–1165, 2006)

NEURONS THAT SYNTHESIZE and release GnRH form the final common pathway for the central regulation of fertility. It has now been just over 16 yr since we first learned that these neurons navigate an unusual developmental path (1, 2), migrating from their place of birth in the nasal compartment (NC) to their final destinations scattered in the basal forebrain in most vertebrates (*i.e.* perhaps not lamprey) (3, 4). The characterization of GnRH neuronal system development and function has become more complicated because there are many different forms of GnRH, some of which likely do not contribute to pituitary gonadotropin regulation. It is likely that neurons making different forms within the same species may have different developmental origins (5, 6). GnRH neurons that regulate the reproductive axis (sometimes referred to as GnRH-1) originate anteriorly in the NC in or around the presumptive vomeronasal organ (VNO) and then associate with the vomeronasal nerve (VNN) to travel across the nasal septum and through the cribriform plate (for previous reviews see Refs. 7–9). The exact site of origin in the NC also may depend on species (10, 11). As the VNN defasciculates after entering the brain, GnRH neurons maintain their association with a subpopulation of fibers of the VNN that take a caudal and ventral turn into the basal forebrain (12). As the migration of GnRH neurons draws to a close they dissociate from their guiding fibers to reach their final destinations (13). Thus, the migratory route has at least three distinct domains: within the NC, crossing the cribriform plate, and within the anterior forebrain. All of these regions likely have distinct molecular signatures. Deciphering the manner and the method with which GnRH neurons traverse this diversely constituted pathway is critical for understanding the development of neurons essential for reproduction. Furthermore, there may be key molecular mechanisms used in common with other migrating neurons that travel long tangential distances through varied milieu (*e.g.* ganglionic eminence to cortex) (14).

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Abbreviations: CNS, Central nervous system; FGF, fibroblast growth factor; FGFR, FGF receptor; GABA, γ-aminobutyric acid; NC, nasal compartment; VNN, vomeronasal nerve; VNO, vomeronasal organ.

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Movement and Migrations

The characterization of the migratory route and movement of GnRH neurons from their place of birth in the NC to their final destinations in the preoptic area and anterior hypothalamus has been inferred in the majority of studies by immunohistochemical comparisons from one stage of development to another (1, 2, 15, 16), after DiI labeling (17), and after olfactory ablations (18–20). *In vitro*, immortalized cell lines (21–23), explants (24, 25), and mouse head slices (26, 27) have all contributed to understanding aspects of GnRH neuron development. Mice in which living GnRH neurons are detectable by GnRH promoter-specific expression of green fluorescent protein (28) make it possible to observe GnRH neurons moving in real time (29).

We used our slice preparation that recapitulates relatively normal migration across all the compartments found *in vivo* (26) with GnRH-green fluorescent protein mice to visualize changes in GnRH neuron migratory behavior as they leave the NC to enter the forebrain (29). Early in their developmental journey, GnRH neurons in the NC move intermittently (33% of 5-min time-sampling periods), attaining rel-

atively low average rates of movement (12–13 μm/h). Their movements follow exactly along the trajectory of VNN fibers by which they are guided (12, 13, 30, 31). As they enter the brain, they increase their frequency of movement (61% of 5-min time-sampling periods). There is a significant increase in turning behavior that likely partially reflects the defasciculation of the VNN as it turns caudally (12, 32, 33) and partially reflects the release of GnRH neurons from caudal VNN fibers (13) to find their final destinations. Interestingly, the speed of movement for GnRH neurons when they are moving remains relatively constant; only the percentage of time in motion changes. Therefore, GnRH neuron movement may be governed by diverse factors that engage a common migratory mechanism.

In addition to GnRH neurons, cortical interneurons have also been shown to traverse a long tangential migratory route through a changing molecular milieu that starts in the ganglionic eminence and extends to the layers of the cerebral cortex (14, 34–36). There may be significant and interesting similarities in aspects of GnRH neuron and cortical interneuron migration. For example, cortical interneurons synthesize γ-aminobutyric acid (GABA) (37), similar to some migrating GnRH neurons (38). GABA may influence both tangential cortical interneuron (39, 40) and GnRH neuron migration (13, 41). Cortical interneurons follow axonal guides for the major portion of their journey and change their mode of movement as they come close to their target regions in the cerebral cortex (14, 35). GnRH neurons follow a portion of the VNN that uniquely turns caudally after entering the central nervous system (CNS) (12) and then may change their mode of migration after releasing from those fibers (13). This change in mode of migration is evident in live video experiments of GnRH neurons by the increased turning behavior and frequency of movement of GnRH neurons in the brain *vs.* the NC and cribriform plate compartments. Thus, the migration of neurons that traverse great distances may share important characteristics, and the study of GnRH neurons may serve as a model for long distance tangential migration within the CNS.

Migration of GnRH neurons also shares many attributes with migration of neural crest cells in mice. Interestingly, few intrinsically expressed molecules that influence murine cranial neural crest cells have been identified (42). Those that are shared with cells in the developing olfactory system and with migrating GnRH neurons include members of the ephrin/Eph family, netrin1/deleted in colorectal cancer (DCC), fibroblast growth factor (FGF) receptors (FGFRs), polysialylated neural cell adhesion molecule, stromal cell-derived factor, and Dlx expression. Ephrin/Eph signaling is important to segregate streams of migrating neural crest cells (43), and based on recent data may also be important for the migration of GnRH neurons exiting the NC (44). Enteric-derived neural crest cells use netrin-1 chemoattraction for their migration (45) and netrin-1 and its receptor DCC are important for GnRH neuron migration (32, 33). Cells fail to enter the second branchial arch in FGFR1 null mice (46), and FGFR1 is now thought to be of major importance for development of the GnRH neuronal system based on studies of Kallmann's syndrome patients (Ref. 47 and see below). Reduced polysialylated neural cell adhesion molecule may contribute to the reduction of cells in sensory organs of splotch mice (48) and alters migration of GnRH neurons (49). Ectopic expression of Dlx2 causes significant decreases in migration of neural crest cells and was recently found to alter GnRH gene expression in development (50). Recently, endothelin-1, a peptide known for developmental roles in neural crest cell migration, was shown to influence the proliferation and movements of an immortalized GnRH cell line (FNC-B4) (51).

Chemical Signals and Molecular Mechanisms

Although GnRH neurons are known to have many features in common, it has also become clear they are phenotypically heterogeneous (Fig. 1 and Table 1). GnRH neurons are heterogeneous for virtually every characteristic that they have ever been examined for, and recent single-cell PCR experiments further amplify this point (*e.g.* Refs. 52–55). GnRH neurons use vomeronasal axons as guides to migrate

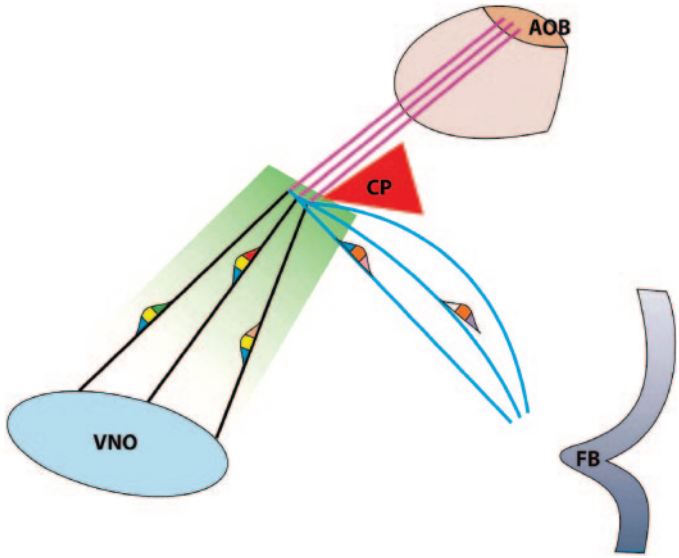

FIG. 1. GnRH neurons share many features but are also phenotypically heterogeneous. GnRH neurons use vomeronasal axons (*black lines*) as guides to migrate from the VNO to the ventral forebrain (FB) during embryonic development. Netrin-1 (*shaded gradient* in forebrain) attracts a subset of VNO axons to the ventral forebrain, but little is known about the proteins (*blue segments* of GnRH neurons) on the surface of GnRH neurons that are necessary to track along the correct axons as they migrate from the nose to the brain. These adhesion molecules may be down-regulated as neurons detach from axons in the forebrain. GnRH neurons possess necessary cytoskeletal proteins and motor functions to migrate over long distances. To explain their directed migration from the VNO across the cribriform plate (CP), one possibility would be the expression of a chemoattractant in an increasing gradient from the VNO to the rostral forebrain (shown in *green*). Such a mechanism would require that GnRH neurons express the appropriate receptor (*yellow* on GnRH neurons) as they migrate through the gradient but lose the expression or function of that receptor after migrating past the gradient. In addition, subsets of GnRH neurons are known to express a variety of other proteins (*pink*, *green*, *orange*, and *purple*, segments of neurons) that modulate their relative mobility during the course of the migration from nose to brain. Therefore, factors in the rostral forebrain caudal to the cribriform plate (*red triangle*) may regulate defasciculation of the VNN into two main branches in the CNS. Finally, axons (*blue*) targeting the ventral forebrain branch from axons (*purple*) that target the accessory olfactory bulb (AOB), not far from the cribriform plate.

TABLE 1. Genes or proteins thought to influence GnRH neurons

Gene/protein	Ref.
Molecules secreted that may influence GnRH neurons	
GABA	38
Norepinephrine and serotonin	59
Cholecystokinin	60
FGF	62, 63
Netrin-1	33
Gas6	7, 22
Hepatocyte growth factor	80
Brain-derived neurotrophic factor	64
Stromal derived factor-1	Our unpublished observation
Proteins on GnRH neuron cell surfaces	
$GABA_A$ receptor, many possible subunits	52, 54, 55
Glutamate receptor (multiple types)	81
cMet, receptor for hepatocyte growth factor	80
Ephrin A5 tyrosine kinase	44
FGFR	47, 62, 63, 79
Norepinephrine and serotonin receptors	59
Cholecystokinin receptor	60
Adhesion-related (tyrosine) kinase	7, 22, 83
Deleted in colorectal cancer (receptor for Netrin-1)	32
Polysialylated neural cell adhesion molecule	49
NELF, nasal embryonic LHRH factor	82
GnRH receptor	89
N-VGCC, N-type voltage-gated calcium channel	67
1B2 immunoreactive lactosamine as a terminal carbohydrate on proteins or lipids	9
Tag-1, transient axonal (surface) glycoprotein	12
Proteins on cell surfaces other than GnRH neurons	
Anosmin (Kal-1)	75–78
Polysialylated neural cell adhesion molecule	49
Deleted in colorectal cancer (receptor for Netrin-1)	32
Extracellular matrix	
Heparan sulfate	77
Proteins in GnRH neuron cytoplasmic compartment	
Extracellular signal-regulated protein Kinase/MAPK	7
GAP-43	31
Galanin	90
Stathmin	91
Nuclear factors	
MEFs, myocyte enhancer factors	83
Oct1, Dlx, Msx, NSCL2, Ebf2, GATA4, SCIP, and Brn2, homeodomain, helix-loop-helix, and zinc finger transcription factors	50, 74, 84–87
Estrogen receptor-β	88

GnRH is the only molecular entity selectively expressed in GnRH neurons and then perhaps not all of the time.

from the VNO to the ventral forebrain during embryonic development in most mammalian species studied. Netrin-1 plays an important role in attracting a subset of VNO axons to the ventral forebrain (32, 33), but little is known about the proteins on the surface of GnRH neurons that are necessary to track along the correct axons as they migrate from the nose to the brain. These adhesion molecules may be down-regulated as neurons detach from axons in the forebrain. Furthermore, although it is clear that all GnRH neurons possess the necessary complement of cytoskeletal proteins and motor functions to migrate over long distances, no mechanisms have been identified that explain their directed migration from the VNO across the cribriform plate. One possibility would be the expression of a chemokine or chemoattractant in an increasing gradient from the VNO to the rostral forebrain (Fig. 1, shown in *green*). Such a mechanism would require that all GnRH neurons express the appropriate chemokine receptor(s) as they migrate through the gradient but lose the receptor expression, change the function of that receptor after migrating past the chemokine gradient, or engage a new ligand-receptor signaling system. Such mechanisms have been invoked in other locations in the CNS where crossing the midline is a crucial aspect of axon guidance (56). Subsets of GnRH neurons are known to express a variety of other proteins that modulate their relative mobility during the course of the migration from nose to brain (Fig. 1 and Table 1). In addition, evidence suggests that axons targeting the ventral forebrain branch off of axons that target the accessory olfactory bulb not far from the cribriform plate. These data suggest that factors in the rostral forebrain caudal to the cribriform plate (*red triangle* in Fig. 1) regulate defasciculation of the VNN into two main branches in the CNS. Future research is expected to greatly increase our knowledge of the factors expressed by GnRH neurons as well as along the surfaces of cells and fibers along their migratory route.

Alterations of the GnRH neuronal migratory pathway, specifically the VNN, impact GnRH neuronal migration in several ways. First, changing the trajectory of the VNN changes the migration of GnRH neurons *in vitro* (9) and *in*

vivo (32, 33). Particular molecular characteristics of olfactory fibers are absolutely necessary for migration in the NC (57). These findings are consistent with a human case of Kallmann's syndrome in which olfactory fiber disorientation in the NC was associated with failure of GnRH neurons to enter the brain (58). In explant cultures of olfactory placode, GnRH neurons continue to migrate along presumptive VNN fibers (24), likely all containing peripherin (25). Similarly, in slice cultures, GnRH neurons migrate along peripherin-containing fibers (13, 29) derived from the VNN as they do *in vivo* (30). When VNN fibers are disrupted *in vitro* at the cribriform plate, the behavior of GnRH neurons in the NC is altered distal to the actual site of disruption. In the absence of evidence of changes in the VNN fibers themselves at locations distal to the disruption at the cribriform plate region, this suggests that the use of VNN fibers by GnRH neurons for guidance entails selective signaling in addition to mechanical guidance.

A number of experiments have been conducted in many laboratories to examine the influence of different chemical factors on GnRH neuron movements using several paradigms. Many factors may influence GnRH neuron migration (Table 1), including neurotransmitters (*e.g.* serotonin or norepinephrine) (59), neuropeptides (*e.g.* cholecystokinin) (60), growth factors (61–64), classical chemoattractants (*e.g.* netrin-1) (32, 33), or chemorepellents (65). Our primary experiments followed early studies of the influence of GABA on GnRH neuron migration (13, 41). Live video microscopy (see movies at http://endo.endojournals.org/cgi/content/full/en.2004-0838/DC1) showed that the $GABA_A$ receptor inhibitor bicuculline caused an increase in the percentage of frames in which GnRH neurons were moving and a decrease in the percentage of frames across which they were turning (29). Previous work had suggested that activation of the $GABA_A$ receptor caused a decrease in GnRH neuron movement (13, 41). Therefore, the result of direct observation directly supports the earlier data and extends this work to suggest specific physical mechanisms by which GnRH neuron movements are affected. Previous work also suggested that bicuculline treatment, in particular, might drive GnRH neurons apart from guiding fibers (13). The finding of a change in turning behavior in the live video experiments may be indicative of such a change in neuron/fiber interactions. Because of the heterogeneity of GnRH neurons, it will be important to test the influence of many factors directly on the behavior of GnRH neurons.

External modulators of GnRH neuron function likely converge on cascading signal transduction pathways that provide mechanisms that regulate neuronal migration (Fig. 2). Therefore, multiple signals may converge on calcium signaling as a general regulator of neuronal migration (66). Recent *in vitro* studies suggest that GnRH neurons specifically may use N-type calcium channels for such a purpose (67). The following three agents are examples of factors that may exert influences on GnRH neuron migration via convergent pathways. Activation of the $GABA_A$ receptors influences GnRH neuron movement (13, 41), and this action is likely through calcium-dependent mechanisms (67, 68). GnRH itself might influence GnRH neuron migration via an autocrine mechanism that involves calcium signaling (69).

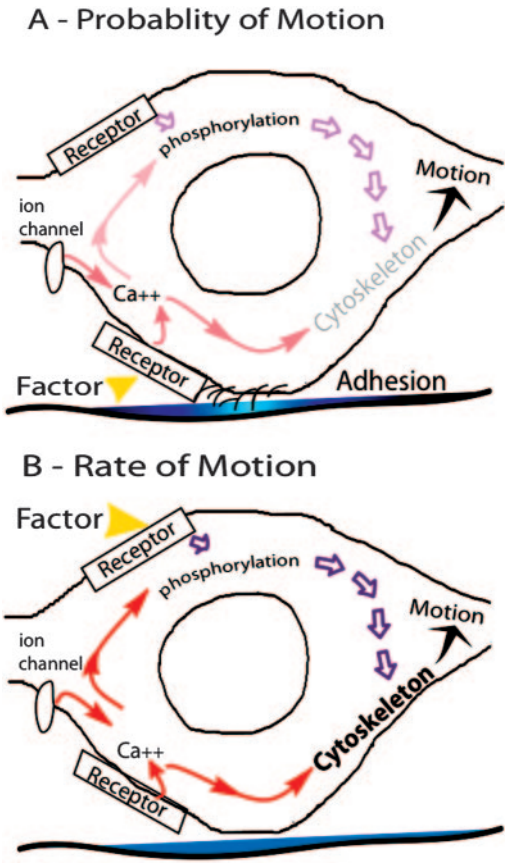

FIG. 2. External modulators may converge on central cellular regulatory mechanisms that regulate GnRH neuronal migration as well as gene transcription in GnRH neurons that also includes GnRH gene transcription. The schematic diagram represents a multitude of cell surface receptor mechanisms that signal through the cytoplasm via calcium- and phosphorylation-dependent cascades to result in either altered gene transcription or cytoskeletal changes that can ultimately result in motion that, when directed, can be seen as migration. Factors that alter the probability of motion (A) likely engage different mechanisms than those that influence the rate of motion (B).

The chemokine stromal cell-derived factor is a known regulator of cell migration (70, 71), a potential regulator of GnRH neuron migration (Schwarting, G. A., and S. A. Tobet, unpublished observations), and likely uses a calcium signal transduction pathway through its CXCR4 receptor (72). Similarly, phosphorylation cascades beginning with cell surface receptor kinases provide multiple routes to the regulation of both gene transcription and cytoskeletal reorganization that would lead to cell movement and ultimately migration (7). All of the signaling mechanisms for GnRH neuronal movement must ultimately converge on mechanisms of cell adhesion and cytoskeletal function to be able to modulate migration (73).

The migratory responses of neurons followed by live video microscopy show two types of responses to external factors (Fig. 2). These different types of responses may help determine molecular mechanisms mediating the process of GnRH neuron migration. For example, altering $GABA_A$ receptor signaling may cause changes in the probability of motion (29) or in the rate of motion (as it can in the hypothalamus) (91). One potential difference in the factors that alter the proba-

bility of motion that might differ from those that influence the rate of motion could be effects on adhesion *vs.* effects on molecular motors or specific aspects of cytoskeletal function (*e.g.* nucleokinesis). As noted earlier for GnRH neurons, GABA might be particularly likely to influence their adhesion to fibers (13) and thereby the probability of motion.

From Theory to Practice

Kallmann's syndrome provides an important bridge between basic and clinical studies (74); it is characterized by anosmia, hypogonadotrophic hypogonadism, as well as other neurological problems. The anosmia likely results from a failure to form connections between the olfactory epithelium and the olfactory bulbs. The gonadal dysfunction is the result of a deficiency in GnRH secretion. In one case of X-linked Kallmann's syndrome, these defects were directly linked to the inability of cells and axons originating in the olfactory epithelium to migrate or grow into the olfactory bulb and forebrain early in development (58). The first candidate Kallmann gene named Kal-1 (75) codes for anosmin-1, a putative adhesion molecule that may modulate neurite outgrowth (76). More recently, mutation analyses have shown that alterations in the autosomal gene coding for FGFR1 provide an additional cause of Kallmann's syndrome and the designation of FGFR1 as Kal-2 (47). At the same time, basic studies on the role of FGF in GnRH neuron development are beginning to define roles of FGFs in the specification of GnRH neuron identity (62, 63). Other studies are linking anosmin-1 as a potential coligand for FGFR1 (77), in concert with heparan sulfate, as mediators of neurite outgrowth that may yet connect to mechanisms of GnRH system development. More studies are needed to determine whether and how anosmin-1 (78) or FGFR1 plays a direct role in regulating GnRH cell migration. As for other factors influencing GnRH neurons, it appears that FGFR signaling will account for only a subpopulation of GnRH neuronal influences (63). New clinical data, however, suggest that multiple combinations of mutations in anosmin-1 and in FGFR1 will identify a greater percentage of individuals with idiopathic hypogonadotropic hypogonadism than ever before (79).

In summary, GnRH neurons, essential for reproduction in all vertebrates, migrate over long distances and through different environments. Previous studies have provided strong clues for the types of molecules and motions that one might expect along the migratory route. New studies using live video microscopy provide direct indications of the changing behavior of GnRH neurons in their different environments. Between the increasing number of molecular candidates for regulating GnRH neuron migration and the number of useful *in vitro* models to evaluate the influences of specific molecules that may be important for their migration, the coming years are likely to bring significantly more clarity to the development of the GnRH neuronal system.

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References

1. **Schwanzel-Fukuda M, Pfaff DW** 1989 Origin of luteinizing hormone-releasing hormone neurons. Nature 338:161–164
2. **Wray S, Grant P, Gainer H** 1989 Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. Proc Natl Acad Sci USA 86:8132–8136
3. **King JC, Sower SA, Anthony EL** 1988 Neuronal systems immunoreactive with antiserum to lamprey gonadotropin-releasing hormone in the brain of *Petromyzon marinus*. Cell Tissue Res 253:1–8
4. **Tobet SA, Chickering TW, Sower SA** 1996 Relationship of gonadotropin-releasing hormone (GnRH) neurons to the olfactory system in developing lamprey (*Petromyzon marinus*). J Comp Neurol 376:97–111
5. **Gorbman A, Sower SA** 2003 Evolution of the role of GnRH in animal (Metazoan) biology. Gen Comp Endocrinol 134:207–213
6. **Amano M, Okubo K, Yamanome T, Oka Y, Kawaguchi N, Aida K, Yamamori K** 2004 Ontogenic development of three GnRH systems in the brain of a pleuronectiform fish, barfin flounder. Zool Sci 21:311–317
7. **Wierman ME, Pawlowski JE, Allen MP, Xu M, Linseman DA, Nielsen-Preiss S** 2004 Molecular mechanisms of gonadotropin-releasing hormone neuronal migration. Trends Endocrinol Metab 15:96–102
8. **Wray S** 2001 Development of luteinizing hormone releasing hormone neurones. J Neuroendocrinol 13:3–11
9. **Tobet SA, Bless EP, Schwarting GA** 2001 Developmental aspect of the gonadotropin-releasing hormone system. Mol Cell Endocrinol 185:173–184
10. **el Amraoui A, Dubois PM** 1993 Experimental evidence for an early commitment of gonadotropin-releasing hormone neurons, with special regard to their origin from the ectoderm of nasal cavity presumptive territory. Neuroendocrinology 57:991–1002
11. **Whitlock KE, Wolf CD, Boyce ML** 2003 Gonadotropin-releasing hormone (GnRH) cells arise from cranial neural crest and adenohypophyseal regions of the neural plate in the zebrafish, *Danio rerio*. Dev Biol 257:140–152
12. **Yoshida K, Tobet SA, Crandall JE, Jimenez TP, Schwarting GA** 1995 Migration of luteinizing hormone-releasing hormone neurons in the developing rat is associated with a transient, caudal projection of the vomeronasal nerve. J Neurosci 15:7769–7777
13. **Bless EP, Westaway WA, Schwarting GA, Tobet SA** 2000 Effects of γ-aminobutyric $acid_A$ receptor manipulation on migrating gonadotropin-releasing hormone neurons through the entire migratory route *in vivo* and *in vitro*. Endocrinology 141:254–262
14. **Marin O, Rubenstein JL** 2003 Cell migration in the forebrain. Annu Rev Neurosci 26:441–483
15. **Ronnekleiv OK, Resko JA** 1990 Ontogeny of gonadotropin-releasing hormone-containing neurons in early fetal development of rhesus macaques. Endocrinology 126:498–511
16. **Tobet SA, Crandall JE, Schwarting GA** 1993 Relationship of migrating luteinizing hormone-releasing hormone neurons to unique olfactory system glycoconjugates in embryonic rats. Dev Biol 155:471–482
17. **Murakami S, Arai Y** 1994 Direct evidence for the migration of LHRH neurons from the nasal region to the forebrain in the chick embryo: a carbocyanine dye analysis. Neurosci Res 19:331–338
18. **Akutsu S, Takada M, Ohki-Hamazaki H, Murakami S, Arai Y** 1992 Origin of luteinizing hormone-releasing hormone (LHRH) neurons in the chick embryo: effect of the olfactory placode ablation. Neurosci Lett 142:241–244
19. **Murakami S Kikuyama S, Arai Y** 1992 The origin of the luteinizing hormone-releasing hormone (LHRH) neurons in newts (*Cynops pyrrhogaster*): the effect of olfactory placode ablation. Cell Tissue Res 269:21–27
20. **Norgren RB Jr., Gao C, Ji Y, Fritzsch B** 1995 Tangential migration of luteinizing hormone-releasing hormone (LHRH) neurons in the medial telencephalon in association with transient axons extending from the olfactory nerve. Neurosci Lett 202:9–12
21. **Fang Z, Xiong X, James A, Gordon DF, Wierman ME** 1998 Identification of novel factors that regulate GnRH gene expression and neuronal migration. Endocrinology 139:3654–3657
22. **Allen MP, Linseman DA, Udo H, Xu M, Schaack JB, Varnum B, Kandel ER, Heidenreich KA, Wierman ME** 2002 Novel mechanism for gonadotropin-releasing hormone neuronal migration involving Gas6/Ark signaling to p38 mitogen-activated protein kinase. Mol Cell Biol 22:599–613
23. **Giampietro C, Luzzati F, Gambarotta G, Giacobini P, Boda E, Fasolo A, Perroteau I** 2005 Stathmin expression modulates migratory properties of GN-11 neurons *in vitro*. Endocrinology 146:1825–1834
24. **Terasawa E, Quanbeck CD, Schulz CA, Burich AJ, Luchansky LL, Claude P** 1993 A primary cell culture system of luteinizing hormone releasing hormone neurons derived from embryonic olfactory placode in the rhesus monkey. Endocrinology 133:2379–2390

25. **Fueshko S, Wray S** 1994 LHRH cells migrate on peripherin fibers in embryonic olfactory explant cultures: an in vitro model for neurophilic neuronal migration. Dev Biol 166:331–348
26. **Tobet SA, Hanna IK, Schwarting, GA** 1996 Migration of neurons containing gonadotropin releasing hormone (GnRH) in slices from embryonic nasal compartment and forebrain. Dev Brain Res 97:287–292
27. **Tobet SA, Walker HJ, Seney ML, Yu KW** 2003 Viewing cell movements in the developing neuroendocrine brain. Integr Comp Biol 43:794–801
28. **Suter KJ, Song WJ, Sampson TL, Wuarin JP, Saunders JT, Dudek FE, Moenter SM** 2000 Genetic targeting of green fluorescent protein to gonadotropin-releasing hormone neurons: characterization of whole-cell electrophysiological properties and morphology. Endocrinology 141:412–419
29. **Bless EP, Walker HJ, Yu KW, Knoll JG, Moenter SM, Schwarting GA, Tobet SA** 2005 Live view of gonadotropin-releasing hormone containing neuron migration. Endocrinology 146:463–468
30. **Wray S, Key S, Qualls R, Fueshko SM** 1994 A subset of peripherin positive olfactory axons delineates the luteinizing hormone releasing hormone neuronal migratory pathway in developing mouse. Dev Biol 166:349–354
31. **Livne I, Gibson MJ, Silverman AJ** 1993 Biochemical differentiation and intercellular interactions of migratory gonadotropin-releasing hormone (GnRH) cells in the mouse. Dev Biol 159:643–656
32. **Schwarting GA, Kostek C, Bless EP, Ahmad N, Tobet SA** 2001 Deleted in colorectal cancer (DCC) regulates the migration of luteinizing hormone-releasing hormone neurons to the basal forebrain. J Neurosci 21:911–919
33. **Schwarting GA, Raitcheva D, Bless EP, Ackerman SL, Tobet S** 2004 Netrin 1-mediated chemoattraction regulates the migratory pathway of LHRH neurons. Eur J Neurosci 19:11–20
34. **Anderson SA, Eisenstat DD, Shi L, Rubenstein JL** 1997 Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. Science 278:474–476
35. **Nadarajah B, Parnavelas JG** 2002 Modes of neuronal migration in the developing cerebral cortex. Nat Rev Neurosci 3:423–432
36. **Ang ES Jr., Haydar TF, Gluncic V, Rakic P** 2003 Four-dimensional migratory coordinates of GABAergic interneurons in the developing mouse cortex. J Neurosci 23:5805–5815
37. **Xu Q, de la Cruz E, Anderson SA** 2003 Cortical interneuron fate determination: diverse sources for distinct subtypes? Cereb Cortex 13:670–676
38. **Tobet SA, Chickering TW, King JC, Stopa EG, Kim K, Kuo-Leblank V, Schwarting GA** 1996 Expression of γ-aminobutyric acid and gonadotropin-releasing hormone during neuronal migration through the olfactory system. Endocrinology 137:5415–5420
39. **Soria JM, Valdeolmillos M** 2002 Receptor-activated calcium signals in tangentially migrating cortical cells. Cereb Cortex 12:831–839
40. **Lujan R, Shigemoto R, Lopez-Bendito G** 2005 Glutamate and GABA receptor signalling in the developing brain. Neuroscience 130:567–580
41. **Fueshko SM, Key S, Wray S** 1998 GABA inhibits migration of luteinizing hormone-releasing hormone neurons in embryonic olfactory explants. J Neurosci 18:2560–2569
42. **Trainor PA** 2005 Specification of neural crest cell formation and migration in mouse embryos. Semin Cell Dev Biol 16:683–693
43. **Robinson V, Smith A, Flenniken AM, Wilkinson DG** 1997 Roles of Eph receptors and ephrins in neural crest pathfinding. Cell Tissue Res 290:265–274
44. **Gamble JA, Karunadasa DK, Pape JR, Skynner MJ, Todman MG, Bicknell RJ, Allen JP, Herbison AE** 2005 Disruption of ephrin signaling associates with disordered axophilic migration of the gonadotropin-releasing hormone neurons. J Neurosci 25:3142–3150
45. **Jiang Y, Liu MT, Gershon MD** 2003 Netrins and DCC in the guidance of migrating neural crest-derived cells in the developing bowel and pancreas. Dev Biol 258:364–384
46. **Trokovic R, Trokovic N, Hernesniemi S, Pirvola U, Vogt Weisenhorn DM, Rossant J, McMahon AP, Wurst W, Partanen J** 2003 FGFR1 is independently required in both developing mid- and hindbrain for sustained response to isthmic signals. EMBO J 22:1811–1823
47. **Dode C, Levilliers J, Dupont JM, De Paepe A, Le Du N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, Pecheux C, Le Tessier D, Cruaud C, Delpech M, Speleman F, Vermeulen S, Amalfitano A, Bachelot Y, Bouchard P, Cabrol S, Carel JC, Delemarre-van de Waal H, Goulet-Salmon B, Kottler ML, Richard O, Sanchez-Franco F, Saura R, Young J, Petit C, Hardelin JP** 2003 Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat Genet 33:463–465
48. **Glogarova K, Buckiova D** 2004 Changes in sialylation in homozygous Sp2H mouse mutant embryos. Birth Defects Res A Clin Mol Teratol 70:142–152
49. **Yoshida K, Rutishauser U, Crandall JE, Schwarting GA** 1999 Polysialic acid facilitates migration of luteinizing hormone-releasing hormone neurons on vomeronasal axons. J Neurosci 19:794–801
50. **Givens ML, Rave-Harel N, Goonewardena VD, Kurotani R, Berdy SE, Swan CH, Rubenstein JL, Robert B, Mellon PL** 2005 Developmental regulation of gonadotropin-releasing hormone gene expression by the MSX and DLX homeodomain protein families. J Biol Chem 280:19156–19165.
51. **Romanelli RG, Barni T, Maggi M, Luconi M, Failli P, Pezzatini A, Morelli A, Maggi R, Zaninetti R, Salerno R, Ambrosini S, Marini M, Rotella CM, Vannelli GB** 2005 Role of endothelin-1 in the migration of human olfactory gonadotropin-releasing hormone-secreting neuroblasts. Endocrinology 146: 4321–4330
52. **Pape JR, Skynner MJ, Sim JA, Herbison AE** 2001 Profiling γ-aminobutyric acid ($GABA_A$) receptor subunit mRNA expression in postnatal gonadotropin-releasing hormone (GnRH) neurons of the male mouse with single cell RT-PCR. Neuroendocrinology 74:300–308
53. **Parhar IS, Ogawa S, Sakuma Y** 2004 Laser-captured single digoxigenin-labeled neurons of gonadotropin-releasing hormone types reveal a novel G protein-coupled receptor (Gpr54) during maturation in cichlid fish. Endocrinology 145:3613–3618
54. **Todman MG, Han SK, Herbison AE** 2005 Profiling neurotransmitter receptor expression in mouse gonadotropin-releasing hormone neurons using green fluorescent protein-promoter transgenics and microarrays. Neuroscience 132: 703–712
55. **Temple JL, Wray S** 2005 Developmental changes in GABA receptor subunit composition within the gonadotrophin-releasing hormone-1 neuronal system. J Neuroendocrinol 17:591–599
56. **Woods CG** 2004 Neuroscience. Crossing the midline. Science 304:1455–1456
57. **Murakami S, Kamiya M, Akutsu S, Seki T, Kuwabara Y, Arai, Y** 1995 Straying phenomenon of migrating LHRH neurons and highly polysialylated NCAM in the chick embryo. Neurosci Res 22:109–115
58. **Schwanzel-Fukuda M, Bick D, Pfaff DW** 1989 Luteinizing hormone-releasing hormone (LHRH)-expressing cells do not migrate normally in an inherited hypogonadal (Kallmann) syndrome. Mol Brain Res 6:311–326
59. **Pronina T, Ugrumov M, Calas A, Seif I, Tramu G** 2003 Influence of monoamines on differentiating gonadotropin-releasing hormone neurones in foetal mice. J Neuroendocrinol 15:925–932
60. **Giacobini P, Kopin AS, Beart PM, Mercer LD, Fasolo A, Wray S** 2004 Cholecystokinin modulates migration of gonadotropin-releasing hormone-1 neurons. J Neurosci 24:4737–4748
61. **Gibson MJ, Ingraham L, Dobrjansky A** 2000 Soluble factors guide gonadotropin-releasing hormone axonal targeting to the median eminence. Endocrinology 141:3065–3071
62. **Gill JC, Moenter SM, Tsai PS** 2004 Developmental regulation of gonadotropin-releasing hormone neurons by fibroblast growth factor signaling. Endocrinology 145:3830–3839
63. **Tsai PS, Moenter SM, Postigo HR, El Majdoubi M, Pak TR, Gill JC, Paruthiyil S, Werner S, Weiner RI** 2005 Targeted expression of a dominant-negative fibroblast growth factor (FGF) receptor in gonadotropin-releasing hormone (GnRH) neurons reduces FGF responsiveness and the size of GnRH neuronal population. Mol Endocrinol 19:225–236
64. **Cronin AS, Horan TL, Spergel DJ, Brooks AN, Hastings MH, Ebling FJ** 2004 Neurotrophic effects of BDNF on embryonic gonadotropin-releasing hormone (GnRH) neurons. Eur J Neurosci 20:338–344
65. **Wong K, Park HT, Wu JY, Rao Y** 2002 Slit proteins: molecular guidance cues for cells ranging from neurons to leukocytes. Curr Opin Genet Dev 12:583–591
66. **Komuro H, Kumada T** 2005 Ca^{2+} transients control CNS neuronal migration. Cell Calcium 37:387–393
67. **Toba Y, Pakiam JG, Wray S** 2005 Voltage-gated calcium channels in developing GnRH-1 neuronal system in the mouse. Eur J Neurosci 22:79–92
68. **Bolteus AJ, Bordey A** 2004 GABA release and uptake regulate neuronal precursor migration in the postnatal subventricular zone. J Neurosci 24:7623–7631
69. **Romanelli RG, Barni T, Maggi M, Luconi M, Failli P, Pezzatini A, Pelo E, Torricelli F, Crescioli C, Ferruzzi P, Salerno R, Marini M, Rotella CM, Vannelli GB** 2004 Expression and function of gonadotropin-releasing hormone (GnRH) receptor in human olfactory GnRH-secreting neurons: an autocrine GnRH loop underlies neuronal migration. J Biol Chem 279:117–126
70. **Lazarini F, Tham TN, Casanova P, Arenzana-Seisdedos F, Dubois-Dalcq M** 2003 Role of the α-chemokine stromal cell-derived factor (SDF-1) in the developing and mature central nervous system. Glia 42:139–148
71. **Belmadani A, Tran PB, Ren D, Assimacopoulos S, Grove EA, Miller RJ** 2005 The chemokine stromal cell-derived factor-1 regulates the migration of sensory neuron progenitors. J Neurosci 25:3995–4003
72. **Tran PB, Ren D, Veldhouse TJ, Miller RJ** 2004 Chemokine receptors are expressed widely by embryonic and adult neural progenitor cells. J Neurosci Res 76:20–34
73. **Schaar BT, McConnell SK** 2005 Cytoskeletal coordination during neuronal migration. Proc Natl Acad Sci USA 102:13652–13657
74. **Gonzalez-Martinez D, Hu Y, Bouloux PM** 2004 Ontogeny of GnRH and olfactory neuronal systems in man: novel insights from the investigation of inherited forms of Kallmann's syndrome. Front Neuroendocrinol 25:108–130
75. **Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, Carrozzo R, Maestrini E, Pieretti M, Taillon-Miller P, Brown CJ, Willard HF, Lawrence C, Persico MG, Camarino G, Ballabio A** 1991 A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature 353:529–536
76. **Soussi-Yanicostas N, Faivre-Sarrailh C, Hardelin JP, Levilliers J, Rougon G, Petit C** 1998 Anosmin-1 underlying the X chromosome-linked Kallmann syndrome is an adhesion molecule that can modulate neurite growth in a cell-type specific manner. J Cell Sci 111:2953–2965
77. **Gonzalez-Martinez D, Kim SH, Hu Y, Guimond S, Schofield J, Winyard P, Vannelli GB, Turnbull J, Bouloux PM** 2004 Anosmin-1 modulates fibroblast

growth factor receptor 1 signaling in human gonadotropin-releasing hormone olfactory neuroblasts through a heparan sulfate-dependent mechanism. J Neurosci 24:10384–10392
78. **Cariboni A, Pimpinelli F, Colamarino S, Zaninetti R, Piccolella M, Rumio C, Piva F, Rugarli EI, Maggi R** 2004 The product of X-linked Kallmann's syndrome gene (KAL1) affects the migratory activity of gonadotropin-releasing hormone (GnRH)-producing neurons. Hum Mol Genet 13:2781–2791
79. **Pitteloud N, Acierno JS, Meysing A, Metzger D, Hayes FJ, Dwyer AA, Hughes VA, Yialamas M, Hall JE, Grant E, Mohammadi M, Crowley Jr WF,** Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci, USA, in press
80. **Giacobini P, Giampietro C, Fioretto M, Maggi R, Cariboni A, Perroteau I, Fasolo A** 2002 Hepatocyte growth factor/scatter factor facilitates migration of GN-11 immortalized LHRH neurons. Endocrinology 143:3306–3315
81. **Simonian SX, Herbison AE** 2001 Differing spatially restricted roles of ionotropic glutamate receptors in regulating the migration of GnRH neurons during embryogenesis. J Neurosci 21:934–943
82. **Kramer PR, Wray S** 2000 Novel gene expressed in nasal region influences outgrowth of olfactory axons and migration of luteinizing hormone-releasing hormone (LHRH) neurons. Genes Dev 14:1824–1834
83. **Allen MP, Xu M, Zeng C, Tobet SA, Wierman ME** 2000 Myocyte enhancer factors-2B and -2C are required for adhesion related kinase repression of neuronal gonadotropin releasing hormone gene expression. J Biol Chem 275: 39662–39670
84. **Lawson MA, Mellon PL** 1998 Expression of GATA-4 in migrating gonadotropin-releasing neurons of the developing mouse. Mol Cell Endocrinol 140: 157–161
85. **Wolfe A, Kim HH, Tobet S, Stafford DE, Radovick S** 2002 Identification of a discrete promoter region of the human GnRH gene that is sufficient for directing neuron-specific expression: a role for POU homeodomain transcription factors. Mol 16:435–449
86. **Corradi A, Croci L, Broccoli V, Zecchini S, Previtali S, Wurst W, Amadio S, Maggi R, Quattrini A, Consalez GG** 2003 Hypogonadotropic hypogonadism and peripheral neuropathy in Ebf2-null mice. Development 130:401–410
87. **Kruger M, Ruschke K, Braun T** 2004 NSCL-1 and NSCL-2 synergistically determine the fate of GnRH-1 neurons and control necdin gene expression. EMBO J 23:4353–4364
88. **Sharifi N, Reuss AE, Wray S** 2002 Prenatal LHRH neurons in nasal explant cultures express estrogen receptor-β transcript. Endocrinology 143:2503–2507
89. **Martinez-Fuentes AJ, Hu L, Krsmanovic LZ, Catt KJ** 2004 Gonadotropin-releasing hormone (GnRH) receptor expression and membrane signaling in early embryonic GnRH neurons: role in pulsatile neurosecretion. Mol Endocrinol 18:1808–1817
90. **Key S, Wray S** 2000 Two olfactory placode derived galanin subpopulations: luteinizing hormone-releasing hormone neurones and vomeronasal cells. J Neuroendocrinol 12:535–545
91. **Dellovade TL, Davis AM, Ferguson C, Sieghart W, Homanics GE, Tobet SA** 2001 GABA influences the development of the ventromedial nucleus of the hypothalamus. J Neurobiol 49:264–276

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