

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

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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, DiI 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 (Halliday 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

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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 et al., 1995). 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 from 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 2–4  $\mu$ l of the 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  $\times 10^6$  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 MgCl<sub>2</sub> and 1.25 mM EGTA in 0.1 M PIPES buffer, pH 7.2. Brains were removed and submerged in fixative overnight at 4°C, then transferred to 30% sucrose in PBS at 4°C until they sank. Brains were sectioned at 100  $\mu$ m 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 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 divaricated apical dendrites. Alternatively, these neurons may not show apical 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 IV. 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. Bipolar neurons typically have single dendrites extending from superior and inferior poles to form narrow, vertically oriented dendritic trees. The dendrites of multipolar neurons, on the other hand, may arise from any surface of the cell body without a preferred orientation. Finally, bitufted cells demonstrate multiple dendrites extending as tufts from opposite poles of an ovoid or elongated cell body.

In AP-injected brains the locations of labeled cells were recorded by photography and/or camera lucida drawings. Rostrocaudal location was determined by counting section number and multiplying by the section thickness (100  $\mu$ m). The rostral tip of the olfactory bulb was designated as the origin. These values were used to calculate anteroposterior (A-P)

dispersion. Dispersion perpendicular to the A-P axis (in the mediolateral plane as well as the superoinferior plane) was regarded as tangential dispersion. Tangential dispersion within cortical clones was measured directly on low-power camera lucida drawings of whole sections. Maximal A-P and tangential dispersion plots (shown in Fig. 8) were created using the Mathematica software program. This program was also used to perform linear regressions for different clone types (see Fig. 8, legend).

Consistent with previous analyses, neuronal clusters in the striatum were defined as cells separated by <500  $\mu$ m (Krushel et al., 1993). Most clusters were composed of similar appearing, similarly situated neurons.

**Clonal analysis with PCR.** Tissue analysis was performed by preparing DNA samples from labeled cells for amplification by PCR, as presented elsewhere (Walsh and Cepko, 1992; Walsh, 1995). Briefly, coverslips were removed in a 50 ml centrifuge tube filled with sterile water. Small tissue fragments (~100  $\times$  200  $\times$  200  $\mu$ m) containing the nucleus of each labeled cell were dissected using a fresh razor blade edge. Tissue fragments were digested in 10  $\mu$ l of proteinase K (0.2 mg/ml) in 1 $\times$  PCR buffer (in mM: 2.5 MgCl<sub>2</sub>, 50 Tris buffer, pH 8.3, 25 KCl, and 0.5% Tween 20) at 65°C for 4–24 hr. Each well was covered with 30  $\mu$ l of mineral oil to prevent evaporation. Samples were then heated to 85°C for 20 min to inactivate proteinase K and then 95°C for 5 min to denature the DNA. A nested PCR protocol was used to increase the sensitivity and specificity of amplification and is described elsewhere (Walsh and Cepko, 1992; Walsh, 1995). At least 10% of all PCR reactions were negative controls, consisting either of unlabeled tissue or reagents alone. None of the negative controls in this study showed amplified DNA.

**Analysis of PCR products.** PCR products from the second PCR reaction were separated on 3:1% NuSieve–Seakem agarose gels to determine tag sizes. Each tag was then digested with *CfoI*, *RsaI*, *AluI*, *MseI*, and *MspI*. Digested DNA samples of similar predigest size were run side by side on agarose gels to allow direct comparison of restriction fragments. PCR products of similar initial size and restriction digest pattern were interpreted as indicating a common progenitor. Dissimilar tag size or restriction patterns were interpreted as indicating separate progenitors.

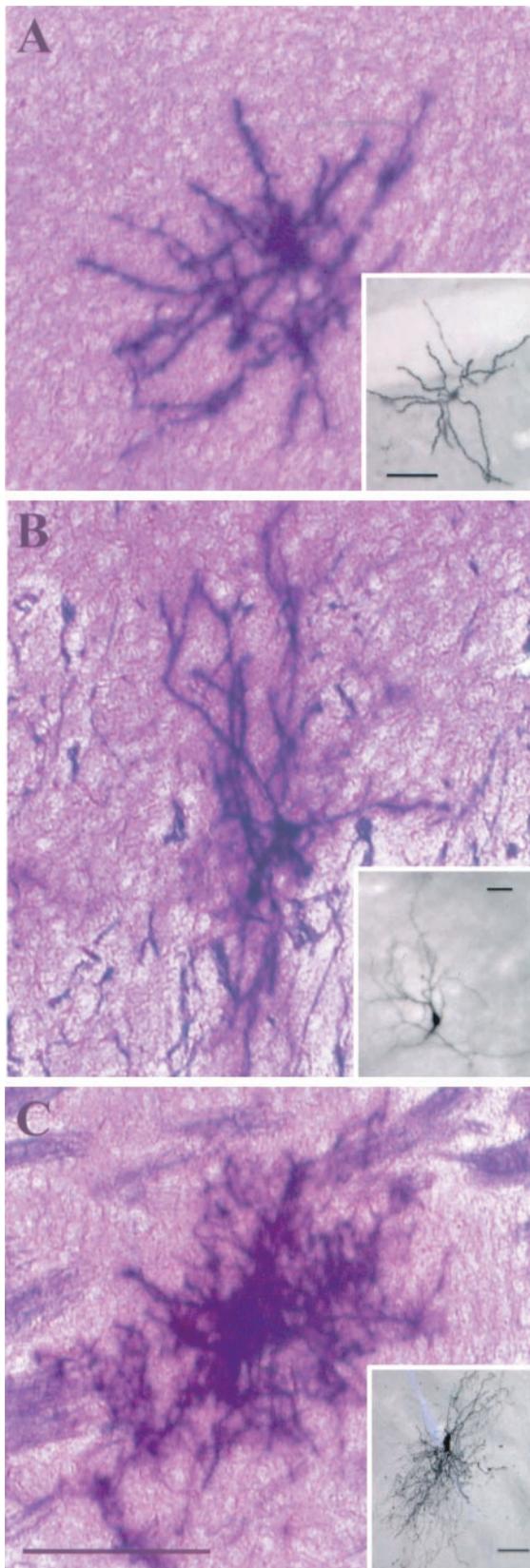
## RESULTS

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 >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  $\mu$ m 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  $\mu$ m 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



**Figure 1.** Retrovirally encoded AP labels a variety of normal neuronal morphologies in the mature striatum. *A–C* show three E14-labeled neurons with divergent cell morphologies. Neurons depicted in the *insets* were filled with biocytin after chemical and physiological characterization. Retrovirally encoded AP allowed for the demonstration of morphological

bodies ( $\sim 20\text{--}35\ \mu\text{m}$  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  $500\ \mu\text{m}$  in the dorsoventral and mediolateral axes and  $750\text{--}1000\ \mu\text{m}$  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  $500\ \mu\text{m}$  (Table 1). Consistent with

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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,  $100\ \mu\text{m}$ ; *insets*,  $50\ \mu\text{m}$ .

**Table 1. Cluster analysis of E14-labeled striatal cells**

	Cluster	Location	Rostrocaudal distance	Orientation
Experiment 1	1	CPu	7800, 7800	Radial
	2	CPu	8000, 8200	Horizontal
	3	CPu	<u>8200</u>	Astrocyte
	4	CPu	8200, 8300	Horizontal
	5	CPu	<b>8500, 8500</b> , 8500, 8500 8500, <b>8600</b> , 8600	Radial Radial
	6	CPu	8600, 8700	Horizontal
	7	CPu	8900, 9000	Horizontal
	8	CPu	8900, <u>8900</u>	Horizontal
	9	CPu	9000, 9100	Horizontal
	10	CPu	9200, 9200, <b>9300</b> , 9400	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	<b>9600, 9600</b> , 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
Experiment 2	26	CPu	7700, 7700, 7700, 7900	Radial
Experiment 3	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
Experiment 4	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
Experiment 5	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	<b>9800, 9900, 9900, 9900, 9900</b>	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.

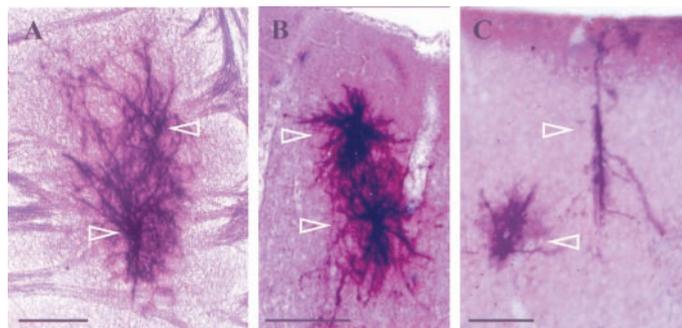
previous studies (Krushel et al., 1993), these clusters ranged in size from two to four neurons (average, 2.14) (Table 1). Only 25% of striatal neurons labeled at later stages of development formed similar appearing clusters (Table 2).

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  $\leq 200 \mu\text{m}$  along the A-P axis. Analogous clusters in the cortex have been termed horizon-

**Table 2. Cluster frequency by age at injection**

	Radial clusters	Horizontal clusters
<b>E14</b>		
% of clusters	23%	77%
Range	2–7	2–3
Average	3.6 ± 1.5	2.06 ± 0.25
<b>E19</b>		
% of clusters	0%	100%
Range	—	2
Average	—	2



**Figure 2.** Horizontal clusters labeled at E14. *A* shows a cluster of AP-labeled neurons (*white triangles*) in the lateral caudate–putamen adjacent to the corticostriatal border. The neurons within this cell cluster are notable for their shared morphological features and the similar orientation of their mutually overlapping cell processes. *B* and *C* depict analogous clusters from cerebral cortex. *B* contains two layer II/III neurons (*white triangles*) that possess features consistent with multipolar neurons. *C* contains contrasting nonpyramidal cell types: a stellate-appearing layer IV neuron and a layer III bipolar neuron. Photomicrographs are oriented such that the pia is at the top. Scale bar, 100  $\mu$ m.

tal clusters (Mione et al., 1997). In addition to horizontal cell clusters and isolated single neurons, we observed radial clusters, oriented perpendicular to the ventricle and to the A–P axis, that were composed of neurons at various distances from the ventricle.

### Horizontal clusters

Horizontal clusters occurred throughout the striatum without any obvious rostrocaudal or mediolateral bias (Table 1). Horizontal clusters represented 77% of clusters labeled at E14 and were almost always (94%) comprised of two neurons at similar distance from the lateral ventricle (average, 2.06 ± 0.24). Horizontal clusters occasionally contained three labeled neurons (6%), consistent with earlier analyses of E14-labeled striatal neurons (Krushel et al., 1993). In this study, neurons in horizontal clusters often occupied virtually identical positions in two adjacent sections (76%) or were located on the same section (9%) within 300  $\mu$ m (Fig. 2*A*). Fifteen percent of horizontal clusters spanned more than two adjacent sections. Clusters spanning three sections always involved one neuron per section on three consecutive sections. Although less common, horizontal clusters labeled by E19 injection invariably contained two similar appearing neurons and were otherwise indistinguishable from those labeled by E14 injections.

### Radial clusters

Radially oriented clusters comprised 23% of clusters identified in this study and were composed of cells located at progressive

distances from the lateral ventricle (Table 1, Fig. 3). Radial clusters were larger than horizontal clusters and included an average of 3.6 labeled cells (range, two to seven cells). Although radial clusters represented only 23% of clusters, they accounted for more than one-third of labeled striatal cells. Radial clusters were oriented along the trajectories of antecedent radial glial processes and spanned up to 90% of the striatal thickness. In spite of their significant radial dispersion, radial clusters showed very little lateral displacement. In this study, 50% of radial clusters spanned <100  $\mu$ m along the A–P axis. By way of comparison, only 9% of horizontal clusters were dispersed <100  $\mu$ m anteroposteriorly. Accordingly, the average A–P diameter of the radial clusters was less than that of horizontal clusters, even though radial clusters contained more cells. Analogous radial clusters, composed principally of pyramidal neurons and spanning the entire cortical plate have, likewise, been described in cerebral cortex (Kornack and Rakic, 1995; Soriano et al., 1995; Mione et al., 1997; Tan et al., 1998; Ware et al., 1999) (Fig. 4).

### Radial clusters included multiple cell types and neuronal morphologies

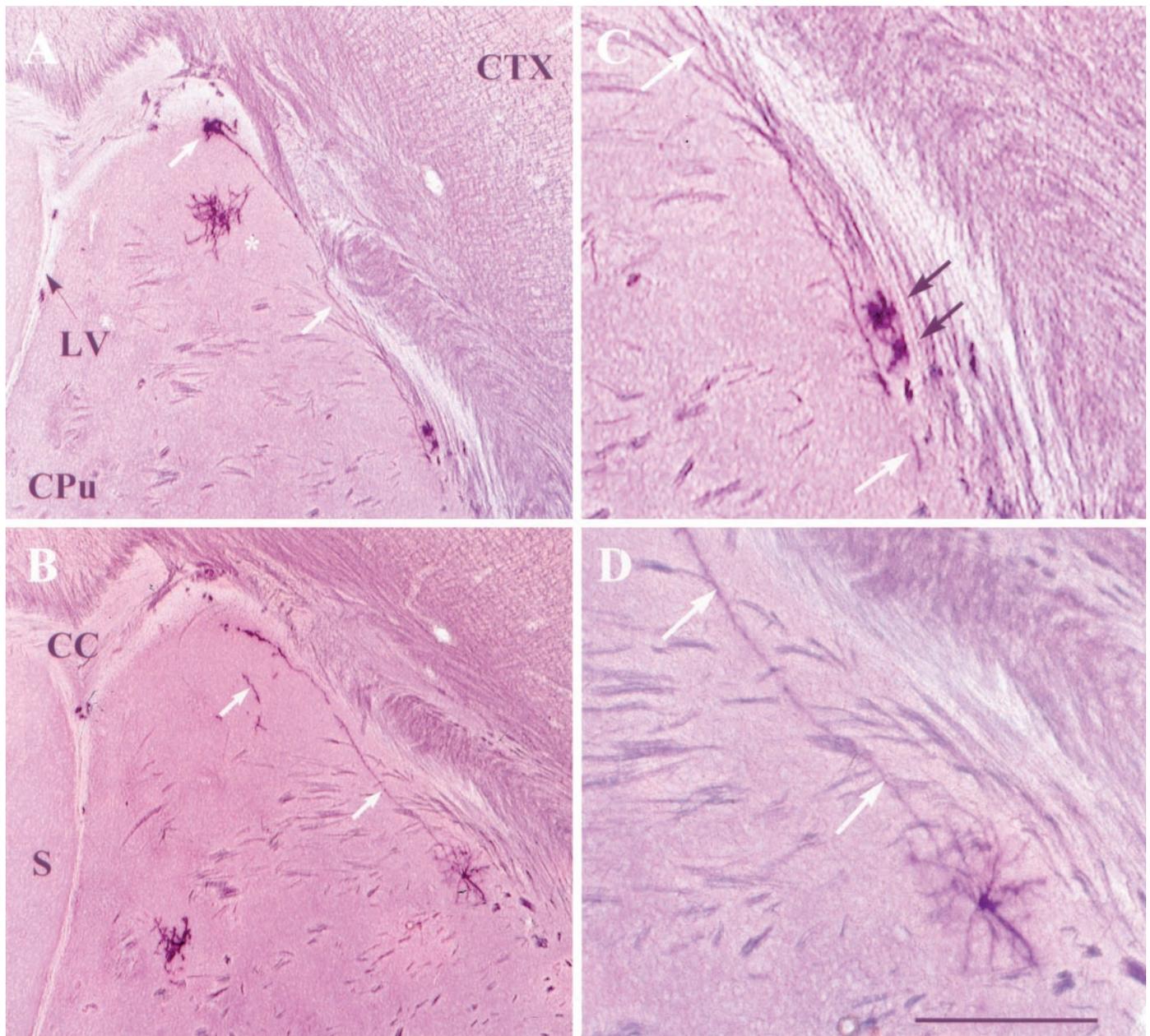
In this study, radial glia comprised <10% of AP-labeled cells. Over the course of development, radial glial cells are known to differentiate into astrocytes. Nevertheless, 40% of radial clusters included cells whose phenotypes were consistent with a radial glial identity. As many as three radial glia 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 mitotic 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., 1995), 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



**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 the another glial process (*D*). Scale bar: *A, B*, 500  $\mu\text{m}$ ; *C, D*, 200  $\mu\text{m}$ .

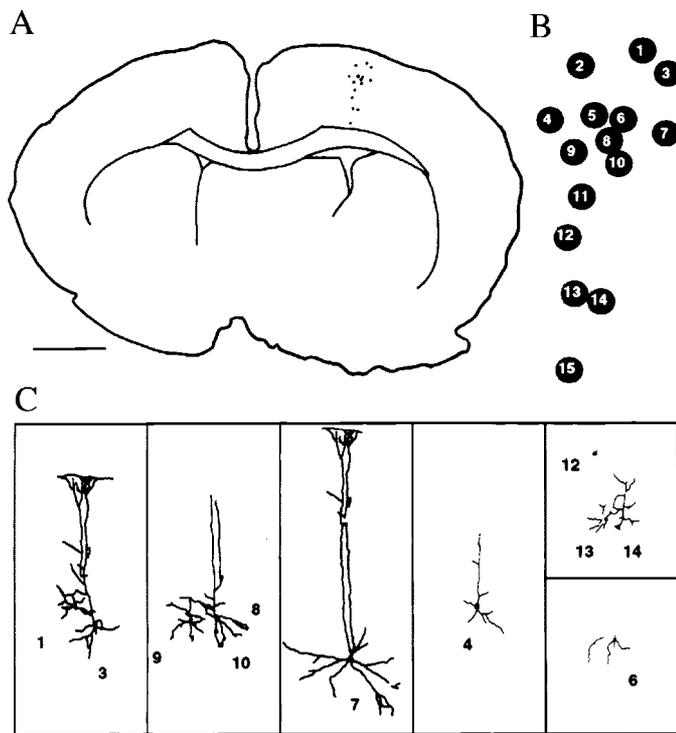
cortical neurons (42%) (Table 3). As a result, this experiment offered novel insights into striatal development. PCR was also successful in five additional hemispheres (experiments 7–12) containing labeled cortical interneurons.

#### 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 4.** Radial cluster in E14-labeled cerebral cortex. *A* depicts the relative positions of 15 AP-labeled neurons as black dots spanning cortical layers II–VI. Other than a cluster of glia in the hippocampus, these were the only labeled cells in the hemisphere. Neurons comprising the radial cluster were dispersed over 800  $\mu\text{m}$  in the A–P axis (sections 71 to section 79), but for clarity are shown together superimposed on a low-power camera lucida drawing of section 75. The radial appearance of this cell cluster is reminiscent of the “stripes” seen in chimera studies. *B* shows these same dots, numbered 1–15, at higher magnification. *C* features camera lucida drawings of 11 neurons according to their numbering in *B*. The majority of these cells show morphological features consistent with a layer-appropriate, pyramidal neuron phenotype. DNA tags were successfully amplified from seven neurons in the radial cluster. Six belonged to a single clone, whereas one neuron in the cluster (*4*) contained a different tag.

100, 91, and 83% of the A–P dispersion displayed in these clones, respectively. Striatocortical dispersion, therefore, accounted for most of the rostrocaudal spread.

Multicell clones also showed significant dispersion within the confines of the striatum and ventral telencephalon. Clones 1 and 3 were dispersed 1.6 mm and 0.7 mm within the striatum anteroposteriorly or >25 and 11% of the rostrocaudal dimension of the adult striatum, respectively. These clones also showed significant mediolateral dispersion. Clone 1 spanned 1.5 mm in the mediolateral plane, whereas clone 3 spread 1.1 mm mediolaterally.

#### Widespread striatal clones included cortical interneurons

Combined analysis of striatal and cortical neurons suggested that 11 of 12 (>90%) of PCR-positive striatal neurons were members of clones that also included cortical interneurons. Thus, striatal clones identified by PCR in this study were recognized as part of larger mixed striatal neuron–cortical neuron clones. It was apparent, therefore, that a high proportion of striatal progenitors labeled at E14 also gives rise to cortical neurons. In fact, PCR-positive striatal neurons were more likely to be related to cortical interneurons than to other striatal neurons (92 vs 83%). Furthermore, 5 of 15 (33%) of cortical interneurons represented in experiment 6 were related to striatal cells.

The largest striatal clone, clone 1, contained two PCR-positive cortical interneurons in the entorhinal cortex at 14,200 and 17,000  $\mu\text{m}$ . Another clonally related neuron was located in neocortex at 14,200  $\mu\text{m}$ . Clone 1 also contained seven striatal neurons: four neurons between 7300 and 7500  $\mu\text{m}$ , a single striatal neuron at 8400  $\mu\text{m}$ , and two neurons at 8900  $\mu\text{m}$  (Fig. 6). Clone 2 included a bipolar interneuron at 14,400 and a single striatal neuron. Clone 3 contained a cortical neuron in parasubicular cortex at 15,700  $\mu\text{m}$ . 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  $\mu\text{m}$  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 neocortex, 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

**Table 3. PCR-defined clones labeled at E14**

	Clone	Cell location	A-P distance ( $\mu\text{m}$ )	Tangential dispersion	
Experiment 6	1	CPu, CPu, CPu, CPu	7300, 7500, 7500, 7500	2.6 mm	
		CPu, CPu, CPu	8400, 8900, 8900		
		VI(N), Ent, Ent	14200, 14200, 17000		
	2	CPu, II	7900, 14400		
		3	OT, NAcSh NAcSh		7600, 8000, 8300
	4	PSub	15700		
		CPu	7300		
	5	III, <u>CPu</u> , AMYG	6800, <u>12000</u> , 13100		
	6	II(P), PRh, PRh	12400, 15700, 15800		4.7 mm
	7	V, V, V(P)	10100, 10100, 10300		1.3 mm
	8	V, VI	5700, 9500		0.9 mm
9	II, IV(P)	6600, 9300	2.5 mm		
10	<u>II-VI</u> , IV	<u>92-9400</u> , 9400	0.2 mm		
11	III, II	10800, 10800	0.3 mm		
Experiment 7	31	V(P), III, VI(N), III	7400, 9000, 9000, 9100	4.3 mm	
		IV, VI(P)	9900, 10300		
	32	III, II(P), II(P), III(P)	9000, 12300, 12300, 14900	8.25 mm	
		Rh	15500		
	33	III, PRh(P), V(N), II(P)	6700, 8400, 12500, 13000	8.0 mm	
		Ent, II, III(P), <u>HC</u>	13700, 13900, 14000, <u>14800</u>		
	34	III, Rh(P)	13900, 14000	3.7 mm	
	35	IV, IV, III(P)	4500, 4500, 7800	3.1 mm	
36	<u>II</u> , <u>III</u> , <u>IV</u> , AOB	5600, 5700, 5800, 6000	7.3 mm		
	III, Rh	8000, 13300			
37	<u>III</u> , <u>III</u> , <u>III</u> , II	5300, 5400, 5500, 10500	1 mm		
40	I, III(P), III(P)	12300, 13300, 13300	1.6 mm		
Experiment 9	54	III, III	8000, 8500	1.1 mm	
	55	I, III	10700, 11300	1.1 mm	
	57	III(N), Ent, Ent	4100, 13500, 13700	3.3 mm	
	58	III(P), III	6600, 11900	2.1 mm	
	61	Pyr, Pyr	9400, 10000	0.3 mm	
	62	IV, PSub	7000, 13500	3.0 mm	
Experiment 10	82	II(P), V	8300, 10900	3.9 mm	

Interneuron-containing clones from six experiments labeled at E14 and analyzed at P14. Anatomical location and rostrocaudal distance from the rostral tip of the olfactory bulb are listed for each neuron or glial cell cluster. The overall tangential (mediolateral and superio-inferior) dispersion for each clone is also shown. Cortical interneurons are designated by the layer in which they appeared (I–VI). P, Cortical pyramid; N, neuron (not definitively pyramid or interneuron); AMYG, amygdala; CPu, caudate–putamen; NAcSh, nucleus accumbens shell; Ent, entorhinal cortex; Psub, parasubicular cortex; OT, olfactory tubercle; Pyr, pyriform cortex; PR, perirhinal cortex; R, rhinal cortex. Underlining designates astrocyte clusters.

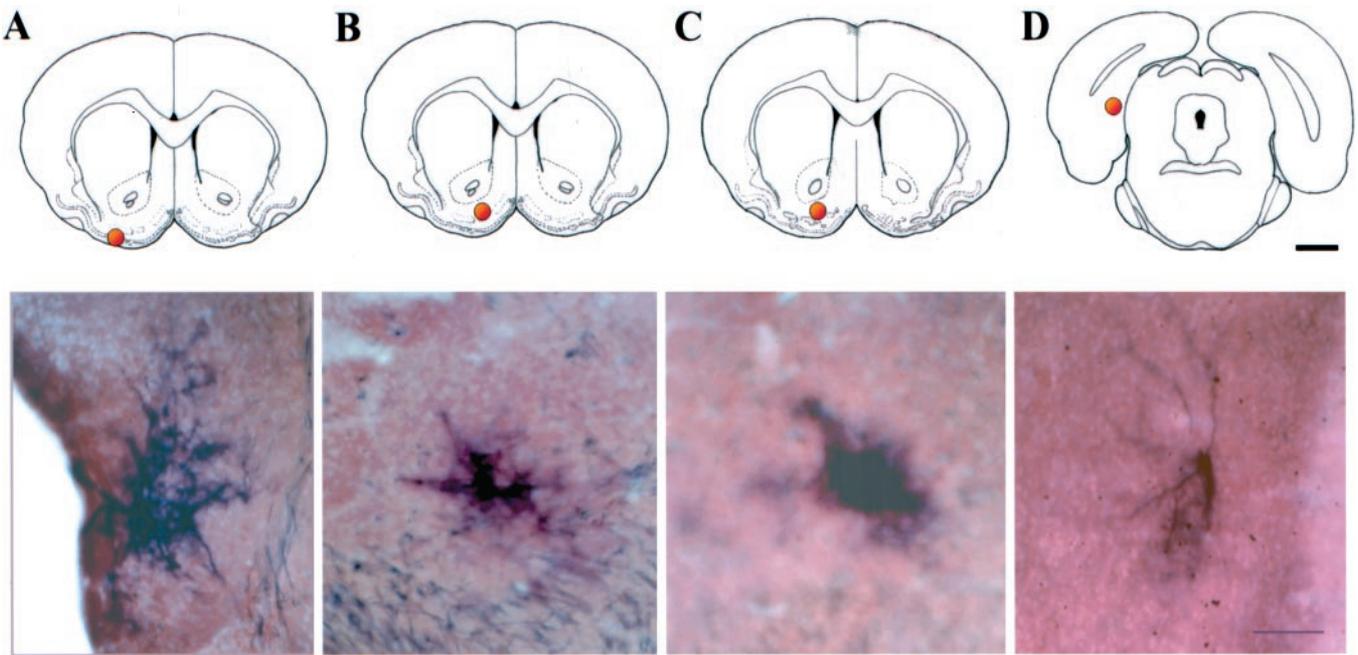
tangentially. The pattern of widespread dispersion in interneuron-containing clones did, however, vary significantly with clonal composition ( $p < 0.001$ ). In this study, 19 clones were identified as containing exclusively pyramids, exclusively interneurons, or both pyramidal neurons and interneurons. 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

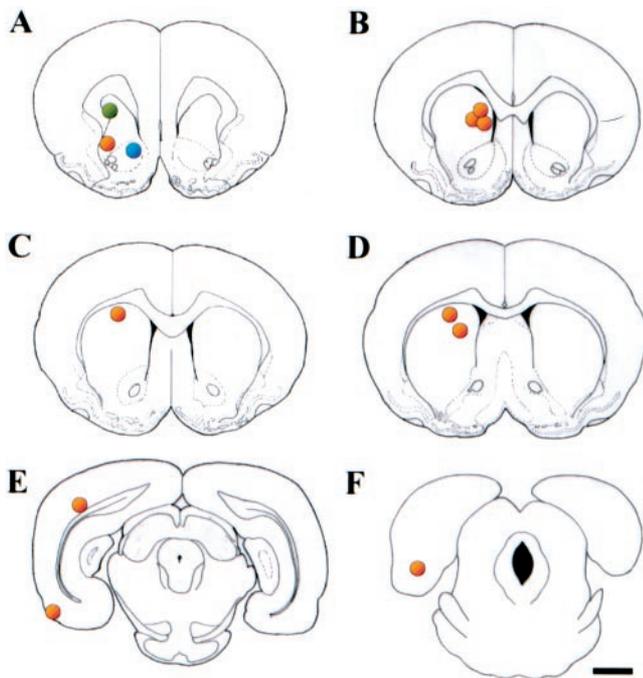
that cortical interneurons showed multiple modes of generation: mixed pyramidal and interneuron clones, mixed striatal–interneuron clones, and exclusive interneuron clones.

## 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



**Figure 5.** Schematic representation of clone 3. *A–D* depict tracings of representative sections from the P14 rat brain. The *yellow dots* indicate the relative positions of neurons in clone 3 as they were observed to disperse within the telencephalon of experiment 6. Clone 3 includes three ventral striatal neurons at 7600, 8000, and 8300  $\mu\text{m}$ , and a cortical neuron in parasubicular cortex at 15,700. High-power photomicrographs depicting these neurons are oriented according to their appearance in coronal sections. The total A-P dispersion observed in clone 3 was 8.1 mm. Scale bar, 2 mm.



**Figure 6.** Schematic representation of clone 1 (Table 3). Clone 1 included 7 striatal neurons: one at 7300  $\mu\text{m}$  (*A*), three neurons at 7500  $\mu\text{m}$  (*B*), one at 8400  $\mu\text{m}$  (*C*), and two neurons at 8900  $\mu\text{m}$  (*D*). A PCR-negative neuron is represented by the *green dot* in *A*. The *blue dot* represents a striatal neuron shown by PCR to be part of a separate striatal clone. Clone 1 also included a layer VI cortical neuron and a superficial perirhinal neuron at 14,200 (*E*). The most caudal cell in clone 1 was a deep entorhinal neuron at 17,000 (*F*). Thus, the total A-P dispersion observed in clone 1 was 9.7 mm. Scale bar, 2 mm.

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  $>300 \mu\text{m}$ . 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. 2*A*).

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. 2*B,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 symmetric 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

**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	45%
Related to interneurons only	29%
Related to glia	19%
Related to striatal cells	12%

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).

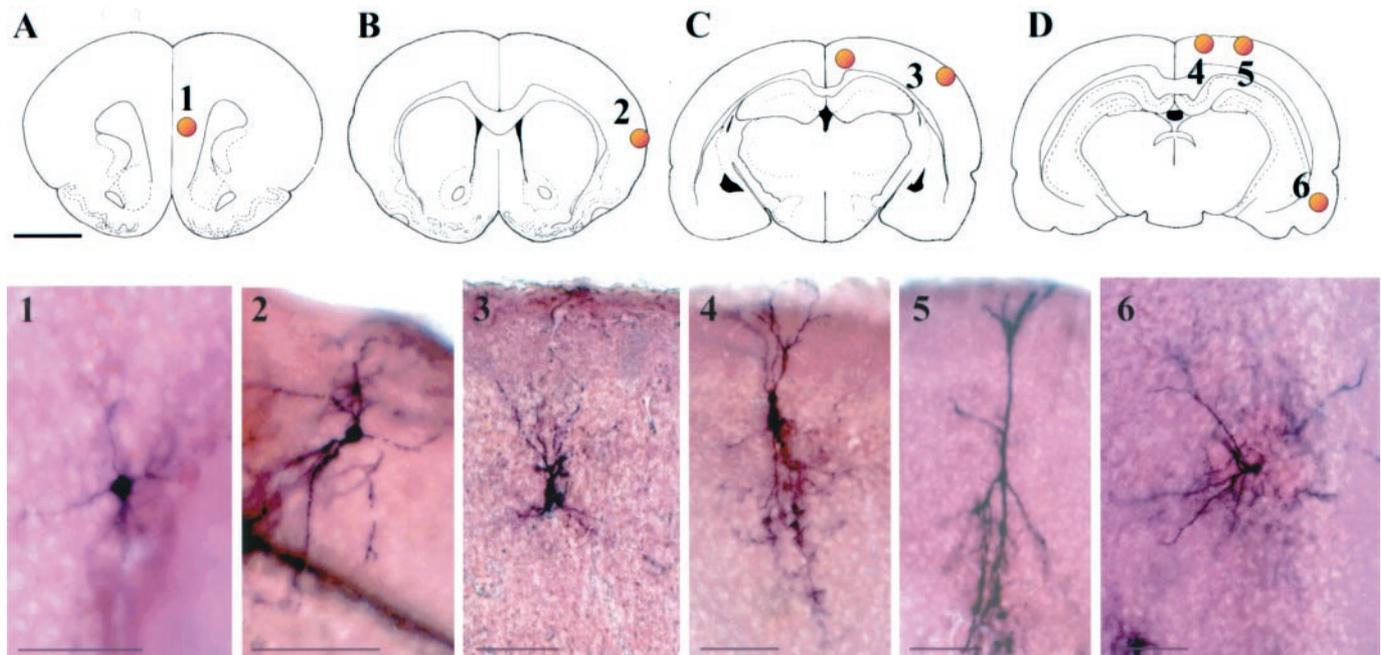
We observed radial clusters in the striatum only when progenitors were labeled at relatively early stages of development so that

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.

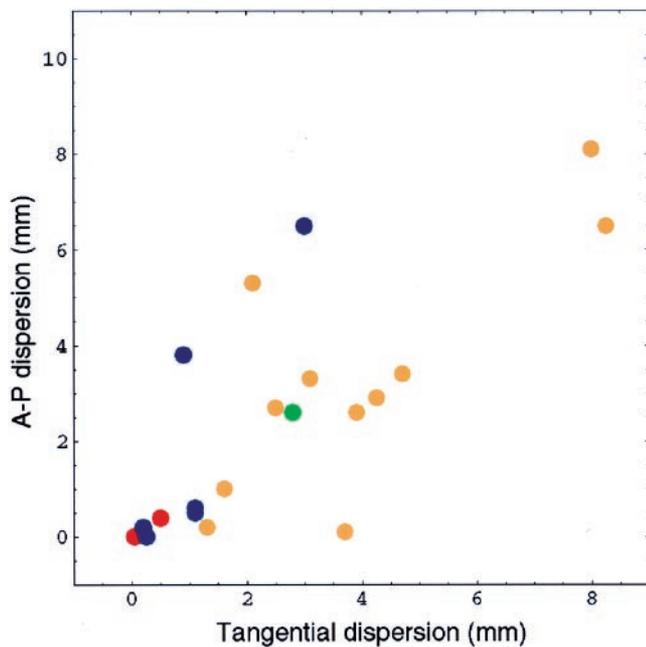
#### Radial glia support clonal units in developing striatum

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-



**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. 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  $\mu$ m.



**Figure 8.** Spatial representation of interneuron-containing clones in the cerebral cortex (Table 3). A-P dispersion is plotted along the y-axis, whereas tangential dispersion (dispersion in the mediolateral and superior-inferior plane) is shown on the x-axis. Each colored dot represents a cortical clone in which cell morphologies allowed classification as an exclusively interneuron (blue dots), mixed pyramidal–interneuron (yellow dots), exclusively pyramidal (red dots), or striatal–cortical clone (green dot). Clones containing both interneurons and pyramidal neurons tended to be dispersed equally in both dimensions, whereas interneuron-only clones were clustered or else dispersed rostrocaudally more than tangentially. The distribution of clones containing both interneurons and pyramidal neurons was statistically distinct from that of exclusive interneuron clones ( $p \leq 0.001$ ). Linear regression analysis indicates that the line best fitting the distribution of interneuron clones (blue dots) is described by the quadratic equation:  $A-P = 2.22T - 0.5$ , whereas the line best fitting the mixed clones (yellow dots) was described by the equation:  $A-P = 0.823T + 0.034$ .

cally dividing radial glial cells that give rise to a neuron and another radial glia cell.

### 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 observed between striatum and cerebral cortex in this study stands in stark contrast to a lack of dispersion between cortex and bulb (Reid et al., 1999). These data are consistent with the notion that the ganglionic eminence contains separate pools of interneurons destined for distinct brain regions (Anderson et al., 1997a,b 1999; Sussel et al., 1999; Wichterle et al., 2001).

### 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 nonfunctional 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; Parnavelas, 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.

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