Differing, Spatially Restricted Roles of Ionotropic Glutamate Receptors in Regulating the Migration of GnRH Neurons during Embryogenesis

Sharon X. Simonian and Allan E. Herbison

Laboratory of Neuroendocrinology, The Babraham Institute, Cambridge, CB2 4AT, United Kingdom

We have examined here the role of glutamate in regulating the process of tangential neuronal migration during embryogenesis by investigating the roles of AMPA and NMDA receptors in the migration of the gonadotropin-releasing hormone (GnRH) neurons from the nose to the hypothalamus. We first determined that GluR1–4 subunit mRNAs were present from embryonic day (E) 12.5 along the complete nose–brain migratory pathway of the GnRH neurons, whereas that of the obligatory NMDAR1 transcript was present only in brain regions of GnRH migration. *In vivo* studies revealed that AMPA receptor antagonism between E12.5 and E16.5 resulted in a significant ($p < 0.05$) accumulation of GnRH neurons in the nose adjacent to the cribiform plate. In contrast, NMDA receptor antagonism over E12.5–E16.5 or E13.5–E16.5 caused a selective increase ($p < 0.05$) in the number of GnRH neurons located in their final resting place within the diagonal band of Broca and preoptic

area. Dual-labeling studies using GnRH promoter–LacZ transgenic mice, which facilitate the identification of receptors in GnRH neurons, identified the presence of NMDAR1 receptors in ~6% of embryonic GnRH neurons located throughout the migratory pathway. Postnatally, the percentage of GnRH neurons expressing NMDAR1 increased to 50%. These results indicate that tonic AMPA receptor activation enhances the migration of GnRH neurons from the nose into the brain, whereas that of NMDA receptor activation slows the final phase of GnRH migration within the forebrain. These *in vivo* observations demonstrate differing, spatially restricted roles for AMPA and NMDA receptor activation in the process of tangential neuronal migration.

Key words: AMPA; glutamate; GnRH; LHRH; migration; mouse; NMDA; transgenics

Neuronal migration is vital for the correct development and functioning of the nervous system and occurs through at least two fundamentally different processes. Radial migration is the principal mode of neuronal migration and uses radial glial cell scaffolds and complex cell–cell interactions to direct the positioning of neurons (Rakic, 1990, 1995; Hatten 1993; Pearlman et al., 1998). In contrast, the more recently recognized process of tangential migration appears to depend more on interactions with gradients of diffusible molecules acting as chemoattractants or repellants (Pearlman et al., 1998). Although molecules such as astrotactin and reelin are clearly implicated in the process of radial migration (Zheng et al., 1996; Curran and D’Arcangelo, 1998), and Slit may have a role in both forms of migration (Zhu et al., 1999), much has yet to be done to establish factors critical for tangential migration (Pearlman et al., 1998).

One of the most dramatic examples of tangential migration in the mammalian brain is that provided by the gonadotropin-releasing hormone (GnRH) neurons. These cells are born outside the brain in the olfactory placode and migrate through the nasal septum to reach the forebrain and, ultimately, the hypothalamus during embryogenesis (Schwanzel-Fukuda and Pfaff, 1989; Wray et al., 1989). Although GnRH neurons are known to migrate

along olfactory axon bundles to enter the brain (Wray et al., 1994; Yoshida et al., 1995) and molecules involved in this process are now beginning to be identified (Fueshko et al., 1998; Yoshida et al., 1999; Bless et al., 2000; Kramer and Wray, 2000), the mechanisms underlying their motility and correct spatiotemporal migration remain largely unknown.

Recent studies have indicated that glutamate has an important role in the process of radial migration within the cerebellum (Komuro and Rakic, 1993) and neocortex (Behar et al., 1999). *In vitro* investigations in both of these regions have shown that the rate of radial migration is clearly dependent on the selective activation of NMDA receptors, and it has been suggested that the tonic activation of these receptors by glutamate may serve to provide the optimal range of intracellular calcium levels essential for neuronal migration (Komuro and Rakic, 1998).

Because cultured embryonic GnRH neurons express functional glutamate receptors (Kusano et al., 1995) and display calcium oscillations (Terasawa et al., 1999) similar to migrating granule cells (Komuro and Rakic, 1996), we hypothesized that the “neurophilic” or tangential migration of this phenotype may also be regulated by extracellular glutamate concentrations. We first determined the temporal and spatial profile of GluR1–4 and NMDAR1 subunit mRNAs along the GnRH migratory pathway during embryonic development and then used specific NMDA and AMPA receptor antagonists to determine their role in GnRH migration *in vivo*. Studies using GnRH promoter–LacZ (GNLZ) transgenic mice then assessed expression of NMDAR1 protein in the migrating GnRH neurons. Using this approach, we provide evidence for a spatially selective role of NMDA receptors in the tangential migration of GnRH neurons through the

Received Aug. 23, 2000; revised Nov. 10, 2000; accepted Nov. 16, 2000.

This work was supported by the Biotechnological and Biological Sciences Research Council (UK). We thank Dr. M. J. Skynner for help with primer design, Dr. R. Benoit for the LR1 antibody, and Dr. R. J. Bicknell for critical reading of this manuscript.

Correspondence should be addressed to Allan E. Herbison, Laboratory of Neuroendocrinology, Department of Neurobiology, The Babraham Institute, Babraham, Cambridge, CB2 4AT, UK. E-mail: allan.herbison@bbsrc.ac.uk.

Copyright © 2001 Society for Neuroscience 0270-6474/01/210934-10\$15.00/0

forebrain during embryogenesis and further identify an unexpected role for AMPA receptors in regulating their entry into the brain.

MATERIALS AND METHODS

NMDA and AMPA receptor subunit mRNA expression along the GnRH migratory pathway

Timed-pregnant (CBA/Ca × C57BL/6J) mice, bred and housed at the Babraham Institute under UK Home Office regulations, were killed by cervical dislocation, and embryos were dissected out and decapitated at embryonic day (E) 12.5, E14.5, E16.5, and E18.5 ($n = 3$ at each age; morning of vaginal plug is E0.5). Postnatal female mice were cervically dislocated at postnatal day (P) 5, P15, and P30, and brains were removed. Whole embryonic heads or postnatal brains were then placed in ice-cold Krebs' solution, and 600- μ m-thick sagittal (embryonic heads) or coronal (postnatal brains) sections were cut using a vibroslice (Campden Instruments, Sibley, UK). With the aid of a dissecting microscope, discrete tissue samples containing the nose, medial septum (MS), or preoptic area (POA) were dissected from E14.5–E18.5 embryonic slices, and either the nose or whole forebrain samples were collected from E12.5 mice. In the same manner, the MS and POA were dissected from coronal postnatal brain slices.

Total RNA was then extracted from each tissue sample (SV total RNA isolation system; Promega, Madison, WI), and 10 μ l of RNA was added to 5 μ l of water containing 250 ng of random hexanucleotides (Amersham Pharmacia Biotechnology, Piscataway, NJ) and incubated at 65°C for 10 min followed by snap-cooling on ice. Complementary DNA (cDNA) synthesis was then performed at 37°C for 1 hr after addition of 5 μ l of reverse transcriptase mixture, pH 8.3, containing (in mM): 50 Tris-HCl, 75 KCl, 3 MgCl₂, 10 DTT, 0.5 dNTPs, and 4 U Omniscript Reverse Transcriptase (Qiagen, Hilden, Germany). Reactions were stored at –20°C.

The PCR for GnRH, GluR1–4, and NMDAR1 was undertaken by adding 1 μ l of cDNA from each sample to a 25 μ l PCR mixture, pH 9.0, containing 50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl₂, 0.2 μ M dNTPs,

Figure 1. Camera lucida-type drawings of (A) sagittal head and (B–E) coronal brain sections of E16.5 mice showing levels of analysis for GnRH neuron location along the GnRH migratory pathway. AC, Anterior commissure; DBB, diagonal band of Broca; LV, lateral ventricle; MS, medial septum; OB, olfactory bulb; POA, preoptic area; rDBB, rostral DBB; 3V, third ventricle.

Figure 2. Composite gels showing amplicons from a set of embryonic (E) to postnatal (P) tissue samples taken from the nose, and medial septum (MS) and preoptic area (POA) brain regions comprising the GnRH migratory pathway that underwent RT-PCR for GnRH (top row), GluR1 (second row), GluR2 (third row), GluR3 (fourth row), GluR4 (fifth row), and NMDAR1 (bottom row). A DNA 1 kb size ladder was placed before and after the tissue samples in each row. The bands of ~50 bp seen in the top row represent primer-dimer amplicons. Note that although each nose and brain region contains GnRH and GluR1–4 transcripts, NMDAR1 mRNA expression is limited to brain regions only.

1 μ M of each primer pair (see Table 1), and 0.6 U of Taq DNA polymerase (Amersham Pharmacia Biotechnology). PCR was performed in a Robocycler (Stratagene, La Jolla, CA) as follows: 94°C for 3 min followed by 30 cycles of 94°C for 1 min, 64°C for 1 min, and 72°C for 1 min. This was followed by a final 5 min incubation at 72°C. Resulting amplicons were resolved on 1.5% agarose gels and visualized using ethidium bromide staining.

The identity of PCR amplicons was confirmed by purification of cDNA from agarose gels using a QIAquick gel extraction kit (Qiagen) followed by fluorescent dideoxy-Sanger sequencing using an ABI fluorescent sequencer at the Babraham Institute Microchemical Facility.

Effect of AMPA and NMDA receptor antagonism on GnRH neuron migration in vivo

Experimental Group 1: NMDA and AMPA receptor antagonism between E12.5 and E16.5. The developmental period between E12.5 and E16.5 is the time during which the majority of GnRH migration from the nose to the brain occurs in mouse (Schwanzel-Fukuda and Pfaff, 1989; Wray et al., 1989). Between E12.5 and E16.5, groups of three timed-pregnant mice received one of four different treatments: (1) twice daily injections (10:30 A.M. and 6 P.M.) of the selective AMPA receptor antagonist 6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3-dione disodium (NBQX; Tocris, Ballwin, MO) [30 mg/kg body weight (BW), i.p.], (2) twice daily injections of vehicle (isotonic saline; 8 μ l/gm BW, i.p.), (3) daily injection (10:00 A.M.) of the competitive NMDA receptor antagonist *cis*-4-(phosphonomethyl)piperidine-2-carboxylic acid (CGS-19755; RBI, Natick, MA; 5 mg/kg BW, i.p.), or (4) daily injection of isotonic saline (4 μ l/gm BW, i.p.). Drugs, doses, and methods of administration were the

Table 1. Sequences of primers used in PCR experiments

Primer	Sequence	Accession number	Location (bp)
GnRH (f)	GACATGATCCTCAAACGTATGG	M14872	1587–1608
GnRH (r)	CAATGTTATACTCGGGTGTGTGG	M14872	4559–4582
GluR1 (f)	ACTCAAGCGTCCAGAATAGAACC	NM_008165	1465–1487
GluR1 (r)	CCATTTCATAAGGACTGAAACGGC	NM_008165	1936–1958
GluR2 (f)	CAGCGACAATTAAGTATACTTCGG	X57498	1071–1094
GluR2 (r)	CAATAGTCAGCTTGTACTTGAATC	X57498	1575–1598
GluR3 (f)	GTTATCACTACATGCTTGCTAACC	AB022342	761–784
GluR3 (r)	CGACACAATAGCCTTCATAGCGC	AB022342	1383–1405
GluR4 (f)	CCATTGCACCTCTGACAATCACG	AB022913	1703–1725
GluR4 (r)	GTCAGGAATGCAGCCAGATTAGC	AB022913	2131–2153
NMDAR1 (f)	AAGTATGCAGATGGAGTGACTGG	NM_008169	1078–1100
NMDAR1 (r)	GTCAGGAATGCAGCCAGATTAGC	NM_008169	1663–1685

Primer locations are given according to sequence numbering in GenBank.

same as those shown to be effective in previous *in vivo* experiments on excitatory amino acid manipulation in the brain (Namba et al., 1994; Jackson et al., 1998; Minger et al., 1998; Sadikot et al., 1998). Importantly, both antagonists cross the placenta and blood–brain barrier (Minger et al., 1998; Sadikot et al., 1998). NBQX was administered twice daily because of its relatively short half-life (Gill et al., 1992).

Pregnant dams were weighed daily and killed by cervical dislocation at 10:30 A.M. on E16.5, and three embryos were dissected out at random from each dam (total $n = 9$ for each treatment group). In addition, three nontreated pregnant dams were killed by cervical dislocation to provide three E14.5 embryos. Whole embryo heads were removed and immersion-fixed in 4% paraformaldehyde (PFA) for 6 hr before being transferred to 30% sucrose in TBS at 4°C where they were stored until sectioning 2–4 d later. Frozen whole-head sections were cut in the sagittal plane on a cryostat (15 μ m thick; Bright, Huntingdon, UK) and collected as five sets of sections corresponding to gestational day (GD) 16 sagittal plates (SAG) 6–12 or GD 14 SAG 1–7 of Schambra et al. (1992) and thaw-mounted onto Superfrost Plus slides (Merck, Poole, UK). Sections were kept at 4°C (for immunocytochemistry) or –70°C (for *in situ* hybridization).

Experimental Group 2: NMDA receptor antagonism between E13.5 and E16.5. The majority of GnRH neuron migration within the brain occurs between E13.5 and E16.5. Groups of three dams received either (1) daily injection (10:00 A.M.) of CGS-19755 (5 mg/kg BW, i.p.) or (2) daily injection of isotonic saline (4 μ l/gm BW, i.p.). Embryos were obtained at E16.5 and treated exactly the same as described above with the exception that whole heads were cut in the coronal plane.

Immunocytochemistry

One set of sections from all treatment groups underwent immunocytochemical staining for GnRH as reported previously (Skynner et al., 1999). Briefly, sections were fixed for 20 min in 4% PFA and incubated in a polyclonal rabbit antisera directed against GnRH-I (LR1; 1:20,000; gift of R. Benoit, McGill University, Montreal, Canada) for 72 hr at 4°C. This was followed by biotinylated goat anti-rabbit antibodies (1:200; Vector Laboratories, Peterborough, UK) and streptavidin-biotinylated horseradish peroxidase complex (1:200; Amersham Pharmacia Biotechnology) each for 90 min at room temperature. GnRH immunoreactivity was visualized using nickel-enhanced diaminobenzidine staining.

A quantitative analysis of GnRH neuron numbers was undertaken by an investigator blind to the experimental groups using a Leitz DM-RB microscope (Leitz, Wetzlar, Germany). The most efficient manner for analyzing GnRH migration is through the use of midline sagittal brain sections where the whole extent of the nose to brain GnRH neuron distribution can be assessed. Sagittal sections were analyzed by determining the three midline sections containing the highest numbers of GnRH neurons and cells allocated to five regions. The nose was divided into two equal regions, and cells were designated as being located in either the “rostral nose” or “caudal nose” (Fig. 1A). Cells located in the brain were allocated to either the olfactory bulbs (OBs), MS, or diagonal band of Broca (DBB)/POA (Fig. 1A). A line stretching from the anterior commissure to the most ventrocaudal aspect of the olfactory bulb was used to distinguish MS from DBB/POA cells (Fig. 1A). Total numbers of

cells and percentages in each of the five regions were then calculated for each animal to give experimental group $\pm$ SEM values.

Although sagittal brain sections are highly advantageous for evaluating the whole GnRH neuronal migratory pathway, coronal brain sections provide the best method for examining the final stages of the GnRH migration into the hypothalamus when these cells start to move laterally away from the midline. The analysis of coronally cut embryonic sections was undertaken by counting GnRH neurons in four brain regions. Cells located in the four sections immediately rostral to the joining of the two hemispheres were classified as OB cells (Fig. 1B). Cells located between this juncture and the appearance of the third ventricle were designated as being either in the MS, if they were situated in the dorsal half of the area between the base of the brain and the dorsal most GnRH neurons (Fig. 1C), or in the rostral (r) DBB, if they were situated in the ventral half (Fig. 1C). In sections caudal to the appearance of the third ventricle, GnRH neurons located in the ventral half were classified as being in the POA, which included the caudal DBB and the area around the organum vasculosum of the lamina terminalis (Fig. 1D,E). The most caudal sections analyzed in the series were typified by fusing of the lateral ventricles (Fig. 1E), and few GnRH neurons were seen at this level. Percentages and total numbers of cells counted in each region were calculated for each animal and combined to give group means $\pm$ SEM. Statistical analysis between saline- and antagonist-treated groups was undertaken using the nonparametric Mann–Whitney *U* test.

In situ hybridization

To assess potential effects of NMDA and AMPA antagonists on GnRH mRNA expression, one set of sections from E12.5–E16.5 treated animals underwent *in situ* hybridization for GnRH mRNA using an antisense oligonucleotide complementary to the sequence encoding the last 15 amino acids of exon II of the mouse GnRH gene (Skynner et al., 1999). The probe was labeled with 35 S to a specific activity of $\sim 10^9$ cpm/ μ g, and slides were hybridized as reported previously (Simonian et al., 2000). Hybridization specificity was assessed by use of competition experiments in which radiolabeled probes were hybridized to sections in the presence of an excess (25-fold) of unlabeled probe.

A quantitative analysis of cellular silver grain density was undertaken by an investigator blind to the experimental groups. Using a Seescan analyzer (Seescan, Cambridge, UK), the number of silver grains overlying cells in the excess unlabeled probe control was determined, and in experimental sections, only those cells expressing numbers of silver grains greater than five times the control value were used for analysis. GnRH neurons were allocated to either the nose or brain, and 30 cells were analyzed in each region from two to three sections from each embryo, and an average silver grain count per micrometer squared per cell was determined. These values were combined to give experimental group means $\pm$ SEM, and statistical analysis was undertaken using the nonparametric Mann–Whitney *U* test.

Immunocytochemical analysis of NMDAR1 protein expression by embryonic and postnatal GnRH neurons

Embryonic tissue. Nuclear-located X-galactosidase (gal) or β -gal staining in GNLZ transgenic mice provides a convenient marker for the GnRH

Figure 3. Photomicrographs showing GnRH immunoreactivity within sagittal sections in the nose and olfactory bulbs (*A, B*) and forebrain (*C, D*) of E16.5 mice treated from E12.5 to E16.5 with saline twice daily (*A*), NBQX twice daily (*B*), saline once daily (*C*), and CGS-19755 once daily (*D*). Arrowheads in *A* and *B* indicate the location of the cribriform plate. Note that more GnRH neurons are located in the caudal nose in NBQX-treated mice (*B*) and that more GnRH neurons are located within the DBB-POA of CGS-19755 treated mice (*D*), compared with saline-matched controls (*A, D*). The sections are oriented so that the nose is to the left and the brain is to the right. Scale bars, 100 μ m.

phenotype and can be used in conjunction with immunocytochemistry to enhance the detection of cytoplasmic- or membrane-located antigens in GnRH neurons (Simonian et al., 2000). Whole heads of E14.5, E16.5, and E18.5 GNLZ-560 embryos ($n = 6$ embryos at each age) were immersion-fixed in 2% PFA in 0.1 M phosphate buffer for 6 hr at room temperature followed by an overnight wash in TBS. Whole heads (E14.5, E16.5) or brains (E18.5) were embedded in 8% gelatin and refixed in 2% PFA overnight and then cut at 100 μ m thickness in the coronal plane on a vibroslice. For each animal, every section through the nose (except E18.5) and forebrain was collected and processed for dual-labeling histochemistry.

Tissue sections were placed into X-gal solution [2 mM $MgCl_2$, 4 mM $K_3Fe(CN)_6$, 4 mM $K_4Fe(CN)_6$, and 4 mg/ml 5-bromo-4-chloro-3-indoyl- β -D-galactosidase in TBS] overnight at room temperature to reveal transgene-expressing cells. All sections from individual GNLZ-560 embryos were then processed for free-floating immunocytochemistry by placing in 1% hydrogen peroxide, 40% methanol, TBS solution for 5 min followed by either GnRH (LR1; 1:20,000) or NMDAR1 (1:500; Santa Cruz Biotechnology) antisera for 5 d at 4°C. Immunoreactivity was then revealed using the ABC method described above. The total number of single- and double-labeled cells was counted in all X-gal-GnRH and X-gal-NMDAR1 sections and allocated to either nose, OB, MS, rDBB, or POA as described above (Fig. 1*B–E*). Individual animal counts were combined to give mean $\pm$ SEM values.

Postnatal tissue. Previous studies in the laboratory with GNLZ mice have shown that the most sensitive method for the detection of receptor proteins in postnatal GnRH neurons requires the immunostaining for the receptor to be undertaken first, followed by visualization of the transgene by β -gal immunocytochemistry. P5 GNLZ-560 mice ($n = 4$ at each age) were decapitated, fixed, and sectioned in the same manner as embryonic brains. P15 and P30 GNLZ-560 mice were administered an overdose of Avertin (tribromoethanol and 2-methylbutan-2-ol in 10% ethanol; 0.2 ml/20 gm BW, i.p.) and perfused directly through the left ventricle of the heart with 15 or 20 ml of 4% PFA, respectively. Brains were post-fixed for 1 hr and then immersed in 30% sucrose in TBS overnight.

The following day, 25- μ m-thick coronal sections were cut through the rostral forebrain of P15 and P30 mice using a freezing microtome. Postnatal sections were incubated in the NMDAR1 antibody (1:500) for 48 hr at 4°C, and immunoreactivity was revealed using the ABC method as above. Sections were then treated with the hydrogen peroxide-methanol-TBS solution to inactivate any remaining peroxidases and incubated in a polyclonal rabbit antisera specific for β -gal (1:8000; ICN Biomedicals, Postfach, Germany) (Skynner et al., 1999) for 48 hr followed by biotinylated goat anti-rabbit antibodies (Vector; 1:200, 90 min) and Vector Elite avidin-peroxidase substrate (1:100, 90 min) before reacting with the diaminobenzidine chromagen.

Postnatal sections were examined using a Leitz DM-RB microscope. For each P5 mouse, numbers of single- and double-labeled β -gal cells

were counted and allocated to either OB, MS, rDBB, or POA (Fig. 1*B–E*). For each P15 and P30 GNLZ-560 mouse, four sections containing the MS-rDBB and four with the POA were selected, and the number of single- and double-labeled β -gal cells was counted in each area in each animal. Individual animal counts were combined to give mean $\pm$ SEM values.

Liquid-phase adsorption control experiments were undertaken by incubating the NMDAR1 antibody at working dilution (0.4 μ g/ml) with the synthetic peptide used to produce the antibody (40 μ g/ml; Santa Cruz Biotechnology). Other controls consisted of the omission of either NMDAR1 or β -gal antisera from the dual-labeling immunostaining protocol.

RESULTS

Differential expression of NMDA and AMPA receptor transcripts along the GnRH migratory pathway

The GnRH, GluR1, GluR2, GluR3, GluR4, and NMDAR1 amplicons were ~320, 494, 528, 645, 451, and 608 bp, respectively (Fig. 2), as predicted by the primers (Table 1), and each was demonstrated to be authentic after sequencing. No amplicons resulted from the PCR of genomic DNA with any of the primer sets, and water blanks were found to be consistently negative.

As expected, the nose and dissected brain regions were found to contain GnRH mRNA at all of the developmental ages examined (Fig. 2). Similarly, all regions at all developmental ages were found to contain GluR1, GluR2, GluR3, and GluR4 transcripts (Fig. 2). NMDAR1 transcripts were also found in all brain regions from E12.5 onward but were never detected within the nose at any developmental age (Fig. 2). The same results were obtained from three independent sets of samples, although positive bands were only observed in two of the three samples for three of the transcripts (e.g., NMDAR1 in E18.5 POA) (Fig. 2). These findings suggested that both AMPA and NMDA receptors could be involved in GnRH migration through the brain, whereas only AMPA receptors might influence migration through the nose.

Differential effects of AMPA and NMDA receptor antagonists on GnRH neuron migration

The overall distribution of GnRH-immunoreactive neurons in all embryos was identical to that reported previously (Schwanzel-Fukuda et al., 1989; Wray et al., 1989). The GnRH neurons

located in nasal regions formed cords, with cell bodies and processes in close proximity to one another (Fig. 3*A,B*), whereas those situated within the forebrain exhibited a more dispersed topography (Fig. 3*C,D*). The numbers of GnRH-immunoreactive neurons identified in the nose and brain of animals treated from E12.5 to E16.5 were not different: NBQX twice daily (121.8 ± 8.5 GnRH neurons per three midline sections), saline twice daily (118.0 ± 10.9), CGS-19755 once daily (114.0 ± 7.6), and saline once daily (85.7 ± 9.2).

NBQX-treated mice, however, exhibited a significantly higher percentage of GnRH neurons located in the caudal nose compared with saline-treated controls ($p < 0.05$) (Figs. 3*A,B*, 4*A*), suggesting a delayed entry of GnRH neurons into the brain after AMPA receptor antagonism. There was a nonsignificant trend for fewer GnRH neurons to be located in each of the three brain regions of AMPA-treated embryos (Fig. 4*A*). When the GnRH neuron location in the brain was evaluated as a percentage of total brain GnRH neurons, no differences were found in their relative distribution within the OB (saline vs NBQX; 25 ± 7 vs $25 \pm 5\%$), MS (46 ± 7 vs $47 \pm 4\%$), or DBB/POA (27 ± 3 vs $32 \pm 4\%$), indicating that migration through the brain was not altered by NBQX treatment.

In contrast, in CGS-19755-treated mice, a significantly higher percentage of GnRH neurons was found to be located in the DBB-POA compared with saline-treated controls ($p < 0.05$) (Figs. 3*C,D*, 4*B*), suggesting augmented migration of GnRH neurons through the MS. The same pattern was observed when analysis was undertaken on GnRH neurons as a percentage of total brain GnRH neurons (saline vs CGS-19755: OB, 32 ± 6 vs $25 \pm 4\%$; MS, 44 ± 4 vs $40 \pm 3\%$; DBB/POA, 24 ± 4 vs $39 \pm 5\%$; $p < 0.05$). No significant differences in the percentage of neurons located in any other region were seen between saline-treated and CGS-19755-treated mice (Fig. 4*B*).

A comparison of the distribution of GnRH-immunoreactive neurons identified in sagittal sections of E14.5 (136 ± 8 GnRH neurons per three midline sections) and E16.5 (saline once and twice daily groups combined) mice demonstrated that a significantly higher percentage of GnRH neurons were located in the DBB-POA of E16.5 mice compared with that of E14.5 mice (21 ± 2 vs $5 \pm 1\%$, respectively; $p < 0.001$). This was reflected by a significantly lower percentage of GnRH neurons located in the MS of E16.5 mice compared with E14.5 mice (33 ± 3 vs $46 \pm 2\%$, respectively; $p < 0.05$). As noted previously (Schwanzel-Fukuda and Pfaff, 1989; Wray et al., 1989), this demonstrates that a substantial degree of GnRH neuron migration occurs from the MS to DBB/POA over E14.5 to E16.5.

NMDA receptors are involved in the migration of GnRH neurons through the medial septum

The first study suggested that NMDA antagonism enhanced the migration of GnRH neurons from the MS into the DBB and POA. Using coronal brain sections, which enable better medio-lateral spatial analysis of GnRH neuron location, we repeated this experiment by investigating the effects of NMDA receptor antagonism over the shorter period of E13.5 to E16.5 when most of the MS to DBB/POA migration occurs.

The total number of GnRH neurons counted in 12 coronal brain sections from each embryo was not different between saline-treated (182.0 ± 8.5 ; $n = 9$) and CGS-19755-treated (184 ± 8.4 ; $n = 9$) mice. However, a significantly higher percentage of GnRH neurons were found to be located in the rDBB compared with saline-treated controls ($p < 0.05$) (Fig. 5*A–C*), and this was

Figure 4. Histograms showing the percentage of GnRH neurons located within five regions along the GnRH migratory pathway within sagittal sections from animals treated from E12.5 to E16.5 with (*A*) saline (light bars) or NBQX (dark bars) or (*B*) saline (light bars) or CGS-19755 (dark bars) ($n = 8$ –9 embryos per group). NBQX treatment resulted in a higher percentage of GnRH neurons in the caudal nose compared with that after saline treatment (*A*). CGS-19755 treatment resulted in a higher percentage of GnRH neurons in the rostral forebrain compared with that after saline treatment. *c*, Caudal; *DBB*, diagonal band of Broca; *MS*, medial septum; *OB*, olfactory bulb; *POA*, preoptic area; *r*, rostral. * $p < 0.05$, ** $p < 0.02$ by Mann–Whitney *U* test.

paralleled by a significantly lower percentage of GnRH neurons located in the MS of CGS-19755-treated mice compared with controls ($p < 0.02$) (Fig. 5*A–C*). Combined, the MS and DBB contained ~50% of all GnRH neurons in both treatment groups. This indicates that the process of migration from the MS to DBB over E13.5–E16.5 is especially sensitive to NMDA antagonism.

No behavioral consequences of NMDA or AMPA receptor antagonism were observed in any animal from any treatment group; however, the mean body weights of dams at day 16.5 of pregnancy were lower (30.8 ± 1.2 gm) after twice daily treatment with NBQX from E12.5 to E16.5 compared with saline twice

Figure 5. *A, B*, Photomicrographs showing GnRH immunoreactivity within E16.5 coronal sections through the medial septum (MS) and rostral diagonal band of Broca (rDBB) of mice treated from E13.5 to E16.5 with saline (*A*) or CGS-19755 (*B*). *C*, Histograms showing the percentage of GnRH neurons located within five regions along the GnRH migratory pathway ($n = 9$ embryos per group). Significantly more GnRH neuron migration occurred from the MS to the rDBB in mice treated with CGS-19755 compared with saline treatment. AHA, Anterior hypothalamic area; OB, olfactory bulb; POA, preoptic area. Scale bars, 150 μ m. * $p < 0.05$, ** $p < 0.02$.

daily (36.0 ± 3.0 gm), saline once daily (35.8 ± 2.0 gm), or CGS-19755 once daily (35.7 ± 2.2 gm). No difference was seen in the mean body weight of dams at day 16.5 of pregnancy treated daily from E13.5 to E16.5 with saline (36.7 ± 0.03 gm) or CGS-19755 (32.5 ± 3.9 gm).

AMPA and NMDA receptor antagonists have no effect on cellular levels of GnRH mRNA expression

After *in situ* hybridization, dense clusters of silver grains were identified over individual cells exhibiting a distribution identical to that observed with immunocytochemistry. Hybridization in the presence of an excess of unlabeled probe resulted in complete absence of silver grain clusters.

In animals treated with NBQX, the silver grain density of hybridized cells in the nose (1.70 ± 0.04 silver grains per micrometer squared per cell) and brain (1.98 ± 0.04 silver grains per micrometer squared per cell) was not different from the silver grain density of cells in the nose (1.75 ± 0.05 silver grains per micrometer squared per cell) and brain (1.99 ± 0.04 silver grains per micrometer squared per cell) of saline-treated animals. Similarly, in animals treated with CGS-19755, the silver grain density of hybridized cells in the nose (1.80 ± 0.04 silver grains per micrometer squared per cell) and brain (1.96 ± 0.05 silver grains per micrometer squared per cell) was not different from the silver grain density of cells in the nose (1.77 ± 0.06 silver grains per micrometer squared per cell) and brain (1.98 ± 0.03 silver grains per micrometer squared per cell) of animals treated with saline.

GnRH neurons express the NMDAR1 subunit

To evaluate the possibility that glutamate acts directly on GnRH neurons as they migrate through the brain, we used GNLZ mice to evaluate the expression of the NMDAR1 subunit, which is obligatory for functional NMDA receptors, in GnRH neurons.

The polyclonal NMDAR1 antibody is directed against epitopes of the intracellular C terminus of human NMDA receptor subunit R1, which are identical to those in the mouse (Santa Cruz Biotechnology). Liquid-phase adsorption experiments and omission of the primary antibody resulted in an absence of immunoreactivity. In postnatal mice, immunoreactive cells were found dis-

tributed throughout the brain in a heterogeneous manner, in good agreement with previous *in situ* hybridization (Van den Pol et al., 1994) and immunocytochemical (Brose et al., 1993; Petralia et al., 1994) studies for NMDAR1 in the postnatal rat. In general, NMDAR1 immunoreactivity was much stronger in P15 and P30 mice compared with P5 animals. In embryonic mice, the same overall pattern of NMDAR1 immunoreactivity as that seen in postnatal mice was observed, but staining was predominantly of the neuropil; however, distinct cellular staining was observed (Fig. 6*D*) within areas of neuropilar staining, with the number of immunoreactive cell profiles increasing with embryonic age. Staining of a mostly neuropilar nature was seen throughout the developing OB, MS, DBB, and POA.

The analysis of transgene expression by X-gal histochemistry in embryonic GNLZ-560 brains revealed the presence of X-gal-positive cells in the classical distribution of migrating GnRH neurons (Fig. 6*A*) as well as in the lateral septum, bed nucleus of the stria terminalis (BNST), and developing tectum. Within the distribution of the classical migrating GnRH neurons, dual-labeling X-gal-GnRH immunostaining in coronal sections of E14.5 embryos ($n = 6$) confirmed the presence of X-gal reaction product in 94 ± 2 and $95 \pm 3\%$ of GnRH-immunoreactive neurons located in the nose and brain, respectively (Fig. 6*A–C*). As in other GnRH-LacZ lines (Skynner et al., 1999), the LacZ reporter provides a good index of the GnRH phenotype within the MS, DBB, and POA in GNLZ-560 mice. Transgene expression in the lateral septum, BNST, and tectum represents cells expressing low levels of GnRH that do not migrate from the nose (Skynner et al., 1999).

In total, 1457, 1878, and 2031 X-gal-positive cells located in the OB, MS, DBB, and POA, corresponding to migrating GnRH neurons, were examined for NMDAR1 immunoreactivity at E14.5, E16.5, and E18.5, respectively ($n = 6$ embryos at each age). Dual-labeled cells, however, characterized by a blue nucleus with brown NMDAR1 staining within the same plane of focus (Fig. 6*E*), were rare and represented $<6\%$ of all X-gal-GnRH neurons at each embryonic age (Fig. 7).

The same distribution of transgene-expressing GnRH cells was

Figure 6. *A–C*, Photomicrographs of GNLZ-560 coronal brain sections through the rostral preoptic area of E14.5 mice dual-labeled with X-gal (blue nuclei) and GnRH (brown cytoplasm). Note that virtually all GnRH neurons have blue nuclei. *D*, NMDAR1 immunoreactivity in the olfactory bulb of an E16.5 mouse. Arrows indicate areas with neuronal staining. *E*, Dual-labeled cell (arrow) displaying X-gal (blue nuclei) and NMDAR1 immunoreactivity (brown staining) in the preoptic area of an E16.5 mouse. *F–I*, Dual labeling with NMDAR1 (black staining) and β -gal (brown nuclei) in the preoptic area of P30 mice (*F–G*), medial septum (*H*), and diagonal band of Broca (*I*) of P5 mice. Arrows in *E–I* indicate double-labeled cells. Scale bars: *A*, *B*, 200 μ m; *C*, *D*, 50 μ m; *E*, *G–I*, 10 μ m; *F*, 20 μ m.

observed within the OB, MS, DBB, and POA of postnatal brains. In total 1622, 352, and 237 β -gal-immunoreactive neurons were examined for NMDAR1 immunoreactivity at P5, P15, and P30, respectively ($n = 4$ per developmental age). Dual-labeled β -gal-NMDAR1 cells in the OB, MS, DBB, and POA were characterized by a light brown nucleus (β -gal) and a dark brown/black cytoplasmic stain (NMDAR1) within the same plane of focus

(Fig. 6*F–I*). At P5, dual-labeled cells represented $\sim 10\%$ of all β -gal-GnRH cells, whereas at both P15 and P30 dual-labeled cells represented $\sim 40\text{--}50\%$ of all β -gal-immunoreactive cells in the MS-rDBB and POA (Fig. 7). These results suggest that substantial numbers of GnRH neurons express detectable NMDAR1 immunoreactivity only after birth and that a marked increase occurs between P5 and P15.

Figure 7. Histogram showing the percentage of X-gal- (embryonic, *E*; *n* = 6 each age) or β -galactosidase- (β -gal; postnatal, *P*; *n* = 4 each age) positive cells representing GnRH neurons of nasal origin, dual-labeled with NMDAR1 immunoreactivity. Note an approximate fivefold increase in the percentage of GnRH neurons expressing NMDAR1 protein from P5 to P15 and P30.

DISCUSSION

We provide here evidence for a role of both AMPA and NMDA receptors in the tangential migration of the GnRH neurons during embryogenesis. Intriguingly, glutamate appears to use different receptor subtypes to modulate GnRH migration in a tight, spatially restricted manner. In good agreement with the temporal and spatial profiling of AMPA and NMDA receptor mRNA expression along the migratory pathway, a selective NMDA antagonist was found to enhance the final stages of GnRH migration within the brain but to have no effect on migration through the nose. In contrast, and despite the expression of all four AMPA receptors along the whole of the GnRH migratory pathway, an AMPA receptor antagonist was found to influence the migration of these cells only from the nose into the brain. In addition to providing evidence for a role of glutamate in tangential neuronal migration, these observations also suggest that glutamate exerts a multifaceted influence on this process by activating different glutamate receptor subtypes in a location-dependent manner to both slow and enhance the migration of GnRH neurons.

Regulation of GnRH neuron migration by NMDA receptors

Our results suggest that NMDA receptors normally function to restrain the migration of the GnRH neurons as they pass ventrolaterally from the MS into the DBB. As shown by others (Schwanzel-Fukuda and Pfaff, 1989; Wray et al., 1989) and confirmed here, the most of the final phase of GnRH migration into the DBB and POA occurs between E14.5 and E16.5. We found that *in vivo* treatment with a specific NMDA receptor antagonist between either E12.5–E16.5 or E13.5–E16.5 resulted in a reduction in GnRH cell number in the MS and an accumulation of GnRH neurons in the DBB and POA. The most likely explanation for this result is that the final-stage migration of GnRH neurons from the MS into these structures was enhanced after NMDA antagonism. Although the first experiment was undertaken using brain sections cut in the sagittal plane to view the entire migratory pathway, the second experiment (E13.5–E16.5) used coronal sections to provide better spatial resolution for the MS to DBB/POA migration. This study demonstrated that the

principal effect of CGS-19755 was to enhance GnRH migration from the MS into the DBB. Interestingly, the route of GnRH neuron migration through the brain is principally midline until they leave the MS, at which point they move both ventrally and laterally to establish their final bilateral topography. Together, these observations suggest that the tonic activation of NMDA receptors between E13.5 and E16.5 normally restrains the ventrolateral migration of GnRH neurons from the MS into the DBB/POA.

The first evidence for a role of glutamate in neuronal migration was provided by Komuro and Rakic (1993) when they demonstrated that the radial migration of cerebellar granule cells was inhibited by NMDA but not by other glutamate receptor antagonists. Subsequent work has provided similar evidence for a selective role of NMDA receptors in facilitating radial migration in the cortex (Behar et al., 1999), although the injection of NMDA agonists in hamsters was found to inhibit migration in the cortex (Marret et al., 1996). We now provide evidence for a role of NMDA receptors in the process of tangential, neurophilic neuronal migration within the brain. Although this has features in common with radial glial migration, notably the selectivity for involvement of NMDA rather than AMPA receptors, the principal difference appears to be that tonic NMDA receptor activity slows rather than enhances GnRH neuron migration. It is thought that NMDA receptor activation facilitates neuronal motility in the cerebellum by enhancing calcium entry into migrating cells (Komuro and Rakic, 1998). Although embryonic GnRH neurons exhibit spontaneous calcium oscillations (Terasawa et al., 1999), the effect of NMDA receptor activation on calcium flux in these cells is not known. It is possible, for example, that NMDA-dependent calcium influx may dampen the profile of calcium oscillations in these cells and slow their migration. Despite evidence for intracellular calcium having a role in the maturation of neurotransmitter synthesis (Spitzer, 1994), we found no effects of NMDA receptor antagonism on GnRH mRNA expression during migration.

Although glutamate is thought to regulate granule and cortical cell migration by acting on NMDA receptors expressed by these cells (Komuro and Rakic, 1993; Behar et al., 1999), it is not entirely clear whether the same situation exists for the GnRH neurons. We show here that NMDAR1 subunit mRNA is present in cells located along the brain GnRH migratory pathway from E12.5 onward, but we have failed to provide evidence for the expression of NMDAR1 protein in more than ~5% of migrating GnRH neurons. On one hand, it is possible that only a few migrating GnRH neurons express NMDA receptors and that this is sufficient to influence the whole population. Alternatively, it is possible that the predominantly neuropilar nature of NMDAR1 immunoreactivity in embryonic brains may not have enabled us to visualize all of the receptors expressed by GnRH neurons, particularly if they are located on distal dendrites. In this case, it remains possible that direct actions of glutamate may occur on greater numbers of the migrating GnRH neurons. In support of this later hypothesis, it has been demonstrated that older cultured embryonic GnRH neurons express functional glutamate receptors (Kusano et al., 1995), and immortalized mouse GnRH neurons, which likely represent embryonic cells, express NMDAR1 mRNA (Urbanski et al., 1994; Mahesh et al., 1999). It is also evident from this study that a very substantial upregulation in the expression of NMDAR1 by GnRH neurons occurs between P5 and P15 before puberty.

Finally, it is worth noting that NMDAR1 receptors were de-

tected in GnRH neurons, regardless of their location within the migratory pathway. Because NMDA receptors are only involved in regulating the final stages of GnRH migration, changes in extracellular glutamate gradients, NMDA receptor composition (Rossi and Slater, 1993; Monyer et al., 1994), or cooperativity between NMDA and GABA_A receptors (Ben-Ari et al., 1997) may underlie the regional specificity of NMDA receptor antagonist action.

Regulation of GnRH neuron migration by AMPA receptors

Surprisingly, given the lack of evidence for AMPA receptor involvement in radial migration (Komuro and Rakic, 1993; Behar et al., 1999), we found here a distinct, spatially discrete effect of NBQX on GnRH migration *in vivo*. We observed that specific AMPA receptor antagonism between E12.5 and E16.5 resulted in a marked accumulation of GnRH neurons within the nose adjacent to the cribriform plate and that this was associated with lower numbers of neurons in the brain. The most likely explanation of this finding is that AMPA receptor antagonism delayed GnRH migration into the brain. Although we have not been able to address directly whether embryonic GnRH neurons express AMPA receptors, our RT-PCR data demonstrate that all four GluR mRNAs are expressed by cells in the nose and basal forebrain of mice from E12.5 onward. Previous studies in the rat have demonstrated that AMPA receptor subunit mRNA is present in the embryonic midbrain from E14 (Monyer et al., 1991), and subunit protein has been detected in whole embryonic heads from E15.5 (Martin et al., 1998). Thus, it is possible that some of the AMPA receptor expression in the nose originates from GnRH neurons.

Although previous developmental studies have not highlighted a role for AMPA receptors in neuronal migration, it is interesting to note the expression of AMPA receptors throughout the developing brain (Monyer et al., 1991), as well as evidence for the functional expression of AMPA receptors in embryonic hypothalamic and hippocampal cells (Van den Pol et al., 1995; Diabira et al., 1999). Together these studies suggest the possibility of widespread effects of glutamate transmission through AMPA receptors on neuronal development. Our current work highlights a role for tonic AMPA receptor activation in the process of tangential migration within the brain and suggests that this receptor has a role in modulating the passage of GnRH neurons quite specifically from the nose into the developing telencephalon. It remains to be determined whether this role is specific for tangential migration or, indeed, the movement of GnRH neurons between the nose and brain. This transition represents one of radical change for the GnRH neurons as they enter a neuronal environment, and recent work has suggested that they may pause at this juncture (Mulrenin et al., 1999) and, further, that this delay is regulated by GABA (Fueshko et al., 1998). In this respect it is intriguing that AMPA and GABA_A receptor activation appear to exert opposite effects and may represent the two sides of a mechanism regulating the timing of GnRH entry into the brain.

In conclusion, we provide evidence that glutamate acts through AMPA receptors to enhance the migration of GnRH neurons from the nose into the brain, whereas tonic NMDA receptor activation slows the final phase of GnRH migration into the DBB and POA. These findings indicate discrete spatially restricted roles for tonic AMPA and NMDA receptor activation in the process of GnRH neuronal migration and provide the first evi-

dence for a role of glutamate within the process of neurophilic, tangential neuronal migration.

REFERENCES

- Behar TN, Scott CA, Greene CL, Wen X, Smith SV, Maric D, Liu Q-Y, Colton CA, Barker JL (1999) Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. *J Neurosci* 19:4449–4461.
- Ben-Ari Y, Khazipov R, Leinekugel X, Caillard O, Gaiarsa J-L (1997) GABA_A, NMDA and AMPA receptors: a developmentally regulated 'menage a trois'. *Trends Neurosci* 20:523–529.
- 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* 141:1254–1262.
- Brose N, Gasic GP, Vetter DE, Sullivan JM, Heineman SF (1993) Protein chemical characterization and immunocytochemical localization of the NMDA receptor subunit NMDA R1. *J Biol Chem* 268:22663–22671.
- Curran T, D'Arcangelo G (1998) Role of reelin in the control of brain development. *Brain Res Rev* 26:285–294.
- Diabira D, Hennou S, Chevassus-au-Louis N, Ben-Ari Y, Gozlan H (1999) Late embryonic expression of AMPA receptor function in the CA1 region of the intact hippocampus *in vitro*. *Eur J Neurosci* 11:4015–4023.
- 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.
- Gill R, Nordholm L, Lodge D (1992) The neuroprotective actions of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) in a rat focal ischaemia model. *Brain Res* 580:35–43.
- Hatten ME (1993) The role of migration in central nervous system. *Curr Opin Neurobiol* 3:38–44.
- Jackson A, Mead AN, Rocha BA, Stephens DN (1998) AMPA receptors and motivation for drug: effect of the selective antagonist NBQX on behavioural sensitization and on self-administration in mice. *Behav Pharmacol* 9:457–467.
- 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.
- Komuro H, Rakic P (1993) Modulation of neuronal migration by NMDA receptors. *Science* 260:95–97.
- Komuro H, Rakic P (1996) Intracellular Ca²⁺ fluctuations modulate the rate of neuronal migration. *Neuron* 17:275–285.
- Komuro H, Rakic P (1998) Orchestration of neuronal migration by activity of ion channels, neurotransmitter receptors, and intracellular Ca²⁺ fluctuations. *J Neurobiol* 37:110–130.
- Kusano K, Fueshko S, Gainer H, Wray S (1995) Electrical and synaptic properties of embryonic luteinizing hormone-releasing hormone neurons in explant cultures. *Proc Natl Acad Sci USA* 92:3918–3922.
- Mahesh VB, Zamorano P, Sevilla LD, Lewis D, Brann DW (1999) Characterization of ionotropic glutamate receptors in rat hypothalamus, pituitary and immortalized gonadotropin-releasing hormone (GnRH) neurons (GT1-7 cells). *Neuroendocrinology* 69:397–407.
- Marret S, Gressens P, Evrard P (1996) Arrest of neuronal migration by excitatory amino acids in hamster developing brain. *Proc Natl Acad Sci USA* 93:15463–15468.
- Martin LJ, Furuta A, Blackstone CD (1998) AMPA receptor protein in developing rat brain: glutamate receptor-1 expression and localization change at regional, cellular, and subcellular levels with maturation. *Neuroscience* 83:917–928.
- Minger SL, Geddes JW, Holtz ML, Craddock SD, Whiteheart SW, Siman RG, Pettigrew LC (1998) Glutamate receptor antagonists inhibit calpain-mediated cytoskeletal proteolysis in focal cerebral ischemia. *Brain Res* 810:181–199.
- Monyer H, Seeburg PH, Wisden W (1991) Glutamate-operated channels: developmentally early and mature forms arise by alternative splicing. *Neuron* 6:799–810.
- Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12:529–540.
- Mulrenin EM, Witkin JW, Silverman A-J (1999) Embryonic development of the gonadotropin-releasing hormone (GnRH) system in the chick: a spatiotemporal analysis of GnRH neuronal generation, site of origin and migration. *Endocrinology* 140:422–433.
- Namba T, Morimoto K, Sato K, Yamada N, Kuroda S (1994) Antiepileptogenic and anticonvulsant effects of NBQX, a selective AMPA receptor antagonist, in the rat kindling model of epilepsy. *Brain Res* 638:36–44.
- Pearlman AL, Faust PL, Hatten ME, Brunstrom JE (1998) New directions for neuronal migration. *Curr Opin Neurobiol* 8:45–54.
- Petralia RS, Yokotani N, Wenthold RJ (1994) Light and electron micro-

- scope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. *J Neurosci* 14:667–696.
- Rakic P (1990) Principles of neural cell migration. *Experientia* 46:882–891.
- Rakic P (1995) Radial versus tangential migration of neuronal clones in the developing cerebral cortex. *Proc Natl Acad Sci USA* 92:11323–11327.
- Rossi DJ, Slater NT (1993) The developmental onset of NMDA receptor-channel activity during neuronal migration. *Neuropharmacology* 32:1239–1248.
- Sadikot AF, Burhan AM, Belanger M-C, Sasseville R (1998) NMDA receptor antagonists influence early development of GABAergic interneurons in the mammalian striatum. *Dev Brain Res* 105:35–42.
- Schambra UB, Lauder JM, Silver J (1992) Atlas of the prenatal mouse brain. San Diego: Academic.
- Schwanzel-Fukuda M, Pfaff DW (1989) Origin of luteinizing hormone-releasing hormone neurons. *Nature* 338:161–163.
- Simonian SX, Skynner MJ, Sieghart W, Essrich C, Luscher B, Herbison AE (2000) Role of GABA_A receptor $\gamma 2$ subunit in the development of the gonadotropin-releasing hormone neurons *in vivo*. *Eur J Neurosci* 12:3488–3496.
- Skynner MJ, Slater R, Sim JA, Allen ND, Herbison AE (1999) Promoter transgenics reveal multiple gonadotropin-releasing hormone-I-expressing cell populations of different embryological origin in mouse brain. *J Neurosci* 19:5955–5966.
- Spitzer NC (1994) Spontaneous Ca²⁺ spikes and waves in embryonic neurons: signaling systems for differentiation. *Trends Neurosci* 17:115–118.
- Terasawa E, Schanhofer WK, Keen KL, Luchansky L (1999) Intracellular Ca²⁺ oscillations in luteinizing hormone-releasing hormone neurons derived from the embryonic olfactory placode of the rhesus monkey. *J Neurosci* 19:5898–5909.
- Urbanski HF, Fahy MM, Daschel M, Meshul C (1994) N-methyl-D-aspartate receptor gene expression in the hamster hypothalamus and in immortalized luteinizing hormone-releasing hormone neurons. *J Reprod Fertil* 100:5–9.
- Van den Pol AN, Hermans-Borgmeyer I, Hofer M, Ghosh P, Heinemann S (1994) Ionotropic glutamate-receptor gene expression in hypothalamus: localization of AMPA, kainate, and NMDA receptor RNA with *in situ* hybridization. *J Comp Neurol* 343:428–444.
- Van den Pol AN, Obrietan K, Cao V, Trombley PQ (1995) Embryonic hypothalamic expression of functional glutamate receptors. *Neuroscience* 67:419–439.
- 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.
- 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.
- Yoshida K, Tobet SA, Crandall JE, Jimenez TP, Schwarting GA (1995) The 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.
- 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.
- Zheng C, Heintz N, Hatten ME (1996) CNS gene encoding astrotactin, which supports neuronal migration along glial fibres. *Science* 272:417–419.
- Zhu Y, Li H-S, Zhou L, Wu JY, Rao Y (1999) Cellular and molecular guidance of GABAergic neuronal migration from an extracortical origin to the neocortex. *Neuron* 23:473–485.