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# Distinct cortical migrations from the medial and lateral ganglionic eminences

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## SUMMARY

**Recent evidence suggests that projection neurons and interneurons of the cerebral cortex are generally derived from distinct proliferative zones. Cortical projection neurons originate from the cortical ventricular zone (VZ), and then migrate radially into the cortical mantle, whereas most cortical interneurons originate from the basal telencephalon and migrate tangentially into the developing cortex. Previous studies using methods that label both proliferative and postmitotic cells have found that cortical interneurons migrate from two major subdivisions of the developing basal telencephalon: the medial and lateral ganglionic eminences (MGE and LGE). Since these studies labeled cells by methods that do not distinguish between the proliferating cells and those that may have originated elsewhere, we have studied the contribution of the MGE and LGE to cortical interneurons using fate mapping and genetic methods. Transplantation of BrdU-labeled MGE or LGE neuroepithelium into the basal telencephalon of unlabeled telencephalic slices enabled us to follow the fate of neurons derived from each of these primordia. We have determined that early in neurogenesis GABA-expressing cells from the MGE tangentially migrate into the cerebral cortex, primarily via the intermediate zone, whereas cells from the LGE do not. Later in neurogenesis, LGE-derived cells also migrate into the cortex, although this migration occurs primarily through the subventricular zone. Some of these LGE-derived cells invade the cortical plate and express GABA, while others remain within the cortical proliferative zone and appear to become mitotically active late in gestation. In addition, by comparing the phenotypes of mouse mutants with differential effects on MGE and LGE migration, we provide evidence that the MGE and LGE may give rise to different subtypes of cortical interneurons.**

Key words: Cortex, Interneuron, Neuronal migration, GABA, Ganglionic eminence, Neuropeptide Y, Subventricular zone, Mouse

## INTRODUCTION

GABAergic neurons constitute roughly 20% of cortical neurons (Hendry et al., 1987; Meinecke and Peters, 1987). These local circuit neurons (interneurons) have essential roles in providing inhibitory tone to cortical projection (glutamatergic) neurons. There are many types of cortical interneurons based upon their neurochemical contents, their dendritic and axonal morphologies, and their physiological properties (Jones, 1975; Houser et al., 1983; Fairen et al., 1984; Lund and Lewis, 1993; De Felipe, 1993; Gupta et al., 2000). The mechanisms that regulate the generation of cortical interneuron subtypes are not known.

Several lines of evidence suggest that cortical GABAergic and glutamatergic neurons arise from distinct lineages. Experiments using retroviruses or mouse chimeras indicate that clones of GABAergic neurons tend to be tangentially dispersed, whereas clones of projection neurons generally are radially arranged (Parnavelas et al., 1991; Luskin et al., 1993; Mione et al., 1994; Tan et al., 1998). The tangential dispersion of cortical GABAergic neurons is consistent with the observation that these cells are generated in the progenitor zones of the basal ganglia, the lateral and medial ganglionic eminences (LGE, MGE). Studies using methods that label both proliferating and postmitotic cells within the LGE or MGE have demonstrated that cells migrate tangentially into the cerebral cortex from both regions (de Carlos et al., 1996; Anderson et al., 1997b; Tamamaki et al., 1997; Chapouton et al., 1999; Sussel et al., 1999; Lavdas et al., 1999; Wichterle et al., 1999; Tamamaki et al., 1999). Double-labeling experiments demonstrate that at least some of these cells are GABAergic (Anderson et al., 1997b; Lavdas et al., 1999; Wichterle et al., 1999). However, since the methods used in these studies label both proliferating cells and cells that may be passing through the MGE or LGE from other regions, the origins of these cells has remained unclear.

Genetic methods have further refined our understanding of the generation of tangentially migrating GABAergic neurons. Mice lacking the function of transcription factors that regulate either regionalization (*Nkx2.1*) or differentiation (*Dlx1*, *Dlx2*, *Mash1* (*Ascl1* – Mouse Genome Informatics)) in the basal ganglia, reduce the production of cortical GABAergic neurons. In double mutants of the *Dlx1* and *Dlx2* homeobox genes (*Dlx1/2* mutants), differentiation of LGE and MGE basal ganglia neurons beginning around E12.5 is blocked, and these mutants have roughly a fourfold reduction in cortical GABAergic neurons (Anderson et al., 1997a; Anderson et al., 1997b; Marín et al., 2000). Mutants of the *Mash1* basic-helix-

loop-helix gene block earlier stages of LGE and MGE differentiation, and also exhibit a reduction in cortical GABAergic neurons (Casarosa et al., 1999; Horton et al., 1999). Mutants of the *Nkx2.1* homeobox gene show evidence of a ventral-to-dorsal respecification of MGE progenitor cells, such that they take on molecular properties of LGE progenitors (Sussel et al., 1999). These mutants show a roughly twofold reduction in the number of cortical interneurons. This suggests that the MGE plays a role in the generation of these cells (Sussel et al., 1999), although abnormalities in cell migration from the *Nkx2.1* mutant basal telencephalon have not previously been demonstrated.

In this report, we provide evidence that progenitor cells within both the LGE and MGE produce neurons that migrate to the cerebral cortex. Tangential migrations from the MGE begin before migrations from the LGE, and MGE-derived cells appear to have a less coherent migration pathway within the cerebral cortex. Analyses of *Dlx1/2* mutants demonstrate that these mice have defects in tangential migrations from both the LGE and MGE, and that these defects are cell autonomous. Analyses of the *Nkx2.1* mutants demonstrate that these mice have a deficit in the MGE migration, but maintain the LGE migration. Unlike the *Dlx1/2* mutants, *Nkx2.1* mutants appear to completely lack cortical interneurons that express neuropeptide Y (NPY), nitric oxide synthase (NOS) and somatostatin. Thus, we suggest that the heterogeneity of cortical interneurons may arise, in part, due to the distinct origins of interneuron subtypes from different basal ganglia progenitor zones. In addition, we provide evidence that, late in gestation, the basal telencephalon may seed the cortical proliferative zones with DLX-expressing, mitotically active cells that contribute to cell proliferation postnatally.

## MATERIALS AND METHODS

### Animals

CD-1 mice were used for the organotypic slice culture experiments involving only wild-type animals. In addition, mouse mutant strains with null alleles of *Nkx2.1* (Kimura et al., 1996), and both *Dlx1* and *Dlx2* (Qiu et al., 1997), were used. These mutant strains were maintained by backcrossing to C57BL/J6 mice for at least ten generations. Genotyping was determined by morphological assessment (cleft palate in *Dlx1/2* nulls and absence of lungs in *Nkx2.1* nulls), and confirmed by PCR as previously described (Anderson et al., 1997a; Sussel et al., 1999). For staging of embryos, midday of the day of vaginal plug formation was considered as embryonic day 0.5 (E0.5). Mouse colonies were maintained at UCSF in accordance with NIH and UCSF guidelines.

### Organotypic slice transplants

Organotypic slice cultures of embryonic mouse telencephalon were prepared as previously described (Anderson et al., 1997a).

In transplantation experiments, BrdU (Sigma, 10μg/μl) was added to the neurobasal/B27 medium of donor slice cultures and then removed after 2 hours with two changes of medium. Portions of the LGE or MGE from donor slices were excised using microdissecting scissors and transplanted into host slices using a Pipetman (Rainin). After incubation for various times, the slices were fixed in 4% PFA for 1 hour at room temperature and either processed for BrdU immunofluorescence as whole slices, or cryoprotected in 30% sucrose for 1 hour, embedded in OCT and cryosectioned at 10-12 μm. Cryosections were processed for immunofluorescence as described below, using primary antibodies and dilutions as follows: rabbit anti-DLX2 (Porteus et al., 1994), 1:200; rabbit anti-calbindin (Swant, Bellinzona, Switzerland), 1:2000; rabbit anti-GABA (Sigma), 1:2000; mouse anti-PCNA (Vector, Burlingame, CA), 1:1000; mouse anti-βIII tubulin (Sigma), 1:1000. Each transplantation experiment reported in the results was repeated on a minimum of eight slices.

### Immunohistochemistry single labeling

E18.5 embryos, removed by Caesarean section, and neonates were anesthetized by cooling and perfused with 4% paraformaldehyde (PFA) and postfixed for 2-4 hours at 4°C. Sections were then cut on a vibratome at 50 μm. Free-floating sections were then preincubated in 5% normal serum of the species in which the secondary antibody was raised, 1% bovine serum albumin (BSA) and 0.3% Triton X-100 in phosphate-buffered saline (PBS) for 1 hour at room temperature, and subsequently incubated with the primary antisera for 36 hours at 4°C in 2% normal serum and 0.3% TX in PBS. The following antibodies were used: rabbit anti-CB (Swant), diluted 1:5000; rabbit anti-CR (Chemicon), 1:5000; rabbit anti-NPY (Incstar, Stillwater, MN), 1:3000; rat anti-SOM (Chemicon), 1:250; and rabbit anti-NOS (Zymed, San Francisco, CA), 1:1000. III tubulin (1:2000; run on free floating sections fixed in 2% acrolein in PBS). Sections were then incubated in biotinylated secondary antibodies (Vector), 1:200, and processed by the ABC histochemical method (Vector). In each experiment, sections from homozygous mutants and their wild-type or heterozygous littermates were processed together. Primary antiserum omission controls were used to further confirm the specificity of the immunohistochemical labeling.

### BrdU immunodetection and double labeling

BrdU-labeled cells were detected in both whole and resectioned slices. Sections were treated with 2N HCL for 20 minutes at 37°C, followed by five rapid rinses in PBS. Sections were then preincubated in 5% normal goat serum for 30 minutes, and anti-BrdU antibody overnight, (anti-mouse, 1:200, Chemicon). The primary antibodies were detected with Alexa (Molecular Probes) fluorescent secondary antibodies. Double-labeling was performed by first incubating in the GABA (1:2000), Calbindin (1:2000) or DLX2 (1:200) antisera overnight at 4°C. Sections were then rinsed in PBS, incubated in Alexa anti-rabbit fluorescent secondary antibodies, rinsed again, and fixed for 20 minutes in PFA. After fixation, sections were rinsed in PBS and processed for BrdU immunofluorescence as above. Sections were coverslipped with Prolong Antifade mounting medium (Molecular Probes) and imaged using a BioRad 1024 confocal microscope. Double labeling for rabbit anti-DLX1 (1:10) and mouse anti-PCNA (1:1000, Vector), or DLX1 and mouse anti-βIII tubulin, was run on cryosections following unmasking by microwaving in 10 mM sodium citrate buffer, pH 6.0.

### In situ hybridization

In situ hybridization experiments were performed using $^{35}S$ riboprobes on 10 μm cryostat sections as described previously (Bulfone et al., 1993). The Lhx6 cDNA was kindly provided by V. Pachnis.

## RESULTS

### Migration of MGE-derived cells into the cerebral cortex

Our previous studies on slice cultures from fetal mouse telencephalons revealed that DiI crystals placed within the LGE label cells that migrate into the cortex, and that some of these cells express GABA and calbindin (Anderson et al., 1997b). Subsequent studies provided evidence that at least some of these cells have their origin in the MGE (Lavdas et

al., 1999; Sussel et al., 1999; Wichterle et al., 1999). To examine the relative contributions of the LGE and MGE to these tangential migrations at E12.5, DiI crystals were placed into the LGE in coronal sections at different rostrocaudal levels of the telencephalon. At rostral levels, where no morphologically apparent MGE is present, cells do not migrate tangentially into the cortex, whereas they do migrate radially into the striatal mantle zone (Fig. 1A,A′). At a slightly more caudal level, where the MGE has begun to appear, a few cells are seen migrating from the LGE to cortex (Fig. 1B,B′). Migration from LGE to cortex is greatest at the rostrocaudal level where the MGE is largest (Fig. 1C,C′). DiI within the caudal extension of the ganglionic eminences (CGE) also labels cells migrating into the cortex, (Fig. 1D,D′).

Since slices that contain the MGE show the most robust migration of cells from the basal telencephalon to the cortex, DiI crystals were placed into the MGE of slices derived from E11.5-E16.5 embryos. While some tangentially migrating cells are detected moving into the cortical primordium at E11.5 + 60 hours in vitro (data not shown), the most robust migrations are observed at later stages (E12.5-E16.5; Fig. 2 and data not shown), consistent with previous studies in the rat and mouse (Lavdas et al., 1999; Sussel et al., 1999; Wichterle et al., 1999).

The migration pathways used by the tangentially migrating cells derived from the MGE appear to change over time. Early in gestation, the cells migrating from the basal telencephalon to the cortex follow a pathway that largely courses between the proliferative zone and the postmitotic zone, also known as the intermediate zone (Fig. 2A,A′). Later in gestation, the cells course mainly within the proliferative zones (ventricular zone, VZ; subventricular zone, SVZ) of the MGE and LGE into the neocortical proliferative and intermediate zones (Fig. 2B).

### At E12.5, the MGE but not the LGE gives rise to cortical GABA-expressing cells in coronal telencephalic slices

We used transplantation of BrdU-labeled MGE or LGE to determine which primordium generates cells that tangentially migrate into the cerebral cortex. Telencephalic slices were incubated in serum-free medium containing BrdU for 2 hours. Following this, portions of the MGE and LGE were transplanted into host slices at the same stage of development, and were subsequently cultured for 24-72 hours (Fig. 3A,B). The slices were then fixed, sectioned at 12 μm, and processed for immunofluorescence. At E12.5, transplantation of a BrdU-labeled MGE into either the LGE or MGE consistently gives rise to cells that migrate into the cortex (<50 cells per 12 μm hemisection in 11 of 12 experiments; Fig. 3C, right side and data not shown). Some of these BrdU-labeled cells also express markers of immature GABAergic interneurons including GABA, calbindin and DLX2 (arrows in Fig. 3D′,E′,F′). The percentage of BrdU double-labeled cells are: calbindin 30±6%; GABA 12±6%; DLX2 15±8% (average±standard deviation of two sections from each of three transplantation experiments). The percentage of double-labeled cortical cells varied considerably between experiments, possibly due to variations in tissue quality, the effect of HCl-treatment (for the BrdU detection) and weak immunolabeling with the DLX2 and GABA antibodies. In contrast to the results of MGE transplantations, experiments using E12.5 BrdU-labeled LGE as donor tissue does not produce cells that migrate tangentially into the cortex ($n$=11), although they do give rise to cells that migrate radially into the region of the striatal mantle (Fig. 3C, left side). Furthermore, the cortex in these specimens shows almost no expression of markers of immature GABAergic neurons (GABA, calbindin and DLX2; Fig. 3D,E,F). These results suggest that at E12.5 the MGE, but not the LGE, gives rise to GABA-, calbindin- and DLX2-expressing cells that migrate laterally through the LGE into the cortex.

### Later-born LGE-derived cells migrate into the cerebral cortex via a distinct pathway

In contrast to results obtained at E12.5, from E14.5 through E16.5, BrdU-labeled transplants of both the LGE and the MGE give rise to cells that migrate into the cortex (Fig. 4A; E15.5 and data not shown). Migration from the LGE to the cortex, however, tends to be less robust than from the MGE. For instance, LGE transplants at E15.5 ($n$=9), have 5-20 cortical BrdU labeled cells/hemisection, whereas MGE transplants ($n$=10), each contained greater than 40 cortical BrdU labeled cells/hemisection. In addition, at these developmental stages LGE and MGE donors produce cells that have very different migratory behaviors within the cortex. MGE-derived cells disperse widely throughout the cortex within 48 hours of transplantation (Fig. 4A, right side). In contrast, LGE-derived cells follow a more restricted migratory pathway. Within 48 hours of the transplantation, most of the LGE-derived cells that have migrated into the cortex are located within the cortical proliferative zones (Fig. 4A, left side).

Both MGE and LGE transplants give rise to cells that express GABA. GABAergic cells are detectable within 48 hours from MGE-derived transplants (Fig. 4E), whereas the detection of GABA-immunoreactive cells requires more time (~60 hours) from LGE derived transplants (Fig. 4F-I). This may be due to the fact that after 48 hours, the LGE-derived cells are still in a periventricular location (Fig. 4A), and require additional time to migrate into the cortical mantle zone (Fig. 4F-I). Unlike E12.5 MGE transplants, calbindin is not expressed by cortical cells derived from either the E15.5 MGE or LGE transplants (data not shown).

The observation of MGE and LGE-derived cells migrating into the cortical proliferative zone in E15.5 slices (Fig. 4A) correlates with the emergence of DLX2-positive cells in this region in vivo (Fig. 4J). Initially (~E12.5-E13.5) DLX-positive cells in the cortex are not in the proliferative zone; rather they are found in the intermediate and marginal zones (Porteus et al., 1994; Anderson et al., 1997b). Then, around E14.5, a few DLX1- and DLX2-expressing cells are observed in the cortical proliferative zone near the striatopallial angle (data not shown), and by E16.5 the number of these cells has greatly increased (Fig. 4J). These cells do not appear to be mitotically active, based on their lack of PCNA expression (Fig. 4J); based on their expression of βIII-tubulin, they appear to be immature neurons (Fig. 4L,M) (Menezes et al., 1994).

At progressively later stages, there are increasing numbers of DLX-positive cells in the cortical proliferative zone (e.g. see results at P0 in Fig. 4K). By this age, many of these cells appear to be proliferating, based on their expression of PCNA (Fig. 4K). Thus, these results support a model that DLX-positive cells begin to migrate from the proliferative zone of the basal telencephalon into the cerebral cortex around E14.5. Sometime

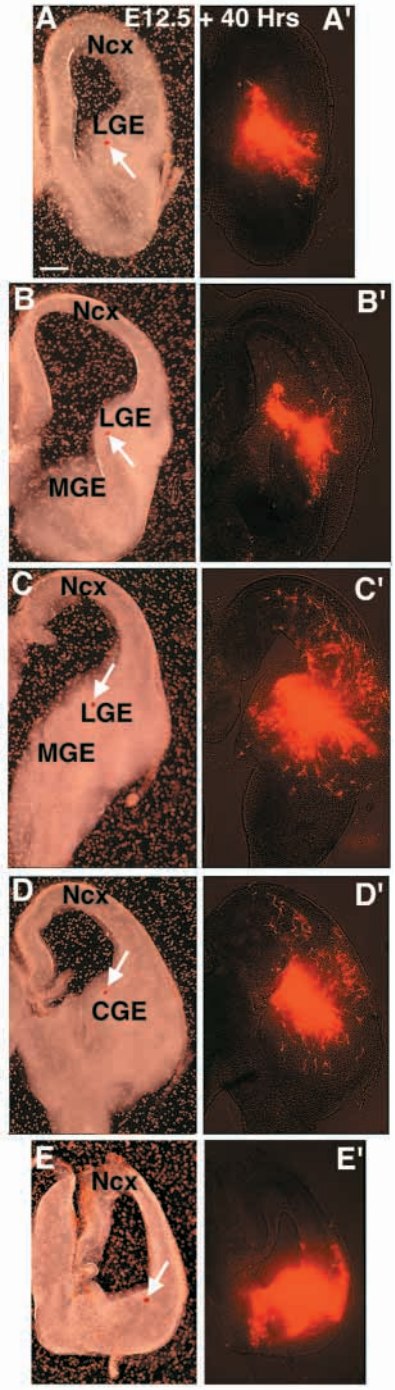


**Fig. 1.** Cell migration from the lateral ganglionic eminence (LGE) to the neocortex (Ncx) at E12.5. (A-E′) The right telencephalic hemispheres of five coronal slices in a rostral to caudal sequence. The left panel of each lettered pair shows a bright-field image of the slice and the site of DiI placement (white arrows) prior to culturing. The right panel shows an image of DiI fluorescence from the same slice after 40 hours in vitro. Note that the most robust tangential migration to the cortex occurred in slices that have a medial ganglionic eminence (MGE). There is also significant migration into the cortex from the caudobasal telencephalon (CGE) (D,D'), which contains the caudal extension of the *Nkx2.1* and *Dlx* domains (Puelles et al., 2000). However, cells do not migrate from the LGE to Ncx at the most rostral level (A), where no morphological medial ganglionic eminence (MGE) is present. E shows retrogradely labeled cells in the lateral cortex from a DiI crystal placed in caudal pallial neuroepithelium. Scale bar: 200 μm

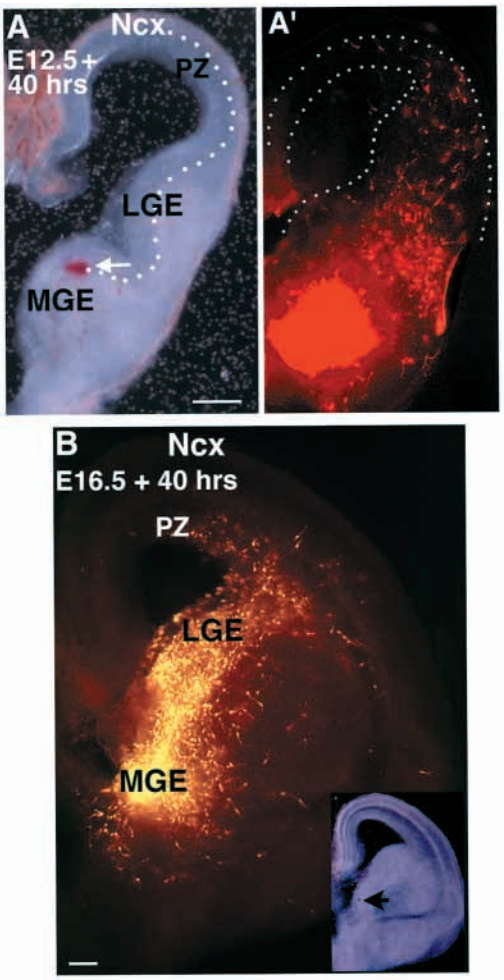


**Fig. 2.** Cell migration from the MGE to neocortex (Ncx). (A) Bright-field image of the right telencephalic hemisphere of an E12.5 slice with a DiI crystal in the MGE (white arrow). (A′) shows an image of DiI fluorescence from the same slice after 40 hours in culture. Many cells migrate dorsally into the Ncx, and most follow a course outside of the proliferative zone (PZ, demarcated by the white dots in A). (B) A slice cultured at E16.5 for 40 hours. The inset shows a bright-field image of the same slice prior to culture, and the black arrow indicates the location of the DiI. Note that while many cells migrate from the MGE to the Ncx at this age, they appear to course more closely to the ventricle and many enter the neocortical PZ. Scale bars: 200 μm.

between E16.5 and P0 DLX-positive cells in the cortical proliferative zone are mitotically active. Presently, the fate of these DLX-positive cells in the cortical proliferative zone is uncertain, but they may correspond to the precursors of neural stem cells that are described in the postnatal cortex (Luskin, 1993; Weiss et al., 1996; Doetsch et al., 1999).

*Dlx1/2* mutants appear to have a deficit in the development of these cells based on two observations. First, they lack βIII-tubulin-positive cells in the proliferative zone of the cerebral cortex at E16.5 (Fig. 4N). Second, at P0 the cortical subventricular zone of *Dlx1/2* mutants has fewer dividing cells (data now shown). These results suggest that DLX function may be required for the development of some cell types that are found in the cortical subventricular zone.

### Comparative analysis of *Nkx2.1* and *Dlx1/2* mutants supports the model that both the MGE and the LGE contribute interneurons to the cerebral cortex

Analysis of mutant mice that have abnormalities in MGE and LGE development provides information on the differential neuronal contributions made by these progenitor zones to the cerebral cortex. *Nkx2.1* mutant mice exhibit a ventral-to-dorsal shift in molecular properties of MGE progenitor cells, resulting in a loss of MGE-derived cells and a ventral expansion of LGE properties (Sussel et al., 1999). Thus, *Nkx2.1* mutants are useful in assessing the contribution of the MGE to the

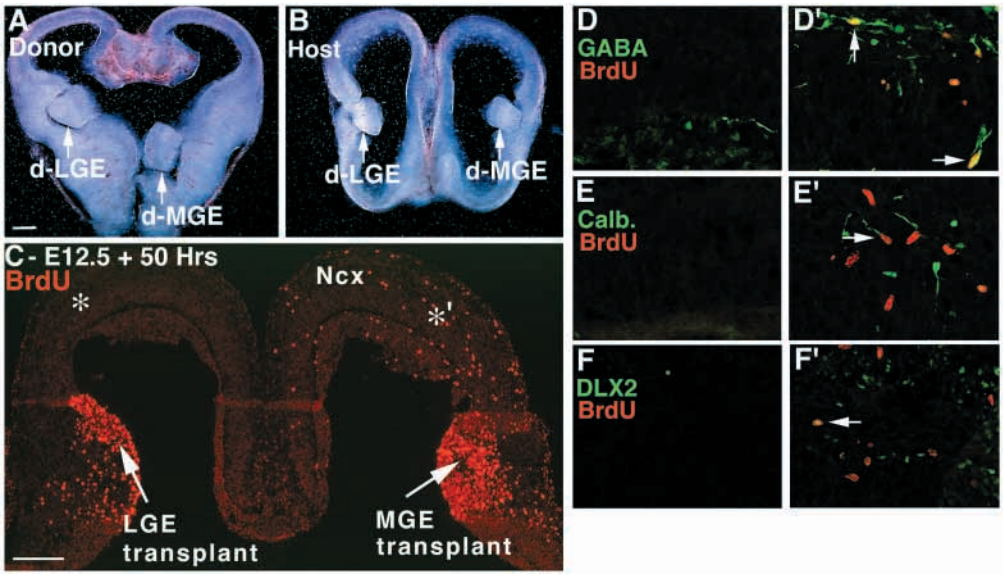


**Fig. 3.** Proliferating cells transplanted from the MGE but not LGE migrate into the neocortex at E12.5. (A) A donor slice (d) was grown in vitro with BrdU (10 μg/ml) for two hours prior to transplanting the left LGE (d-LGE) and the right MGE (d-MGE) into the host slice (B). Note that this host slice was from a rostral level at which no morphological MGE is present, and thus no LGE to Ncx migration would occur in slices (see Fig. 1A). After incubation for 50 hours, the slice was fixed and resectioned, and sections were immunolabeled for BrdU. Many cells from the MGE transplant (C, right) have migrated into the Ncx, while cells from the LGE transplant (left side) have remained within the ventral telencephalon. Similar MGE-to-cortex migration occurs when MGE transplants are placed into either the MGE or LGE of coronal host slices at more caudal levels (data not shown). The asterisks (* and *′) in C indicate the neocortical regions that were studied by immunofluorescence for BrdU (red) and GABA, calbindin and DLX2 (green, D,D′, E,E′, F,F′ respectively). On the left side of the slice, which contains the LGE transplant (D-F), the Ncx had little or no labeling for GABA, calbindin (Calb.) or DLX2. In contrast, the right side of the slice, which contains the MGE transplant (D′-F′), contains many cells in the Ncx that label for each of these markers, and some cells are double-labeled with BrdU (arrows). Scale bars: 200 μm.

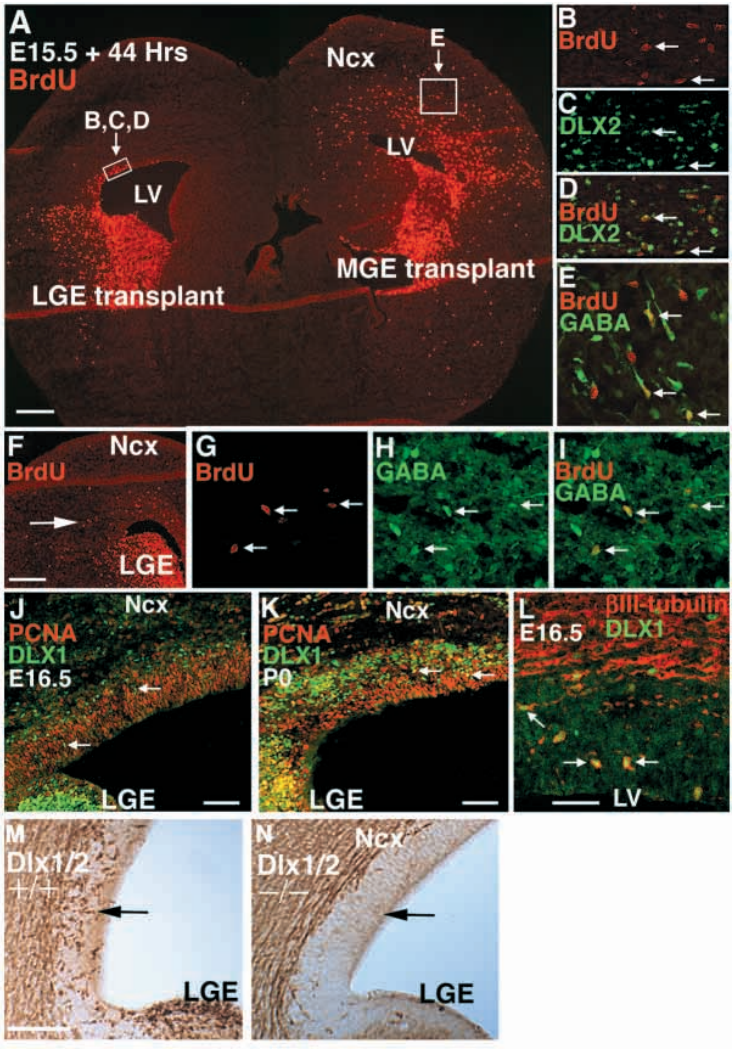


**Fig. 4.** Proliferating cells transplanted from both the MGE and LGE migrate into the Ncx at E15.5. (A) BrdU immunofluorescence of a 12 μm section from an E15.5 slice cultured for 44 hours. The left side received a BrdU-labeled LGE, whereas the right side received a BrdU-labeled MGE. Some cells from the LGE transplant have migrated into the Ncx, primarily within the proliferative zone. The boxed region is shown at higher power (B-D). Some of the LGE transplanted cells (red, B,D) in the neocortical proliferative zone also express DLX2 (green, C,D). The right side of this slice received a BrdU-labeled MGE, and cells from this transplant have migrated into the neocortical proliferative, intermediate and mantle zones. The boxed region of the Ncx indicates the approximate location from an adjacent section that is shown in E; this panel shows double-labeling for BrdU and GABA (arrows). (F-I) After 60 hrs in culture, some cells from the LGE express GABA in the Ncx. (F) A low-magnification view of BrdU labeling in an E15.5 slice that received a BrdU labeled LGE transplant and was subsequently cultured for 60 hours. The large white arrow indicates the position of the cells shown in (G) BrdU, (H) GABA and (I) merged BrdU/GABA. (J) At E16.5, DLX1 immunolabeled cells (green) are found in the cortical proliferative (PCNA positive) zone (red). Unlike DLX1-positive cells in the proliferative zone of the LGE, cortical DLX1-positive cells do not appear to be proliferating (PCNA negative). (K) At P0, however, many DLX1-positive cells (green) in the cortical PZ also express PCNA (red). (L) Double labeling for β-III tubulin (red) and DLX1 (green) at E16.5. Many DLX1-positive cells in the cortical proliferative zone also express β-III tubulin (white arrows). (M,N) Immunohistochemistry for β-III tubulin in wild-type and *Dlx1/2*$^{-/-}$ mice at E15.5. In the wild-type (M), the polarized morphology of many cells in the cortical PZ is consistent with their tangential migration from the LGE into the Ncx. These cells are largely absent in the Dlx1/2 mutant (N). Scale bars: in A, 200 μm for A; in F, 200 μm for F, 100 μm for J,K,M,N, 50 μm for L.

formation of cells that tangentially migrate to the cortex. Consistent with the hypothesis that many cortical interneurons originate in the MGE, the cortex in *Nkx2.1* mutants contains about a 50% reduction in GABA- and DLX2-immunoreactive cells at E18.5 (Sussel et al., 1999).

We took advantage of the MGE defect in the *Nkx2.1* mutants to confirm whether the LGE produces cells that tangentially migrate to the cerebral cortex. DiI crystals were placed either into a dorsal or ventral position in the proliferative zone of the basal telencephalon in coronal slices obtained from *Nkx2.1* mutant mice at E13.5 and E16.5. In agreement with our previous experiments, very few DiI-labeled cells are detected migrating tangentially into the cortex of *Nkx2.1* mutants at E13.5, whereas some cells migrate radially into the striatal mantle (Fig. 5A). The greatly attenuated contribution of early-born, MGE-derived cells to the cortex is also supported by the lack of DLX2- and calbindin-expressing cortical cells in *Nkx2.1* mutants at E13.5 (Fig. 5B-E).

While early tangential migrations are lost in *Nkx2.1* mutants, these migrations were present at E16.5. Cells migrating from the basal telencephalon to the cortex could be detected from either ventral or dorsal DiI placements (Fig. 6A). Since the *Nkx2.1* mutants appear to be devoid of normal MGE tissue, this later set of experiments supports the idea that cortical interneurons derive from the MGE early (e.g. E11.5-E14.5), and from both the LGE and the MGE at later times (e.g. after E14.5).

### Development of a specific subset of cortical interneurons requires *Nkx2.1* function in the MGE

The cerebral cortex contains many interneuron subtypes with distinct chemical, morphological and physiological characteristics (Houser et al., 1983; Jones et al., 1975; Lund and Lewis, 1993; De Felipe, 1993; Gupta et al, 2000). It is possible that different subtypes of cortical interneurons may arise from different subdivisions of the basal telencephalon. To address this issue, we examined the cortex of E19.0 *Nkx2.1* mutants for the presence of interneurons that expresses NOS, somatostatin and NPY. In mature cortex, NPY- and NOS-expressing cells are largely part of a somatostatin-expressing interneuron subpopulation (Kubota and Kawaguchi, 1994; Gonchar and Burkhalter, 1997). Interestingly, although GABA and DLX2 immunoreactivity are reduced by about 50% in the *Nkx2.1* cortex (Sussel et al., 1999), NOS-, NPY- and somatostatin-expressing cells are undetectable (Fig. 6B-D and data not shown). In contrast, NPY-, NOS- and somatostatin-expressing cells are rare (reduced over 80%), but present in the *Dlx1/2* mutant cortex (data not shown) and in the *Dlx1/2* mutant striatum (Marín et al., 2000).

The reduction of calbindin-expressing interneurons in the cortex of *Nkx2.1* mutants at E19.0 is also disproportionately higher than that of GABAergic cells (~90% reduction of calbindin versus ~50% reduction of GABA; Sussel et al., 1999). This finding, together with the lack of neocortical calbindin-expressing cells at E13.5 (Fig. 5D,E), suggests that most of these cells derive from the MGE as well. These results imply that distinct subtypes of cortical interneurons are derived from distinct progenitor zones (the MGE versus the LGE).

### Analysis of *Dlx1/2* mutants also suggests that the MGE and LGE contribute interneurons to the cortex

*Dlx1/2* mutant mice have a severe reduction in the tangential migration of cells from the LGE to cortex in vitro, and also have an approximately 75% reduction of cortical GABA-immunoreactive cells at P0 (Anderson et al., 1997b). These defects result from a block in neuronal differentiation in the basal telencephalon (Anderson et al., 1997a). Although abnormalities in the SVZ of the LGE of this mutant have been most carefully studied (Anderson et al., 1997a), SVZ defects in the MGE are also apparent. These defects include reduced proliferation and reduced expression of *Dlx5* and *Dlx6* (Anderson et al., 1997a; Marín et al., 2000). Thus, we tested whether tangential migrations from the MGE are also defective in *Dlx1/2* mutants. DiI crystals were placed in the MGE in slices from *Dlx1/2* mutants that ranged in age from E11.5 to E16.5. In these experiments, little tangential migration is observed, although some radial migrations are present in the slices from younger embryos (Fig. 7A,B).

The lack of MGE to cortex migration in slices from *Dlx1/2* mutants is closely paralleled by alterations in the expression of the Lim-homeodomain gene *Lhx6* (Grigoriou et al., 1998). Normal expression of *Lhx6* is found in cells migrating from the MGE through the LGE and into the cortex (Fig. 7C; Sussel et al., 1999; Lavdas et al., 1999). In contrast, the number of Lhx6-positive cortical cells is reduced over 90% in *Dlx1/2* mutants (Fig. 7D). Nevertheless, *Lhx6* expression does spread to the striatum in a pattern similar to that of cells migrating from the mutant MGE, suggesting that this part of the tangential migration is less severely affected (Fig. 7B,D).

Thus, the phenotype observed in the cortex of *Dlx1/2* mutants appears to reflect deficits in migration from both the LGE and the MGE. However, these deficits may be only partial, as neither *Lhx6* expression, nor expression of any of the markers for interneuron subtypes that we have studied (calbindin, NOS, NPY, somatostatin) is completely absent in the *Dlx1/2* mutant cortex (Anderson, 1997b, and data not shown).

### The defect in the tangential migration from the basal telencephalon to the cerebral cortex in the *Dlx1/2* mutants is cell autonomous

Transplantation assays were used to determine whether the migration defects found in the *Dlx1/2* mutants were due to intrinsic abnormalities of the migrating cells, or to defects in their environment. Isochronic transplants of portions of the MGE or LGE were inserted into either the MGE or LGE of host telencephalic slices. To test for the cell autonomy of the defect, mutant tissue was transplanted into a wild-type host. Alternatively, to test whether extracellular factors contributed to the migration defect, wild-type tissue was transplanted into *Dlx1/2* mutant slices. In most cases the transplants contained both the VZ and SVZ and were placed into the proliferative zone of the host (see right side of Fig. 8A,B). In an additional series of experiments, transplants containing only the SVZ were placed into the SVZ/intermediate zone of host slices (see left side of Fig. 8A,B). Similar results were found from either type of transplant.

Transplantation of wild-type LGE or MGE into *Dlx1/2* mutant hosts showed tangential migrations similar to those found when transplanted into wild-type hosts (compare Fig. 8C,D with Fig. 4A). However, transplantation of *Dlx1/2* mutant LGE or MGE into wild-type hosts showed no evidence of tangential migrations into the cerebral cortex (Fig. 8E,F).

However, some radially directed migration did occur from the *Dlx1/2* mutant LGE transplanted into the wild-type or heterozygous hosts (arrow in 8F). These results suggest that the inability of cells to migrate from the ganglionic eminences into the cortex in *Dlx1/2* mutants is a cell-autonomous defect.

## DISCUSSION

We have presented evidence that many cortical GABAergic neurons (cortical interneurons) in neonatal mice are derived from two distinct subcortical sources: the LGE and the MGE. According to this model, only the basal ganglia primordia produce GABAergic cells prenatally. This implies that the same processes that control the specification of telencephalic subdivisions are linked to the specification of the major neuronal subtypes. Accordingly, while the subcortical telencephalon is the source of GABAergic neurons, the cortical telencephalon appears to be the primary source of glutamatergic neurons. Furthermore, there is evidence that rostroventral regions of the subcortical telencephalon are the source of cholinergic neurons (Marín et al., 2000).

This model is based on results derived using a combination of in vivo and in vitro experiments. Lack of *Nkx2.1* function leads to an apparent ablation of MGE derivatives (Sussel et al., 1999), which enabled us to assess the differential contributions of the MGE and LGE in the production of cells that tangentially migrate to the cerebral cortex. In addition, we used mice lacking *Dlx1* and *Dlx2* function to study the effect of blocking the tangential migration of neurons from both the MGE and LGE (Anderson et al., 1997a; Anderson et al., 1997b). In conjunction with these genetic approaches, we used two in vitro methods involving telencephalic slice cultures (transplantation of BrdU-labeled tissues and DiI labeling) to follow the fate of cells derived from the LGE and MGE. Insights derived from these experiments are discussed below.

### GABAergic neurons derived from the MGE and LGE have distinct properties

Our results support a model that cortical GABAergic neurons derived from the MGE and LGE have different properties based on the timing of their production, their routes of tangential migrations and their neurochemical phenotypes.

Several lines of evidence show that the MGE is the primary source of tangentially migrating cells during early stages of telencephalic development (E11.5-E14.5). First, DiI placement in the MGE shows tangential migrations to the cortex beginning around E11.5 (data not shown). Second, DiI placement at E12.5 in different rostrocaudal positions of the basal telencephalon reveal tangential migrations only in section planes containing a MGE (Fig. 1). Third, early tangential migrations are lacking in mice with a defective MGE (*Nkx2.1* mutants). These observations are also consistent with the fact that the MGE matures earlier than the LGE (Smart, 1985; Kohtz et al., 1998). Migration from the LGE to the cortex, on the other hand, appears to be primarily restricted to later stages of neurogenesis (E14.5-E16.5; Figs 3, 4).

The routes taken by subcortical cells migrating into the cortex varies depending on both the source and the age of the migration (Fig. 9). Early-born, MGE-derived cells migrate primarily within the intermediate zone of the ganglionic eminences, and disperse throughout the cortical layers. In contrast, later-born cells from both the MGE and LGE migrate largely within the subcortical proliferative zones. However, cells from the later MGE and the LGE appear to behave differently within the cortex. MGE cells disperse into the cortical plate shortly after their arrival in the cortex, whereas most LGE-derived cells appear to initially migrate within the cortical proliferative zone (compare the left and right sides of Fig. 4A).

The Dlx genes also appear to regulate this tangential migration into the cortical proliferative zone. Starting at E14.5, there are DLX1- and DLX2-positive cells present in the cortical proliferative zone, primarily in the location of the subventricular zone (Fig. 4J and data not show). In *Dlx1/2* mutants, which have reduced tangential migration out of the LGE (Anderson et al., 1997a; Anderson et al., 1997b), βIII-tubulin-positive cells are lost in the cortical proliferative zone (Fig. 4N). The extent to which these cells will ultimately become cortical interneurons, or will assume other fates, remains to be determined.

*Nkx2.1* mutants, in which much of the MGE appears to be replaced by LGE-like tissue, lack detectable cortical GABAergic neurons that express NPY, NOS and somatostatin (Fig. 7 and data not shown), whereas about 50% of cortical GABAergic interneurons are still present (Sussel et al., 1999). *Nkx2.1* mutants also lack NPY-, NOS- and somatostatin-expressing striatal interneurons that tangentially migrate from the MGE to the striatum (Marín et al., 2000). In the mature cortex, it has been proposed that most interneurons can be grouped into three classes, those that express calretinin, parvalbumin or somatostatin (Kubota and Kawaguchi, 1994; Gonchar and Burkhalter, 1997). NOS- and the NPY-expressing interneurons are mainly part of the somatostatin-expressing class (Kubota and Kawaguchi, 1994; Gonchar and Burkhalter, 1997). Thus, these results have important implications for the generation of cortical interneuron heterogeneity, because it suggests that specific types of cortical interneurons are produced in distinct regions of the basal telencephalon. The fact that many GABA-expressing cells are present in the *Nkx2.1* mutant cortex suggests that other classes of interneurons may be less dependent on a normal MGE for their generation. Unfortunately, the *Nkx2.1* mutant animals die at birth, so that assessment of the calretinin- and parvalbumin-expressing classes cannot be made in these mutants.

These results do not rule out the possibility that important aspects of interneuron specification are regulated by the environment through which they migrate, or by the environment where they make synaptic contacts. Indeed, there are several lines of evidence suggesting that local cortical cues play important roles in determining interneuron phenotype (Mione et al., 1994; Jones et al., 1994; Götz et al., 1995; Marty et al., 1997; Wahle et al., 2000).

We think it unlikely that the loss of cortical NPY, NOS and somatostatin expression is due only to a *Nkx2.1*-dependent defect in the transcription of these genes. In wild type mice, *Nkx2.1* expression appears to be downregulated in these cells as they leave the basal telencephalon, suggesting that Nkx2.1 function is not normally required for the maintenance of NPY or somatostatin expression (Marín et al., 2000). Thus, it is more likely that the loss of NPY-, NOS- and somatostatin-expressing cortical cells is due to a block in their production.

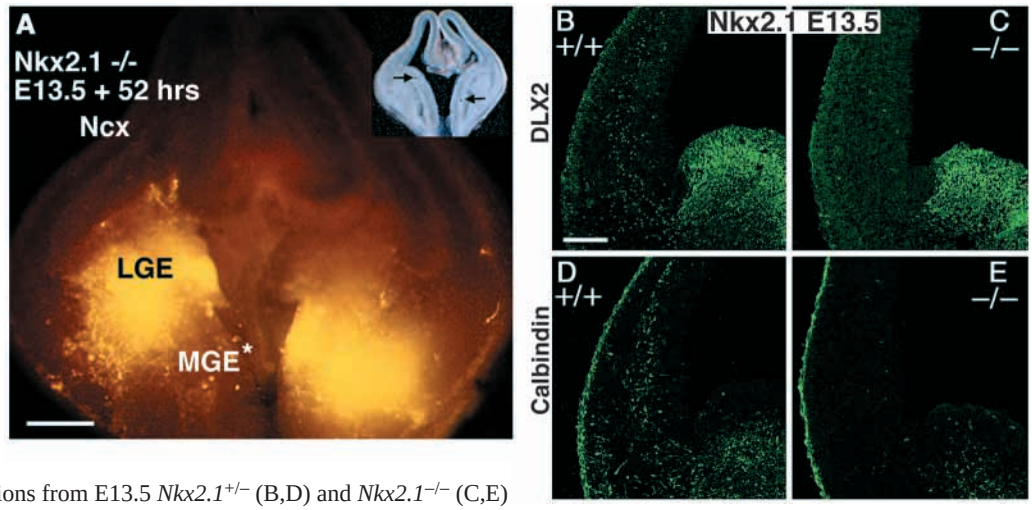


**Fig. 5.** *Nkx2.1* mutants lack tangential migration from the LGE and MGE to the neocortex at E13.5. (A) *Nkx2.1*$^{-/-}$ slice cultured for 52 hours. Inset figure shows the same slice prior to culturing, and the position of the DiI crystal placed into the left LGE and the right MGE*. During this relatively early stage of telencephalic development, *Nkx2.1* mutants have very little tangential migration to the Ncx compared with wild-type mice (compare with Fig. 2). Note that radial migrations in the basal telencephalon are grossly intact. (B,C) DLX2 and (D,E) calbindin immunofluorescence in coronal sections from E13.5 *Nkx2.1*$^{+/-}$ (B,D) and *Nkx2.1*$^{-/-}$ (C,E) embryos. Very few DLX2- or calbindin-expressing cells are present in the *Nkx2.1* mutant Ncx at this age. Scale bars: in A, 300 μm for A; in B, 100 μm for B-E.

### The production of tangentially migrating cells from the LGE appears to be independent of the MGE

Despite the lack of a normal MGE, development of the LGE, including the production of neurons that migrate to the cerebral cortex, continues in the *Nkx2.1* mutants (Fig. 6; Sussel et al., 1999). This suggests that the MGE does not play a major role in the patterning, proliferation and differentiation of the LGE. This point is particularly germane in that *Nkx2.1* mutants have a major reduction in the expression of sonic hedgehog in the telencephalon (Sussel et al., 1999), a protein that can regulate regionalization, cell-type specification and proliferation (Ericson et al., 1997; Martí et al., 1995; Wechsler-Reya and Scott, 1999).

### Evidence that the *Dlx1/2* mutants lack tangential migrations from the basal telencephalon to the cortex due to a cell autonomous defect

Several transcription factors are now implicated in controlling basal telencephalon patterning (*Nkx2.1*, Gsh2) and differentiation (*Dlx1*, *Dlx2* and *Mash1*), and thereby regulate the production of cortical GABAergic neurons (Anderson et al., 1997a; Anderson et al., 1997b; Szucsik et al., 1997; Sussel et al., 1999; Casarosa et al., 1999; Horton et al., 1999; Fode et al., 2000). Genes that affect patterning probably reduce the number of cortical GABAergic cells because the mispecified progenitor cells can not produce these cells. However, genes that affect differentiation of these neurons could block their tangential migration either through cell autonomous and/or non-autonomous effects. Using a transplantation assay we found that *Dlx1/2* mutant cells were unable to migrate in wild-type slices (Fig. 8E,F), demonstrating that these cells have a cell-autonomous defect in migration. Conversely, wild-type cells, transplanted into *Dlx1/2* mutant slices did migrate (Fig. 8C,D), providing evidence that loss of *Dlx1/2* function does

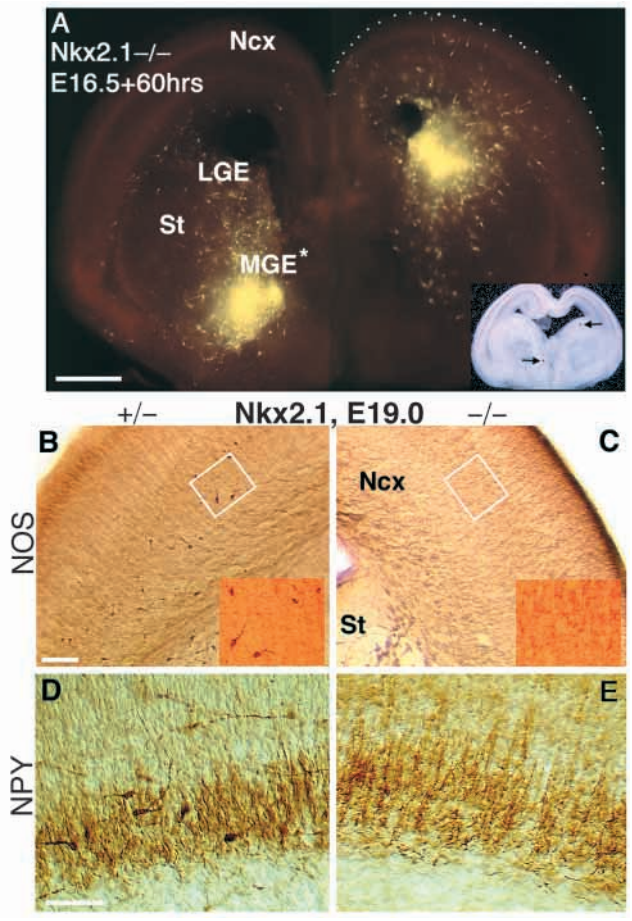


**Fig. 6.** (A) *Nkx2.1* mutants have tangential migration to the Ncx from the LGE and MGE* at E16.5. Inset shows a bright field view of the slice prior to culturing for 60 hours, and the black arrows indicate the position of DiI crystals. Some of the cells from the LGE injection (right side) appear to migrate radially through the cortical plate. St, striatum. (B-E) Absence of a subset of neocortical interneurons in *Nkx2.1* mutants. Sections from *Nkx2.1*$^{+/-}$ animals at E19.0 have NOS- (B) and NPY- (D) expressing cells in deeper portions of the cortical plate and subplate. *Nkx2.1* mutants lack expression of NOS (C) and NPY (E) in neocortical cells, although NPY expression is maintained in fibers penetrating the mutant cortical plate (E). Insets show higher magnification of the boxed regions. Scale bars: 400 μm in A; 100 μm in B,C; 50 μm in D,E.

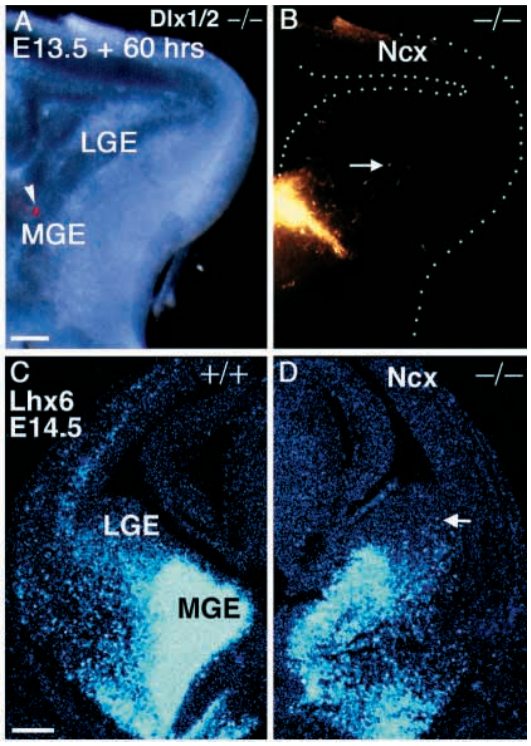


**Fig. 7.** Migration from the MGE to Ncx is greatly diminished in slices from *Dlx1/2* mutants. (A,B) Dark field and epifluorescence views, respectively, of a slice from an E13.5 *Dlx1/2*–/– embryo cultured 60 hours. The white arrowhead in A marks the location of a DiI crystal placed prior to culture. In the *Dlx1/2* mutant slice, a few cells have migrated from the MGE dorsolaterally into the striatal mantle, but not into the Ncx (compare with wild-type in Fig. 2B). (C,D) Reduction of *Lhx6*-expressing cells in the Ncx of *Dlx1/2* mutants at E14.5, as assessed by in situ hybridization. Scale bars: 200 μm.

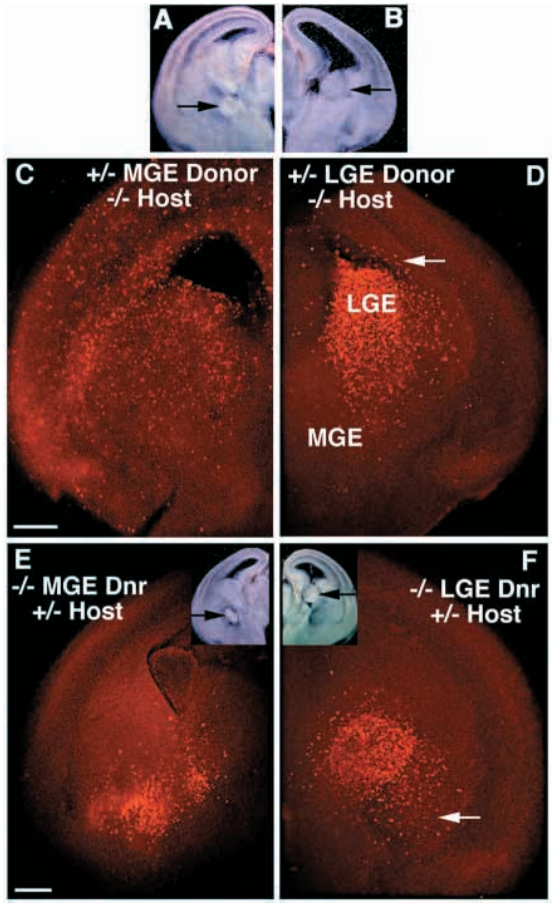


**Fig. 8.** The lack of GE-Ncx migration in *Dlx1/2* mutants appears to be a cell-autonomous defect. (A,B) Dark field photomicrograph of an E15.5 mutant recipient slice before (A) and after (B) regions of heterozygote (+/–) BrdU-labeled MGE (left) or LGE (right) were transplanted (arrows). (C,D) After 60 hours incubation, the distribution of BrdU-positive wild-type cells (detected by immunofluorescence) reveals that tangential (and radial) migrations can take place in the *Dlx1/2* mutant slice from either (+/–) MGE (C) or LGE (D) donors. The transplanted (+/–) MGE cells migrate into all layers of the Ncx (C), while many of the transplanted (+/–) LGE cells migrate into the neocortical proliferative zone (arrow in D). Transplantation of *Dlx1/2*$^{-/-}$ MGE and LGE cells into the (+/–) slices (E,F, respectively) do not migrate into the Ncx although some radial migration of cells appears to occur (white arrow in F).

not block the production of signals required for directing the basal telencephalon to cortex migration. The wild-type cells migrate to the cortex in the *Dlx1/2* mutant slices, despite decreased expression of netrin 1 and slit 1, two potential regulators of this migration (data not shown).

## Are all cortical GABAergic neurons derived from the basal ganglia?

Our results support the model that most neonatal GABAergic neurons originate from progenitors in the basal telencephalon. Furthermore, we have evidence that all cortical regions, including the olfactory bulb (Anderson et al., 1997a; Bulfone et al., 1998), olfactory cortex (S. A. A., O. M. and J. L. R. R, unpublished observations), cortex (Anderson et al., 1997a; Anderson et al., 1999; Sussel et al., 1999; Lavdas et al., 1999; Casarosa et al., 1999; this paper) and hippocampus (Anderson

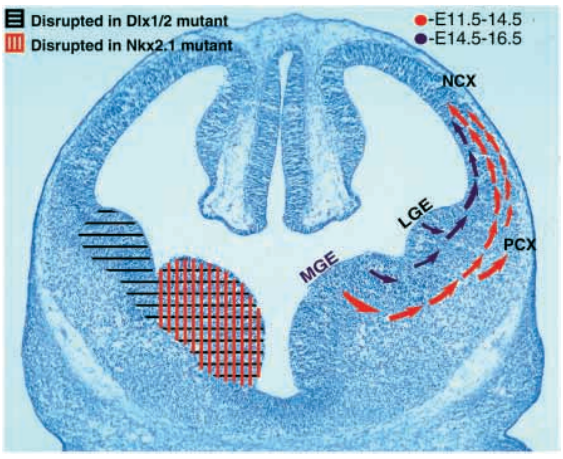


**Fig. 9.** Origins and migratory pathways of cortical interneurons derived from the basal telencephalon. Early in the period of cortical neuronogenesis (E11.5-E14.5, red arrows), cells migrate from the MGE into the cortex via a route that avoids the periventricular proliferative zone (PZ). Later in neuronogenesis (E14.5-E16.5, purple arrows), cells migrate from both the MGE and the LGE into the cortex via a route that largely tracts within the PZ. NCX, neocortex; PCX, piriform cortex.

et al., 1997b; Pleasure et al., 2001) obtain GABAergic neurons from the basal telencephalon.

It remains unclear, however, whether a subset of cortical interneurons derives from the cortical proliferative zone. The *Dlx1/2* mutant appears to lack virtually all ventral telencephalic to cortical migration in coronal slice cultures, yet a substantial number of interneurons remain. Interestingly, cortical cells expressing *Lhx6*, which may derive from the MGE (Sussel et al., 1999; Lavdas et al., 1999; Marín et al., 2000), are not completely lacking in the *Dlx1/2* mutant (Fig. 7D). This implies that some MGE-derived cells may indeed migrate into the *Dlx1/2* mutant cortex. Conceivably, this could occur via a rostrodorsal pathway (Gadisseux et al., 1992; Meyer et al., 1998). In addition, other regions of the basal telencephalon, such as the *Nkx2.1*- and *Dlx1/2*-expressing neuroepithelium of the septum and caudal ganglionic eminence, may be sources of cortical interneurons. Ongoing studies are now testing whether all cortical GABAergic neurons in the mature mammalian cortex are in fact derived from the basal telencephalon.

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