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Directional guidance of interneuron migration to the cerebral cortex relies on subcortical Slit1/2-independent repulsion and cortical attraction

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SUMMARY

Tangential migration from the basal telencephalon to the cortex is a highly directional process, yet the mechanisms involved are poorly understood. Here we show that the basal telencephalon contains a repulsive activity for tangentially migrating cells, whereas the cerebral cortex contains an attractive activity. In vitro experiments demonstrate that the repulsive activity found in the basal telencephalon is maintained in mice deficient in both *Slit1* and *Slit2*, suggesting that factors other than these are responsible for this activity. Correspondingly, in vivo analysis demonstrates that interneurons migrate to the cortex in the absence of *Slit1* and *Slit2*, or even in mice simultaneously lacking *Slit1*, *Slit2* and *netrin 1*. Nevertheless, loss of *Slit2* and, even more so, *Slit1* and *Slit2* results in defects in the position of other specific neuronal populations within the basal telencephalon, such as the cholinergic neurons of the basal magnocellular complex. These results demonstrate that whereas *Slit1* and *Slit2* are not necessary for tangential migration of interneurons to the cortex, these proteins regulate neuronal migration within the basal telencephalon by controlling cell positioning close to the midline.

Key words: Cell migration, Interneuron, Cholinergic neurons, Basal magnocellular complex, Telencephalon, Cortex, Basal telencephalon, medial ganglionic eminence, Mouse, *Slit1*, *Slit2*, *netrin 1*

INTRODUCTION

Neurons are most frequently born at a distance from the place where they finally become integrated in a specific neuronal circuit, so that they have to migrate to reach their final destination. The process of cell migration requires young neurons to perfectly synchronize multiple actions, including the timing for initiation and cessation of the movement as well as the appropriate responses to multiple guidance cues encountered through their trajectory. Directional guidance of neurons appears to be governed by mechanisms similar to those that control the guidance of growing axons, i.e. contact guidance (permissive and non-permissive substratum for migration) and diffusible gradients (attractive and repulsive cues) (Tessier-Lavigne and Goodman, 1996).

Neurons with disparate migratory behaviors may arise from common progenitor zones within the neural tube, suggesting that distinct cell populations have intrinsic mechanisms to distinguish the molecular cues that are relevant to their directional guidance. An extreme example of this circumstance is found in the subpallial telencephalon, where the lateral and medial ganglionic eminences (LGE and MGE, respectively) give rise to multiple neuronal populations with disparate migratory patterns. During mid-embryonic stages, for example, the LGE gives rise primarily to cells that migrate radially to differentiate as γ-aminobutyric-containing (GABAergic) projection neurons in the striatum, whereas the basal telencephalon (MGE and adjacent regions of the telencephalon stalk) are the source of cells that migrate tangentially towards the striatum, neocortex and hippocampus, where they differentiate as GABAergic interneurons (Anderson et al., 1997; Anderson et al., 2001; Lavdas et al., 1999; Pleasure et al., 2000; Sussel et al., 1999; van der Kooy and Fishell, 1987; Wichterle et al., 1999; Wichterle et al., 2001). In addition, the LGE appears to simultaneously contribute to cells that migrate rostrally into the developing olfactory bulb, whereas the MGE also gives rise to neurons that remain within the basal telencephalon (Marín et al., 2000; Wichterle et al., 2001). It is clear, therefore, that a precise control of the migratory behavior of each one of these neuronal populations must exist to guarantee the correct wiring of the telencephalon.

The mechanisms that control the migration of the different

cell populations derived from the subpallium are poorly understood. Repulsion from the ventricular zone of the subpallium has been suggested to play a role both in the tangential migration of interneurons to the cortex and olfactory bulb (Hu, 1999; Wu et al., 1999; Zhu et al., 1999), as well as in the radial migration of projection neurons into the developing striatum (Hamasaki et al., 2001). It seems unlikely, however, that the same mechanism of repulsion from the ventricular zone can account alone for such extremely divergent migratory patterns in vivo, and it is therefore expected that additional mechanisms exist to delineate each of the different routes of migration. In line with this expectation, a repulsive activity for migrating cortical interneurons in the developing striatum has been shown to contribute to the channeling of migrating cells into specific paths on their way towards the cortex (Marín et al., 2001).

As for the mechanisms involved, the molecular nature of the cues that direct neuronal migrations in the subpallium is largely unknown. Slit1, a diffusible guidance protein, has been shown to repel GABAergic cells derived from the LGE in vitro, and it has been suggested that this repulsion provides the directional guidance for neurons migrating from the LGE to the cortex (Zhu et al., 1999). Another diffusible guidance protein, netrin 1, has similarly been implicated in the repulsion of cells from the ventricular zone of the LGE towards the developing striatum (Hamasaki et al., 2001). Hepatocyte growth factor (HGF) has been shown to act as a motogen (i.e. a factor that stimulates migration) for cells tangentially migrating to the cortex (Powell et al., 2001), although it is not known whether the same factor provides any directional guidance to this migration. Antibody-blocking experiments suggest that interaction between migrating interneurons and cell adhesion molecules (including TAG1) may contribute to regulating the migrations (Denaxa et al., 2001). Finally, repulsion from the developing striatum by class 3 (secreted) semaphorins helps sort subsets of tangentially migrating interneurons towards the cortex (Marín et al., 2001). However, these insights fail to provide a cohesive understanding about the mechanisms that set the direction of the migration from the subpallium towards the cortex.

These studies have provided several important candidates for molecules directing subpallial migrations and, in the case of class 3 semaphorin and cell adhesion molecule involvement, direct tests of the functions of these molecules have been obtained. Nonetheless, the full complement of cues directing these migrations is still undefined, and the specific involvement of Slit and netrin proteins, which has been suggested to be key to directing these cells, has not been tested directly. The goals of the present study were therefore to define the developmental mechanisms that direct the migration of interneurons from the basal telencephalon to the cortex, and to test the roles of the Slit and Netrin proteins in this process. Through the development of new slice culture assays that test the behavior of tangentially migrating cells, we show that the basal telencephalon contains a repulsive activity for these cells whereas the cerebral cortex contains an activity that attracts them. Analysis of mice carrying loss-of-function alleles for *Slit1*, *Slit2* and *netrin 1* (*Ntn1*) demonstrate that, contrary to expectation, these proteins are not necessary parts of the repulsive activity found in the basal telencephalon and, in addition, do not appear to play a significant role in controlling tangential migration of interneurons to the cerebral cortex. However, Slit proteins are important regulators of neuronal positioning within the basal telencephalon, controlling cell migration across the midline and establishing the bilateral location of specific cell groups, such as the cholinergic basal magnocellular complex.

MATERIALS AND METHODS

Mouse colonies and genotyping

All animals were treated according to protocols approved by the Committee on Animal Research at the University of California, San Francisco. Embryos and newborn fetuses were obtained from matings between *Slit1*$^{-/-}$, *Slit2*$^{+/-}$ or *Slit1*$^{-/-}$; *Slit2*$^{+/-}$ animals. E18.5 fetuses were also obtained from matings between two *Slit1*$^{-/-}$; *Slit2*$^{+/-}$; *Ntn1*$^{+/-}$ mice. Mutant animals were initially recognized by detection of bright GFP fluorescence resulting from GFP transgene insertion in the *Slit* locus during generation of the mutant allele (Plump et al., 2002), or by X-gal staining of the product of the *lacZ* gene inserted into the *Ntn1* locus (Serafini et al., 1996). Additional genotyping was performed by PCR as described elsewhere (Plump et al., 2002). Embryos with generalized expression of GFP were obtained from matings of wild-type and GFP heterozygous mice, as described before (Marín et al., 2001).

Slice culture experiments

Organotypic slice cultures of embryonic mouse telencephalon were prepared as previously described (Anderson et al., 1997). Briefly, embryos (E12.5-16.5) were removed by Caesarean section and decapitated. Brains were removed, embedded in 4% low-melt point agarose, and 250 μm thick coronal sections were cut on a vibratome. The sections were then transferred to polycarbonate culture membranes (13 mm diameter, 8 μm pore size; Corning Costar, Cambridge, MA) in organ tissue dishes containing 1 ml of medium with serum (Gibco MEM with glutamine, 10% fetal calf serum, penicillin, and streptomycin). They were subsequently incubated for 1 hour in a sterile incubator (37°C, 5% CO_2), after which the medium was changed to Neurobasal/B-27 (Gibco BRL, Life Technologies Inc, Gaithersburg, MD). Transplantation was performed immediately after this step, as described before (Marín et al., 2001). In other cases, DiI placements were used to study tangential migration, as described elsewhere (Anderson et al., 1997). DiI crystals (C-16 DiI; Molecular Probes) were placed into the tissue with an insect pin and slices were returned to the incubator for the appropriate time, then fixed with 4% PFA and mounted on slides.

Matrigel explants

For co-culture experiments, E13.5 brains were embedded in 4% low melting point agarose in PBS and vibratome sections were obtained as described above. Small pieces of the cortex and MGE were dissected from approximately the same rostrocaudal level of the telencephalon and incubated for 1 hour in 1 ml of medium with serum. To set up the co-cultures, 25 μl of Matrigel® solution (BD Biosciences, NJ) was pipetted onto the bottom of four-well dishes (Nunc, Roskilde, Denmark) and allowed to gel for about 45 minute. Explants were then placed onto this base and 25 μl of collagen were added on top. Collagen co-cultures consisted of a piece of neocortex and a piece of MGE separated by approximately 400 μm. After a period of 45 minutes, to allow the matrigel to gel, Neurobasal/B-27 medium was added. Explants were cultured for 36 hours in a sterile incubator (37°C, 5% CO_2). Cell migration from MGE explants was semiquantified as described before (Zhu et al., 1999).

In situ hybridization

^{35}S-riboprobes were used for in situ hybridization as described

previously (Marín et al., 2000). Probes used for *GAD67*, *Lhx6*, *Slit1*, *Slit2*, *Ntn1* and *Isl1* have been previously described (Brose et al., 1999; Grigoriou et al., 1998; Pfaff et al., 1996; Serafini et al., 1994).

Immunohistochemistry

Embryos were obtained by Caesarean section, anesthetized by cooling, perfused with 4% PFA in PBS and postfixed in PFA for 2-8 hours. Following postfixation, brains were cryoprotected in 30% sucrose and cut in a freezing sliding microtome at 40 μm. Free-floating sections were preincubated in 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 24-36 hours at 4°C in 0.5% BSA and 0.3% Triton X-100 in PBS. The following antibodies were used: rabbit anti-calbindin (Swant, Bellinzona, Switzerland; diluted 1:5000), rabbit anti-calretinin (Chemicon; diluted 1:5000), rabbit anti-NPY (Incstar; diluted 1:3000), and rabbit anti-GABA (Sigma; diluted 1:2000). Sections were then incubated in biotinylated secondary antibodies (Vector; diluted 1:200) and processed by the ABC histochemical method (Vector). The sections were then mounted onto Superfrost Plus slides (Fisher), dried, dehydrated, and coverslipped with Permount (Fisher).

Cell counting

Cells were counted on images obtained from 40-60 μm sections acquired in a Spot2 cooling CCD camera attached to a conventional microscope. For cell counting in the cerebral cortex, a standardized box (40,000 μm^2) was used to delineate the appropriate areas. Three sections at different rostrocaudal levels in the telencephalon were used in each case, whereas two different levels were employed for cell counting in the hippocampus, striatum and basal magnocellular complex. For slice migration experiments, quantification of the different cell populations was expressed as a percentage of the total number of cells per slide. For the inverted cortex experiments illustrated in Fig. 3, cells were counted in two standardized areas (22,500 μm^2) of the medial and lateral regions of the cortex at the same distance (600-850 μm, depending on the case) from the center of the MGE graft. One-way ANOVA was used to estimate significant differences among cell populations in all experiments.

RESULTS

Previous studies have shown that migration of interneurons from the basal telencephalon to the cortex is a highly directional process (Anderson et al., 1997; Lavdas et al., 1999; Wichterle et al., 2001). Thus, although interneurons use layer-specific migratory routes at different developmental stages, the direction of migration, from subcortical to cortical territories, is maintained (reviewed by Marín and Rubenstein, 2001). This directionality could be explained by the presence in the basal telencephalon of a repulsive activity that forces interneurons towards the cortex, by the presence in the cortex of an attractive activity, or by a combination of repulsion and attraction. We therefore tested for the presence of such activities.

The basal telencephalon contains a repulsive activity for cells tangentially migrating to the cortex

In a first series of experiments we sought to determine whether the basal telencephalon contains a repulsive activity for tangentially migrating cells. To study the tangential migration of cells, we prepared slice cultures from E13.5 wild-type mouse embryos, and transplanted into these hosts portions of the MGE from green fluorescent protein (GFP)-expressing transgenic mice; to ensure synchrony, GFP and non-GFP embryos were always littermates (Marín et al., 2001). We first tested the behavior of tangentially migrating cells in the absence of the cortex. The entire cortex was removed unilaterally and a piece of the MGE obtained from GFP-expressing slices (MGE^{GFP}) was transplanted homotypically and ipsilaterally into the host slice (Fig. 1A). After 48 hours in culture, GFP cells had migrated dorsally, accumulating close to the edge of slice (n=19). As in control experiments, GFP cells never migrated ventrally (Fig. 1B,C and data not shown). Thus, information present in the subpallial telencephalon is sufficient to direct tangential migration of MGE cells towards the cortex.

To test whether different regions of the subpallium differentially influence the migration of MGE-derived cells, we transplanted small pieces of MGE^{GFP} into the mantle zone at different dorsoventral positions within the subpallium (Fig. 1D; n=16 for each case). Cells derived from the MGE migrated dorsally into the cortex when transplanted into the mantle of the LGE (the striatum or the mantle region superficial to it; position 1 in Fig. 1D and data not shown) or into the mantle of the MGE (the globus pallidus or the mantle region superficial to it; position 2 in Fig. 1D,E). In contrast, when the MGE^{GFP} was grafted into the most ventral regions of the subpallium (position 3 in Fig. 1D,F), very few cells left the graft and none of them reached the cortex (Fig. 1F). This domain of the basal telencephalon, which includes part of the septum at rostral telencephalic levels and the preoptic area (POa) at caudal telencephalic levels, therefore appears to prevent the ventral migration of MGE cells.

To test whether the repulsive activity found in the most ventral aspect of the subpallium was present in a gradient, we designed an experiment in which MGE cells were forced to migrate towards the ventral midline. The cortex was removed ipsilateral to the side where a piece of MGE^{GFP} was transplanted into the pallial-subpallial boundary (Fig. 1G). After 48 hours in culture, GFP cells had invaded the subpallium, although they did not migrate uniformly within this tissue (Fig. 1H). Thus, most GFP cells remained within the mantle of the LGE, with very few cells migrating into the MGE mantle and virtually none close to the ventral midline of the basal telencephalon (Fig. 1H,I; n=25). The distribution of GFP cells in the slice cultures did not vary significantly when the slices were maintained for up to four days in vitro (n=8; data not shown), suggesting that tangentially migrating cells derived from the MGE respond to a graded repulsive activity with its peak concentration in the most ventral region of the basal telencephalon.

The cerebral cortex contains an attractive activity for cells tangentially migrating to the cortex

Tangential migration from the basal telencephalon to the cortex could also require attraction from the cerebral cortex. To test this hypothesis, we analyzed the effect of an ectopic cortex on the tangential migration of cells derived from the MGE. In these experiments, we removed the entire contralateral subpallium and placed the contralateral cortex close to the basal telencephalon. To study the behavior of MGE cells, a piece of MGE^{GFP} was homotypically transplanted into the intact side of the slice (Fig. 2A; n=22). After 48 hours in culture, GFP cells migrated normally on the intact side of the

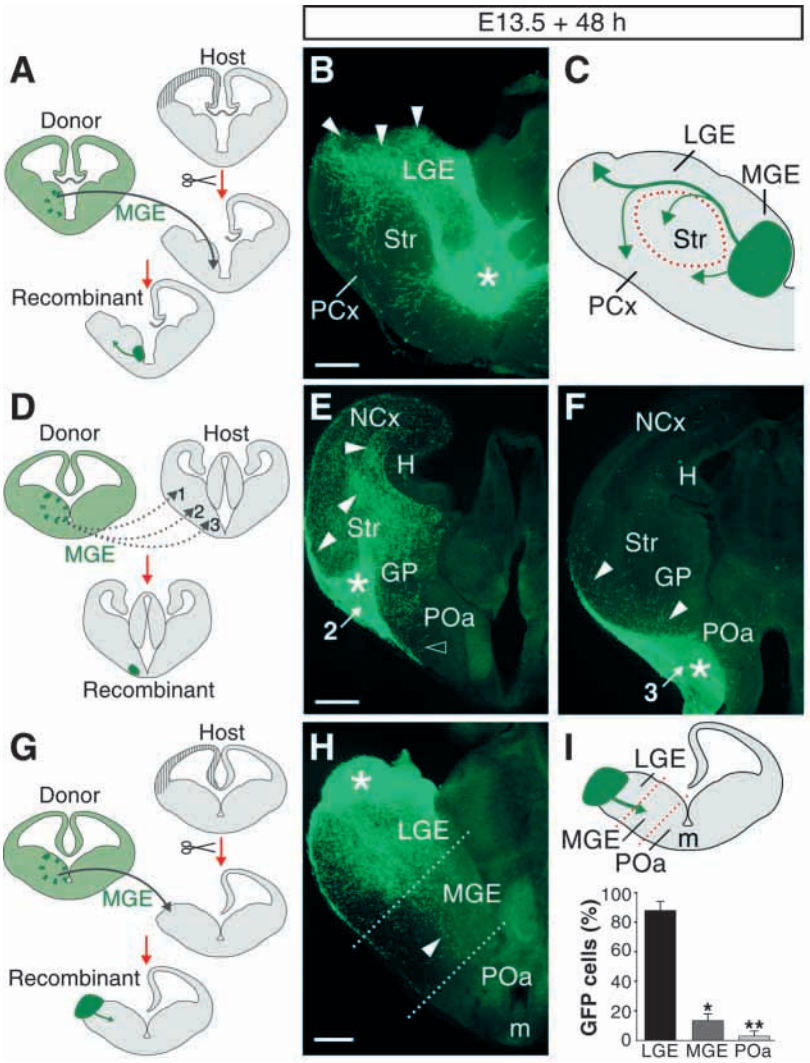

Fig. 1. The basal telencephalon contains a repulsive activity for tangentially migrating cells. (A) Schematic of the slice transplantation paradigm used to analyze the migration of MGE^{GFP}-derived cells in the absence of cortex. (B,C) Migration of MGE^{GFP}-derived cells (asterisk) after 48 hours in culture, labeled with GFP (B), and schematic representation of the routes followed by the MGE^{GFP}-derived cells (C). Arrowheads point to cells in the pallial/subpallial boundary, dorsal to the striatum. (D) Schematic of the slice transplantation paradigm used to analyze the migratory behavior of MGE^{GFP}-derived cells transplanted into dorsal (striatal; position 1), intermediate (pallidal; position 2) or ventral (preoptic; position 3) regions within the subpallium. (E,F) Migration of MGE^{GFP}-derived cells after 48 hours in culture, labeled with GFP. Examples of migratory behavior of cells derived from transplants (asterisks) into intermediate (E) or ventral (F) regions the subpallium. Arrowheads point to cells migrating from the transplants. Note the limited number of cells migrating from the transplant in position 3. (G) Schematic of the slice transplantation paradigm used to analyze the behavior of MGE^{GFP}-derived cells forced to migrate towards the preoptic area. (H) Migration of MGE^{GFP}-derived cells (asterisk) after 48 hours in culture, labeled with GFP. Note that very few cells migrate into the MGE mantle (arrowhead) and virtually none into the POa. (I) Analysis of the number of cells that migrated in to the mantle of the LGE, MGE or POa [which contains the ventral midline (m)] in the experiments described in G. Total number of cells (n=25): 5240 (LGE), 711 (MGE), 123 (POa). Histograms show averages±s.d. Asterisks denote significant differences in migration between the LGE and MGE (*) and between the MGE and POa (**). P<0.001 in both cases. LGE, lateral ganglionic eminence; m, ventral midline; MGE, medial ganglionic eminence; NCx, neocortex; PCx, piriform cortex; POa: preoptic area; Str, striatum. Scale bars: 300 μm.

slice, with many cells reaching the ipsilateral cortex (Fig. 2B). As in previous experiments (Fig. 1), however, GFP cells derived from the MGE did not migrate ventrally into the POa. Thus, this initial experiment failed to reveal an attractive activity in cortex, but it was not conclusive since attraction could have been obscured by the repulsive activity in the POa. In a second series of experiments, we therefore tested the behavior of the tangentially migrating cells derived from the MGE in a similar paradigm but in the absence of the POa. We removed the entire contralateral subpallium as well as the ipsilateral POa and placed the contralateral cortex close to the MGE, which contained a piece of MGE^{GFP} (Fig. 2C; n=14). After 48 hours in culture, GFP cells migrated normally on the intact side of the slice, with many cells reaching the ipsilateral cortex (Fig. 2D). In addition, however, many GFP cells also migrated into the ectopic cortex (Fig. 2D). These experiments reinforced the notion that the POa contains a repulsive activity for tangentially migrating cells.

If the cortex contains an attractive activity for tangentially migrating cells, then it might have been expected that in the previous experiments more MGE-derived cells would have migrated into the ectopic cortex than toward the ipsilateral cortex, since the ectopic cortex is closer to the MGE. However, the large number of GFP cells present in those experiments prevented quantification of cell numbers. We therefore performed a similar experiment using DiI crystals to label a small cohort of MGE-derived cells (Fig. 3A; n=18). In these experiments, the number of DiI-labeled cells that invaded the ectopic cortex was ~60% larger than the number of cells that migrated towards the ipsilateral cortex (Fig. 3B,C). This result suggests that the cortex is attractive to and/or permissive for tangentially migrating cells.

We performed additional experiments to further characterize the apparent cortical attractive activity. First, we transplanted MGE^{GFP} into the neocortex of wild-type slices (Fig. 3D; n=16), and studied the behavior of the tangentially migrating cells after 48 hours in culture. MGE cells disperse in both lateral and medial directions, but virtually none of them migrated into the subpallium (Fig. 3E,F). Interestingly, GFP cells tended to migrate preferentially towards the medial cortex rather than to the lateral cortex (13 out of 16 cases). This experiment suggest that the attractive activity present in the cortex may be

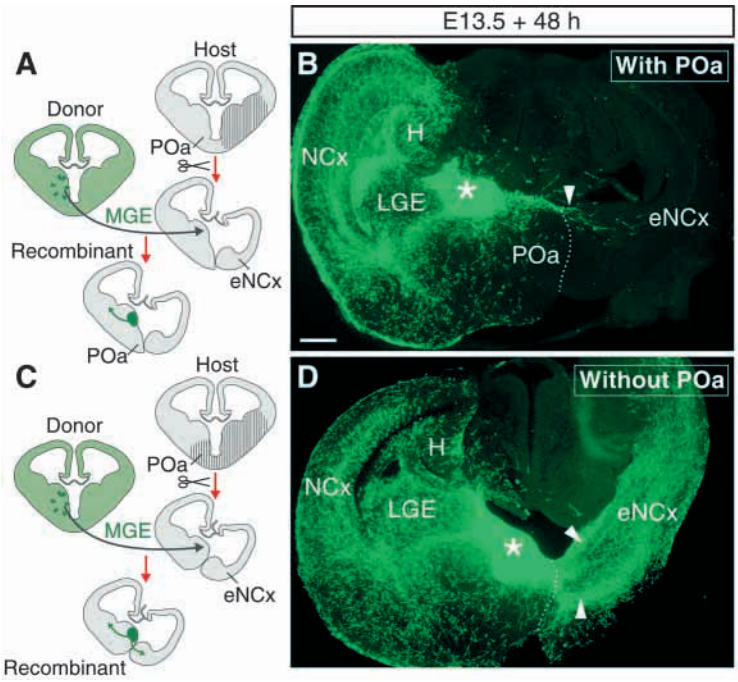

Fig. 2. The preoptic area prevents tangential migration towards an ectopic cortex. (A) Schematic of the slice transplantation paradigm used to analyze the migration of MGE^{GFP}-derived cells towards an ectopic cortex. (B) Migration of MGE^{GFP}-derived cells (asterisk) after 48 hours in culture, labeled with GFP. Most cells migrate towards the ipsilateral cortex, whereas very few cells migrate medially towards the ectopic cortex (arrowhead). (C) Schematic of the slice transplantation paradigm used to analyze the migration of MGE^{GFP}-derived cells towards an ectopic cortex in the absence of the preoptic area. (D) Migration of MGE^{GFP}-derived cells (asterisk) after 48 hours in culture, labeled with GFP. A large number of cells migrate towards the ectopic cortex in the absence of the preoptic area (arrowheads). eNCx, ectopic neocortex; H, hippocampus; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; NCx, neocortex; POa, preoptic area; Str, striatum. Scale bar: 300 μm.

distributed in a gradient increasing from lateral to medial regions of the cortex. Alternatively, the medial cortex is more permissive than the lateral cortex for the tangentially migrating cells.

If the cortex contains an attractive activity that is expressed in a medial to lateral gradient, then MGE cells would preferentially migrate towards the medial cortex if both regions of the cortex were found at the same distance from the source of migrating cells. To explore this possibility, we inverted the orientation of the neocortex (i.e., excluding the piriform cortex and the hippocampus) in wild-type slices and transplanted MGE^{GFP} homotypically to study the behavior of MGE cells (Fig. 3G; n=12). Quantification of the number of migrating cells found in medial and lateral regions of the inverted cortex located at the same distance from the center of the source of migrating cells (m and l boxes in Fig. 3H) revealed that significantly more MGE cells migrated to medial than lateral regions of the cortex [Fig. 3H,I; n=12, cells per 22,500 μm^2; medial (m) 237.25 (±67.35 s.d.), lateral (l) 149.92 (±69.46), P=0.0049]. The fact that more cells preferentially migrate towards the medial cortex in this experiment suggests that the cortex not only constitutes a highly permissive substratum for migration but also that it contains a chemoattractive activity that influences the behavior of tangentially migrating cells.

To provide direct evidence for the existence of a diffusible cortical chemoattractant for cells migrating from the MGE, we co-cultured small explants of MGE and neocortex on a permissive substratum and analyzed the distribution of cells that migrated out of the MGE explants after 36 hours in culture (Fig. 4A). In matrigel matrix, cells migrating out the MGE are preferentially oriented toward the cortical explant in co-culture experiments (n=38 explants; Fig. 4B,C). In contrast, migration of cells was similar from all sides of the explant when isolated pieces of the MGE were cultured (data not shown). Thus, the developing cortex releases a diffusible attractive activity that influences the migration of MGE cells.

Interneurons migrate to the cortex in *Slit1;Slit2* double mutants

The previous experiments suggest that tangential migration from the MGE to the cortex is controlled by coordinated repulsive and attractive activities present in the basal telencephalon and cortex, respectively. It has been previously shown that, *in vitro*, Slit proteins can repel GABAergic cells derived from the ganglionic eminences, and it has been hypothesized that *Slit1* expression in the ventricular zone repels tangentially migrating cells to the cortex (Zhu et al., 1999). These data, along with the strong expression of *Slit2* in the most ventral region of the subpallium during the period of interneuron migration to the cortex (Bagri et al., 2002) [and data not shown; *Slit3* is not expressed in the subpallium during this period (Marillat et al., 2001)], suggest that Slit proteins could be responsible for the repulsive activity found in the basal telencephalon (Figs 1, 2).

To directly address the role of Slit proteins in the guidance of cells tangentially migrating from the subpallium to the cortex, we studied mice carrying loss-of-function alleles for both *Slit1* and *Slit2* (Plump et al., 2002). We first examined the distribution of tangentially migrating interneurons in the embryonic cortex, as revealed by the expression of *GAD67*, *Lhx6* and *Dlx2*, three genes that identify embryonic GABA interneurons (Anderson et al., 1997; Lavdas et al., 1999). Comparison of the expression of these markers at different rostrocaudal levels within the cortex of wild-type and *Slit1;Slit2* double mutants, and at different embryonic stages (E12.5 and E14.5) showed no obvious differences in the number or laminar distribution of *GAD67*, *Lhx6* and *Dlx2* expressing cells within the cortex (n=3; Fig. 5A-D and data not shown), suggesting that *Slit1* and *Slit2* are not necessary for the migration of interneurons to the embryonic cortex. In agreement with these experiments, analysis of tangential migration in slice cultures using DiI crystals to label cells derived from the basal telencephalon revealed no significant differences between slices

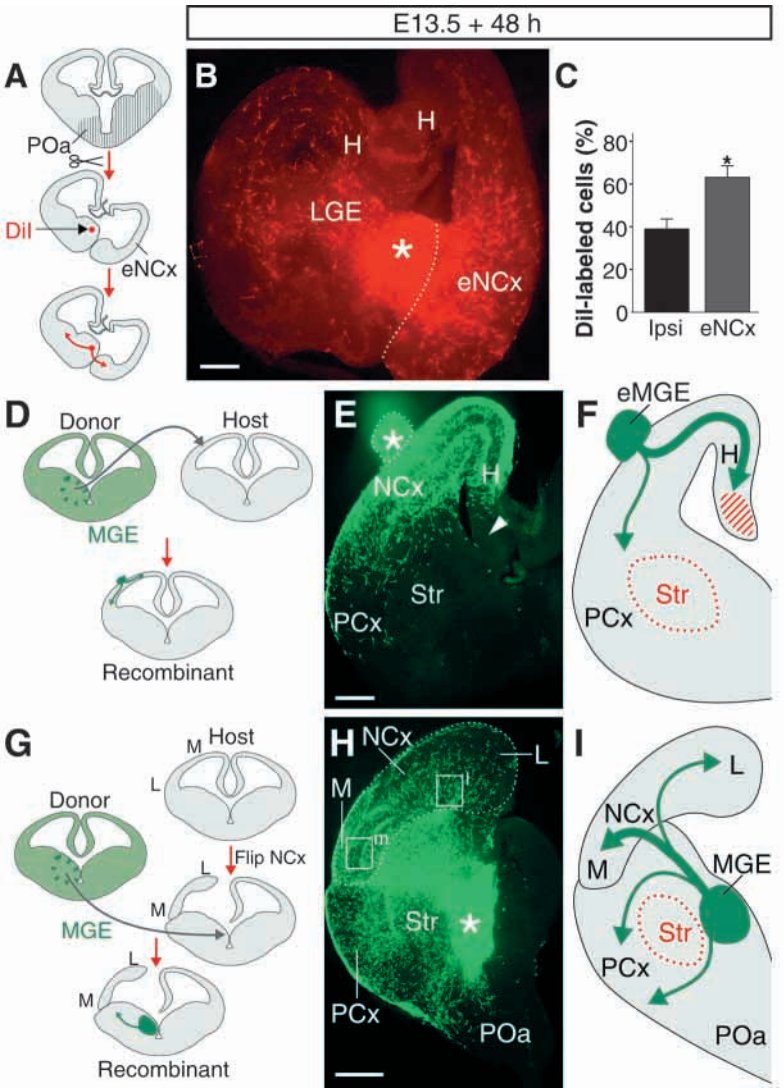

Fig. 3. The cortex contains an attractive activity for tangentially migrating cells derived from the MGE. (A) Schematic of the experimental paradigm used to quantify the migration of MGE-derived cells towards an ectopic cortex. (B) Migration of DiI labeled cells from the MGE (asterisk) after 48 hours in culture. Most cells migrate towards the ectopic cortex. (C) The number of DiI-labeled cells that migrated into the ipsilateral side of the slice (ipsi) or into the ectopic cortex (eNCx). The number of cells in the ipsilateral side includes those in the cortex and those en route towards the cortex (MGE and LGE). Total number of cells (*n*=18): 1590 (ipsi), 2582 (eNCx). Histograms show averages ±s.d. **P*<0.001. (D) Schematic of the slice transplantation paradigm used to analyze the migration of MGE^{GFP}-derived cells when transplanted ectopically into the dorsal cortex. (E,F) Migration of MGE^{GFP}-derived cells (asterisk) after 48 hours in culture, labeled with GFP (E), and schematic representation of the routes followed by the MGE^{GFP}-derived cells (F). Note that cells fail to migrate into the more medial aspect of the embryonic hippocampus (i.e. the developing dentate gyrus and cortical hem; arrowhead in E, hatched region in F) (see also Polleux et al., 2002). (G) Schematic of the slice transplantation paradigm used to analyze the migration of MGE^{GFP}-derived cells towards an inverted dorsal cortex. (H,I) Migration of MGE^{GFP}-derived cells (asterisk) after 48 hours in culture, labeled with GFP (H), and schematic representation of the routes followed by the MGE^{GFP}-derived cells (I). The boxes shown in H (m and l) indicate the regions from which cell counting was made. eNCx, ectopic neocortex; H, hippocampus; L, lateral region of the dorsal cortex; LGE, lateral ganglionic eminence; M, medial region of the dorsal cortex; MGE, medial ganglionic eminence; NCx, neocortex; PCx, piriform cortex; POa, preoptic area; Str, striatum. Scale bars: 300 μm.

obtained from wild-type or *Slit1;Slit2* double mutants (*n*=17 for each case; data not shown).

As expected from the results obtained during embryonic stages (Fig. 5), analysis of the distribution of interneurons in the cortex and hippocampus of newborn *Slit1;Slit2* double mutants was indistinguishable from that in wild-type mice. For example, the number of calbindin immunoreactive cells found in the neocortex (*n*=3; Fig. 6A-D) and hippocampus (Fig. 6G-J) was comparable in wild type and *Slit1;Slit2* double mutants [cells per 40,000 μm^2 in the neocortex: control 90.22 (±5.0 s.d.), *Slit1/2*$^{-/-}$ 86.44 (±4.2), *P*=0.372; in the hippocampus: control 108.33 (±10.6), *Slit1/2*$^{-/-}$ 101.33 (±9.6), *P*=0.749]. Likewise, no significant differences were found in the number of GABA, calretinin or neuropeptide Y (NPY) immunoreactive cells in the cortex of wild-type and *Slit1;Slit2* double mutants (*n*=3; data not shown). Thus, *Slit1* and *Slit2* do not seem to be required in vivo for tangential migration from the subpallium to the cortex.

Slit1 and Slit2 are not essential components of the repulsive activity present in the basal telencephalon

The absence of defects in the migration of interneurons to the cortex in *Slit1;Slit2* double mutants could have at least two explanations: (1) the attractive activity present in the cortex (Figs 3, 4) may be enough to compensate for the lack of repulsion from the basal telencephalon in *Slit1;Slit2* double mutants; or (2) the repulsive activity found in the basal telencephalon (Figs 1, 2) may not be mediated by Slit proteins. To distinguish between these possibilities, we first analyzed the migratory behavior of MGE-derived cells in the absence of cortex using the slice culture assay. The entire cortex was removed from one side of E13.5 wild-type slices, DiI crystals were inserted into the MGE of both sides of the slices, and the migration of labeled cells was closely monitored every 4 hours (Fig. 7A). In all cases (*n*=13), MGE-labeled cells reach the pallial-subpallial boundary at the same time (roughly after 24 hours; Fig. 7B), suggesting that basal telencephalic repulsion plays a greater role than cortical attraction in the initial parts of the migration.

In a second series of experiments, we tested whether the repulsive activity found in the basal telencephalon was present in *Slit1;Slit2* double mutants by doing similar experiments to those used initially to describe the location of this activity. First, the entire cortex was removed ipsilaterally from E13.5

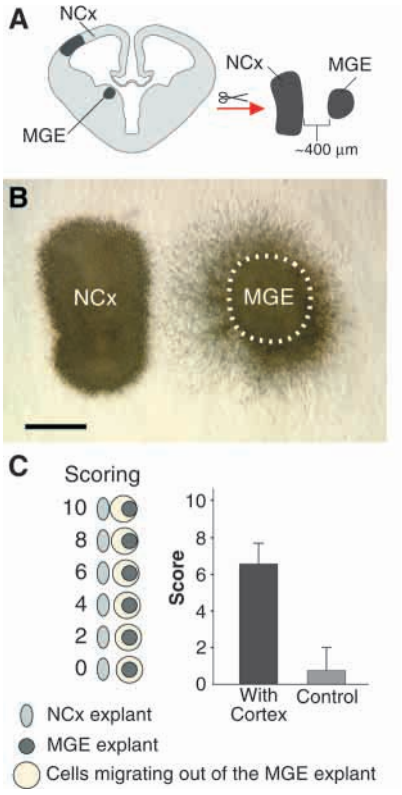

Fig. 4. Chemoattractive activity of the neocortex on cells migrating from the MGE. (A) Schematic of the experimental paradigm used for E13.5 cells. (B) An example of the distribution of neurons originating in the MGE after 36 hours of co-culture with a neocortical explant. The presence of more MGE cells in the regions proximal to the cortex than distal to it indicates an attractive effect of the cortex on MGE cells. (C) Diagram of the scheme used to semiquantify the effects of the cortex on MGE migration [modified from Zhu et al. (Zhu et al., 1999)] and score of migration in co-culture experiments (with cortex) or in MGE alone experiments (control). MGE, medial ganglionic eminence; NCx, neocortex. Scale bar: 200 μm.

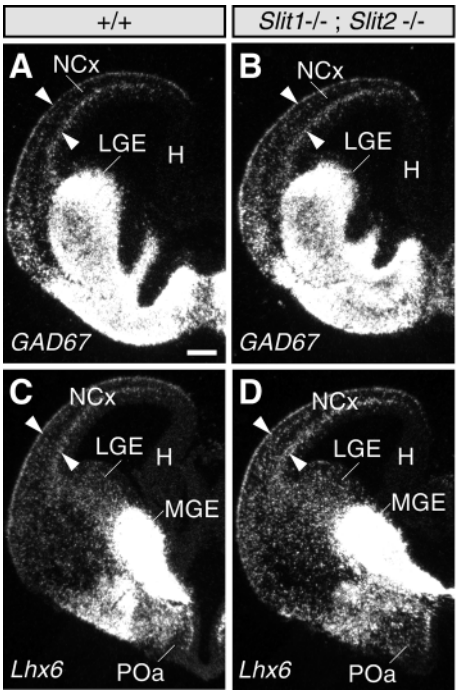

Fig. 5. Tangential migration to the cortex of *Slit1;Slit2* double mutants. Coronal sections through the telencephalon of E14.5 embryos showing expression of *GAD67* (A,B) and *Lhx6* (C,D) in wild-type (A,C) and *Slit1;Slit2* mutant (B,D) mice. Arrowheads point to cells migrating in the marginal and intermediate zones of the cortex. H, hippocampus; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; NCx, neocortex; POa, preoptic area; Str, striatum. Scale bars: 500 μm.

slices obtained from *Slit1;Slit2* double mutants and a piece of the MGE obtained from GFP-expressing slices (MGE^{GFP}) was transplanted homotypically into the host slice (Fig. 7C). After 48 hours in culture, GFP cells had migrated towards the cortex (Fig. 7D; n=12), in a manner indistinguishable from those of wild-type slices (Fig. 1A-C). As in control experiments, GFP cells never migrated ventrally in slices derived from *Slit1;Slit2* double mutants (Fig. 7D), suggesting that Slit1 and Slit2 proteins are not required for the repulsive activity found in this region. Identical results were found in similar experiments in which DiI was used to label migrating cells from the MGE (data not shown), to rule out the possibility that the small amount of Slit1 present in the MGE^{GFP} graft was able to repel tangentially migrating cells towards the cortex. In another series of experiments, the cortex was removed ipsilaterally from E13.5 slices obtained from *Slit1;Slit2* double mutants and a piece of MGE^{GFP} was transplanted into the pallial-subpallial boundary (Fig. 7E; n=11). After 48 hours, GFP cells migrated into the subpallium but, as in the experiments using wild-type slices (Fig. 1G,I), they were unable to migrate ventrally to the mantle of the MGE (Fig. 7F). Together, these experiments suggest that Slit1 and Slit2 are not essential components of the repulsive activity for tangentially migrating neurons found in the most ventral region of the subpallium.

Simultaneous loss of Slit1, Slit2 and Ntn1 does not prevent interneuron migration to the cortex

The previous experiments suggest that molecules other than Slit1 and Slit2 account for the repulsive activity present in the subpallium. Recently, it has been shown that, in vitro, Ntn1 can repel GABAergic cells derived from the ganglionic eminences (Hamasaki et al., 2001). *Ntn1* is abundantly expressed in all regions of the subpallium, including the POa (Tuttle et al., 1999), suggesting that it may have a synergistic effect with Slit proteins in controlling cell migration in the basal telencephalon. To test this hypothesis, we first generated *Slit1;Ntn1* double mutants. In contrast to *Slit1* mutants, which survive into adulthood and have roughly normal telencephalic development (Bagri et al., 2002; Plump et al., 2002), *Slit1;Ntn1* double mutants died at birth. Analysis of cortical interneuron markers at E18.5, however, failed to reveal a difference in the number of cortical interneurons between wild type, *Slit1* and *Slit1;Ntn1* mutants. For example, despite the smaller size of the cortex in *Slit1;Ntn1* double mutants, the density of calbindin immunoreactive cells in the cortex was similar in the two genotypes [Fig. 8A-F; n=3. Cells per 40,000 μm^2; control 91.45 (±2.27s.d.), $Slit1/2^{-/-}$ 97.78 (±7.08), P=0.214]. Similar

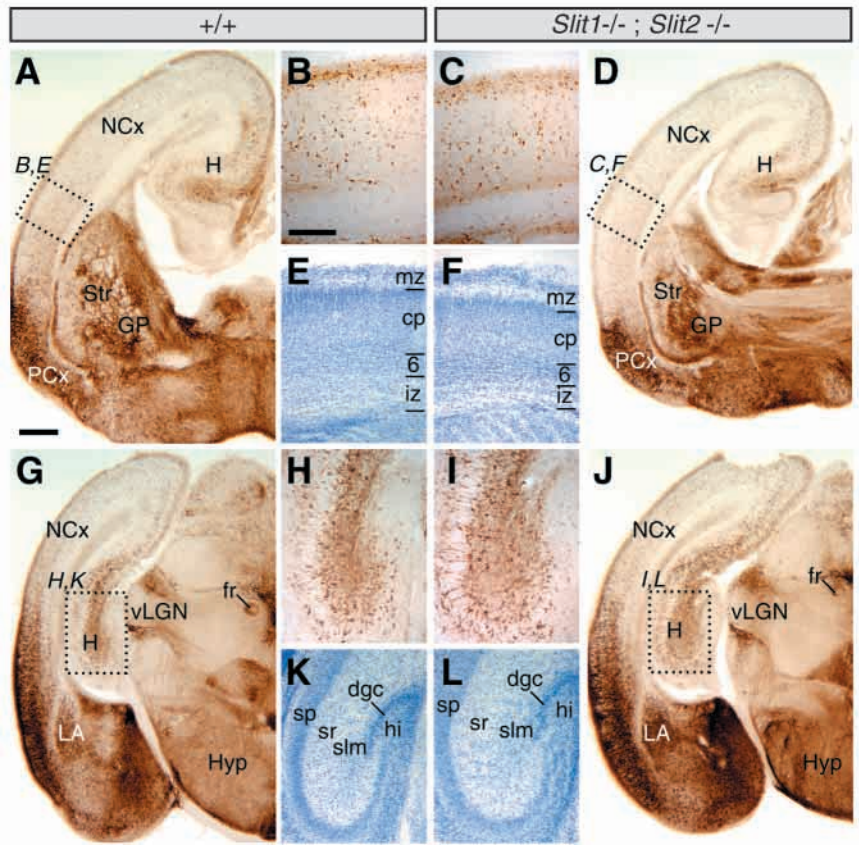

Fig. 6. Normal number of interneurons in the neocortex and hippocampus of *Slit1;Slit2* double mutants. Coronal sections through intermediate (A-F) and caudal (G-L) levels of the telencephalon of E18.5 wild-type (A,B,G,H) and *Slit1;Slit2* mutant (C,D,I,J) mice stained with anti-calbindin antibodies. (B,C) High magnification of the regions boxed in A and D, respectively. (E,F) Nissl staining of sections adjacent to B and C, respectively. (H,I) High magnification photographs of the regions boxed in G and J, respectively. (K,L) Nissl staining of sections adjacent to H and I, respectively. cp, cortical plate; dgc, dentate gyrus cell layer; fr, fasciculus retroflexus; GP, globus pallidus; H, hippocampus; hi, hilus; Hyp; hypothalamus; iz, intermediate zone of the cortex; LA, lateral nucleus of the amydgala; mz, marginal zone of the cortex; NCx, neocortex; PCx, piriform cortex; slm, stratum lacunosum moleculare; sp, stratum piramidale; sr, stratum radiatum; Str, striatum; vLGN, ventral lateral geniculate nucleus; 6, layer 6 of the cortex. Scale bars: 300 μm (A,D,G,J); 200 μm (B,C,E,F,H,I,K,L).

results were obtained when the distribution of GABA-, *GAD67*-, *Lhx6*- or *Dlx2*-expressing cells was analyzed (*n*=3; data not shown). In addition, calbindin immunohistochemistry also showed that there was not a severe defect in the generation of striatal neurons in *Slit1;Ntn1* mutants.

Since *Slit2* is also expressed in the preoptic region (Bagri et al., 2002) (and data not shown), we next generated *Slit1; Slit2;Ntn1* triple mutants. Analysis of the distribution of GABA interneurons in the cortex of *Slit1; Slit2;Ntn1* triple mutants, at E18.5, as revealed by in situ hybridization for *GAD67* and *Lhx6*, showed no significant differences compared to controls (Fig. 8H-K; *n*=3). Thus, interneurons migrate from the subpallium to the cortex and hippocampus, and integrate into cortical layers, in mice simultaneously lacking *Slit1*, *Slit2* and *Ntn1*.

Slit proteins control neuronal migration close to the midline in the basal telencephalon

Our previous analysis on the role of *Slit1* and *Slit2* in the guidance of forebrain axons in vivo suggested that, despite their broad expression within the telencephalon, Slit proteins appear to exert their primary function close to the midline (Bagri et al., 2002). To determine whether Slit proteins play a role in the guidance of any neuronal populations within the basal telencephalon, we analyzed the distribution of specific neuronal populations that are normally located close to the ventral midline of the telencephalon in mice deficient in *Slit2* or in both *Slit1* and *Slit2*. One of these neuronal populations is the basal magnocellular complex, which contains large cholinergic neurons that distribute in bilateral groups in the preoptic area (Fig. 9A,G,H). In *Slit2* and *Slit1;Slit2* mutants there is a periventricular ectopic collection of cholinergic neurons, with some of their processes crossing the midline (Fig. 9B,E and data not shown). Normally, cholinergic neurons and their processes are not present in these locations (Fig. 9A,C). These defects are more prominent in *Slit1;Slit2* double mutants than in *Slit2* mutant mice, suggesting that *Slit1* and *Slit2* have partially redundant contributions in controlling cell positioning close to the ventral midline. The collection of ectopic cholinergic neurons appear to be misplaced from the basal magnocellular complex, since the number of neurons remaining within this nucleus was reduced, in particular at caudal levels [Fig. 9G,J; *n*=3. Cells per 40,000 μm^2; control 170.17 (±17.46), *Slit1/2*$^{-/-}$ 69.5 (±5.63), *P*<0.001]. In contrast, the number of cholinergic neurons within the striatum was similar in control and *Slit1;Slit2* mutant mice [Fig. 9D,F; *n*=3. Cells per 40,000 μm^2; control 103 (±6.56), *Slit1/2*$^{-/-}$ 96 (±16.52), *P*=0.532].

Analysis of the distribution of other chemically defined neuronal populations within the basal telencephalon of *Slit2* and *Slit1;Slit2* mutants revealed similar defects. For example, neurons containing NPY were found in ectopic locations associated with the anterior commissure in the POa of *Slit1;Slit2* double mutants (data not shown). As in the case of the cholinergic neurons, the ectopic midline NPY neurons may be misrouted cells. In this case, they may correspond to cells that should have migrated to the dorsolateral striatum, since in *Slit1;Slit2* double mutants this structure contained fewer GABA/NPY interneurons than normal mice [*n*=3, cells per 40,000 μm^2; control 84.67 (±15.31), *Slit1/2*$^{-/-}$ 47.33 (6.81),

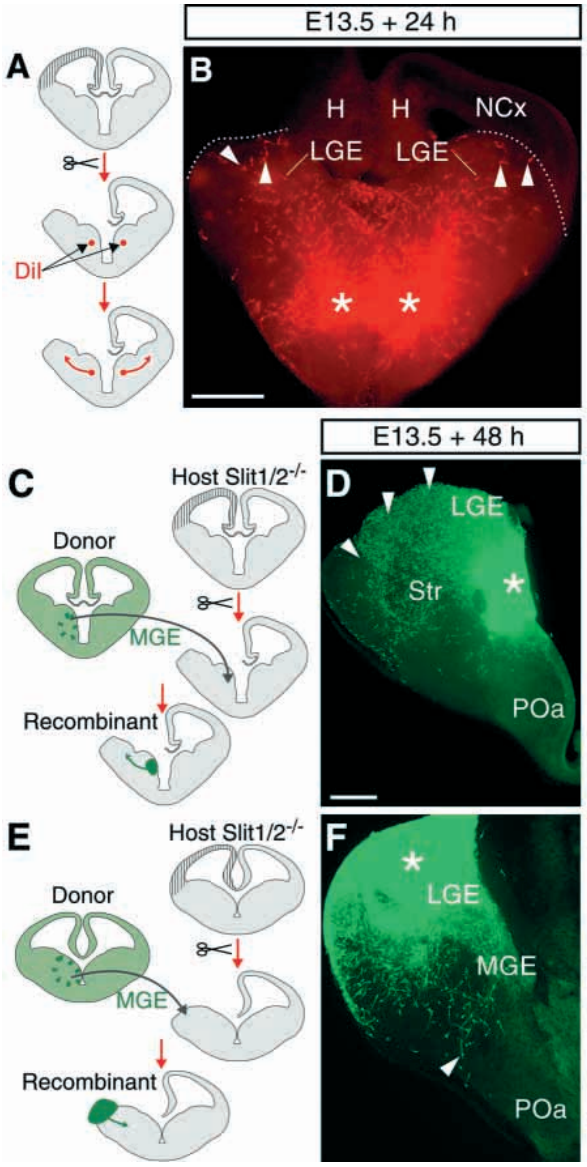

Fig. 7. The repulsive activity present in the basal telencephalon is maintained in *Slit1;Slit2* double mutants. (A) Schematic of the experimental paradigm used to analyze the migration of MGE-derived cells in the presence or absence of cortex. (B) Migration of DiI-labeled cells from the MGE (asterisks) after 24 hours in culture. Labeled cells (arrowheads) approach the pallial/subpallial boundary (dotted white line) at the same time in the side of the slice without cortex (left) as in the side with cortex (right). (C) Schematic of the slice transplantation paradigm used to analyze the migration of MGE^{GFP}-derived cells in the absence of cortex in slices obtained from *Slit1;Slit2* double mutants. (D) Migration of MGE^{GFP}-derived cells (asterisk) revealed after 48 hours in culture, labeled with GFP. Arrowheads point to cells in the pallial/subpallial boundary, dorsal to the striatum. (E) Schematic of the slice transplantation paradigm used to analyze the behavior of MGE^{GFP}-derived cells forced to migrate towards the preoptic area in slices obtained from *Slit1;Slit2* double mutants. (F) Migration of MGE^{GFP}-derived cells (asterisk) after 48 hours in culture, labeled with GFP. Note that very few cells migrate into the MGE mantle (arrowhead) and none into the preoptic area. H, hippocampus; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; NCx, neocortex; POa, preoptic area; Str, striatum. Scale bars: 300 μm.

$P<0.02$]. Thus, *Slit1* and *Slit2* control neuronal positioning within the basal telencephalon, in the vicinity of the ventral midline.

DISCUSSION

A substantial number of cortical GABAergic interneurons are born in the subpallial telencephalon and migrate tangentially to reach their final destination in the neocortex and hippocampus (reviewed by Corbin et al., 2001; Marín and Rubenstein, 2001). Because of their essential roles in cortical function (McBain and Fisahn, 2001), as well as the impact that abnormal neuronal migration has on human neurological conditions (Ross and Walsh, 2001), the mechanisms underlying the tangential migration of cortical interneurons are of considerable interest.

Little is know about the nature of the cues that provide directionality to the migration of interneurons from the basal telencephalon to the cortex. In vitro experiments have demonstrated that Slit proteins repel GABA neurons derived from the subpallium, leading to the suggestion that these molecules guide interneurons from the subpallium into the cortex (Zhu et al., 1995). In an attempt to clarify the source and nature of the cues that control this process, we have used slice culture experiments as well as analysis of mice carrying loss-of-function alleles for *Slit1*, *Slit2* and *Ntn1* to study interneuron migration to the cortex. Three main conclusions can be drawn from our experiments: (1) both attractive and repulsive activities direct interneuron migration to the cortex; (2) Slit1, Slit2 and Ntn1 are not required in vivo for interneuron migration; and (3) Slit proteins control neuronal positioning near the midline in the basal telencephalon.

Coordinate attractive and repulsive activities control interneuron migration to the cerebral cortex

Our experiments indicate that both attractive and repulsive cues exert considerable influence on the guidance of tangentially migrating cells from the subpallial telencephalon to the cortex. In slice culture experiments, we have shown that the most ventral region of the telencephalon is repulsive for tangentially migrating cells (Fig. 1), whereas the developing cortex is attractive for cells directed towards this region (Figs 2, 3). Early migration in the subpallium may be more dependent upon repulsion from the basal telencephalon, whereas extension through the pallium may rely more directly on cues present in the cerebral cortex. In line with this hypothesis, initial migration towards the cortex is largely independent of the presence of the cortex itself, since cells migrate dorsally in the absence of its target and they reach the pallial/subpallial boundary (the dorsal border between the striatum and the cortex) roughly at the same time in the presence or absence of the cortex (Fig. 1).

It has been previously suggested that migration of interneurons from the LGE to the cortex is mediated by a repulsive activity present in the ventricular zone of the subpallium (Zhu et al., 1999). Zhu et al. hypothesized that a gradient of repulsive activity, with the strongest repulsion at the medial side of the striatal primordium, drives GABAergic interneurons to migrate laterally into the neocortex. However, since most cortical interneurons appear to derive from

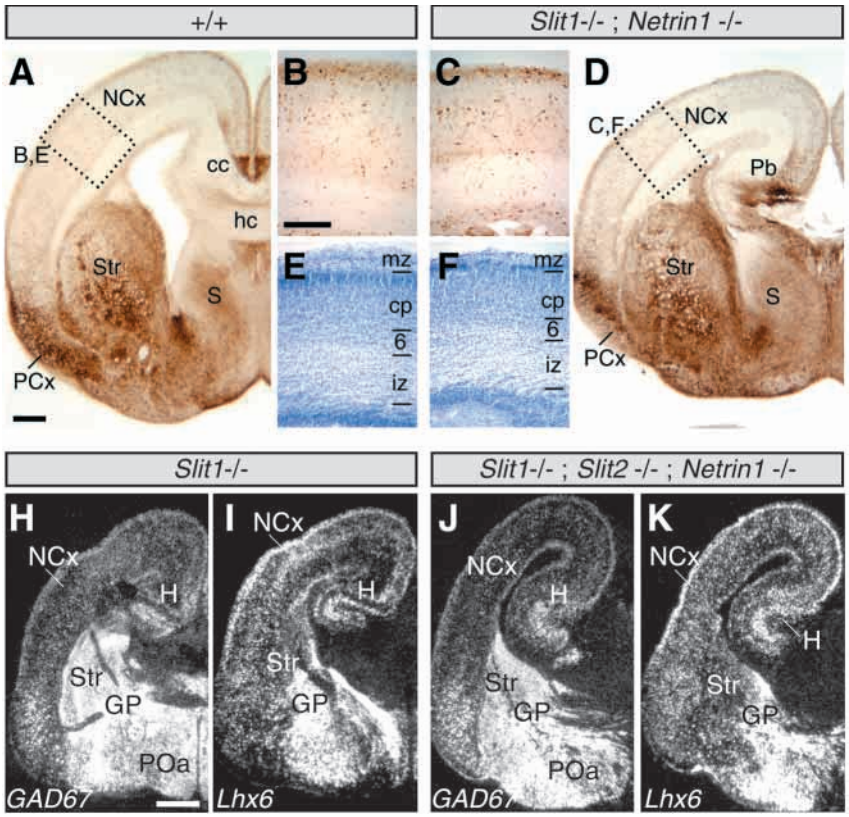

Fig. 8. Interneurons migrate to the cortex in *Slit1;Ntn1* and *Slit1;Slit2;Ntn1* mutants. Coronal sections through the telencephalon of E18.5 fetuses showing calbindin immunohistochemistry in wild-type (A,B) and *Slit1;Ntn1* (C,D) mutant mice. (B,C) High magnification photographs of the regions boxed in A and D, respectively. (E,F) Nissl staining of from sections adjacent to B and C, respectively. (H-K) Coronal sections through the telencephalon of E18.5 embryos showing expression of *GAD67* (H,J) and *Lhx6* (I,K) in *Slit1* (H,I) and *Slit1;Slit2;Ntn1* (J,K) mutant mice. cc, corpus callosum; cp, cortical plate; GP, globus pallidus; H, hippocampus; hc, hippocampal commissure; iz, intermediate zone of the cortex; mz, marginal zone of the cortex; NCx, neocortex; PB, Prost bundle; PCx, piriform cortex; POa, preoptic area; S, septum; Str, striatum; Scale bars: 300 μm (A and D); 200 μm (B, C, E, and F); 500 μm (H-K).

subpallial regions ventral to the LGE, such as the MGE (Anderson et al., 2001; Lavdas et al., 1999; Sussel et al., 1999; Wichterle et al., 1999; Wichterle et al., 2001), with the LGE giving rise primarily to neurons that remain in the striatum (Hamasaki et al., 2001; Wichterle et al., 2001), it seems more reasonable that the repulsive activity for cells tangentially migrating to the cortex should be located ventral to the MGE. In agreement with this notion, our experiments suggest that tangentially migrating cells derived from the MGE are repelled by an activity present in the mantle of the most ventral region of the basal telencephalon (Figs 1, 2, 6). This activity appears to inhibit the motility of tangentially migrating cells, directing

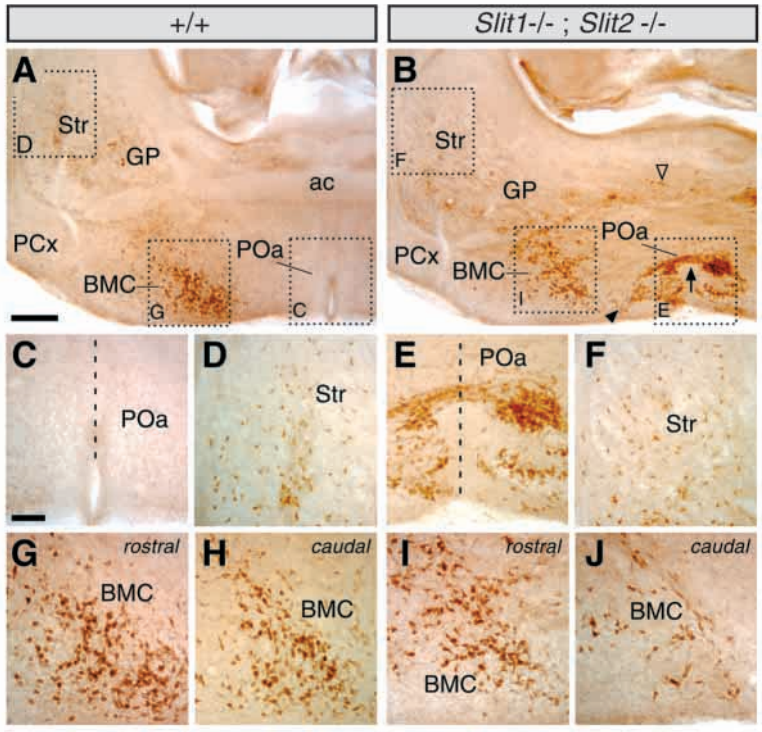

Fig. 9. Cholinergic neurons from the basal magnocellular complex are displaced in the midline of *Slit1;Slit2* double mutants. Coronal sections through the telencephalon of newborn fetuses showing choline acetyltransferase (ChAT) immunohistochemistry in wild-type (A,C,D,G,H) and *Slit1;Slit2* mutant (B,E,F,I,J) mice. (A,B) Distribution of cholinergic cells at the level of the anterior commissure in wild-type (A) and *Slit1;Slit2* mutant (B) mice. In the preoptic area, note the collection of ectopic cholinergic neurons close to the midline, where some cells and fibers cross the midline in *Slit1;Slit2* mutant mice (arrow in B). Ectopic cells appear to be continuous with other cholinergic neurons in the basal magnocellular complex (arrowhead). Additional ectopic cells are present in the anterior commissure (open arrowhead). (C,D,E,F,G,I) High magnification the boxed areas in A and B. (H,J) High magnification photographs of the basal magnocellular complex at caudal levels in wild-type (H) and *Slit1;Slit2* mutants (J). Dashed lines in C and E indicate the midline. ac, anterior commissure; BMC, basal magnocellular complex; GP, globus pallidus; PCx, piriform cortex; POa, preoptic area; Str, striatum; Scale bars: 300 μm (A,B); 100 μm (C-J).

their migration towards the cortex. However, repulsion from the ventricular zone might be necessary to facilitate radial migration of cells away from the progenitor zones of the subpallium towards the developing basal ganglia, as suggested for the striatum (Hamasaki et al., 2001).

Our experiments also suggest that the cortex influences tangential migration from the basal telencephalon (Figs 2-4). Since migrating cells are able to reach the pallial/subpallial boundary in the absence of the cortex (Fig. 7B), the activity present in the cortex may primarily function to facilitate the lateral to medial dispersion of tangentially migrating cells. A good candidate molecule for this attractive activity is HGF, which acts as a motogen (i.e. a factor that stimulates migration) for tangentially migrating cells (Powell et al., 2001). It is still unknown whether HGF can also function as a chemoattractive molecule for cortical interneurons, but the fact that it can function as a chemoattractant for developing motor axons (Ebens et al., 1996) is at least consistent with this possibility. Ntn1, a guidance molecule that has been shown to be attractive for migrating cells in other systems (Alcántara et al., 2000; Bloch-Gallego et al., 1999; Yee et al., 1999), is expressed at low levels in the developing hippocampus, but is completely absent from the rest of the cortex (Livesey and Hunt, 1997; Serafini et al., 1994). However, both *Ntn1* and *DCC* mutants seem to have normal numbers of cortical interneurons (Anderson et al., 1999). Other molecules that are known to be expressed in the developing cortex have been reported to be attractive for migrating cells in a variety of different systems. These factors include chemokines, EGF, FGF and TGFβ-related molecules (Branda and Stern, 2000; Caric et al., 2001; Lehmann, 2001; Zou et al., 1998), but their potential role in controlling the tangential migration of interneurons remains to be investigated.

Our slice experiments suggest that the cortical attractive activity is present in a gradient with the strongest attraction in the medial cortex (Fig. 3). A gradient of increased attraction from lateral to medial regions of the cortex would direct migration towards medial regions of the cortex, suggesting that the cortex fills up with incoming interneurons from medial to lateral regions. This hypothesis is consistent with the observation that interneurons in the hippocampus are generally born earlier than interneurons in the neocortex (Soriano et al., 1989a; Soriano et al., 1989b), although additional experiments would be required to confirm this observation. Despite the presence of an attractive activity controlling the direction of migration within the cortex, an additional mechanism seems necessary to ensure balanced distribution of interneurons throughout the cortex, e.g. to prevent all interneurons from accumulating in the hippocampus. The nature of such a mechanism remains to be elucidated.

Roles of Slit1, Slit2 and Ntn1 in cell migration in the telencephalon

In this study, we have focused on the initial characterization of cues responsible for the repulsive activity present in the basal telencephalon. Candidate molecules for this activity included Slit1 and Slit2, which are expressed in the subpallium during the time of interneuron migration and in vitro can repel GABAergic cells derived from the ganglionic eminences (Zhu et al., 1999). Our analysis of *Slit1;Slit2* double mutants suggests, however, that these proteins are not necessary for tangential migration from the basal telencephalon to the cortex (Figs 5, 6). Moreover, despite the fact that Ntn1 can also repel subpallial GABAergic cells (Hamasaki et al., 2001), analysis of *Slit1;Slit2;Ntn1* triple mutants demonstrates that a cooperative action of these three proteins is not required for the subpallial-to-pallial interneuron migrations (Fig. 8). Thus, it is likely that other factor(s) provide the repulsive information.

How can one reconcile the apparently contradictory results obtained in vitro [Slit repulsion of GABAergic cells derived from the subpallium (Zhu et al., 1999)] and in vivo (lack of migration defects in the absence of Slit1 and Slit2; present study)? One possibility would be that other molecules cooperate with Slits in repelling interneurons towards the cortex. Our slice experiments, however, suggest that the repulsive activity found in the basal telencephalon is not altered in *Slit1;Slit2* mutants, suggesting that the contribution of Slits to this activity is not significant (Fig. 7). A second possibility is that the results obtained in vitro do not reflect the effect of Slit proteins on cells tangentially migrating to the cortex, but rather on neurons that normally remain within the basal ganglia. Indeed, our experiments show that Slit proteins are required for the migration of subsets of subcortical GABAergic (NPY) and cholinergic neurons. This interpretation would suggest that the heterogeneity of cell populations present in migration assays should be taken into account in future in vitro experiments. Finally, it is also possible that tangentially migrating cells indeed respond to Slits, but they only normally do so once the cells arrive in the cortex. For example, *Slit1*, *Slit2* and *Slit3* are expressed in very restricted laminar patterns in the early postnatal cortex (Marillat et al., 2001), suggesting that Slits may play a role in controlling the layer destination of GABAergic interneurons, a possibility that we have not yet explored.

Previous experiments have suggested a role for Slits and Ntn1 in neuronal migration in the striatum. Projection neurons in the striatum are largely derived from the LGE (Wichterle et al., 2001), with early-born cells destined for the patch compartment and later-generated cells directed towards the matrix (van der Kooy and Fishell, 1987). Interestingly, *Slit1* and *Ntn1* are co-expressed in the ventricular zone of the LGE, and in vitro experiments have shown that both molecules influence the migration of cells derived from the LGE (Hamasaki et al., 2001; Zhu et al., 1999). In particular, Slit1 and Ntn1 mimic the repulsive activity present in the ventricular zone of the LGE, leading to the suggestion that these molecules may play a crucial role in the outward migration of postmitotic cells away from the ventricular zone towards the developing striatum (Hamasaki et al., 2001). Nevertheless, our analysis of *Slit1;Ntn1* double mutants shows that a striatum of roughly normal appearance forms in the absence of these cues (Fig. 8). This does not exclude the possibility of more subtle defects in the development of the striatum and other subpallial structures that could be revealed through a more detailed analysis of *Slit1;Ntn1* mutants.

Despite the lack of evidence supporting a role in vivo for Slit1 and Slit2 in the tangential migration of cells from the basal telencephalon to the cortex, analysis of *Slit* mutants demonstrates that these proteins play a significant role in controlling cell positioning in the mammalian telencephalon. The distribution of specific neuronal populations, such as the cholinergic basal magnocellular complex, is affected in mice

lacking *Slit2*, and even more so in mice lacking both *Slit1* and *Slit2* (Fig. 9). Thus, loss of Slit function appears to impair the ability of some neurons to migrate away from their progenitor zone particularly in the region of the ventral telencephalic midline. Alternatively, since several axonal pathways are disrupted in the telencephalon of *Slit2* and *Slit1/2* mutants (Bagri et al., 2002), it is conceivable that the defects in the position of cholinergic and NPY neurons could be secondary to alterations of the ventral midline caused by the accumulation of ectopic fibers. This possibility seems less likely, however, because the cholinergic neurons of the basal forebrain are born at least 2 days before the arrival of cortical and thalamic axons to the basal telencephalon (Brady et al., 1989). Thus, in the telencephalon, Slits appear to be required to regulate the guidance of neurons at the midline, a function that parallels previous observations in *Drosophila*, where Slit was shown to be required for the migration of muscle precursors away from the midline (Kidd et al., 1999); so, the role of Slit proteins in controlling cell migration appears to be highly conserved throughout evolution.

A model for the directional guidance of cortical interneurons

As it has been demonstrated for growing axons (Zou et al., 2000), long-range neuronal migrations, such as the migration of cells from the subpallium to the cerebral cortex, appear to be controlled through a number of carefully choreographed guidance commands (Marín and Rubenstein, 2003). In this case, for example, interneurons migrating from the MGE are first directed dorsally through repulsion by an unknown activity. Cortical interneurons are then instructed to avoid the developing striatum through repulsion involving semaphorin/neuropilin interactions (Marín et al., 2001). In addition, interneurons are drawn towards the cortex by a graded attractive activity that may also facilitate their dispersion through all cortical territories. Finally, additional cues may be necessary to direct other aspects of interneuron patterning in the cortex, such as lamina-specific positioning. Considering the impact that defects in radial migration of cortical projection neurons have in the etiology of multiple human neurological conditions (Ross and Walsh, 2001), it is reasonable to hypothesize that disruption of the tangential migration of interneurons may also underlie some human neurological disorders. Identification of the attractive and repulsive factors that direct this migration will help test this hypothesis.

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