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SHORT COMMUNICATION
Tangential migration of cells from the basal to the dorsal telencephalic regions in the chick

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Abstract

The evolutionary relationship between telencephalic regions of the avian and mammalian brains has been a long-standing issue in comparative neuroanatomy. Based on various criteria, a number of homologous regions have been proposed. Recent studies in mammals have shown that basal regions of the telencephalon give rise to neurons that migrate dorsally and populate the cerebral cortex. In the present study we demonstrate that, similar to mammals, neurons from a ventricular region of the palaeo-striatal complex – the dorsal subpallial sulcus – of the chick telencephalon migrate dorsally to populate the developing pallium. Further characterization of these cells revealed that they express the neurotransmitter γ-aminobutyric acid, but not the calcium-binding protein calbindin. These findings provide evidence that the mouse and chick basal regions are not only homologous in terms of gene expression patterns and connectivity, but they both also contribute inhibitory interneurons to dorsal regions of the developing telencephalon.

Introduction

The establishment of homologies between telencephalic regions of different vertebrates has been a subject of debate in comparative neuroanatomy. Disagreements have arisen mainly because of the different criteria used by researchers to define homologies. Similarities in functional connectivity and homology in gene expression patterns have been used extensively. For example, similarities in sensory connectivity led to the proposal that several telencephalic regions are homologous, e.g. the dorsal neuroepithelium of the vertebrate forebrain (which will give rise to the laminated neocortex in mammals and to the non-laminated pallium in non-mammals), the mammalian isocortex and the avian dorsal ventricular ridge, and the mammalian ganglionic eminences and the avian palaeostriatal complex (Karten, 1997; Medina & Reiner, 2000; Streidter & Keefer, 2000). This suggests a common evolutionary and developmental origin of these regions. The relative expression patterns of homologous genes provide an alternative and accurate tool to compare potential homologous areas. Thus, several genes have been identified in the mouse brain that mark different domains in the developing telencephalon. By comparing the expression patterns of genes such as Dlx2, Nkx2.1, Pax-6, Tbr-1 and Emx-1, Puelles and colleagues (Puelles *et al*., 2000) were able to verify homologies between mammalian and avian telencephalic regions.

During corticogenesis in mammals, young neurons generated in the cortical ventricular zone migrate to their positions in the neocortex in an 'inside-out' sequence, with the earlier-born neurons positioned in the deeper layers and the later-born cells migrating past them and occupying more superficial layers (Angevine & Sidman, 1961; Caviness & Sidman, 1973; Rakic, 1974). Recent evidence indicates that in the developing telencephalon, populations of neurons with distinct neurotransmitter phenotypes are derived from progenitors that are located in different regions. Thus, glutamate-containing pyramidal neurons are produced only in pallial areas, whereas the vast majority of γ-aminobutyric acid (GABA)ergic interneurons are generated in the subpallium (Parnavelas, 2000). The site of origin of cholinergic neurons seems to be the ventral-most region of the subpallial telencephalon (Marin *et al*., 2000; Marin & Rubenstein, 2001). In particular, GABA-containing interneurons arise in the basal regions of the telencephalon – the medial ganglionic eminence (MGE) and the lateral ganglionic eminence (LGE) (Anderson *et al*., 1997 – commented in Lumsden & Gulisano, 1997; Lavdas *et al*., 1999; Sussel *et al*., 1999; Wichterle *et al*., 1999). These cells follow tangential migratory paths to reach the cortex (for reviews, see Marin & Rubenstein, 2001; Nadarajah & Parnavelas, 2002).

Earlier studies in the avian brain, using cumulative labelling, have suggested that the dorsal telencephalon develops in an 'outside-in' sequence, with young neurons always accumulating in deeper layers (La Vail & Cowan, 1971; Primmet *et al*., 1989). More recent experiments that utilized BrdU (Striedter & Keefer, 2000) have provided evidence that in the avian wulst, the likely homologue of the mammalian neocortex, young neurons migrate past older cells, suggesting that this pattern is not unique to mammals. Furthermore, Cobos *et al*. (2001), using quail and chick grafts, found that the vast majority of the calbindin- and GABA-immunoreactive neurons in the avian pallium have a subpallial origin, possibly migrating tangentially into the dorsal ventricular ridge and cortical areas. Using fluorescent tracers, we demonstrate here that neurons originating from a singular ventricular

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subpallial region in the chick migrate tangentially and populate pallial regions. Specifically, we provide evidence that the ventricular dorsal subpallial sulcus (dss), a region that corresponds to the avian homologue of the LGE, contributes GABAergic neurons to dorsal pallial regions.

Materials and methods

Organotypic cultures

White Leghorn chicken embryos were incubated at 38 °C and in relative humidity of 50–80%. Hatching time under these conditions is

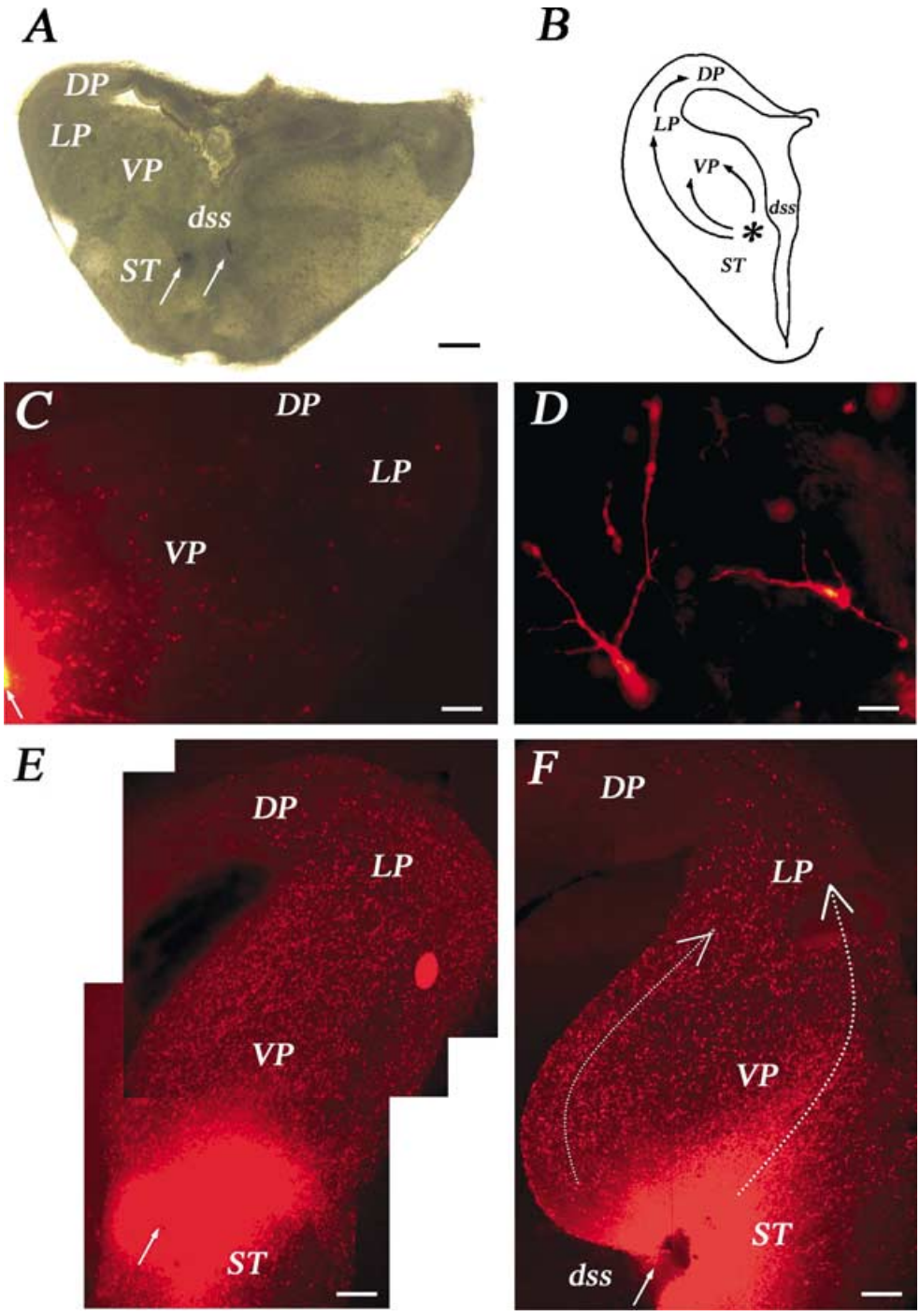

FIG. 1. Tangential migration of neurons in the developing chick telencephalon. Fluorescent dyes were placed in the dss [arrows in (A), (C), (E) and (F); asterisk in (B)]. Placement of DiI in an HH32 slice (A) and (C) resulted in streams of labelled cells migrating towards the DP through the VP and LP (B) and (C). Under high magnification (D), DiI-labelled migrating cells in the pallidum typically displayed a long and thick leading process. (E) and (F) Confocal images of CMTMR-labelled cells emanating from the dss of slices prepared from HH29 (E) and HH32 (F) brains. A robust stream of cells can be seen crossing the pallial–subpallial boundary and migrating through the VP towards the LP and DP [schematic representation in (B)]. In (F), note that the majority of the cells seem to respect the pallial–subpallial boundary (arrowhead) and migrate laterally within the subpallial region [the long right arrow in (F)], while a small number of cells seem to migrate in the more ventricular region [the long left arrow in (F)] [see schematic representation in (B)]. DP, dorsal pallidum; dss, dorsal subpallial sulcus; LP, lateral pallidum; VP, ventral pallidum; ST, striatum. Scale bars: (A) 250 μm; (C) 125 μm; (D) 20 μm; (E) and (F) 150 μm.

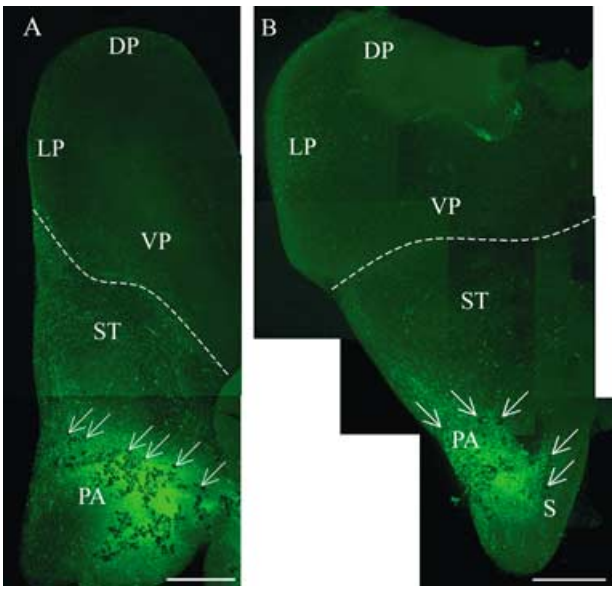

FIG. 2. Confocal images of CMFDA labelled cells emanating from the pallidum (arrowhead) of slices prepared from HH 29 in (A) and HH32 in (B). No cells can be seen crossing the pallial–subpallial boundary (sketched line). DP, dorsal pallidum; dss, dorsal subpallial sulcus; LP, lateral pallidum; PA, pallidum; S, septum; ST, striatum; VP, ventral pallidum. Scale bars: 300 μm.

approximately 21 days. After 6–8 days of incubation, embryos staged according to the Hamburger–Hamilton scale HH27–HH35 were rapidly removed and placed in ice-cold Ringer buffer (pH 7.3; supplemented with 6.5 mg/mL D-glucose). The following procedures were performed under sterile conditions. The brains were removed and embedded in 3% agar in phosphate-buffered saline (PBS), pH 7.2. They were then cut in the coronal plane at 300 μm with a Vibratome. Slices were placed onto millicell CM membranes (Millipore) in 30 mm Petri dishes containing 1 mL DMEM/F12 defined medium (Sigma), supplemented with 6.5 mg/mL D-glucose, 0.1 mM L-glutamine, 50 mg/mL penicillin/streptomycin and 10% heat-inactivated foetal calf serum (Life Technologies). After 1 h, they were transferred into neurobasal medium (Life Technologies) supplemented with 1 : 50 B27 (Life Technologies), 6.5 mg/mL D-glucose, 0.1 mM L-glutamine and 50 mg/mL penicillin/streptomycin.

Application of fluorescent tracers

To follow the migration of neurons, crystals of 1,1′-dioctodecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI; Molecular Probes) were placed with the aid of glass micropipettes in various regions of the palaeo-striatal complex. Alternatively, tungsten particles coated with 4-chloromethyl benzoyl amino tetramethyl rhodamine (CMTMR; Molecular Probes) or 5-chloromethylfluorescein diacetate (CMFDA; Molecular Probes) were placed in the same region (Alifragis *et al.*, 2002).

Following placement of the tracer, the slice cultures were incubated for a further 24–48 h in neurobasal medium, and then fixed in 4% paraformaldehyde in PBS for 3 h. They were subsequently rinsed in PBS, mounted and examined with a fluorescent microscope (Leica) or a laser-scanning confocal microscope (Leica DMR).

Immunohistochemistry

Cultured brain slices labelled with CMTMR were fixed in 4% paraformaldehyde in PBS overnight, cryoprotected in 20% sucrose/PBS and sectioned at 20 μm using a cryostat. To characterize the phenotype of migrating cells, sections were blocked with 10% normal goat serum and 0.3% Triton X-100 in PBS for 1 h and then incubated in primary antibodies against GABA (rabbit polyclonal, 1 : 750; Sigma) or calbindin (rabbit polyclonal, 1 : 10000; Swant) at 4 °C overnight. Following washes in PBS, sections were incubated with fluorescein isothiocyanate (FITC) -conjugated secondary antibodies (1 : 500; Molecular Probes) at room temperature for 2 h. Sections were then washed, mounted with Vectashield (Vector) and examined using a Leica dual-channel confocal microscope (Leica DMR). To reveal co-localization of fluorescent tracer and antibody staining, stacks of images were collected sequentially in the z-plane using Argon and Krypton lasers. Images were subsequently reconstructed using Metamorph imaging software.

Results

Migration of telencephalic neurons

Slice cultures were prepared from chick embryonic brains and labelled in the palaeo-striatal complex (Fig. 1A–D) using crystals of DiI (HH27–29 $n = 80$, HH30–32 $n = 100$ and HH33–35 $n = 70$). This

TABLE 1A. List of labelled slices brain in the palaeo-striatal complex (pallium and septum), using CMTMR, CMFDA or DiI

Stage of labelling	Number of slices labelled in pallidum and septum with CMTMR or CMFDA	Number of slices labelled in pallidum and septum with DiI	Total number of slices labelled in pallidum and septum	Number of slices with cells migrating into the lateral and ventral pallidum	Number of slices with cells migrating into the dorsal pallidum
HH27–29	5	25	30	0	0
HH30–32	5	45	50	2*	0
HH33–35	2	8	10	0	0

*Very few cells have been detected (two and three cells, respectively).

TABLE 1B. List of labelled slices brain in the palaeo-striatal complex (dorsal subpallial sulcus), using CMTMR, CMFDA or DiIB

Stage of labelling	Number of slices labelled in dorsal subpallial sulcus with CMTMR or CMFDA	Number of slices labelled in dorsal subpallial sulcus with DiI	Total number of slices labelled dorsal subpallial sulcus	Number of slices with cells migrating into the lateral and ventral pallidum	Number of slices with cells migrating into the dorsal pallidum
HH27–29	5	45	50	49	30
HH30–32	15	35	50	50	42
HH33–35	2	57	60	38	5

All the slices are examined for cells migration into lateral pallidum, ventral pallidum and dorsal pallidum.

fluorescent marker allows visualization of cell bodies of living neurons and their processes (Fig. 1D). Alternatively, we used CMTMR- or CMFDA-coated tungsten particles (HH27–29 $n = 10$, HH30–32 $n = 20$ and HH33–35 $n = 10$; Table 1A and B) (Figs 1E–F, 2 and 3). The major advantage of this dye is its low toxicity compared with DiI, which allows for the survival of the majority of labelled cells. In addition, CMTMR enables subsequent aldehyde fixation and further processing of the tissue for immunohistochemistry (Alifragis *et al.*, 2002). After 24–48 h *in vitro*, slices displayed groups of labelled cells emerging from the lateral and ventral pallium and heading towards the

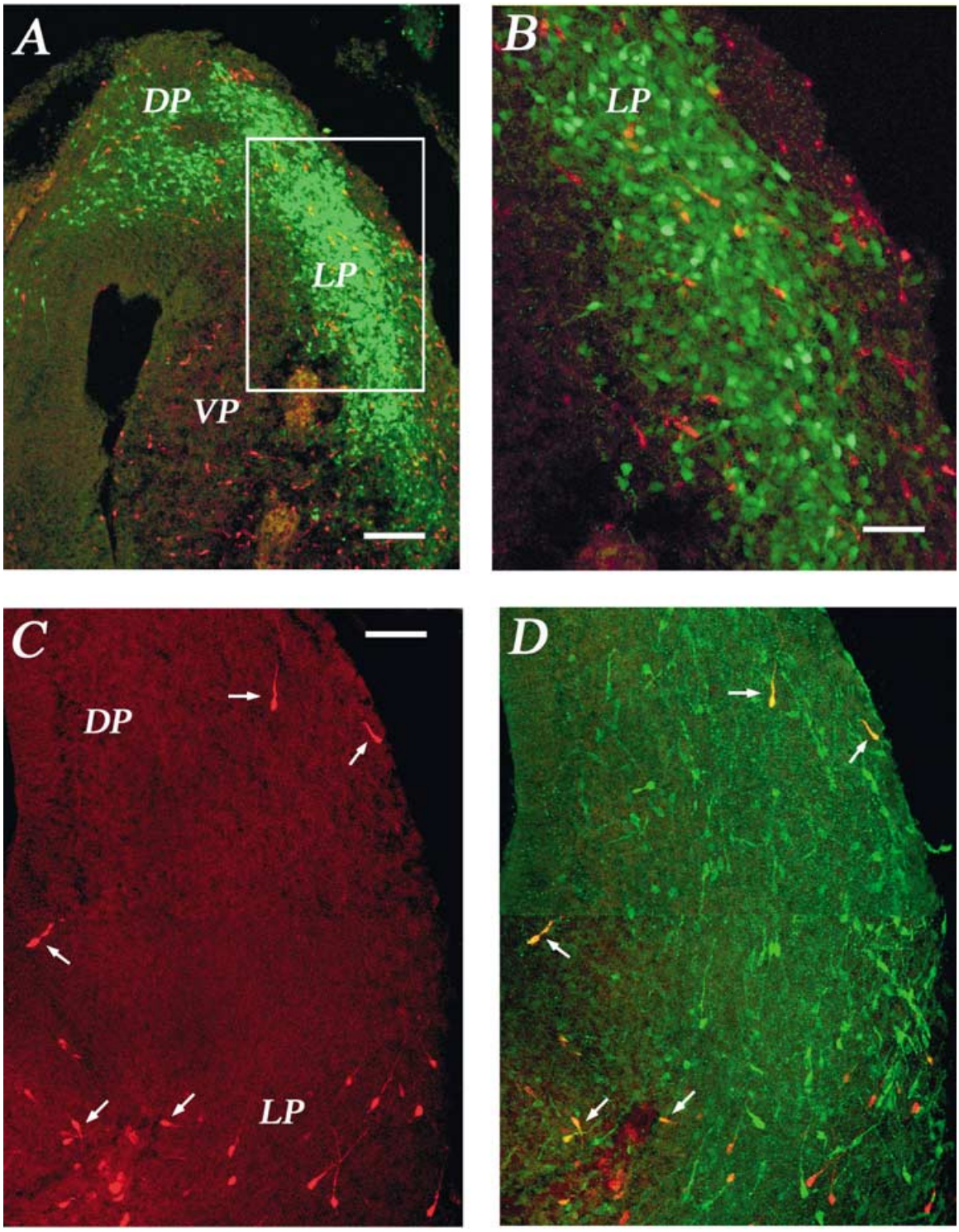

FIG. 3. Phenotypic characterization of CMTMR-labelled cells in HH32 chick slice cultures. (A), (B) and (D) are z-projections of images that were collected by sequential confocal laser scanning. Immunostaining of CMTMR-labelled cells with antibodies revealed that migrating neurons from the dss (red) were negative for calbindin [(A), (B); green], but contained GABA [(D); green; arrows]. (B) is a high-magnification image of the boxed area in (A). DP, dorsal pallidum; dss, dorsal subpallial sulcus; LP, lateral pallidum; ST, striatum; VP, ventral pallidum. Scale bars: (A) 200 μm; (B) 75 μm; (C) 80 μm.

dorsal pallium (Fig. 1B and C). A stream of migrating cells directed towards the dorsal pallium was only seen when the dye was placed in a ventricular region considered to be the avian homologue of the mammalian LGE ($n = 30$ slices at HH30–32; Table 1A and B) (Fig. 1A and B). This region, the dss, was characterized by morphological features such as lack of a ventricular bulge and the presence of a shallow depression on its ventricular surface (Puelles *et al*., 2000). When the marker was applied to more basal ventricular regions (pallidum and septum), the labelled migrating cells were restricted to the basal telencephalic structure ($n = 30$ at HH27–29, $n = 50$ slices at HH30–32, $n = 10$ at HH33–35; Table 1A and B) (Fig. 2).

A stream of cells was observed to emerge from the ventricular dss in slices taken from HH29–32 brains (Fig. 1), with a smaller number of cells emerging from this region at HH27–28 and HH33–35 (data not shown). Labelled cells had the characteristic appearance of migrating neurons, displaying a long and thick leading process and a thin trailing process. The direction of the thick leading process we considered as the direction of the cell migration (Fig. 1D). Slices labelled with CMTMR displayed streams of migrating cells towards the developing ventral, lateral and dorsal pallium, as depicted in Fig. 1B and as shown by the long arrows in Fig. 1F. Cells that migrated into the dorsal pallium appeared dispersed in different layers including the ventricular zone (Figs 1; 3A and C). To ascertain that the labelling was due to active migration and not due to passive diffusion of the fluorescent dye, control slices were incubated with 2 μg/μL cytochalasin D, a potent inhibitor of cell motility (Marin *et al*., 2000), following the application of the dye. These slices were observed to be devoid of labelled cells (data not shown).

Immunohistotochemical characterization of migrating neurons

We characterized the phenotype of migrating cells from the subpallium to the dorsal pallium of the chick using GABA and calbindin immunohistochemistry. These are markers found to be expressed in neurons generated in the mammalian ventral telencephalon and migrating tangentially to the neocortex (Anderson *et al*., 1997; Lavdas *et al*., 1999). Thus, slice cultures prepared from HH29–32 embryos, in which fluorescent tracers had been placed in the subpallium, were sectioned and processed for immunohistochemistry. Analysis of double-labelled sections revealed strong calbindin immunoreactivity in cells located in the lateral and dorsal pallium (Fig. 3A and B). However, although CMTMR-labelled cells were seen intermixed with calbindin-immunoreactive neurons, none appeared to contain calbindin. GABA-positive neurons were also observed in the dorsal and lateral pallial regions of the telencephalon (Fig. 3C and D). Many of these cells appeared to be migrating from the subpallium as they contained the fluorescent tracer CMTMR (Fig. 3D).

Discussion

The origin and migration of neurons along tangential paths from ventral telencephalic regions to the dorsal telencephalon has been demonstrated to be an important mechanism in mammalian forebrain development. The recent discovery of subpallial-derived neurons in the developing avian telencephalon (Cobos *et al*., 2001) suggests that such migratory paths may be a highly conserved trait in cortical evolution. Furthermore, it may also suggest common developmental mechanisms for the origin and migration of cortical interneurons that evolved prior to the evolution of cortical layer formation.

In rodents, three different streams of interneurons originating in the ventral telencephalon have been observed to round the corticostriatal notch and follow tangentially orientated routes to enter the neocortex: an early cohort (E12 mouse), originating in the MGE, invades mainly the cortical marginal zone. A second and more prominent cohort (E13–15 mouse), composed chiefly of MGE cells, migrates through the intermediate zone (IZ). At the later stages of corticogenesis (E15–17 mouse), neurons originating in the LGE and MGE appear in the subventricular zone (Anderson *et al*., 2001; Marin & Rubenstein, 2001). Using fluorescent tracers at HH28 and HH35 (E6–8) stages in chick, we have shown here that neurons from the ventricular dss, a region that is phylogenetically related to the mammalian LGE (Puelles *et al*., 2000), migrate dorsally to populate the developing pallium. Within the dorsal pallium, migrating neurons were identified both in the ventricular zone and throughout the developing layers.

Cobos *et al*. (2001), using quail–chick grafts at HH8, have recently reported that about 90% of GABA- and calbindin-positive pallial neurons migrate between HH25 and HH35 from the subpallial region. They found that the pallidum contributed three times more to this cell population than the striatum. Based on gene expression studies (and in particular Nkx2.1), Puelles *et al*. (2000) proposed that in the chick telencephalon, the pallidum is the homologue of the murine MGE and the dss is the homologue of the murine LGE.

In our present experiments, we were able to discriminate an individual population of migrating cells that originated from the ventricular part of dss and gave rise to the dorsally migrating stream between HH28 and HH35. We also observed that the neurons originating in the dss were GABA-positive, but calbindin negative.

Different methodological approaches may account for the differences reported in our study and that of Cobos *et al*. (2001). Taken together, our data support the hypothesis of homology between mammalian and bird telencephalon, and suggest that the mouse LGE and the chick dss are not only homologous because they share gene expression patterns and connectivity, but also because they both contribute cells to the dorsal regions of the developing telencephalon.

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Abbreviations

CMFDA, 5-chloromethylfluorescein diacetate; CMTMR, 4-chloromethyl benzoyl amino tetramethyl rhodamine; DiI, 1,1′-dioctodecyl-3,3,3′,3′-tetramethylindocarbocyanine; dss, dorsal subpallial sulcus; GABA, γ-aminobutyric acid; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; PBS, phosphate-buffered saline.

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