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Striatal precursors adopt cortical identities in response to local cues

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SUMMARY

One of the early steps in the regionalization of the CNS is the subdivision of the forebrain into dorsal and basal telencephalic ventricular zones. These ventricular zones give rise to the cortex and striatum respectively, in the mature brain. Previous work suggests that while neural precursors are able to move within both the dorsal cortical and basal striatal ventricular zones, they are unable to cross the boundary area between them. To determine if the regional identities of the cells in these ventricular zones are restricted, cells from the basal striatal ventricular zone were either transplanted back into their original environment or into the dorsally adjacent cortical ventricular zone. Use of in vitro explants of mouse telencephalon demonstrated that striatal precursors are able to integrate heterotopically within 12 hours of being placed onto the surface of cortical ventricular zone. To examine whether heterotopically placed neural precursors have phenotypes appropriate to their host or donor environment, in vivo transplants in rats were performed. Striatal ventricular zone cells transplanted to a striatal environment adopt morphologies and axonal projections characteristic of striatal cells. In contrast, striatal ventricular zone cells transplanted in vivo to a cortical environment acquired morphologies and axonal projections specific to cortex. These findings suggest that within forebrain, position-specific cues play an instructive role in determining critical aspects of regional phenotype.

Key words: telencephalon, heterotopic transplants, regionalization, mouse, rat

INTRODUCTION

Although the neurons that populate both dorsal and basal forebrain become postmitotic over a similar time course (Angevine and Sidman, 1961; van der Kooy and Fishell, 1986), their regional organization and cellular diversity in the adult are strikingly different. The major basal structure of the forebrain, the striatum, has a nuclear organization and is largely composed of neurons with a single neuronal morphology, the medium spiny cell (Kemp and Powell, 1971). In contrast, the cortex, which forms the dorsal aspect of the forebrain, has a laminar organization and contains a diverse variety of neuronal morphologies, including pyramidal, stellate and fusiform cells (Shatz, 1992). These two regions also differ in their patterns of axonal projections. The majority of striatal efferents project to either the globus pallidus or substantia nigra. Cortical projections, while considerably more diverse, can be divided up into three basic categories; subcortical projections to spinal chord, brainstem and pontine nuclei, commissural projections to the contralateral cortex and associational projections to the ipsilateral cortex (Shatz, 1992).

Critical to the understanding of how these areas are established is the determination of the initial events underlying their divergence in regional patterning. Despite their differences in mature organization, the striatum and cortex share a common history for much of their early development. Superficially the process of regionalization resembles the segmentation of the rhombocephalon. However, unlike the hindbrain, their early development does not involve the establishment of an obvious neuromeric boundary (Keynes and Lumsden, 1990). The earliest indicators of differences in their regional organization appear a few days prior to the first wave of neurogenesis, with the emergence of discrete dorsal (cortical) and basal (striatal) ventricular zones (VZs) (Johnston, 1923; Smart and Sturrock, 1979), each having a characteristic pattern of gene expression (reviewed by Puelles and Rubenstein, 1993). The dorsal cortical VZ has a sheet-like epithelial structure and is molecularly characterized by the expression of a variety of genes including *Emx-1* and *2*, and *Wnt-7b*. In contrast, the striatal VZ has a bulb-like structure (often referred to as the lateral ganglionic eminence) and is molecularly characterized by the expression of genes including *Dlx-1* and *2*, *MASH-1* and *Nkx-2*.

Previous experiments have shown that while neural precursors are able to move within both the dorsal and basal VZs, they are unable to cross the border region between them (Fishell et al., 1993). This raises the question of whether the cells situated on either side of this boundary area are restricted in terms of their regional identity (Steinberg, 1970, Gutherie et al., 1993) or whether their spatial movements are confined by a mechanical barrier to lateral dispersion. To assess whether the establishment of this border signifies that the cells on either side of it are regionally determined, I have transplanted striatal

precursors to examine whether they are able to integrate and differentiate within the adjacent cortical environment. The results of these experiments suggest that striatal precursors can modify their regional phenotype within the telencephalon in response to local cues. Together these experiments suggest that local cues shape the regional architecture of these distinct areas of forebrain.

MATERIALS AND METHODS

Isolation of striatal precursors

Donor cells were isolated directly from E15 mice, by microdissection of the striatal VZ, the lateral ganglionic eminence, which forms a swelling in the ventrolateral wall of the cerebral vesicles (Fig. 1). Pieces of striatal ventricular zone were dissected using electrolytically sharpened tungsten needles. To prevent contamination by adjacent ventricular zones, only the protruding head of the striatal ventricular zone was collected. Care was taken to remove both postmitotic cells and underlying entorhinal cortex from pieces of striatal ventricular zone. For each preparation, between 4 and 16 striatal ventricular zones were collected. The tissue was dissociated using sequential treatments of 0.08% and 0.25% trypsin in a CMF-PBS solution containing 0.02% EDTA. Dissociated cells were labeled with the lipophilic dye PKH-26-GL (Zynaxis). A number of lines of evidence support the supposition that the cells isolated by this method are neural precursors. First, in excess of 95% of these cells are immunopositive for Nestin, a marker for proliferating neural precursors (Friederikson and McKay, 1988). Second, when these cells were placed in culture they assume shapes closely resembling progenitor cells (Temple, 1989).

In vitro transplantation of striatal precursors onto telencephalic explants

Explants of the lateral wall of the E15 mouse telencephalon were prepared by making an incision across the dorsomedial surface of the forebrain. The wall of the telencephalic vesicle was subsequently amputated by making an incision between the lateral and medial ganglionic eminence. The resulting explant was maintained in vitro for periods up to 12 hours. Immunostaining and patterns of proliferation support the idea that the cellular behavior within the explant retains in vivo characteristics (Fishell et al., 1993). For in vitro experiments the explants were positioned ventricular side up on Millipore filters. Labeled donor cells were placed onto the ventricular zone using a 1.0 µl Hamilton syringe. Explants were cultured in DMEM:F12 medium (supplemented with glutamine and glucose) containing 10% heat inactivated horse serum.

In vivo transplantation of striatal precursors into the lateral ventricle of forebrain

Embryonic transplantations were done using techniques described in Fishell and van der Kooy (1987). E16.5 to E17.5 rats (roughly comparable in their development to E15 mice) were used for the in vivo recipients. Rats were given a laparotomy and the uterine horns were exposed. The position of embryos was determined by transillumination using a fiber optic device. Using the calvarian sutures as a landmark, a 0.5 µl injection of cells was delivered through the uterine wall into the cerebral ventricles of host embryo forebrains. The hole in the uterine wall was sealed using cyanoacrylic adhesive. Every embryo within a time-pregnant animal was injected. Subsequent to injections pregnant females were sutured and monitored for a 24-hour recovery period. Approximately 80% of the injected embryos were delivered normally. Estimates of the total number of cells that integrated within host animals were made by counting cells within individual sections of the host brains, calculating an average number of integrated cells per section and multiplying by the total number of sections. Given that 10,000 cells were on average injected, a rough estimate of the efficiency of integration based on this method is approximately 10-20%. This estimate makes the assumption that proliferation among the transplanted cells is minimal. As the dye used to mark cells is diluted to below detectable levels within three cellular divisions (see below), proliferation could (depending upon amount) either result in overestimation or underestimation of cellular incorporation.

The possibility that incorporation of LGE cells into the cortex results from contamination of the donor cells with cortical cells is unlikely. Apart from the fact that the dissection can be performed cleanly, if a 'sorting' process of LGE cells and 'contaminating' cortical cells were the explanation to these results, host animals that received transplants from a single batch of LGE precursors would be expected to have a similar percentage (dictated by the degree of 'cortical' contamination) of precursors incorporating into the cortex and the striatum respectively. This was not the case. Within a single litter of animals, all of which received transplants from a single pool of LGE cells, individual animals showed every variation from 100% of the incorporating cells ending up in the striatum to 100% ending up in the cortex.

Examination of dye-transfer from viable and non-viable cells

The reliability of this approach depends on the assumption that dye from the donor cells doesn't transfer into cells in the host animal. Two experiments were done as controls for the possibility of dye transfer. First, labeled cells were made non-viable through a combination of osmotic lysis and repeated freeze/thawing prior to in vitro or in vivo transplantation. When labeled non-viable cells were used as the donor tissue, negligible labeling was seen in the host animals regardless of the length of survival period. Second, when C6 cells, a glial cell line with a characteristic astrocytic morphology, were used as the donor cells, the only labeled cells in the hosts, after either in vitro or in vivo transplantation, were cells displaying a glial morphology, which closely resembled the in vitro morphology of C6 cells.

Retrograde tracing of axonal projections of transplanted cells

One day before being killed (at P6), animals that had previously received an embryonic transplant of striatal VZ cells were given stereotactic injections of two distinguishable retrograde tracers. To label commissural projecting neurons, two 0.3 µl injections of a 3.0% solution of the retrograde axonal tracer Fluoro-Gold (Fluorochrome Inc.) were given into the corpus callosum on the side of the forebrain contralateral to the site of transplantation. To label descending projections to the spinal cord, two 0.3 µl (one medial and one lateral) injections of a 5.0% solution of the retrograde axonal tracer Fast Blue (Sigma) were given into the descending corticospinal tract at the level of the metencephalon, ipsilateral to the site of transplantation.

Processing and host tissue

Both explants and host brains were fixed in a PBS solution containing 4% paraformaldehyde and 0.1% glutaraldehyde. Immersion fixation was used for explants, whereas host brains were transcardially perfused. Tissue was postfixed overnight in a PBS solution of 4% paraformaldehyde and 10% sucrose. Tissue was embedded in agarose and vibrotome sectioned coronally. Tissue was mounted in dabco/PBS. PKH-26 labeled donor cells were imaged with a Zeiss Axiovert microscope fitted with a Biorad MRC 600 confocal scan head and argon laser, with DIC/fluorescent (488/550 nm excitation) dual channel illumination or a Hammamatsu c4880 cooled CCD camera using fluorescent (550 nm excitation) illumination.

RESULTS

In vitro transplantation of striatal precursors onto cortical VZ

This experiment examined the behavior of striatal VZ cells when transplanted to a cortical environment. The use of in vitro explants allowed the timecourse and manner by which striatal cells integrate into a cortical VZ to be examined. Striatal VZ cells were isolated (Fig. 1) and labeled with the lipophylic dye PKH-26 (Gao and Hatten, 1993). They were then placed onto the cortical VZ surface of age matched forebrain explants. Although the cortical ventricular zone was intact, striatal cells were able to integrate into this heterotopic environment.

After a 4-hour incubation, striatal VZ cells positioned on the surface of the cortical VZ extended a process into the underlying VZ. Eight hours after being placed onto the surface of the cortical VZ, many of the cell bodies of striatal VZ cells had followed their neuritic processes into the cortical VZ. After a 12-hour incubation, some of the striatal VZ cells had migrated deeper into the cortical VZ and extended processes to both the pial and ventricular surfaces (Fig. 2). In a small number of cases it was noted that two cells were found integrated within the VZ, in clone proximity. While, it is possible that these cases represent instances where two donor cells integrated together, an interesting alternative explanation is that donor cells can at least occasionally re-enter the mitotic cycle after integrating into the host tissue.

To examine whether homotopic transplants had a higher affinity for reintegration than the heterotopically placed striatal precursors, the experiment was repeated using E15 cortical cells as the donor population. Again these preparations were fixed and examined after 4-, 8- and 12-hour survival periods. In these experiments, although cortical cells are derived from the host environment, their ability to integrate into the host ventricular zone were neither quicker nor more efficient.

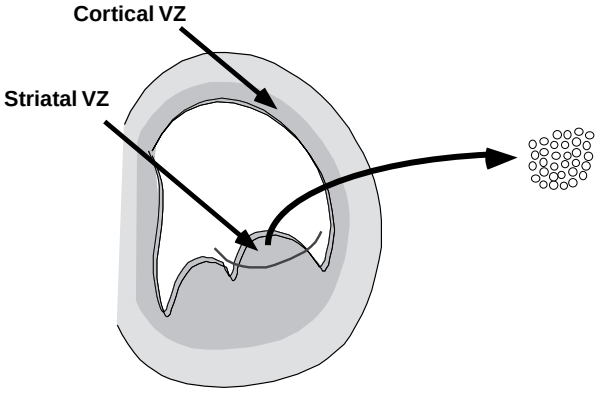

Fig. 1. A schematic representation of the dissection of the striatal VZ used for donor cells in all experiments. Within a coronal section of the E15 mouse, two eminences (medial and lateral) are apparent within the basal forebrain. The lateral eminence (hence the name lateral ganglionic eminence) comprises the striatal VZ. Using electrolytically sharpened tungsten needles the part of the eminence that extends into the lateral ventricle of the forebrain can be dissected cleanly yielding a pure population of striatal progenitors. After trypsinization, these progenitors can be gently dissociated for use as donor cells for transplant experiments.

Further support for the notion that cortical versus striatal precursors do not have an innate preference for associating with homotopic areas of the telencephalon at E15 came from experiments where striatal VZ was substituted for cortical VZ as the host environment. These results exactly paralleled the earlier findings, demonstrating that, regardless of whether cortical or striatal cells were used as the donor population, similar numbers of cells integrated. This in vitro analysis suggests that cells transplanted into the cerebral ventricles at this age are able to integrate into the VZs of forebrain within 12 hours of in vivo transplantation.

In vivo transplantation of striatal precursors onto cortical VZ

To address whether the striatal cells that integrated into the cortical VZ follow a program of development appropriate for their host or donor environment, in vivo transplants of these cells into the cerebral ventricles of the forebrain were performed. As rats are more tolerant than mice to in utero manipulations these experiments were performed using E16.5 rats (roughly comparable in stage of development to E15 mice) as both donors and recipients. Animals receiving transplants of striatal VZ cells were examined up to 15 days after in utero surgery (Fig. 3). Most animals receiving transplants had numerous cells integrated into both the striatal and cortical VZs. This allowed the fate of cells that integrated within the cortex to be directly compared with those that incorporated into the striatum.

Homotopic integration of striatal precursors

Transplanted striatal VZ cells that reincorporated homotopically within basal forebrain differentiated into neurons with a morphology typical of medium spiny striatal cells (Figs 3, 4). To test whether these neurons also made axonal projections appropriate to striatum, the retrograde neuronal tracer Fast Blue was injected into the substantia nigra, an area normally innervated by the striatum. In accordance with their position and morphology, many of transplanted cells were double-labeled with Fast Blue and PKH-26, suggesting that the transplanted cells had established efferent projections to the substantia nigra (data not shown).

Not only are the organization and morphology of the mature neuronal population within the cortex distinctly different than those seen in the striatum, so are the developmental events that lead to their establishment. Unlike striatal development, where postmitotic neurons coalesce into a nuclear structure, cortical development is characterized by a predictable series of steps in which the cells sequentially migrate, assemble and differentiate to form an ordered set of cortical laminae. Striatal VZ cells that incorporated into the cortex were examined during each of these phases to determine how closely their developmental progression follows normal cortical maturation.

Three days after transplantation, at E20, labeled cells were seen migrating through the intermediate zone and into the cortical plate. The profile of migrating cells was reminiscent of those described both in vivo (Rakic, 1972) or in vitro (Edmondson and Hatten, 1987; Hatten, 1990; O'Rourke et al., 1992). Interestingly, as reported by O'Rourke et al. (1992), while a majority of migrating cells had an orientation parallel to the radial glia, a small population of cells migrating within the intermediate zone were oriented orthogonal to them. In

contrast, to all later time points, at E20, donor cells which incorporated into the cortex, looked similar to those seen in the striatum. As shown in Fig. 3, both display a morphology that appears to be generically characteristic of all migrating neurons (Hatten, 1990). Five days after transplantation, at P1-P2, the transplanted striatal VZ cells that incorporated into the cortex were positioned in cortical laminae and had a morphology similar to developing pyramidal or stellate cells (Peinado and Katz, 1992). At P6-7, after a 10-day survival period, transplanted cells within the cortex produce a variety of typical mature 'cortical' morphologies including pyramidal, stellate and fusiform (Figs 3, 5).

Analysis of the specific morphologies of cells within the cortex after the 10-day survival period demonstrated that they were appropriate for their location within the cortical laminae (Ramon Y Cajal, 1990). In the deeper layers of cortex (layers V and VI), most cells (>85%) resembled cortical pyramidal cells, with an apical dendrite and a descending axon. In the middle layers of cortex (layer IV), stellate cells with a radial array of dendrites and an axon projecting either to the deeper or more superficial cortical laminae predominated (65%, with the remainder having a pyramidal morphology). In the most superficial cortical laminae, transplanted cells took on a fusiform morphology (>95%, with the remainder having a stellate morphology). In total, of the cells within the dorsolateral cortex, 68% had a pyramidal morphology, 23% had a stellate morphology and 9% had a fusiform morphology (quantitation was based on an analysis of 500 cells examined in 8 animals at P7). Both the percentages of the various cell morphologies and their distribution among the cortical laminae

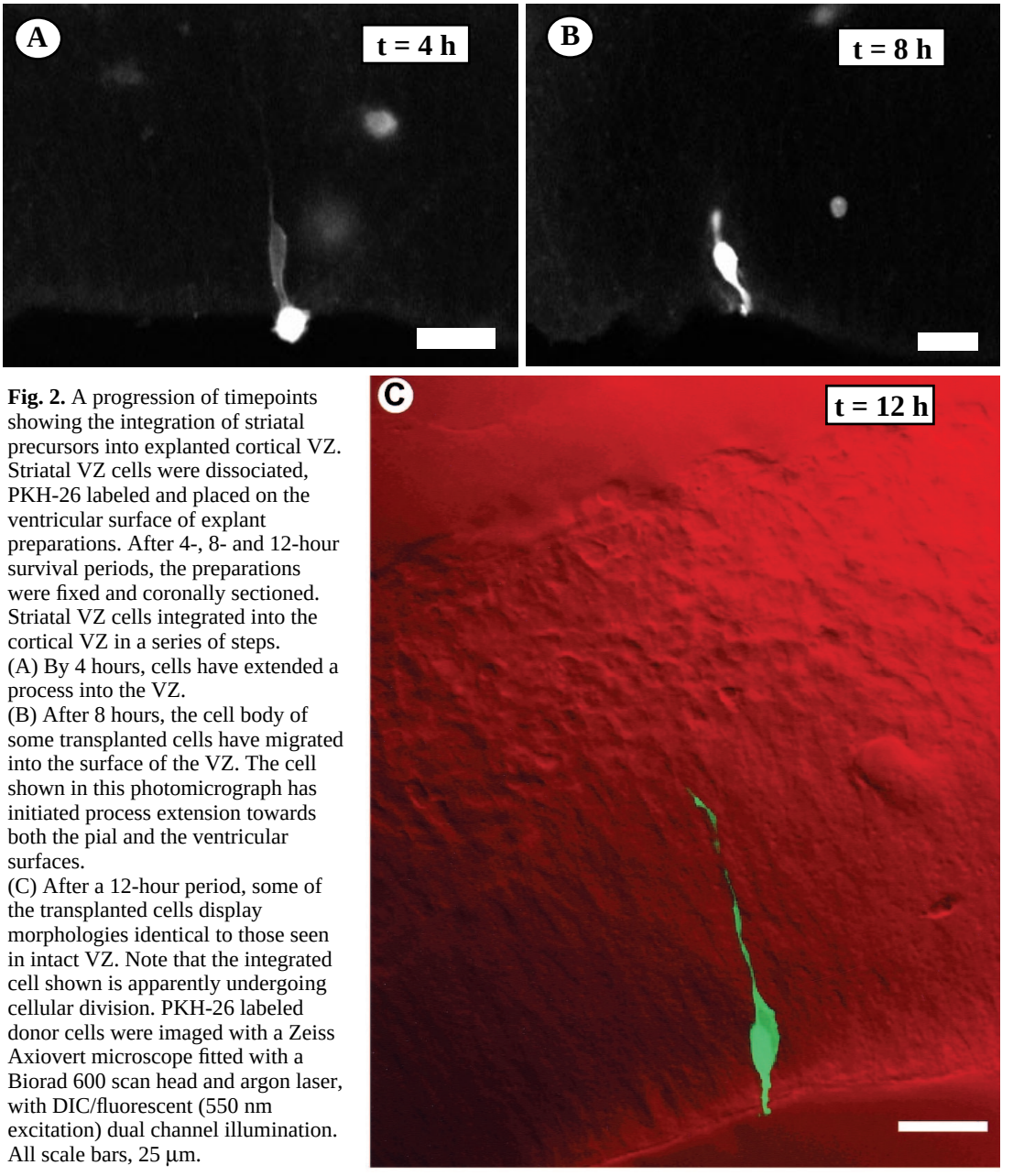

Fig. 2. A progression of timepoints showing the integration of striatal precursors into explanted cortical VZ. Striatal VZ cells were dissociated, PKH-26 labeled and placed on the ventricular surface of explant preparations. After 4-, 8- and 12-hour survival periods, the preparations were fixed and coronally sectioned. Striatal VZ cells integrated into the cortical VZ in a series of steps.
(A) By 4 hours, cells have extended a process into the VZ.
(B) After 8 hours, the cell body of some transplanted cells have migrated into the surface of the VZ. The cell shown in this photomicrograph has initiated process extension towards both the pial and the ventricular surfaces.
(C) After a 12-hour period, some of the transplanted cells display morphologies identical to those seen in intact VZ. Note that the integrated cell shown is apparently undergoing cellular division. PKH-26 labeled donor cells were imaged with a Zeiss Axiovert microscope fitted with a Biorad 600 scan head and argon laser, with DIC/fluorescent (550 nm excitation) dual channel illumination. All scale bars, 25 μm.

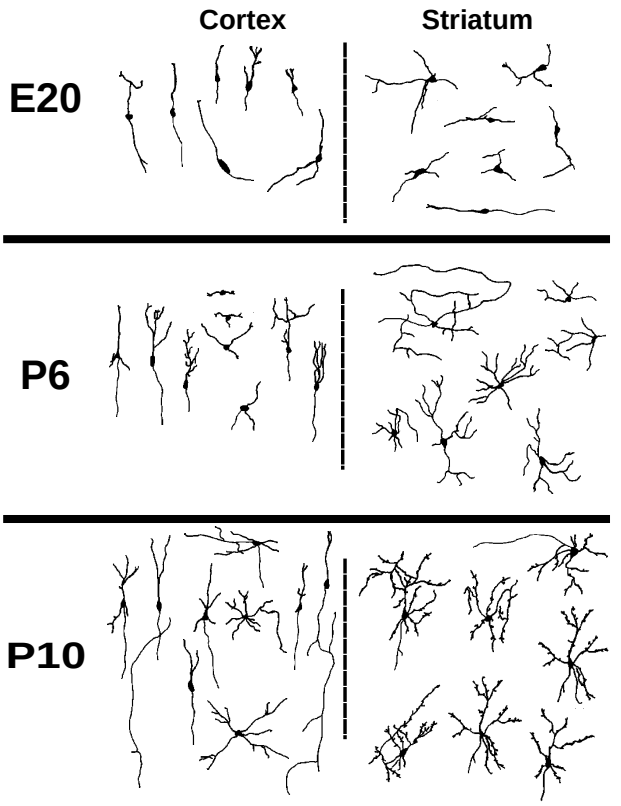

Fig. 3. The morphology of transplanted striatal VZ cells which incorporated into the cortex and the striatum respectively, at three successive timepoints, as shown by camera lucida drawings. At E20, transplanted cells within both the striatum and cortex have minimal axonal or dendritic arborization. While cells within the striatum generally show a higher degree of dendritic arborization, the morphology of the cells in these two regions appear remarkably similar. Regardless, of which area within the forebrain they integrated, transplanted cells have a profile similar to that seen in migrating neurons in vivo. By P6, region specific morphologies have become evident. Within cortex, while cells with a pyramidal morphology predominate, significant numbers of cells with either stellate or fusiform morphologies are also present (see text for statistical analysis). By P10, donor cells with regional morphologies characteristic of mature cortex or striatum are observed. Within the cortex, donor cells can easily be classified as pyramidal, stellate or fusiform in shape. While long axonal processes are seen emanating from cells of all morphology, this was most evident in donor cells that resembled pyramidal neurons. In contrast, within striatum, cells almost exclusively took on a morphology appropriate to medium spiny neurons. Interestingly, donor cells have begun to develop the 'spiny' dendritic appearance characteristic of mature striatal neurons.

closely match that seen in cerebral cortex (Sloper, 1973; Sloper et al., 1979; Rockel et al., 1980).

By P7, cortical pyramidal neurons would be expected to have established connections with their targets (O'Leary and Terashima, 1988). Pyramidal cells within the cortex comprise two major classes of projection neurons, those forming commissural projections to the contralateral cortex and those forming descending subcortical projections. To examine the axonal projections of striatal VZ cells that have incorporated into the cerebral cortex, two distinguishable retrograde tracers were injected into areas that receive innervation from commissural and descending cortical projections, respectively (Koester and O'Leary, 1993). At the time that transplanted cells entered the cortical laminae, the majority of deep layer neurons have already initiated their descending projections, while the majority of neurons that reside in the more superficial layers of cortex are still in the process of generating a commissural projection to the contralateral cortex (Koester and O'Leary, 1993). In accordance with this, transplanted double-labeled (i.e. PKH-26 and retrograde tracer labeled) neurons within the cortex that formed commissural projections greatly outnumbered those forming subcortical projections (Fig. 6). The presence of numerous PKH-26 labeled axons (Fig. 6B) coursing within the corpus callosum (the path taken by commissural projecting cortical neurons) further supported the establishment of a robust callosal projection. As striatal cells do not form axonal projections to either of these targets, this strongly suggests that the projection patterns of the transplanted cells are a result of these cells acquiring fates appropriate for their cortical 'host' environment.

DISCUSSION

Fate mapping of mammalian forebrain and markers of striatal VZ suggest that regional specification is established within the E15 mouse telencephalon (Grove et al., 1993; Luskin et al., 1988; Walsh and Cepko, 1992, 1993; Price et al., 1992; Simone et al., 1992; Bulfone et al., 1993). Recently, further support for this idea has come from examination of telencephalic explants (Fishell et al., 1993). These experiments indicated that neural precursors within E15 telencephalon are restricted from crossing from one VZ to another. In the hindbrain, (Fraser et al., 1990); the inability of cells from neighboring rhombomeres to mix (Gutherie and Lumsden, 1991) appears to be related to adhesion differences (Gutherie et al., 1993) and the early acquisition of a determined cell state (Gutherie et al., 1992; Lumsden et al., 1994). However, an alternative interpretation of the present observations is that rather than indicating that these cells are committed to a specific region, they are physically confined to their VZ as result of a chemical or mechanical barrier to lateral dispersion. Heterotopic transplantation of striatal precursors within mammalian telencephalon provided a test of this hypothesis, by examining the ability of local environments to specify regional identity. The present experiments directly challenged the regional fate of precursors within forebrain and yielded two major findings. First, that striatal precursors can integrate into heterotopic regions of telencephalic VZ. Second, that although regionally specified, striatal precursors are not uniformly committed to a specific regional fate within the telencephalon. On the basis of these observations, I propose that the mechanisms which establish regional identity within forebrain are markedly different than those that act in the hindbrain. The present results are consistent with a model of forebrain regionalization where local cues play a critical role in determining regional fate within the telencephalon. Therefore, in contrast to hindbrain where the precursors are thought to be regionally determined early in development (Lumsden and Keynes, 1989; Keynes and Lumsden, 1990; Lumsden et al., 1994), the cortical and striatal VZs appear to be allocation territories with no irreversible difference in cell state.

Instructive versus selective events in regional differentiation of striatal precursors

Two models could explain the results of the present experiments. In one model, specified precursors change their regional identity in response to local cues. Alternatively, in the other model, a selective event results in a subpopulation of the donor cells, which are regionally unspecified, incorporating heterotopically. Given that only a fraction of the donor cells are ever accounted for in the present experiments, they cannot satisfactorily address whether the results of heterotopic transplants are a product of instructive or selective events or a combination of both. Resolution of this issue would require a degree of donor cell book-keeping which at present is not feasible.

Further complicating the interpretation of these results is the possibility that transplanted cells undergo proliferation within the host animal. In vitro work has demonstrated (unpublished observation) that within three cell divisions the PKH-26 dye is completely undetectable, therefore the large numbers of cells in host regions cannot be explained by proliferation of a small subpopulation of donor cells after transplantation. Nonetheless it remains possible that donor cells undergo at least one cellular division within the host animal and that this is an obligatory step towards adopting the regional fate of the host region. I am presently undertaking experiments to address this issue by labeling host animals with mitotic markers (i.e. BrdU) after transplantation.

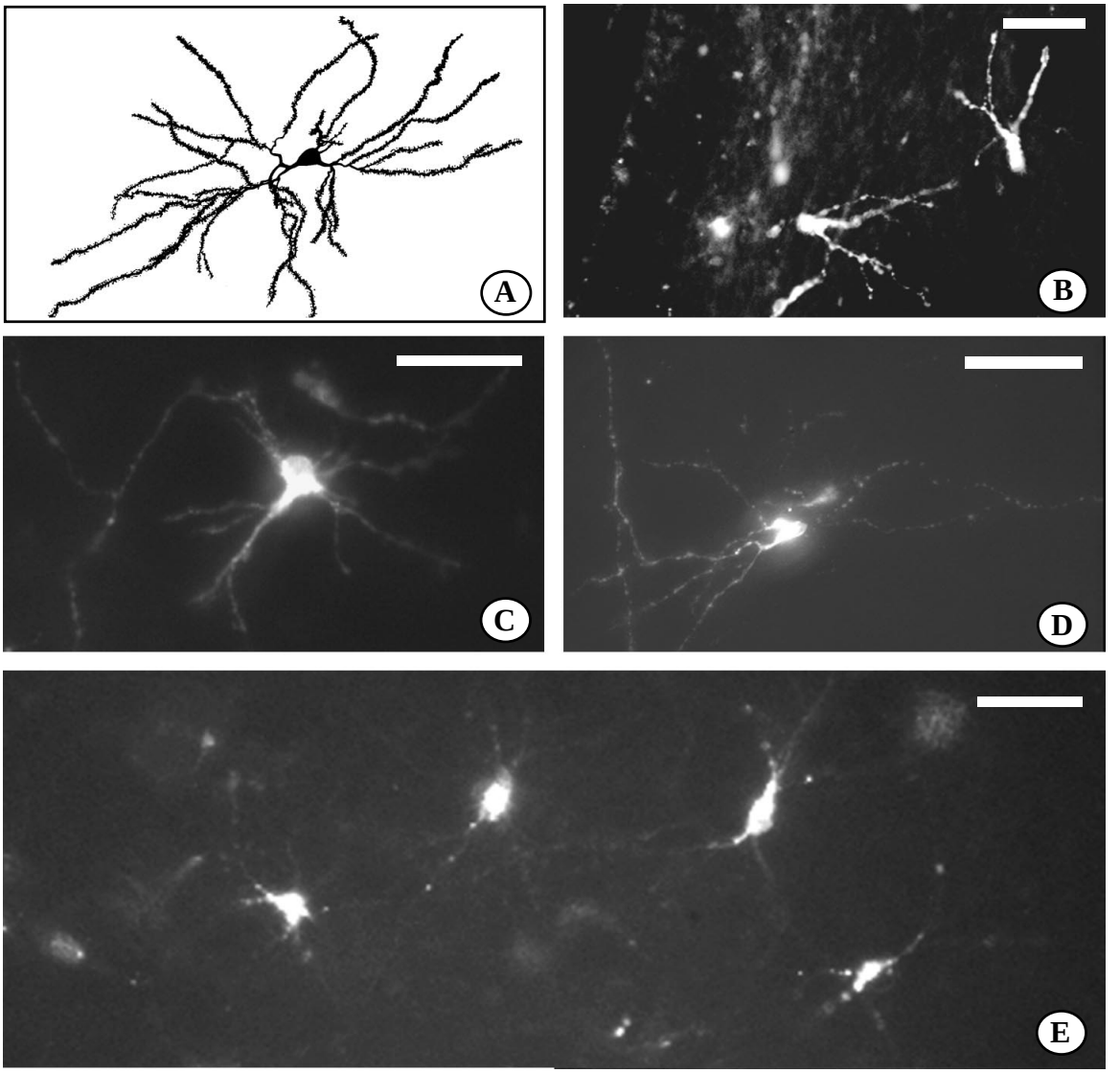

Fig. 4. Striatal VZ cells that incorporate homotopically into striatum in vivo develop with the morphology of medium spiny neurons. (A) A camera lucidia drawing of a typical adult medium spiny neuron (adapted with permission from Penny et al., 1988). B and E show the morphology of transplanted striatal VZ cells at P2 (after a 5-day survival period), which have incorporated into the striatum. These cells, like those in normal striatum, have begun to differentiate but as yet have only developed short dendrites that lack the spiny appearance of adult striatal cells. C and D are two examples of transplanted striatal VZ cells within the striatum, at P7 (after a 10-day survival period). These cells, like those within normal striatum, have now adopted a much more mature morphology. Dendritic processes can be seen to radiate out in all directions and these cells have begun to adopt a spiny appearance. PKH-26 labeled donor cells were imaged with a Zeiss Axiovert microscope fitted with a Hammamatsu c4880 cooled CCD camera using fluorescent (550 nm excitation) illumination. All scale bars, 25 μm.

Recent work by Campbell et al. (1994) find that earlier transplants of E13.5-14 mouse striatal VZ into the forebrain ventricle of age matched rats, consistently result in striatal incorporation as detected using the mouse neuron-specific marker, M6. However, they do observe incorporation into other forebrain structures such as the septum and to a lesser extent the cortex. The differences in the patterns of incorporation between the present study and this one could be a result

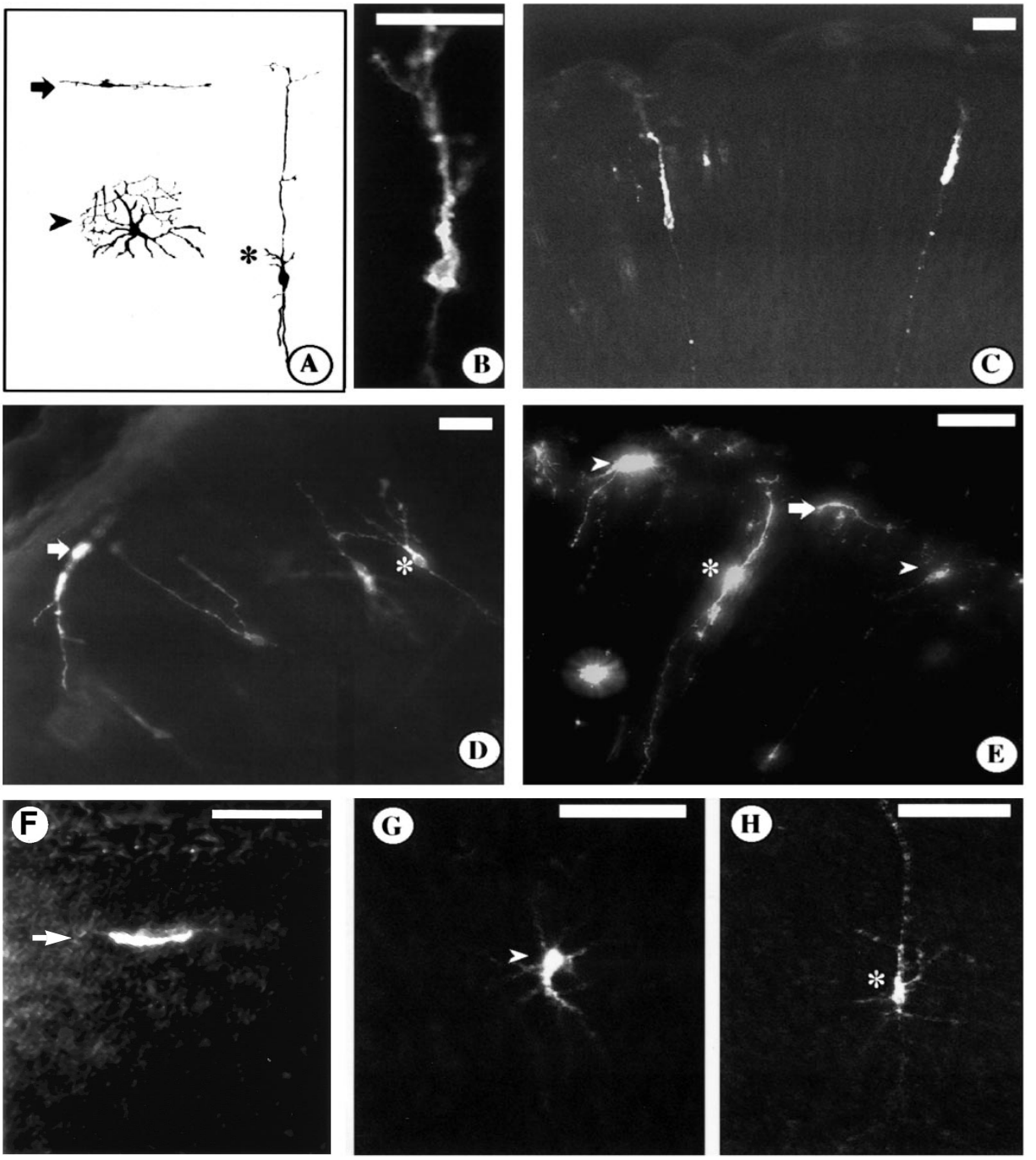

Fig. 5. Striatal VZ cells that incorporate heterotopically into the cortex in vivo adopt morphologies characteristic of cortical cells. (A) A schematic drawing of the three basic morphologies of cortical neurons, pyramidal (asterisk), fusiform (arrow) and stellate (arrowhead), adapted from the drawings of Ramon y Cajal (1990). B and C show the morphology of transplanted striatal VZ cells within the cortex, at P2 (after a 5-day survival period). Cells have adopted an elongated pyramidal-shape, oriented parallel to cortical columns, with a relatively unbranched apical dendrite. Work by Katz suggests that these pyramidal-shaped cells can eventually develop into cells with a pyramidal or stellate morphology (Peinado and Katz, 1990). D and E show two cortical areas containing transplanted striatal VZ cells, at P7 (after a 10-day survival period). Cells have developed morphologies that can now be classified as fusiform (arrows in D, E and F), stellate (arrowheads in E and G) or pyramidal (asterisks in D, E and H). Higher magnification photomicrographs of individual cells show a detailed view of typical (F) fusiform, (G) stellate and (H) pyramidal morphologies, at P7. Labeled transplanted cells were visualized as described in Fig. 4. All scale bars, 25 μm.

of developmental timing differences between the two studies or the selective proliferation of integrated cells (i.e. the use of a proliferation-sensitive marker in my study (dye) versus the proliferation insensitive marker used in their work (M6)). Interestingly, as in the present study, cells that incorporated heterotopically appeared to undergo site-specific differentiation appropriate to the region in which they incorporated (personal communication, K. Campbell).

Specification versus determination in mammalian forebrain

It has been suggested that neural patterning and histogenesis result from a series of progressive restrictions occurring as the cells proceed through development (Anderson, 1989; Hatten, 1993). Recent evidence from a variety of experiments suggests that the determination of cell type, laminar fate and axonal projection occur at various times during neuronal development (Bronner-Fraser and Fraser, 1988; Macklis, 1993; McConnell and Kaznowski, 1991; Stanfield and O'Leary, 1985). While the present experiments suggest striatal VZ cells are not fully committed, the fact that molecular markers of basal forebrain are expressed in these cells prior to the stage used for transplantation to cortex suggests that the process of regional specification has already been initiated (Puelles and Rubenstein, 1993). It is as yet unclear whether after transplantation striatal VZ cells regulate their regional patterns of gene expression to match their host environment. Given that these markers are suggestive of regional specification, it will be of interest to examine whether regional markers of basal forebrain such as *Dlx-2*, are shut-off and regional markers of dorsal forebrain,

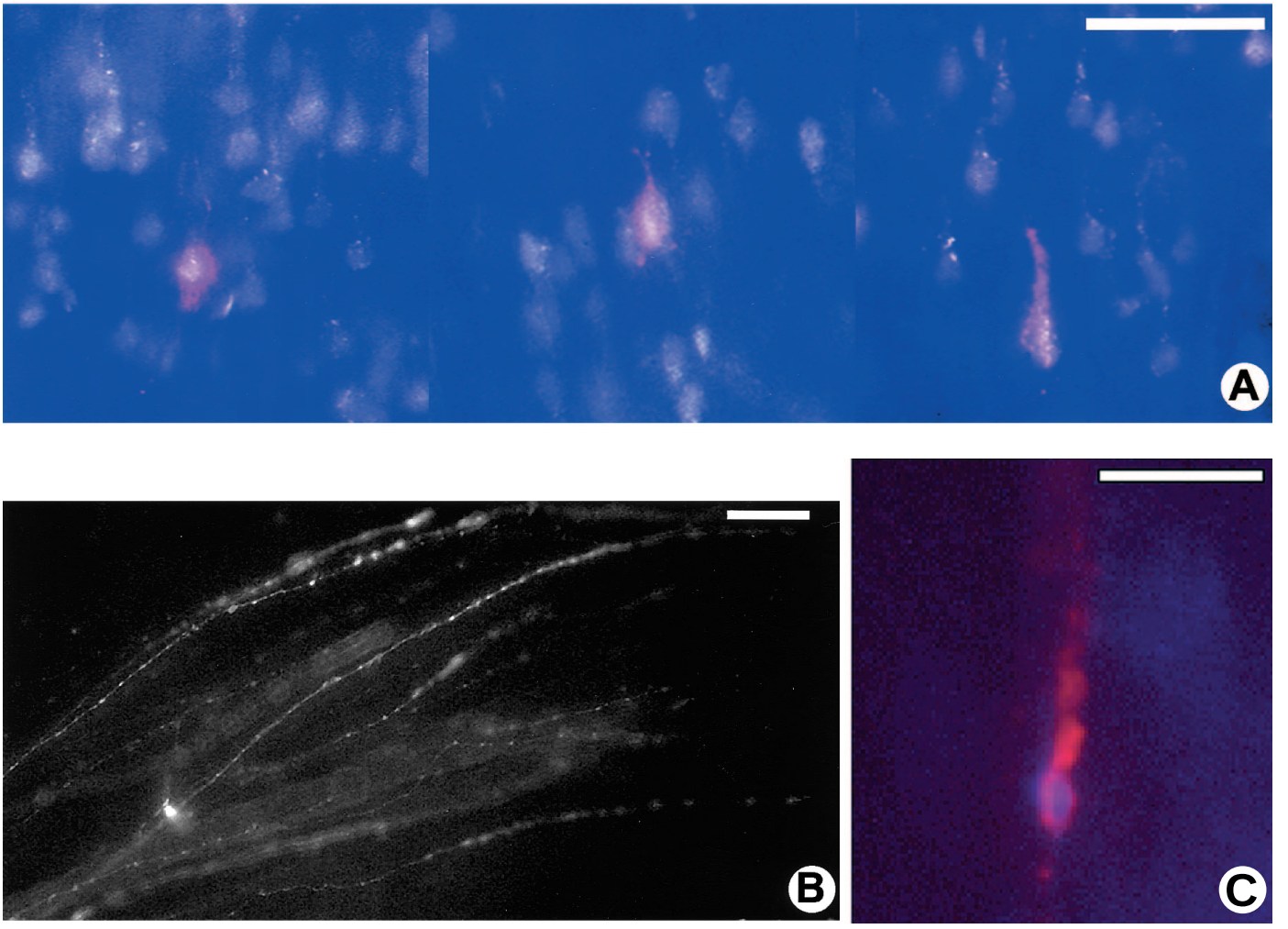

Fig. 6. Striatal VZ cells that incorporate heterotopically into the cortex in vivo make projections appropriate to cortical but not striatal neurons. (A) Three examples of striatal VZ cells that have incorporated into cortex, assumed a pyramidal morphology and made commissural projections to the contralateral cortex. Retrograde tracing of the cortical commissural projection was done by injection of Fluro-Gold into the contralateral corpus callosum, 1 day prior to the animal's death. Each of these PKH-26 labeled transplanted cells is double labeled with Fluro-Gold. B shows PKH-26 labeled axons within the corpus callosum, originating from donor striatal VZ cells, which have incorporated into the cortex. (C) A rare striatal VZ cell that incorporated into the cortex and sent a descending projection into the corticospinal tract. Retrograde tracing of the corticospinal projection was done by injection of Fast Blue into the ipsilateral corticospinal tract at the level of the metencephalon. In all cases, injection of the retrograde tracer was made 1 day prior to the animal's death All scale bars, 25 μm.

such as *Emx-1*, are expressed in cells moved from the striatal to the cortical VZ. Regardless of the outcome, the present experiments suggest restriction of regional identity probably occurs later in development than has been suggested by other investigators on the basis of fate mapping (Groves et al., 1993, Luskin et al., 1993). This suggests that while the process of regional specification within these cells has begun at the time of transplantation, they are not yet regionally determined. Hence even after dorsal versus basal regions of VZ can be distinguished by both their characteristic shape and a variety of molecular markers, critical aspects of regional phenotype remain responsive to environmental cues.

The role of local cues within forebrain

If the regional fate of forebrain VZ cells is controlled by local cues, how then are these cues established within the CNS? It seems likely that local interactions between communities of cells within specific regions of the CNS act synergistically to provide positional cues. Evidence in support of this idea has come from experiments that have investigated the fate of intact pieces of tissue moved to new positions within the neuroaxis. These studies revealed that when intact tissue is transplanted to novel sites, it can retain the positional information of the donor environment (Barbe and Levitt, 1991; Bally-Cuif et al., 1992; Guthrie et al., 1992; Cohen-Tannoudji et al., 1994). Further support for the idea that cell communities establish positional information within the neuraxis has come from experiments using immortalized cell lines as a means to probe local cues during development. This work has demonstrated that transplantation of immortalized cells to either the early postnatal cerebellum or hippocampus can induce these cells to adopt regional phenotypes in response to cues in their host environment (Snyder et al., 1992; Renfranz et al., 1991; Gao and Hatten, 1994). Together with our previous findings (Fishell et al, 1993) these experiments supports a model of development where boundaries isolate cell communities within forebrain, allowing positional cues to specify cell communities within distinct regions of the telencephalon.

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