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## Live view of gonadotropin-releasing hormone containing neuron migration

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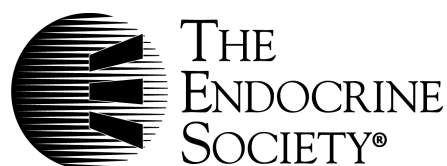
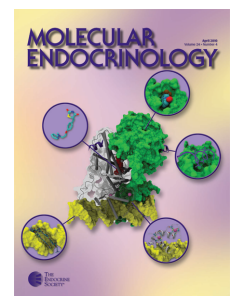
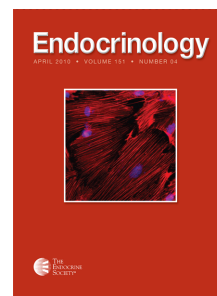
# Endocrinology

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Elizabeth P. Bless, Heather J. Walker, Kwok W. Yu, J. Gabriel Knoll, Suzanne M. Moenter, Gerald A. Schwarting and Stuart A. Tobet

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# Live View of Gonadotropin-Releasing Hormone Containing Neuron Migration

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**Neurons that synthesize GnRH control the reproductive axis and migrate over long distances and through different environments during development. Prior studies provided strong clues for the types of molecules encountered and movements expected along the migratory route. However, our studies provide the first real-time views of the behavior of GnRH neurons in the context of an *in vitro* preparation that maintains conditions comparable to those *in vivo*. The live views 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 changes in direction after they enter the brain. Perturbations of guiding fibers distal to moving GnRH neurons in the nasal compartment influenced movement without detectable changes in the fibers in the immediate vicinity of moving GnRH neurons. This suggests that the use of fibers by GnRH neurons for guidance may entail selective signaling in addition to mechanical guidance. These studies establish a model to evaluate the influences of specific molecules that are important for their migration. (Endocrinology 146: 463–468, 2005)**

**T**HE NEURONS THAT synthesize and release GnRH form the final common pathway for the central regulation of fertility. These neurons navigate an unusual developmental path, migrating from their place of birth in the nasal compartment to their final destinations scattered in the basal forebrain. GnRH neurons originate anteriorly in the nasal compartment in or around the presumptive vomeronasal organ and then associate with the vomeronasal nerve (VNN) to travel across the nasal septum and over the cribriform plate (reviewed in Refs. 1–3). As the VNN defasciculates once in 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 (4). Toward the end of their migratory route, GnRH neurons dissociate from their guiding fibers to find their final destinations (5). This migratory route thus has the following three distinct domains: within the nasal compartment, crossing the cribriform plate, and within the anterior forebrain. All of these domains have distinct molecular and cellular profiles. Therefore, 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 aspects in common with other migrating neurons that travel long tangential distances through varied milieux (*e.g.* ganglionic eminence to cortex) (6).

In the past, we and others have ascertained information about the timing of GnRH migration (7–10) and effects of

different molecules (5, 11, 12) on GnRH development, but conclusions were based on techniques that depend on differences between a starting point in one animal and an end point in another animal. Recently, we have taken advantage of transgenic mice that contain green fluorescent protein driven by the GnRH promoter (13) to create organotypic slices that allow us to follow live GnRH neurons along their migratory route. This technique has given us the opportunity to collect information on the distinctive behaviors displayed by GnRH neurons as they move through different compartments toward their final destinations.

## Materials and Methods

### *Animals and collection of organotypic slices*

Homozygous breeding pairs of GnRH-GFP transgenic mice were maintained at the Shriver Center on a 14-h light, 10-h dark cycle and provided food and water *ad libitum*. All procedures were approved by the Animal Care and Use Committees of The Shriver Center at The University of Massachusetts Medical School (Waltham, MA) and Colorado State University (Fort Collins, CO). The animals were time-bred, with the presence of a vaginal plug designated as embryonic d 0 (E0). Pregnant mice were deeply anesthetized on the d 13 of gestation (or E13) with 80 mg/kg ketamine and 8 mg/kg xylazine. Embryos were removed one at a time for dissection, and slices were cut through the whole head as previously described (5, 14). The baseline data presented was taken from 27 slices derived from 14 litters. Briefly, pups were decapitated into cold Krebs cutting buffer, the skin was removed to the eyes, and heads were then embedded in 8% agarose (Type VII-A; Sigma Chemical Co., St. Louis, MO). All heads were cut in Krebs buffer in the parasagittal plane at 250  $\mu$ m on a vibrating microtome (model VT1000S; Leica, Bannockburn, IL). The sections from each head were then placed in individual 60-mm dishes with 10 ml of cold sterile filtered Krebs buffer containing 1 ml HEPES, 1 ml penicillin/streptomycin (100X; Sigma), and 200  $\mu$ l gentamycin (Sigma). All sections remained in the sterile Krebs for at least 15 min but for no more than 2 h, at which time slices were washed with 4 ml of media [94.3 ml DMEM F12 Phenol Red Free (GIBCO-Invitrogen Corporation, Carlsbad, CA), 2 ml B-27 supplement (GIBCO-Invitrogen), 1 ml glutamate, 1.33 ml penicillin/streptomycin (Sigma),

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Abbreviations: E0, Embryonic d 0; GABA,  $\gamma$ -aminobutyric acid; GFP, green fluorescent protein; NGS, normal goat serum; RT, room temperature; VNN, vomeronasal nerve.

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248  $\mu$ l L-glutamine (Sigma), and 1.1 ml D-glucose] followed by a 35-min incubation in a 36 C incubator at 5% CO<sub>2</sub>. After incubation, the slices were again washed with 10 ml of the previously mentioned media and plated in the center of 25-mm round vitrogen-coated coverslips followed by a 1-h incubation period. The slices were covered with a sterile filtered Vitrogen overlay [1 ml Vitrogen-100 (Cohesion Technologies, Inc., Palo Alto, CA), 125  $\mu$ l of 10 $\times$  phenol red free MEM (Cellgro, Herndon, VA), 23  $\mu$ l penicillin/streptomycin, and 33  $\mu$ l 1 M NaHCO<sub>2</sub>] and incubated for 1.5 h until the overlay solidified, at which point 1 ml of media [serum-free Neurobasal medium with B27 supplement (Invitrogen, Carlsbad, CA)] and supplemented with 134 U/ml penicillin, 0.13 mg/ml streptomycin, 1.34 mM glutamine, and 0.5% glucose) was pipetted into the dish. Slices were maintained in Neurobasal media until use for video microscopy (at least 18 h post plating and up to 3 d). For one additional experiment, slices from E14 embryos were treated as outlined earlier for a 2-h collection of baseline video data (seven cells from two slices across two litters), and then bicuculline was added (target concentration of 20  $\mu$ M after addition every 30 min), and data collection was continued for an additional 2-h period.

### Video microscopy

For video microscopy (15), coverslips containing slices were washed three times with warm (36 C) sterile filtered Krebs containing 11 mM glucose and 25 mM NaHCO<sub>2</sub> and placed into the video viewing apparatus with fresh Krebs buffer constantly circulating over the slice and the temperature of the bath maintained at 35–37 C. GnRH neurons were visualized using a fluorescein isothiocyanate filter set on a Nikon TE200 microscope (Nikon USA, Melville, NY) using a 20 $\times$  plan Apo phase objective (0.75 numerical aperture), and images were digitized using a Dage RC300 camera (Dage-MTI, Michigan City, IN). Every cell was checked for specific GFP fluorescence by examining each field using a tetramethyl rhodamine isothiocyanate filter. Any cell fluorescing in both channels (indicating nonspecific autofluorescence) was not analyzed. An IPLab Spectrum (Scanalytics, Inc., Fairfax, VA) imaging script was created to take a z-series of three images at 5- $\mu$ m intervals through the tissue, at 5-min periods over the duration of the recording period (2–6 h). After video recording fluorescent data from any one slice, a series of bright-field images was taken of the tissue at several magnifications to demarcate the location of the viewed cells within their correct compartment.

### Video analysis

Each z-series was flattened into a single frame by selecting the brightest pixel value from the stack for each x-y coordinate. These frames then comprised a video sequence for motion analysis. Video sequences were adjusted for slice movement by matching nondrifting background objects (nonspecific autofluorescent surface objects) that appeared in each frame. Cells demonstrating specific GFP fluorescence were analyzed for absolute distance and net distance traveled, average speed and maximum speed, percentage of migratory time, and the linearity of movement. Absolute distance was calculated as the sum of distances traveled from frame to frame. Net distance was calculated as the distance between a cell's starting position (location in first frame) and ending position (location in last frame observed). Speed was calculated by dividing the duration of time that a cell was visible in a video sequence by the absolute distance traveled by that cell. Maximum speed was calculated by dividing the greatest distance one cell traveled between two consecutive frames by the time interval between those frames (5 min). The percentage of migratory time of each cell was defined as the ratio of movement frames to idle frames, where a movement frame was defined as a frame in which a cell traveled a distance greater than or equal to 1  $\mu$ m from the previous frame. There were no notable differences in the characteristic of cell motion within specific compartments viewed on d 1, 2, or 3 after plating. Therefore, the data presented is summarized by compartment and not by day after plating.

### Immunocytochemistry

All slices were fixed in 4% paraformaldehyde for 15 min after video recording. They were then stored in 0.1 M phosphate buffer until used for immunocytochemistry. Slices were carefully removed from their

respective coverslips and were placed in fresh Petri dishes. The slices were then washed three times in 0.05 M PBS (pH 7.4), treated with 0.5% sodium borohydride for 2 h, and then washed twice for 5 min each before incubating in blocking reagent [5% normal goat serum (NGS), 0.3% Triton X-100, 1.0% H<sub>2</sub>O<sub>2</sub> in PBS]. All slices went through two 1-h blocking steps before a six-night incubation in primary antiserum, either GFP (1:10,000; Molecular Probes, Eugene, OR) or the GnRH antibody LR-1 (1:10,000; generously provided by Dr. Robert Benoit, McGill University, Montreal, Quebec, Canada), in 5% NGS and 0.3% Triton X-100 in PBS. Prior health of GnRH neurons was indicated by immunocytochemical results showing fusiform morphology and extended processes as opposed to rounded cells. After washing slices in 1% NGS and 0.02% Triton X-100 in PBS, the slices were incubated overnight in biotinylated secondary antibody. The following morning, slices were washed four times at room temperature (RT) in 0.02% Triton X-100 in PBS and then incubated at RT in Vectastain ABC reagent for 3 h (Vector Laboratories, Burlingame, CA). After further washes in PBS, slices were incubated for 15 min in 0.025% 3,3'-diaminobenzidine in PBS, and then 10  $\mu$ l of H<sub>2</sub>O<sub>2</sub> was added (final, 0.02% H<sub>2</sub>O<sub>2</sub>), and the reaction was allowed to run for 20 min at RT to produce a brown reaction product. Washing in PBS terminated the reaction. To assess the integrity of slices for which video sequences were recorded, we used peripherin immunocytochemistry (16). This allowed us to view whether or not vomeronasal fibers were intact. These slices were incubated with the primary antiserum for peripherin (1/3000; Chemicon, Temecula, CA) at 4 C for three additional nights. The slices were then washed four times in 0.02% Triton X-100 in PBS and incubated overnight in Cy3-conjugated goat antirabbit secondary antibody (Jackson ImmunoResearch, West Grove, PA). Fluorescent slices were washed several times and then mounted between two coverslips using Advantage Aqueous Permanent Mounting Media (Accurate Chemical & Scientific Corporation, Westbury, NY).

## Results

### *GFP-containing live GnRH neurons in vitro maintain the same shape as those after fixation and immunocytochemical detection of GnRH*

GnRH neurons were identified based on GFP fluorescence in the nasal compartment, cribriform plate region, and anterior forebrain of slices taken from E13 mouse heads and maintained *in vitro* for up to 3 d (Fig. 1, A–C). Fluorescent cells had the same fusiform morphology *in vitro* as immunoreactive cells examined after perfusion fixation *in vivo* (Fig. 1D). This was true whether the tissue was reacted for GFP (Fig. 1D) or GnRH (data not shown). Localization of immunoreactive peripherin showed that the VNN was intact across the cribriform plate in a majority of head slices (Fig. 1, E and F). In the cases where peripherin immunocytochemistry indicated fiber disruption at the cribriform plate, movement data were summarized separately (see below, *e.g.* Fig. 4). The ability to detect fluorescence in live GnRH neurons *in vitro* from embryonic slices was notably less than the sensitivity of *post hoc* immunocytochemistry for either GnRH or GFP. This was partially due to physical limitations of optical resolution through a 250- $\mu$ m-thick slice and partially due to the enhanced sensitivity inherent in the amplification process in immunocytochemistry.

### *The rate of GnRH neuron movement depends on location along the migratory route*

GnRH neurons in the brain moved faster than GnRH neurons in the nasal compartment. The average rate of motion for GnRH neurons in the nasal compartment was  $12.7 \pm 1.5$   $\mu$ m/h, and in the cribriform plate, it was  $11.7 \pm 1.8$   $\mu$ m/h, whereas the average rate of motion of GnRH neurons in the

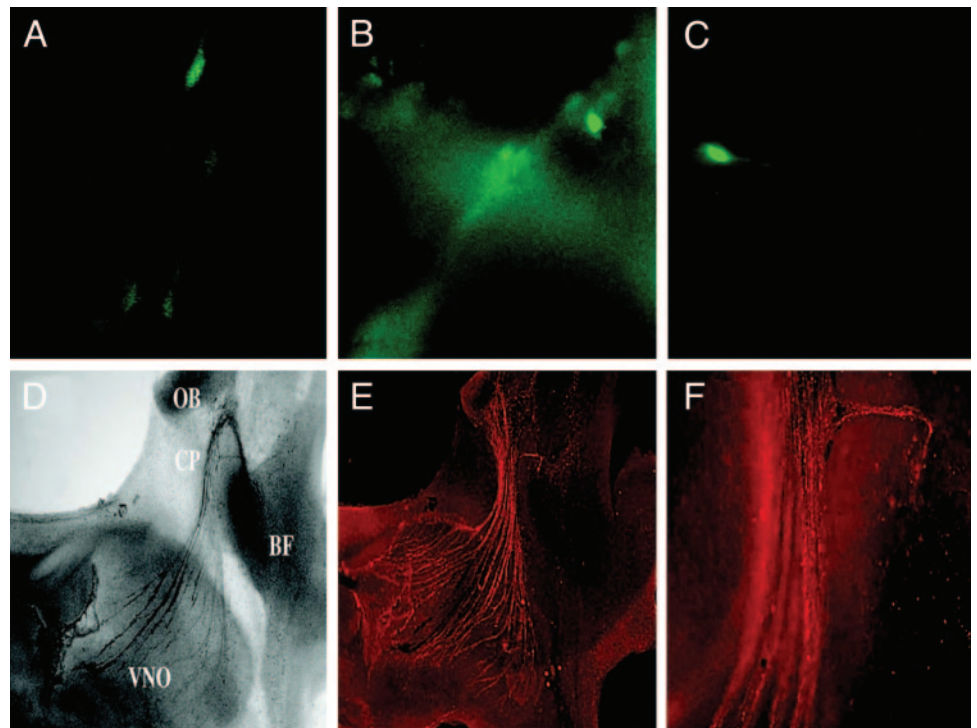


FIG. 1. Live GFP-labeled GnRH neurons maintain similar morphology *in vitro* as that seen *post hoc* via GFP (and GnRH, data not shown) immunocytochemistry. A–C, Live GFP-labeled GnRH neurons in 250- $\mu$ m-thick slices through the nasal compartment (A), cribriform plate (B), and forebrain (C). D, The entire GnRH migratory route in a 250- $\mu$ m-thick slice of an E13 mouse head after GFP immunocytochemistry. E and F, Peripherin immunocytochemistry demonstrating an intact cribriform plate in the same E13 mouse head slice as that shown in D. CP, Cribriform plate; BF, basal forebrain; VNO, vomeronasal organ; OB, olfactory bulb.

brain was approximately twice as fast at  $23.8 \pm 3.45 \mu\text{m}/\text{h}$  (Fig. 2A;  $F_{(2,51)} = 7.35$ ;  $P < 0.05$ ). To view sample videos, see supplemental movies, published on The Endocrine Society's Journals Online web site at <http://endo.endojournals.org>. This difference in apparent rate of motion was due to a difference in the number of instances in which there was frame-to-frame movement. Another analysis was performed using  $12 \mu\text{m}/\text{h}$  as a cutoff to determine incidence of movement (as  $12 \mu\text{m}$  represents the size of a typical GnRH neuron and motion defined as greater than 1-cell diameter). This additional analysis demonstrated that there was a significant difference ( $F_{(2,51)} = 13.65$ ;  $P < 0.0001$ ) in the percentage of frames in which cells moved in the nasal compartment ( $33 \pm 0.03\%$ ) and the cribriform plate ( $32.6 \pm 0.04\%$ ) *vs.* the forebrain ( $61 \pm 0.07\%$ ) (Fig. 2B). If the motionless frames are omitted, then the average rate of motion for GnRH neurons across frames when they were moving was similar from across all compartments (approximately  $30 \mu\text{m}/\text{h}$ ). The maximum speeds (distance over a 5-min period) also did not differ by compartment and were approximately  $75 \mu\text{m}/\text{h}$ .

#### *The course of GnRH neuron movement depends on location along the migratory route*

GnRH neurons were more likely to travel a straight-line path in the nasal compartment than in the forebrain. Examination of schematic diagrams of the motion of representative GnRH neurons over time (Fig. 3A) shows notably more turning behavior in neurons tracked in the forebrain. To quantify this behavior for any given movement, the angle (in degrees) was calculated between the cell position in one frame and the cell position in the previous frame. If there was a  $90^\circ$  or more difference between this angle and the angle of the previous frame-to-frame movement, then the point arbitrarily counted

as a direction change point. The direction change percentage was calculated as the ratio of direction change points to total movement points. The average direction change percentage for neurons in the brain ( $44 \pm 0.05\%$ ) was significantly greater than that in the nasal compartment ( $26 \pm 0.05\%$ ) (Fig. 3B;  $F_{(2,51)} = 3.82$ ;  $P < 0.05$ , Tukey-Kramer *post hoc* comparison,  $P < 0.05$ ), demonstrating a quantitative increase in the turning behavior of GnRH neurons in the brain.

#### *The course of GnRH neuron movements in the nasal compartment depends on fiber extension at the cribriform plate*

GnRH neuron movements in the nasal compartment were usually straightforward (Fig. 3A). However, when the slice plating process disrupted VNN fibers, there were notable effects on GnRH neuron movement. Rather than move in their normal approximation of a straight line, GnRH neurons on or adjacent to disrupted fibers meandered more. In particular, the total distance moved was no longer similar to the net distance moved (nasal compartment with intact fibers, absolute distance/net distance =  $1.93 \pm 0.2$ ; nasal compartment with disrupted fibers, absolute distance/net distance =  $3.21 \pm 0.68$ ;  $t_{(30)} = 2.42$ ;  $P < 0.05$ ; Fig. 4). Other than the cut ends at the cribriform plate region, peripherin immunocytochemistry revealed no discernible differences in the VNN fibers at the point where GnRH neurons were behaving differently.

#### *GnRH neuron movements in the brain compartment were altered by antagonism of $\gamma$ -aminobutyric acid ( $\text{GABA}$ )<sub>A</sub> receptor function using bicuculline*

As noted earlier, GnRH neuron movements in the brain compartment from slices started at E13 were more frequent

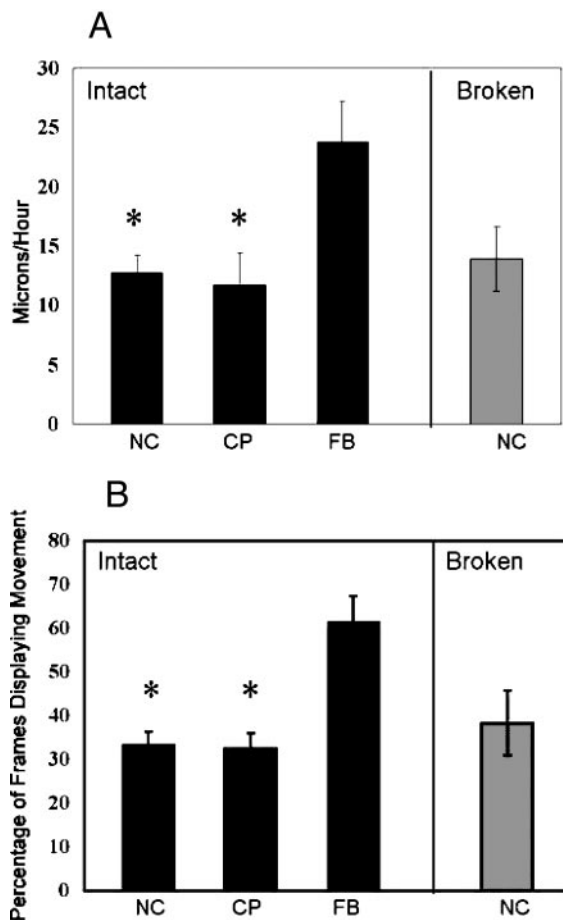


FIG. 2. The rate of movement and the percentage of time spent in motion were altered as GnRH neurons moved through different compartments. A, The average speed of GnRH neurons was significantly faster in the forebrain (FB;  $n = 19$ ) compared with that in the nasal compartment (NC;  $n = 23$ ) or cribriform plate (CP;  $n = 12$ ). B, The percentage of frames in which GnRH neurons were in motion (moving  $1 \mu\text{m}$ ) was significantly greater in the forebrain compared with the nasal compartment or cribriform plate. \*,  $P < 0.05$  compared with forebrain (Tukey-Kramer multiple comparison test). Broken refers to slices in which the fiber connections between the nasal compartment and the basal forebrain were disrupted as a function of plating.

and included more turns than movements in the nasal compartment (Fig. 3A). In an initial experiment to evaluate the potential influence of GABA on GnRH neuronal migration, we examined movements of GnRH neurons in the brain compartment from slices started on E14 when a greater percentage of GnRH neurons are already in the brain compartment. After a 2-h baseline and after the addition of bicuculline, there was a significant increase in the percentage of frames with movement ( $F_{(1,6)} = 7.10$ ;  $P < 0.05$ ; Fig. 5A) and a significant decrease in the percentage of frames with turns ( $F_{(1,6)} = 7.14$ ;  $P < 0.05$ ; Fig. 5B).

### Discussion

The characterization of the migratory route and movement of GnRH neurons from their place of birth in the nasal compartment to their final destination in the preoptic area and anterior hypothalamus has been inferred previously by immunohistochemical comparisons from one stage of development

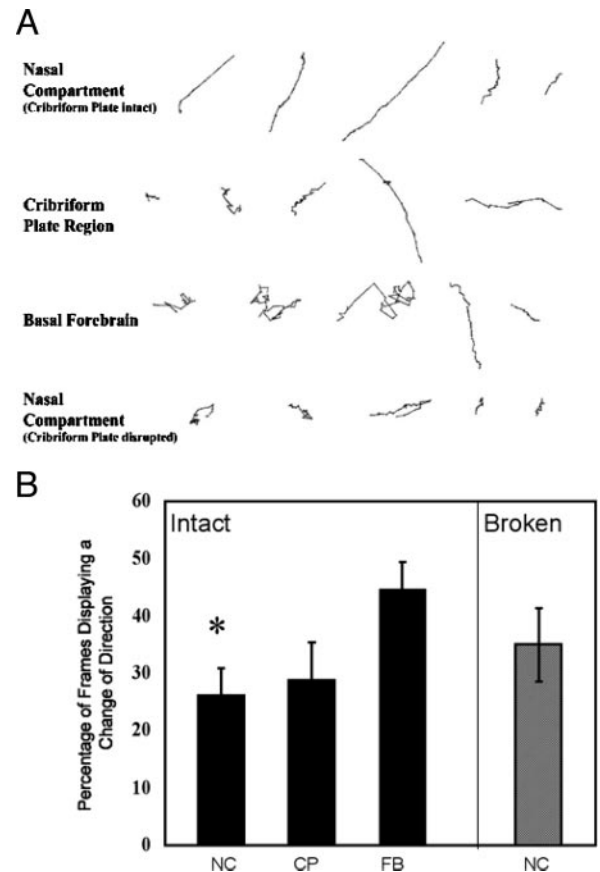


FIG. 3. The trajectory of GnRH neurons is altered as they move farther along their migratory route. A, Schematic diagram of the course of five different GnRH neurons in each compartment analyzed demonstrating different trajectories between compartments. B, The percentage of frames showing a change of direction (details in *Results*) is significantly greater in neurons in the forebrain (FB) compared with neurons in the nasal compartment (NC). \*,  $P < 0.05$  compared with forebrain (Tukey-Kramer multiple comparison test). CP, Cribriform plate.

to another (7–10), with 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) labeling (17), and olfactory ablations (18–20). Here for the first time, we provide direct insight into the manner by which these crucial neurons travel over their long and unique route. By taking advantage of a line of mice in which living GnRH neurons are detectable by GnRH promoter-specific expression of GFP (13), GnRH neurons were observed directly in a slice preparation that recapitulates relatively normal migration across all the compartments found *in vivo* (14). The results show GnRH neuron migratory behavior viewed in real-time changes as they leave the nasal compartment to enter the forebrain.

Early in their developmental journey, GnRH neurons in the nasal compartment move intermittently (33% of 5-min time-sampling periods), attaining relatively low average rates of movement (12–13  $\mu\text{m}/\text{h}$ ). Their movements follow exactly along the trajectory of VNN fibers by which they are guided (4, 5, 16, 21). 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 (4, 22, 23) and partially reflects the release of GnRH neurons from caudal VNN fibers (5) to find their final destinations.

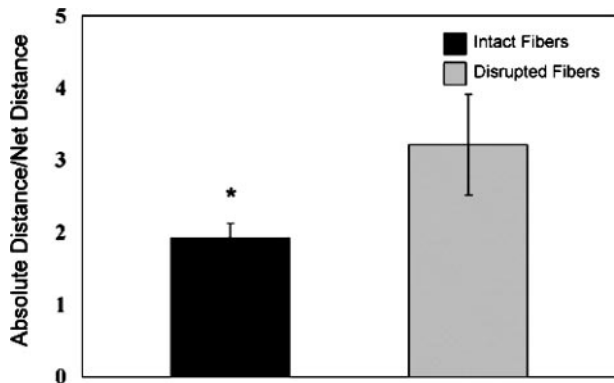


FIG. 4. The course of GnRH neurons in the nasal compartment is altered if the fibers of the vomeronasal nerve are disrupted across the cribriform plate. The absolute/net distance traveled is greater for neurons in slices where the cribriform plate was broken ( $n = 9$ ) compared with slices with an intact cribriform plate ( $n = 23$ ). \*,  $P < 0.05$  (Student's  $t$  test).

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 (6, 24–26). There may be significant and interesting similarities in aspects of GnRH neuron and cortical interneuron migration. For one, cortical interneurons synthesize GABA (27), similar to

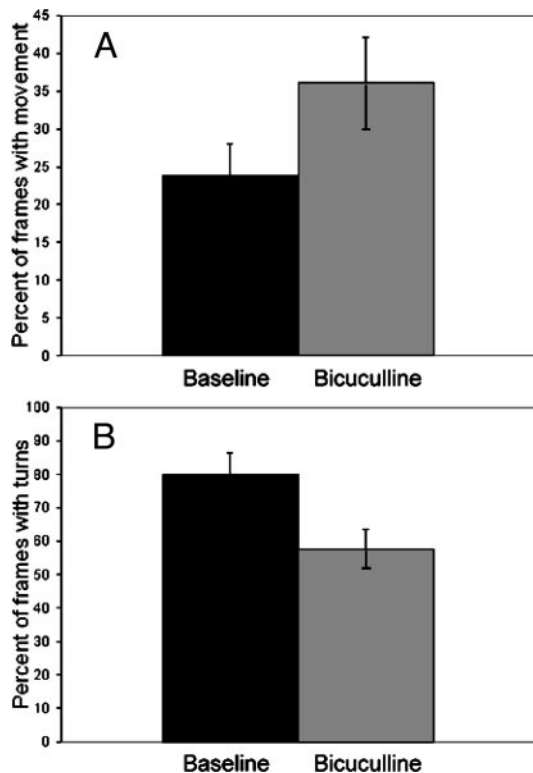


FIG. 5. The movements of GnRH neurons in the forebrain compartment are altered if the slices are treated with bicuculline (target concentration,  $20 \mu\text{M}$ ). With each cell providing baseline and bicuculline data ( $n = 7$ ), the percentage of frames with motion was significantly increased (A), and the percentage of frames with turns significantly decreased (B) after blockade of GABA<sub>A</sub> receptors.  $P < 0.05$  (repeated measures ANOVA).

some migrating GnRH neurons (28). GABA may influence both tangential cortical interneuron migration (29) and GnRH neuron migration (5, 30). Cortical interneurons may follow axonal guides for the major portion of their journey and change modes as they come close to their target regions in the cerebral cortex (6, 25). GnRH neurons follow a portion of the VNN that uniquely turns caudally after entering the CNS (4) and then may change their mode of migration after releasing from those fibers (5). This change in mode of migration was evident in the current experiment by the increased turning behavior and frequency of movement of GnRH neurons in the brain *vs.* the nasal compartment 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.

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* (3) and *in vivo* (22, 23). Particular molecular characteristics of olfactory fibers are absolutely necessary for migration in the nasal compartment (31). These findings are consistent with a human case of Kallmann syndrome in which olfactory fiber disorientation in the nasal compartment was associated with failure of GnRH neurons to enter the brain (32). In explant cultures of olfactory placode, GnRH neurons continue to migrate along presumptive VNN fibers (33), likely all containing peripherin (34). Similarly, in slice cultures, GnRH neurons migrate along peripherin-containing fibers (see Ref. 5 and the current study) derived from the VNN, as they do *in vivo* (16). The current study contained an illuminating and important perturbation experiment. When VNN fibers were disrupted at the cribriform plate, the behavior of GnRH neurons in the nasal compartment was altered significantly, and this alteration was seen distal to the actual site of disruption. This occurred despite any 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 may entail selective signaling in addition to mechanical guidance.

We have begun experiments to examine the influence of different chemical factors on GnRH neuron movements using the slice video paradigm. Our initial experiment was designed to follow up previous studies of the influence of GABA on GnRH neuron migration (5, 30). In the current experiment, the GABA<sub>A</sub> 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. Previous work has suggested that activation of the GABA<sub>A</sub> receptor causes a decrease in GnRH neuron movement (5, 30). Therefore, the complementary current result directly supports the earlier data and extends this work to suggest specific physical mechanisms by which GnRH neuron movements are affected. Previous work also has suggested that bicuculline treatment, in particular, may drive GnRH neurons apart from guiding fibers (5). The finding of a change in turning behavior in the current experiment may be indicative of such a change in neuron/fiber interactions. Many other secreted factors may

influence GnRH neuron migration including other neurotransmitters (e.g. serotonin or norepinephrine) (35), neuropeptides (e.g. cholecystokinin) (36), growth factors (37, 38), classical chemoattractants (e.g. netrin-1) (22, 23), or chemorepellents (39). It will be important to test the influence of such factors directly on the behavior of GnRH neurons.

In summary, GnRH neurons, which are essential for reproduction in all vertebrates, migrate over long distances and different environments. Prior studies have provided strong clues for the types of molecules and motions that one might expect along the migratory route. However, the current study provides the first actual view of the behavior of GnRH neurons in the context of an *in vitro* preparation that provides for movements comparable to those *in vivo* (5, 14). The live view provides direct indications of the changing behavior of GnRH neurons in their different environments and establishes a model to evaluate the influences of specific molecules that may be important for their migration.

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### References

- MacColl G, Quinton R, Bouloux PM 2002 GnRH neuronal development: insights into hypogonadotropic hypogonadism. *Trends Endocrinol Metab* 13:112–118
- Wray S 2001 Development of luteinizing hormone releasing hormone neurons. *J Neuroendocrinol* 13:3–11
- Tobet SA, Bless EP, Schwarting GA 2001 Developmental aspect of the gonadotropin-releasing hormone system. *Mol Cell Endocrinol* 185:173–184
- 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
- Bless EP, Westaway WA, Schwarting GA, Tobet SA 2000 Effects of  $\gamma$ -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
- Marin O, Rubenstein JL 2003 Cell migration in the forebrain. *Annu Rev Neurosci* 26:441–483
- Schwanzel-Fukuda M, Pfaff DW 1989 Origin of luteinizing hormone-releasing hormone neurons. *Nature* 338:161–164
- 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
- Ronnekleiv OK, Resko JA 1990 Ontogeny of gonadotropin-releasing hormone-containing neurons in early fetal development of rhesus macaques. *Endocrinology* 126:498–511
- 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
- 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
- Drapkin PT, Monard D, Silverman AJ 2002 The role of serine proteases and serine protease inhibitors in the migration of gonadotropin-releasing hormone neurons. *BMC Dev Biol* 2:1
- 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
- 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
- Tobet SA, Walker HJ, Seney ML, Yu KW 2003 Viewing cell movements in the developing neuroendocrine brain. *Integr Comp Biol* 43:794–801
- Wray S, Key S, Qualls R, Fueshko SM 1994 A subset of peripheral positive olfactory axons delineates the luteinizing hormone releasing hormone neuronal migratory pathway in developing mouse. *Dev Biol* 166:349–354
- 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
- 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
- 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
- Norgren Jr RB, Gao C, Ji Y, Fritzsche 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
- Live N, 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
- 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
- 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
- Anderson SA, Eisenstat DD, Shi L, Rubenstein JL 1997 Interneuron migration from basal forebrain to neocortex: dependence on *Dlx* genes. *Science* 278:474–476
- Nadarajah B, Parnavelas JG 2002 Modes of neuronal migration in the developing cerebral cortex. *Nat Rev Neurosci* 3:423–432
- Ang Jr ES, Haydar TF, Gluncic V, Rakic P 2003 Four-dimensional migratory coordinates of GABAergic interneurons in the developing mouse cortex. *J Neurosci* 23:5805–5815
- Xu Q, de la Cruz E, Anderson SA 2003 Cortical interneuron fate determination: diverse sources for distinct subtypes? *Cereb Cortex* 13:670–676
- Tobet SA, Chickering TW, King JC, Stopa EG, Kim K, Kuo-Leblank V, Schwarting GA 1996 Expression of  $\lambda$ -aminobutyric acid and gonadotropin-releasing hormone during neuronal migration through the olfactory system. *Endocrinology* 137:5415–5420
- Soria JM, Valdeolmillos M 2002 Receptor-activated calcium signals in tangentially migrating cortical cells. *Cereb Cortex* 12:831–839
- 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
- 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
- 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
- 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
- Fueshko S, Wray S 1994 LHRH cells migrate on peripheral fibers in embryonic olfactory explant cultures: an *in vitro* model for neurophilic neuronal migration. *Dev Biol* 166:331–348
- 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
- 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
- Gibson MJ, Ingraham L, Dobrjansky A 2000 Soluble factors guide gonadotropin-releasing hormone axonal targeting to the median eminence. *Endocrinology* 141:3065–3071
- Gill JC, Moenter SM, Tsai PS 2004 Developmental regulation of gonadotropin-releasing hormone neurons by fibroblast growth factor signaling. *Endocrinology* 145:3830–3839
- 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