Evidence of Common Progenitors and Patterns of Dispersion in Rat Striatum and Cerebral Cortex

Christopher B. Reid1,2 and Christopher A. Walsh3,4,5

1Department of Pharmacology and 2Neuroscience Program, F. Edward Hebert School of Medicine, Uniformed Services University, Bethesda, Maryland 20814, 3Division of Neurogenetics, Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, Boston, Massachusetts 02115, and 4Program in Neuroscience and 5Program in Biological and Biomedical Sciences, Harvard Medical School, Boston, Massachusetts 02115

To correlate clonal patterns in the rat striatum with adult neuronal phenotypes, we labeled striatal progenitors between embryonic day 14 (E14) and E19 with a retroviral library encoding alkaline phosphatase. In the adult striatum, the majority of E14-labeled neurons (87%) were members of discrete horizontal or radial cell clusters. Radial clusters accounted for only 23% of cell clusters but >34% of labeled cells. Striatal clones also demonstrated an unexpected widespread pattern of clonal dispersion. The majority of striatal clones were widely dispersed within the striatum, and 80% of clones were part of even larger clones that included cortical interneurons. Finally, we observed that PCR-positive cortical interneurons were members of clones containing both interneurons and pyramids (44%), exclusively interneuron clones (24%), or combined striatal–cortical clones (16%), consistent with the view that cortical interneurons have multiple origins in differentially behaving progenitor cells. Our data are also consistent with the notion that similar mechanisms underpin striatal and cortical development.

Key words: cortex; striatum; radial migration; tangential migration; clonal analysis; pyramidal neuron; nonpyramidal neuron; interneurons; radial glia

In spite of striking differences in overall organization, striatum and cortex are increasingly seen as developmentally interconnected. The striatum, comprised of caudate–putamen, nucleus accumbens, and olfactory tubercle, is remarkable for its relatively homogenous structure. The striatum lacks the laminar organization displayed in the cerebral cortex. Striatal neurons are defined, instead, according to their location in patch or matrix compartments. Patch and matrix regions are interspersed, but identifiable, according to their contrasting neurochemical profiles and connectivity (Graybiel and Ragsdale, 1978; Herkenham and Pert, 1981; Gerfen, 1984, 1985). The striatum is composed principally of medium spiny, projection neurons (DiFiglia et al., 1976; Wilson and Groves, 1980) that account for 90% of the cellular complement (Kemp and Powell, 1971) and represent the major target of cortical inputs (Somogyi et al., 1981). Interneurons of various classes comprise the remaining 10%.

Neurogenesis in striatum and cortex occurs over the same developmental epoch, and the striatum appears to be a source of cortical neurons. Involvement of tangential migration in the distribution of cortical interneurons was first suggested by microscopic examination of developing forebrain (Van Eden et al., 1989; De Diego et al., 1994). More recently, DI tracing techniques directly demonstrated migration from lateral ganglionic eminence (LGE) to cerebral cortex (De Carlos et al., 1996; Anderson et al., 1997b; Tamamaki et al., 1997). Finally, migration of presumptive neurons was observed from medial ganglionic eminence (MGE) (Lavdas et al., 1999; Sussel et al., 1999; Anderson et al., 2001). Cells arriving to the cortex from LGE express Dlx-1 and Dlx-2 and appear to differentiate into GABAergic interneurons (Anderson et al., 1997b, 1999). In light of these studies, striatal development can now be recognized as much more complex than previously thought, however the basic patterns of migration and differentiation underlying striatal development have not been fully elucidated.

The migration of neurons from ganglionic eminence to cortex raises fundamental questions about neuronal precursors in striatal proliferative zones. We wondered, what, for instance, is the potential of striatal progenitors. Do common progenitors produce cells destined for both striatum and cerebral cortex, or does the ganglionic eminence contain a mixed population of progenitors restricted to a cortical or striatal fate?

Although striatal cell lineage has been previously studied (Haldyad and Cepko, 1992; Krushel et al., 1993) the present study was undertaken to determine clonal relationships among dispersed cells in the mature brain. We successfully labeled striatal and cortical neurons in nine experiments between embryonic day 14 (E14) and E19. We frequently observed neuronal clusters after E14 injection and P14 analysis, but only occasional clusters after E19 injections. PCR confirmed sibling relationships among clustered neurons and revealed widespread dispersion of clonally
related cells in the mature striatum. Amplification of DNA tags also demonstrated a high frequency of clones dispersing between striatum and cortex and involving cortical interneurons in a single experiment. Finally, the pattern of dispersion in clones containing cortical interneurons suggests that cortical interneurons have multiple origins in developing telencephalon.

MATERIALS AND METHODS

Alkaline phosphatase-encoding retroviral library. The preparation and composition of the alkaline phosphatase (AP) retroviral library used for these experiments is described elsewhere (Reid and Walsh, 1992). In brief, the AP library was derived from 3400 clones containing genomic DNA fragments cloned into the XhoI site of the DAP vector (Fields-Berry et al., 1992). The AP library contains between 100 and 400 distinct retroviral constructs at approximately equal titer.

Animal surgery. Timed-pregnant Long-Evans rats were purchased from Charles Rivers Laboratories (Wilmington, MA). Pregnancies were timed by the day after breeding (E0). Birth usually occurred on E21. Surgical procedures and injection of the retroviral supernatant into the lateral ventricles of the fetal rat brains are described in detail elsewhere (Walsh and Cepko, 1992). To label early-born as well as late-born striatal neurons, we made retroviral injections at the earliest feasible stages of development. Progenitors for the various striatal cell types were infected by injecting 4–6 μl of AP encoding retroviral library into the lateral ventricles of fetal rats between E14 and E19. The titer of retroviral supernatants varied from 1 to 20×10^6 colony-forming units/ml. The half-life of the virus at 37°C was ~4 hr; therefore, retroviral infection can be reasonably regarded as occurring during brief time windows.

Histology and analysis of clones. Animals were killed 14 d after birth by an overdose of Nembutal and perfused with 2–4% paraformaldehyde in 2 mM MgCl2 and 1.25 mM EGTA in 0.1 M Pipes buffer, pH 7.2. Brains were removed and submerged in fixedative overnight at 4°C, then transferred to 30% sucrose in PBS at 4°C until they sank. Brains were sectioned at 100 μm thickness using a Bright cryostat, and sections were mounted onto gel-coated glass slides. Sections were later processed for AP activity according to protocols presented elsewhere (Cepko et al., 1995). Labeled striatal cells were detected by microscopic examination of tissue sections. AP expression in cell bodies and processes usually allowed identification of the most labeled cells as neurons or glia.

Cortical cell types were frequently discernible in AP-stained sections according to standard morphological criteria (for review, see Peters and Jones, 1984). The pyramidal neurons of layer III and V were identified according to their dominant apical dendrites that give off branches as they extend from the cell body to the pial surface. These neurons were also identified according to the shape of their cell bodies and by the presence of basal dendrites. The apical dendrites of most layer III and V pyramidal neurons end in a spray of terminal branches at the pia, however other "modified" pyramidal neurons in layer II may display very short and/or divergated apical dendrites. Alternatively, these neurons may not have dendrites at all, but instead possess layer I terminal tufts that arise directly from the cell body. Other "modified" pyramidal neurons found in layer VI often show apical dendrites that arise from the lateral aspect of the side of the cell body or from a basal dendrite before extending as far as layer V. Finally the pyramidal neurons of layer IV often display round cell bodies, relatively thin apical dendrites, and thick dendrites radiating in all directions (Peters and Jones, 1984).

In contrast to the morphologies described above, nonpyramidal neurons are so called because they lack those characteristics typical of pyramidal neurons. They appear in a large variety of sizes, have a variety of dendritic and axonal configurations, and may have sparsely spiny, very spiny, or smooth processes (Peters and Jones, 1984). Nonpyramidal neurons were, nevertheless, often recognizable in our preparations as bipolar neurons, basket cells, or bitufted neurons. Bitufted neurons typi-

RESULTS

Striatal progenitor cells were infected by injections of the AP retroviral library into the lateral ventricles of fetal rats on E14, E17, E19. When injected animals were analyzed at postnatal day 14 (P14), mature neuronal and glial phenotypes were detected in the striatum as well as in adjacent forebrain regions such as amygdala, cerebral cortex, and olfactory bulb. The AP retroviral library produced efficient AP expression and robust staining of striatal cell processes.

As in previous studies of cortical (Reid et al., 1995; 1997) and olfactory bulb development (Reid et al., 1999), AP expression and staining allowed for reliable identification of greater than 95% of labeled striatal cells as neuronal or glial using standard morphologic criteria. The AP protein accumulates over time in the membranes of newly born neurons (Halliday and Cepko, 1992) and by P14, the vast majority show heavy labeling. Still, striatal neurons with divergent neurochemical phenotypes often display comparable morphological characteristics. As a result, specific neuronal cell types could not be reliably assigned on the basis of morphology alone.

AP-labeled cells show normal morphologies

The appearance of many AP-expressing neurons was, nevertheless, similar to those seen in postmortem studies of fixed tissue. Cells resembling medium spiny neurons had small cell bodies (10–20 μm in diameter) and three to five smooth dendritic trunks that branched into spiny secondary and higher order dendrites (Fig. 1A) (Kemp and Powell 1971; DiFiglia et al., 1976; Dimova et al., 1980; Wilson and Groves 1980; Chang et al., 1982). Their dendrites branch in all directions, filling an approximately spherical volume, 300–500 μm in diameter (Wilson et al., 1990). Other labeled neurons (Fig. 1B), had an appearance similar to cholinergic aspiny neurons: large spherical, oval, or elongated cell
bodies (~20–35 μm in diameter), thick, smooth, or sparsely spined primary dendrites, and thin dendrites that branch to form widely distributed second and higher order processes. Often bifurcating at long distances from the cell body (Wilson et al., 1990; Kawaguchi 1992, 1993), the dendrites of aspiny neurons typically occupy a space whose width is 500 μm in the dorsoventral and mediolateral axes and 750–1000 μm in the rostrocaudal plane (Bolam et al., 1984; Phelps et al., 1985; Graybiel et al., 1986; Wilson et al., 1990; Kawaguchi, 1992, 1993). AP-expressing interneurons with extensively ramified dendrites resembling the parvalbumin-immunopositive neurons were also seen in this study (Fig. 1C). Their dendrites are smooth proximally and become varicose distally (Kemp and Powell, 1971; DiFiglia et al., 1976; Dimova et al., 1980; Chang et al., 1982; Cowan et al., 1990; Kita et al., 1990; Lapper et al., 1992). Retrovirally labeled interneurons with this appearance were typically observed in dorsolateral striatum, consistent with their most common location in immunohistochemical studies (Kita et al., 1990).

The AP retroviral library also labeled large numbers of striatal subventricular zone cells. Many of these cells demonstrated spindle-shaped cell bodies with thick leading processes and thin trailing processes. As such, they were indistinguishable from migratory postmitotic cells destined for the olfactory bulb (Alvarez-Buylla, 1990; Luskin, 1993; Lois et al., 1996; Doetsch et al., 1997; Reid et al., 1999; Kornack and Rakic, 2001). The variety of recognizable striatal cell types labeled in this study suggests that retroviral labeling did not drastically alter normal cellular differentiation or affect progenitor cell behavior. AP-labeled cortical neurons could likewise often be classified according to standard morphological criteria (Peters and Jones, 1984) (see Materials and Methods).

**Spatial analysis of retrovirally labeled cells**

Although clonal analysis with PCR provides more definitive information about clonal relationships, the distribution of retrovirally labeled neurons gives general information about whether labeled cells cluster and has been used in the past to infer clonal relationships among striatal cells (Halliday and Cepko, 1992; Krushel et al., 1993).

In this study, glial cells always formed multicell clusters. Astrocyte clusters varied in size from two cells to several hundred cells. The largest of these glial clusters spread over multiple sections and into adjacent forebrain regions in an unpredictable manner. Labeled astrocytes had short, overlapping cell processes that usually obscured their cell bodies. As a result, precise determination of cell number was not possible. Oligodendrocyte clusters were much less commonly labeled than astrocytes, usually contained many fewer cells, and were almost always restricted to white matter tracts. The relative paucity of oligodendrocytes probably reflects the fact that many of these cells are born after P14 (Parnavelas, 1999).

AP-labeled striatal neurons also showed a strong tendency to cluster. More than 87% of striatal neurons labeled at E14 were members of geographically discrete cell clusters defined by the location of these cells within 500 μm (Table 1). Consistent with features consistent with these known cell classes. Insets depict a medium spiny neuron (A), a giant cholinergic neuron (B), and a parvalbumin-positive neuron (C). The AP reaction product does not appear to alter neuronal differentiation or striatal development. Insets are used with permission (Bennett and Wilson, 1998). Scale bars, 100 μm; insets, 50 μm.

![Figure 1](https://example.com/figure1.jpg)
previous studies (Krushel et al., 1993), these clusters ranged in size from two to four neurons (average, 2.14) (Table 1). Only 25% of striatal neurons labeled at later stages of development formed similar appearing clusters (Table 2).

After E14 injections, neuronal cell clusters usually contained two or three morphologically similar cells located at a similar distance from the lateral ventricle (Fig. 2A). Most often, these clustered cells were found occupying virtually identical positions on two adjacent sections separated by $\approx 200 \mu m$ along the A-P axis. Analogous clusters in the cortex have been termed horizon-

| Cluster | Location   | Rostrocaudal distance | Orientation |
|---------|------------|-----------------------|-------------|
| 1       | CPU        | 7800, 7800            | Radial      |
| 2       | CPU        | 8000, 8200            | Horizontal  |
| 3       | CPU        | 8200                  | Astrocyte   |
| 4       | CPU        | 8200, 8300            | Horizontal  |
| 5       | CPU        | 8500, 8500, 8500, 8500| Radial      |
| 6       | CPU        | 8600, 8700            | Horizontal  |
| 7       | CPU        | 8900, 9000            | Horizontal  |
| 8       | CPU        | 8900, 8900            | Horizontal  |
| 9       | CPU        | 9000, 9100            | Horizontal  |
| 10      | CPU        | 9200, 9200, 9300      | Radial      |
| 11      | CPU        | 9200, 9200, 9200      | Radial      |
| 12      | CPU        | 9200, 9300            | Horizontal  |
| 13      | NAcSh      | 9300, 9400            | Horizontal  |
| 14      | NAcSh      | 9300, 9400            | Horizontal  |
| 15      | CPU        | 9600, 9700            | Horizontal  |
| 16      | NAcSh      | 9600, 9700            | Horizontal  |
| 17      | CPU        | 9600, 9600, 9700      | Radial      |
| 18      | CPU        | 9800, 9800            | Horizontal  |
| 19      | CPU        | 10000, 10100          | Horizontal  |
| 20      | CPU        | 10500, 10600          | Horizontal  |
| 21      | LPO        | 11100, 11100          | Horizontal  |
| 22      | CPU        | 11100, 11200          | Horizontal  |
| 23      | CPU        | 11500, 11600          | Horizontal  |
| 24      | CPU        | 12800, 12800          | Horizontal  |
| 25      | LAMYG      | 13300, 13400          | Horizontal  |
| 26      | CPU        | 7700, 7700, 7700, 7900| Radial      |
| 27      | CPU        | 9700, 9800            | Horizontal  |
| 28      | CPU        | 9900, 10000           | Horizontal  |
| 29      | CPU        | 10600, 10700          | Horizontal  |
| 30      | CPU        | 10800, 10900          | Horizontal  |
| 31      | CPU        | 12100, 12200          | Horizontal  |
| 32      | CPU        | 8700, 8700, 8700      | Radial      |
| 33      | CPU        | 8700, 8800, 8900      | Horizontal  |
| 34      | CPU        | 8800, 8800            | Radial      |
| 35      | NAcSh      | 9100, 9200            | Horizontal  |
| 36      | NAcSh      | 9700, 9800            | Horizontal  |
| 37      | NAcSh      | 9800, 9900            | Horizontal  |
| 38      | CPU        | 11000, 11100, 11200  | Horizontal  |
| 39      | CPU        | 11600, 11800          | Horizontal  |
| 40      | CPU        | 6500, 6600            | Horizontal  |
| 41      | CPU        | 7200, 7300            | Horizontal  |
| 42      | CPU        | 7300, 7400            | Horizontal  |
| 43      | CPU        | 7300, 7400            | Horizontal  |
| 44      | CPU        | 7300, 7400, 7400      | Radial      |
| 45      | CPU        | 7600, 7700, 7800      | Horizontal  |
| 46      | CPU        | 8700, 8800            | Horizontal  |
| 47      | CPU        | 9800, 9900, 9900, 9900| Radial      |
| 48      | LAMYG      | 13500, 13600, 13700  | Horizontal  |

Topographic and morphologic analysis of striatal cell clusters labeled with the AP retroviral library at E14. Neuronal and glial clusters are indicated along with their anatomical location, orientation, and position along the rostrocaudal axis. CPu, Caudate–putamen; NAcSh, nucleus accumbens shell; LAMYG, lateral amygdala; LPO, lateral preoptic area. Radial glia cells are indicated by boldface type. Underlining designates astroglia.
Table 2. Cluster frequency by age at injection

| Age | Radial clusters | Horizontal clusters |
|-----|----------------|---------------------|
| E14 | % of clusters  | 23%                 | 77%                |
|     | Range          | 2-7                 | 2-3                |
|     | Average        | 3.6 ± 1.5           | 2.06 ± 0.25        |
| E19 | % of clusters  | 0%                  | 100%               |
|     | Range          | —                   | 2                  |
|     | Average        | —                   | 2                  |

Radial clusters included multiple cell types and neuronal morphologies

In this study, radial glia comprised <10% of AP-labeled cells. Over the course of development, radial glial cells are known to differentiate into astrocytes. Nevertheless, 40% of radial clusters included cells whose phenotypes were consistent with a radial glial identity. As many as three radial glia cells appeared in a single radial cluster. These cells showed a cell body in the proliferative zone, often, a short apical process contacting the ventricular surface, and a fine, radial process extending toward the pial surface (Smart and Sturrock, 1979). Other AP-labeled cells forming radial clusters included mature neurons, immature neurons, and migratory cells that were found in close association with, or even contacting, labeled radial glial processes (Fig. 3). This close association was consistent with the hypothesis that some AP-labeled cells migrate from the striatal ventricular zone along the processes of clonally related radial glia (Noctor et al., 2001). The presence of immature neurons and migrating cells in these radial clusters suggests that radial cluster progenitors are mitotically shortly before analysis at P14.

In contrast to horizontal clusters, cells in radial clusters frequently showed variable levels of AP expression: typically higher in cells near the ventricle and lower in cells far away. This might reflect a general gradient in birth dates, because older cells accumulate higher levels of AP.

Clonal analysis by PCR

To determine clonal relationships among AP-labeled cells, we performed PCR amplification of retrovirally encoded DNA tags. Because PCR analysis is <100% successful, clone size, clonal dispersion, and clonal diversity are generally underestimated. Given the complexity of the AP retroviral library (Reid et al., 1992), the likelihood of two progenitors being coincidentally infected with the same tag is <5% for experiments with fewer than four clones and <40% for experiments with fewer than eight clones (Walsh and Cepko, 1992).

Overall, PCR success rates were significantly lower in striatal neurons as compared with cortical neurons. This discrepancy was thought to be attributable to the heavier AP expression and staining noted in striatal neurons whose dendritic processes frequently overlap their cell bodies. Experiments with PCR success rates <40% were excluded from further analysis. In experiment 6, however, PCR efficiency in striatal neurons was equal to that of
cortical neurons (42%) (Table 3). As a result, this experiment offered novel insights into striatal development. PCR was also successful in five additional hemispheres (experiments 7–12) containing labeled cortical interneurons.

**Multicell clones**

PCR-defined clones varied widely in size and composition. Multicell clones contained as few as one neuron and as many as 10 neurons. The majority of PCR-positive striatal neurons labeled at E14 were members of multicell clones. These clones consisted of 2–10 neurons for an average of four neurons per multicell clone. The size of E14-labeled clones was therefore similar to the size of clones labeled by much earlier injections at E9.5 (McCarthy et al., 2001).

After E14 injection, 4/5 (80%) of multicell clones represented in the striatum were widely dispersed. The least dispersed clone, clone 5, was dispersed 6.3 mm along the A-P axis. Clones 1 and 3, the most widely dispersed clones (Figs. 5, 6), were dispersed 9.7 and 8.1 mm rostrocaudally. On average, multicell clones dispersed 55% of the rostrocaudal dimension of the P14 forebrain. In addition, all spread beyond the borders of the striatum into the cerebral cortex. In clones 1, 2, 3, and 5, the distance between the nearest clonally related striatal and cortical cells accounted for 55,

---

**Figure 3.** A radial cluster in the P14 striatum. Low-power photographs of two adjacent sections show radially clustered cells in the caudate–putamen (CPu) in relation to the lateral ventricle (LV), corpus callosum (CC), and cerebral cortex (CTX). Radial clusters, in contrast to horizontal clusters, frequently involved divergent cell types. White arrows indicate radial glia cell bodies and their processes. A mature, heavily stained neuron (white asterisk) is located relatively close to the lateral ventricle and the cell bodies of the radial glia cells (A, B). High-power photographs reveal immature cells in close association with one radial glial cell process (C, black arrows) and a neuron that appears to be in contact with another glial process (D). Scale bar: A, B, 500 μm; C, D, 200 μm.
The largest striatal clone, clone 1, contained two PCR-positive cortical interneurons in the entorhinal cortex at 14,200 and 17,000 μm. Another clonally related neuron was located in neocortex at 14,200 μm. Clone 1 also contained seven striatal neurons: four neurons between 7300 and 7500 μm, a single striatal neuron at 8400 μm, and two neurons at 8900 μm (Fig. 6). Clone 2 included a bipolar interneuron at 14,400 and a single striatal neuron. Clone 3 contained a cortical neuron in parasubicural cortex at 15,700 μm. This cell represented one PCR-positive neuron from a tight cluster of similar appearing cells. Clone 5 contained a multipolar neuron at 6800, a neuron in the amygdala, and a cluster of astrocytes in the striatum.

Within mixed striatal–cortical clones, cortical neurons were always located far posterior to labeled striatal neurons, and with the exception of clone 5, posterior to all AP-labeled striatal cells. The relative location of clonally related striatal and cortical neurons implies the existence of caudally dispersing intermediate progenitors. The predominantly caudal migration suggested by this study is in contrast to the lateral migration emphasized by slice culture experiments (Anderson et al., 1997b; Lavdas et al., 1999).

In summary, 4 of 10 (40%) multicell cortical clones in experiment 6 also contained striatal cells and provided direct evidence of common striatal/cortical progenitors on embryonic day 14. It is notable that all but one of the cortical neurons that were related to striatal neurons were identified as interneurons.

### Composition of interneuron clones

The unexpected and frequent clonal relationship between striatal neurons and cortical interneurons prompted us to extend our analysis to all cortical neurons. We performed clonal analysis among AP-labeled cortical cells in five additional hemispheres (experiments 7–12) and identified a total of 25 multicell clones that contained at least one cortical interneuron (Table 3). Among multicell clones, a surprisingly high percentage (44%) of interneuron-containing clones also included pyramidal neurons (Table 4). Multicell clones restricted to interneurons were less common and accounted for only 24% of interneuron clones. A comparable proportion of multicell, interneuron-containing clones included labeled astrocytes. The varied composition of these clones highlights the wide variety of clonal patterns involving cortical interneurons. One clone (clone 10) included a cluster of astrocytes located 200 μm from a single cortical interneuron. Another clone (clone 33) (Fig. 7) contained multiple pyramidal neurons, multiple interneurons, and an astrocyte cluster in the hippocampus. A third clone (clone 36) included a neuron in the accessory olfactory bulb, a large cluster of astrocytes in the neo-cortex, and a neuron in the deep layers of rhinal cortex.

Interneuron-containing clones varied in size, so we also calculated the percentage of individual cortical interneurons related to various other cell types. Predictably, 76% of cortical interneurons were related to other cortical interneurons, however the vast majority were also related to other cell types: 45% were clonally related to pyramidal neurons, 19% were related to glia, and 12% were related to striatal cells. Only 29% of cortical interneurons were related only to other cortical interneurons.

### Interneuron clones showed contrasting patterns of dispersion

Cortical clones containing interneurons frequently showed widespread dispersion. In this experiment 88% of interneuron-containing clones were dispersed >1 mm rostrocaudally and...
The pattern of widespread dispersion in interneuron-containing clones did, however, vary significantly with clonal composition (*p* < 0.001). In this study, 19 clones were identified as containing exclusively pyramids, exclusively interneurons, or both pyramidal neurons and interneurons. Clones containing strictly pyramidal neurons always formed clusters including a large radial cluster. Clones containing both cell types showed comparable dispersion tangentially and rostrocaudally, and were always dispersed at least 1 mm tangentially. Exclusive interneuron clones, on the other hand, frequently formed smaller clusters. Otherwise they showed even greater A-P dispersion than tangential dispersion (Fig. 8).

Within mixed pyramidal–interneuron clones, pyramidal neurons were sometimes clustered, but more often than not, pyramidal cells dispersed in a manner indistinguishable from interneurons. There were also no obvious rules governing the spatial relationship between clonally related pyramidal neurons and interneurons. Mixed pyramidal–interneuron clones and exclusive interneuron clones, therefore appeared to represent distinct patterns of neuron generation in the cerebral cortex. We concluded that cortical interneurons showed multiple modes of generation: mixed pyramidal and interneuron clones, mixed striatal–cerebral cortex clones, and exclusive interneuron clones.

**DISCUSSION**

The present study provides new information about clonal organization in the striatum. Our data confirm the tendency of E14-labeled neurons to form uniform cell clusters (Krushel et al., 1993) and extended these results by demonstrating larger, widespread striatal clones that incorporate more than one cluster. Some horizontal cell clusters were subclones of widely dispersing clones in striatum and cerebral cortex. Although the existence of mixed striatal–cortical clones has been suggested indirectly (Tan et al., 1998; Marin et al., 2001), this report demonstrates directly that single progenitor cells can give rise to striatal and cortical neurons. Previously we reported rare clones dispersing between striatum and cortex (Walsh and Cepko, 1993; Reid et al., 1995), however these clones could not be related to overall patterns of striatal development. It is evident from this study that mammalian forebrain contains progenitors that contribute not only to widely dispersed and mixed cell clusters, but also to clonal patterns of neuron generation in both the striatum and cerebral cortex.
disparate portions of striatum, but to cortex, and likely other subcortical regions like amygdala and preoptic area.

Symmetrical and asymmetrical divisions
Cluster analysis of nine experiments labeled between E14 and E19 showed a strong preponderance of horizontal clusters overall (80%). In horizontal clusters, labeled cells occupied very similar positions, usually in adjacent sections (76%) and never separated by >300 μm. In fact, 94% of horizontal clusters were two-cell clusters in which neurons showed similar morphology, similar levels of AP expression, and equal distance from the lateral ventricle (Table 1, Fig. 2A).

Analogous horizontal cell clusters in cerebral cortex contain cells located at similar distances from the ventricle, occupy either one or two adjacent layers, and typically show similar, layer-appropriate morphologies (Fig. 2B,C). In contrast to radial clusters, which are thought to be derived from cell divisions over a protracted period of neurogenesis, horizontal clusters have generally been interpreted as the progeny of late, symmetrically dividing progenitors (Kornack and Rakic, 1995; Mione et al., 1997). It is likely that horizontal clusters in rat striatum also represent progeny of late symmetrical divisions.

Radial clusters were observed in caudate–putamen as well as nucleus accumbens and involved a significant proportion of labeled cells (34%). Although radial clusters accounted for <25% of the clusters recognized in this study, they typically contained more cells (Tables 1, 2). They were also less dispersed rostrocaudally than horizontal clusters and, therefore, contained a higher density of cells in the A-P dimension (Table 1).

Embryonic analysis within days of retroviral injection captured the beginnings of radially oriented clusters within the striatal proliferative zones (Halliday and Cepko, 1992; Marin et al., 2000). However, radial clusters were not observed to span the mature striatum when cellular migration and differentiation were
complete (Krushel et al., 1993; Tan et al., 1998). The current study suggests that radial clusters represent an important element of striatal development and an enduring feature of the mature striatum.

Radial clusters have also been described in cerebral cortex (Tan and Breen, 1993; Tan et al., 1995) where they span multiple cell layers and are thought to form by migration of clonally related progeny along one or a small number of similarly situated radial glia cells. Composed predominantly of pyramidal neurons (Mione et al., 1997; Tan et al., 1998), the largest radial clusters so far observed contain hundreds of neurons (Tan et al., 1998). Recent analysis using PCR has demonstrated that similarly large clusters are in fact polyclonal (McCarthy et al., 2001).

We observed radial clusters in the striatum only when progenitors were labeled at relatively early stages of development so that the progeny of multiple divisions could be surveyed together as a whole. In this study, radial clusters appeared in four of five experiments after E14 injection, but were never observed in brains injected after E14 (Table 2). The same requirement for early labeling has been demonstrated in ferret cortex (Ware et al., 1999). It should be noted that radially oriented clusters have also been reported in optic tectum (Gray et al., 1988; Gray and Sanes, 1991), retina (Turner and Cepko, 1987; Holt et al., 1988; Williams and Goldowitz, 1992), and spinal cord (Leber et al., 1990) and may represent an invariant feature of the developing nervous system.

Radial glia support clonal units in developing striatum

Although most radial glial cells are thought to differentiate into astrocytes in the mature brain (Culican et al., 1990), 40% of radial clusters in the striatum contained radial glia in addition to mature neurons and undifferentiated, migratory cells. These clusters often extended to the corticostriatal border (>2 mm), or to ventral pallidum. In addition, some mature and immature cells appeared to maintain close proximity to labeled radial glial cell processes even at long distances (>1 mm) from the ventricle.

Although generally thought to represent a distinct cell population, radial glia cells have been increasingly implicated as neuronal progenitors (Chanas-Sacre et al., 2000; Malatesta et al., 2000). Time-lapse microscopy has lent direct support to the view that cortical radial glia divide to produce neurons (Noctor et al., 2001). In this study, we demonstrated that some radial glia cells and neurons in the developing striatum derive from common progenitors (Fig. 3). It should be noted that the appearance of some clusters was compatible with a model involving asymmetri-

Table 4. Interneuron sibling relationships

| Interneuron clones          |     |
|----------------------------|-----|
| Containing pyramids        | 44% |
| Interneuron only           | 24% |
| Containing glia            | 20% |
| Containing striatal cells  | 16% |
| Interneurons               |     |
| Related to interneurons    | 76% |
| Related to pyramids        | 43% |
| Related to interneurons only| 29% |
| Related to glia            | 19% |
| Related to striatal cells  | 12% |

Figure 7. Mixed morphology clone: the numbered, yellow dots in A–D illustrate the positions of neurons according to their location in the P14 rat brain (scale bar, 3 mm). Numbers associated with the dots correspond to high-power photomicrographs of the six clonally related neurons shown below. One layer V neuron whose position is indicated in C is not illustrated. With the exception of the neuron depicted in D (6), corresponding photomicrographs are oriented such that the pia is at the top. The photograph of neuron 6 is oriented as it appeared in the coronal section. The three of the PCR-positive neurons in this clone demonstrated features consistent with pyramidal neurons (2, 3, and 5) whereas the remaining clone members (1, 4, and 6) did not. Camera lucida drawings demonstrated that the layer II neuron depicted in D (4) possessed multiple ascending and descending dendrites emanating from the superior and inferior margins of the cell body, respectively. The neuron illustrated in panel 6 displayed dendrites radiating in all directions consistent with a multipolar neuron in lateral entorhinal cortex. This large clone also contained a cluster of hippocampal glial cells (not depicted). Scale bars, 100 μm.
Comparison with other brain regions

Analysis of cell lineage in developing striatum revealed patterns of migration and clonal organization that were evocative of cerebral cortex. Radial clusters were oriented parallel to the trajectories of radial glia in the developing brain (Mione et al., 1997), whereas smaller horizontal clusters were demonstrated to be part of larger widespread clones (Reid et al., 1995). The progenitors of widely dispersing striatal clones were clearly multipotential, involving neurons in cerebral cortex, as well as cells in striatum (Table 3).

The mechanisms underlying neuronal migration to cerebral cortex and olfactory bulb from the striatal proliferative zone appear to involve separate progenitors. Frequent dispersion of radial glia in the vertebrate brain (Mione et al., 1997), whereas smaller horizontal clusters were demonstrated to be part of larger widespread clones (Reid et al., 1995). The progenitors of widely dispersing striatal clones were clearly multipotential, involving neurons in cerebral cortex, as well as cells in striatum (Table 3).

The mechanisms underlying neuronal migration to cerebral cortex and olfactory bulb from the striatal proliferative zone appear to involve separate progenitors. Frequent dispersion of radial glia in the vertebrate brain (Mione et al., 1997), whereas smaller horizontal clusters were demonstrated to be part of larger widespread clones (Reid et al., 1995). The progenitors of widely dispersing striatal clones were clearly multipotential, involving neurons in cerebral cortex, as well as cells in striatum (Table 3).

Genetic control of striatal neurogenesis

Multiple genes have been implicated in controlling the migration of MGE-derived interneurons. Slit-1, for instance, is expressed in the ventricular zone (VZ) of LGE and is repellent for striatal interneurons and olfactory bulb neurons (Itoh et al., 1998; Mason et al., 2001). The effect of Slit-1 on both GABAergic and non-GABAergic neurons is blocked in explants by the addition of a non-functional Slit-1 receptor (Zhu et al., 1999). A similar repulsive activity has been demonstrated by semaphorins 3A and 3F, however their repellent effect is selective for GABAergic interneurons destined for cerebral cortex (Marin et al., 2001). Netrin-1 is another diffusible factor expressed in the striatal VZ. The repulsive activity of Netrin-1 on migrating SVZ neurons in the striatum is blocked in the presence of antibodies against Deleted-in-colorectal cancer, its putative receptor (Hamasaki et al., 2001). In the context of the present study, it appears that individual progeny of striatal progenitors labeled at E14 may show contrasting sensitivities to factors such as slit-1, netrin-1 or the semaphorins. Therefore, expression of receptors for diffusible signals may be environmentally regulated or may instead reflect inherited differences assigned through asymmetric cell divisions.

Multiple modes of cortical interneuron generation

A consensus view has emerged that cortical interneurons and pyramidal neurons have distinct origins (Tan et al., 1998; Anderson et al., 1999; Parnavas, 2000). When neuronal migration from basal telencephalon to cerebral cortex is blocked, there is a dramatic decrease in GABA reactivity in the cerebral cortex (Anderson et al., 1997b). A strict separation of pyramidal and interneuron progenitors is, however, difficult to resolve with chimera and many retroviral labeling experiments. Immunohistochemical examination of E16-labeled cortical clones in rat cerebral cortex, for instance, demonstrated clusters containing both glutamate-positive and GABA-positive neurons (Lavdas et al., 1996). This result leaves open the possibility that some progenitors produce both GABAergic and glutamatergic neurons.

On the basis of their representation in chimera or retroviral experiments, it has been suggested that pyramidal neurons arise in the cortical VZ and form radial clusters, whereas GABAergic neurons generated in MGE become scattered in the cerebral cortex. It should be noted, however, that one-third of scattered neurons in the cortices of unbalanced mouse chimeras were glutamate-positive and presumably pyramidal neurons (Tan et al., 1998). Conversely, it seems likely that some proportion of cortical interneurons derives from progenitors restricted to a cortical fate because labeled GABAergic cells were seen in the cortices of chimeric animals even when labeling was not observed in underlying striatum (Tan et al., 1998). Because it is not yet possible to follow individual cells from their point of origin to their final state of differentiation, the possibility remains that some fraction of interneurons originates in the cortical VZ.

REFERENCES

Alvarez-Buylla A (1990) Commitment and migration of young neurons in the vertebrate brain. Experientia 46:879–882.
Anderson SA, Qiu M, Bulfone A, Eisenstat DD, Meneses J, Pedersen R, Rubenstein JL (1997a) Mutations of the homeobox genes Dlx-1 and Dlx-2 disrupt the striatal subventricular zone and differentiation of late born striatal neurons. Neuron 19:27–37.
Anderson SA, Eisenstat DD, Shi L, Rubenstein JL (1997b) Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. Science 278:474–476.
Anderson SA, Mione M, Yun K, Rubenstein JLR (1999) Differential origins of neocortical projection and local circuit neurons; role of Dlx genes in neocortical interneuronogenesis. Cereb Cortex 9:646–654.
clonally related cells in the primate neocortex: relationship to distinct mitotic lineages. Neurosci 15:311–321.
Kornack DR, Rakic P (2001) The generation, migration, and differenti-
ation of olfactory neurons in the adult primate brain. Proc Natl Acad Sci USA 98:4752–4757.
Krushel JA, Johnson PG, Fishell G, Tibshirani R, van der Kooy D (1993) Spatially localized neuronal cell lineages in the de veloping mammalian forebrain. Neuroscience 53:1035–1047.
Lapper SR, Smith Y, Sadikot AF, Parent A, Bolam JP (1992) Cortical input to parvalbumin-immuneactive neurons in the putamen of the squirrel monkey. Brain Res 596:215–224.
Lavdas AA, Mione MC, Parnavelas JG (1996) Neuronal clones in the cerebral cortex show morphological and neurotransmitter heterogeneity during development. Cereb Cortex 6:490–497.
Lavdas AA, Grigoriou M, Mione V, Parnavelas JG (1999) The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J Neurosci 19:7881–7888.
Leber SM, Bredevold SM, Sanes JR (1990) Lineage, arrangement, and death of clonally related motoneurons in chick spinal cord. J Neurosci 10:2451–2462.
Lois C, Garcia-Verdugo JM, Alvarez-Buylla (1996) Chain migration of neuronal precursors. Science 271:978–981.
Luskin MB (1993) Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron 11:173–189.
Malatesta P, Hattfuss E, Gotz M (2000) Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. Development 127:5253–5263.
Marin O, Anderson SA, Rubenstein JL (2000) Origin and molecular specification of striatal interneurons. J Neurosci 20:6063–6076.
Marin O, Yaron A, Bagri A, Tessler-Lavigne M, Rubenstein JLR (2001) Survival of striatal and olfactory bulb neurons is regulated by semaphorin-neurpinin interactions. Science 293:872–875.
Mason HA, Ito S, Corfas G (2001) Extracellular signals that regulate the tangential migration of olfactory bulb neuronal precursors: inducers, inhibitors, and repellents. J Neurosci 21:7654–7665.
McCarty M, Turnbull DH, Walsh CA, Fishell G (2001) Telencephalic neural progenitors appear to be restricted to regional and glial fates before the onset of neurogenesis. J Neurosci 21:6772–6781.
Mione MC, Cavanagh JFR, Harris B, Parnavelas JG (1997) Cell fate specification and laminar organization of striatal divisions in the developing cerebral cortex. J Neurosci 17:2018–2029.
Noctor SG, Flint AC, Weissman TA, Dammerman RS, Kriegstein AR (2001) Neurons derived from radial glial cells establish radial units in neocortex. Nature 409:714–720.
Parnavelas JG (1999) Glial cell lineages in the rat cerebral cortex. Exp Neurol 156:418–429.
Parnavelas JG (2000) The origin and migration of cortical neurons: new vistas. Trends Neurosci 23:126–131.
Peters A, Jones EG (1984) Classification of cortical neurons. In: Cerebral Cortex 1:107–122.
Phelps PE, Houser CR, Vaughan JE. (1985) Immunocytochemical local-
ization of choline acetyltransferase within the rat neostriatum: a cor-
relation with light and electron microscopic study of cholinergic neurons and synapses. J Comp Neurol 238:280–307.
Reid CB, Liang I, Walsh C (1995) Systematic widespread clonal organi-
sation in cerebral cortex. Neuron 15:299–310.
Reid CB, Tavaoze IF, Walsh CA (1997) Clonal dispersion and evi-
dence for asymmetric cell division in ferret cortex. Development 124:2441–2450.
Reid CB, Liang I, Walsh CA (1999) Clonal mixing, clonal restriction, and specification of cell types in the developing rat olfactory bulb. J Comp Neurol 403:106–118.
Smart HM, Sturrock RR (1979) Ontogeny of the neostriatum. In: The neostriatum. New York: Pergamon.
Somogyi P, Bolam JP, Smith AD (1981) Monosynaptic cortical input and axon collaterals of identified striatoni gral neurons: a light and electron microscopic study using the Golgi-peroxidase transport-degeneration procedure. J Comp Neurol 195:567–584.
Soriano E, Dumesnil N, Auladell C, Cohen-Tannoudji M, Sotelo C (1995) Molecular heterogeneity of progenitors and radial migration in the developing cerebral cortex revealed by transgene expression. Proc Natl Acad Sci USA 92:11676–11680.
Sussel L, Marin O, Kimura S, Rubenstein JL (1999) Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular re-
specification within the basal telencephalon: evidence for a transfor-
mation of the pallidum into the striatum. Development 126:3339–3370.
Tamamaki N, Fujimori KE, Takauji R (1997) Origin and route of tan-
gentially migrating neurons in the developing neocortical interior.
J Neurosci 17:831–843.
Tan SS, Breen S (1993) Radial mosaicism and tangential cell dispersion both contribute to mouse neocortical development. Nature 362:638–640.
Tan SS, Faulkner-Jones B, Breen SJ, Walsh M, Bertram JF, Reese BE
(1995) Cell dispersion patterns in different cortical regions studied with an X-inactivated transgenic marker. Development 121:1029–1039.

Tan SS, Kalloniatis M, Sturm K, Tam PP, Reese BE, Faulkner-Jones B (1998) Separate progenitors for radial and tangential cell dispersion during development of the cerebral neocortex. Neuron 21:295–304.

Turner D, Cepko CL (1987) Cell lineage in the rat retina: a common progenitor for neurons and glia persists late in development. Nature 328:131–136.

Van Eden CG, Mrzljak L, Voorn P, Uylings HB (1989) Prenatal development of GABA-ergic neurons in the neocortex of the rat. J Comp Neurol 289:213–227.

Walsh C (1995) PCR-based techniques for utilizing retroviruses as cell lineage markers. Methods in molecular genetics, Vol 4, Molecular virology (Adolph KW, ed), pp 280–295. Orlando, FL: Academic.

Walsh C, Cepko CL (1992) Widespread dispersion of neuronal clones across functional regions of the cerebral cortex. Science 255:434–440.

Walsh CA, Cepko CL (1993) Widespread clonal dispersion in proliferative layers of cerebral cortex. Nature 362:632–635.

Ware ML, Tavazoie SF, Reid CB, Walsh CA (1999) Coexistence of widespread clones and large radial clones in early embryonic ferret cortex. Cereb Cortex 9:636–645.

Wichterle H, Turnbull DH, Nery S, Fishell G, Alvarez-Buylla A (2001) In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. Development 128:3759–3771.

Williams RW, Goldowitz D (1992) Structure of clonal and polyclonal cell arrays in chimeric mouse retina. Proc Natl Acad Sci USA 15:1184–1188.

Wilson CJ, Groves PM (1980) Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: a study employing intracellular inject of horseradish peroxidase. J Comp Neurol 194:599–615.

Wilson CJ, Chang HT, Kitai ST (1990) Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum. J Neurosci 10:508–519.

Zhu Y, Li H, Zhou L, Wu JY, Rao Y (1999) Cellular and molecular guidance of GABAergic neuronal migration from an extracortical origin to the neocortex. Neuron 23:473–485.