Control of cortical interneuron migration by neurotrophins and PI3-kinase signaling

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SUMMARY

During telencephalic development, cells from the medial ganglionic eminence (MGE) are thought to migrate to the neocortex to give rise to a majority of cortical GABAergic interneurons. By combining time-lapse video-microscopy, immunofluorescence and pharmacological perturbations in a new in vitro migration assay, we find that MGE-derived cells migrate through the entire extent of the cortex and into the CA fields of the hippocampus, but avoid the dentate gyrus. Migrating neurons initially travel within the marginal zone and intermediate zone, and can enter the cortical plate from either location. Tangential migration is strongly stimulated by BDNF and NT4 and attenuated by the Trk-family inhibitor, K252a, suggesting that migration is regulated by TrkB signaling. Furthermore, TrkB-null mice show a significant decrease in the number of calbindin-positive neurons migrating tangentially in the embryonic cortex. BDNF and NT4 cause rapid activation of PI3-kinase in MGE cells, and inhibition of PI3-kinase (but not of MAP kinase or PLCγ) dramatically attenuates tangential migration. These observations suggest that TrkB signaling, via PI3-kinase activation, plays an important role in controlling interneuron migration in the developing cerebral cortex.

Key words: Neurotrophins, Mouse, CNS/migration, Interneuron

INTRODUCTION

While the importance of cell migration in cortical development has long been known, the cellular and molecular basis of cortical cell migration is only now beginning to be understood. Until recently, the dominant view was that most cortical neurons left the ventricular zone after their last mitosis and migrated along radial glial fibers to occupy a specific laminar position within the cortical plate. While this form of migration certainly takes place in the developing cortex, recent observations suggest that radial migration may not provide an adequate description of the cellular events that underlie cortical development. The first evidence to suggest the need for a revised model came from observations of tangential dispersion of precursors or post-mitotic neurons in the developing cortex (de Carlos et al., 1996; Fishell et al., 1993; O'Rourke et al., 1997; O'Rourke et al., 1992; O'Rourke et al., 1995; Parnavelas et al., 1991; Tan and Breen, 1993; Tan et al., 1995). The widespread distribution of clonally related cells also suggested the possibility of nonradial migration in the cortex (Walsh and Cepko, 1992). The source and destination of these putative tangentially migrating cells, however, remained a mystery until experiments by Anderson et al. suggested that neurons migrated from the ganglionic eminence (GE) to the cortex and gave rise preferentially to GABAergic interneurons (Anderson et al., 1997). This conclusion was based mainly upon the

observation that there are virtually no neocortical GABAergic neurons in *Dlx1/Dlx2* double knockout mice, two homeobox genes that are expressed in the ventricular and subventricular zones of the GE (Anderson et al., 1997). It has been suggested that the majority of radially migrating neurons generated in the dorsal part of the telencephalon give rise to excitatory pyramidal neurons, whereas neurons migrating tangentially from the ventral to the dorsal part of the telencephalon give rise to GABAergic interneurons (Parnavelas et al., 1991; Parnavelas, 2000).

Although tangential migration has been extensively studied in the past few years, little is known about the dynamics and molecular control of this process. Most of the experiments performed so far to study the migration of GE-derived neurons have studied the role of transcription factors in the specification of the distinct phenotypes of cortical and striatal interneurons, and have relied on methods such as DiI injection (Anderson et al., 1997; Anderson et al., 2001; Lavdas et al., 1999; Tamamaki et al., 1997) and adenovirus- or electroporation-mediated GFP expression (Chapouton et al., 1999; Marin et al., 2001). As only a small proportion of tangentially migrating cells are labeled by these methods, we wanted to design a biological assay whereby large numbers of tangentially migrating neurons could be visualized, allowing quantitative analysis of the dynamics of tangential migration. The sampling limitation in the analysis of migrating cells has hampered molecular

investigation of tangential migration, although it is noteworthy that both neurotransmitters released by corticofugal axons as well as the axonal guidance cues Slit, Sema3a and Sema3f have recently been implicated in regulating the migration of cells from GE to the neocortex (Métin et al., 2000; Zhu et al., 1999; Marin et al., 2001).

To characterize the migration of neurons from the GE to the neocortex, we have developed a co-culture assay in which explants of the GE from GFP-expressing mouse embryos are cultured adjacent to cortical slices from wild-type mice. At various times after culture, the migration of GE cells into the neocortex was analyzed by examining GFP-positive neurons within the cortical slice. This assay allows the dynamics of migrating cells to be examined by fluorescence video microscopy, and also allows the morphology and fate of the migrating cells to be assessed by immunofluorescence. In addition, the accessibility of the culture system allows perturbation experiments that permit investigation of the molecular control of cell migration. Using this assay, we have been able to obtain detailed information about the extent and dynamics of tangentially migrating neurons, and have discovered that TrkB and PI3-kinase signaling play an important role in the control of the tangential migration of interneurons from the GE to the cortex.

MATERIALS AND METHODS

Isochronic and heterochronic co-cultures

Embryonic day 14 (E14) to E16 mouse embryos were isolated from timed-mated pregnant wild-type female (E1=midnight after the day after the plug is detected), bred with heterozygous transgenic males expressing enhanced green fluorescent protein (GFP) under the control of a β-actin promoter and a CMV enhancer (Okabe et al., 1997). The resulting litters contained on average 50% wild-type and 50% GFP+ heterozygous embryos. Embryos were quickly dissected in ice-cold HBSS (Life Technologies), embedded in 3% low melting point agarose (Sigma) diluted in HBSS and sectioned on a vibratome at 250 μm in the coronal plane. The neocortex or neocortex plus hippocampal formation was trimmed from sections from wild-type mice and placed adjacent to GE explants from GFP-expressing mice on porous PETC membranes (Falcon 1 μm pore size). The co-cultures were maintained in an air-interface culture system as described previously (Polleux et al., 2000). For heterochromic co-cultures, E14-E16 GE explants from GFP-expressing mice were cultured with rat P2-4 cortical slices isolated and sectioned as described above. Isochronic and heterochronic GE-GFP co-cultures showed similar patterns and dynamics of cell migration.

Time-lapse video-microscopy

The migration of GE-GFP cells was monitored using an inverted Zeiss (Axiovert 135) microscope equipped with a heated stage and a 6% CO_2 chamber. Images were obtained with a silicon-intensified target (SIT – 2400-08) video-camera (Hamamatsu, Japan) and recorded onto the hard drive of PC computer before being analyzed using a digital image processor (METAMORPH 3.51, Universal Imaging, West Chester, PA) and stored onto CD-ROM. Time-lapse imaging of migrating cells was performed on co-cultures on Millipore inserts (Millicell-CM; 0.4 μm pore size) mounted on 60 mm glass-bottom dishes (MatTek Corporation). Multiple locations on several co-cultures were imaged during any one session by piloting a three-axis motorized stage (MultiControl 2000, Marhauzer). The effects of neurotrophins (BDNF, NT3 and NT4; obtained from Amgen) on

migration was tested directly by pipetting 20 μl (diluted at 20 ng/ml in slice culture medium-control vehicle) onto the explants.

The speed of each migrating cell was computed with the METAMORPH software, using the 'track-point' function to generate series of points representing the position of each cell body at 15 minute intervals. The average rate of migration of each cell was calculated using Excel 98 software, and the differences of the mean velocities were analyzed for statistical significance using one-way ANOVA using StatView 5 software.

Immunofluorescence

After 4% paraformaldehyde fixation overnight, double immunofluorescence labeling was performed as described earlier (Polleux et al., 2000). The following antibodies were used. Mouse monoclonals: GFP (1:1000; Molecular Probes), MAP2 (1:2000; Sigma), TuJ1 (1:1000; Sigma), NeuN (1:600; Sigma), BrdU (1:400; Sigma), Nestin (rat 401; 1:20; Developmental Hybridoma Bank) and neurofilament 165 kDa (NF165 kDa; 1:20; Developmental Hybridoma Bank). Rabbit polyclonals: GFP (1:3000; Molecular Probes), GABA (1:500; Chemicon) and TrkB (1:600; Oncogene). Primary antibodies were visualized using Cy3- (red) or Cy2- (green) conjugated secondary antibodies (goat anti-mouse or goat anti-rabbit; 1:600; Jackson ImmunoResearch). Immunofluorescent labeling was observed using a Leica TSC-SP confocal microscope mounted on a Leica DMR microscope. For the detection of NT4 by immunofluorescence, we used a chick anti-human NT4 polyclonal IgY antibody (1:400; Promega, Madison WI). We then used an affinity-purified biotin-conjugated anti-chicken IgY secondary antibody (1:600, Promega, Madison WI) followed by incubation in Cy3-conjugated streptavidin (1:1000; CyDye-Amersham Pharmacia Biotech).

Pharmacology

We used LY 294002 [2-(4-Morpholinyl)-8phenyl-4H-1-benzopyran-4-one; Biomol] at a final concentration of 50 μM to inhibit PI3-kinase activity (Vlahos et al., 1994); K252a (Calbiochem) at a final concentration of 50 nM to inhibit Trk receptor autophosphorylation (Berg et al., 1992; Nye et al., 1992; Tapley et al., 1992); U73122 ([1-(6-((17b)-3-methoxyestra-1,3,5(10)-trien-17-yl)amino-hexyl)-1H-pyrrole-2,5-dione]; Calbiochem) at a final concentration of 1 μM to inhibit phospholipase C (Smith et al., 1996); and U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene; Calbiochem) at a final concentration of 10 μM to inhibit MAP kinase kinase (MEK1/2) (Favata et al., 1998). In each case, the final concentration represented 1:1000 dilution of a stock solution in DMSO. Control cultures were treated with DMSO at a 1:1000 dilution.

Analysis of TrkB mutant mice

Wild-type mice and mice with a targeted deletion of the TrkB gene (Ntrk2 – Mouse Genome Informatics) (Klein et al., 1993) were individually genotyped by PCR performed on proteinase K-digested tissue. Embryos were fixed at E15 and their brains were immersed in fresh paraformaldehyde 4% overnight, then removed from the skull, postfixed overnight and cryoprotected in 30% sucrose in phosphate buffer for 2 days before sectioning. Serial 46 μm frozen sections were obtained in the coronal plane. Polyclonal antibodies raised against calretinin (1:10,000, Swant, Switzerland) and calbindin 28K (1:20,000, Swant, Switzerland) were used on free-floating sections, as previously described (Lebrand et al., 1996). Briefly, sections were incubated overnight with the primary antibody diluted in PBS+ (0.1 M PBS with 0.2% gelatin and 0.25% Triton X-100). Then, sections were washed in PBS+ and incubated with secondary antibodies (biotinylated goat anti- rabbit; 1:200; DAKO, Denmark) for 2 hours at room temperature. Sections were washed in PBS+ and incubated with a streptavidin-biotin-peroxidase complex (1:200; Amersham, Arlington Heights, IL) for 2 hours at room temperature. Sections were then reacted with a solution containing 0.02% diaminobenzidine and

0.003% H_2O_2 in PBS, pH 7.6. All sections were mounted on TESPA-coated slides, air-dried overnight, dehydrated and coverslipped in DePeX.

Phospho-AKT western blots

E14 GE explants were cultured for 2 days in vitro as described above. They were then pretreated for 30 minutes with 50 µM LY294002 (Biomol) or vehicle (DMSO, 1:1000) followed by a 30 minutes stimulation with 50 ng/ml BDNF or NT4. Slices were lysed in Tris-buffered saline containing, 1% nonidet P-40, 10% glycerol, 2 mM EGTA, 1 mM sodium vanadate, 1 mM PMSF, 2 µg/ml pepstatin, 5 µg/ml aprotinin, 10 g/ml leupeptin, cleared by centrifugation at 13,000 *g* at 4°C for 10 minutes, and loaded on a 10% polyacrylamide gel for electrophoretic separation. Phosphorylation of Akt was detected by western blotting using a rabbit polyclonal phospho-Akt (Ser473) antibody (Cell Signaling Technology). Membranes were stripped and reprobed with rabbit polyclonal Akt antibody (Cell Signaling Technology) to control for loading.

RESULTS

Characterization of tangential migration of GE cells into the cortex

Our initial experiments were directed at characterizing the trajectory and target specificity of migrating GFP-expressing GE cells (GE-GFP cells) using the GE-cortex co-culture assay (Fig. 1A,B). When E14-E16 GE explants were placed next to isochronic cortical slices, there was a massive influx of GE-GFP cells into the cortical slice within 24 hours. Tangential migration was restricted principally to two zones of the cerebral wall: the intermediate zone (IZ) and the marginal zone (MZ) (Fig. 1C,D). Between 2 and 3 days in vitro (DIV), large numbers of GE-GFP cells could be seen invading the cortical plate (CP) both from the IZ and the MZ (Fig. 1E). The migrating cells had characteristic and striking morphologies. Virtually all of these cells had an oblong cell body and one major leading process that was tipped with a growth cone (Fig. 1F,G). The leading process had a gradually tapering morphology and was typically 10-15 cell body diameters in length (100-150 µm) (Fig. 1F,G; see Figs 3, 4). In contrast to axons, which follow stereotypical trajectories to their targets (and often are tightly fasciculated with each other), the orientation of leading processes of migrating neurons was highly variable, suggesting that once within the cerebral wall, the GE-GFP cells often change their direction of local migration (Fig. 1E).

GE-GFP cells continued to migrate to the cortex in large numbers during the first few days in culture. To determine the extent of this corticofugal migration, we analyzed the distribution of GE-GFP cells in co-cultures of GE explants with telencephalic slices that contained the entire lateral and dorsal extent of the neocortex as well as the hippocampal formation (Fig. 1A,B). In these co-cultures, GE-GFP cells routinely migrated all the way into the hippocampus, suggesting that at least some hippocampal neurons originate in the GE (Fig. 1B,H-N). Interestingly, there were spatial restrictions to migration within the hippocampus. Although GE-GFP cells readily populated the presumptive CA1-CA3 subfields (pCA, Fig. 1H), there was a clear and consistent lack of GE-GFP neurons within the dentate gyrus (DG, Fig. 1H). The GE-GFP cells avoided the dentate gyrus even when the GE explants

were placed immediately adjacent to a hippocampal slice where GE-GFP cells would have easy access to the dentate gyrus (Fig. 1I-N). It was also striking that GE-GFP cells migrated right up to the border between CA3 and DG without invading the DG proper (Fig. 1H), suggesting that the inhibitory effect of DG on GE-GFP cells was likely to be due to a cell surface-associated inhibitory signal, rather than a diffusible signal.

To determine the specificity of cell migration out of the GE, we carried out co-culture experiments in which GE explants were cultured next to the dorsal thalamus, another forebrain structure. As shown in Fig. 1O, GE-GFP cells showed no migration into thalamic slices, indicating that GE cells migrate only into specific target structures such as the cortex and hippocampus. We also examined the ability of neurons from regions other than the GE to migrate into the cortex. For these experiments, explants of various brain regions from GFP-expressing mice were cultured adjacent to neocortical slices. In contrast to the GE explants, which were a rich source of migratory cells, cells from other regions showed little or no migration. For example, thalamic explants from GFP-expressing mice readily extended axons into cortical explants, but thalamic GFP neurons never migrated into the neocortex (data not shown). These experiments indicate that the migration of GE-GFP cells into cortex and hippocampus is both a consequence of the migratory potential of GE neurons and the substrate properties of the target tissues.

Origin of tangentially migrating cells

While the GE-cortex co-culture experiments showed that GE cells migrate in large numbers into the cortex, they did not reveal the contribution of the lateral ganglionic eminence (LGE) and the medial ganglionic eminence (MGE) to the tangentially migrating population. To determine if cells from the LGE or MGE migrated equally well to the cortex, we cultured LGE or MGE explants from GFP-expressing mice next to unlabeled cortical slices. As shown in Fig. 2A-B, GFP-expressing cells from both the LGE and MGE were effective in migrating into the cortical slice, but the number of cells migrating out of the MGE was consistently two to three times greater than that migrating out of the LGE, indicating that most of the GE cells that migrate into the cortex at E14-E16 come from the MGE.

It is formally possible that the cells we see migrating from the MGE or LGE to the cortex are generated in some other structure and simply pass through these structures en route to the cortex. To determine if the cells from the MGE and LGE that migrate into the cortex undergo their final mitosis in those structures, we labeled E14 explants of the LGE or MGE from GFP-expressing mice with BrdU for 8 hours before washing off the BrdU and positioning cortical slices next to the explants. After 3 days in culture, we scored the number of BrdU+/GFP+ cells in the cortex, which represents the population of cells that underwent their final mitotic division during the labeling period and then migrated to the cortex. While only about 3% of the LGE-GFP cells in the cortex were double labeled for BrdU, about 15% of the MGE-GFP cells were double labeled (Fig. 2C-H). Thus, at E14-E16, the majority of tangentially migrating GE cells originate in the MGE.

To examine whether MGE cells migrating to the cortex continue to proliferate while migrating, we labeled MGE-
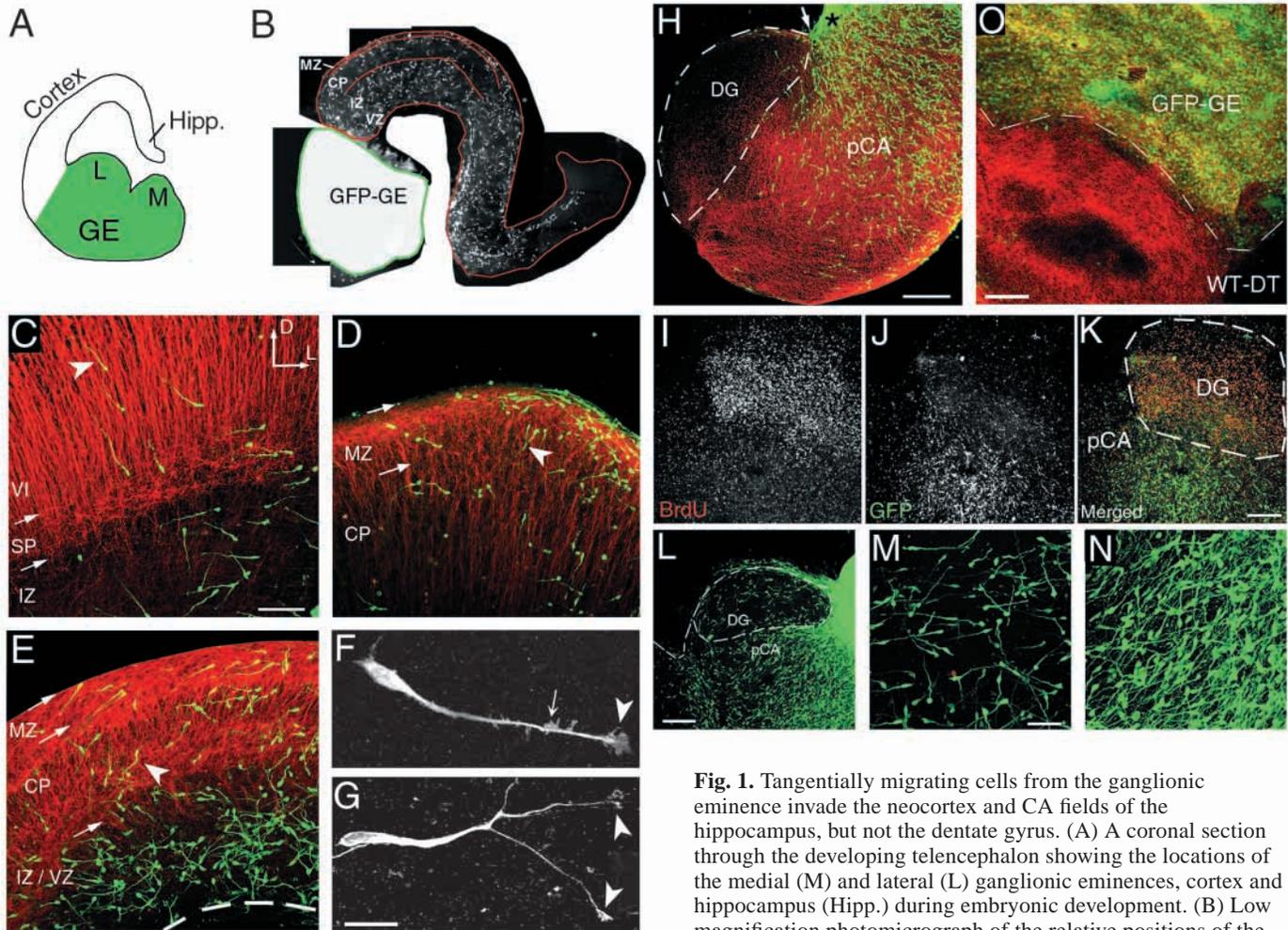
Fig. 1. Tangentially migrating cells from the ganglionic eminence invade the neocortex and CA fields of the hippocampus, but not the dentate gyrus. (A) A coronal section through the developing telencephalon showing the locations of the medial (M) and lateral (L) ganglionic eminences, cortex and hippocampus (Hipp.) during embryonic development. (B) Low magnification photomicrograph of the relative positions of the GE from GFP-expressing mice (GE-GFP), cortex and hippocampus in a co-culture assay. The white label in the cortex indicates the location of GE-GFP cells that have migrated from the GE to the cortex. Note the extensive invasion of the cortex and hippocampus by the tangentially migrating cells. (C,D) Analysis of the spatial distribution of E15 GE-GFP cells that have migrated into the cortex after 20 hours in vitro indicates that GFP cells are found mainly in the intermediate zone (IZ) and the marginal zone (MZ) of co-cultured cortical slices. In these zones, cells are mainly oriented tangentially. Note that some GFP cells have already invaded the cortical plate (VI, CP). Cells that enter the CP from the IZ typically have leading processes directed towards the pial surface (arrowhead, C), while those entering the CP from the MZ have leading processes directed away from the pial surface (arrowhead, D), indicating that cell bodies follow the leading process as the cells migrate into the CP. The dorsal (D) and lateral (L) aspects of the cortical slice are indicated in C. In (C,D), the GE explants are located to the right of the cortical explant shown. (E) By 36 hours in vitro, large numbers of E16 GE-GFP cells have migrated into the cortex, most of which travel through the IZ. Progressively more cells are found in the CP but they no longer show a clear radial orientation in the CP, suggesting that cells alter their trajectories of migration after entering the CP. The broken line in E indicates the edge of the slice on the ventricular side. In C-E, the red channel correspond to MAP2 immunofluorescence. (F,G) Examples of the morphology of tangentially migrating cells. The cells typically have a leading process that is 10-15 times the cell body diameter. The leading process is always tipped by a prominent growth cone (arrowheads) and often contains multiple filopodia (arrow in F). Many of the migrating cells also have branched leading processes (G), which may participate in the mechanics of altering trajectories of migration (see also Fig. 5). (H) In GE-cortex co-cultures, E16 GE-GFP cells migrate tangentially up to the most medial aspect of the telencephalon by 3 days in vitro where they accumulate in the developing hippocampus but avoid the developing dentate gyrus (DG). Cells in the marginal zone (star) of the putative CA regions (pCA) stop migrating sharply at the interface between the pCA and DG (arrow) identified as being TuJ1 negative (red channel). (I-K) The region of the developing hippocampus avoided by E16 GE-GFP cells (green channel, I) is a highly proliferative zone (delineated by the broken line in K; red channel, BrdU), which is typical of the DG anlage. (L-N) The avoidance of the developing DG by GE-GFP cells can be observed even when an explant of E16 MGE-GFP (to the right in L) is placed directly adjacent to the DG for 3 days in vitro. Higher magnification images show a marked difference in GFP cell density in DG (M) and pCA (N) regions located 250 μm away from the interface with the MGE explant. (O) GE-GFP cells do not migrate into wild-type dorsal thalamus (WT-DT) slices in a co-culture assay, indicating that GE cells are selective about their target zones of migration. All panels in this and other figures are from isochronic co-cultures, unless otherwise indicated. CP: cortical plate; SP: subplate; IZ: intermediate zone; VZ: ventricular zone; DG: developing dentate gyrus; pCA: putative CA regions; VI: cortical layer VI. Scale bars: 150 μm in C-E; 30 μm in F,G; 250 μm in H; 350 μm in I-K; 300 μm in L; 75 μm in M,N; 200 μm in O.

Fig. 2. The majority of cells migrating tangentially into the cortex are generated in the MGE. (A,B) Comparison of the migration of GFP-positive cells from LGE-GFP (A) and MGE-GFP (B) explants into co-cultured P2 cortical slices at 3 days in vitro. Note that many more GFP-positive cells migrate into the cortex from the MGE (B) compared with the LGE (A). (C-H) E14 MGE explants (C-E) or E14 LGE explants (F-H) were exposed to BrdU in vitro for 8 hours before being co-cultured with P2 cortex for 3 days without BrdU. GFP-positive cells migrating from the MGE were five times more frequently double labeled for BrdU than were GFP-positive cells migrating from the LGE (red arrowheads in C-H; see text for quantification), indicating that the majority of GE-derived cells that migrate into the cortex originate in the MGE. (I) MGE-derived cells do not proliferate after migrating to the cortex. E14 GFP-expressing MGE explants were co-cultured with P2 cortex for 48 hours and pulsed labeled with BrdU for 4 hours just before fixation. No GFP-positive cells in the cortex were labeled with BrdU, indicating that MGE-derived cells migrate into the cortex after their final mitosis. Scale bars: 600 μm in A,B; 100 μm in C-H; 120 μm in I.
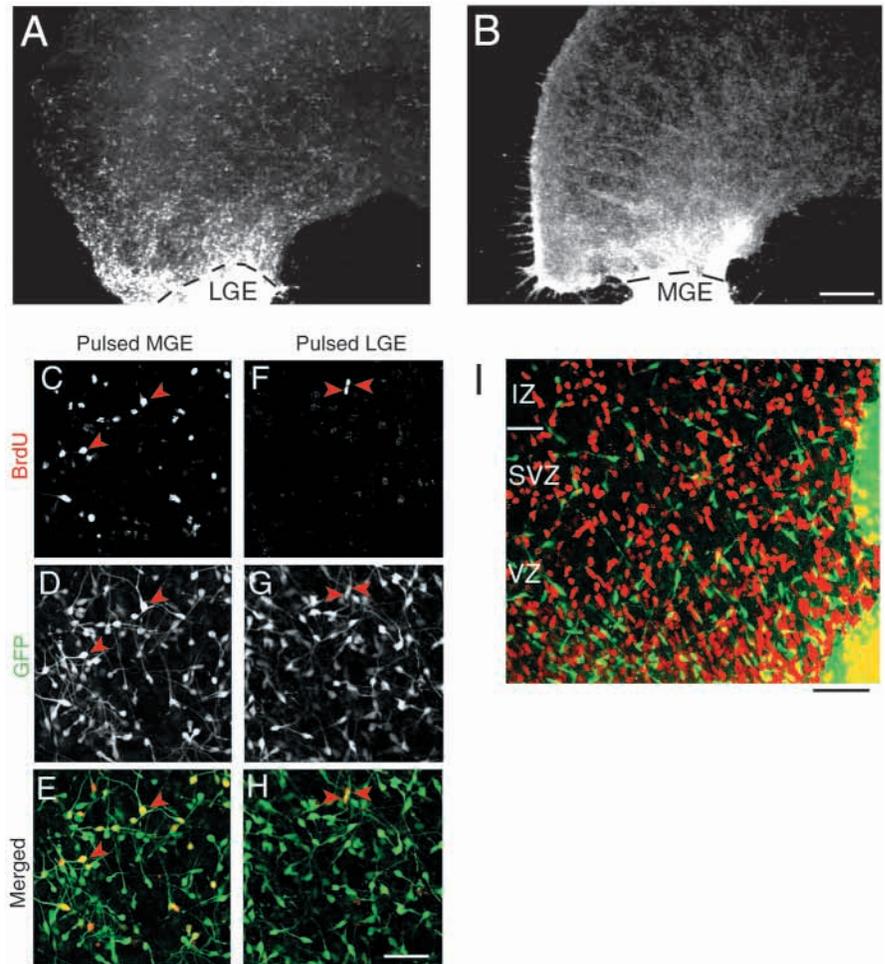
cortex co-cultures at 2 days in vitro with BrdU for 4 hours before fixation. Of 504 MGE-GFP cells (from four explants) scored that had migrated to the cortex, not a single one was BrdU labeled, indicating that cells migrating from the MGE to the cortex do not continue to proliferate during migration (Fig. 2I). As the MGE appears to be the major source of cells that migrate into the cortex, in the remainder of study we primarily used MGE-GFP explants in co-culture experiments.

Fate of tangentially migrating cells

We next wanted to determine the differentiated fate of MGE-derived cells that migrate into the cortex. As shown in Fig. 3A-C, the vast majority of E14-16 MGE-GFP cells express the neuronal marker MAP2 after 2 days in vitro (85.2±7.3%; $n=322$ cells from three independent experiments), indicating that most if not all of the tangentially migrating cells differentiate into neurons. These cells also express other neuronal markers such as NeuN (68.6±8.8%; $n=128$; Fig. 3D-F) but do not express the progenitor and radial glial marker nestin (Fig. 4). Analysis of neurotransmitter phenotypes indicated that about a third of E14-16 MGE-GFP cells express GABA after 7 days in vitro when co-cultured with isochronic cortex (37±5.6%; $n=223$; Fig. 3G-I). Owing to the relatively late postnatal maturation of the GABAergic neuronal phenotype, we examined whether older cortical slices would induce a larger fraction of MGE-GFP cells to differentiate into

GABAergic neurons. Culturing E14-16 MGE-GFP explants with P2-P4 cortical slices led to about 40% of the MGE-GFP neurons to differentiate into GABAergic cells at 7 days in vitro. Thus, between 35 and 40% of the MGE-GFP cells differentiate into GABAergic cells independent of the age of the recipient cortex.

Cellular substrates of tangential migration

It has previously been shown that radially migrating neurons use radial glial fibers as a substrate to migrate from the ventricular zone to the cortical plate. The variability in the orientation of the leading processes of migrating cells suggests that it is unlikely that these cells use a single cellular substrate for migration within the cerebral wall. The zone through which they migrate contains cortical efferent and afferent axons, radial glial fibers, other tangentially migrating neurons and dendrites of cortical neurons (once the cells enter the cortical plate). To determine if migrating GE-GFP cells preferentially associated with one of these cellular elements, we carried out double immunofluorescence analysis using cell type-specific antibodies. About 10% of GE-derived cells found in the CP after 36 hours in vitro migrate radially towards the pial surface making numerous close contacts with radial glial fibers (Fig. 4A,B), but the orientation of the leading process is not always aligned with the radial glial processes, suggesting that MGE-GFP cells probably do not migrate along radial glia. GE-GFP
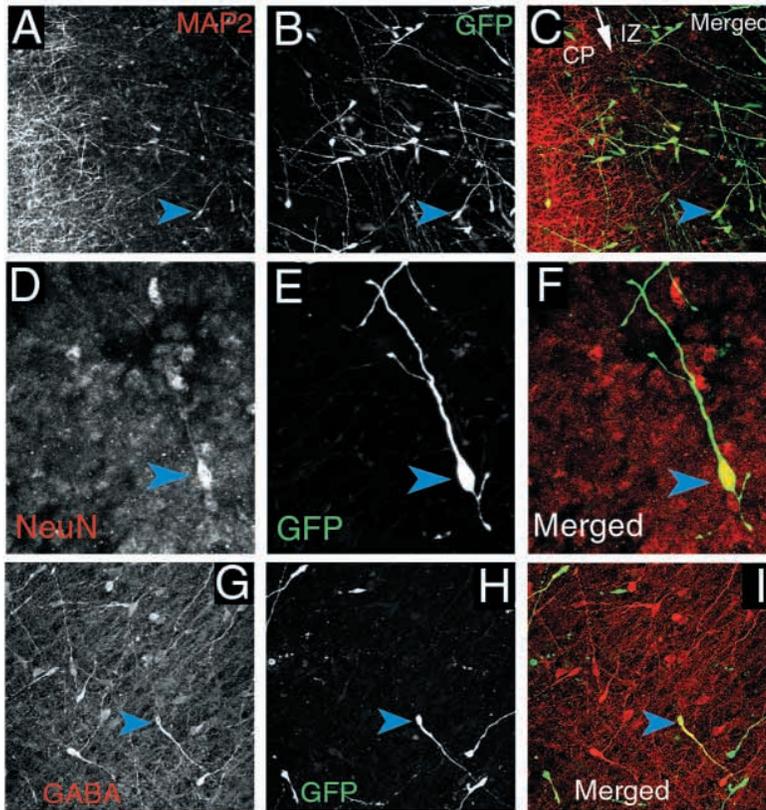
Fig. 3. Fate of MGE-derived tangentially migrating cells. (A-C) After 2 days in vitro, about 85% of E14 MGE-GFP cells (green, B) that migrate into the intermediate zone (IZ) express the neuronal marker MAP2 (A, red in C). The arrow in C delineates the border between the cortical plate (CP) and the IZ. (D-F) After 3 days in vitro, about 65% of E14 MGE-GFP cells (E, green in F) migrating in the IZ express the nuclear neuronal marker NeuN (D, red in F). (G-I) After 7 days in vitro, about 35% of E14 MGE-GFP cells (H, green in I) found in the cortex express the neurotransmitter GABA (G, red in I). Blue arrowheads indicate examples of double-labeled cells. Scale bars: 150 µm in A-C,G-I; 60 µm in D-F.

Fig. 4. Cellular interactions of MGE-derived tangentially migrating neurons. (A,B) Relationship between the leading process of E14 GE-GFP cells and the radial glial fibers in the intermediate zone (red, nestin). Cells migrating tangentially in the intermediate zone make several contacts with radial glial fibers, whereas some of the cells invading the cortical plate that migrate radially (arrow in A, shown at higher magnification in B) make multiple close contacts with the nestin+ glial fibers (arrowheads in B). In each image, the dorsal (D) and lateral (L) aspects are indicated. (C) Example of a migrating E14 GE-GFP cell making a few close contacts with axons (red, neurofilament 165 kDa) within the cortical intermediate zone. The insets numbered 1 (red, NF 165 kDa), 2 (green, GFP) and 3 (1 and 2 merged) represent a X-Z orthogonal section taken at the level indicated by the arrowhead in C. The blue arrows (3) indicate the location of close contact between an axon (red) and the leading process (green) of the tangentially migrating cell shown in C. (D) Example of an E14 GE-GFP cell migrating radially in the cortical plate towards the pia. This GE-GFP neuron makes contacts with cortical apical dendrites, revealed by MAP2 immunofluorescence (red). The insets numbered 1 (red, MAP 2), 2 (green, GFP) and 3 (1 and 2 merged) represent a X-Z orthogonal section taken at the level indicated by the arrowhead in D. The blue arrows (3) indicate the location of a close contact between an apical dendrite and the leading process of the GFP+ cell shown in D. Scale bars: 80 µm in A; 20 µm in B,C; 30 µm in D.

cells also make occasional contacts with axons in the intermediate zone (Fig. 4C) and cortical neuron apical dendrites within the CP (Fig. 4D), but the spatial relationship between the migrating cells and the axons or dendrites suggests that GE cells can migrate within the cerebral wall without having to grow along an existing cellular substrate.

Dynamics of tangential migration in the cortex

In the next series of experiments we examined the dynamics of MGE-GFP neurons as they migrated into the cortex. For this, we used time-lapse video-microscopy to image about 300 E14-E16 MGE-GFP cells migrating through isochronic wild-type cortex. The period of observation ranged from 3 hours to 3 days. The first striking feature of MGE-derived cells is their fast rate of migration, which averages 58±8.2 µm per hour and can reach instantaneous rates of migration of about 140 µm/hour for periods up to 30 minutes. Fig. 5 shows examples of the dynamics of migration of MGE-GFP cells as they migrate from the marginal zone (Fig. 5A-D) or the intermediate zone (Fig. 5E-H) into the cortical plate. We found examples of
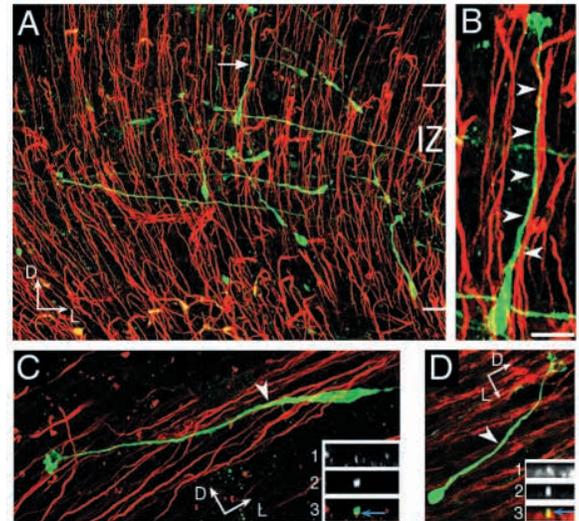
MGE-GFP cells migrating from the MZ to the CP (Fig. 5A), and also observed cells migrating from the CP to the MZ (Fig. 5B). In both cases, the migratory cells displayed a prolonged pause (50-70 minutes) at the CP/MZ interface before crossing over into the new zone. Many of the migrating cells were characterized by a saltatory mode of translocation, alternating between fast and slow instantaneous rates of migration (Fig. 5C). Fig. 5D shows that GE-derived cells found in the MZ typically move coherently in a lateral-to-medial direction, indicating that there is a preferred direction of migration within the MZ.

MGE-GFP cells in the IZ most frequently invaded the cortex by making sharp 90° turns (Fig. 5E). Unexpectedly, these sharp

Fig. 5. Dynamics of tangentially migration MGE-GFP neurons. (A,B) Time-lapse analysis of MGE-GFP cells in isochronic co-cultures moving radially down from the MZ to the CP (A) and from the CP to the MZ (B). The position and morphology of the migrating cells is shown at 15 minute time intervals. Note that in each case, the cells pause at the interface between the MZ and the CP before crossing into the new zone. (C) Illustration of the kinetics of migration of an E15 GE-GFP cell within the MZ. Each circle represents the position of the cell body every 15 minutes and reveals that the cell body translocates according to a saltatory mode of migration. (D) Trajectories of 23 MGE-GFP cells migrating within the MZ. Twenty out of these 23 cells migrate coherently from the lateral (left-hand side) to medial (right-hand side) aspect of the cortex. One cell (#3) migrates mediolaterally, one cell (#1) migrates down into the CP and two cells (#13 and #2) migrate from the CP to the MZ. (E,F) Morphological dynamics of the leading process during a change in the direction of migration. These cells, migrating within the IZ, make 90° (E) or 180° (F) changes in their trajectory in less than 2 hours (time interval is indicated below each drawings). Note that in each case, the turn is not initiated by the growth cone of the leading process, but instead by a second leading process that emerges from the cell body. (G) Example of an MGE-GFP cells crossing from the IZ to the CP. Note that the cell initially migrating in the IZ pauses for a prolonged period (arrowhead) before changing its trajectory by 90° and entering the CP. The arrows indicate the direction of migration. Conventions as in C.

(H) Trajectories of six GE-GFP cells migrating within the IZ. Note that most cells migrate from a lateral (left) to medial (right) direction. One cell can be seen changing trajectories to enter the CP. Scale bar: 50 µm in A-D,G-H; 100 µm in E,F.
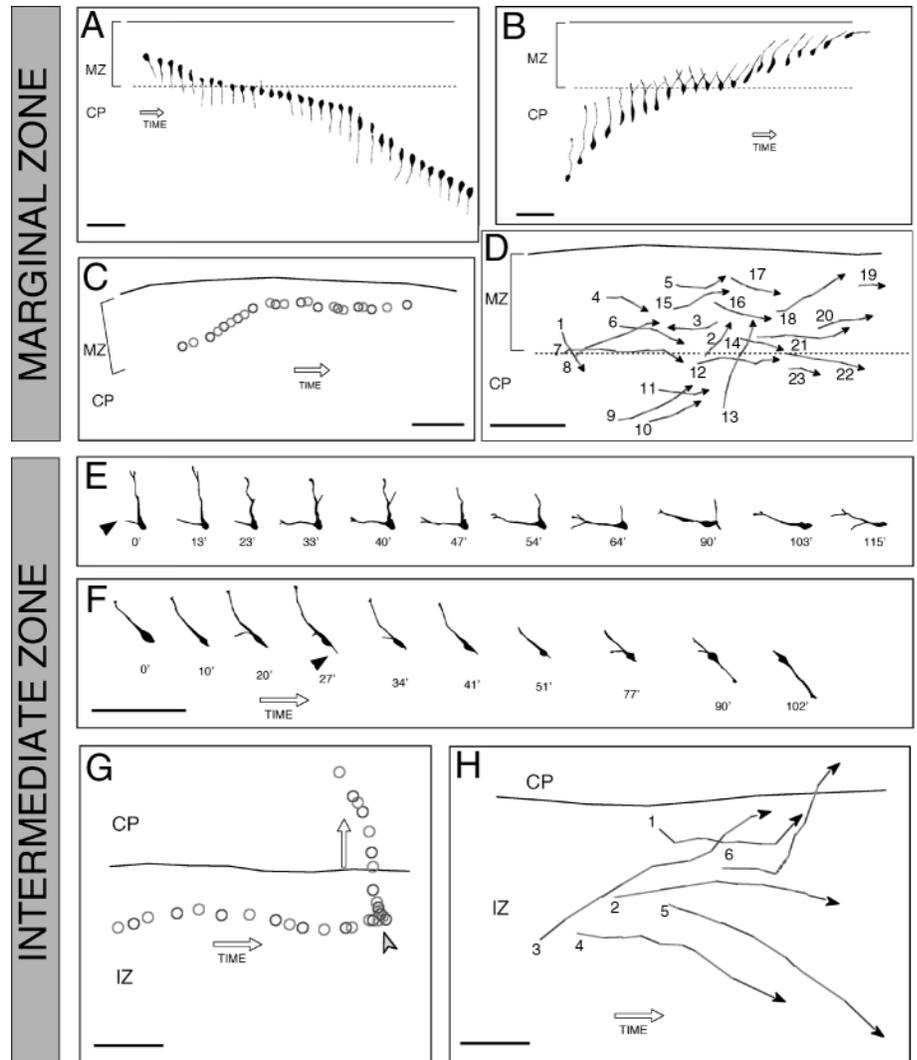
turns were usually not made by the pre-existing leading process, but instead involved the generation of a new leading process in the new direction of migration (see arrowheads in Fig. 5E,F). While the cell changes its direction of migration, the cell body pauses and suspends translocation as a new leading process is generated (Fig. 5E,F). These pauses lasted for up to 2 hours before migration resumed in the new direction (arrowhead in Fig. 5G). As in the case of cells migrating in the MZ, GE-derived cells migrating in the IZ moved coherently in a lateral-to-medial direction and only a small subpopulation invaded the CP at any given time (Fig. 5H).

Effects of BDNF and NT4 on tangential cell migration

To determine the influence of extracellular factors on the rate and extent of migration of MGE-GFP neurons to the cortex, we examined the effects of various factors on the MGE-GFP cell migration in our co-culture assay. We report on our investigations of the role of neurotrophins in regulating tangential cell migration. The neurotrophins were tested for

three main reasons: (1) they are expressed in the developing cortex (Maisonpierre et al., 1990; Timmusk et al., 1993; Friedman et al., 1998; Fukumitsu et al., 1998); (2) it has previously been suggested that some of their in vivo effects could be due to a potential action on neuronal migration (Behar et al., 1997; Brunstrom et al., 1997); and (3) their tyrosine kinase receptors, TrkB and TrkC, are expressed in cortical interneurons (Klein et al., 1990; Gorba and Wahle, 1999). To test the effects of neurotrophins on the migration of MGE-GFP neurons into the cortex, we treated MGE-cortex co-cultures with control vehicle solutions or with BDNF, NT3 or NT4 at a concentration of 50 ng/ml. As shown in Fig. 6A-C, just 24 hours of stimulation with either BDNF or NT4 led to a marked increase in the number of MGE-GFP neurons that migrate into the cortex. NT3 had no effect on tangential migration (data not shown). Thus TrkB ligands potently promote MGE cell migration into the cortex.

As BDNF and NT4 had similar effects on tangential migration and act via similar signaling mechanisms, for the remainder of the study we restricted our analysis to the effects

of NT4. To assess quantitatively the effects of NT4 on cell migration, we defined a migration index as cell density (number of cells/10^4 μm^2) in the intermediate zone at various distances from the MGE-cortex interface (Fig. 6D). Quantitative analysis indicated that NT4 (50 ng/ml) induced a threefold (ANOVA F=28.53; $P<0.0001$) increase in the number of MGE-GFP cells at any given distance from the interface
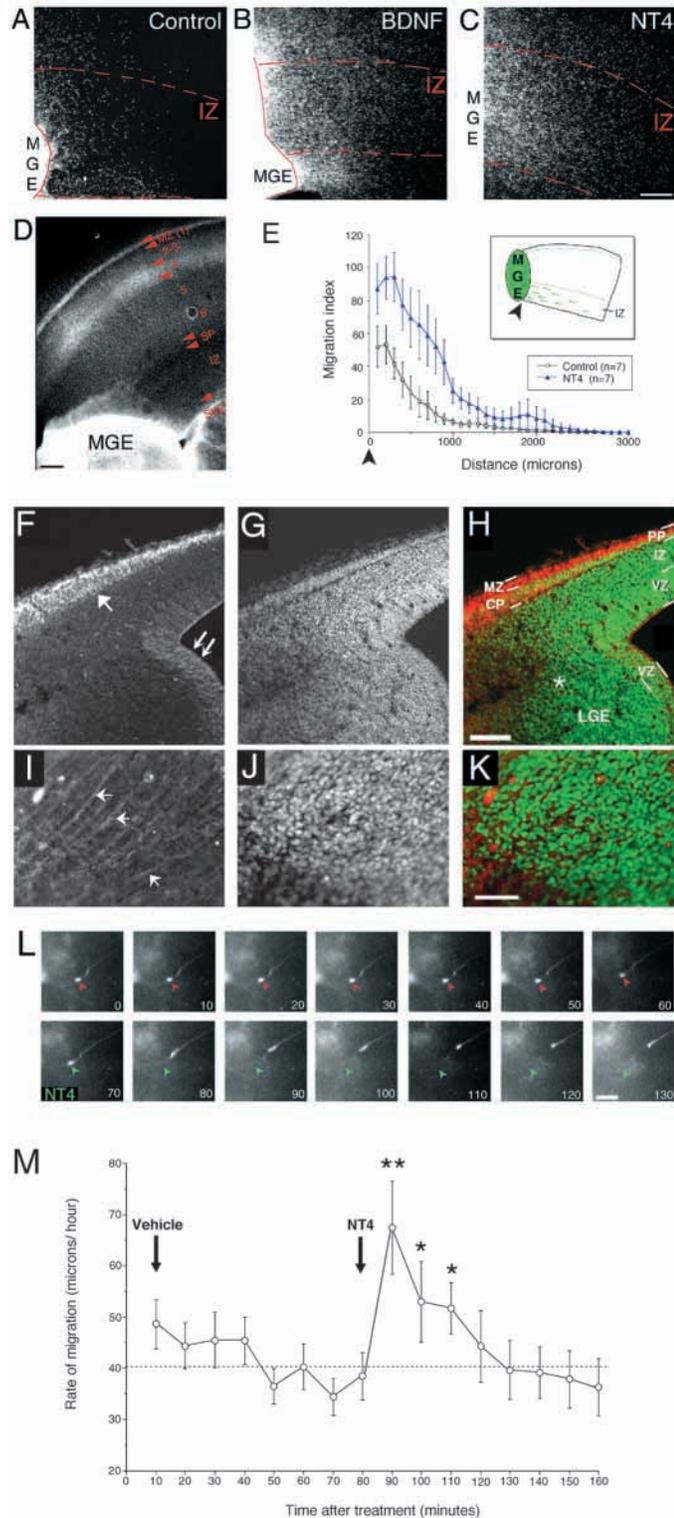
between the MGE and the cortex (Fig. 6E). Moreover, at distances greater than 2500 μm from this interface, MGE-derived cells were found in the IZ of the cortex treated with NT4 but not in the controls, suggesting that NT4 can influence the extent of tangential migration.

We then sought to determine the pattern of expression of NT4 at the protein level during telencephalic development as it had previously not been described. Fig. 6F-K summarize the pattern of expression of NT4 at E15 in the ventral and the dorsal telencephalon. NT4 is expressed at moderate levels in the ventricular zone of the GE (equally in the lateral and the medial parts, data not shown) and at higher levels in the cortical plate and marginal zone of the dorsal telencephalon. At low magnification, the mantle region of the ventral telencephalon and the intermediate zone of the dorsal telencephalon, which correspond to the migratory pathway of MGE-derived neurons, seemed devoid of NT4 labeling (star in Fig. 6H). However, at

Fig. 6. BDNF and NT-4 regulate tangential migration of MGE-derived cells to the cortex. (A-C) E14 MGE-GFP explants were co-cultured with P2-P4 cortical slices for 1 day in the presence of control vehicle (0.1 M PBS, A), recombinant BDNF (50 ng/ml, B), or recombinant NT-4 (50 ng/ml, C) in the slice culture medium. Both BDNF and NT4 treatment lead to an increase the total number of MGE-GFP cells that migrate into the cortex. (D) Nuclear counterstaining (Hoechst 33258) reveals the cellular organization of the cortex in MGE-cortex co-cultures. (E) Quantification of the effects of NT4 on migration of MGE neurons into the cortex in MGE-cortex co-cultures (experiment shown in A,C). The migration index represents the density of GFP+ cells (number of cells per 10^4 μm^2) found in 100 μm wide stripes of intermediate zone at indicated distances from the MGE-cortex interface (arrowhead on the inset). Note that NT4 treatment (blue triangles) leads to an increase in the number of cells that migrate into the cortex and in the maximum distance of migration. Quantification was performed on nine explants taken from three independent experiments. NT4 treatment showed a statistically significant increase of the migration index compared with the control according to an ANOVA test ($P<0.0001$). (F-K) Expression pattern of NT4 in the developing telencephalon. Coronal sections were processed for NT4 immunofluorescence (F,I) and counterstained with Hoechst (G,J). The double immunofluorescence images are shown in (H,K). (F-H) At low magnification, NT4 can be detected in the ventricular zone of the GE (double arrows in F), as well as in the cortical plate and marginal zone of the cortex (single arrow in F). (I-K) With a higher magnification image through the cortical intermediate zone, NT4 immunofluorescence can be detected in radially oriented processes (arrows in I), which are likely to be radial glial processes. (L) NT4 can acutely induce the motility of MGE-derived cells. Time-lapse video images of an E15 MGE-GFP cell located in the IZ of a P2 cortical slice. The cell was not moving for the first 60 minutes (red arrowhead) before the application of NT4. Application of NT4 (green arrowhead; 20 μl at 20 ng/ml) induces migration in the MGE-GFP cell. The arrowhead in each panel indicates the initial position of the cell body. Numbers at the bottom right indicate time in minutes. (M) Recombinant NT4 can acutely increase the rate of migration of MGE-derived cells. E14 MGE-GFP cells migrating in the IZ of P2 cortical slices were imaged by time-lapse video-microscopy for 70 minutes in the presence of control vehicle (PBS) and for 80 minutes after application of recombinant NT4 (20 ng/ml). The instantaneous rate of migration (μm per hour) of MGE-GFP cells (n=77) is plotted as a function of time. Quantification was performed on eight explants taken from two independent time-lapse video-microscopy experiments. Scale bar: 250 μm in A-C; 400 μm in D; 150 μm in F; 40 μm in L. *, $P<0.05$; **, $P<0.003$.

higher magnification, NT4 could be detected in radial processes, which may be radial glia (Fig. 6I). This is interesting considering that tangentially migrating cells make close orthogonal or longitudinal contacts with nestin+ radial glial processes during their migration (Fig. 4A,B). Therefore, NT4 is expressed along the migratory path of MGE-derived cells in vivo, consistent with a possible role for NT4 in tangential migration.

NT4 could influence tangential migration via one or both of two mechanisms: (1) by inducing the motility of MGE neurons as they exit the ventricular zone in the GE; or (2) by acutely affecting the rate of cell migration of MGE neurons along their tangential pathway. To determine if NT4 can acutely influence the rate of MGE cell migration, we used time-lapse video-microscopy to image MGE-GFP cells before and after exposure to NT4. In the first set of experiments, we examined the ability of NT4 to induce the motility of MGE-GFP cells that were not migrating. To do this, we selected E14 MGE-GFP cells in the intermediate zone of P2 cortex after 24-36 hours in vitro that had not been migrating for at least 60 minutes (Fig. 6L). Cells that respond to this criteria are rare. Sixty minutes after the beginning of recording, the cells were stimulated with 50 ng/ml of recombinant NT4 applied directly onto the slice. This treatment reliably induced migration in cells that were previously stationary (n=7/7 cells; Fig. 6L), indicating that NT4 is sufficient to induce motility on non-migrating MGE cells.

We next tested whether NT4 could induce an increase in the rate of migration of MGE-GFP cells. To do this, we performed time-lapse video analysis of MGE-derived cells migrating in the IZ of P2 cortical slices. After 80 minutes of observation under control conditions, we applied recombinant NT4 (20 ng/ml) directly onto the slice and monitored changes in the rate of migration of 77 cells (Fig. 6M). This experiment revealed that as little as 10 minutes of stimulation with recombinant NT4, led to a 70% increase in the rate of migration of MGE-derived cells. An ANOVA analysis revealed that NT4 treatment has a significant effect on the rate of migration (F=3.123; P<0.003), whereas the control (vehicle) treatment showed no significant effect (F=1.392; P=0.205). Further analysis using PLSD Fisher test demonstrated that after a brief exposure to NT4 the rate of migration was affected for about 30 minutes after treatment, and then returned to baseline (broken line in Fig. 6M). Thus, NT4 acutely affects the rate of migration of MGE-derived cells.

Role of Trk-family receptors in controlling tangential cell migration

As NT4 and BDNF act as ligands for the TrkB receptor tyrosine kinase, we used double immunofluorescence to examine the expression of TrkB in MGE neurons that migrate to the cortex. As shown in Fig. 7A-C, MGE-GFP neurons that had migrated into the cortex expressed TrkB. Strikingly, TrkB was localized in these migrating cells along the entire leading process, while little, if any, was present on the cell bodies. Localization of TrkB on the migrating cells suggests that endogenous TrkB ligands are likely to be involved in regulating the migration of MGE neurons.

To test if activation of Trk-family receptors by endogenous ligands was involved in regulating the migration of MGE neurons to the cortex, we examined the effects of treating the cultures with K252a, an inhibitor of the Trk family receptor tyrosine kinases (Knusel and Hefti, 1992). As shown in Fig. 7D-E, treatment of MGE-cortex co-cultures with K252a sharply reduced the number of MGE-GFP cells that migrate into the cortex. Quantitative analysis indicated that K252a treatment led to a marked reduction in both the number of migrating cells and the distance within the cortex to which the cells migrate (Fig. 7F). These experiments suggest that activation of Trk-family receptors by endogenous ligands is involved in regulating migration of MGE neurons, and supports a role for neurotrophins in regulating the migration of MGE cells to the cortex.

To determine if TrkB plays a role in vivo in the control of tangential migration from the MGE to the cortex, we examined embryos with a targeted deletion of the TrkB gene (Klein et al., 1993). We performed immunocytochemical labeling for two Ca^{2+} binding protein, Calbindin 28K (Fig. 7G-J) and calretinin (data not shown), which are two early markers of tangentially migrating interneurons (Fonseca et al., 1995), in E15 wild-type and TrkB$^{-/-}$ embryos. Inspection of calbindin 28K labeling at low magnification revealed no gross abnormalities in the cytoarchitecture of the ventral and dorsal telencephalon of TrkB null mice compared with control littermates (Fig. 7G,H). At higher magnification in both wild-type and TrkB$^{-/-}$ mice, calbindin+ neurons migrate tangentially in three distinct layers of the dorsal telencephalic wall (Fig. 7I,J): (1) the sub-ventricular zone, (2) the interface between the upper part of the intermediate zone and the subplate, and (3) the marginal zone. At this stage only few calbindin+ neurons have invaded the cortex, which is reminiscent of our in vitro data (Fig. 1B-E). Quantitative analysis revealed that there is a significant reduction (32%) in the number of calbindin+ neurons that migrate into the developing cortex in TrkB null compared with wild-type cortex (Fig. 7K).

In contrast to the effects of TrkB deletion on the distribution of calbindin+ neurons, we did not find any significant difference in the distribution of calretinin+ interneurons migrating tangentially in the cortex of control (data not shown). The difference in the effects of TrkB deletion on the distribution of calbindin+ and calretinin+ interneurons suggests that TrkB signaling may regulate the migration of specific subpopulations of interneurons into the cortex.

Role of PI3-kinase signaling in regulating tangential migration

Stimulation of Trk receptors leads to activation of several intracellular signaling pathways, of which the best-characterized effectors are (1) MEK1/2-MAP kinase, (2) PLCγ, and (3) PI3-kinase (reviewed by Kaplan and Miller, 2000). Pharmacological perturbation experiments show that inhibition of MAP kinase or PLCγ does not significantly affect tangential migration (Fig. 8F), and raises the possibility that TrkB signaling might control migration via activation of PI3-kinase. One of the main downstream effectors of PI3-kinase is AKT, which is rapidly phosphorylated upon PI3-kinase activation. Phosphorylation of AKT, which can be detected by phospho-AKT-specific antibodies, therefore serves as a sensitive assay for PI3-kinase activation. To determine if stimulation of MGE neurons with BDNF or NT4 led to the activation of PI3-kinase, we stimulated MGE explants with neurotrophins, and examined AKT phosphorylation by western
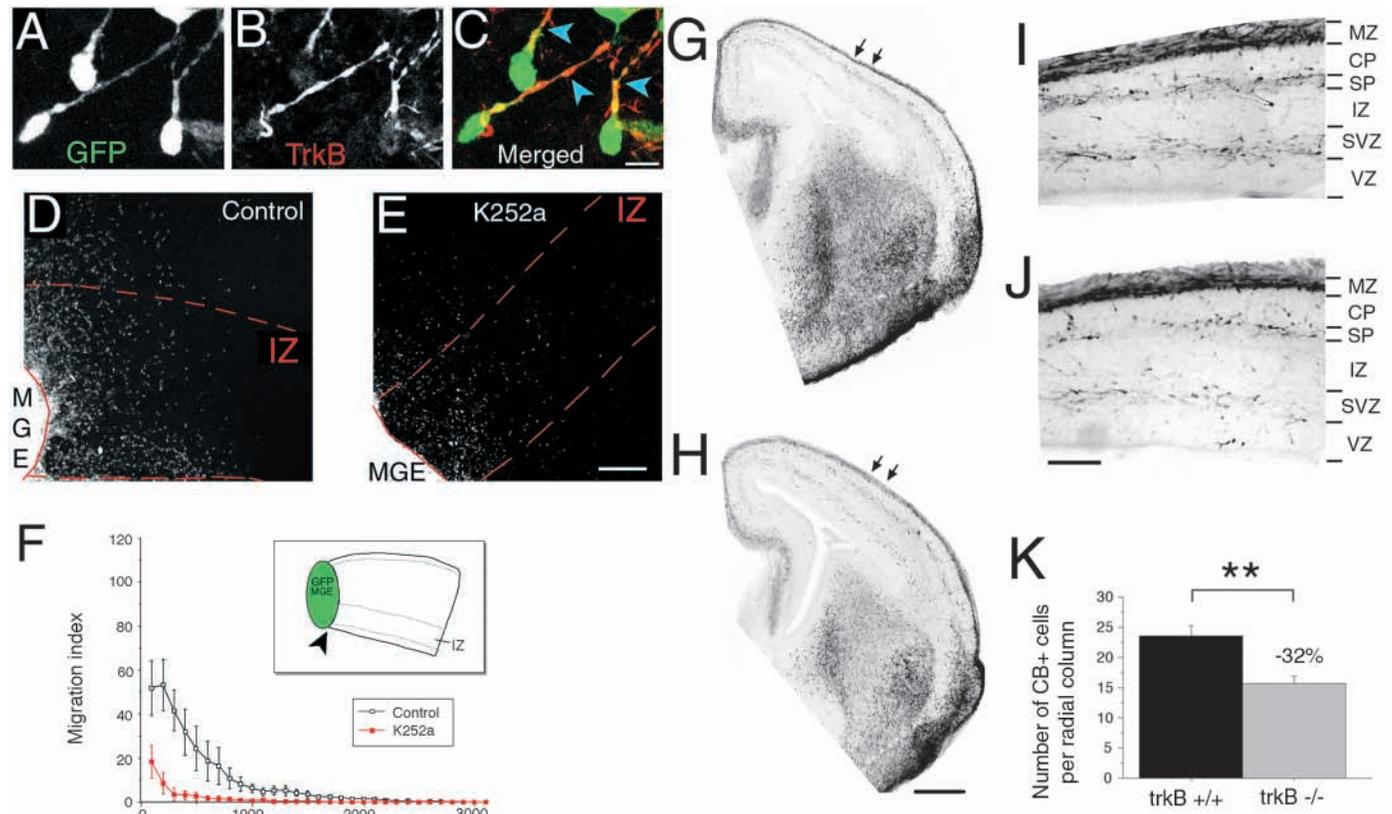
Fig. 7. Localization and function of TrkB receptors in tangential migration. (A-C) Double immunofluorescence for GFP (A) and TrkB (B) indicates that E14 MGE-GFP cells migrating in the cortical intermediate zone express the TrkB receptor (merged image in C). Note that the TrkB receptor is localized to the leading processes (arrowheads in C) of MGE-GFP cells. (D-F) Effect of inhibiting Trk receptors on migration of GE-GFP cells. (D,E) Confocal images of E14 MGE-GFP cells migrating tangentially into the IZ of early postnatal cortex in the presence of control vehicle (DMSO 1:1000; D) or 50 nM of the Trk tyrosine kinase inhibitor K252a (E). (F) Quantification of the experiments shown in D,E from six slices taken from three independent experiments. Conventions are the same as in Fig. 6E. K252a treatment (red) significantly inhibits migration according to an ANOVA test (F=28.53; P<0.0001). (G-J) Analysis of tangential migration in wild-type and TrkB null mice. (G-H) Low magnification photographs of coronal sections of E15 wild-type (G) and TrkB$^{-/-}$ mice (H) stained for calbindin 28K. At this rostrocaudal level of section, the global cytoarchitecture of the telencephalon in the two genotypes is indistinguishable. (I-J) High magnification photographs of the lateral cortex of sections shown in G,H. Tangentially migrating calbindin+ cells are found mainly in the subventricular zone (SVZ), the upper part of the intermediate zone (IZ), the subplate (SP) as well as in the marginal zone (MZ) in both wild-type (I) and TrkB$^{-/-}$ (J) cortex. (K) Quantification of the number of calbindin+ cells per 200 μm wide radial column (indicated by double arrows in G,H). This analysis reveals a significant decrease in the number of tangentially migrating calbindin+ cells in TrkB$^{-/-}$ cortex compared with TrkB$^{+/+}$ cortex at E15 [–32%; **P<0.005 – Mann-Whitney test; 12 sections from three wild-type and three knockout mice]. Scale bars: 15 μm in A-C; 250 μm in D,E, 400 μm in G,H; 120 μm in I,J.

blot analysis. BDNF and NT4 stimulation both led to a rapid and robust increase in AKT phosphorylation, which was prevented by pretreatment of the explant with the PI3-kinase inhibitor LY294002 (Fig. 8A). Thus, BDNF and NT-4 lead to PI3-kinase activation in MGE cells.

To determine if PI3-kinase function was involved in regulating the migration of cells from the MGE to the cortex, we examined the effects of LY294002 treatment on the migration of MGE-GFP neurons to the cortex in our co-culture assay. As shown in Fig. 8B-D, LY294002 treatment completely suppressed the ability of NT4 to stimulate migration of GE neurons into the cortex, indicating that PI3-kinase function is required by NT4 to exert its effect on migration. Because the K252a experiment suggested that endogenous neurotrophins are important in determining the extent of tangential migration, we quantified the LY294002 treatment experiment to see if migration was reduced to a level similar to that seen with

K252a. Indeed, quantitative analysis showed that LY294002 treatment (with or without NT4, Fig. 8E) reduces migration to levels comparable to K252a treatment. These experiments strongly suggest that TrkB ligands regulate migration of GE cells into the cortex via activation of the PI3-kinase signaling pathway.

Finally, as PI3-kinase has been previously shown to be involved in the control of neuronal survival, we wanted to rule out the possibility that our effect of PI3-kinase inhibition was due to an increased level of apoptosis of MGE-derived cells. To do this, we examined the percentage of apoptotic nuclei found in the IZ of P2 cortical slices treated with NT4 and LY294002, as described above. The percentage of nuclei showing condensed chromatin staining by Hoechst 33258 was less than 1% in control, NT4-treated and NT4+LY294002-treated slices (data not shown). Furthermore, fewer than 0.1% of the MGE-GFP cells show condensed chromatin labeling
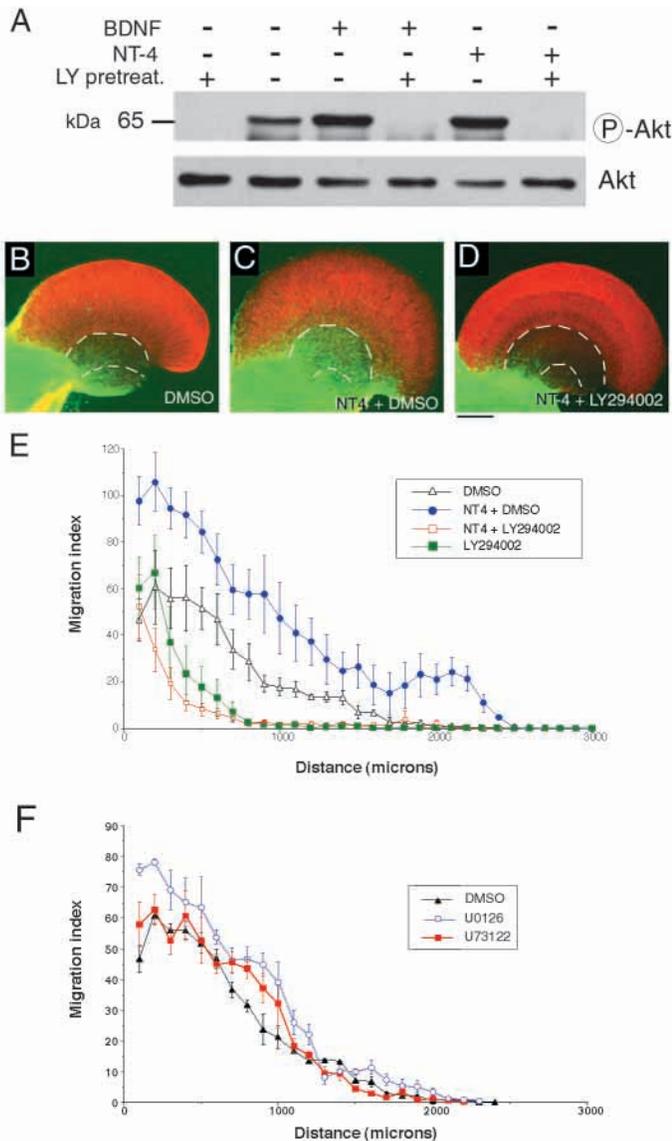
maturation of cortical neurons. The present results indicate that in addition to these previously identified cellular functions, BDNF and NT4 play an important role in regulating tangential migration of MGE cells into the cortex and thereby contribute to the final distribution of cortical interneurons.

Pattern of migration of GE-derived cells

We find that neurons that migrate into the cortex are preferentially generated in the MGE, and not the LGE. This observation is consistent with recent results that identify the MGE as the principal source of embryonically generated cortical interneurons. Anderson et al. labeled groups of LGE or MGE neurons in slice cultures and found that MGE-derived cells preferentially invaded the cortex (Anderson et al., 2001). Similar results were obtained by Wichterle et al., who showed that transplanted MGE cells, but not LGE cells, migrated into the cortex in large numbers (Wichterle et al., 2001). Thus, evidence obtained from several independent approaches indicates that the MGE is the principal source of cells that migrate into the cortex.

MGE-derived neurons migrate along the IZ and MZ to access the dorsal telencephalon. This is also consistent with the findings of Anderson et al. (Anderson et al., 2001) and Wichterle et al. (Wichterle et al., 2001). By time-lapse video-microscopy we were able to follow the trajectories of MGE neurons migrating through the neocortex, and found that tangentially migrating neurons could invade the CP from either the MZ or the IZ. The migration of cells from the MZ to the CP was unexpected and is reminiscent of the migration of granule cells from the external granule layer (EGL) to the internal granule layer (IGL) in the developing cerebellum (reviewed by Hatten and Mason, 1990). It will be of interest to

typical of apoptotic cells in the three experimental conditions, suggesting that NT-4 stimulation and inhibition of PI3-kinase do not affect the survival of migrating MGE cells. These experiments indicate that the effects of PI3-kinase inhibition on migration are not an indirect consequence of an effect on cell viability, and support a direct role for PI3-kinase in the control of tangential cell migration.

DISCUSSION

We have developed a new in vitro assay that recapitulates the tangential migration of cells from the GE to the cortex, and have used this assay to explore the cellular and molecular factors that control tangential migration in the developing telencephalon. Our observations indicate that GE cells show great specificity in their trajectories of migration, and that tangential migration is regulated by neurotrophins and PI3-kinase signaling. Neurotrophins have previously been implicated in regulating survival, differentiation and

determine if the migration of EGL neurons and MGE neurons is regulated by similar mechanisms.

We find that MGE-derived neurons migrate throughout the lateromedial extent of the developing telencephalon and invade the hippocampal primordium. This observation suggests that some, if not all, hippocampal interneurons are generated in the GE. Migration of GE cells to the hippocampus has also been recently been suggested by Rubenstein and colleagues, based on a lack of GABAergic cells in the hippocampus in *Dlx1/Dlx2*-null mice (Pleasure et al., 2000). We find that tangentially migrating cells specifically avoid the developing dentate gyrus. Although interneurons found in the dentate gyrus are generated during largely overlapping periods (E12 to E18 in the mouse) with interneurons found in the rest of the hippocampus (Lubbers et al., 1985; Soriano et al., 1989), our experiments suggest that interneurons that populate these two hippocampal subdivisions have distinct origins (Galceran et al., 2000; Grove and Tole, 1999; Tole et al., 1997; Tole et al., 2000). The DG also differs from the rest of the telencephalon in its ability to produce new neurons throughout adulthood (Eckenhoff and Rakic, 1988; Gould et al., 1999; Rakic, 1985). Our results indicate that signals expressed in the DG act as potent chemorepulsive factors for GE-derived migrating neurons, and prevent them from invading the DG. The identity of this signal is not yet known, but it is worth noting that several axonal chemorepulsive signals are expressed exclusively in the dentate gyrus, and may also function as chemorepellants for migrating interneurons.

Dynamics of tangential migration

The use of GFP-expressing cells in our co-culture assay allowed us to examine the dynamics of tangential migration. The rate of migration of MGE-derived cells with the cerebral wall is 50-60 µm/hour, which is three to ten times faster than cells migrating along radial glial fibers (Komuro and Rakic, 1993; Komuro and Rakic, 1992; Miller, 1999). In vivo transplantation experiments also indicate that MGE cells have rapid migration rates in the neocortex (Wichterle et al., 2001). Interestingly, the dynamics of migration described in this study for embryonic GE-derived cells are similar to that described for migration of cells from the postnatal sub-ventricular zone of the cortex or striatum, which mainly give rise to astrocytes (Kakita and Goldman, 1999). Therefore common mechanisms may regulate the motility of non-radially migrating cells throughout telencephalic development.

An unexpected feature of the tangentially migrating neurons is the way they change direction. Instead of following a leading process that turns, in most cases MGE cells change direction by extending a new leading process (originating along the pre-existing leading process or on the cell body). This mode of turning differs importantly from the mode used by axons where steering decisions originate from, and are executed by the growth cone at the tip of the axon. It appears that for tangentially migrating neurons the entire leading process can act as a sensor, and that a signal from the leading process can be propagated to the cell body to initiate a new leading process. The fact that TrkB, which controls the motility of these cells, is expressed along the entire leading process of these migrating neurons, and that membrane ruffles or branches can originate anywhere along the leading process support the notion that the entire leading process is capable of making steering decisions in tangentially migrating neurons.

The fact that MGE neurons often enter the CP by extending a new leading process that emerges from the existing leading process raises the possibility that factors that control the invasion of GE-derived neurons into the CP may act as branching factors for these cells. A signal that may be involved in regulating branching of the leading process is Slit, which is expressed in the developing cortical plate and has a branching activity (Wang et al., 1999; Whitford et al., 2002). Slit has also been implicated in regulating LGE cell migration (Zhu et al., 1999), but so far we have not detected a chemotropic effect of Slit1 or Slit2 on MGE cell migration (K. W. and A. G., unpublished).

Regulation of migration by neurotrophins and PI3-kinase signaling

Our investigations into the molecular control of tangential cell migration indicate that BDNF and NT4, the high-affinity ligands for the TrkB receptor tyrosine kinase, stimulate the migration of MGE-derived neurons. While a role for neurotrophins in cell migration had been previously suggested (Behar et al., 1997; Brunstrom et al., 1997; Schwartz et al., 1997; Ringstedt et al., 1998), there has been little or no direct evidence showing that neurotrophins can acutely regulate cell motility or migration. We find that BDNF and NT4 greatly increase the number of MGE cells that migrate into the cortex. Time lapse imaging experiments show that NT4 induces motility in stationary cells and acutely increases the rate of migration in cells already migrating. We also find that inhibiting the Trk receptors drastically reduces tangential migration and that tangential migration of calbindin+ interneurons is reduced in TrkB-null mice. These experiments strongly support a role for TrkB ligands in regulating migration MGE cells to the cortex.

As many of our conclusions are based on the effects of neurotrophins on the number of MGE-GFP cells present in the cortex, it is important to consider whether these effects may in part reflect effects of neurotrophins on the survival or proliferation of MGE cells. To examine the effects of neurotrophin treatment on the survival of MGE cells, we counted apoptotic profiles (shrunken or fragmented nuclei) in MGE-GFP cells in the absence or presence of NT4. Quantitative analysis showed that less than 1% of MGE-derived cells have apoptotic nuclei under either condition, suggesting that a survival effect of NT4 does not account for the great increase in MGE-GFP cells found in the cortex after NT4 treatment (F. P. and A. G., unpublished). Similarly, the addition of K252a did not increase the percentage of apoptotic profiles among MGE-GFP cells, indicating that the reduction of MGE-GFP cells in the cortex in the presence of K252a is not due to increased cell death. BrdU-labeling experiments indicated that tangentially migrating cells were postmitotic and did not retain any dividing potential (Fig. 2 and data not shown), suggesting that neurotrophin-induced proliferation of migrating cells does not contribute to the increase in the MGE-GFP cells in the cortex after neurotrophin treatment. If proliferation were to play a role in the increased number of MGE-derived cells found in the cortex after NT4 stimulation, this effect would have to be exerted entirely before cells began migrating out of the MGE. This seems unlikely because the average cycle time of GE precursors in the mouse at E14 is about 20 hours, and a very significant increase in the number

of MGE-GFP cells in the cortex is seen within 24 hours. These observations, together with the experiments demonstrating acute effects of neurotrophins on cell motility (Fig. 6), suggest that the principal effect of BDNF and NT4 on MGE-GFP cells is to regulate their migration.

Neurotrophin stimulation of the TrkB receptor leads to activation of the MAP kinase, PLCγ and PI3-kinase signaling pathways (reviewed by Kaplan and Miller, 2000). Our investigations of the contribution of these pathways to neuronal migration suggest that MAP kinase and PLCγ are not required for MGE cell migration. However, we find that PI3-kinase is activated in MGE explants by neurotrophins and that PI3-kinase function is required for tangential migration. In recent years PI3-kinase has been implicated in a large number of biological responses downstream of receptor tyrosine kinases, including cell survival, proliferation and migration (reviewed by Vanhaesebroeck et al., 1997). The class Ia PI3-kinases are heterodimers that consist of a catalytic subunit of relative molecular mass 110 kDa (p110α,β,δ) in complex with an adaptor molecule (p85α, p85β, p55γ or splice variants) that contains Src-homology (SH2) domains. It has recently been shown that in macrophages, colony-stimulating factor 1 (CSF1)-induced proliferation requires the activation of p110α, whereas CSF1-induced actin reorganization and migration depends on the activity of p110β and p110δ (Vanhaesebroeck et al., 1999). It will be of interest to determine if the effects of TrkB activation on neuronal migration and survival are also mediated via distinct forms of PI3-kinase. The mechanism by which PI3-kinase activation regulates migration of GE cells is not known, but one possibility is that it might be mediated by Rho-family GTPases. PI3-kinase activation leads to the formation of PIP2 and PIP3 at the cell membrane, which can act as docking sites for exchange factors of the Rho GTPase family (Han et al., 1998; Ma et al., 1998). Rho proteins, in turn, could locally influence the actin cytoskeleton to regulate cell motility. Such a mechanism, which is independent of changes in gene expression, would allow local changes in neurotrophin concentration to acutely influence neuronal migration.

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