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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).

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 Dil 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 defas-
ciculation 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 inter-neuron 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.

Migrating GnRH neurons also share many attributes with migration of neural crest cells in mice. Interestingly, few intrinsically expressed molecules that influence murine cran-
ial 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), fi-
broblast growth factor (FGF) receptors (FGFRs), polysialyl-
lated neural cell adhesion molecule, stromal cell-derived factor, and Dlx expression. Ephrin/Eph signaling is import-
ant 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 chemotraction 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 develop-
ment of the GnRH neuronal system based on studies of Kallmann’s syndrome patients (Ref. 47 and see below). Re-
duced polysialylated neural cell adhesion molecule may con-
tribute 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 fea-
tures in common, it has also become clear they are pheno-
typically 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...
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.

**TABLE 1. Genes or proteins thought to influence GnRH neurons**

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

GnRH is the only molecular entity selectively expressed in GnRH neurons and then perhaps not all of the time.
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 influence GnRH neuron migration (Table 1), including neurotransmitters (e.g. serotonin or noradrenaline) (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/endo.2004-0838/DC1) showed that the GABAA 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 GABAA 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). These different types of responses may help determine molecular mechanisms mediating the process of GnRH neuron migration via convergent pathways. Activation of the GABAA 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).

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 GABAA 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-
ibility 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, hypogonadotropic hypogonadism, as well as other neurological problems. The anosmia likely results from a failure to form connections between the olfactory epithelium and the olfactory bulb. 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 FGF 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 account for only a subpopulation of GnRH neuronal influences (63).

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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