Restriction of Late Cerebral Cortical Progenitors to an Upper-Layer Fate

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Summary

Early in development, neural progenitors in cerebral cortex normally produce neurons of several layers during successive cell divisions. The laminar fate of their daughters depends on environmental cues encountered just before mitosis. At the close of neurogenesis, however, cortical progenitors normally produce neurons destined only for the upper layers. To assess the developmental potential of these cells, upper-layer progenitors were transplanted into the cerebral cortex of younger hosts, in which deep-layer neurons were being generated. These studies reveal that late cortical progenitors are not competent to generate deep-layer neurons and are instead restricted to producing the upper layers.

Introduction

The mammalian cerebral cortex is organized into layers of neurons that share similar functional properties and patterns of connectivity. The layers are generated in an inside-out pattern during development, with proliferating cells in the ventricular zone first producing the deep layers 6 and 5, and later generating neurons destined for layers 4, 3, and 2. Previous studies have explored the developmental potential of early cortical progenitor cells, normally destined to give rise to the deep-layer neurons of layer 6, by transplanting these cells into older brains at the time of upper-layer neurogenesis (McConnell and Kaznowski, 1991). These experiments reveal that the laminar fates of deep-layer neurons are specified by the time of mitosis, since cells transplanted late in the cell cycle migrate to their normal deep-layer positions even in an older environment. Progenitors transplanted earlier during the S phase of the cell cycle, however, are multipotent: their daughters migrated to the upper cortical layers, assuming the laminar fate typical of host neurons. This multipotency is consistent with retroviral lineage studies which have shown that cortical progenitors infected early in development produce neurons of several layers over the course of successive cell divisions (Walsh and Cepko, 1988; Luskin et al., 1988; Price and Thurlow, 1988; Rakic, 1988; Reid et al., 1995); thus, at the onset of neurogenesis, progenitors are normally multifated.

In contrast, retroviral infections performed late in neurogenesis label a more restricted set of progeny, since neurons born at these times migrate exclusively to the last-generated layers, 3 and 2 (Rakic, 1988; Walsh and Cepko, 1988; Luskin et al., 1988; Price and Thurlow, 1988; Reid et al., 1995). Not only do late neural progenitor cells normally produce a smaller subset of laminar phenotypes, but these cells also display molecular differences compared with ventricular cells early in development: late progenitor cells express only low levels of the homeodomain gene *Otx1*, while early progenitors express *Otx1* at high levels (Frantz et al., 1994). Together, these findings raise the possibility that the developmental potential of late progenitors may be distinct from that of their earlier counterparts. Should this prove to be the case, the mechanisms that specify the laminar fates of cortical neurons might change over time during development. Although there are now many examples of cell–cell interactions that induce the production of specific cell types in the vertebrate nervous system (e.g., Shah et al., 1994; Roelink et al., 1995; Cepko et al., 1996), the possible contribution of lineage-based mechanisms is less well characterized. Here, we explore the mechanisms of laminar determination by late cortical progenitor cells and find that these cells are committed to the production of upper-layer neurons in a manner that is independent of environmental cues and heritable through successive cell divisions.

Results

To assess their developmental potential experimentally, upper-layer progenitor cells were transplanted back into the ventricular zone of younger host brains, during the time that neurons of the deep layers are being generated. Ferret cerebral ventricular cells were removed on postnatal day 0 (P0), which corresponds to embryonic day 42 (E42), at the onset of layer 2/3 neurogenesis (McConnell, 1988; Jackson et al., 1989). The cells were dissociated, labeled with [^{3}H]thymidine, and then transplanted into ferret host brains on E32, when the deep-layer neurons of layer 5 are being born (McConnell, 1988; Jackson et al., 1989). In addition to heterochronic transplants, control ventricular cells from E32 donors were transplanted isochronically into E32 hosts. In both cases, donor cells were labeled with [^{3}H]thymidine during dissociation, immediately before transplantation; thus, cells marked with this tag were both labeled and transplanted during S phase, a time at which early cortical progenitor cells are multipotent (McConnell and Kaznowski, 1991). The positions of both heavily and lightly labeled progeny of transplanted cells were examined at a variety of times after transplantation.

Integration of Progenitor Cells into Embryonic Host Brains

Cells were transplanted either directly into the host ventricular zone or by injection into the lateral ventricle. Both methods resulted in the integration of labeled cells specifically into the ventricular zone (Figure 1A). Cells were recovered from regions encompassing the entire neocortex, including posterior regions underlying occipital cortex, midcortical regions dorsal to the hippocampus, and anterior regions overlying the developing striatum. During the week following transplantation, labeled

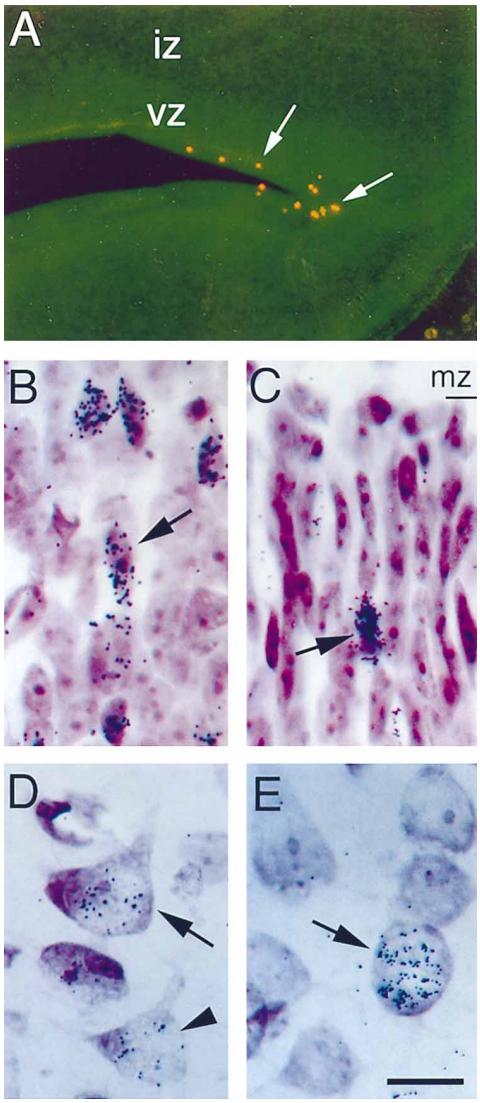

Figure 1. Positions of Isochronically and Heterochronically Transplanted Cells in Host Brains

(A) DiI-labeled, isochronically transplanted cells (arrows) in the cortical ventricular zone (vz) of an E32 ferret immediately after transplantation.

(B and C) Positions of [^{3}H]thymidine-labeled cells in the developing telencephalon before the onset of cortical upper-layer neurogenesis. (B) Transplanted cells (arrow) with an elongated morphology in the cortical plate following isochronic transplantation (8 day survival). (C) Labeled cell (arrow) with an elongated, migratory morphology at the top of the cortical plate after heterochronic transplantation (9 day survival). Because most labeled cells will eventually populate the upper layers, the neurons of which are only beginning to be generated in the host, it is likely that this transplanted cell will continue to migrate outward as new cells are added to the top of the cortical plate.

(D and E) Final positions of differentiated [^{3}H]thymidine-labeled transplanted cells in the adult cortex. (D) Heavily labeled pyramidal neuron (arrow) and lightly labeled neuron (arrowhead) in layer 5 of an isochronic transplant recipient. (E) Heavily labeled neuron (arrow) in layer 2/3 of a heterochronic transplant recipient. Abbreviations: iz, intermediate zone; mz, marginal zone. Scale bar in (E) represents 200 μm for (A) and 20 μm for (B)–(E).

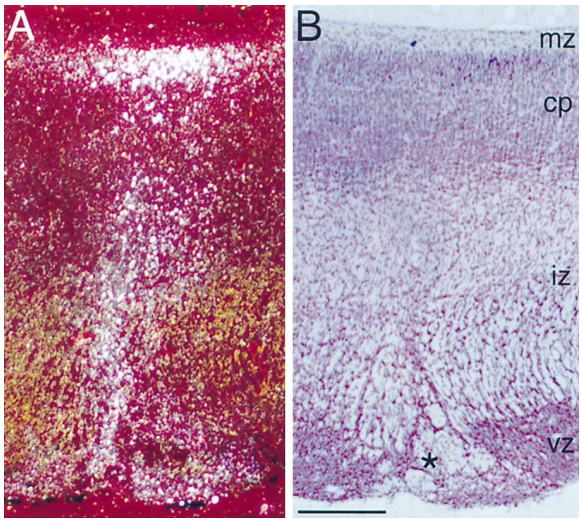

Figure 2. Migration of Transplanted Neurons in the Host Cerebral Wall

Low power photomicrographs of an isochronic host animal, 8 days after transplantation. (A) Dark-field view in which [^{3}H]thymidine-labeled cells are visible as white dots. Many labeled cells have entered the cortical plate (cp), where they accumulate underneath the marginal zone (mz). Other labeled cells are found in the intermediate zone (iz) and in an injection site in the ventricular zone (vz). (B) Bright-field view of the same section, stained with cresyl violet. The injection site is visible as a small bulge at the lumenal surface (asterisk). Scale bar represents 300 μm.

cells migrated away from the ventricular zone, through the intermediate zone, and into the cortical plate (Figure 2). During these stages, the cells displayed the elongated morphology characteristic of young migrating neurons (Figures 1B and 1C) (Rakic, 1971; Edmondson and Hatten, 1987; O'Rourke et al., 1992). By 6 weeks postnatal, when all the cortical layers are well differentiated in ferret, transplanted cells had differentiated within the cortex and had assumed a neuronal morphology (Figures 1D and 1E). Although the great majority of labeled neurons were found in the neocortex (5032 cells total in isochronic and heterochronic transplants), some were also found in subiculum (180 cells), entorhinal cortex (223 cells), hippocampus (927 cells), and in clusters within the anterior subventricular zone and leading into the olfactory bulb (168 cells). Although some labeled cells were recovered in clusters in the anterior subventricular zone, a structure that contributes to the formation of the olfactory bulb (Luskin, 1993), cells derived from the neocortical ventricular zone never migrated into the olfactory bulb, confirming the previous observations of Zigova et al. (1996). No labeled cells were observed in the thalamus, hypothalamus, or striatum of animals that received either isochronic or heterochronic transplants, although 4 cells were found in the septum of one heterochronic host. These experiments reveal little of the extensive incorporation of cells into noncortical regions as was seen when striatal precursors were transplanted into the lateral ventricle (Brüstle et al., 1995; Campbell et al., 1995).

When cells were injected directly into the ventricular zone, a substantial fraction of labeled cells remained in

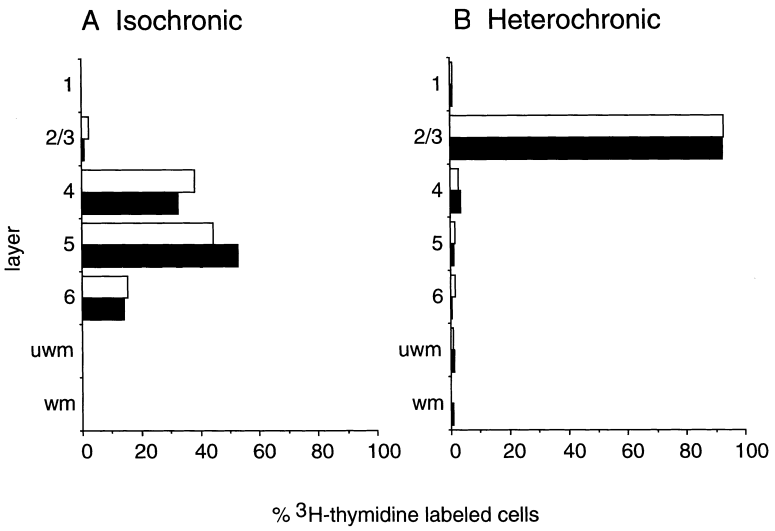

Figure 3. Final Laminar Positions of [^{3}H]Thymidine-Labeled, Transplanted Cells That Have Migrated into the Adult Host Cortex

Closed bars show the positions of cells heavily labeled with [^{3}H]thymidine (20 silver grains/cell), and open bars show the positions of lightly labeled cells (<20 grains/cell). (A) Isochronic transplants: heavily labeled cells (n = 310 cells from 3 brains) are found predominantly in layer 5, while lightly labeled cells (n = 367) populate slightly more superficial positions. There was no statistically significant difference between these two distributions by χ^2 analysis (p = 0.858). (B) Heterochronic transplants: both heavily labeled (n = 590 cells from 10 brains) and lightly labeled cells (n = 139) are found almost exclusively in layer 2/3, the position appropriate for their origin. The distribution of each of these populations differs significantly from those of isochronic controls (χ^2 analysis, p = 0.0001), but not from one another (p = 0.679). Abbreviations: wm, white matter; uwm, former subplate cells in the white matter directly underlying layer 6.

injection sites, identifiable as cell-sparse regions that disrupted the normal architecture of the ventricular zone (Figure 2). In adults that received isochronic transplants (all of which received only cell injections targeted to the ventricular zone), an average of 74.3% of all labeled cells migrated away from injection sites and into the cortex; in heterochronic transplants that received similarly targeted injections, an average of 64.5% of labeled cells migrated away from injection sites. Thus, the ability of cells to migrate was comparable in isochronic and heterochronic transplants. However, a smaller fraction of cells migrated in animals that received both intraventricular and targeted injections (average, 30.6%). This smaller figure reflected the presence of many small aggregates of labeled cells at the ventricular surface after intraventricular injections (data not shown). Although a smaller percentage of labeled cells migrated in these transplants, intraventricular injections produced very large numbers of labeled cells in the cortex, presumably because cells were able to enter the ventricular zone at multiple sites and with less scarring of ventricular tissue.

Laminar Destinations of Transplanted Neurons

Within neocortex, the final laminar positions of [^{3}H]thymidine-labeled neurons differed markedly between isochronic and heterochronic transplants. In control isochronic transplants, cells obtained from E32 donors were transplanted into same-aged hosts. Heavily labeled neurons were found predominantly in layer 5 (Figure 3A), a site consistent both with their time of origin and with the laminar destination of host neurons being generated at the time of transplantation (McConnell, 1988; Jackson et al., 1989). In occipital cortex, heavily labeled neurons were found primarily in layers 5 and 6, whereas cell positions were more superficial (in layers 5 and 4) in rostral cortex, consistent with the overall anterior-to-posterior gradient of neurogenesis in the developing cortex (McConnell, 1988; Jackson et al., 1989). Lightly labeled neurons that went through a subsequent round of cell division in the host environment migrated to layers 5 and 4 (Figure 3A), adopting the same fate as host neurons generated on the day or two subsequent to transplantation (McConnell, 1988; Jackson et al., 1989). These experiments show that transplanted cells derived from young donors are capable of populating the deep cortical layers in a manner that is both consistent with their origin and appropriate for the host environment.

If progenitor cells derived from older animals maintain the broad developmental potential characteristic of younger ventricular cells (McConnell and Kaznowski, 1991), they should be capable of generating deep-layer neurons. If, however, their developmental potential has narrowed to include only the last-generated cortical phenotypes, heterochronically transplanted neurons should migrate exclusively to the upper layers 2 and 3. Remarkably, the heavily labeled daughters of late progenitors transplanted to a younger environment displayed the phenotype characteristic of their origin and not of their new environment: over 90% of cells in neocortex were found in layer 2/3 (Figure 3B), a layer that was almost never populated by isochronically transplanted neurons (Figure 3A). These results suggest that late cortical progenitor cells are restricted to an upper-layer fate. An alternative explanation, however, is that late progenitors are indeed multipotent but that laminar specification occurs during a point in the cell cycle that precedes the time of transplantation. Were this the case, transplanted cells that undergo cell division in a younger environment should be exposed to cues that trigger deep-layer neurogenesis during the appropriate time in the cell cycle; thus, lightly labeled neurons should migrate to cortical layers 5 and 4 (as do lightly labeled cells in isochronic transplants: Figure 3A). In contrast with this prediction, neurons lightly labeled with [^{3}H]thymidine in heterochronic transplants migrated specifically to layer 2/3 (Figure 3B). Even after one or more rounds of cell division in a novel environment, neurons derived from late progenitor cells displayed a commitment to adopting the upper-layer fates typical of their

origin. Late cortical progenitors are thus not competent to respond to environmental cues present in younger brains that signal the production of the deep-layer neurons, and instead display a more restricted developmental potential.

Mitotic Potential of Late Progenitor Cells

In addition to producing cells of a restricted laminar phenotype, late cortical ventricular cells normally proceed through fewer cell divisions than do early progenitors (Rakic, 1988; Walsh and Cepko, 1988; Luskin et al., 1988; Price and Thurlow, 1988; Reid et al., 1995; Caviness et al., 1995). Do late progenitors have an intrinsically restricted mitotic potential? The extent of proliferation can be estimated crudely by counting and comparing the numbers of heavily and lightly labeled cells (the latter of which have undergone at least one additional round of division subsequent to transplantation) in heterochronic versus isochronic transplants. Heterochronic transplants produced fewer lightly labeled cells (19.1% of 729 labeled cells in cortex) than did isochronic controls (54.2% of 677 cells), suggesting that heterochronically transplanted progenitors divided fewer times, even in younger environments that normally support many more rounds of cell division. However, an excessively large number of divisions could produce the same result by diluting the radioactive label below the limits of detection. This possibility prevents us from making any definitive conclusions about the mitotic potential of late progenitors. It would be preferable to address this issue using retroviral lineage tracers to assess clone sizes directly following transplantation, as has been done in the developing retina, where potential appears also to be restricted in later progenitors (Fekete et al., 1990, Soc. Neurosci., abstract).

Migration of Transplanted Neurons into the Upper Layers

The results described above raise the question of how cells restricted to an upper-layer fate find their normal destination when placed in a younger brain. At E32, layer 2/3 does not exist as a target for migration: the first neurons normally destined for this layer are generated at the time of birth, about 10 days later; it takes another 10–14 days for these cells to reach their final positions at the top of the cortical plate (McConnell, 1988; Jackson et al., 1989). There are at least two ways that presumptive upper-layer neurons might find their appropriate positions following heterochronic transplantation. Transplanted cells might remain in the ventricular zone until the onset of upper-layer neurogenesis, and only then initiate migration along with host upper-layer neurons (Figure 4, solid cells). Alternatively, transplanted neurons might begin to migrate immediately and continue until the upper layers form around them, at which point the cells are signaled to stop and differentiate (Figure 4, hatched cells). These possibilities were distinguished by examining the positions of labeled cells at intermediate times, from 7–16 days after transplantation (E39–P7).

At 7–9 days post-transplantation (E39–E41), the host is generating layer 4 neurons; layer 2/3 neurogenesis

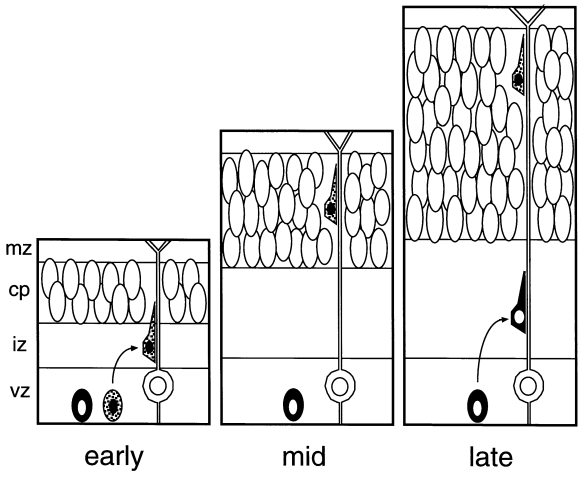

Figure 4. Possible Mechanisms for the Targeted Migration of Upper-Layer Cells Following Transplantation into a Younger Host Brain

Each panel shows a successively later time in development. One possibility (stippled cells) is that transplanted cells remain in the ventricular zone until the onset of upper-layer neurogenesis in the host, and only then migrate. Alternatively (solid cells), cells might initiate migration immediately after transplantation, but continue to migrate until upper-layer host neurons arrive in the cortical plate. Abbreviations: mz, marginal zone; cp, cortical plate; iz, intermediate zone; vz, ventricular zone.

begins several days later (McConnell, 1988; Jackson et al., 1989). Thus, at these ages, transplanted cells that wait for the host layer 2/3 to form before migrating should be confined to the ventricular zone; if an extended process of migration is at work, migrating cells should be found in more superficial locations. In both isochronic controls and heterochronic transplants, large numbers of transplanted cells have left the ventricular zone at 7–9 days after transplantation (cf. Figure 2). When the positions of migrating cells were plotted in isochronic transplants (Figure 5A), most labeled cells were found in the cortical plate, including the upper regions where newly arrived cortical neurons are found; the majority displayed the morphological features of migrating neurons (Figure 1B). The distribution of migrating cells in heterochronic transplants was similar after 8–9 day survivals: cells with a migratory morphology (Figure 1C) were distributed throughout the cerebral wall including the cortical plate (Figure 5B). Thus, labeled cells had clearly left the ventricular zone; they did not postpone their migration until the onset of layer 2/3 neurogenesis, although they did appear to migrate more slowly than did cells in isochronic transplants.

Examination of longer survivals revealed that heterochronically transplanted neurons migrated for extended periods. Twelve days after transplantation, isochronic neurons occupied their final positions in the deep and midcortical plate (Figure 5C); at this age (P2), unlabeled layer 4 neurons occupy the superficial cortical plate (McConnell, 1988; Jackson et al., 1989). Transplanted cells in the cortical plate displayed a rounded rather than elongated morphology, consistent with neuronal differentiation. Heterochronically transplanted cells, in contrast, were distributed throughout the cortical plate

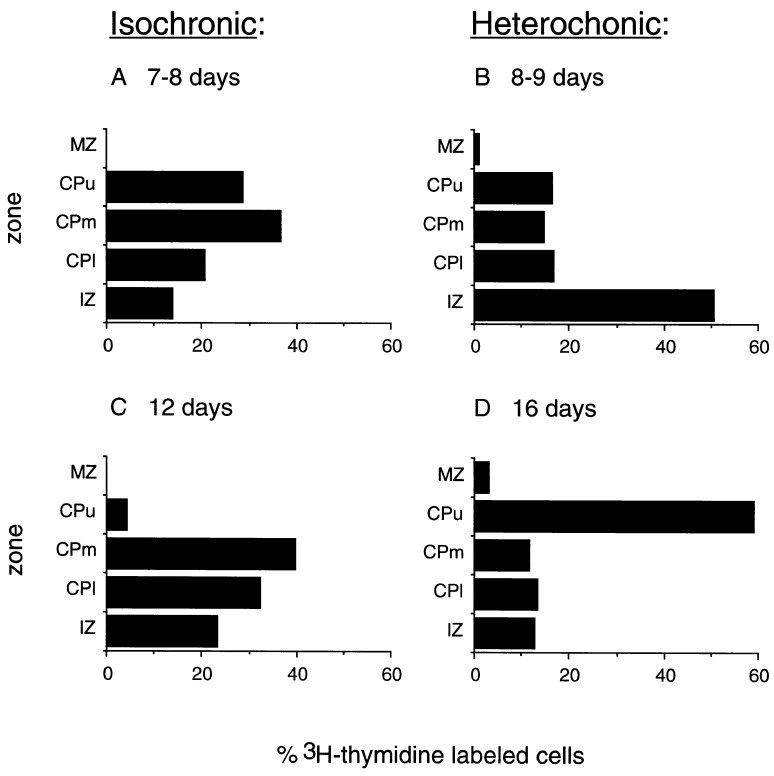

Figure 5. Positions of Migrating [^{3}H]Thymidine-Labeled Cells at Various Times after Transplantation

(A) and (B) show times that precede the onset of upper-layer neurogenesis, while (C) and (D) show times at which deep-layer neurons are differentiating and upper-layer neurons are just beginning to enter the cortical plate. Cells remaining at injection sites are not included (see Experimental Procedures). (A) Isochronic transplants, 7–8 day survival: 86% (n = 419 cells, 2 brains) of migrating transplanted cells have entered the cortical plate. (B) Heterochronic transplants, 8–9 day survival: 50% (n = 507 cells, 3 brains) of migrating cells are in the cortical plate. This distribution differs significantly from that of the comparably aged isochronic controls shown in (A) (χ^2 analysis, p = 0.0001). (C) Isochronic transplants, 12 day survival: roughly 72% (n = 395 cells, 4 brains) of cells that have migrated away from injection sites are in their final positions within the lower two-thirds of the cortical plate. (B) Heterochronic transplants, 16 day survival: 87% (n = 470 cells, 1 brain) of migrating transplanted cells are in the cortical plate; the majority occupy its upper zone. This distribution differs significantly from that shown in (C) (χ^2 analysis, p = 0.0001). Abbreviations: MZ, marginal zone; CPu, upper portion of the cortical plate, containing newly arrived neurons; CPm, middle portion of the cortical plate; CPl, lower portion of the cortical plate; IZ, intermediate zone.

even 16 days after transplantation (P6) and were concentrated at the top of the cortical plate among newly arrived neurons (Figure 5D). Labeled cells in all regions of the cortical plate maintained an elongated morphology (similar to that shown in Figure 1C), suggesting that presumptive upper-layer neurons transplanted into a younger environment actively seek the upper layers through an extended period of migration in which they continue to migrate into the top of the cortical plate until the host upper layers form around them. Only then, 1–2 weeks later, do the transplanted neurons round up and differentiate (data not shown). The mechanism that underlies this laminar homing is not known.

Discussion

While early cortical ventricular cells are both multifated and directly multipotent with respect to the laminar phenotypes of their progeny (Walsh and Cepko, 1988; Luskin et al., 1988; Price and Thurlow, 1988; Rakic, 1988; McConnell and Kaznowski, 1991; Reid et al., 1995), late progenitors that normally give rise only to upper-layer neurons are restricted to the production of these phenotypes. When transplanted heterochronically into a much younger cortical environment, late progenitor cells produce neurons that migrate exclusively to the upper-layer positions appropriate for their origin, even after an additional round of cell division in the new environment. Newly generated neurons initiate migration without regard to whether their target layer is present; laminar homing is accomplished through an extended migration that lasts for at least 3 weeks, until the host upper layers form and provide a site for differentiation. These results suggest that the competence of cortical progenitor cells to respond to cues signaling the production of deep layers is lost over time in development.

The mechanism that produces this restriction of developmental potential by late progenitor cells is unclear. A loss of competence could result from active, inductive signals that promote the production of a committed lineage, as during neural crest cell development (Shah et al., 1994; Lo and Anderson, 1995). Alternatively, a cell-autonomous change might be triggered by, for example, an internal clock that counts cell divisions, a mechanism that may contribute to the differentiation of oligodendrocytes from O2A progenitor cells (Temple and Raff, 1986). We also do not know whether the change in competence of cortical ventricular cells is progressive or sudden. While transplantation experiments have revealed that early E29 progenitors are capable of producing either deep- or upper-layer neurons, the developmental potential of ventricular cells in mid-neurogenesis is unknown. It would be interesting to ascertain whether layer 4 progenitors are capable of producing all the remaining layers (4, 3, and 2), but not the earlier-generated layers 5 and 6. Such a finding would suggest that progenitor cells lose successively the capacity to produce previously formed cell types, as proposed for the developing retina (Cepko et al., 1996). Transplantation studies in the developing neural crest suggest that late-emigrating neural crest cells also have a more restricted developmental potential than do their earlier counterparts (Artinger and Bronner-Fraser, 1992). It is thus possible that, in both the CNS and neural crest,

the amount of time that a cell spends within the neural tube before initiating migration may provide it with increasingly limited options for its subsequent development. It is not clear, however, whether the loss of potential seen in the neural crest results from an intrinsic change within a single lineage, or from the loss of a multipotent sublineage over time in development.

Not only is the developmental potential of late cortical progenitors more restricted than that of their earlier counterparts, but early and late ventricular cells also exhibit molecular differences. The homeodomain gene *Otx1* is expressed at high levels by early rat ventricular cells during the time of deep-layer neurogenesis, and *Otx1* expression is maintained by neurons of layers 5 and 6 neurons in the adult cortex (Frantz et al., 1994). During late embryogenesis, when upper-layer neurons are produced, *Otx1* mRNA is much less abundant in the ventricular zone. These observations raise the possibility that *Otx1* expression confers a deep-layer identity onto early generated cells, or that its expression is required for deep-layer neurogenesis in the presence of appropriate environmental cues, ideas that remain to be tested experimentally.

While this study has focused on laminar determination, the fate of a cortical neuron has many facets, including the neurotransmitter phenotype, axonal connections, and synaptic inputs of the cell. Recent evidence suggests that different aspects of neuronal fate can be determined by distinct mechanisms operating at distinct times during development. Several investigators have suggested that multipotent precursor cells in cortex may generate committed sublineages of excitatory pyramidal and inhibitory nonpyramidal neuronal progenitors (Parnavelas et al., 1991; Reid et al., 1995). Yet both pyramidal and nonpyramidal neurons within a clone can occupy different layers, suggesting that the specification of laminar identity can be uncoupled from neurotransmitter phenotype. The present results add a twist to this story, showing that the mechanisms that determine even a single aspect of cell fate, the laminar identity of the neuron, can change over time. Early in cortical neurogenesis, multipotent cells respond to what appear to be instructive environmental cues that trigger the production of deep-layer neurons (McConnell and Kaznowski, 1991). By the end of neurogenesis, however, through mechanisms that remain unclear, the lineage becomes restricted to the production of the last-generated cell type, the neurons of the upper cortical layers. It remains to be seen whether identical rules apply to both the pyramidal and nonpyramidal neuronal sublineages and whether a loss of developmental competence over time proves a general feature of many regions in the developing neural tube.

Experimental Procedures

Transplantation

Donor brains were sliced in the coronal plane at 400 μm thickness (Roberts et al., 1993) and placed in Hank's balanced salt solution (HBSS). The ventricular and subventricular zones were dissected using fine knives; cells were then dissociated enzymatically with papain (McConnell and Kaznowski, 1991) and labeled with [^{3}H]thymidine in vitro during dissociation using 1 μCi [^{3}H]thymidine/ml enzyme solution, to ensure that progenitors would be in S phase at the time of transplantation. In some cases, cells were also labeled with DiI (1,1′-dioctadecyl-3,3,3,3′-tetramethylindocarbocyanine perchlorate, DiIC18(3), Molecular Probes; 40 μl of a 2.5 mg/ml solution in 100% EtOH was added to 2 ml of culture medium for 10 min). Cells were washed and resuspended in HBSS, loaded into a 5 μl Unimetrics syringe equipped with a 30G needle, and injected into the brains of E32 host ferrets, using methods for fetal surgery identical to those published in Luskin and Shatz (1985). Two types of injections were made: all animals received injections that were targeted to the ventricular zone by inserting the needle into temporal cortex and then aiming the needle dorsally until the needle tip was visible through the skull, at which point 0.3 μl of cell suspension was injected by pressure. This was repeated at different sites until 2–3 injections were made per hemisphere. Some hosts also received injections of 0.3 μl of cell suspension directly into the lateral ventricle, a procedure that allowed cell incorporation throughout the ventricular zone (Brüstle et al., 1995; Campbell et al., 1995; Fishell, 1995). Donor brains that received transplants were sectioned and analyzed by autoradiography for the positions of neurons heavily labeled with [^{3}H]thymidine, using methods described previously (McConnell, 1988). The integration of labeled cells from intraventricular injections appeared to result from the attachment of small aggregates of transplanted cells to the host ventricular surface. There was no difference in the final laminar destinations of cells that were transplanted into the host by direct injection into the ventricular zone versus intraventricular injection.

To determine criteria for the appropriate targeting of transplanted cells to the host ventricular zone, several host brains were examined shortly after transplantation. Labeled cells were confined strictly to the ventricular zone in successful transplants (Figure 1A). In a small number of animals, labeled cells were clustered in the marginal zone (future layer 1) immediately after transplantation, revealing that the injections were mistargeted. For this reason, one adult host that showed large numbers of labeled cells in layer 1 was excluded from the analysis; labeled cells in layer 1 were observed only rarely in all remaining hosts.

Migration Patterns of Transplanted Neurons

Animals received transplants of P0 or E32 cells, as described above, but were perfused at a variety of times after transplantation (isochronic transplants: 7–8 day survival, 2 brains; 12 day survival, 4 brains; heterochronic transplants: 8–9 day survival, 3 brains; 16 day survival, 1 brain). The positions of labeled cells were determined as described above. At these intermediate timepoints, many cells in the ventricular or subventricular zones occupied injection sites that were easily identifiable. The fraction of cells that migrated away from injection sites was inexplicably variable in the isochronic controls (only 8.4% of 419 total cells were in injection sites at 7–8 days, but 58.7% of 395 cells occupied injection sites after a 12 day survival). For this reason, only the positions of those cells that migrated away from injection sites are shown in Figure 5. However, the fractions of cells found at injection sites in heterochronic transplants at 8–9 days (41.6%, $n = 507$ cells total) and at 16 days (43.8%, $n = 470$ cell total) were nearly identical, showing that cells do not delay migration until the onset of host upper-layer neurogenesis.

Statistical Analysis

The laminar positions of heavily and lightly labeled cells were determined for both isochronic and heterochronic transplants at a variety of ages. The distributions adopted by cells under these different conditions and at these different times were compared using the χ^2 contingency table analysis of the Macintosh StatView 512+ statistics and graphics program.

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