Conserved pattern of tangential neuronal migration during forebrain development

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Origin, timing and direction of neuronal migration during brain development determine the distinct organization of adult structures. Changes in these processes might have driven the evolution of the forebrain in vertebrates. GABAergic neurons originate from the ganglionic eminence in mammals and migrate tangentially to the cortex. We are interested in differences and similarities in tangential migration patterns across corresponding telencephalic territories in mammals and reptiles. Using morphological criteria and expression patterns of *Darpp-32*, *Tbr1*, *Nkx2.1* and *Pax6* genes, we show in slice cultures of turtle embryos that early cohorts of tangentially migrating cells are released from the medial ganglionic eminence between stages 14 and 18. Additional populations migrate tangentially from the dorsal subpallium. Large cohorts of tangentially migrating neurons originate ventral to the dorsal ventricular ridge at stage 14 and from the lateral ganglionic eminence from stage 15. Release of GABAergic cells from these regions was investigated further in explant cultures. Tangential migration in turtle proceeds in a fashion similar to mammals. In chimeric slice culture and in ovo graft experiments, the tangentially migrating cells behaved according to the host environment – turtle cells responded to the available cues in mouse slices and mouse cells assumed characteristic migratory routes in turtle brains, indicating highly conserved embryonic signals between these distant species. Our study contributes to the evaluation of theories on the origin of the dorsal cortex and indicates that tangential migration is universal in mammals and sauropsids.

KEY WORDS: Cerebral cortex, Neocortex, GABAergic neurons, Nkx2.1, Tbr1, Darpp-32, Tangential migration, DVR, MGE, LGE, Turtle

INTRODUCTION

Most parts of the brain are remarkably conserved across amniotes, a group including reptiles, birds and mammals. In all species examined, the telencephalon consists of a dorsal half, called the pallium, that comprises several subregions including the neocortex in mammals, and a ventral half, called the subpallium (Nieuwenhuys et al., 1998; Butler and Hodos, 2005). Molecular and genetic studies in mouse have shown that telencephalon development is regulated by genes expressed from early embryonic stages in specific subdivisions of the pallium and subpallium: Pax6 and Tbr1 are expressed in the pallium, Dlx and Mash1 (also known as Ascl1 – Mouse Genome Informatics) in the subpallium, Nkx2.1 (also known as Titf1) and Lhx6 in the ventral division of the subpallium known as the medial ganglionic eminence (MGE) (Bulfone et al., 1995; Shimamura et al., 1995; Sussel et al., 1999; Lavdas et al., 1999; Stoykova et al., 2000; Puelles et al., 2000; Hevner et al., 2001). Comparative studies in mammals, birds and reptiles (Smith-Fernandez et al., 1998; Puelles et al., 2000) show conserved patterns of gene expression, suggesting homologies between regions in distant species.

In mammals, the two main functional populations of cortical neurons are generated in distinct regions (Parnavelas, 2000). Excitatory pyramidal neurons are born in the pallium and migrate into the cortical plate according to an 'inside-first, outside-last' order

(Angevine and Sidman, 1961). The precursors of inhibitory GABAergic interneurons arise in a Nkx2.1-expressing subpallial region, and migrate tangentially into the neocortex (De Carlos et al., 1996; Tamamaki et al., 1997; Anderson et al., 1997; Ladvas et al., 1999; Wichterle et al., 1999; Nery et al., 2002; Tanaka et al., 2003; López Bendito et al., 2004). Adult turtle cortex contains the same two broad functional categories of neurons as the cortex of mammals: excitatory pyramidal and inhibitory stellate neurons (Connors and Kriegstein, 1986). Glutamatergic neurons migrate radially within the pallium and occupy their position in the cortex according to an 'outside-first to inside-last' neurogenetic gradient (Goffinet, 1983; Goffinet et al., 1986). In turtle dorsal cortex, GABAergic neurons represent only 6% of all neurons (Blanton et al., 1987), compared with a 10-20% representation in rodents and primates (Parnavelas, 2000; Hendry et al., 1987). As in mammals, GABAergic neurons are tangentially oriented in the embryonic turtle cortex (Blanton and Kriegstein, 1991b), but the origin of these pallial GABAergic cells in reptiles is not known in spite of its obvious relevance for understanding cortical evolution.

The pallium of mammals differs from reptiles in (1) the presence of the six-layered neocortex in the dorsal pallium of mammals, whereas only three layers are present in pallial areas in sauropsids (birds and reptiles), and (2) the absence of the large dorsal ventricular ridge (DVR) in mammals (Striedter, 2005; Butler and Hodos, 2005). The DVR is the hallmark of the lateral part of the pallium in sauropsids (see Ulinski, 1983; Bruce and Neary, 1995; Striedter, 1997). The large DVR that develops in reptiles dorsally to the palliostriatal boundary could influence tangential migration streams between the ventral and dorsal halves of the telencephalon (Puelles et al., 2000; Molnár and Butler, 2002a; Molnár and Butler, 2002b; Molnár et al., 2006). Indeed, this pallial-subpallial junction in mammals is a complex boundary zone that guides the migratory route of radially migrating subpallial cells (Marin and Rubenstein, 2003), controls the tangential migration of pallial cells ventrally to the striatum, and might influence cell migration between subpallium and pallium (Fishell et al., 1993;

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Chapouton et al., 1999). Because the origin, direction and timing of migrating cell populations during brain development determine the morphological organization of adult structures, we asked whether fundamental differences in morphology between mammals and reptiles are correlated to differences in migration patterns across corresponding telencephalic territories.

To examine the source and pattern of the tangentially migrating neurons during early development of the turtle pallium, we analyzed the regional expression of selected developmental genes and studied the migratory patterns in embryonic organotypic slice and explant cultures from stages 14 through 20. We also developed chimeric transplantation methods in vitro and in ovo to assay the behavior of mammalian tangentially migrating neurons in a reptilian environment and vice versa. We report that tangential migration exists in reptiles and a proportion of tangentially migrating neurons generated in the subpallium are GABA positive. Restricted sectors of the dorsal and ventral subpallium express distinct genes and are responsible for the production of migrating cells. The DVR itself does not contribute any tangentially migrating neurons, but the region lying ventral to it appears to be a major source. Cells from diverse areas in the subpallium differ in morphology and in their specific migratory routes. Xenograft experiments demonstrate that both reptilian and mammalian transplanted tangentially migrating cells integrate into the brain tissue of the other species, suggesting a common evolutionary past and the conservation of guidance mechanisms in distant species.

MATERIALS AND METHODS

Turtle eggs
All experimental procedures comply with EU and institutional regulations. Permission from the French Ministry of Ecology was given to C.P. to capture gravid females and collect limited numbers of turtle eggs every year in France. This study was performed over several years because only a limited number of eggs are accessible each year during a short period of time (4 weeks). A total of 420 eggs of *Emys orbicularis*, ranging from embryonic stages 13 to 20, were used. Eggs were collected as described by Dorizzi et al. (Dorizzi et al., 1996) and incubated either at 25°C or 30°C to modulate the development rate. Developmental stages were evaluated according to the timetable of Yntema in *C. serpentina* (Yntema, 1968) adapted to *E. orbicularis* by Pieau and Dorizzi (Pieau and Dorizzi, 1981).

Mouse embryos
For the MGE graft experiments, we used transgenic mice with reporter gene expression (Zambrowicz et al., 1997). Embryos that ubiquitously express the GFP or the β-galactosidase transgene were bred in the laboratory as described previously (Bellion et al., 2003; Bellion and Métin, 2005).

Fixation of turtle embryonic brains
Embryos were chilled on ice, removed from the shell and decapitated. At later embryonic stages, the skulls were hemisected above the brain to provide better access for the fixative, and then the whole heads were fixed in 4% paraformaldehyde in 0.12 mM phosphate buffer (pH 7.4) (PAF) at 4°C, and kept in fixative until histological processing. Brains destined for GABA immunostaining were fixed in 0.5% glutaraldehyde in PAF at 4°C for 3 hours and then stored in PAF at 4°C until histological processing.

Immunohistochemistry in fixed tissue
Fixed turtle brains were either sectioned with a slicer (Vibratome, Campden Instruments, UK) or with a cryostat (Jung CM3000, Leica, Germany) following cryoprotection in PBS containing 15% (w/v) sucrose. Sections prepared with the slicer (80-100 μm) were reacted whilst free floating, whereas sections prepared with the cryostat (10-20 μm) were reacted on the slide.

Sections and cultures were immunostained using the same protocols. If diaminobenzidine (DAB) was the final reaction product, tissues were pretreated for 1 hour in PBS containing 1% H_2O_2. For Nkx2.1, Tbr1, Pax6, GABA, Darpp-32 (also known as Ppp1r1b), β-tubulin (TuJ1; also known as βIII-tubulin and Tubb3) or GFP immunostaining, cultures or slices were preincubated 1 hour in PGT (PBS containing 2 g/L gelatin and 0.2% Triton X-100) and incubated overnight with the primary antibody (diluted in PGT): rabbit anti-Nkx2.1 serum (Biopat, 1/2000); rabbit anti-Tbr1 serum (generous gift of R. Hevner, University of Washington, Seattle, WA; 1/2500); mouse anti-Pax6 IgG (Developmental Studies Hybridoma Bank, 1/200); rabbit anti-GABA serum (Sigma, 1/10,000); rabbit anti-Darpp-32 serum (generous gift of J. A. Girault, Institut du Fer à Moulin, Paris, France; 1/60,000); mouse anti-TuJ1 (Babco, 1/5000); rabbit anti-GFP (Molecular Probes, 1/1000). Secondary antibodies were diluted 1/200 in PGT: Cy3-conjugated or Alexa 488-coupled goat anti-mouse or anti-rabbit IgG (Jackson Laboratories, Bar Harbor, ME) and biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA). The biotinylated antibodies were detected using the Vectastain ABC Elite Kit (Vector Laboratories) and DAB as a chromagen. Sections and cultures were mounted in Mowiol (0.1% in glycerol 25% in Tris buffer 0.1 M at pH 8.5) and observed on a Zeiss Axioskop microscope (Zeiss, Germany) or a Leica DMR microscope (Leica Microsystems, Germany). Grafted slices were observed using a confocal microscope (Leica, DMRE TCS-SP2).

Organotypic slice culture
Embryos were rinsed in cold PBS, decapitated and their heads transferred to cold Leibovitz (L15) medium supplemented with 50 U penicillin/ml, 50 μg streptomycin/ml (P/S). After removing the brain from the skull, embryonic brains were embedded in 2% type-VII agar (Sigma-Aldrich) in culture medium and sectioned using a manual slicer (Campden Instruments, WPI). Coronal sections (200 or 300 μm) comprising both the pallium and the subpallium were collected in cold (4°C) L15 culture medium containing P/S. Slices were transferred to Millicell chambers (inserts PICMORG50, 30 mm in diameter, pore size 0.4 μm, Millipore, Bedford, MA) on complete culture medium [DMEM/F12 medium supplemented with 2 mM L-glutamine, 33 mM D-Glucose, 3 mM sodium bicarbonate, 10 mM HEPES buffer (pH 7.4), P/S, 5% fetal calf serum and N2 and B27 supplement, all from Gibco BRL] and cultured for 3-5 days at 35°C in a 5% CO_2 humidified incubator.

Culture of explants
Small explants of the ventricular zone (VZ) were dissected with tungsten needles from defined subregions of pallium and subpallium in coronal sections under the binocular microscope. These explants were numbered as a function of their slice of origin (anterior, medium, posterior, see Fig. 2N). Four explants of the same origin were deposited on each poly-L-lysine/laminin-coated glass coverslip and cultured for 2 or 3 days in complete culture medium without fetal calf serum. In each experiment, two coverslips with explants from the same origin were cultured: one coverslip was fixed with PAF containing 33 mM sucrose and the other with PAF containing 33 mM sucrose and 0.1% glutaraldehyde for GABA immunohistochemistry.

Labeling and grafting in slice cultures
Cells migrating in slices were labeled by small deposits of lipophilic carbocyanine (DiI) or green fluorescent cell tracker (CMFDA, CFSE, Molecular Probes, OR) at various dorsoventral levels. Surface landmarks in the curvature of the ventricle were used to place tracers in pallial (DC, DVR) or subpallial (LGE, MGE) sites and at the palliostriatal boundary (PSB, see Fig. 2N). In our hands, DiI-labeled cultures with long incubation periods started to show additional undesirable retrograde labeling [also observed in fixed embryonic turtle brains (Cordery and Molnár, 1999)] and therefore analyses were mostly performed on slices labeled with CMFDA- or CFSE-impregnated bamboo fibers (see Table 1 for detailed numbers of the various paradigms). After 3-5 days in vitro, slices were fixed by immersion in PAF or in PAF containing 0.5% glutaraldehyde and conserved in PAF at 4°C. Homografts were performed with turtle explants incubated in F12-DMEM containing 40 μg/ml DiI for 1 hour at 35°C, and rinsed three times in F12-DMEM before grafting. Xenografts were performed with GFP- or β-galactosidase-expressing VZ explants dissected in the neocortex or in the MGE of E12.5 or E13.5 mouse embryos as explained elsewhere (Bellion et al., 2003; Bellion et al., 2005). Grafted slices were cultured and fixed as described above.

Table 1. Number of turtle forebrain slices that received cell tracker (CMFDA or CFSE) or DiI placements into the subpallium or pallium at each embryonic stage

Embryonic stage	Total	CMFDA/CFSE injection		DiI injection	
		Pallium	Subpallium	Pallium	Subpallium
13 to early14 (65-80 mg)	9	–	9	–	–
14 (75-90 mg)	30	3	13	5	9
15 (100-140 mg)	35	1	34	–	–
16 (150-190 mg)	33	3	13	7	10
17 to early 18 (200-240 mg)	22	–	22	–	–
18 (250-300 mg)	19	3	8	2	6
19 (300-390 mg)	23	–	23	–	–
20 (400-500 mg)	11	–	–	5	6

The range in weight at each stage is given in milligrams (mg) and follows the timetable of Pieau and Dorizzi (Pieau and Dorizzi, 1981). On average, three slice cultures were prepared from every telencephalon. The same proportion of anterior, medial and posterior slices was injected at each stage.

In ovo transplants

GFP-expressing VZ explants dissected from the neocortex or the MGE of E12.5 mouse embryos were stained with Fast Green so as to be able to verify the position of the transplanted tissue block after injecting them under direct visual control with a glass micropipette into the lateral ventricle of stage 17 turtle embryos. The shell was then closed with transparent tape and eggs returned to the incubator (humidified, 35°C, 5% CO_2).

RESULTS

Tangentially oriented GABA-immunopositive cells in the developing pallium and Nkx2.1-immunopositive cells in the developing striatum and DVR of *Emys orbicularis*

In mice, the distribution of GABAergic neurons in the developing cortex can provide insights into the timing and major routes of the tangential migration from the basal telencephalon to the cortex (Tanaka et al., 2003; López-Bendito et al., 2004), although a significant proportion of neurons migrating tangentially do not express GABA and some GABAergic cortical neurons might differentiate locally (Polleux et al., 2002; Bellion et al., 2003; Gulacsi and Lilien, 2003). To assess the timing of tangential migration in the turtle embryo, we followed the differentiation of GABAergic cells in the developing telencephalon. In early turtle embryos (stages 13/14, Fig. 1A) the thin pallium was devoid of GABA staining, whereas the thicker subpallium contained darkly stained GABA-immunopositive cells. At later stages, the dorsal pallium was invaded by GABAergic cells extending long and branched processes oriented tangentially to the pial surface (Fig. 1B). These processes were directed toward the dorsal cortex and ended with complex growth cones (Fig. 1B, white arrows). As embryonic development proceeded, the distribution of GABAergic cells progressed toward the dorsomedial border of the pallium. From stage 19, the density of GABA-immunopositive cells specifically decreased in the DVR, clearly showing the ventral border of the pallium (Fig. 1C, arrowhead).

In mice, a large proportion of GABAergic cortical neurons originate from the Nkx2.1-positive MGE (Shimamura et al., 1995; Sussel et al., 1999). In turtle embryos, cells in the ventricular zone (VZ) and mantle of the ventral subpallium were found to strongly express Nkx2.1 protein (Fig. 1D and Fig. 2A-C). At embryonic stages when GABAergic neurons largely colonize the dorsal cortex, Nkx2.1-positive cells became numerous in the mantle of the dorsal subpallium, suggesting that they migrated dorsally from the ventral subpallium (Fig. 1E,F). At stage 20, Nkx2.1-positive cells were also observed in the DVR and lateral cortex (LC), and some

even in the dorsal cortex (DC) (Fig. 1F). From stage 16, we observed low level Nkx2.1 protein expression in pallial progenitors and postmitotic cells.

Developmental gene expression in turtle telencephalon

To establish homologies between morphological subdivisions of the embryonic turtle and mouse telencephalon, we analyzed the distribution of Pax6, Tbr1 and Nkx2.1 proteins in turtle embryos (Fig. 2). In mice, Tbr1 is restricted to postmitotic cells in the pallium, at the pial surface of the ventral subpallium, and in the caudal ganglionic eminence (CGE) (Sussel et al., 1999; Puelles et al., 2000). Tbr1 is a good marker of the pallial-subpallial boundary (PSB) because its expression stops abruptly at this frontier. By contrast, Pax6 is strongly expressed in the VZ of the pallium. It is also expressed in the VZ of the embryonic striatum [or lateral ganglionic eminence (LGE)] and in postmitotic cells at the surface of the LGE (Sussel et al., 1999; Stoykova et al., 2000; Puelles et al., 2000).

In stage 14 turtle embryos, Nkx2.1 protein expression was restricted to the ventral subpallium. In the rostral telencephalon, the dorsal limit of Nkx2.1 expression (Fig. 2A-C, arrows) was slightly dorsal to a small groove dividing the subpallium into two dorsal and ventral ridges – the LGE and MGE, respectively (Fig. 2, dashed line). Ventrally, the Nkx2.1-positive domain extended until the ventral midline across the presumptive preoptic and entopeduncular areas (Fig. 2A-C). The LGE was limited ventrally by the Nkx2.1-positive domain and extended dorsally until the PSB identified by the ventral limit of Tbr1 expression in pallial cells (Fig. 2D-F, arrows). Morphologically, this boundary coincides with a change in curvature of the ventricular neuroepithelium and with a change in thickness of the telencephalon. In the LGE, Pax6 immunoreactivity was faintly expressed in the ventricular zone according to a decreasing dorsoventral gradient and strongly expressed in postmitotic cells at the pial surface of the mantle zone (Fig. 2G-I, white arrowheads).

From stage 16, the borders of the LGE were determined dorsally by the ventral limit of the Tbr1-positive pallial domain (Fig. 2K) and ventrally by the dorsal limit of the Nkx2.1-positive VZ (Fig. 2M). The LGE contained a dense and large cluster of Darpp-32-positive cells (Fig. 2J,L). Darpp-32 is known to be expressed in embryonic rodent striatum (Guennoun and Bloch, 1992) and in the adult striatum of both turtles and mammals (Ouimet et al., 1983; Smeets et al., 2003). Therefore, Darpp-32 expression was used to localize the LGE in cultured slices (see Fig. 3). The DVR differentiates dorsal to the PSB (Fig. 2G-I, dorsal to arrows).

Fig. 1. Distribution of GABA and Nkx2.1 immunoreactivity in the developing turtle telencephalon. (**A-C**) Detection of GABA in coronal sections of embryonic turtle brains from the level close to the optic chiasm region. At stage 14 (A), cells above the VZ of the pallium are GABA immunonegative. By contrast, the VZ of the subpallium shows an intense neuropil labeling and the mantle contains darkly stained cells. At stage 16 (B), tangentially oriented GABA-immunoreactive cells are present throughout the entire pallium with the exception of the dorsal-most sector. (Inset) Enlarged view showing neurites ending with large growth cones (white arrows). By this stage, the density of GABA-positive cells is increased in the VZ of the dorsal and medial ridges (the LGE and MGE, respectively) of the subpallium, which are separated by a slight depression (dashed line). At stage 19 (C), GABA-immunopositive cells distribute more medially in the dorsal pallium. A clear transition in GABA staining occurs at the pallial-subpallial boundary dorsal to the sulcus between the enlarged DVR and LGE (arrowhead). GABA-positive cells still concentrate in the VZ of the LGE. (**D-F**) Detection of Nkx2.1 in coronal sections of embryonic turtle brains using fluorescent secondary antibodies. The depression or groove (dashed line) separating the LGE and MGE correlates well with the dorsal limit of Nkx2.1 expression in the subpallial VZ (white arrow). Nkx2.1-positive cells distribute in the mantle of the MGE from stage 14 (D), in the mantle of the LGE at stage 17 (E), and in the striatum, DVR and LC at stage 20 (F). (Inset) Enlarged view showing sparse immunopositive cells in the DC. DC, dorsal cortex; DVR, dorsal ventricular ridge; LC, lateral cortex; LGE, lateral ganglionic eminence; m, mantle; MGE, medial ganglionic eminence; P, pallium; SP, subpallium; Str, striatum; VZ, ventricular zone. Scale bars: 500 μm.
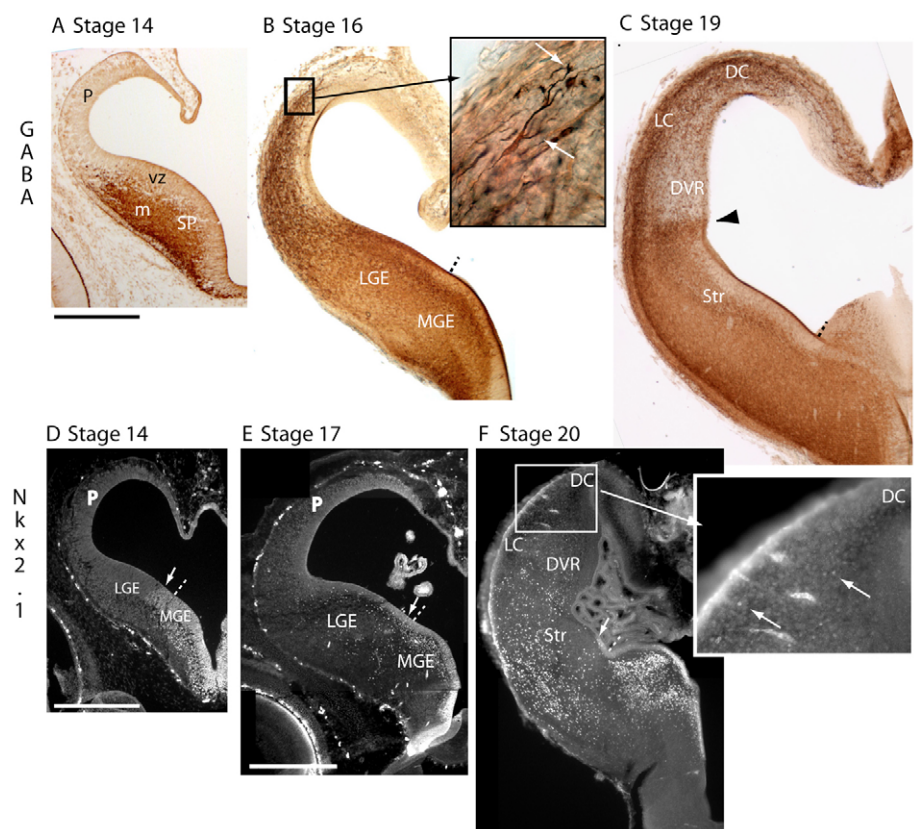
Origin and timetable of tangential migrations in the turtle forebrain

GABA and Nkx2.1 immunostaining suggested that subpallial cells might start to migrate tangentially toward the dorsal pallium beginning at stage 14 in turtle embryos. We investigated the source of these cells in areas in the subpallium. We also searched for evidence of tangential migration from the DVR and dorsal cortex.

Injection sites were classified using Darpp-32 staining in cultured slices. Injections within the VZ of the ventral ridge of the subpallium were located below the Darpp-32-positive embryonic striatum and were classified as 'MGE placement sites'. Injections within the VZ of the dorsal ridge of the subpallium were located at the ventricular side of the Darpp-32-positive region, and were classified as 'LGE tracer placement sites'. Injections at the limit of the thin pallium and the thick subpallium were located above the Darpp-32-positive region and below the DVR that protruded and filled the ventricle in cultured slices, and these were classified as 'PSB placement sites'. Table 1 summarizes the number of slices injected at each stage and Fig. 4 gives the number of slices injected in DVR, PSB, LGE and MGE. We also examined a few CGE placements that are not shown.

In stage 13 and 14 slices, a few tangentially migrating cells were labeled from the ventral half of the subpallium (presumptive MGE). At a slightly older stage (stage 14/15, see Fig. S1B′-C′ in the supplementary material), tracer placements in the ventral subpallium labeled a vast number of tangentially oriented cells in the dorsal telencephalon, confirming that the tangential migration of MGE cells to the pallium starts at late stage 14. Labeled cells followed specific routes along the ventricle in the LGE and DVR as in older slices (see Fig. S1 in the supplementary material and Fig. 4). Injections into the dorsal half of the subpallium (presumptive LGE) labeled radially distributed cells in embryonic striatum. Injections at the PSB labeled cells near the pial surface both dorsal and ventral to the injection site.

At stages 15 and 16, injection of the tracer CMFDA into the same site gave a consistent pattern of labeling and so these stages were analyzed together. Subpallial CMFDA placements labeled numerous cells in the pallium, and MGE placement labeled the most numerous wave of tangentially migrating cells. Cells from the MGE (Fig. 3C,D) distributed along the ventricle in both the ventral and dorsal telencephalon, and colonized the whole lateral cortex and the lateral part of the dorsal cortex, but were not observed in the hippocampus. Surprisingly, the cell density within the mantle zone of the DVR was lower than in the neighboring cortex or in the ventricular zone.

LGE injections labeled numerous cells in the Darpp-32-positive sector of the striatum (Fig. 3B-B″). As in stage 14 slices, CMFDA placements performed in the PSB (Fig. 3A) labeled numerous cells in the dorsal telencephalon. Again, labeled cells were less numerous within the core of the DVR than along the pia or the ventricle. Cells labeled from the PSB also distributed to the ventral embryonic striatum.
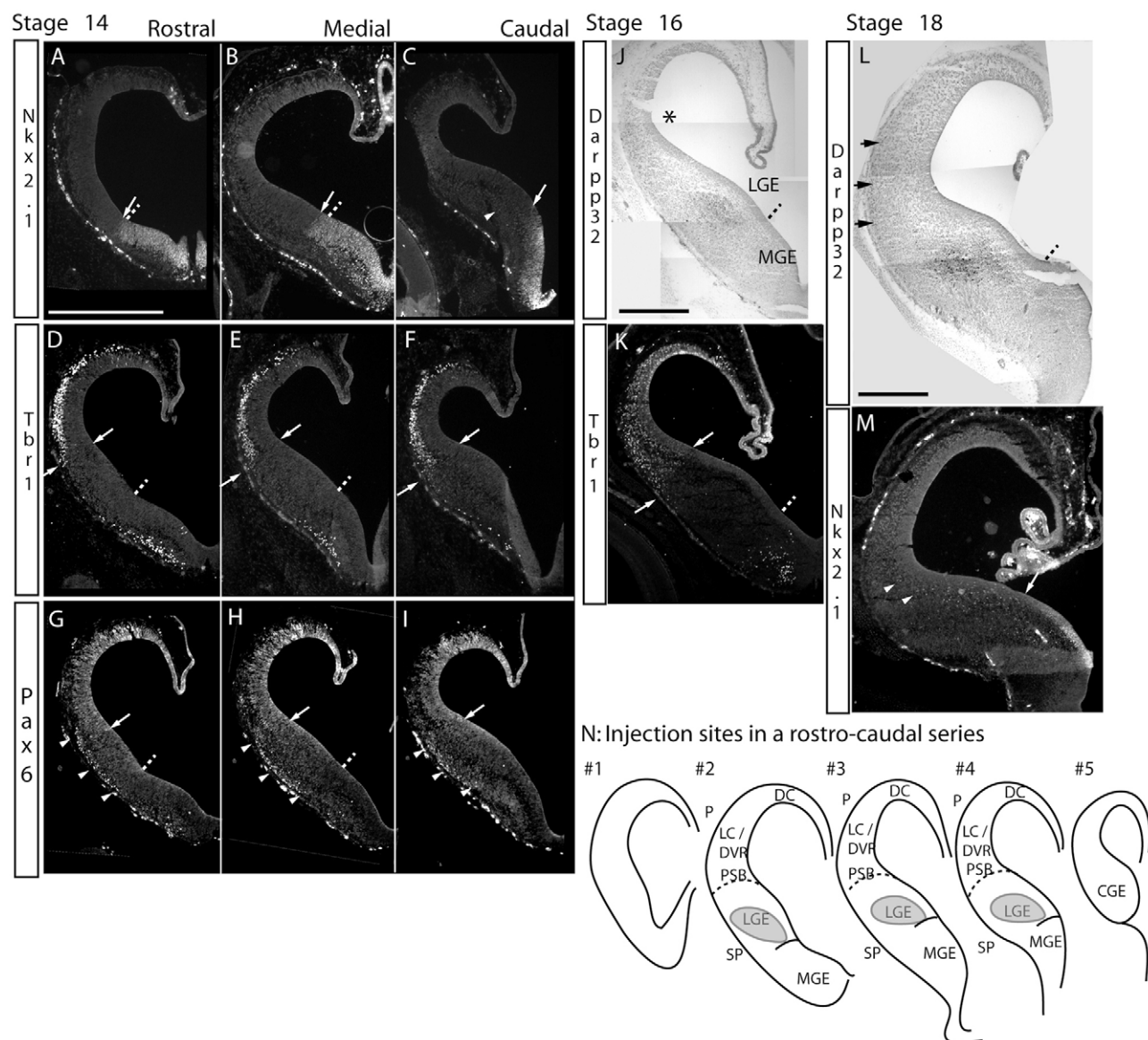
Fig. 2. Regionalized expression of Nkx2.1, Tbr1, Pax6 and Darpp-32 in the telencephalon of turtle embryos. Nkx2.1 (A-C,M), Tbr1 (D-F,K), Pax6 (G-I) and Darpp-32 (J,L). (**A-I**) Coronal sections of stage 14 turtle embryos at three distinct rostrocaudal levels. (**J-M**) Stage 16 (J,K) and stage 18 (L,M) coronal sections. (J,L) Darpp-32-immunoreactive cells form a compact region in the embryonic striatum (mantle zone of the LGE) and distribute under the pia in the presumptive lateral cortex (arrows in L). Scale bars: 500 μm. (**N**) Drawings of the rostrocaudal series of thick (200-300 μm) coronal sections of a stage 16 turtle telencephalon showing limits of the presumptive MGE, LGE, LC and DVR (LC/DVR) and DC. The Darpp-32-positive area is indicated in gray.

By contrast, cells labeled in the dorsal telencephalon did not migrate to the subpallium. Cells from the ventricular zone of the DVR remained within the DVR. DiI crystal placements into the DVR and/or the dorsal cortex labeled cortical cells that might be retrogradely labeled (not illustrated), which can also be observed in fixed material (Cordery and Molnár, 1999).

From stage 17, the number of cells labeled from the dorsal half of the subpallium (LGE placements) increased (Fig. 3F). Cells labeled from the LGE distributed throughout the whole pallium (DVR and dorsal cortex), although their density was highest along the ventricle. At stage 17, MGE placements labeled fewer cells, mostly in the ventricular zone of the ventral and dorsal telencephalon (Fig. 3G). At later stages (18-19), MGE tracer placements rarely labeled cells outside the MGE, whereas LGE placements still labeled high numbers of cells in the pallium (Fig. 4). Tracing from the PSB labeled cells that were more likely to be distributed to the lateral cortex and the pial surface of the DVR (Fig. 3H).

Telencephalic explants from turtle embryos release long-distance migrating cells in vitro

Cells labeled from the MGE distributed along the VZ of the LGE and PSB, suggesting that they migrated along the ventricle. Therefore, tracer placement into the VZ of the LGE or PSB in slices might have labeled migrating MGE cells in addition to LGE and PSB cells. In spite of this possibility, we observed that cells labeled from the PSB, LGE or MGE differed in their distribution patterns at each stage examined (Fig. 4).
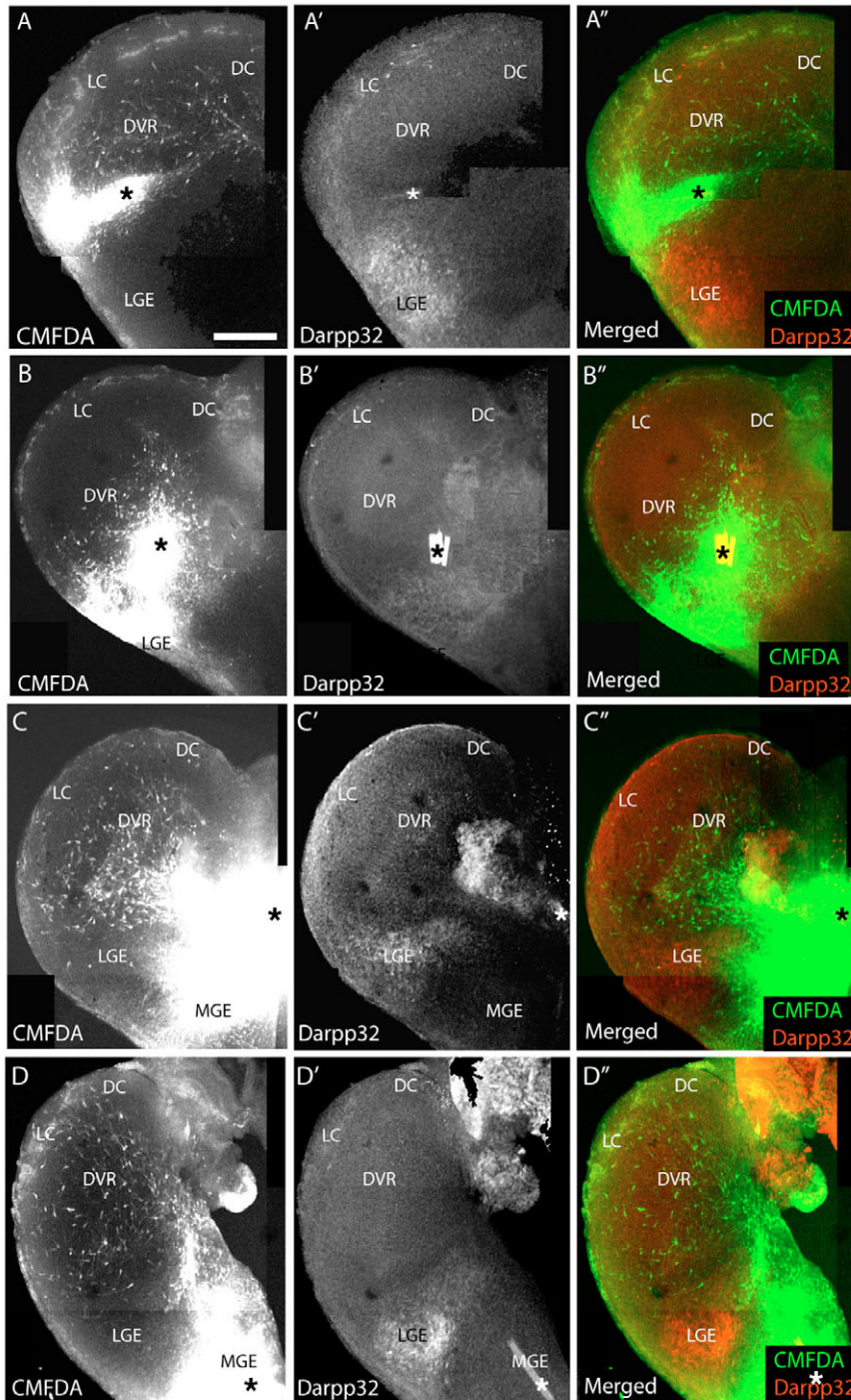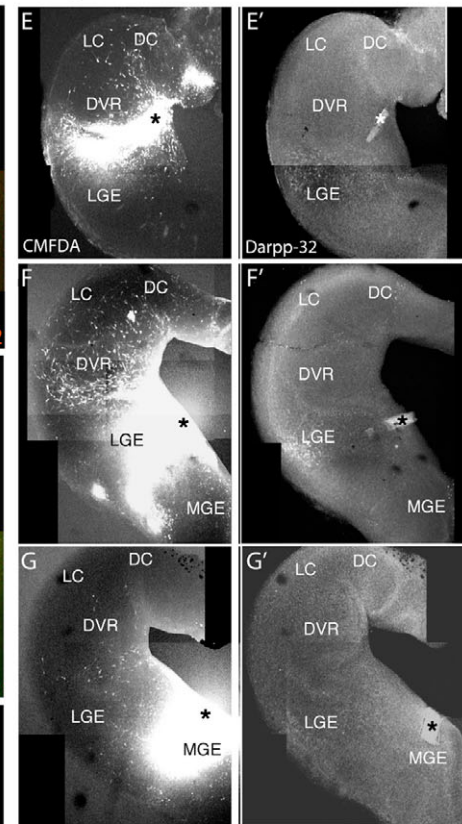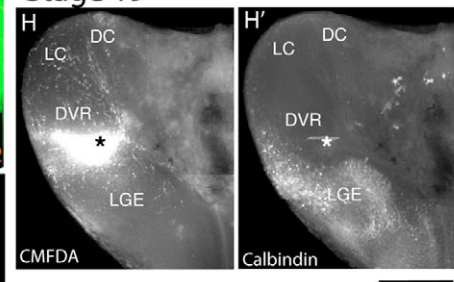
Fig. 3. CMFDA tracer injections into the subpallium and pallial-subpallial boundary (PSB) label cells in the pallium. (**A-D″**) Four coronal slices from stage 15 turtle embryos were labeled from the VZ of the PSB (A), LGE (B) or MGE (C, rostral and D, caudal) with CMFDA-coated bamboo fibers (black or white stars), cultured for 5 days, fixed in PAF and immunoreacted with Darpp-32 antibodies (A′,B′,C′,D′). (A″,B″,C″,D″) Merged images (CMDFA, green; Darpp-32, red). (A) A PSB injection ventral to the DVR and dorsal to the Darpp-32-positive LGE area (A′) labeled cells in the DVR and LC but not in the DC. (B) Tracer placement into the LGE labeled numerous cells oriented radially in the Darpp-32-positive area (B′) and few cells in the pallium. (C,D) MGE injections ventral to the Darpp-32-positive area (C′,D′) labeled cells between the Darpp-32-positive LGE and DVR, in the LC and along the VZ of the DVR (C″,D″). A few labeled cells are present in the DC. Scale bar: 200 μm. (**E-H′**) Four coronal slices from turtle embryos at stage 17 (E-G′) or 19 (H,H′) were labeled with CMDFA-coated bamboo fibers (black or white stars) placed into the VZ of the PSB (E,H), LGE (F) or MGE (G) and stained for Darpp-32 (E′-G′) or calbindin (H′) immunoreactivity after fixation. PSB placements labeled a very large number of cells in the PSB (E,H), and labeled scattered cells in the DVR and cortex (LC, DC). LGE placements at stage 17 (F) labeled numerous cells in the pallium, at the PSB and along the pallial VZ. By contrast, MGE placements (G) no longer labeled cells in the pallium, and labeled few cells along the PSB. Scale bars: 200 μm in E-G′; 500 μm in H,H′.
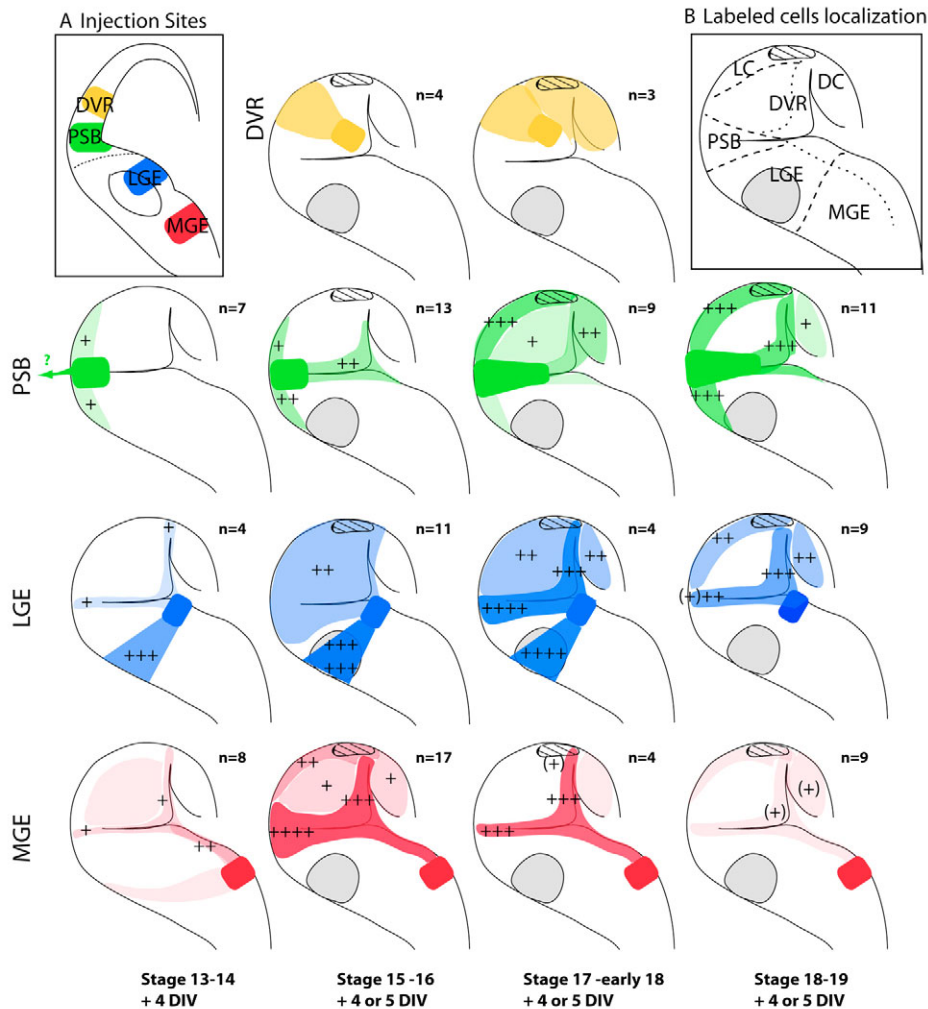
Fig. 4. Summary of migration patterns from different sectors in embryonic turtle forebrain at various stages. CMDFA placement sites are represented by a color code (see **A**). In labeled and cultured slices, the density of labeled cells was evaluated in sectors (as defined in **B**). The dotted line in the DVR, LGE and MGE separates the VZ and postmitotic areas that were analyzed separately. Gray and striped areas show the location of Darpp-32-positive cells in the LGE and the lateral cortex, respectively. Each scheme summarizes the mean distribution of labeled cells as observed in a number (n) of slices labeled from the same area at same stage. Injection sites are arranged into rows and stages are arranged in columns with the stages indicated at the bottom. The analysis was semi-quantitative: in each sector of each injected slice, the number of labeled cells was evaluated as: +++, high; ++, medium; +, low; (+), half that of low. The mean number of labeled cells in each area was multiplied by two and translated into a color code proportional to the cell density (see text).

To further characterize the source of tangentially migrating cells, we examined migration from telencephalic ventricular zone pieces explanted at five different dorsoventral levels and cultured on a laminin substrate for 2 days (Fig. 5A-C and Table 2).

From stages 14 and 16, MGE explants were surrounded by and released far more cells than dorsal cortex explants at any stage (Fig. 5A,B). At stage 18, the number of cells released by MGE explants decreased, whereas cell migration from LGE explants that started at stage 16 increased (Fig. 5C). PSB explants that already released a significant number of cells at stage 14, released an increasing number of cells at later stages, as observed around LGE explants.

By contrast, explants from pallial origin (DVR or DC) released very few cells at any stage. Variations in cell number were observed around DVR explants, possibly owing to slight variation in explant position with regard to the ventral limit of the pallium. Cortical explants extended long and numerous axons that were consistently denser and longer than axons growing from PSB and LGE explants (Fig. 5A).

The morphology of cells surrounding MGE and PSB explants differed significantly at all stages examined. PSB explants released cells with large soma and long leading processes (Fig. 5D). By contrast, cells migrating from MGE explants exhibited smaller cell bodies and extended short neurites tipped by very large growth cones (Fig. 5E). At stage 16, cells around LGE explants resembled cells around PSB explants, although we also observed cells with short leading processes and large growth cones, as observed around MGE explants. This suggested that cells migrating from the MGE had already reached the LGE at stage 16.

Similarly, cells labeled from MGE tracer placement sites in organotypic slices exhibited shorter processes and smaller cell bodies than cells labeled from the PSB (Fig. 5H-I″). Moreover, MGE and PSB cells differed in density and distribution. To assess the GABAergic phenotype of cells labeled from both sites, CMFDA-injected slices were immunostained with GABA antibodies. Large proportions of double-labeled cells were observed in all reacted slice cultures (Fig. 5H″,I″).

Altogether, the above observations strongly suggest that two different populations of tangentially migrating cells are produced in the basal telencephalon of turtle embryos: (1) cells extending short processes ending with large growth cones in vitro are mostly produced from stages 14 to 18 in the MGE of the basal telencephalon; and (2) cells extending longer processes ending with thinner growth cones in vitro are produced in the dorsal subpallium from early embryonic stages. The origin of the latter population might be initially restricted to an area close to the PSB, which then extends to the rest of the LGE as embryonic development proceeds.
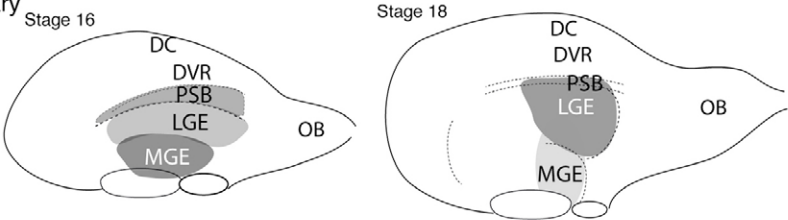
Fig. 5. Morphology and migration properties of cells released from the PSB or MGE explants in culture. (**A-G**) Cell migration and axonal outgrowth from stage 14 (A), 16 (B) and 18 (C) telencephalic turtle explants cultured on a laminin substrate. Explants prepared from the DC, DVR, PSB, LGE or MGE were cultured for 2 days and stained for GABA or β-tubulin (TuJ1). Large numbers of GABAergic neurons migrated from MGE explants at stage 14 and 16, and from LGE explants at stage 18. PSB explants released a significant number of cells from stage 14 to 18. Cells around the DVR and cortical explants were always few in number. Long and dense axons were only observed around pallial explants. At high magnification, cells released from PSB (D) explants show longer neurites and larger somas than cells released from MGE (E) explants, which show large growth cones. (F) Summary of semi-quantitative analysis of the cell migration and axonal outgrowth (by the method explained in Fig. 4 legend). At least six explants of the same origin were analyzed at each stage. (G) Diagrams showing lateral views of stage 16 and 18 hemispheres summarizing the origin of explants that released migrating cells in vitro. Regions in dark gray released high cell densities, those in light gray released few cells. As development proceeds, LGE and PSB explants produce more cells, whereas this capacity decreased in the MGE. (**H-I″**) CMFDA placement (asterisk) in the PSB (H) of a stage 16 slice labeled cells with thick leading neurites and frequent bifurcations (H′, arrows). Placement in the MGE (I) of a stage 16 slice labeled densely packed cells that showed simpler and thinner neurites (I′). GABA immunostaining (red in H″,I″) revealed numerous processes and cell bodies in slices. In optical sections obtained with confocal microscopy at the surface of each slice (H″,I″), a significant proportion of cells labeled from the PSB or MGE with CMFDA are GABA-immunopositive (arrowheads). Scale bars: 50 μm in C,D,H-I″; 100 μm in E.
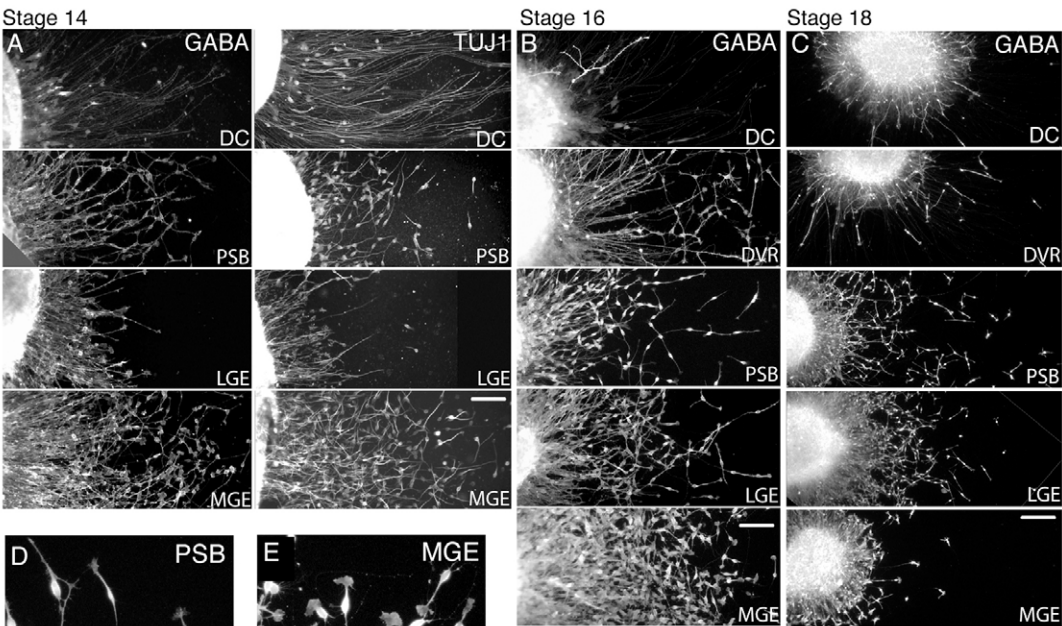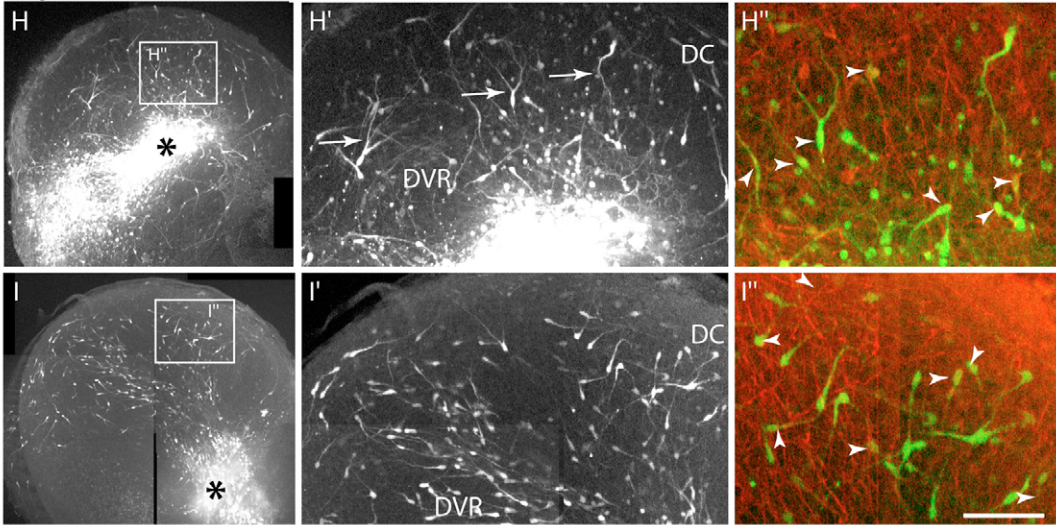

F Semi-quantitative analysis of cell migration

Stage	GABAergic cells density			Axons density	
	14	16	18	14	16-18
Dorsal CX	+	(+)	(+)	+++	+++
DVR		+	(+)		++
PSB (ant.)	++	++	+++	+	+
PSB (post.)		++	+		(+)
LGE	+	++	+++	+	+
MGE	+++	+++	+	(+)	(+)

Table 2. Embryonic stage and origin of turtle forebrain explants cultured on laminin

Explant origin	Stage		
	14	16	18
Dorsal cortex	8	12	7
DVR	–	13	5
PSB (ant.)	8	12	6
PSB (post.)	–	8	3
LGE	8	10	6
MGE	7	10	5

Shown is the number of explants originating for subregions of the pallium and subpallium at stages 14, 16 and 18 and cultured for 2 days on laminin-coated glass coverslips.
ant., anterior.
post., posterior.

In ovo and trans-species grafting experiments

We examined whether migration patterns observed in turtle embryos are governed by conserved mechanisms in distant species by combining mammalian and reptilian embryonic brain tissue in transplant experiments. DiI-labeled explants from stage 16 to 18 turtle subpallium grafted into coronal slice cultures from E12.5-13.5 mouse embryos (Fig. 6A-B″, Table 3) released cells that showed characteristic migration patterns in the mouse host forebrain depending on their site of origin. Turtle LGE or PSB explants grafted close to the mouse PSB released cells that migrated along this boundary and accumulated ventrally at the pial surface in the piriform cortex and/or presumptive amygdala (Fig. 6A). Very few turtle cells were observed in the lateral cortex, and no cells were observed in the host dorsal cortex. Turtle MGE explants placed in the mouse MGE released individual cells that migrated tangentially to the cortex of the mouse host slice (Fig. 6B′). Some grafted turtle cells began to develop dendritic arbors, indicating that the mammalian host cortex offered a permissive environment for reptilian cell maturation (Fig. 6B″). Thus, turtle subpallial cells exhibit the same specific migratory properties in rodent and turtle brains, revealing that they retain their migratory properties outside their brain of origin and that the same migratory cues were present in the mammalian host brain.

Conversely, we tested whether the response of mouse MGE cells to guidance cues present in the turtle dorsal telencephalon had been retained between these two distant species during evolution. GFP-expressing MGE explants from E12.5-13.5 mouse embryos were grafted into stage 16, 18 and 20 turtle slices (Table 3). GFP-expressing mouse cells spread dorsally throughout the whole turtle forebrain (Fig. 6C). They colonized the lateral and dorsal turtle pallium where they exhibited the characteristic polarized morphology of migrating cells. Grafted mouse MGE cells were less numerous in the core of the DVR, in the medial pallium (the presumptive hippocampus) and septum. By comparison, mouse cortical explants released very few migrating cells into turtle slices (Fig. 6E).

To follow the cells for longer in a native three-dimensional environment, we performed grafting experiments in turtle embryos in ovo. A turtle hemisphere was injected at stage 17 with a GFP-expressing E12.5 mouse MGE explant. Following 13 days incubation in ovo, the MGE explant was no longer visible, but dispersed GFP-expressing cells were remarkably restricted to the dorsal telencephalon (Fig. 6D). They distributed into the DVR, the LC and the lateral-most part of the DC, but did not invade the dorsomedial cortex. Only a few cells distributed along the VZ and the pial surface of the embryonic striatum. In the DVR, very few

mouse cells colonized the central core nucleus, as found in turtle cortical slice cultures. As observed in turtle slices, mouse cortical cells did not migrate very far when injected into the lateral ventricle of a stage 17 turtle embryo (Fig. 6F).

These experiments show that MGE cells from mouse and turtle embryos are able to migrate to and colonize the dorsal telencephalon (in a specific manner) of the other species in vitro. Additionally, the mouse cortex offers a permissive environment for the maturation of GABAergic cells released from turtle MGE explants. Moreover, mouse MGE cells distribute to the same pallial areas of the turtle brain that turtle MGE cells do – to the LC and DC, but not to the core of the DVR, although this structure is absent in mammals. Thus, MGE cells respond to similar local guidance cues in the embryonic dorsal telencephalon of these two distant species.

DISCUSSION

Our study investigated whether the tangential mode of migration exists in reptiles and, if so, how it contributes to embryonic forebrain development. We show that a large population of cells migrates tangentially to the dorsal telencephalon in the turtle embryo whose genesis is regulated in time and is restricted to defined subregions corresponding to the PSB and to the LGE and MGE in the basal forebrain. These sectors generate and release GABAergic neurons that have the capacity to migrate tangentially to the lateral and dorsal cortex. Therefore, similar to avian (Cobos et al., 2001a; Cobos et al., 2001b; Tuorto et al., 2003) and mammalian (Parnavelas, 2000; Corbin et al., 2001; Marin and Rubenstein, 2003) brains, tangential migration of GABAergic neurons of subpallial origin are present in the reptilian cortex.

The number and location of sectors producing tangentially migrating cells and migratory pathways are remarkably similar in reptiles and mammals and they express similar patterns of developmental genes. This suggests that conserved mechanisms are responsible for the genesis of subpopulations of tangentially migrating cells. Chimeric graft culture and in ovo transplant experiments suggest that conserved signals control the migratory routes of these cells. By contrast, the number of tangentially migrating cells generated at the PSB is higher in reptiles than in mammals, according to differences in the developmental rules that orchestrate the development of the corticostriatal boundary in the two species (Puelles et al., 2000).

Common subpallial origin of GABAergic neurons in the telencephalon of reptiles and mammals

Although the dorsal cortex of sauropsids (birds and reptiles) only contains a fraction of the cell types found in the mammalian isocortex (Reiner, 1991; Reiner, 1993), the two antagonistic functional classes of neurons – GABAergic and glutamatergic – are present, suggesting that they were already present in the common ancestor (Goffinet, 1983; Goffinet et al., 1986; Blanton et al., 1987; Blanton and Kriegstein, 1991a; Blanton and Kriegstein, 1991b; Reiner, 1991). The percentage and distribution of inhibitory GABAergic neurons in avian and reptilian pallial regions resemble those seen in mammals (Veenman and Reiner, 1994; Jarvis et al., 2005).

In rodents, most GABAergic cortical interneurons derive from the Nkx2.1- and Lhx6-positive MGE (Ladvas et al., 1999; Sussel et al., 1999; Wichterle et al., 1999; Xu et al., 2004). In the turtle embryo, early cohorts of tangentially migrating cells are released from a Nkx2.1-immunopositive region between stages 14 and 18. Later cohorts originate in the LGE, a Darpp-32- and Pax6-immunopositive region dorsal to the MGE and ventral to the PSB, as reported in
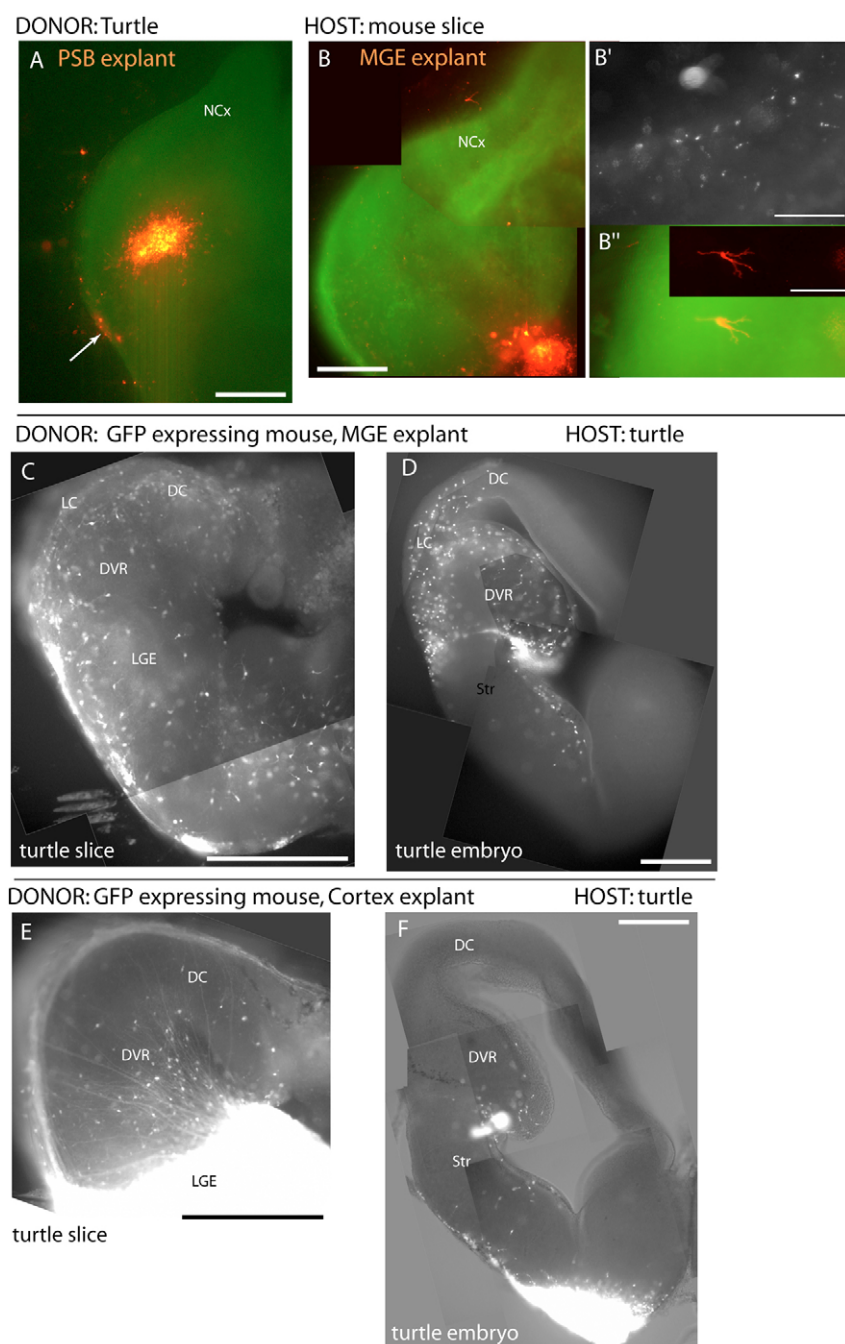
Fig. 6. Mouse-turtle interspecies grafting experiments reveal conserved guidance mechanisms in mammals and reptiles. (**A-B'**) When DiI-labeled explants of PSB and MGE of stage 16 to 18 turtle embryos were grafted into E13.5 mouse forebrain slices, the released labeled cells (red) migrated within the host tissue. Cells from PSB explants grafted into the corticostriatal boundary (A) migrated along the corticostriatal boundary and accumulated ventrally (arrow), whereas MGE cells grafted into the basal telencephalon (B,B') migrated in the orthogonal direction, across the corticostriatal boundary. Turtle MGE cells dispersed and migrated as individuals to colonize the mouse cortex (B') and some developed branched neurites in the host tissue (B"). (**C-F**) GFP-expressing MGE explants from E12.5 mouse embryos grafted into stage 16 turtle slices (C) or into stage 17 telencephalic vesicles in ovo (D) were no longer visible after a few days. Individual GFP-positive mouse MGE cells migrated long distances within the telencephalon of turtle embryos, colonized the entire slice (C), or the pallium in the in ovo experiments (D). By contrast, cortical explants (E,F) still formed a compact mass of tissue several days after grafting and released very few cells. The few cells that were released did not migrate very far, either in grafted slice (E) or grafted hemisphere (F). Scale bars: 500 μm in A,B,C-F; 200 μm in B'; 40 μm in B".

mouse (De Carlos et al., 1996; Marin and Rubenstein, 2003). Injections into the subpallium of caudal slices label large numbers of cells in the DVR and dorsal cortex (C.M. and Z.M., unpublished), suggesting that the turtle CGE releases cells migrating tangentially to the pallium as described in the mouse (Nery et al., 2002; Yozu et al., 2005). Similarly, the PSB in turtle releases cells that migrate dorsally and ventrally along the pial surface of the telencephalon (Bielle et al., 2005). In contrast to mice, these cells are numerous at early embryonic stages in turtle (stage 14) and their number increases as embryonic development proceeds. This confirms that cell production at the PSB junction differs strikingly between turtles and mice during embryonic development (Fernandez et al., 1998). In birds, it has been demonstrated from chick-quail graft studies and organotypic slice culture experiments that, similar to rodents, most

GABAergic interneurons originate in the ventral telencephalon (Cobos et al., 2001a; Cobos et al., 2001b; Tuorto et al., 2003). However, the relative contribution of cohorts generated in the ventral pallidum (MGE) and in the dorsal subpallium (paleostriatum, LGE) to the GABAergic population in the dorsal telencephalon is emphasized slightly differently in these studies (Cobos et al., 2001a; Cobos et al., 2001b; Tuorto et al., 2003).

Migratory routes: specificity and conserved properties between distant species

In turtle embryos, streams of tangentially migrating cells are situated in locations predicted from immunohistochemical studies (Blanton and Kriegstein, 1991b) (this study). Cells labeled from sites in the dorsal (LGE) or ventral (MGE) subpallium initially follow common

Table 3. Turtle-mouse interspecies graft experiments

| | | | | Turtle | | | |
| | | | | Stage 16-18 | | Stage 20 | |
Explant donor	Mouse E13.5	Explant donor		Pallium	Subpallium	Pallium	Subpallium
Turtle stage 16-18							
MGE	5	E12.5 mouse *EGFP*	Cortex	1	3	6	3
LGE	2		MGE	2	2	2	5
PSB	4	E12.5 mouse *lacZ*	Cortex	3	3	3	4
DVR	3		MGE	5	6	7	4

To the left is shown the number of E13.5 mouse forebrain slices grafted with turtle explants. To the right is shown the number of turtle forebrain slices (stages 16-18 or stage 20) grafted with E12.5 mouse explants expressing either *EGFP* or *lacZ*. Turtle explants were grafted in homotopic positions in mouse slices (DVR explants were placed in the lateral cortex). Mouse explants were placed in pallial (DVR or dorsal cortex) or subpallial (MGE or LGE) areas in turtle slices.

tangential migratory pathways, then reorient radially at the subpallium-DVR boundary, and distribute again tangentially to the ventricle in the pallium. Migratory routes show the same orientation with regard to pallial and subpallial molecular domains as described in birds (Tuorto et al., 2003) and mammals (De Carlos et al., 1996; Tanaka et al., 2003; López-Bendito et al., 2004). In both turtle and mouse, cells from the PSB also migrate radially along the PSB boundary and tangentially in the marginal zone (Bielle et al., 2005).

Nevertheless, cells labeled from the MGE, LGE and PSB in turtle slices show some specificity in their distribution patterns. In contrast to MGE cells that occasionally enter the central core of the DVR, cells from the LGE distribute within the whole DVR. Furthermore, unlike MGE and LGE cells, PSB cells do not migrate ventrally into the subpallium and do not distribute along the ventricle in the pallium. Therefore, the tangentially migrating cells generated in PSB, LGE and MGE each exhibit some specific migratory properties. However, in our experiments, cell movements were limited in the coronal plane and we did not investigate rostrocaudal migrations that are known to occur in rodents (Tanaka et al., 2006).

Our chimeric mouse-turtle slice culture and in ovo graft experiments indicate that the signals required for tangential migration and integration in a specific fashion are indeed highly conserved. The ability of the mammalian MGE cells to read developmental signals in the turtle embryonic brain is fascinating, considering the phylogenetic distance between the two species. Moreover, this process seems to be independent of the development of the DVR, which lies dorsal to the PSB. Indeed, mouse MGE cells behave as turtle MGE cells when they avoid the turtle DVR. Further chimeric graft experiments could help in testing current ideas on homologies. Our study suggests that the subcortical generation of the GABAergic interneurons and their tangential migration to the dorsal telencephalon during embryonic development is universal in mammals and sauropsids. GABAergic neurons in fixed embryonic turtle brains and cells labeled in cultured slices show branched leading processes similar in morphology to mammalian tangentially migrating neurons. This suggests common migratory mechanisms (Bellion et al., 2005). In addition, the capacity of cells generated in the subpallium to migrate tangentially over long distances is conserved at adult stage in both mammals and sauropsids (Perez-Canellas and Garcia-Verdugo, 1996; Perez-Canellas et al., 1997; Lois and Alvarez-Buylla, 1994).

Fate of tangentially migrating neurons
Our results support the view that several cohorts of tangentially migrating cells are generated in distinct sectors of the subpallium in reptiles, and that these cohorts distribute along specific migratory pathways. Differences in the neuritic morphology of tangentially migrating cells produced in the MGE and in the PSB strongly support the hypothesis that distinct populations of

tangentially migrating cells are produced in ventral and dorsal sectors of the subpallium. In mammals, recent studies have established a correlation between the spatiotemporal segregation of the neurogenesis and the generation of different populations of GABAergic neurons (Butt et al., 2005; Yozu et al., 2005). Accordingly, morphological subclasses of GABAergic interneurons have been observed in the turtle cortex (Blanton et al., 1987). It would be interesting to determine whether they originate in distinct cohorts and whether cells generated in the PSB contribute to the population of Cajal-Retzius cells in the turtle forebrain (Goffinet, 1983), as recently established in mammals (Bielle et al., 2005). Further studies that incorporate transcription factor and interneuron marker expression are required but might prove difficult because cells with strong Nkx2.1 expression are observed neither in mouse nor turtle pallium (Sussel et al., 1999) (this study). The molecular mechanisms underlying the production of tangentially migrating cohorts are not understood in turtle, but it is likely that some principles are conserved between sauropsids and mammals. In mammals, local and temporal variations in cell specification are likely to rely on the combinatorial expression of different transcription factors in different subregions (MGE, LGE and CGE) of the neuroepithelium (Nery et al., 2002) (for a review, see Métin et al., 2006). Many of these transcription factors have orthologs expressed in the ventral forebrain of reptiles and birds (Fernandez et al., 1998; Puelles et al., 2000) (this study). Therefore, it is likely to be a conserved genetic pathway that specifies the areas that produce different populations of GABAergic neurons in the turtle.

Implications for our current theories on the origin of the mammalian neocortex
In lizard, turtle and bird, the dorsal cortex appears rudimentary compared with that in mammals, but the DVR hosts most of the neurons required for the information-processing circuits that are homologous to the mammalian neocortex (Karten, 1969; Karten, 1997; Ulinski, 1983; Manger et al., 2002). This led Karten (Karten, 1997) to propose that a considerable population of mammalian neurons is generated outside the cortex and migrates into the cortex during development (Northcutt and Kaas, 1995; Karten, 1997). However, neurons migrating tangentially to the neocortex in mammals are mostly GABAergic and originate from domains that do not coincide with the domain considered homologous to the DVR as defined by Emx gene expression patterns (Fernandez et al., 1998; Puelles et al., 2000). Moreover, previous studies in birds (Cobos et al., 2001a; Cobos et al., 2001b; Tuorto et al., 2003) and our present study in turtle demonstrate tangential migration of GABAergic interneurons generated in a Dlx-expressing subpallial domain in sauropsids.

Therefore, it is more conceivable that changes in the local dorsal cortical neurogenetic program, together with some major rearrangements at the striatocortical junction (Puelles et al., 2000; Striedter, 2005; Molnár and Butler, 2002a; Molnár and Butler, 2002b), provide the foundation for remodeling the mammalian cerebral cortex. It is currently unclear how GABAergic populations increase together with the glutamatergic neurons whose expansion and greater complexity is related to the elaboration of the mitotic compartments in the mammalian germinal zone (Krubitzer and Kahn, 2003; Noctor et al., 2004; Lukaszewicz et al., 2005; Tamamaki, 2005; Molnár et al., 2006; Martinez-Cerdeno et al., 2006). Although the generation of the glutamatergic and GABAergic neurons is to a large extent separated in sauropsids and mammals, the balance between these two cell types is maintained. This must have required the evolution of as yet unknown regulatory mechanisms.

Supplementary material
Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/134/15/2815/DC1

References
Anderson, S. A., Eisenstat, D. D., Shi, L. and Rubenstein, J. L. (1997). Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* **278**, 474-476.
Angevine, J. B. and Sidman, R. L. (1961). Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* **192**, 766-768.
Bellion, A. and Métin, C. (2005). Early regionalisation of the neocortex and the medial ganglionic eminence. *Brain Res. Bull.* **66**, 402-409.
Bellion, A., Wassef, M. and Métin, C. (2003). Early differences in axonal outgrowth, cell migration and GABAergic differentiation properties between the dorsal and lateral cortex. *Cereb. Cortex* **13**, 203-214.
Bellion, A., Baudoin, J. P., Alvarez, C., Bornens, M. and Métin, C. (2005). Nucleokinesis in tangentially migrating neurons comprises two alternating phases: forward migration of the Golgi/centrosome associated with centrosome splitting and myosin contraction at the rear. *J. Neurosci.* **25**, 5691-5699.
Bielle, F., Griveau, A., Narboux-Neme, N., Vigneau, S., Sigrist, M., Arber, S., Wassef, M. and Pierani, A. (2005). Multiple origins of Cajal-Retzius cells at the borders of the developing pallium. *Nat. Neurosci.* **8**, 1002-1012.
Blanton, M. G. and Kriegstein, A. R. (1991a). Morphological differentiation of distinct neuronal classes in embryonic turtle cerebral cortex. *J. Comp. Neurol.* **310**, 558-570.
Blanton, M. G. and Kriegstein, A. R. (1991b). Appearance of putative amino acid neurotransmitters during differentiation of neurons in embryonic turtle cerebral cortex. *J. Comp. Neurol.* **310**, 571-592.
Blanton, M. G., Shen, J. M. and Kriegstein, A. R. (1987). Evidence for the inhibitory neurotransmitter gamma-aminobutyric acid in aspiny and sparsely spiny nonpyramidal neurons of the turtle dorsal cortex. *J. Comp. Neurol.* **259**, 277-297.
Bruce, L. L. and Neary, T. J. (1995). The limbic system of tetrapods: a comparative analysis of cortical and amygdalar populations. *Brain Behav. Evol.* **46**, 224-234.
Bulfone, A., Smiga, S. M., Shimamura, K., Puelles, L., Peterson, A. and Rubenstein, J. L. R. (1995). *T-brain (Tbr-1)*: a homologue of *Brachyury* whose expression defines molecularly distinct domains within the cerebral cortex. *Neuron* **15**, 63-78.
Butler, A. B. and Hodos, W. (2005). *Comparative Vertebrate Neuroanatomy* (2nd edn). Hoboken, NJ: Wiley.
Butt, S. J. B., Fucillo, M., Nery, S., Noctor, S., Kriegstein, A., Corbin, J. and Fishell, G. (2005). The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron* **48**, 591-604.
Chapouton, P., Gartner, A. and Gotz, M. (1999). The role of Pax6 in restricting cell migration between developing cortex and basal ganglia. *Development* **126**, 5569-5579.
Cobos, I., Puelles, L. and Martinez, S. (2001a). The avian telencephalic subpallium originates inhibitory neurons that invade tangentially the pallium (dorsal ventricular ridge and cortical areas). *Dev. Biol.* **239**, 30-45.
Cobos, I., Shimamura, K., Rubenstein, J. L., Martinez, S. and Puelles, L. (2001b). Fate map of the avian anterior forebrain at the four-somite stage, based on the analysis of quail-chick chimeras. *Dev. Biol.* **239**, 46-67.
Connors, B. W. and Kriegstein, A. R. (1986). Cellular physiology of the turtle visual cortex: distinctive properties of pyramidal and stellate neurons. *J. Neurosci.* **6**, 164-177.
Corbin, J. G., Nery, S. and Fishell, G. (2001). Telencephalic cells take a tangent: non-radial migration in the mammalian forebrain. *Nat. Neurosci.* Suppl. **4**, 1177-1182.
Cordery, P. and Molnár, Z. (1999). Embryonic development of connections in turtle pallium. *J. Comp. Neurol.* **413**, 26-54.
De Carlos, J. A., Lopez-Mascaraque, L. and Valverde, F. (1996). Dynamics of cell migration from the lateral ganglionic eminence in the rat. *J. Neurosci.* **16**, 6146-6156.
Dorizzi, M., Richard-Mercier, N. and Pieau, C. (1996). The ovary retains male potential after the thermosensitive period for sex determination in the turtle Emys orbicularis. *Differentiation* **60**, 193-201.
Fernandez, A. S., Pieau, C., Reperant, J., Boncinelli, E. and Wassef, M. (1998). Expression of the Emx-1 and Dlx-1 homeobox genes define three molecularly distinct domains in the telencephalon of mouse, chick, turtle and frog embryos: implications for the evolution of telencephalic subdivisions in amniotes. *Development* **125**, 2099-2111.
Fishell, G., Mason, C. A. and Hatten, M. E. (1993). Dispersion of neural progenitors within the germinal zones of the forebrain. *Nature* **362**, 636-638.
Goffinet, A. M. (1983). The embryonic development of the cortical plate in reptiles: a comparative study in Emys orbicularis and Lacerta agilis. *J. Comp. Neurol.* **215**, 437-452.
Goffinet, A. M., Daumerie, C., Langerwerf, B. and Pieau, C. (1986). Neurogenesis in reptilian cortical structures: 3H-thymidine autoradiographic analysis. *J. Comp. Neurol.* **243**, 106-116.
Guennoun, R. and Bloch, B. (1992). Ontogeny of D1 and Darpp-32 gene expression in the rat striatum: an in situ hybridization study. *Brain Res. Mol. Brain Res.* **12**, 131-139.
Gulacsi, A. and Lillien, L. (2003). Sonic hedgehog and bone morphogenetic protein regulate interneuron development from dorsal telencephalic progenitors in vitro. *J. Neurosci.* **23**, 9862-9872.
Hendry, S. H., Schwark, H. D., Jones, E. G. and Yan, J. (1987). Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. *J. Neurosci.* **7**, 1503-1519.
Hevner, R. F., Shi, L., Justice, N., Hsueh, Y.-P., Sheng, M., Smiga, S., Bulfone, A., Goffinet, A., Campagnoni, A. T. and Rubenstein, J. L. R. (2001). Tbr1 regulates differentiation of the preplate and layer 6. *Neuron* **29**, 353-366.
Jarvis, E. D., Gunturkun, O., Bruce, L., Csillag, A., Karten, H., Kuenzel, W., Medina, L., Paxinos, G., Perkel, D. J., Shimizu, T. et al. (2005). Avian brains and a new understanding of vertebrate brain evolution. *Nat. Rev. Neurosci.* **6**, 151-159.
Karten, H. J. (1969). The organization of the avian telencephalon and some speculations on the phylogeny of the amniote telencephalon. *Ann. N. Y. Acad. Sci.* **167**, 146-179.
Karten, H. J. (1997). Evolutionary developmental biology meets the brain: the origins of mammalian cortex. *Proc. Natl. Acad. Sci. USA* **94**, 2800-2804.
Krubitzer, L. and Kahn, D. M. (2003). Nature versus nurture revisited: an old idea with a new twist. *Prog. Neurobiol.* **70**, 33-52.
Lavdas, A. A., Grigoriou, M., Pachnis, V. and Parnavelas, J. G. (1999). The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. *J. Neurosci.* **19**, 7881-7888.
Lois, C. and Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. *Science* **264**, 1145-1148.
López-Bendito, G., Sturgess, K., Erdelyi, F., Szabo, G., Molnar, Z. and Paulsen, O. (2004). Preferential origin and layer destination of GAD65-GFP cortical interneurons. *Cereb. Cortex* **14**, 1122-1133.
Lukaszewicz, A., Savatier, P., Cortay, V., Giroud, P., Huissoud, C., Berland, M., Kennedy, H. and Dehay, C. (2005). G1 phase regulation, area-specific cell-cycle control and cytoarchitectonics in the primate cortex. *Neuron* **47**, 353-364.
Manger, P., Slutsky, D. and Molnár, Z. (2002). Subdivisions of visually responsive regions of the dorsal ventricular ridge of the iguana (Iguana iguana). *J. Comp. Neurol.* **453**, 226-246.
Marin, O. and Rubenstein, J. L. (2003). Cell migration in the forebrain. *Annu. Rev. Neurosci.* **26**, 441-483.
Martinez-Cerdeno, V., Noctor, S. C. and Kriegstein, A. R. (2006). The role of intermediate progenitor cells in the evolutionary expansion of the cerebral cortex. *Cereb. Cortex.* **16** Suppl. 1, i152-i161.
Métin, C., Baudoin, J.-P., Rakic, S. and Parnavelas, J. G. (2006). Cell and molecular mechanisms involved in the migration of cortical interneurons. *Eur. J. Neurosci.* **23**, 894-900.

Molnár, Z. and Butler, A. B. (2002a). The corticostriatal junction: a crucial region for forebrain development and evolution. *BioEssays* **24**, 530-541.

Molnár, Z. and Butler, A. B. (2002b). Neuronal changes during forebrain evolution in amniotes: an evolutionary developmental perspective. *Prog. Brain Res.* **136**, 21-38.

Molnár, Z., Métin, C., Stoykova, A., Tarabykin, V., Price, D. J., Francis, F., Meyer, G., Dehay, C. and Kennedy, H. (2006). Comparative aspects of cerebral cortical development. *Eur. J. Neurosci.* **23**, 921-934.

Nery, S., Fishell, G. and Corbin, J. G. (2002). The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. *Nat. Neurosci.* **5**, 1279-1287.

Nieuwenhuys, R., ten Donkelaar, H. J. and Nicholson, C. (1998). *The Central Nervous System of Vertebrates*. Berlin: Springer.

Noctor, S. C., Martinez-Cerdeno, V., Ivic, L. and Kriegstein, A. R. (2004). Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat. Neurosci.* **7**, 136-144.

Northcutt, R. G. and Kaas, J. H. (1995). The emergence and evolution of mammalian neocortex. *Trends Neurosci.* **18**, 373-379.

Ouimet, C. C., Miller, P. E., Hemmings, H. C., Walaas, S. I. and Greengard, P. (1983). DARPP-32, a dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein enriched in dopamine-innervated brain regions. *J. Neurosci.* **4**, 11-124.

Parnavelas, J. G. (2000). The origin and migration of cortical neurones: new vistas. *Trends Neurosci.* **23**, 126-131.

Perez-Canellas, M. M. and Garcia-Verdugo, J. M. (1996). Adult neurogenesis in the telencephalon of a lizard: a [3H]thymidine autoradiographic and bromodeoxyuridine immunocytochemical study. *Brain Res. Dev. Brain Res.* **93**, 49-61.

Perez-Canellas, M. M., Font, E. and Garcia-Verdugo, J. M. (1997). Postnatal neurogenesis in the telencephalon of turtles: evidence for nonradial migration of new neurons from distant proliferative ventricular zones to the olfactory bulbs. *Brain Res. Dev. Brain Res.* **101**, 125-137.

Pieau, C. and Dorizzi, M. (1981). Determination of temperature sensitive stages for sexual differentiation of the gonads in embryos of the turtle, *Emys orbicularis. J. Morphol*. **170**, 373-382.

Polleux, F., Whitford, K. L., Dijkhuizen, P. A., Vitalis, T. and Ghosh, A. (2002). Control of cortical interneuron migration by neurotrophins and PI3-kinase signaling. *Development* **129**, 3147–3160.

Puelles, L., Kuwana, E., Puelles, E., Bulfone, A., Shimamura, K., Keleher, J., Smiga, S. and Rubenstein, J. L. (2000). Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. *J. Comp. Neurol*. **424**, 409-438.

Reiner, A. (1991). A comparison of neurotransmitter-specific and neuropeptide-specific neuronal cell types present in the dorsal cortex in turtles with those present in the isocortex in mammals: implications for the evolution of isocortex. *Brain Behav. Evol*. **38**, 53-91.

Reiner, A. (1993). Neurotransmitter organization and connections of turtle cortex: implications for the evolution of mammalian isocortex. *Comp. Biochem. Physiol*. **104A**, 735-748.

Shimamura, K., Hartigan, D. J., Martinez, S., Puelles, L. and Rubenstein, J. L. R. (1995). Longitudinal organization of the anterior neural plate and neural tube. *Development* **121**, 3923-3933.

Smeets, W. J. A. J., Lopez, J. M. and Gonzalez, A. (2003). Immunohistochemical localization of DARPP-32 in the brain of the turtle,

Pseudemys scripta elegans: further assessment of its relationship with dopaminergic systems in reptiles. *J. Chem. Neuroanat*. **25**, 83-95.

Smith-Fernandez, A., Pieau, C., Reperant, J., Boncinelli, E. and Wassef, M. (1998). Expression of the Emx-1 and Dlx-1 homeobox genes define three molecularly distinct domains in the telencephalon of mouse, chick, turtle and frog embryos: implications for the evolution of telencephalic subdivisions in amniotes. *Development* **125**, 2099–2111.

Stoykova, A., Treichel, D., Hallonet, M. and Gruss, P. (2000). *Pax6* modulates the dorsoventral patterning of the mammalian telencephalon. *J. Neurosci*. **20**, 8042-8050.

Striedter, G. F. (1997). The telencephalon of tetrapods in evolution. *Brain Behav. Evol*. **49**, 179-213.

Striedter, G. F. (2005). *Principles of Brain Evolution*. Sutherland, MA: Sinauer Associates.

Sussel, L., Marin, O., Kimura, S. and Rubenstein, J. L. (1999). Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development* **126**, 3359-3370.

Tamamaki, N. (2005). Pyramidal neurons of upper cortical layers generated by NEX-positive progenitor cells in the subventricular zone. *Proc. Natl. Acad. Sci. USA* **102**, 17172-17177.

Tamamaki, N., Fujimori, K. E. and Takauji, R. (1997). Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. *J. Neurosci*. **17**, 8313-8323.

Tanaka, D., Nakaya, Y., Yanagawa, Y., Obata, K. and Murakami, F. (2003). Multimodal tangential migration of neocortical GABAergic neurons independent of GPI-anchored proteins. *Development* **130**, 5803-5813.

Tanaka, D. H., Maekawa, K., Yanagawa, Y., Obata, K. and Murakami, F. (2006). Multidirectional and multizonal tangential migration of GABAergic interneurons in the developing cerebral cortex. *Development* **133**, 2167-2176.

Tuorto, F., Alifragis, P., Failla, V., Parnavelas, J. G. and Gulisano, M. (2003). Tangential migration of cells from the basal to the dorsal telencephalic regions in the chick. *Eur. J. Neurosci*. **18**, 3388-3393.

Ulinski, P. S. (1983). *Dorsal Ventricular Ridge: A Treatise on Forebrain Organization in Reptiles and Birds*. New York: John Wiley.

Veenman, C. L. and Reiner, A. (1994). The distribution of GABA-containing perikarya, fibers, and terminals in the forebrain and midbrain of pigeons, with particular reference to the basal ganglia and its projection targets. *J. Comp. Neurol*. **339**, 209-250.

Wichterle, H., Garcia-Verdugo, J. M., Herrera, D. G. and Alvarez-Buylla, A. (1999). Young neurons from medial ganglionic eminence disperse in adult and embryonic brain. *Nat. Neurosci*. **2**, 461-466.

Xu, Q., Cobos, I., De La Cruz, E., Rubenstein, J. L. and Anderson, S. A. (2004). Origins of cortical interneuron subtypes. *J. Neurosci*. **24**, 2612-2622.

Yntema, C. L. (1968). A series of stages inn the embryonic development of *Chelydra serpentina. J. Morphol*. **125**, 219-251.

Yozu, M., Tabata, H. and Nakajima, K. (2005). The caudal migratory stream: a novel migratory stream of interneurons derived from the caudal ganglionic eminence in the developing mouse forebrain. *J. Neurosci*. **25**, 7268-7277.

Zambrowicz, B. P., Imamoto, A., Fiering, S., Herzenberg, L. A., Kerr, W. G. and Soriano, P. (1997). Disruption of overlapping transcripts in the ROSA beta geo 26 gene trap strain leads to widespread expression of beta-galactosidase in mouse embryos and hematopoietic cells. *Proc. Natl. Acad. Sci. USA* **94**, 3789-3794.