Development 128, 4203-4216 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
DEV9789

EGFRs mediate chemotactic migration in the developing telencephalon

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Accepted 2 August 2001

SUMMARY

Epidermal growth factor receptors (EGFRs) have been implicated in the control of migration in the telencephalon, but the mechanism underlying their contribution is unclear. We show that expression of a threshold level of EGFRs confers chemotactic competence in stem cells, neurons and astrocytes in cortical explants. This level of receptor expression is normally achieved by a subpopulation of cells during mid-embryonic development. Cells that express high levels of EGFR are located in migration pathways, including the tangential pathway to the olfactory bulb via the rostral migratory stream (RMS), the lateral cortical stream (LCS) leading to ventrolateral cortex and the radial pathway from proliferative zones to cortical plate. The targets of these pathways express the ligands HB-EGF and/or TGFα. To test the idea that EGFRs mediate chemotactic migration these pathways, we increased the size of the population of cells expressing threshold levels of EGFRs in vivo by viral transduction. Our results suggest that EGFRs mediate migration radially to the cortical plate and ventrolaterally in the LCS, but not tangentially in the RMS. Within the bulb, however, EGFRs also mediate radial migration. Our findings suggest that developmental changes in EGFR expression, together with changes in ligand expression regulate the migration of specific populations of cells in the telencephalon by a chemoattractive mechanism.

Key words: Cerebral cortex, Rostral migratory stream, HB-EGF, Stem cell

INTRODUCTION

Construction of the telencephalon involves the migration of neurons and glia from proliferative zones to specific sites in the cerebral wall. Different types of neurons and glial cells leave the ventricular zone (VZ) and subventricular zone (SVZ) at specific times during development, follow characteristic routes during their migration, and settle in distinct locations (Berry et al., 1964; Hicks and D'Amato, 1968; Rakic, 1974; Raedler and Raedler, 1978; Smart and Smart, 1982; Bayer and Altman, 1991a). Some cells migrate radially to the cortical plate, using glial cells for guidance, while others migrate tangentially (Rakic, 1995; Walsh and Cepko, 1988; Misson et al., 1988; O'Rourke et al., 1992; Tan et al., 1998; Goldman and Luskin, 1998; de Carlos et al., 1996; Anderson et al., 1997; Lavdas et al., 1999; Parnavelas, 2000). Tangential migration occurs in ventral-to-dorsal, dorsal-to-ventral and caudal-to-rostral directions (deCarlos et al., 1996; Anderson et al., 1997; Lavdas et al., 1999; Tomioka et al., 2000; Luskin, 1993; Reid et al., 1995). The complexity of migration in the telencephalon is reflected in the variety of intrinsic and extrinsic molecules that play a role in its regulation (Reiner, 2000; Trommsdorff et al., 1999; Osterhout et al., 1997; Anton et al., 1997; Rio et al., 1997; Colamarino and Tessier-Lavigne, 1995; Alcantara et al., 2000; Hu, 1999; Wu et al., 1999; Zhu et al., 1999; Jacques et al., 1998). Although some of the molecules that control migration have been identified, little is known about the mechanisms that cause different types of cells to leave proliferative zones at specific times, use distinct modes of migration and stop in appropriate locations.

Epidermal growth factor receptors (EGFRs) have been shown to regulate migration in a variety of cells through direct and indirect mechanisms (Blay and Brown, 1985; Bailly et al., 2000; Chen et al., 1994; Duchek and Rorth, 2001; Miettinen et al., 2000; Hoschuetzky et al., 1994; Chan et al., 2000; Li et al., 1999; Li et al., 2000; Tokumaru et al., 2000; Xie et al., 1998). In the telencephalon, EGFRs are expressed in a precise temporal pattern. At early embryonic stages (before E14 in mice or E16 in rats), low levels of EGFRs can be detected; however, at mid-late embryonic stages, higher levels of EGFR mRNA and protein are expressed by a small heterogeneous population of cells that includes astrocytes, neurons and multipotent stem cells (Morshead et al., 1994; Weikert and Blum, 1995; Seroogy et al., 1995; Weikert and Blum, 1995; Eagleson et al., 1996; Kornblum et al., 1995; Kornblum et al., 1997, Burrows et al., 1997; Lillien and Raphael, 2000). Many of the cells that express a high level of EGFRs are found in migration pathways, including the radial pathway from proliferative zones to cortical plate through the intermediate zone, the tangential pathway from anterior SVZ to olfactory bulb via the rostral migratory stream (RMS), and the ventrolateral pathway from the corner between the cortex and striatum (corticostriatal sulcus) to the lateral and ventral cortex via the lateral cortical stream (LCS) (Bayer et al., 1991). In

EGFR-null mice, progenitor cells appear to accumulate in proliferative zones of the late embryonic telencephalon rather than migrating to the cortical plate or olfactory bulb (Threadgill et al., 1995), suggesting that EGFR-mediated signaling is involved in both radial and tangential modes of migration. Consistent with this phenotype, mis-expressing EGFRs in the embryonic rat telencephalon promoted radial migration to the cortical plate (Burrows et al., 1997), and infusing EGF into the lateral ventricle diverted cells from the rostral migratory stream to the cortex (Craig et al., 1996).

Several ligands that activate the EGFR are expressed in the developing telencephalon, including transforming growth factor α (TGFα) and heparin binding epidermal growth factor (HB-EGF) (Lazar and Blum, 1992; Weikert and Blum, 1995; Kornblum et al., 1997; Kornblum et al., 1999; Nakagawa et al., 1998; Opanshuk et al., 1999). TGFα is expressed in the olfactory bulb, striatum, and choroid plexus, but not in the cerebral cortex, with the exception of ventrolateral cortex (Lazar and Blum, 1992; Weikert and Blum, 1995; Kornblum et al., 1997). By contrast, HB-EGF is expressed in the embryonic cortex, particularly the cortical plate (Nakagawa et al., 1998; Kornblum et al., 1999). Its expression increases during embryonic development, and it continues to be expressed in ventrolateral cortex postnatally (Opanshuk et al., 1999; Kornblum et al., 1999).

Previous studies demonstrated that the level of EGFR expression affects the timing of migration and the settling patterns of cells (Burrows et al., 1997), but did not resolve the underlying mechanism. Migration associated with a high level of EGFR expression could reflect chemoattraction or chemorepulsion. To clarify the underlying mechanism, we have used explants to alter the location and concentration of ligand while monitoring the migratory behavior of cells. These studies demonstrate that a high level of EGFRs characteristic of late embryonic cells mediates chemoattraction. EGFRs and ligands for the EGFR, particularly HB-EGF, are expressed in several migration pathways. To test the idea that EGFRs mediate chemotactic migration in these pathways, we mis-expressed EGFRs in mouse telencephalon in utero at E10.5 and E14.5. This served to increase the size of the population of cells that express a high level of EGFRs. Our findings suggest that temporal and spatial changes in the availability of ligand, together with developmental changes in EGFR expression, regulate the chemotactic migration of a subpopulation of cells radially to the cortical plate and ventrolaterally in the LCS, but not tangentially in the RMS. Within the olfactory bulb, however, EGFRs promote radial migration out of proliferative zones, as in the cortex.

MATERIALS AND METHODS

Culture preparations

Explant cultures

Explants of embryonic telencephalon were prepared from timed-pregnant Sprague-Dawley rats or CD1 mice (Charles River). Developmental stage was determined from crown-rump length and examination of external features (Long and Burlingame, 1938; Theiler, 1972). Brains were dissected in HBSS (Gibco-BRL) and either the dorsolateral cortex (Fig. 1A) or the medial wall and dorsolateral cortex were placed on nucleopore filters (13 mm, 0.2 μm pore, Corning). Meninges were not removed, and explants were initially cultured ventricular surface down. Progenitor cells were infected with either a control virus expressing β-galacatosidase (IZAP; Burrows et al., 1997) or a virus co-expressing β-galactosidase and wild-type human EGFR (Lillien, 1995). Cells were infected by adding 20-30 μl of medium containing virus to the tops of the filters. This results in the selective infection of progenitor cells in the VZ (Burrows et al., 1997). After 1 day, some explants were flipped over (see Fig. 1A). Growth factors were added daily to the medium underneath the filters, beginning 1 day after infection. After a total of 4 days in culture, explants were fixed for immunocytochemistry, X-gal histochemistry or were dissociated to assay for the presence of multipotent stem cells among the infected population of cells (neurosphere culture) (Reynolds and Weiss, 1992). At least three explants per condition were analyzed.

Neurosphere cultures

To determine whether the cells that migrated towards exogenous EGF family ligands included multipotent stem cells, explants of E12 rat dorsolateral cortex were infected with EGFR virus and cultured with HB-EGF or TGFα (1 ng/ml) for 3 days. Explants were then dissociated with 0.4% trypsin (Sigma) for 15 minutes at 35°C, triturated and cultured on uncoated tissue culture dishes (24-well plate, 5×10^4 cells per well; Corning) in serum-free medium (Bottenstein and Sato, 1979) containing 0.1-1 ng/ml EGF (R&D) for 10 days; 0.2 ml of medium containing fresh EGF was added every 4 days. The number of infected cells per culture was counted at the time cells were plated. The number of neurospheres per well was counted after 10 days, and the number of neurospheres derived from infected cells was determined by staining for β-galactosidase expression. As reported previously (Burrows et al., 1997), nearly all of the neurospheres that developed in these cultures were derived from progenitor cells infected with EGFR virus. We could estimate the proportion of infected cells that were stem cells, i.e. generated a neurosphere, by dividing the number of β-gal-positive neurospheres/well by the number of infected cells cultured/well initially.

Immunocytochemistry and X-gal histochemistry

Brains and explants were fixed in 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4) or 3% Pipes buffer for 2 hours at 4°C for immunocytochemistry. For X-gal histochemistry, explants were fixed in 0.5% glutaraldehyde in phosphate-buffered saline (PBS) for 7.5 minutes at room temperature, and brains were fixed in 4% PFA in 3% Pipes buffer for 2 hours at 4°C. For immunocytochemistry, explants and brains were rinsed in PBS, cryoprotected in graded sucrose (10-30%), frozen and sectioned at 20 μm. Sections were blocked in PBS containing 10% fetal bovine serum (FBS) and 0.1% Triton X-100. Primary antibodies were applied overnight in block at 4°C. Primary antibodies included rabbit β-galactosidase (1:200, 5-Prime, 3-Prime), mouse anti-MAP2 (1:200; Sigma), mouse anti-S-100β (1:400; Sigma), mouse anti-GFAP (1:400; Sigma), sheep anti-EGFR (1:50; Upstate Biotechnology) and goat anti-HB-EGF (1:50; R&D and 1:10; Santa Cruz). Antibodies were visualized with donkey anti-rabbit Cy3 or Cy2, anti-sheep Cy3, anti-goat Cy3 or anti-mouse Cy2 (Jackson ImmunoResearch). For X-gal histochemistry, brains and explants were rinsed in PBS after fixation and incubated in X-gal (Molecular Probes) overnight at 37°C. Tissue was rinsed in PBS, cryoprotected, frozen and sectioned at 50 μm. Sections were stained with DAPI (Molecular Probes) and mounted in glycerol-PBS. Sections were examined with a Leica DMR microscope, using brightfield and fluorescence optics. Images were captured using a Sensys digital camera and IPLab and Photoshop software.

Infections in utero

For infections of E14.5 mice, pregnant CD1 mice were anaesthetized with Nembutal (Abbott Laboratories). After midline laparotomy, the mouse was placed in a bath of warm Locke's solution (Burrows et al.,

1997). One uterine horn was exposed and a fiber optic light placed against the uterine wall to visualize the lateral ventricle. Virus (approximately 1 μl, containing 80 μg/ml polybrene, 0.025% Fast Green, and 10% FBS) was injected with a beveled glass micropipet through the uterine wall into the lateral ventricle. The titer of the virus was 0.5-1×10^7 cfu/ml. Three to four embryos per horn were injected, the uterine horn was replaced, and the mouse sutured and allowed to recover for 3-4 days. To harvest embryos, dams were euthanized by cervical dislocation, the injected embryos removed and the brains were fixed for 2 hours in 4% PFA (in 3% Pipes buffer) at 4°C. Brains were then rinsed in PBS and either stained as wholemounts with X-gal or cryoprotected in graded sucrose. After overnight incubation in X-gal, brains were rinsed, cryoprotected in sucrose and frozen. Frozen sections (50 μm) were collected and counterstained with DAPI (Molecular Probes). The location of X-gal-labeled cells was analyzed using a Leica DMR microscope and brightfield optics. All labeled cells in all sections were counted. For infections at E10.5, timed-pregnant mice were anaesthetized with Nembutal. After midline laparotomy, one uterine horn was exposed. A segment of the uterus containing two embryos was gently pulled through a latex gasket attached to a 10 cm petri dish in which a 25 mm hole had been cut. The dish was filled with warm PBS and the lateral ventricles of the embryos were visualized using an ultrasound backscatter probe (Humphrey Instruments) (Olsson et al., 1997). Virus was injected into the lateral ventricle with a beveled glass pipette, using a mineral oil-filled syringe attached to a manual microsyringe pump (Stoelting). Three or four embryos were injected per animal. The uterus was replaced and the dam sutured. Dams were euthanized by cervical dislocation 4-6 day slater, and the embryos collected and processed as described above.

Western blots

Dorsolateral cortex from E16 mouse embryos was lysed in 50 mM Tris, 150 mM NaCl, 1% NP-40, 0.25% deoxycholate and 1 mM EGTA, containing aprotinin, leupeptin, vanadate and phenylmethylsulfonyl fluoride (PMSF). 110 μg of total protein was separated on a 15% gel by SDS-PAGE. Recombinant human HB-EGF (R&D, 100 ng/lane) was used as a positive control. After transfer to nitrocellulose, blots were probed with HB-EGF antiserum (R&D and Santa Cruz). Bands were visualized with donkey anti-goat HRP (Jackson ImmunoResearch) and ECL (Amersham).

Data analysis

In Fig. 2, each data point represents the mean±s.e.m. from at least three explants. For infections at E14.5 (Fig. 6), we analyzed the locations of 2094 cells in five brains infected with control virus and 3103 cells in four brains infected with EGFR virus. For infections at E10.5 (Fig. 8), we analyzed 2757 cells in five brains infected with control virus and 2293 cells in three brains infected with EGFR virus 4 days post-infection. For analysis 6 days post-infection, we counted 7535 cells in four brains infected with control virus and 8431 cells in three brains infected with EGFR virus. To determine the statistical significance of differences, individual brains were compared using a Mann-Whitney test and StatView software. Differences were considered significant at $P<0.05$.

RESULTS

Virally transduced EGFR mediates chemotaxis in cortical explants

In a previous study, mis-expression of EGFRs in the embryonic rat cortex promoted radial migration toward the cortical plate (Burrows et al., 1997). The mechanism underlying this change in migration could involve chemoattraction or chemorepulsion. To distinguish between these mechanisms, we used explants in which the location and concentration of ligand could be controlled. Explants of dorsolateral cortex or medial cortex plus dorsolateral cortex were cultured on nucleopore filters with their ventricular surface down (Fig. 1A). Progenitor cells were infected with either a control virus (expressing the histochemical marker β-gal) or a virus that co-expresses this marker and wild-type EGFRs. These viruses selectively infect dividing cells and can be used to follow their fate. After 1 day, infected cells were located in the lower third of the explants, corresponding to the ventricular zone (VZ) (Burrows et al., 1997). Some explants were cultured for 3 more days in this orientation ('VZ down'), while others were flipped over ('VZ up') 1 day after infection (Fig. 1A). Ligand was added to the medium underneath the filter beginning 1 day after infection. In the VZ-down cultures, infected progenitor cells were therefore close to the source of ligand initially, but in the VZ-up cultures, they began at a distance from the ligand. The final location of infected cells was visualized by X-gal histochemistry after a total of 4 days in culture (Fig. 1). Experiments were performed with tissue from rats and mice.

In explants of E12.5-E13 mouse cortex infected with control virus and cultured without ligand, infected cells were found throughout the explant after 4 days, whether they were grown VZ down or up (Fig. 1B,D). A similar distribution was observed in the presence of exogenous ligand (TGFα or HB-EGF, 10 ng/ml), whether grown VZ down or up (Fig. 1C,E). EGF-family ligands therefore did not alter migration at this age. In explants of E12.5-E13 mouse cortex infected with EGFR virus and cultured without ligand, infected cells were also distributed throughout the explant after 4 days (Fig. 1F). However, when ligand was added to EGFR-infected explants, infected cells were found predominantly at the surface closest to the source of ligand, (Fig. 1G,H), indicating chemoattraction. Chemotactic responsiveness was seen in explants from younger mice (Fig. 1I) and rats (Fig. 1J-M), at low (0.1 ng/ml; Fig. 1K), intermediate (1 ng/ml; Fig. 1G,H,L) and high (10 ng/ml; Fig. 1I,M) concentrations of ligand, in both medial (Fig. 1L) and lateral cortex. Other members of the EGF-ligand family including EGF, amphiregulin and β-cellulin also stimulated chemotaxis (data not shown). These results demonstrate that telencephalic cells can respond chemotactically to EGF-family ligands, but they appear to require a high level of EGFRs for this response.

Chemotaxis is independent of cell type

The population of cells that expresses a high level of EGFRs in the embryonic telencephalon is heterogeneous and includes astrocytes, stem cells and neurons (Seroogy et al., 1995; Weickert and Blum, 1995; Kornblum et al., 1995; Kornblum et al., 1997; Eagleson et al., 1996; Lillien and Raphael, 2000). It has been reported that EGFR stimulation can mediate the chemotactic migration of astrocytes (Elenius et al., 1997). Moreover, many of the progenitor cells infected with EGFR virus that migrated to the cortical plate differentiated preferentially into astrocytes (Burrows et al., 1997). These findings raised the possibility that chemotactic migration could be elicited only after induction of an astrocyte fate. Alternatively, chemotaxis might be elicited in multiple types of cells if they express sufficient numbers of EGFRs. To distinguish between these possibilities, we analyzed the antigenic phenotype of cells that migrated

towards EGF family ligands. We also determined whether this population included multipotent stem cells that could divide in response to EGF to generate neurospheres (Reynolds and Weiss, 1992).

Nearly all of the EGFR-infected cells migrated toward ligand in explants of E12 rat cortex (Fig. 1). This population includes neurons (Fig. 2A-C) and astrocytes (Fig. 2A,D,E). Moreover, after they migrated, approximately 25% of the EGFR-infected cells could be stimulated to divide in response to EGF to generate neurospheres, characteristic of stem cells (Fig. 2A). These findings demonstrate that chemotactic migration does not require that cells differentiate into astrocytes; chemotaxis can also be elicited in neurons and multipotent stem cells, provided they express sufficient numbers of EGFRs.

EGFR level and cellular distribution change during embryonic development

EGFR expression normally increases between E13 (Fig. 3A,C) and E16 (Fig. 3B,D) in the mouse telencephalon. An increase in EGFR expression was also observed in rat cortex during mid-late stages of embryonic development (data not shown). Only a small proportion of cells express this high level of the EGFR, for example, fewer than 1% of the cells in E16 mouse dorsal cortex. The distribution of these cells at E16 is graded, however, with more cells expressing a high level EGFRs in lateral cortex ('L' in Fig. 3B), than in medial cortex ('M' in Fig. 3B). Cells that express a high level of EGFR were found in large numbers in migration pathways, including the radial pathway from proliferative zone to cortical plate via the intermediate zone (Fig. 3B,D), the lateral cortical stream (Fig. 3F-I) and the rostral migratory stream (Fig. 3E,J). The lateral cortical stream contains cells that originate from the proliferative zones near the corticostriatal sulcus (asterisk, Fig. 3) (Bayer et al., 1991; Bayer and Altman, 1991b). These cells migrate laterally and ventrally during mid-late embryonic development to provide neurons and glia to the lateral and ventral cortex (Fig. 3F-I) (Bayer et al., 1991). These regions of the cortex do not have proliferative zones directly beneath them because they lie above the lateral ganglionic eminence (LGE, future striatum). To reach the lateral and ventral cortex, cells migrate around the LGE, via the LCS (Fig. 3F-I). Cells that express a high level of EGFRs are also found in the RMS leading to the olfactory bulb from the anterior SVZ (Fig. 3E,J).

The distribution of the EGFRs on the cell surface also changed during development. At E13, EGFR immunoreactivity was concentrated at the apical surface of cells lining the lateral ventricle (Fig. 3A,C). By contrast, at E16 EGFR staining was distributed more uniformly over the surface of cells (Fig. 3B-D,G-J). Virally transduced EGFR is also distributed over the surface of infected cells, and its expression is only two to three times higher than the average endogenous level observed during late embryonic development (Burrows et al., 1997).

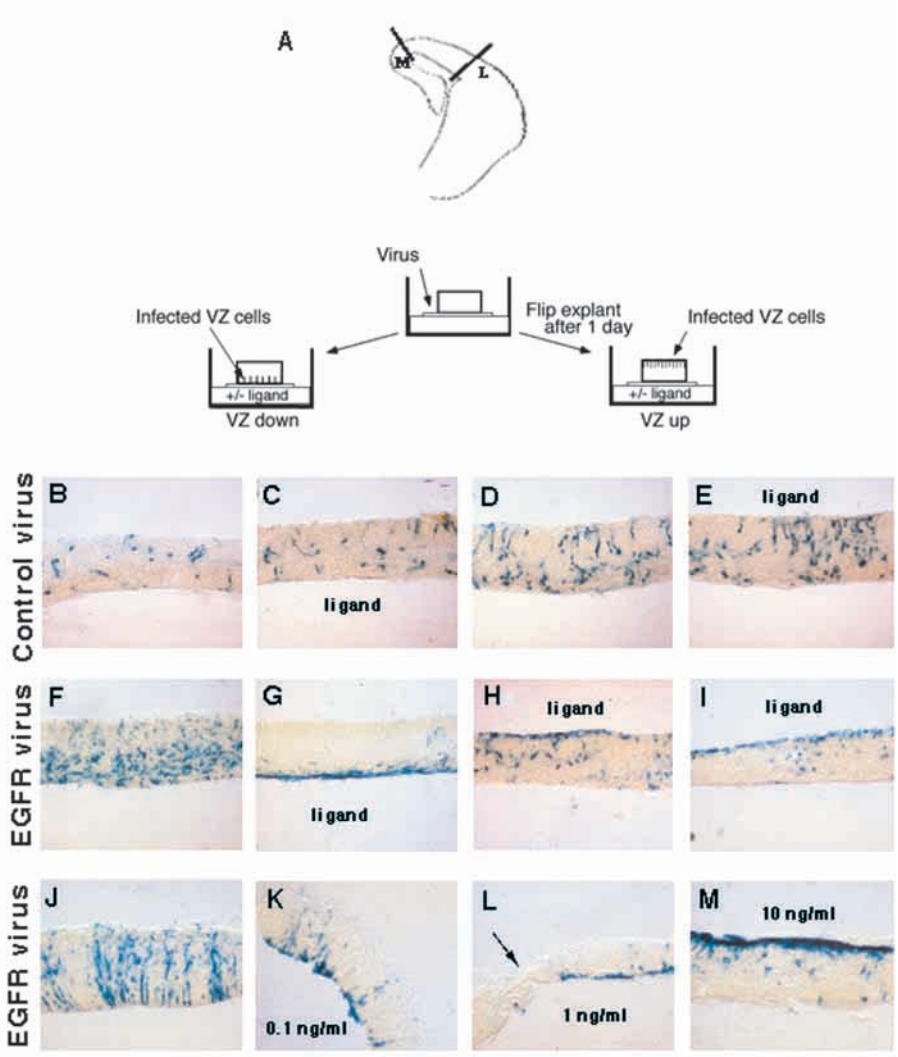

Fig. 1. EGFRs mediate chemotaxis after viral transduction of explants. (A) How the explants were dissected and manipulated to alter the orientation of infected cells relative to ligand. Dorsolateral cortex was used in all experiments except L, where dorsolateral and medial cortex was used. In all micrographs, the ventricular surface is down, whether explants were grown VZ up or down, and the relative location of ligand is indicated. At least three explants were analyzed per condition. Similar distributions of cells were noted in triplicates. (B) E12.5 mouse, control virus, grown VZ down, no ligand; (C) E12.5 mouse, control virus, VZ down, TGFα 10 ng/ml; (D) E12.5 mouse, control virus, VZ up, no ligand; (E) E12.5 mouse, control virus, VZ up, HB-EGF 10 ng/ml; (F) E12.5 mouse, EGFR virus, VZ down, no ligand; (G) E12.5 mouse, EGFR virus, VZ down, HB-EGF 1 ng/ml; (H) E12.5 mouse, EGFR virus, VZ up, HB-EGF 1 ng/ml; (I) E10.5 mouse, EGFR virus, VZ up, HB-EGF 10 ng/ml; (J) E12 rat, EGFR virus, VZ down no ligand; (K) E12 rat, EGFR virus, VZ down, TGFα 0.1 ng/ml; (L) E12 rat, EGFR virus, VZ down, TGFα 1 ng/ml (arrow indicates midline between hemispheres); and (M) E12 rat, EGFR virus, VZ up, TGFα 10 ng/ml.

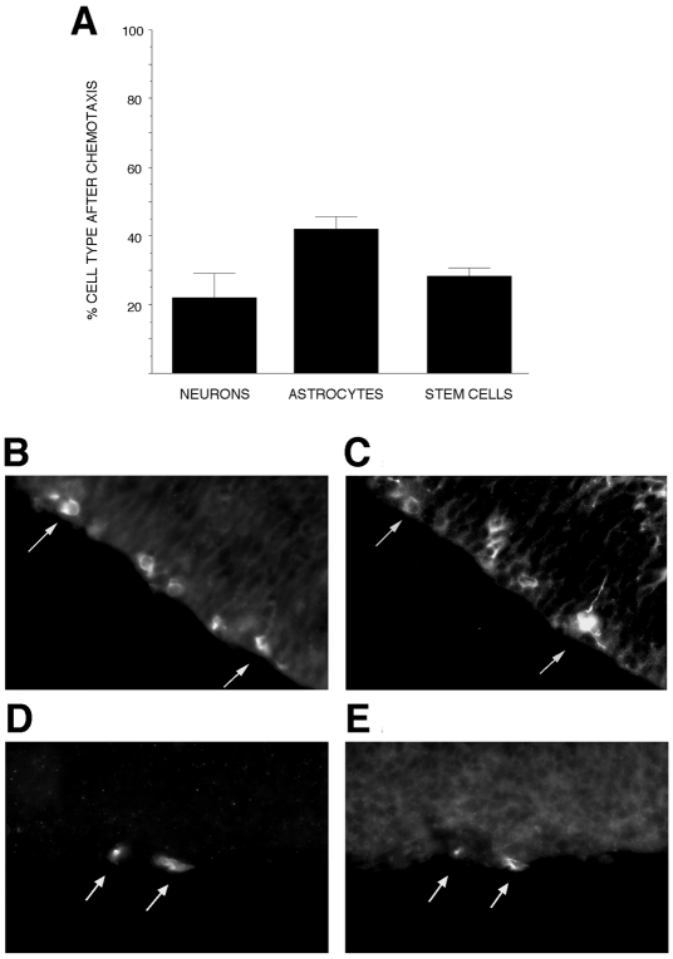

Fig. 2. Chemotaxis is independent of cell type. (A) The identity of cells that chemotax in response to TGFα or HB-EGF (1 ng/ml) was determined in E12 rat cortical explants infected with EGFR virus. MAP2 was used to identify neurons, S-100β and GFAP were used to distinguish astrocytes. EGFR-infected cells that chemotaxed (labeled with anti-β-gal in B,D; arrows) expressed the neuronal marker MAP2 in C or the astrocyte marker GFAP in E (arrows). To determine whether any of the cells that chemotax were stem cells, explants were dissociated after 3 days in ligand and the ability of EGFR-infected cells to divide in response to EGF (0.1-1 ng/ml) to generate neurospheres was assessed after 10 days (A), as described in the Materials and Methods. Approximately 25% of the EGFR-infected cells that chemotax could generate neurospheres, a characteristic of stem cells.

High endogenous EGFRs mediate chemotaxis

If a high level of EGFR expression is required for chemotaxis, and endogenous EGFR expression rises by E16, the cells that express a high endogenous level of EGFRs at E16 should migrate toward a source of ligand. To test this idea, explants of E16 mouse cortex were grown in the absence or presence of HB-EGF and the location of cells that express a high level of endogenous EGFR was assessed 3 days later (Fig. 4). Without exogenous HB-EGF, most of the EGFR+ cells were found diffusely distributed in the lower third of the explants (Fig. 4A). By contrast, after exposure to HB-EGF for 3 days, EGFR+ cells were densely packed in a thin layer close to the source of exogenous ligand (Fig. 4B). Taken together with the results from viral transduction of younger cells, these findings indicate that cells expressing a high level of EGFRs can migrate chemotactically through their normal tissue environment toward a source of ligand. The number of EGFRs necessary for this response is normally achieved between E13-E16 in mice and E15-E18 in rats.

Ligand expression

Several ligands that activate the EGFR, including TGFα and HB-EGF, are expressed in selected regions of the embryonic telencephalon. In rat, TGFα mRNA is expressed in the choriod plexus at least as early as E15 and in the LGE as early as E13 (Kornblum et al., 1997). If TGFα made in choroid plexus is cleaved to a diffusible form it could be released into the cerebral spinal fluid filling the lateral ventricle to act on cells at the cortical ventricular surface. HB-EGF mRNA has been reported to be expressed in proliferative zones of the rat cortex at least as early as E14 (Nakagawa et al., 1998). Staining with anti-HB-EGF antiserum in the E13 mouse revealed immunoreactivity at the ventricular surface (Fig. 5A).

mRNA for HB-EGF, but not TGFα, is expressed in the mid-late embryonic cortical plate in rats (Kornblum et al., 1999). Consistent with this report, we detected HB-EGF immunoreactivity in the dorsal and lateral cortical plate, with the most intense staining in two stripes, corresponding to subplate/white matter and marginal zone in the E16 mouse (Fig. 5B). A similar pattern of staining was observed in E15 rat (data not shown). These are the layers settled preferentially by EGFR-infected cells in mid-embryonic rat (Burrows et al., 1997). More diffuse staining for HB-EGF was also seen in the intermediate zone, especially laterally (Fig. 5B,C). TGFα mRNA is expressed in ventrolateral regions of the cortex by E17 in rat (Kornblum et al., 1997), as is HB-EGF mRNA (Kornblum et al., 1999). Together, HB-EGF and TGFα could create a lateral-high, medial-low gradient of ligand. Consistent with mRNA expression, we noted a gradient of HB-EGF immunoreactivity in E16 mouse cortex that was more intense laterally (Fig. 5B,C).

HB-EGF is initially expressed in membrane-anchored forms which are cleaved to generate diffusible forms. Although earlier reports suggested that the membrane-anchored forms of EGF family ligands were biologically active (Brachman et al., 1989; Wong et al., 1989), recent work indicates that cleavage to generate diffusible forms is more important for migration than originally thought (Dong et al., 1999; Tokumaru et al., 2000). Expression of cleaved, diffusible forms of mouse HB-EGF in E16 dorsolateral cortex was confirmed by western blot. In addition to the higher molecular weight, membrane-associated forms of HB-EGF (25-32 kDa), lower molecular weight (19-24 kDa), diffusible forms (Ono et al., 1994; Gechtman et al., 1999) were observed (Fig. 5D).

Mis-expression of EGFRs in vivo

The patterns of EGFR and ligand expression, together with the demonstration of chemotaxis via EGFRs in explants, suggest that EGFRs could mediate chemotaxis radially to the cortical plate, ventrolaterally via the LCS, and tangentially to the olfactory bulb via the RMS. To test this idea, we mis-expressed the EGFR in vivo. This serves to increase the size of the population of cells that expresses a threshold level of EGFRs needed for chemotaxis. If EGFRs mediate chemotaxis, mis-

expression should promote migration of EGFR-infected cells in these pathways.

We first injected virus into the lateral ventricles of E14.5 mice and analyzed the laminar and regional distribution of infected cells 3-4 days later. Analysis of laminar distribution, an indicator of radial migration to the cortical plate, focused on dorsal cortex because the number of infected cells was greater there than in other regions (Fig. 6A,B). The dorsal cortex was divided into seven layers: ventricular zone (VZ), subventricular zone (SVZ), intermediate zone (IZ), subplate/white matter (sp/wm), lower cortical plate (cp-l), upper cortical plate (cp-u) and marginal zone (MZ) (Fig. 6C-E). Mis-expression of EGFRs at this stage of development promoted radial migration to the cortical plate (Fig. 6C-E), as observed after mis-expression in mid-embryonic rat (Burrows et al., 1997). As in the rat study, these cells settled predominantly in the marginal zone and deep cortical plate (subplate/white matter plus lower cortical plate) (Fig. 6C,E). By contrast, the proportion of control infected cells was greater in the inner layers of the cerebral wall, the VZ, SVZ and IZ (Fig. 6C,D).

We also compared the proportion of control and EGFR-infected cells that migrated ventrolaterally via the LCS and tangentially into the olfactory bulb via the RMS. At this stage of embryonic development, mRNA for several EGF family ligands is expressed in the targets of these pathways, the ventrolateral cortex and the olfactory bulb, respectively (Lazar and Blum, 1992; Kornblum et al., 1997; Kornblum et al., 1999). If EGFRs mediate chemotactic migration in these pathways, we expected to see a greater proportion of EGFR-infected cells in ventrolateral cortex and in the olfactory bulbs. In fact, the proportion of EGFR-infected cells in these regions was not greater than control-infected cells 3-4 days after infection (Fig. 6B). Within the olfactory bulb, however, the laminar distribution of EGFR-infected cells was altered in the same manner as in the dorsal cortex: a greater proportion of the EGFR-infected cells migrated radially out of proliferative zones into the differentiated zone, compared to control-infected cells (Fig. 6F).

To determine whether earlier infections and longer post-

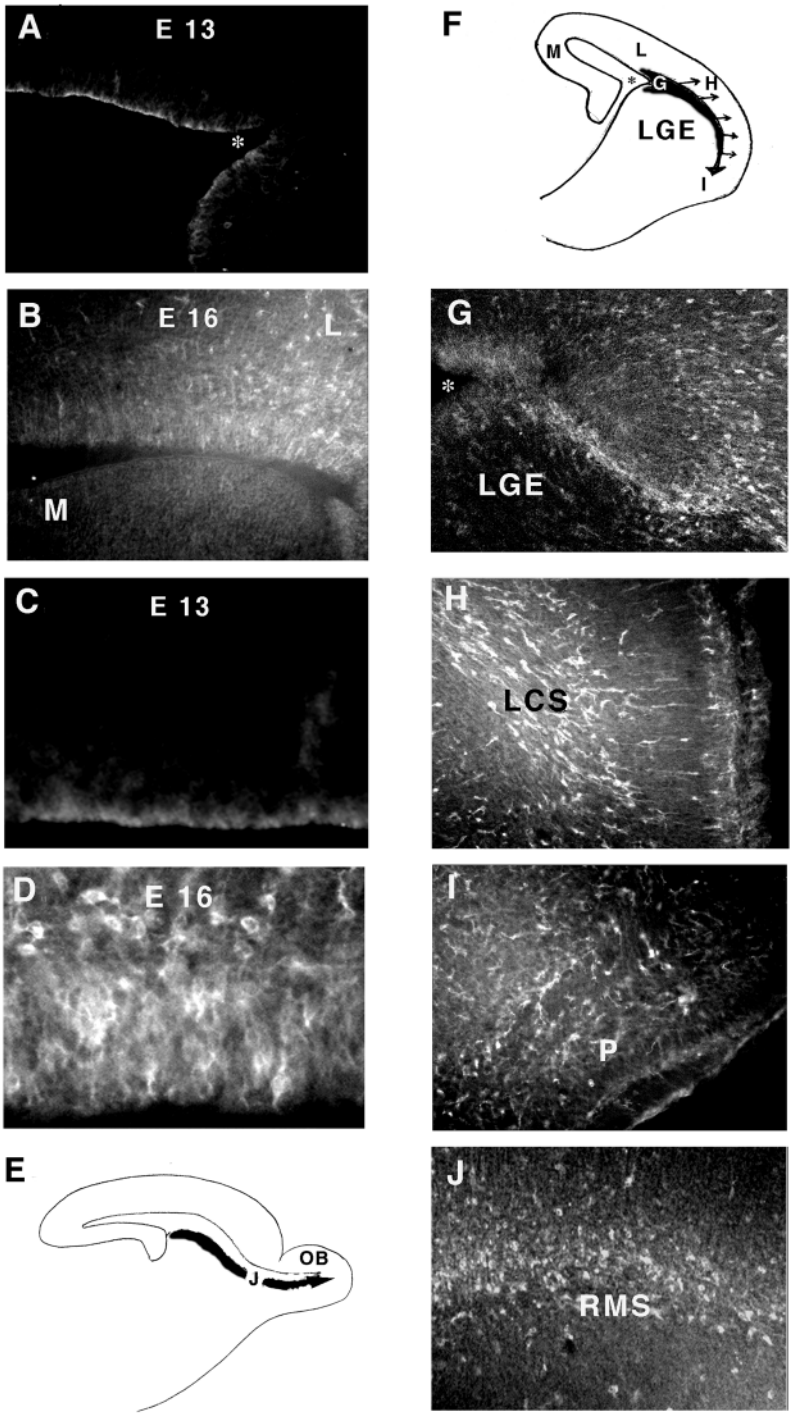

Fig. 3. Expression of EGFRs changes during development. At E13 (A,C) EGFR immunostaining is relatively low, but increases by E16 (B,D) in mouse telencephalon. The distribution of cells expressing a high level of EGFR immunoreactivity was graded in the E16 cortex (B),with more cells expressing a high level of staining in lateral regions (L) than in medial regions (M). Asterisk indicates corticostriatal sulcus. The distribution of EGFRs on the cell surface also changes. At E13, EGFR immunostaining was concentrated at the apical surfaces of cells lining the lateral ventricle (A,C), but at E16 it was distributed more uniformly over the cell surface (B,D). C,D are higher magnifications A,B, respectively. The cells that expressed a high level of EGFRs were seen in proliferative zones (B,D) and in migration pathways, including the intermediate zone (B,D), the lateral cortical stream (LCS) (F-I) and the rostral migratory stream (RMS) (E,J). E illustrates the RMS in a sagittal view, with the letter J indicating the approximate position of the image in J. OB, olfactory bulb. F illustrates the LCS migration route, with letters indicating the approximate positions of images in panels G-I (M and L refer to medial and lateral). G shows EGFR expression at the beginning of the pathway, near the lateral ventricle (corticostriatal sulcus), as the pathway curves around the lateral ganglionic eminence (LGE). H shows the LCS in the ventrolateral cortex, with EGFR-positive cells leaving to migrate radially to the cortical plate. I shows the LCS leading into the piriform cortex (P). J shows EGFR-positive cells in the RMS posterior to the olfactory bulb in a parasagittal section. All other sections are transverse.

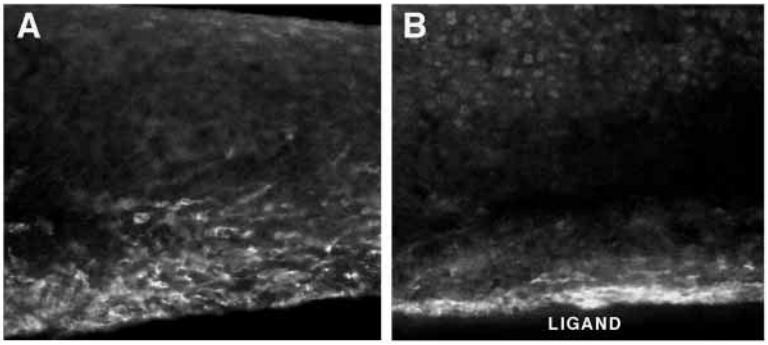

Fig. 4. Endogenous EGFRs mediate chemotaxis. Explants of E16 dorsolateral cortex were cultured VZ down without (A) or with (B) exogenous HB-EGF (10 ng/ml) for 3 days and the location of cells expressing a high endogenous level of EGFRs was determined immunocytochemically.

infection intervals were required to see EGFR-mediated changes in migration in the LCS and RMS, we infected mouse progenitor cells in utero at E10.5, rather than E14.5. To perform these infections, we used ultrasound backscatter imaging to visualize injections into lateral ventricles (Olsson et al., 1997). The location of infected cells was analyzed 4, 5 and 6 days later. For this analysis, the cerebral wall was divided into six regions, as illustrated in Fig. 6A, and the laminar distribution of infected cells was determined as described for E14.5 infections (Fig. 6C). Infected cells were also counted in olfactory bulb.

In brains infected with control virus at E10.5 and analyzed 6 days later, large clusters of cells were observed in the cortical plate in dorsomedial, dorsal, lateral and ventrolateral regions of the cortex (Fig. 7A). In brains infected with EGFR virus, however, more cells were found in the marginal zone and the subplate/white matter in lateral and ventrolateral regions of the cortex (Fig. 7B). In ventrolateral cortex, cells infected with EGFR virus (Fig. 7C,D) or control virus (Fig. 7E,F) were found in the lateral cortical stream (LCS). The EGFR-infected cells were found among cells that express a high level of endogenous EGFRs (Fig. 7E,F and see Fig. 3G-I). By contrast, in dorsomedial cortex, a greater proportion of cells infected with EGFR virus were found in proliferative zones (Fig. 7B). These lateral-medial differences in migration are consistent with the pattern of ligand expression described above (see Fig. 5). Quantitative analysis of the distribution of control and EGFR-infected cells 6 days post-infection is summarized in Fig. 8A-D. Comparing the proportion of EGFR-infected cells in the subplate/white matter and marginal zone layers in ventrolateral, lateral, dorsal and dorsomedial regions highlights the ventrolateral to dorsomedial gradient in their migration to these layers.

We also analyzed infected brains after a shorter interval, 4 days post-infection, and noted several differences in the laminar and regional distributions of infected cells. In dorsal cortex 4 days post-infection, more EGFR-infected cells were still in proliferative zones, compared with control-infected cells (Fig. 8E). This suggests that EGFR-infected cells were initially retained in proliferative zones, but migrated out of proliferative zones rapidly between 4 and 6 days post-infection in dorsal cortex (Fig. 8B,E), but not in dorsomedial cortex (Fig. 8A). This is consistent with temporal and spatial changes in HB-EGF expression described above (see Fig. 5).

The regional distribution of EGFR-infected cells also changed between 4 and 6 days post-infection. The proportion of EGFR-infected cells in ventrolateral cortex rose while the proportion in dorsal cortex declined (Fig. 8F). By contrast, the proportions of EGFR-infected cells in other areas, such as the LGE and hippocampus, did not change between 4 and 6 days (data not shown). Moreover, the proportions of control-infected cells in dorsal and ventrolateral cortex did not change between 4 and 6 days (Fig. 8F). The ventral shift in the distribution of infected cells was therefore specific to cortical cells infected with EGFR virus. This suggested that many of the EGFR-infected cells in dorsal cortex were diverted ventrolaterally between 4 and 6 days. Consistent with this idea, at 5 days post-infection EGFR-infected clones in dorsal cortex contained cells that were aligned radially in proliferative zones, but appeared to be migrating laterally in

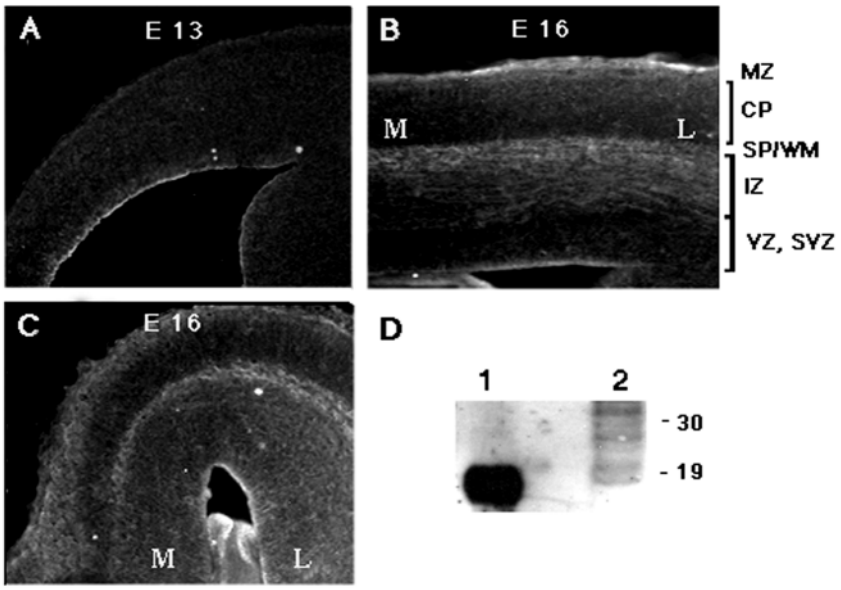

Fig. 5. Temporal and spatial patterns of HB-EGF staining in E13 (A) and E16 mouse (B,C). At E13 (A), HB-EGF immunoreactivity was seen at the ventricular surface. By contrast, at E16 (B), staining was most intense in two additional layers, corresponding to the marginal zone (MZ) and subplate/white matter (SP/WM). Diffuse staining was also visible in the intermediate zone (IZ). VZ, ventricular zone; SVZ, subventricular zone. Staining was more intense laterally (L) than medially (M) (B,C). (D) Western blot of lysates of E16 mouse dorsolateral cortex probed with anti-HB-EGF. Lane 1, recombinant HB-EGF (R&D); lane 2, E16 lysate.

the intermediate zone (Fig. 8G). The lateral cortical stream is a lateral extension of the cortical intermediate zone, and it consists of cells that migrate ventrolaterally (Bayer et al., 1991). The presence of EGFR-infected cells in the lateral cortical stream (Fig. 7C-F) suggests that the dorsal-to-ventral shift in their distribution involves migration via the lateral cortical stream.

We also examined the distribution of infected cells in the olfactory bulb. As observed in brains infected at E14.5, earlier mis-expression of EGFRs did not promote tangential migration into the olfactory bulbs via the RMS (Fig. 8E). Once in the bulbs, however, expression of EGFRs tended to promote radial migration from proliferative zones to the differentiating zone, as noted for E14.5 infections; for example, 6 days post-infection at E10.5, 20% of control-infected cells were still in the VZ of the olfactory bulb. None of the EGFR-infected cells remained in the VZ but were located in the SVZ (35%) or the differentiating zone (65%).

DISCUSSION

Several observations have implicated EGFRs in the control of migration in the developing telencephalon. The phenotype of EGFR-null mice (Threadgill et al., 1995) and the effects of viral transduction of the receptor in the mid-embryonic rat (Burrows et al., 1997; Burrows et al., 2000) suggested that EGFRs were important for the migration of cells from proliferative zones, though it was not clear whether EGFRs mediated chemotaxis or chemorepulsion. The present study demonstrates that the expression of a high level of EGFRs, characteristic of a subpopulation of cells in the mid-late embryonic telencephalon, confers competence to chemotax in response to EGF-family ligands. In vivo, mis-expression of EGFRs promotes migration radially to the cortical plate and ventrolaterally via the

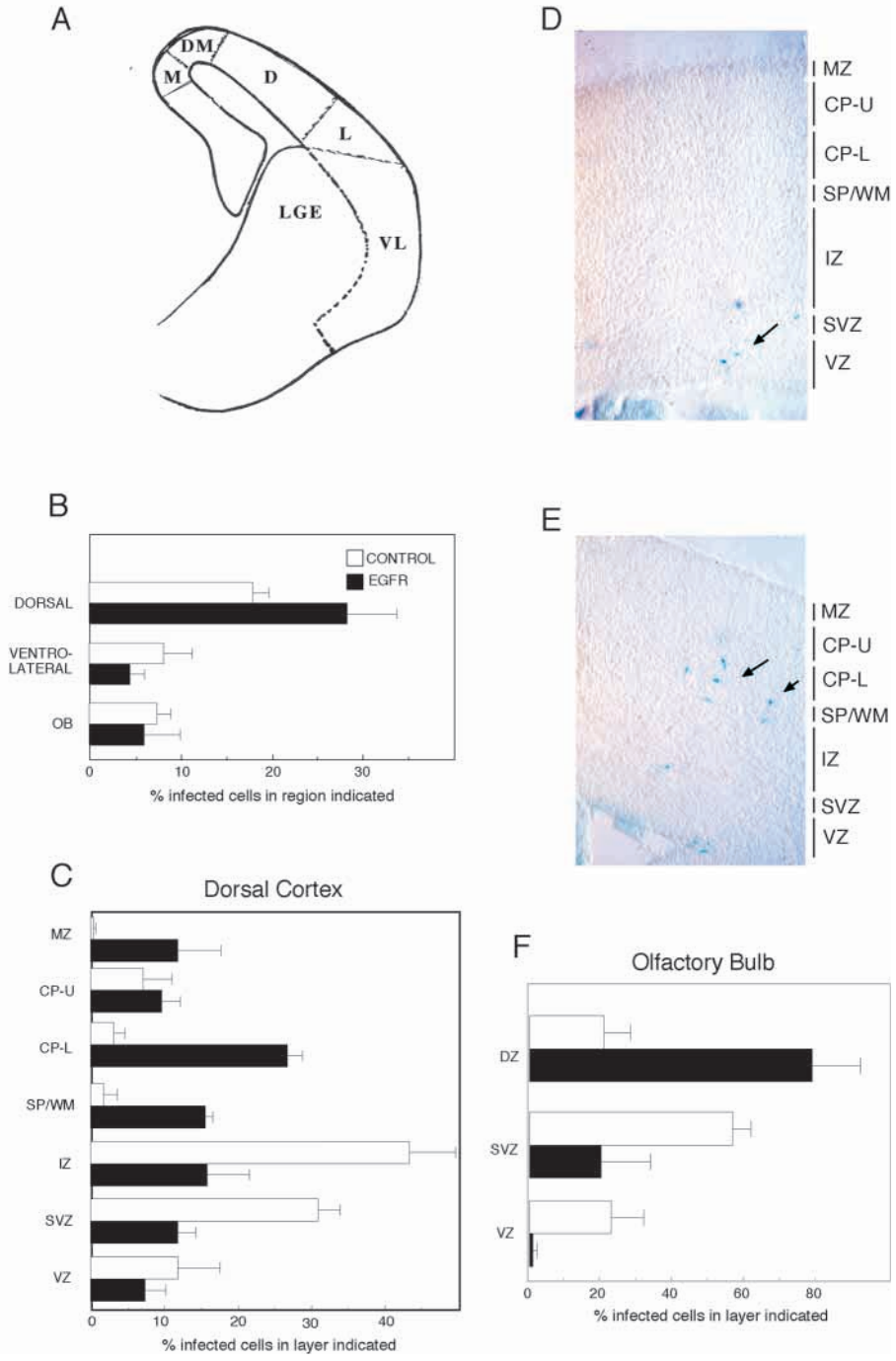

Fig. 6. Mis-expression of EGFRs in vivo at E14.5. The laminar and regional positions of cells infected in utero with control or EGFR virus were determined 3-4 days after infection. (A) Illustration of regional divisions. M, medial; DM, dorsomedial; D, dorsal; L, lateral; VL, ventrolateral. For control virus, 2757 cells in five embryos were counted; for EGFR virus, 3103 cells in 4 embryos were counted. (B) Regional distribution of infected cells in cortex (dorsal, ventrolateral) and olfactory bulb (OB). Note that mis-expression of EGFRs does not promote migration to the olfactory bulb via the RMS or the VL cortex via the LCS. (C) Laminar positions of infected cells in dorsal cortex. VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; sp/wm, subplate/white matter; cp-l, lower half of cortical plate; cp-u, upper half of cortical plate; MZ, marginal zone. More of the EGFR-infected cells were located in sp/wm and MZ than control-infected cells (28.9±7.5 versus 2.2±1.9; P=0.01). By contrast, more control-infected cells were located in the inner half of the cerebral wall (VZ+SVZ+IZ) than EGFR-infected cells (87.4±5.9 versus 35.6±9.9; P=0.01). (D) Micrograph of cells infected with control virus. Several cells in the inner half of the cerebral wall are shown (arrow points to cells in VZ). (E) Micrograph of cells infected with EGFR virus. Several cells in the sp/wm are shown (arrows). (F) The laminar positions of infected cells in the olfactory bulb indicated that within the bulb, EGFR-misexpression promoted migration out of proliferative zones and into the differentiating zone (78.9±13.5 versus 22.3±5.9; P=0.025), as in dorsal cortex.

lateral cortical stream, mimicking the migration of cells that normally express a high level of EGFRs at mid-late stages of embryonic development (Fig. 8H). Cells in the RMS also express a high level of EGFRs normally, and mis-expression of EGFRs promoted radial migration of cells within the bulb, but not tangential migration into the bulb. Our results suggest that temporal and spatial changes in ligand expression in the targets of these pathways, together with an increase in EGFR expression at mid-embryonic stages, regulate the migration and settling of specific populations of cells in developing telencephalon by a chemoattractive mechanism.

Threshold levels of EGFRs mediate chemotaxis in multiple types of cells

Our previous work demonstrated that several responses to EGF family ligands required a high level of EGFR expression. These responses include proliferation as a multipotent stem cell and differentiation into astrocytes (Burrows et al., 1997). A threshold mechanism appeared to determine the choice between these responses, with low concentrations of ligand (0.1-1 ng/ml) favoring proliferation and higher concentrations (10 ng/ml) favoring astrocyte differentiation.

Observations in this study and in previous work (Burrows et al., 1997) support the idea that chemotactic migration towards

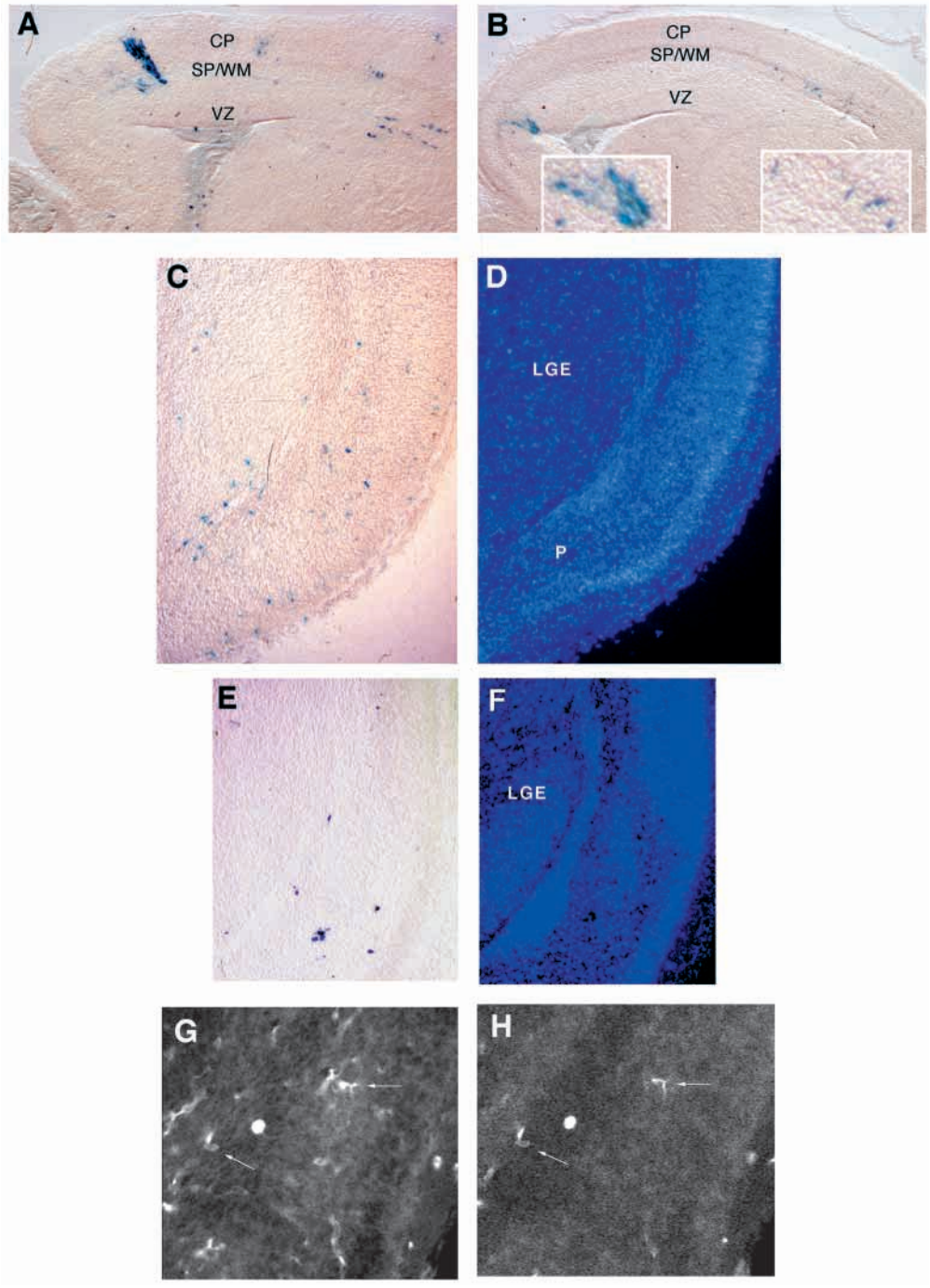

Fig. 7. Infection at E10.5 results in region-specific changes in migration. (A) Micrograph of control-infected cells 6 days after injection at E10.5. Note clusters of cells in cortical plate (CP). (B) Micrograph of EGFR-infected cells 6 days after infection at E10.5. Note cells in subplate/white matter (SP/WM) in lateral and ventrolateral cortex, but not dorsomedial cortex, where they remained in proliferative zones (VZ, ventricular zone). Insets: higher magnification images of cells in dorsomedial (left) and ventrolateral (right) cortex. In ventrolateral cortex, EGFR-infected cells were found in the lateral cortical stream (C,D), migrating toward the piriform cortex (P). (C) Brightfield micrograph of X-gal labeled cells, (D) DAPI. (E,F) Control-infected cells were also found in the lateral cortical stream (E, brightfield; F, DAPI). (G,H) EGFR-infected cells (arrows) in the lateral cortical stream were found among cells that express high endogenous EGFRs. G was stained with an antibody that recognizes both endogenous and virally transduced EGFRs; in H, the EGFR-infected cells could be distinguished by expression of β-galactosidase.

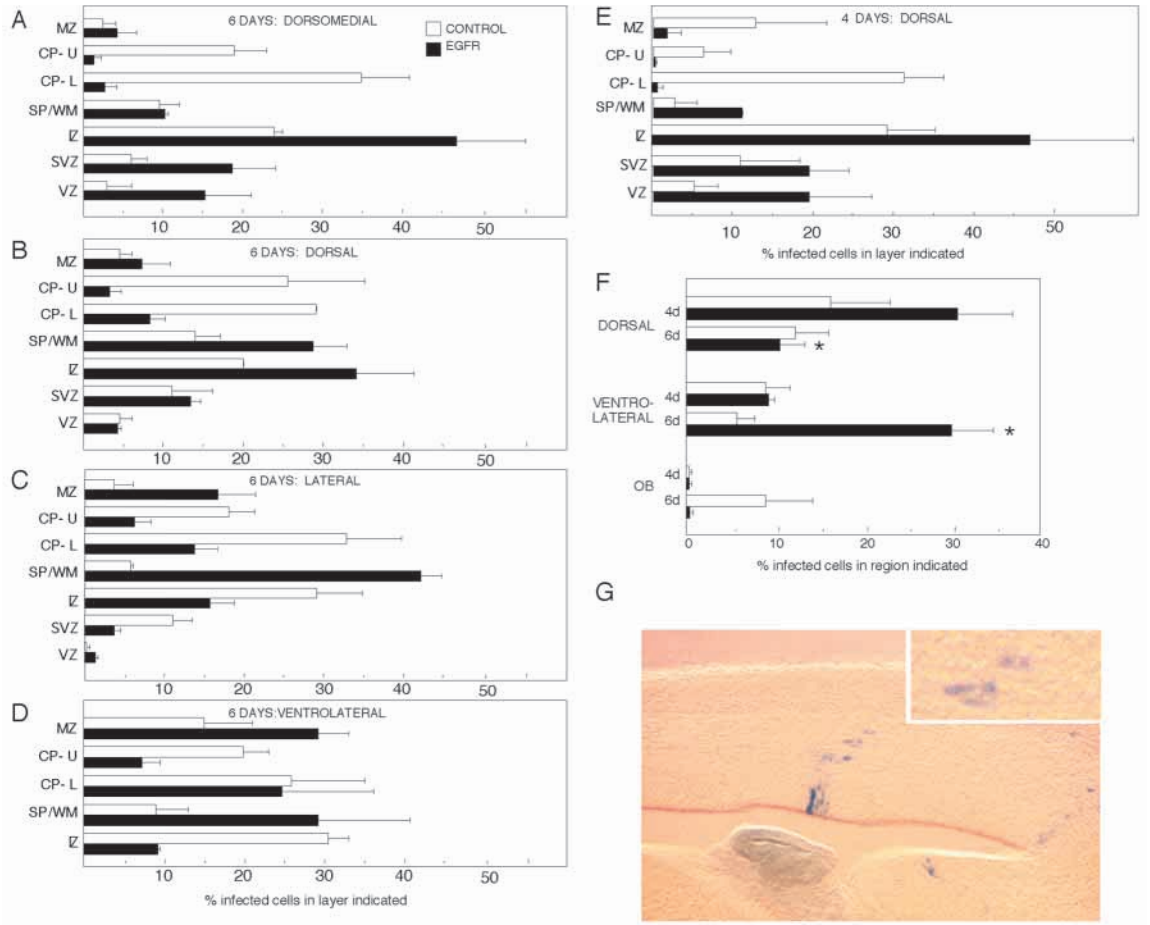

Fig. 8. Laminar and regional distributions of infected cells 6 and 4 days after infections at E10.5. For control virus, 7535 cells in four embryos were analyzed 6 days after infection, and 2757 cells in five embryos were analyzed 4 days after infection. For EGFR virus, 8431 cells in three embryos were analyzed 6 days after infection, and 2293 cells in three embryos were analyzed 4 days after infection. (A) In dorsomedial cortex, the migration of EGFR-infected cells out to the cortical plate was reduced relative to control infected cells, with more of the EGFR-infected cells located in proliferative zones (VZ, SVZ) and the IZ 6 days after infection. By contrast, in dorsal (B), lateral (C) and ventrolateral cortex (D), EGFR-infected cells were found in greater proportions in sp/wm and MZ layers 6 days after infection. (E) In dorsal cortex 4 days post-infection, more of the EGFR-infected cells were located in proliferative zones, compared with control infected cells, suggesting that their migration to the sp/wm and MZ occurred between 4 and 6 days post-infection. (F) Comparison of the proportion of infected cells in different regions 4 and 6 days after infection revealed a dorsal to ventrolateral shift in EGFR-infected cells, but not control-infected cells. This suggests that EGFR-infected cells from dorsal cortex were diverted into the LCS. Asterisk indicates that the difference between the proportion of EGFR-infected cells in dorsal cortex at 4 days versus 6 days was significant (30.5±6.1 versus 10.5±2.9; P=0.04), as was the difference in ventrolateral cortex comparing 4 and 6 days (9.6±0.7 versus 30.1±4.7; P=0.01). (G) Micrograph of a clone of EGFR-infected cells in dorsal cortex 5 days post-infection. Note the radial alignment of cells in proliferative zones and the lateral displacement in the IZ. Inset: higher magnification image of cells in the IZ displaced laterally. (G) Model summarizing migration pathways containing EGFR-positive cells, ligands and the effects of EGFR misexpression. Pink circles represent cells expressing high endogenous EGFR, blue circles represent EGFR-infected cells, stippling represents ligands (HB-EGF and/or TGFα). Left, transverse view; right, sagittal view.

EGF family ligands also requires a high level of EGFR expression. Our results show that chemotaxis can be elicited at low and high ligand concentrations in multiple types of cells, including neurons and stem cells. How can the ability of EGF family ligands to induce astrocytes be reconciled with chemotactic migration of neurons and multipotent stem cells? The concentration of ligand and the state of the cell may be crucial in determining the choice of response. For example, if cells become committed to a neuronal fate before elevation of EGFR expression, they could chemotax as neurons, or as neuron-restricted progenitor cells. If stem cells were exposed to relatively low concentrations of ligand, they could chemotax

in response to EGFR stimulation and remain multipotent. Several recent studies have highlighted the migratory nature of neural stem cells (Noble, 2000). Our findings raise the possibility that EGFRs contribute to their migration.

Cellular mechanism that underlies changes in migration and settling

Early embryonic cortical cells express low levels of the EGFR and respond to EGFR stimulation (Ferri and Levitt, 1995; Eagleson et al., 1996), but they do not chemotax in response to EGF family ligands. This could reflect differences in the coupling of EGFRs to intracellular transduction pathways at different levels of receptor expression. Alternatively, the ability to sense a gradient of chemoattractant could depend on expression of a threshold number of receptors distributed more uniformly over the cell surface. A uniform distribution of EGFRs has been reported for several types of cells that chemotax in response to EGF family ligands (Servant et al., 2000; Bailly et al., 2000), and is evident in the late embryonic mouse cortex (see Fig. 3).

In addition to altering the direction of migration in the telencephalon, the expression of a threshold level of EGFRs affected settling patterns. More of the cells infected with EGFR virus were found in the deepest layers of the cortical plate and the marginal zone, compared with cells infected with control virus, when analyzed at E16.5-E17.5. This settling pattern was also observed after infection of embryonic rat cortex (Burrows et al., 1997). These layers exhibit the strongest immunostaining for HB-EGF. Although HB-EGF promoted chemotactic migration, these settling patterns suggest that very high levels of ligand might act as a stop signal. Consistent with this idea, sustained activation of EGFRs has been shown to inhibit migration in *Drosophila* (Duchek and Rorth, 2001), and sustained activation of Raf, one of the intracellular components of EGFR signaling, was reported to inhibit migration of rat embryo fibroblasts (Slack et al., 1999).

In other types of cells, EGFRs have been shown to modulate motility by a variety of mechanisms. They can act directly to alter migration by associating with molecules that control cell adhesion and cytoskeletal organization (Hoschuetzky et al., 1994; Xie et al., 1998; Li et al., 1999; Li et al., 2000; Sieg et al., 2000; Chan et al., 2000), or indirectly by regulating the level of extracellular molecules that modify the ECM through which cells migrate (Miettinen et al., 2000). The mechanisms by which EGFRs control the migration of cells in the telencephalon remain to be elucidated, but preliminary studies involving transplantation of EGFR-null cells into wild-type hosts suggest that both cell-autonomous (direct) and non-autonomous (indirect) mechanisms contribute to EGFR-mediated migration (J. V. and A. F., unpublished).

Ligand expression

Several members of the EGF ligand family are expressed in the developing telencephalon. TGFα mRNA has been observed in the olfactory bulb, LGE, ventrolateral cortex, thalamus and choriod plexus at the stages of development studied here (Lazar and Blum, 1992; Kornblum et al., 1997; Kornblum et al., 1999; Nakagawa et al., 1998). HB-EGF mRNA was observed in the cortical plate in several studies, and in thalamus, though there are conflicting reports concerning the expression of HB-EGF in proliferative zones of the cortex (Nakagawa et al., 1998; Kornblum et al., 1999). The HB-EGF immunoreactivity we observed in the cortical plate is consistent with mRNA expression, including the higher levels of expression ventrolaterally (Kornblum et al., 1999). As thalamic neurons also express HB-EGF mRNA (Kornblum et al., 1999), it is possible that some of the immunoreactivity seen in the subplate and intermediate zone is derived from thalamic axons. Expression of HB-EGF and TGFα by thalamocortical axons could also contribute to the lateral-medial gradient of immunoreactivity seen at mid-embryonic stages and provide a substratum for cells migrating ventrolaterally (see Fig. 5).

EGF family ligands were reported to be biologically active in their membrane-anchored form (Brachman et al., 1989; Wong et al., 1989), but the importance of ectodomain cleavage for specific biological responses, including migration, has been demonstrated by several recent studies (Dong et al., 1999; Tokumaru et al., 2000). Therefore, even though mRNA or protein is expressed, the ligand may not be active. Viral transduction of EGFRs in cortical progenitor cells confers chemotactic competence and can serve as a probe for biologically active ligand in vivo. The alterations in migration and settling observed among these cells matches the pattern of HB-EGF immunoreactivity, suggesting that much of the staining we observed represents biologically active ligand. Westerns blot analysis confirmed the presence of lower M_r cleaved forms of HB-EGF in the E16 mouse cortex.

In some regions, TGFα and HB-EGF are co-expressed (Kornblum et al., 1997; Kornblum et al., 1999). TGFα binds to erbB1 receptors (EGFR) but HB-EGF can bind to erbB4 as well as erbB1 (Raab and Klagsbrun, 1997; Elenius et al, 1997). In vitro, we observed similar effects on migration using TGFα and HB-EGF, suggesting that chemotaxis is mediated by erbB1. Consistent with this idea, preliminary results using tyrphostins to selectively inhibit the kinase activity of specific members of the EGF receptor family showed that chemotactic responses to HB-EGF could be blocked by the EGFR-specific tyrphostin AG 1478 but not the erbB2-specific tyrphostin AG 825 (L. L., unpublished).

Regional and temporal differences in migration

Some of the changes in migration associated with mis-expression of EGFRs in the E10.5 mouse were similar to those reported previously in the mid-embryonic rat (Burrows et al., 1997). For example, mis-expression of EGFRs promoted radial migration in dorsal cortex, with settling predominantly in deep layers of the cortical plate and the marginal zone (Burrows et al., 1997). There were several notable differences, however, in the effects of mis-expressing EGFRs at an earlier stage. In dorsomedial cortex, fewer EGFR-infected E10.5 cells migrated out of proliferative zones by E16.5 (see Figs 7, 8). By contrast, in dorsal cortex, EGFR-infected cells were initially delayed in their migration, but subsequently migrated out to the cortical plate. EGFR-infected cells in early embryonic dorsomedial and dorsal cortex were able to chemotax in response to HB-EGF in explants (see Fig. 1I-M), suggesting that differences in ligand availability underlie these differences in migration. HB-EGF immunoreactivity is low in dorsal cortical plate at E13 and in dorsomedial cortical plate at E16, but higher in dorsal cortex at E16 (see Fig. 5). Differences in migration therefore correlate with temporal and spatial patterns of ligand expression.

Mis-expression of EGFRs in early embryonic cortex also promoted migration ventrolaterally. Progenitor cells infected at E10.5 with EGFR virus were seen in the LCS at E16 among cells expressing high endogenous EGFR (see Fig. 7). The LCS is a lateral extension of the IZ, and includes neurons and glia that migrate ventrolaterally toward the piriform cortex during late stages of embryonic development (Bayer et al., 1991). Cells in the LCS are believed to originate from the corner between cortex and LGE (corticostriatal sulcus; Bayer et al., 1991; Bayer and Altman 1991b). Cells in the corticostriatal sulcus and the lateral cortical stream normally express a high level of EGFRs by E16, but expression of EGFRs in more medial regions, i.e. dorsal cortex, is still relatively low at E16 (see Fig. 3). By increasing EGFR expression in dorsal cortex prematurely, some of these cells appeared to be diverted from radial migration to the dorsal cortical plate into a more tangential trajectory toward the ventrolateral cortex via the LCS. The ventrolateral cortex, including the piriform cortex, expresses HB-EGF and TGFα during this period of development (Kornblum et al., 1997; Kornblum et al., 1999), suggesting that these ligands diverted the migration of EGFR-infected E10.5 dorsal cells. We did not see an increase in the proportion of EGFR-infected cells in ventrolateral cortex following infections at E14.5 (see Fig. 6B). It is possible that longer periods of time post-infection (i.e. 6 days rather than 3-4 days) are required to see migration in this pathway. The promotion of migration in the LCS by mis-expression of EGFRs suggests that chemotaxis mediated by EGFRs may be important for the normal construction of the lateral and ventral cortex.

It is not clear what substratum cells in the LCS use to migrate to lateral and ventral sites. Some radial glia have been observed to originate at the ventricular surface in lateral cortex and curve ventrolaterally around the LGE (Misson et al., 1988), suggesting a radial mode of migration. Thalamocortical axons are another possible substratum, because of their location and expression of HB-EGF and TGFα (Kornblum et al., 1997; Kornblum et al., 1999). At the same time that cells in the LCS migrate ventrally, interneurons have been shown to migrate dorsally from the lateral and medial ganglionic eminences into the cortex (de Carlos et al., 1996; Anderson et al., 1997; Lavdas et al., 1999). Interneurons and cells in the LCS therefore traverse the same path, but move in opposite directions. The direction of their migration may be specified by distinct signals (Powell et al., 2001), or different types of cells may respond in distinct ways to the same signal, as noted for other guidance molecules (Colamarino and Tessier-Lavigne, 1995; Alcantara et al., 2000).

The RMS also contains cells that express a high level of EGFRs, and the olfactory bulb expresses several EGF family ligands as early as E15 in the rat (Eagleson et al, 1996; Kornblum et al., 1997; Kornblum et al., 1999). Based on expression of ligand and receptor, and the phenotype of EGFR null mice (Threadgill et al., 1995), we expected to observe increased migration of EGFR-infected cells into the olfactory bulb via the RMS, but this did not occur. It is not likely that this reflects inactive ligand in the bulb or inability of these cells to chemotax to HB-EGF, because within the olfactory bulb, EGFR-infection promoted radial migration from proliferative zones to the differentiating zone. Several explanations could account for the failure of EGFR mis-expression to promote tangential migration in the RMS. Cells in the RMS may be so far from the ligands made in the olfactory bulb initially that their migration is not affected until after they have entered the bulb. Other cues might be more important for regulating tangential migration in the RMS (Hu, 1999; Wu et al., 1999; Conover et al., 2000), while EGFRs mediate radial migration within the bulb. It is also possible that EGFRs in the RMS regulate migration by a non-autonomous (indirect) mechanism. For example, EGFR activation could regulate matrix metalloproteinases (Miettinen et al., 2000), and this could explain the migration phenotype in the olfactory bulbs of EGFR-null mice (Threadgill et al., 1995). Preliminary results from transplantation of EGFR null cells into wild-type hosts support this idea (J. V. and A. F., unpublished).

Our findings suggest that the timing of departure from proliferative zones, migration routes and settling patterns are regulated in part by the time at which EGFR expression rises to a threshold level, and in part by temporal and spatial changes in ligand expression in the migration targets. Both receptor and ligand expression increase during mid-embryonic development (Kornblum et al., 1997; Burrows et al., 1997). Our recent work demonstrated that two extrinsic signals regulate the timing of the increase in EGFR expression (Lillien and Raphael, 2000). The expression of HB-EGF mRNA in the cortical plate and the differentiating zone of the olfactory bulb (Kornblum et al., 1999) indicates that neurons are at least one source of this ligand, but the mechanisms that control the temporal and spatial patterns of ligand expression have not yet been determined.

We thank Dr Dan Turnbull for assembling the ultrasound backscatter imaging equipment, Dr Gord Fishell for advice on ultrasound injections, and Drs Cynthia Lance-Jones, Eric Frank, Paula Monaghan and Pat Levitt for their thoughtful comments on the manuscript. This work was supported by the March of Dimes, NIH grant RO1 NS38306 and a Traveling Research Fellowship from The Wellcome Trust.

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