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In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain

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SUMMARY

Recent studies suggest that neurons born in the developing basal forebrain migrate long distances perpendicularly to radial glia and that many of these cells reach the developing neocortex. This form of tangential migration, however, has not been demonstrated in vivo, and the sites of origin, pathways of migration and final destinations of these neurons in the postnatal brain are not fully understood. Using ultrasound-guided transplantation in utero, we have mapped the migratory pathways and fates of cells born in the lateral and medial ganglionic eminences (LGE and MGE) in 13.5-day-old mouse embryos. We demonstrate that LGE and MGE cells migrate along different routes to populate distinct regions in the developing brain. We show that LGE cells migrate ventrally and anteriorly, and give rise to the projecting medium spiny neurons in the striatum, nucleus accumbens and olfactory tubercle, and to granule and periglomerular cells in the olfactory bulb. By contrast, we show that the MGE is a major source of neurons migrating dorsally and invading the developing neocortex. MGE cells migrate into the neocortex via the neocortical subventricular zone and differentiate into the transient subpial granule neurons in the marginal zone and into a stable population of GABA-, parvalbumin- or somatostatin-expressing interneurons throughout the cortical plate.

Key words: Ganglionic eminences, Neocortical development, Tangential neuronal migration, Ultrasound, Fate map, Non-pyramidal, Interneurons, Medium spiny neurons, GABA, Parvalbumin, Somatostatin, DARPP-32, Mouse

INTRODUCTION

Cell migration is an important determinant of brain structure. Most, if not all, nerve cells have to migrate from the sites where they are born to places where they terminally differentiate and integrate into the brain circuitry. Radial migration of young neurons from germinal regions lining lateral ventricles to more superficial layers of the neocortex has been recognized as a prerequisite for proper morphogenesis and function of the cerebral cortex (Caviness and Rakic, 1978; Sidman and Rakic, 1973; Walsh and Goffinet, 2000). Radial migration could explain the radial organization of the cerebral cortex and the inside-out sequence of cortical formation, and as such it has been for many years considered to be the principal mode of cell translocation in the developing cortex (Rakic, 1988).

This view began to change when retroviral lineage tracings revealed that a large number of neurons do not follow the radial path, but disperse tangentially throughout the developing neocortex (Price and Thurlow, 1988; O'Rourke, 1992; Walsh and Cepko, 1992). It has been proposed that tangentially and radially migrating neurons belong to different cell pools that segregate early in the embryonic development (Tan and Breen, 1993; Tan et al., 1998). Finally, several experiments suggested that many of the tangentially migrating neurons are not born in the cortical neuroepithelium as was always assumed, but instead originate outside the neocortex in the basal forebrain in regions called ganglionic eminences (reviewed by Anderson et al., 1999; Parnavelas, 2000). From these findings emerged a new concept of cortical development, which proposes that two separate populations of neurons participate in corticogenesis. One population consists of the radially migrating neurons, which are born in neocortical ventricular zone and probably give rise to the principal pyramidal neurons of the neocortex. The second population includes neurons born in ganglionic eminences that migrate tangentially into the neocortex and probably differentiate into the cortical non-pyramidal neurons (Anderson et al., 1999; Parnavelas, 2000).

The name ganglionic eminences reflects the assumption that these ventral regions give rise to the basal ganglia (Fentress et al., 1981; Shimamura et al., 1995; Smart and Sturrock, 1979). The ganglionic eminences are subdivided into the medial (MGE), lateral (LGE) and caudal ganglionic eminence. The MGE is thought to give rise to pallidum, while the LGE is believed to generate most of the striatal neurons (Olsson et al., 1998). Initial studies of neuronal migration from the basal forebrain to the neocortex implicated LGE as the principal source of tangentially migrating neurons (Anderson et al., 1997; de Carlos et al., 1996; Tamamaki et al., 1997). However, recently, it became apparent that a large proportion of these neurons originate in the most ventral aspect of the telencephalon, in the MGE (Lavdas et al., 1999; Sussel et al., 1999; Wichterle et al., 1999). Analysis of the expression of Lim homeodomain gene *Lhx6* in the embryonic forebrain revealed a stream of cells extending from the MGE to the neocortex (Grigoriou et al., 1998). This, in combination with experiments showing dorsal migration of MGE cells in slice cultures, suggested that Lhx6-positive cells generated in the MGE migrate tangentially to the neocortex (Lavdas et al., 1999). Further evidence for this migration came from the analysis of mice with targeted ablation of Nkx2.1 homeodomain protein, which leads to the respecification of the MGE into the LGE (Sussel et al., 1999). Detailed analysis of cellular defects in *Nkx2.1*$^{-/-}$ mice revealed that subsets of interneurons are depleted in the developing striatum (Marin et al., 2000), neocortex (Sussel et al., 1999) and hippocampus (Pleasure et al., 2000). However, these deficits appear to be less pronounced than in the case of the disruption of Dlx1/2 transcription factors which are expressed in both the LGE and MGE (Anderson et al., 1997), indicating that both these regions may contribute neurons to the developing dorsal telencephalon.

Our studies of LGE and MGE cell migration revealed that the two eminences contain cells with strikingly different migratory behaviors (Wichterle et al., 1999). MGE cells migrate extensively in vitro and have a unique capacity to penetrate and disperse through the adult brain parenchyma. LGE cells, on the other hand, migrate less extensively in cultures and are unable to disperse in the adult brain. However, when LGE and MGE cells are transplanted into the adult subventricular zone, only LGE cells migrate along the rostral migratory stream to the olfactory bulb (Wichterle et al., 1999). These differences in cell migration suggested that LGE and MGE neuronal precursors might follow distinct migratory pathways in their normal environment in the developing brain.

While there are multiple studies of MGE and LGE cell migration in vitro and indirect studies in animals carrying targeted mutations in specific genes, there is currently no study analyzing migratory pathways of these cells directly in vivo. It remains unclear whether the two regions contribute equally to corticogenesis and whether LGE and MGE cells differentiate into the same types of neurons after they reach their final destinations. Most importantly, the perinatal lethality of both the *Dlx1/2* and *Nkx2.1* mutant mice makes it impossible to determine the fate and final phenotypes of tangentially migrating neurons. Although neuronal migration from ganglionic eminences into neocortex has received the most attention, other tangential migratory pathways were described in the developing forebrain. For example, a migratory route connecting the ganglionic eminences with the thalamus (so called gangliothalamic body) has been histologically identified in embryonic human brains (Letinic and Kostovic, 1997; Rakic and Sidman, 1969) and a rostral migration from the subventricular zone (SVZ) to the olfactory bulb has been demonstrated in postnatal and adult rodent brains (Lois and Alvarez-Buylla, 1994; Luskin, 1993). It is therefore plausible that the ganglionic eminences generate neurons destined for other embryonic brain regions besides the dorsal telencephalon.

To address these issues, we analyzed the fate of ganglionic eminence cells directly in the developing mammalian brain. We used biomicroscopy for targeted transplantation of labeled LGE and MGE cells into the embryonic mouse brain in utero (Olsson et al., 1997). We show that homotopically and homochronically grafted 13.5-day-old MGE and LGE cells migrate along distinct routes and give rise to different types of neurons in the embryonic forebrain. We also show that, at the age studied, the MGE is the principal source of tangentially migrating neurons destined for the developing neocortex.

MATERIALS AND METHODS

Donor cell preparation

Ventricular and subventricular zones from ganglionic eminences were dissected from 5-15 E13.5 mouse embryos as described previously (Wichterle et al., 1999). Swiss-Webster (Taconic), CD-1 (Charles River) or transgenic mice expressing human placental alkaline phosphatase in all cells (DePrimo et al., 1996) were used as donor animals. All animals used in this study were maintained and treated according to protocols approved by the Institutional Animal Care and Use Committee at New York University School of Medicine. Tissue was dissected from the anterior region of the LGE and MGE, where sulcus clearly divides these two regions. Bordering tissues between MGE, LGE and neocortex were discarded during dissections to prevent contamination. Explants were mechanically dissociated by repeated pipetting through 200 μl yellow plastic pipette tip (20-30 times). Dissociated cells were washed with 1 ml of L-15 medium (Gibco) containing DNase I (10-100 μg/ml) and pelleted by centrifugation (5 minutes, 800 ***g***). Cells isolated from transgenic animals were resuspended in 5 μl of L-15 medium containing DNase I and kept on ice until further use. Non-transgenic cells were resuspended in 100 μl of Diluent C and fluorescently labeled by incubation with 100 μl of PKH26 dye (2-3 μl of the dye in 100 μl of Diluent C) (Sigma) for 2 minutes. Labeled cells were washed two to three times with Neurobasal medium (Gibco) and resuspended in 5 μl of L-15 medium containing DNase I. Cell suspensions were stored on ice until further use.

Transplantation in utero

High density cell suspension (~800,000 cells/μl) was front-loaded into beveled glass micropipettes (~50 μm diameter) that were prefilled with mineral oil and mounted on a microinjector (modified version of Narishige). Cells were allowed to settle inside the pipette and the excess cell-free medium was expelled. The recipient pregnant mice (E13.5) were anesthetized, uterine horns were exposed through an intraperitoneal incision and animals were mounted under the biomicroscope as described previously (Liu et al., 1998; Olsson et al., 1997). The tip of the micropipette was inserted into the LGE or MGE under real-time ultrasound guidance and 10-20 nl of cell suspension was injected. The position of the embryo and the path of the micropipette insertion was recorded for each embryo. Embryos in which we detected extensive cell leakage to the lateral ventricle (either detected at the time of transplantation by the ultrasound microscope or identified in processed brains as deposits of labeled cells within the choroid plexus and in periventricular regions) were excluded from the subsequent analysis.

Analysis of cell migration

Transplanted embryos (~60% of injected embryos survived) were collected at 1, 2 and 4 days after transplantation or as 3-week and 4-month-old animals. Three to six successfully transplanted animals were analyzed for each time-point and region (~50% of surviving injected animals contained grafted cells). For labeling of proliferating cells, some pregnant mice were injected with BrdU (40 mg/kg) 1-2 hours before sacrifice. Embryos were transcardially perfused with 4% paraformaldehyde 0.25% glutaraldehyde, brains removed and postfixed overnight. Brains were cut coronally into 50-100 μm thick sections using a vibratome. Sections from brains that received cells carrying alkaline phosphatase transgene were inactivated for 30 minutes at 70°C and stained with NBT/BCIP (Boehringer, 1/0.1 mg/ml). Sections were mounted in Krystalon (EM Science) or Aquamount (Polysciences), and photographed using an Olympus microscope and SPOT camera (Diagnostic Instruments). Sections from animals receiving fluorescently labeled cells were wet-mounted and photographed. The density of graft-derived cells in individual brain regions was analyzed using the NIH Image 1.62 software. Images of all available sections from individual brains were analyzed. Brain regions of interest were selected, their areas measured and numbers of graft-derived cells were counted (using the Analyze Particles function). Total numbers of labeled cells found in selected brain regions were used to calculate the distribution of grafted cells. To calculate the cell density in individual regions, we divided the number of cells by the area and thickness of brain slices and averaged results obtained for regions extending across multiple sections.

Immunohistochemistry

Animals were transcardially perfused with 4% paraformaldehyde, brains removed and postfixed overnight. Samples were cryoprotected in 30% sucrose in phosphate-buffered saline (PBS), embedded in Histoprep (Fisher Scientific) and frozen. Brains were sectioned coronally at 20-30 μm using a cryostat, after which sections were air dried and stored at −20 °C. The immunostaining was performed either on cryostat sections or on free-floating 50 μm vibratome sections. The following antibodies were used for immunofluorescence: mouse CR-50 (1:400; Ogawa et al., 1995), rabbit anti-GABA (1:2500, Sigma), rabbit anti-neurofilament-145 (Chemicon, 1:1000), rat anti-BrdU (1:200, Harlan Sera Lab), rabbit anti-somatostatin (1:500, Calbiochem), mouse anti-parvalbumin (1:1000, Sigma), rabbit anti-calretinin (1:500, Chemicon), mouse anti DARPP-32 (1:1000; Ouimet et al., 1984), rabbit anti-human placental alkaline phosphatase (hPLAP) (1:200, Accurate Chemical & Scientific Corp., New York) and sheep anti-hPLAP (1:100, American Research Products, MA). The following secondary antibodies were used: cy3-conjugated donkey anti-mouse, cy3-conjugated donkey anti-rabbit, cy2-conjugated donkey anti-rabbit and cy2-conjugated donkey anti-sheep (all from Jackson ImmunoResearch, PA). Sections were washed in PBS, blocked for 1 hour in PBS containing 10% donkey serum and 0.2% Triton X-100. Sections were then incubated overnight at 4°C in primary antibodies diluted in PBS containing 1% donkey serum and 0.2% Triton X-100, then washed three times in PBS and incubated with secondary antibodies for 1-2 hours at room temperature in the dark. For BrdU double staining, sections were first processed for the hPLAP immunostaining, fixed in 3% PFA for 15 minutes, washed three times in PBS, incubated in 4M HCl at room temperature for 5-10 minutes, washed three times in PBS and stained for BrdU. Fluorescent images were obtained using a cooled-CCD camera (Princeton Instruments) and Metamorph software (Universal Imaging, Pennsylvania) or the SPOT camera.

RESULTS

Ultrasound guided transplantation

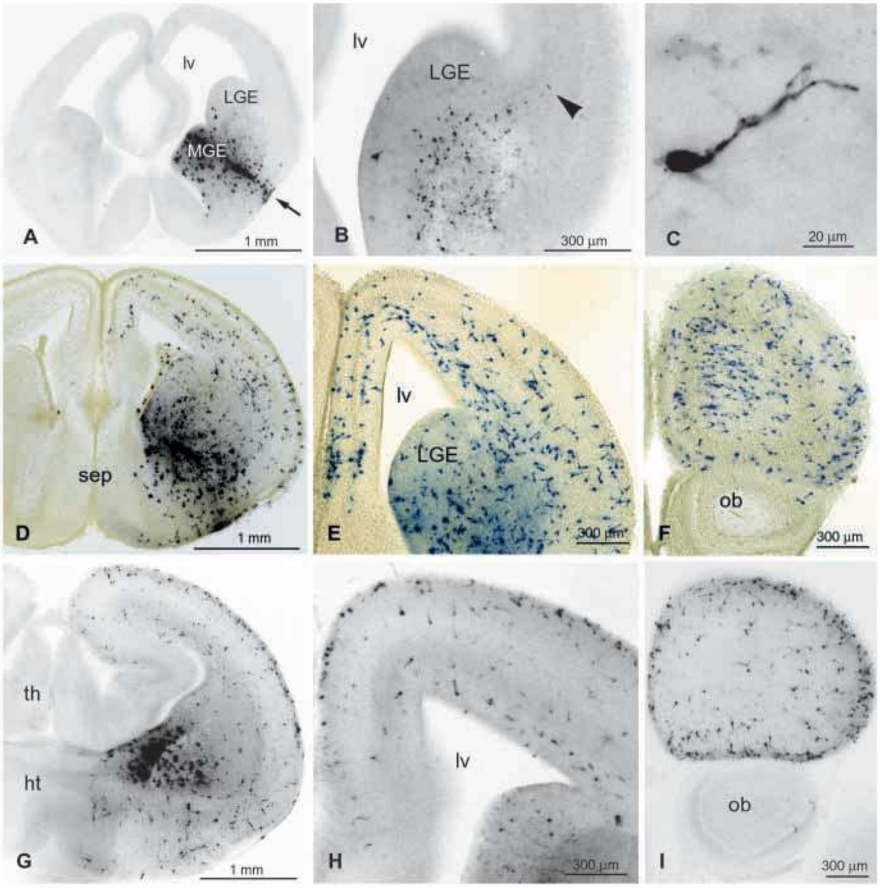
In order to study the migration and fate of MGE and LGE cells directly in the embryonic brain we used a recently developed technique of ultrasound guided microtransplantation (Liu et al., 1998; Olsson et al., 1997). The MGE is a transient structure in the developing mouse brain that appears around embryonic day 10 (E10) and persists until approximately E16, when it fuses with the rapidly growing LGE. We decided to perform our experiments on E13.5 animals – when the MGE is well developed but still separated from the LGE. To capture the timecourse of MGE cell migration, we analyzed transplanted embryos at 1, 2 and 4 days after transplantation. Final distribution of transplanted MGE and LGE cells was analyzed in 3-week-old postnatal animals and the long term survival of MGE-derived cells was analyzed in 4-month-old animals. In order to prevent leakage of grafted cells into the ventricular system, we used a beveled glass micropipette (~50 μm in diameter) and limited our transplantations to 10-20 nl of a dense cell suspension containing between 5000 and 15,000 cells. As some grafted cells were deposited along the track of the micropipette, we approached the ganglionic eminences from different angles and excluded from our analysis regions adjacent to the needle track and brains in which grafted cells had excessively leaked into ventricles (which could be detected by the ultrasound microscope). In an average experiment ~60% of injected embryos survived, but generally only ~50% of these embryos contained grafted cells. This is probably due to the uneven distribution and aggregation of cells within the micropipette during the microtransplantation.

MGE cells migrate via the subventricular zone to the neocortex

In order to determine migratory pathways of MGE cells in the developing forebrain, we analyzed the distribution of grafted cells at 1 (*n*=3), 2 (*n*=5) and 4 days (*n*=4) after homotopic and homochronic transplantation. At 1 day, MGE cells were largely confined to the site of transplantation. Labeled migrating cells were observed within the LGE (developing striatum) and occasionally in the most proximal aspects of the neocortex (close to the LGE/cortical boundary; Fig. 1A,B). One day later, at 2 days after transplantation, large numbers of migrating cells dispersed throughout most of the dorsal forebrain (5600±1000 labeled cells/brain, *n*=3; Fig. 1D-F). The highest concentration of tangentially oriented labeled cells in the neocortex was found within the neocortical SVZ (BrdU-labeled proliferative region adjacent to the cortical VZ (The Boulder Committee, 1970)) (Figs 1E, 2A-C). This region, also known as the lower intermediate zone, has been previously shown to contain a large number of MGE-derived Lhx6-expressing cells (Lavdas et al., 1999). To determine whether MGE cells use corticofugal axons as a substrate for their migration (Metin et al., 2000), we double labeled some sections with antibodies against neurofilament-145 (NF-145). While MGE-derived cells were occasionally aligned with neurofilament immunoreactive fibers within the lower intermediate zone, they were rarely observed within the axon rich upper intermediate zone (Fig. 2C). Tangentially oriented migratory cells were also found in the subplate, cortical plate and marginal zone. Radially oriented cells with their leading processes oriented centrifugally towards the pial surface were detected within the upper intermediate zone and cortical plate. These results indicate that tangentially migrating neurons use primarily substrates in the neocortical subventricular zone for their translocation.

The majority of graft-derived cells at 2 days after transplantation were bipolar with a long leading process which was sometimes bifurcated. The leading process was often tipped

Fig. 1. Distribution of MGE cells one (E14.5, A-C), two (E15.5, D-F) and four (E17.5, G-I) days after homotopic transplantation. Coronal sections of embryonic mouse brains containing MGE cells labeled with PKH26 fluorescent dye before transplantation. Graft-derived cells are black or dark blue. (A) One day after transplantation, most of the grafted cells were located at the site of transplantation in the MGE. No grafted cells were detected in the lateral ventricle (lv). The pipette insertion site is marked by an arrow. (B) At 1 day post transplantation migrating cells were observed in the LGE and at the cortical boundary (arrowhead). (C) Example of a migratory cell with a leading process and a growth cone. (D) Two days after transplantation, multiple cells migrated into the neocortex. Migration is directional as no cells migrate to the septum (sep). (E) The highest density of migrating MGE cells is in the cortical subventricular zone. (F) Tangential section through the anterior neocortex reveals migrating cells in the subventricular zone (central lighter region). No graft-derived cells migrated into the olfactory bulb (ob). (G) Four days after transplantation, most of the MGE-derived cells settled in the neocortical marginal zone. No labeled cells were found in the hypothalamus (ht) and thalamus (th). (H) Cortical subventricular zone contains fewer migrating cells than at 2 days and most of the grafted cells are found in the marginal zone. (I) Disappearance of migrating neurons from the subventricular zone is obvious in the tangentially cut anterior cortex. Olfactory bulb is devoid of labeled cells.



with a prominent growth cone with long filopodia and lamellopodia (Fig. 1C). Most of the labeled cells were oriented with their leading process pointing in the dorsal direction, away from the MGE (Fig. 2A). Interestingly, some MGE-derived cells were oriented in other directions, pointing backward towards the MGE, radially to the cortical plate or with their leading process intercalated into the neocortical ventricular zone. The speed of MGE cell migration inferred from the position of labeled cells at days 1 and 2 after transplantation is remarkable. Many labeled cells were found as far as the border between the neocortex and hippocampus (Fig. 1E) 2 days after transplantation (i.e. >2 mm away from the site of

Fig. 2. MGE cells in the subventricular and marginal zones at two (E15.5) and four (E17.5) days after transplantation. (A) Higher magnification of the boundary between the developing striatum and neocortex at 2 days after transplantation reveals the shape and orientation of tangentially migrating neurons. Most PKH26-labeled MGE neurons migrate close to the neocortical ventricular zone and only few cells are observed in the marginal zone. (B) Double labeling for BrdU reveals most of the tangentially oriented cells in the neocortical SVZ. (C) Tangentially migrating cells do not use corticofugal axons in the upper intermediate zone, which were labeled with antibody against neurofilament-145. (D) Confocal image of differentiating MGE-derived neurons in the marginal zone 4 days after transplantation. (E) Quantification of the density of graft-derived cells demonstrates the shift from the subventricular (SVZ) to marginal zone (MZ) between days 2 and 4. (F) Immunostaining of transplanted MGE cells expressing alkaline phosphatase in one day old pup (7 days after transplantation) reveals that some of the MGE-derived cells (green) express GABA (red). (G) Staining with antibody CR-50 against reelin (red) revealed that MGE-derived cells (green) are found close to clusters of CR-50 positive cells but they are not double-labeled.

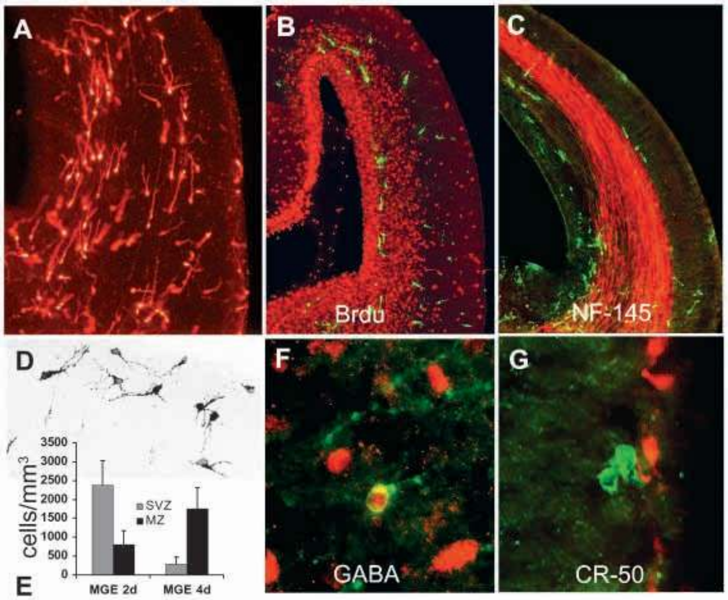


Table 1. Density and distribution of MGE and LGE cells in selected forebrain regions four days after homotopic transplantation

	NCx	Gl	OT	Str	NA	GP	A
MGE 1	+++ (82%)	− (0%)	+ (1%)	+ (9%)	− (1%)	+++ (4%)	+ (2%)
MGE 2	+++ (83%)	− (0%)	++ (2%)	++ (12%)	+ (1%)	+ (1%)	+ (1%)
MGE 3	+++ (87%)	− (0%)	++ (2%)	++ (8%)	+ (1%)	+ (1%)	− (0%)
MGE 4	+++ (88%)	− (0%)	++ (1%)	++ (8%)	− (0%)	+ (1%)	+ (1%)
LGE 1	− (3%)	− (0%)	++++ (6%)	++++ (76%)	+++++ (14%)	− (0%)	− (0%)
LGE 2	− (4%)	− (0%)	++++ (5%)	+++++ (83%)	+++++ (7%)	− (0%)	− (0%)
LGE 3	− (3%)	− (0%)	+++++ (7%)	+++++ (87%)	++ (2%)	− (1%)	− (0%)
LGE 4	− (1%)	+++++ (31%)	+++++ (6%)	++++ (61%)	+ (0%)	− (0%)	− (0%)
LGE 5	− (1%)	+++++ (23%)	+++++ (8%)	+++++ (65%)	++ (1%)	− (0%)	− (0%)

Minus and plus signs correspond to the following densities of grafted cells: (−) <100 cells/mm^3; (+) 100-200 cells/mm^3; (++) 200-400 cells/mm^3; (+++) 400-800 cells/mm^3; (++++) 800-1600 cells/mm^3; (+++++) >1600 cells/mm^3. Distribution of grafted cells among selected areas is shown in parentheses.

A, amygdala; Gl, olfactory bulb granule cell layer; GP, globus pallidus; NA, nucleus accumbens; NCx, neocortex; OT, olfactory tubercle; Str, striatum.

transplantation). These cells had to move with speeds of >80 µm/hour, which is similar to the speed of homotypic, glia-independent neuronal migration in vitro (Wichterle et al., 1997).

MGE cells disperse throughout the developing telencephalon

At 4 days, many graft-derived cells dispersed throughout the dorsal forebrain (6200±800 labeled cells/brain, *n*=4). Most of the migrating cells (~85%) were found in the neocortex (Fig. 1G, Table 1). In contrast to the 2 day survival, many labeled cells were concentrated superficially in the marginal layer of the neocortex (~60% of labeled cells in neocortex) at this time-point (Fig. 1G-I). Two days after transplantation, the density of labeled cells in the neocortical subventricular zone was approx. three times higher than in the marginal zone (Fig. 2E). By contrast, the density of labeled cells in the marginal zone 4 days after transplantation was approx. six times higher than in the subventricular zone (Fig. 2E). These results indicate that subsequent to the initial phase of tangential migration through the SVZ, MGE derived neurons move radially into the cortical plate and marginal zone, where they may further disperse tangentially and differentiate.

Although most MGE cells migrated into the neocortex, some labeled cells were observed within the developing hippocampus, piriform cortex, anterior olfactory nucleus, striatum, globus pallidus and amygdala 4 days after transplantation (Table 1). At this survival time, graft-derived cells had started to extend multiple processes from cell bodies, suggesting that the MGE cells had started to differentiate. In contrast to the basal ganglia, where MGE cells extended longer processes, labeled cells within the marginal zone of neocortex extended only multiple short processes (Fig. 2D). Morphologically immature labeled cells with migratory (bipolar) shape were found primarily in the neocortical SVZ and cortical plate. No labeled cells were found in the contralateral hemisphere. Migration of MGE cells was directional and selective, as they did not disperse into the neighboring hypothalamus, thalamus, septum or olfactory bulb (Fig. 1G,I).

MGE cells differentiate into subpial granule neurons in the marginal zone

To analyze the final phenotype of MGE and LGE-derived neurons, we transplanted genetically labeled cells constitutively expressing human placental alkaline phosphatase (hPLAP) (DePrimo et al., 1996). In our previous studies, we have shown that at least 30% of MGE cells grafted into the adult brain differentiate into GABAergic neurons (Wichterle et al., 1999). It has been also shown that some MGE cells migrating to the neocortex in embryonic brain slices express the CR-50 antigen, which would suggest that they differentiate into Cajal-Retzius cells (Lavdas et al., 1999). To study the phenotype of MGE-derived cells, differentiating in the marginal zone, we double immunostained brain sections of postnatal day 1 (P1) animal, which received an MGE graft on E13.5 for alkaline phosphatase and GABA or CR-50. We found that some MGE-derived cells within the developing cortical plate and marginal zone were immunoreactive for GABA (Fig. 2F). MGE cells in the marginal zone were often found associated with clusters of CR-50 immunoreactive cells, but we never observed double labeled MGE-derived cells stained with CR-50 antibodies (Fig. 2G). These results suggest that E13.5 MGE cells that migrate into the marginal zone give rise to a subset of subpial granule cells but they do not differentiate into Cajal-Retzius neurons.

MGE-derived cells persist in the neocortex into adulthood

Neurons in the marginal zone are believed to undergo apoptosis early in the postnatal period (Spreafico et al., 1999; Spreafico et al., 1995; Super et al., 1998). The long-term survival of cells migrating tangentially into the neocortex has not been studied previously and therefore it was possible that MGE cells form a transient cell population that dies soon after birth. To study the fate of MGE cells, we grafted hPLAP-expressing MGE cells into the MGE of wild-type embryos and analyzed the final distribution of MGE-derived cells in 3-week-old mice (*n*=4) and in 4-month-old adults (*n*=3). There was no apparent difference between MGE cell distribution at these two survival times. The marginal zone was cell sparse and did not contain any graft-derived hPLAP-positive cells. The deeper layers of the neocortex, by contrast, contained numerous hPLAP-labeled cells (Fig. 3). Although the MGE-derived cells were found in all cortical layers, their concentration was highest in layers 5 and 6 (75% of MGE-derived cells).

Enzymatic detection of the alkaline phosphatase resulted in Golgi-like staining of the graft-derived cells and their processes. In the neocortex, MGE-derived cells differentiated into neurons of varying sizes and shapes (Fig. 4A-F). In most cases, the morphology of individual MGE-derived neurons was

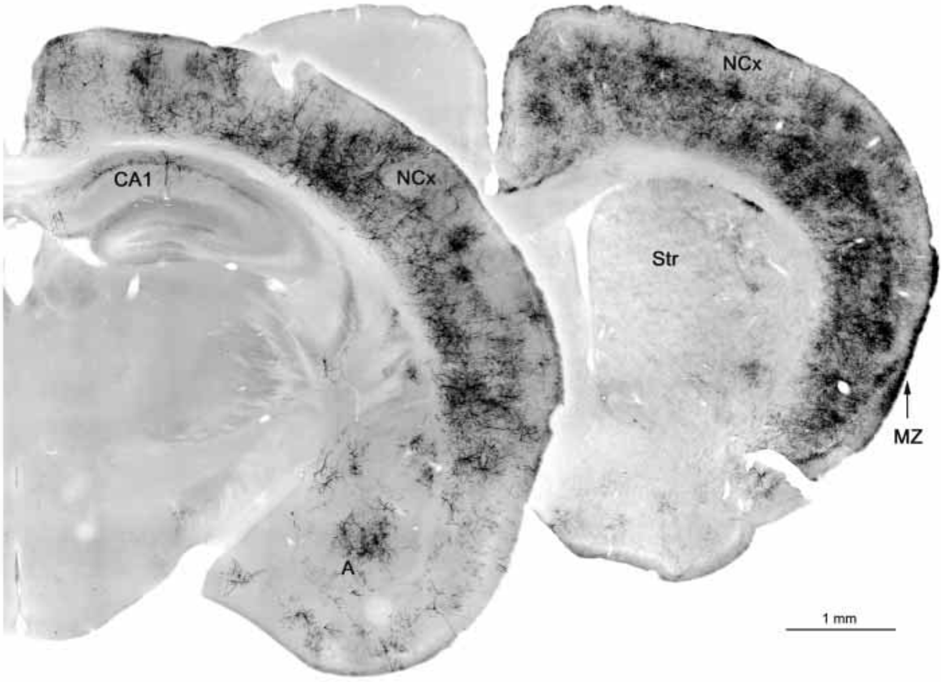


Fig. 3. MGE cells expressing alkaline phosphatase were grafted into the wild-type E13.5 MGE. The majority of graft-derived cells are found in the neocortex in 3-week-old (left, more posterior coronal section) and 4-month-old (right, more anterior coronal section) animals. Some cells in the posterior section are observed in the amygdala and CA1 field of the hippocampus. In contrast to the 4-day survival (Fig. 1G-I), no graft-derived cells are found in the marginal zone (the dark staining in the marginal zone are labeled projections of neurons located deeper in the cortical plate, arrow). A, amygdala; MZ, marginal zone; NCx, neocortex; Str, striatum.

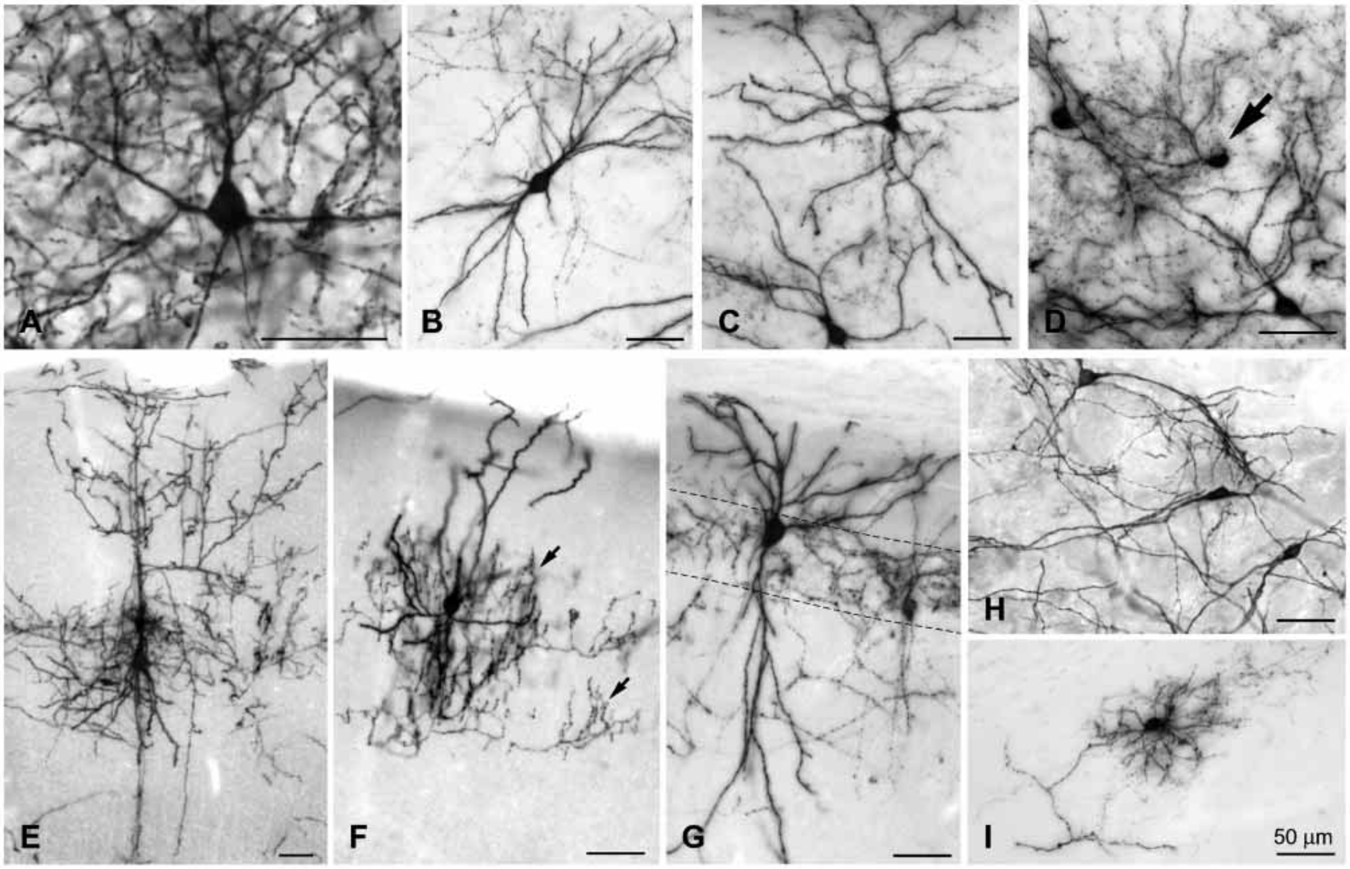


Fig. 4. Morphology of MGE-derived neurons stained for alkaline phosphatase. (A,D,E,H) Neurons in 4-month-old animal; (B,C,F,G,I) neurons in 3-week-old animal; dorsal is towards the top throughout. MGE cells in the neocortex differentiated into: aspiny multipolar neurons (A,C), bi-tufted neurons (B), neurons with a small cell body (D, arrow), basket cells (E) and chandelier cells (F). MGE cells were also observed in: the CA1 field of the hippocampus (basket cells) (G), the lateral globus pallidus (H) and the striatum (I). To capture some of the fine three dimensional processes, two to three photographs taken in different focal planes were fused to produce the final image.

confounded by large numbers of labeled dendrites and axons crisscrossing the field (Fig. 4A). However, one brain contained only relatively small number of grafted cells, which made the analysis of individual neuronal projections possible. Most cells were multipolar aspiny or sparsely spiny neurons with medium to large cell bodies (Fig. 4A,C). We also observed radially oriented bi-tufted or double bouquet cells (Fig. 4B), cells with small cell bodies and less prominent dendritic arbors (Fig. 4D, arrow), basket cells (Fig. 4E) and chandelier cells (Fig. 4F) (Fairen et al., 1984). None of the MGE-derived neurons manifested typical features of pyramidal neurons (triangular cell body extending a thick spiny apical dendrite). None of the labeled processes of MGE-derived neurons, which crisscrossed the neocortex, contained dense spines (Fig. 4A). The processes accumulated particularly in marginal zone (Fig. 3) and layers 5 and 6, but we did not detect any axons projecting into the contralateral hemisphere or extensive axonal projections to subcortical regions. Therefore we concluded that the majority of MGE cells differentiated into a population of locally projecting interneurons, some of which were labeled with antibodies against GABA.

While the highest density of MGE-derived cells was observed in the neocortex, some MGE cells were also detected in the anterior olfactory nucleus, piriform cortex, striatum, lateral globus pallidus (but never in the medial globus pallidus), in the CA1 and occasionally in the caudal aspect of the CA3 fields of the hippocampus, in multiple nuclei of the amygdala and in the bed nucleus of the stria terminalis (Fig. 3, data not shown). MGE-derived cells found in different brain regions had different shapes. MGE neurons differentiating in the CA1 field of the hippocampus had a typical morphology of hippocampal basket cells (large cell bodies and characteristic aspiny projections) (Fig. 4G). Cells in the lateral globus pallidus were medium size neurons with only few projections (Fig. 4H). MGE-derived striatal neurons were either similar to the ones in globus pallidus or smaller neurons with extensive fine dendritic arborizations (Fig. 4I). Although the majority of MGE-derived neurons in all studied areas were aspiny or sparsely spiny, few neurons found at the ventral striatum (close to the site of transplantation) differentiated into medium spiny neurons similar to the ones observed in LGE grafts (see below). As observed at 2 or 4 day survival times, MGE graft-derived cells did not migrate in the thalamus, hypothalamus, septum or olfactory bulb (Fig. 3). A few cells (<10 neurons per brain) were detected in the contralateral hemisphere. These cells, which probably migrated to the other hemisphere along the corpus callosum, differentiated into neocortical neurons close to the midline.

Neocortical non-pyramidal neurons can be subdivided into three categories based on their expression of parvalbumin, calretinin or somatostatin (Gonchar and Burkhalter, 1997; Kubota et al., 1994). In order to identify the types of neocortical interneurons derived from the MGE, we immunostained brain sections with antibodies against these three markers. Approximately 70% of MGE-derived neurons in the neocortex were immunoreactive for parvalbumin (170

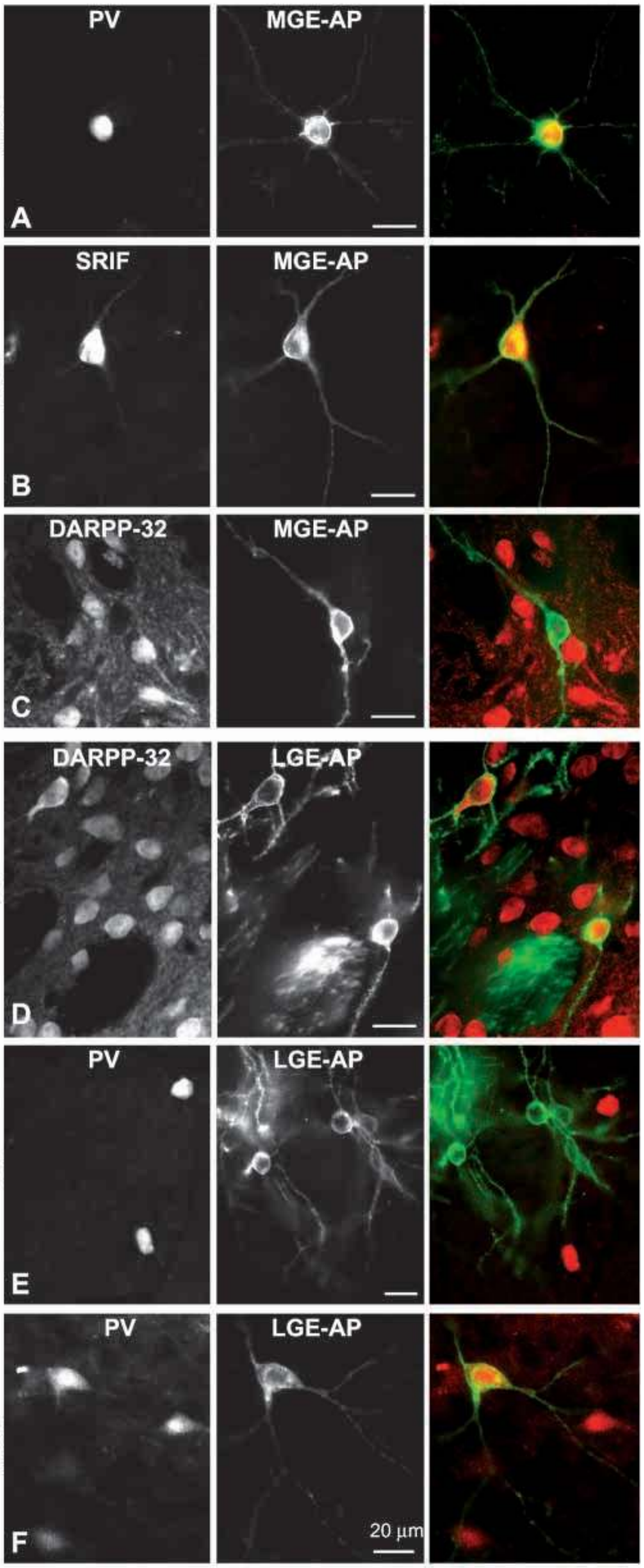


Fig. 5. Immunohistochemical characterization of grafted MGE (A-C) and LGE (D-F) cells in the neocortex (NCx; A,B,F) and striatum (Str; C,D,E). MGE-derived cells are from a 4-month-old animal, LGE-derived cells are from 3-week-old animal. The second column and green color in the third column represent staining for alkaline phosphatase expressed in transplanted cells. (A-C) Many MGE cell in the neocortex expressed parvalbumin (A) and somatostatin (B) but within striatum did not express striatal marker DARPP-32 (C). (D,E) The majority of LGE cells in the striatum expressed DARPP-32 (D) but did not express parvalbumin (E). (F) By contrast, some of the LGE-derived neurons that migrated into the neocortex expressed parvalbumin.

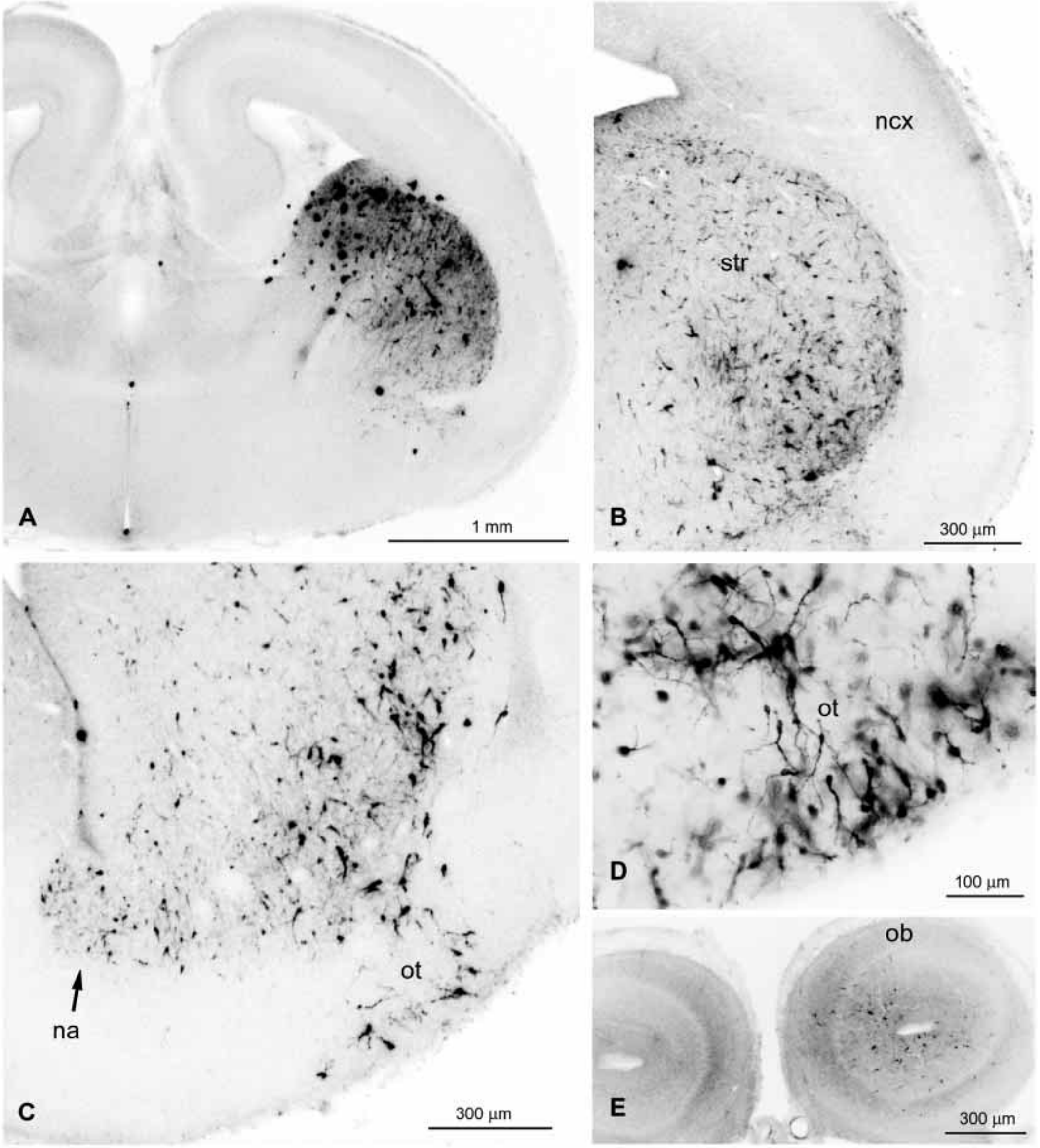


Fig. 6. Homotopic transplants of PKH26-labeled LGE cells. (A) Coronal section of E17.5 embryonic brain four days after transplantation reveals accumulation of LGE cells in the striatum and absence of LGE cells in the neocortex. Some labeled cells leaked into brain ventricles during the transplantation (black dots in choroid plexus of the lateral ventricle). (B) More anterior view reveals selective dispersion of LGE-derived cells throughout the striatum (str). Notice the sharp boundary between the striatum and neocortex (ncx). (C) LGE-derived cells migrate also into the nucleus accumbens (na) and olfactory tubercle (ot) in the basal forebrain. (D) High magnification of labeled cells in the olfactory tubercle reveals multiple processes extending from individual cells. (E) In several animals, labeled LGE cells migrated into the core of the olfactory bulb (ob).

MGE-derived cells analyzed) (Fig. 5A), ~35% were immunoreactive for somatostatin (120 cells analyzed) (Fig. 5B) and <3% of MGE-derived cells were labeled with anti-calretinin antibodies (130 cells analyzed). As mentioned above, some of the MGE cells migrated into the striatum and differentiated there into aspiny neurons. None of these cells expressed DARPP-32 (Fig. 5C), which is a marker of projecting medium spiny neurons (Ouimet et al., 1998), but some of them expressed parvalbumin (data not shown). These results indicate that MGE-derived neurons in striatum retain their biochemical profile and probably represent a distinct population of striatal interneurons derived from the MGE (Marin et al., 2000).

LGE cells remain within the developing basal forebrain

Labeled LGE cells grafted back into the embryonic LGE dispersed within the basal forebrain (1500±500 cells, *n*=5), but only very few cells migrated into the neocortex at four days after transplantation (<5% of grafted cells) (Fig. 6A,B; Table 1). Graft-derived LGE cells were observed to disperse and differentiate within the dorsal and ventral striatum including the nucleus accumbens and olfactory tubercle (Fig. 6C,D; Table 1). None or only very few LGE-derived cells were found in the globus pallidus, amygdala, septum, neocortex or piriform cortex (Fig. 6A; Table 1). Interestingly, the final distribution of LGE-derived cells depended on the precise position of the graft within the developing LGE. When LGE cells were placed in the caudal aspect of the LGE (just anterior to the caudal ganglionic eminence) large numbers of grafted cells migrated into the nucleus accumbens (Fig. 6A-D; LGE 1 and 2 in Table 1). By contrast, LGE cells grafted into the more anterior LGE did not contribute substantially to nucleus accumbens (LGE 3-5 in Table 1). Instead, LGE cells placed in the dorsal anterior LGE migrated in large numbers rostrally into the granule layer of the olfactory bulb (Fig. 6E; LGE 4 and 5 in Table 1). In all brains that received LGE grafts, labeled axons extending along the internal capsule all the way to the substantia nigra were

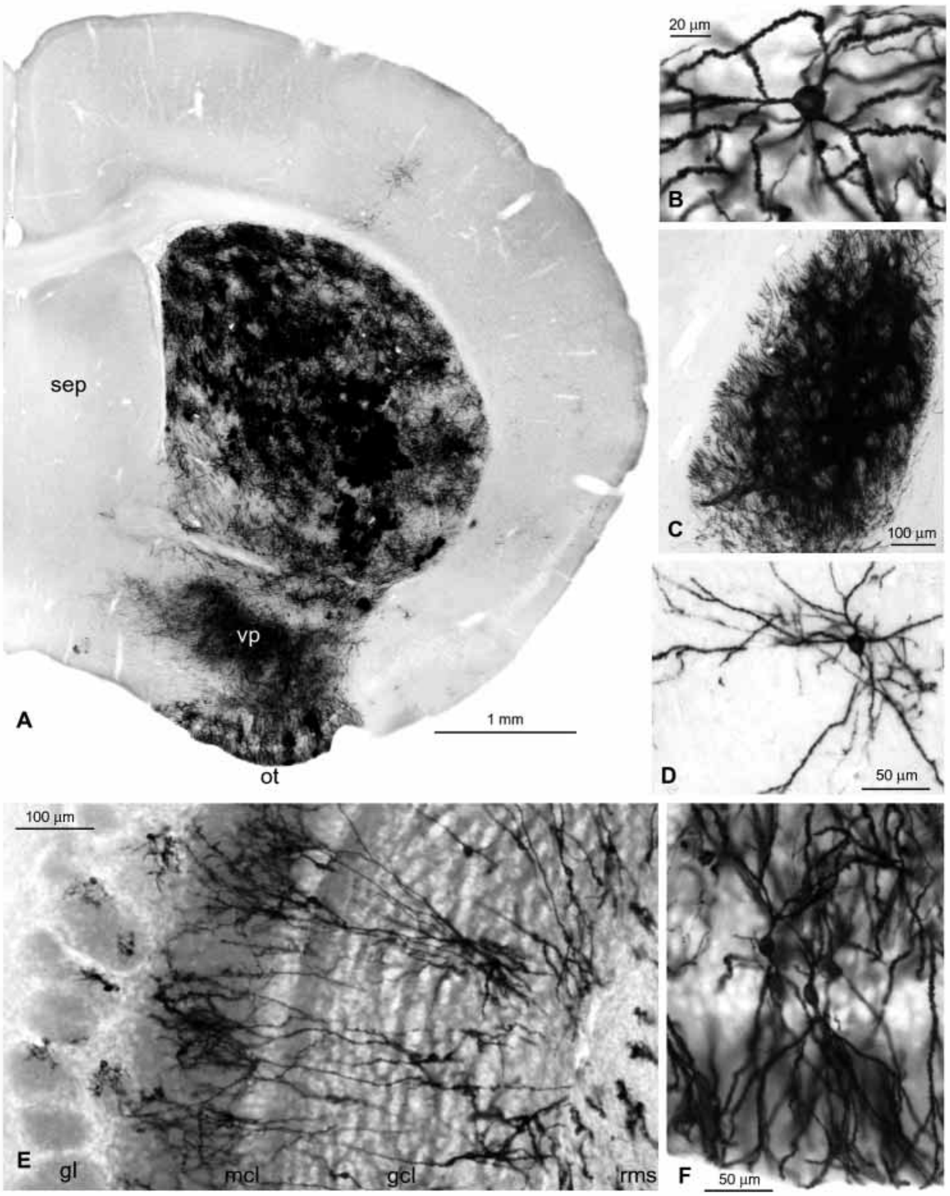


Fig. 7. Alkaline phosphatase-expressing LGE cells in 3-week-old animals. (B,D,E) Two to three fused photographs taken in different focal planes. (A) LGE cells disperse throughout the striatum and migrate ventrally into the olfactory tubercle (ot). Many labeled axons crisscross the ventral pallidum (vp). Notice that this distribution is complementary to the distribution of MGE cells (Fig. 3). No cells are detected in the septum (sep) and only rarely in the neocortex. (B) LGE cells in the striatum differentiated into medium spiny neurons. (C) Projecting axons were found among others in the medial globus pallidus. (D) The few LGE-derived neurons that migrated into the neocortex differentiated into aspiny neurons. LGE cells migrated to the olfactory bulb and differentiated into interneurons in the granule cell layer (gcl) and glomerular layer (gl). (E) Immature neurons were observed in the rostral migratory stream (rms). (F) LGE cells in the olfactory tubercle differentiated into bi-tufted spiny neurons with processes reaching the surface of the brain.

observed as early as 4 days after transplantation. This suggested that LGE-derived cells started to differentiate into the principal striatal projecting neurons.

LGE cells grafted into the MGE do not adopt MGE migratory properties

It has been suggested that some embryonic neural cells can adopt different phenotypes depending on their location in the developing brain (Brustle et al., 1995; Campbell et al., 1995; Fishell, 1995; Olsson et al., 1997). To test whether LGE cells could behave as MGE cells and migrate to the neocortex, we transplanted LGE cells heterotopically into the MGE (n=3). Four days later, LGE cells remained within the basal forebrain. The olfactory tubercle and ventral striatum proximal to the site of transplantation contained the highest density of labeled cells. Fewer labeled cells migrated to the developing nucleus accumbens and piriform cortex. Similar to the case of homotopic LGE grafts, we observed very few labeled cells within the neocortex (~8%, n=3) and amygdala. These findings suggest that E13.5 LGE cells do not adopt the migratory properties of MGE cells after heterotopic transplantation into the developing MGE.

LGE cells differentiate into medium spiny neurons and olfactory bulb interneurons

To study the long-term fate of LGE cells, we grafted transgenic hPLAP-expressing LGE cells into the LGE of wild-type embryos. The final fate of LGE cells was studied in 3-week-old animals (n=2). Similarly, as at 4 days after transplantation, only very few graft-derived cells were found in the neocortex (Fig. 7A). The majority of LGE-derived cells differentiated within the striatum, olfactory tubercle and nucleus accumbens into medium spiny neurons (Fig. 7B). Although within the striatum and nucleus accumbens these cells were multipolar, within the olfactory tubercle they had bi-tufted shape with dendritic processes reaching the ventral surface of the brain

(Fig. 7F). To confirm the phenotype of LGE-derived cells, we immunostained brain tissue with antibodies against DARPP-32, which is expressed in the majority of medium spiny neurons (Ouimet et al., 1998). We observed that ~97% of LGE-derived cells (74 cells analyzed) in striatum, nucleus accumbens and olfactory tubercle expressed DARPP-32 (Fig. 5D). We have shown that many of the MGE-derived cells in the neocortex and striatum expressed parvalbumin (Fig. 5A). By contrast, none of the LGE-derived cells in striatum stained with antibodies against this marker (Fig. 5E) (130 cells analyzed). Multiple labeled LGE-derived axons were observed in the internal capsule, lateral and medial globus pallidus (Fig. 7C), and substantia nigra, which are probably the efferents of the striatal medium spiny neurons. Dense labeled axons were also found in the ventral pallidum (Fig. 7A), which are probably derived from projecting neurons in the nucleus accumbens and olfactory tubercle. In both analyzed animals, LGE-derived cells were also found in the olfactory bulb, where they differentiated into granule and periglomerular interneurons (Fig. 7E). One of the analyzed animals contained labeled neuronal precursors within the subventricular zone (SVZ) and rostral migratory stream leading to the olfactory bulb (Fig. 7E), suggesting that some LGE cells gave rise to stem cells residing in the SVZ (Doetsch et al., 1999).

While most of the LGE cells differentiated into spiny neurons in striatum, nucleus accumbens and olfactory tubercle or interneurons in the olfactory bulb, few LGE-derived cells migrated into the neocortex. These neurons were aspiny or sparsely spiny (Fig. 7D) and they did not express DARPP-32 (data not shown). Interestingly, we found that several of these neurons were immunoreactive for parvalbumin (Fig. 5F), suggesting that LGE cells migrating to the neocortex are biochemically and morphologically similar to the MGE cells. These cells could correspond to MGE cells passing through the LGE at the time of dissection.

LGE and MGE-derived glial cells

Although the majority of MGE and LGE-graft derived cells differentiated into neurons, we observed also some graft-derived glial cells. MGE-derived glia were exclusively located in the proximity to the site of transplantation. Oligodendrocytes were encountered in striatal white matter, in the internal capsule and in the anterior commissure close to the site of transplantation. MGE-derived astrocytes were relatively rare and observed exclusively within the ventral striatum (data not shown). No glial cells were found in the neocortex after homotopic MGE transplantation, suggesting that unlike MGE-derived young neurons, grafted glial or bipotential progenitors did not undergo long distance tangential migration at the ages studied. Similarly, LGE-derived glia did not migrate into the neocortex or olfactory bulb. LGE-derived oligodendrocytes were scattered throughout the striatum and multiple LGE-derived astrocytes (protoplasmic, fibrous and stellate) were observed within the core of the striatum and olfactory tubercle (Fig. 7A, data not shown).

DISCUSSION

We provide direct in vivo evidence indicating that the embryonic MGE is the principal source of neurons migrating tangentially to the developing neocortex. In contrast to previous reports (Anderson et al., 1997; de Carlos et al., 1996; Tamamaki et al., 1997), we did not detect significant migration of E13.5 LGE cells into the neocortex. The few LGE-derived cells that migrated to the neocortex in vivo, probably correspond to contaminating MGE cells passing through the LGE on route to the neocortex. This is supported by our findings that these cells have many characteristics typical of MGE but not of LGE-derived neurons – they are aspiny or sparsely spiny, they do not express DARPP-32, but some of them express parvalbumin.

Interestingly, recent analysis of tangential cell migration in brain slices isolated from wild type and *Nkx2.1*$^{-/-}$ embryos suggested that at least in vitro LGE cells in E14.5 and older embryos do migrate to the neocortex (Anderson et al., 2001). In this study we did not detect significant number of LGE-derived cells in the neocortex, even in those adult animals in which LGE-derived neural stem cells remained in the SVZ and continued to produce neuronal precursors migrating to the olfactory bulb. This indicates that LGE cells migrating rostrally to the olfactory bulb and LGE cells migrating dorsally to the neocortex belong to separate lineages. It remains to be determined if the dorsally migrating cells are derived locally from the LGE ventricular zone cells, or whether they originate outside the LGE and only at later embryonic stages infiltrate this region. The latter would be consistent with the proposition that the postnatal SVZ is divided into the neurogenic (anterior) and gliogenic (posterior) domain (Luskin, 1998). It is possible that the anterior domain containing rostrally migrating neurons is derived from the embryonic LGE, while the posterior domain containing dorsally migrating glial precursors is largely a descendant of the caudal ganglionic eminence (CGE). Currently it is not known how divergent the LGE and CGE are and how sharp a boundary separates the two regions.

The medial and lateral ganglionic eminences do not develop simultaneously. The MGE develops earlier and faster than other parts of the forebrain. Between days E11 and E14, the MGE forms the most conspicuous protuberance in the forebrain vesicle. Later it appears that the growth of the MGE is diminished, and between days E15 and E16 the MGE fuses with the growing LGE. We have shown in vivo that E13.5 MGE cells migrate away from the MGE into the developing neocortex, where they differentiate into cortical interneurons. Although we have observed multiple morphologically and biochemically distinct types of cortical interneurons derived from MGE grafts, it remains to be addressed whether all cortical interneurons are generated outside the neocortex.

MGE cells from animals as young as E11 have a remarkable capacity to migrate in an in vitro tangential migration assay (Wichterle et al., 1999). It is conceivable that MGE cells younger than E13.5 also disperse widely across the developing forebrain, possibly contributing cells to the globus pallidus (Fentress et al., 1981; Shimamura et al., 1995; Smart and Sturrock, 1979), striatum (Olsson et al., 1998; Marin et al., 2000), neocortex (Lavdas et al., 1999; Meyer et al., 1998; Sussel et al., 1999) and hippocampus (Pleasure et al., 2000). Therefore, the MGE might be a transient structure in the developing mammalian forebrain, whose primary function is to generate large quantities of tangentially migrating neurons, perhaps of different phenotypes, depending on the time of their birth.

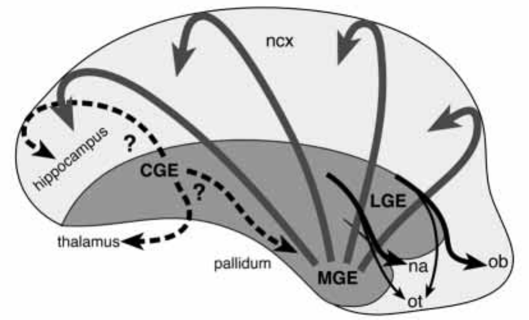


Fig. 8. Model of tangential neuronal migratory pathways in the developing mouse brain. Longitudinal view of the developing telencephalic vesicle with the anterior end pointing to the right. Gray arrows depict the large scale migration of MGE cells into the developing neocortex (ncx). Black arrows depict the migratory pathways from different parts of the LGE. Cells from the dorsoanterior LGE migrate preferentially into the olfactory bulb (ob), while more posteriorly grafted LGE cells migrate preferentially into the nucleus accumbens (na). LGE cells disperse through the striatum and migrate ventrally to the olfactory tubercle (ot). Hypothetical migratory routes from the caudal ganglionic eminence (CGE) are depicted by broken arrows.

Analysis of the MGE cell migration indicates that these cells initially move tangentially, independent of the radial glia. While tangentially oriented MGE cells were observed in all neocortical regions at 2 days after transplantation, the highest concentration was found in the subventricular zone of the LGE and neocortex, similar to the distribution of Lhx6- (Grigoriou et al., 1998; Lavdas et al., 1999) and calbindin- (Del Rio et al., 2000) expressing cells. It has been proposed that tangentially migrating neurons might interact with corticofugal axonal fibers present in the intermediate zone (O'Rourke, 1995; Metin et al., 2000; Poluch et al., 2001). The fact that most of the tangentially migrating cells are concentrated in the axon sparse subventricular or lower intermediate zone and avoid the axon-rich upper intermediate zone suggests that MGE-derived cells use substrates either expressed specifically by the lower intermediate zone axons, or generated by ventricular or subventricular zone cells. We propose that neocortical subventricular zone may be populated in large part by young neurons originating in restricted areas in the basal forebrain and similarly, like the adult SVZ, it might serve as a main corridor for tangential neuronal migration (Fishell, 1993; O'Rourke, 1997).

LGE cells contribute mainly to the dorsal and ventral striatum (including the nucleus accumbens and olfactory tubercle) and the olfactory bulb (Anderson et al., 1997; Olsson et al., 1998; Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). Migrating LGE cells may use both the tangential and radial pathways for their dispersal. Radial glia passing through the LGE curve ventrally and terminate at the olfactory tubercle. Thus, it is likely that LGE cells migrating to the olfactory tubercle use radial glia for their translocation. By contrast, LGE cells migrating to the olfactory bulb and to the medial aspect of the nucleus accumbens move in the direction perpendicular to the orientation of glial fibers. It is likely that this type of migration is similar to the tangential migration of postnatal and adult SVZ cells to the olfactory bulb (Lois et al., 1996; Wichterle et al., 1997). Detailed analysis of the sites of transplantation and regions into which LGE cells migrate suggests a general anteroventral orientation of LGE migration (Fig. 8). Our finding that some LGE cells migrate into the granule layer of the developing olfactory bulb several days before the first granule cells are born (Bayer, 1983) is quite surprising. It is possible that the early SVZ cells specified in the developing LGE move anteriorly and seed the subventricular zone in the olfactory bulb, which later will give rise to the early olfactory bulb interneurons (Anderson et al., 1997; Corbin et al., 2000).

Histological studies of the human and rat fetal brains suggested that cells born in the SVZ adjacent to the developing olfactory bulb migrate through a narrow region at the olfactory peduncle to the surface of the developing brain and give rise to the subpial granule cells in the marginal zone (Gadisseux et al., 1992; Meyer et al., 1998). As these studies relied on histological analysis, it might have appeared that LGE cells migrating into the narrow region of the olfactory tubercle continued in their migration into the marginal zone of the neocortex. Our study demonstrates that E13.5 MGE gives rise to some of the transient neurons in the subpial granule layer. However, it has been shown that many subpial granule cells are born before E13.5 and it remains to be determined whether these earlier cells also originate in the MGE.

Another migratory pathway from ganglionic eminences to the pulvinar nucleus of the developing thalamus has been detected in the fetal human brain (Letinic and Kostovic, 1997; Rakic and Sidman, 1969). It has been suggested that this migratory pathway is present only in humans but not in other vertebrates. Consistent with this hypothesis, we did not observe migration of E13.5 MGE or LGE cells into the diencephalon. However, we did not study the fate or migratory pathways of cells found in the caudal ganglionic eminence. We noticed that caudal ganglionic eminence cells migrate extensively (similar to MGE cells) in an in vitro tangential migration assay (H. W., S. N. and A. A.-B., unpublished). We do not know the fate of caudal ganglionic eminence cells in vivo, but we can speculate that these cells might behave either similarly like MGE cells (migrate dorsally to integrate into the developing hippocampus and caudal neocortex), like LGE cells (migrate into the ventral basal ganglia, possibly pallidum), or alternatively they might be able to cross into the diencephalon like cells in the human brain (Fig. 8).

Because both the LGE and MGE cells migrate through the same regions within the LGE, but then their migration diverges to distinct target areas, the two neuronal populations must use different guidance systems. It has been proposed that LGE cell migration is guided by a chemorepulsive factor Slit1 secreted from the ventricular zone of the LGE (Zhu et al., 1999). It remains to be determined which mechanisms guide the long distance MGE cell migration. Our preliminary studies indicate that MGE cells are guided to the neocortex by the selective permissiveness of dorsal forebrain areas in combination with chemorepulsive and chemoattractive factors secreted from the ventral forebrain and neocortex respectively (H. W. and A. A.-B., unpublished).

MGE cells engage in a migration across most of the embryonic forebrain. Why do these cells undergo such a long journey? As the developing central nervous system is subdivided along its longitudinal axis into anatomically, genetically and functionally distinct areas (Rubenstein et al., 1998), radial and tangential migrations probably play different roles during brain development (Herrup and Silver, 1994). Radial migration provides the basis for radial mosaicism of the developing neocortex (Rakic, 1988). Tangentially migrating neurons, on the other hand, often cross molecularly defined brain boundaries (Puelles et al., 2000). As different regions of the neuroepithelium give rise to different neuronal types, tangential migration might be used to import specific cell types into regions where these cells are not or cannot be normally produced. Therefore, tangential migration might have been co-opted by nature to expand the cellular complexity in particular regions of the developing brain. From the evolutionary perspective, ganglionic eminences belong to older brain structures than neocortex. It is possible that the tangential migration of MGE cells was a prerequisite for the evolution or subsequent expansion of neocortical regions in mammals. Finally, it has been proposed that the early differentiating preplate and marginal zone neurons influence neocortical development (Frotscher, 1998) and that GABA released in the developing neocortex could affect the rate of neocortical progenitors proliferation and their migration (Behar et al., 2000; Haydar et al., 2000; LoTurco et al., 1995). It is therefore conceivable that MGE-derived tangentially migrating GABAergic neurons regulate the early steps of corticogenesis by producing signals affecting the proliferation, migration or commitment of young neurons produced in the neocortical ventricular zone.

In summary, we have shown directly in living E13.5 mouse embryos that the medial ganglionic eminence is the principal germinal region giving rise to neurons that migrate tangentially into the neocortex where they differentiate into several types of cortical interneurons. Cells from the lateral ganglionic eminence at this age did not migrate to the neocortex, but differentiated into striatal medium spiny projecting neurons, or migrated ventrally into the olfactory tubercle, nucleus accumbens and olfactory bulb. It remains to be addressed what mechanisms underlie the differences in the migratory behavior of these two cell populations and whether their migratory properties change during the embryonic development. The ultrasound guided transplantation proves to be a powerful technique to study cell migration in vivo and in the future it should facilitate more extensive fate mapping analysis of the developing mammalian embryo. Hopefully, it will help to elucidate the complex migratory pathways of cells born in other parts of the nervous system or in other embryonic tissues in a similar way to the way in which it aided our study of cell migration during the formation of mammalian forebrain.

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