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The Dispersion of Clonally Related Cells in the Developing Chick Telencephalon

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Lineage analysis in the chick telencephalon was carried out using a library of retroviral vectors. Clones were analyzed in posthatch day 14–21 animals for the phenotype and final locations of sibling cells. Clones often contained multiple types of neurons and glia. Clones of more than four cells almost always crossed functional boundaries. They were dispersed primarily along the rostrocaudal axis or in multiple directions, e.g., along the rostrocaudal and mediolateral axes. In order to begin to understand how the final patterns of dispersion were reached, embryonic tissue was examined. Radial migration, apparently supported by radial glial cells, occurred within the proliferative zones in all clones. In contrast to the migration of cells in the mammalian telencephalon, no tangential migration within the proliferative zones was observed at any age examined. However, beginning at embryonic day 4.5, tangential migration in the mantle zone in multiple directions was observed among the majority of clones. This type of migration occurred as soon as a mantle zone became apparent. It appeared that the tangential migration was not along radial glial processes. As in the mammalian telencephalon and chick diencephalon, dispersion among clonally related cells in the chick telencephalon is frequent, is extensive, and results from tangential migration in a variety of directions. © 1998 Academic Press

INTRODUCTION

Lineage analyses using retroviral vectors and a variety of other labeling methods have allowed an appreciation of the fact that clonally related cells can disperse over great distances during development of the vertebrate central nervous system (CNS) (for review see Cepko *et al.,* 1997). Some of the most striking dispersion has been observed among sibling cells within the mammalian telencephalon (Austin and Cepko, 1990; Walsh and Cepko, 1988, 1992, 1993; Reid *et al.,* 1995, 1997; O'Rourke *et al.,* 1992, 1995, 1997; Fishell, 1997) and the avian forebrain (Balaban *et al.,* 1988; Arnold-Aldea and Cepko, 1996; Golden and Cepko, 1996, 1997; Szele and Cepko, 1996). In rats, cells deriving from a single progenitor have been found to disperse to the extent that they end up occupying several functional domains of the neocortex, hippocampus, and/or olfactory bulb (Walsh and Cepko, 1992; Reid *et al.,* 1995). In the telencephalon of chickens, extensive dispersion was observed as well, with the result that a single clone could populate several functional domains (Szele and Cepko, 1996). One of the most surprising patterns of dispersion was exhibited by the ''RC clones,'' which constituted 60% of the clones of more than four cells in the chick telencephalon. RC clones showed extensive dispersion along the rostrocaudal (RC) axis, with limited dispersion along the mediolateral axis.

We have at least a partial understanding of where and when the migration that leads to the widespread dispersion of clonally related cells in the mammalian telencephalon occurs. Newborn neurons begin their migration from the proliferative zones, the ventricular and subventricular zones (VZ and SVZ, respectively), along radial glia (Rakic, 1972). This type of migration is initially fairly radial, but the radial glial processes can follow circuitous routes, particularly in the lateral regions of the rodent telencephalon (Misson *et al.,* 1991). Sibling cells traveling along radial glia in these regions can become dispersed within the mediolateral plane of the telencephalon through this mechanism alone (Austin and Cepko, 1990; Misson *et al.,* 1991). Dispersion also occurs as a result of tangential migration that appears to be independent of radial glia. DiI labeling has shown that postmitotic neurons within the proliferative zones (O'Rourke *et al.,* 1997) and within the cortical plate (O'Rourke *et al.,* 1992, 1995) can travel in a variety of directions orthogonal to radial glia. Retrovirally marked cells of the rodent telencephalon also were found to disperse along routes orthogonal to radial glia within the cortical plate and/or within the proliferative zones (Austin and Cepko, 1990; Walsh and Cepko, 1993). These observations led to the suggestion that dispersion of mitotic cells in the proliferative zones generates a periodicity in the spacing of cells along the RC axis (Reid *et al.,* 1995). In contrast to the

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variety of routes of migration observed within the cerebral cortex, there is directed migration in the most rostral portion of telencephalon (Luskin, 1993; Lois *et al.,* 1996). The rostral migratory stream of rats and mice comprises neuroblasts which follow a direct path from the anterior subventricular zone to the olfactory bulb path along a glial network (Lois *et al.,* 1996). To date, it has remained unclear whether the routes and/or mechanisms of migration observed in the mammalian telencephalon are used in the forebrain of other types of vertebrates such as birds.

During the evolution of the CNS in vertebrates, the complexity and size of the telencephalon has expanded more rapidly than those of any other part of the brain (Ariëns Kappers *et al.,* 1936). The anatomy of the avian telencephalon appears to be fundamentally different from that of the mammal (Karten, 1969; Shimizu and Karten, 1993). While the mammalian telencephalon is for the most part a laminated structure, the chick forebrain comprises a series of nuclei which are homologous to some of the functional subdivisions of the mammalian telencephalon, such as the hippocampus, cerebral cortex, and basal ganglia. These functionally distinct areas of the bird brain do not have laminae of cytoarchitectonically distinct types of neurons corresponding to those seen in the mammalian cerebral cortex. Also in contrast to mammalian neocortical development, avian telencephalic cells do not have to migrate through proximal laminae in an inside-out fashion, as they do in the neocortex of mammals (Tsai *et al.,* 1981), perhaps indicating that the mechanisms and routes of migration are fundamentally different between birds and mammals.

Because the chick forebrain is so different from that of mammals, it was possible *a priori* that lineal relationships and migration paths would also be fundamentally different. In the current study, we address these issues. We describe those final patterns of dispersion of retrovirally marked clones of the chick telencephalon that are different from the previously reported pattern of RC clones (Szele and Cepko, 1996). In addition, by examining tissue at early times after the introduction of retroviral tags, we begin to elucidate the pathways of migration in the chick telencephalon.

METHODS

Retroviral Infection and Histology

Neurogenesis in the chick telencephalon starts at stage 24 (E4) and ends at E10 (Tsai *et al.,* 1981). Chick embryos were infected at stages 15–20 (E2.5) and sacrificed at E4.5, 5.5, 6.5, 7.5, and 8.5 ($N = 3$; except for E6.5, $N = 4$) for the studies of migration. Eleven chicks infected between Hamburger and Hamilton (1951) stages 15–19 were sacrificed between P14 and P21 and similar clonal patterns were seen in all of them. Chick embryos were infected with the CHAPOL retroviral library encoding human placental alkaline phosphatase and a library of oligonucleotide tags (Golden *et al.,* 1995). Chicks were perfused transcardially with 4% paraformaldehyde in PBS, embryonic brains were dissected out in 4% paraformaldehyde and fixed overnight, and brains were cryoprotected in 30% sucrose. Sixty-micrometer sections were cut on a cryostat in the transverse plane (except for E4.5 and E5.5 brains, which were cut in the horizontal plane). Alkaline phosphatase (AP) activity was detected according to Golden *et al.* (1995).

PCR and Sequencing

Telencephalic cells exhibiting AP activity were analyzed by sequencing the viral oligonucleotide tag following PCR amplification of a small area of the tissue (a ‘‘pick’’). Some clones contained such a high density of cells that it was impossible to pick single AP^+ cells. In these cases, several cells were included per pick. The majority of these picks harbored only one sequence. Cells were not assigned a clonal identity if they were in a pick that contained more than one sequence. To date, the same viral tag has not been recovered from more than one independent infection (with the exception of rare cases of contamination) using the original preparation of viral stock reported in Golden *et al.* (1995) and used in the studies of Golden and Cepko (1996, 1997) and Szele and Cepko (1996). To date, 678 different inserts have been recovered, yielding an even distribution, as each was recovered once, and leading to the prediction of a complexity of $>10^5$ using a Monte Carlo simulation (Walsh *et al.,* 1992).

FIG. 1. Schematic illustration showing the location of 14 representative small clones (two to four cells) in P14–21 chick telencephalon. The entire chick telencephalon is schematized in 400-μm transverse sections (adapted from Kuenzel and Masson, 1988). The clones represented are from three brains. Embryos were infected with the CHAPOL retroviral library. Each symbol in the legend represents an individual clone containing a unique DNA insert. Individual colored dots, circles, and squares in the cross sections represent picks usually containing one cell. Clones were found on both right and left hemispheres, but are depicted only on the right for clarity. In the following list, rostrocaudal distances between the farthest members of the clone are indicated in parentheses. Clone 3, 1 oligodendrocyte and 1 astrocyte (1500 μm); clone 4, 4 astrocytes (240 μm); clone 7, 3 astrocytes (180 μm); clone 25, 1 neuron and 1 oligodendrocyte (1980 μm); clone 31, 2 astrocytes and 2 neurons (3540 μm); clone 38, 4 neurons (60 μm); clone 57, 1 neuron, 2 radial glia (arrows), and 1 unidentified cell (arrowhead) (720 μm); clone 64, 2 neurons (60 μm); clone 84, 3 astrocytes (120 μm); clone 86, 2 neurons (300 μm); clone 88, 2 astrocytes (120 μm); clone 108, 2 neurons (720 μm); clone 109, 1 astrocyte and 1 neuron (60 μm); and clone 125, 1 astrocyte and 1 neuron (1500 μm). Note that many of these clones had cells found in register with each other in the rostrocaudal plane and thus with a few more members would probably fulfill the criteria of a RC clone (more than four cells on three or more adjacent or nearly adjacent 60-μm sections). AA, archistriatum anterior; AI, archistriatum intermedium; APH, area parahippocampalis; Bas, nucleus basalis; CDL, area corticoidea dorsolateralis; E, ectostriatum; HA, hyperstriatum accessorium; HD, hyperstriatum dorsale; HIS, hyperstriatum intercalatum supremum; Hp, hippocampus; HV, hyperstriatum ventrale; INP, nucleus intrapeduncularis; LPO, lobus parolfactorius; N, neostriatum; NI, neostriatum intermedium; PA, paleostriatum augmentatum; PP, paleostriatum primitivum; PVT, paleostriatum ventrale; SL, nucleus septalis lateralis; TO, tuberculum olfactorium; TPO, area temporoparieto-occipitalis; Va, vallecula telencephali.

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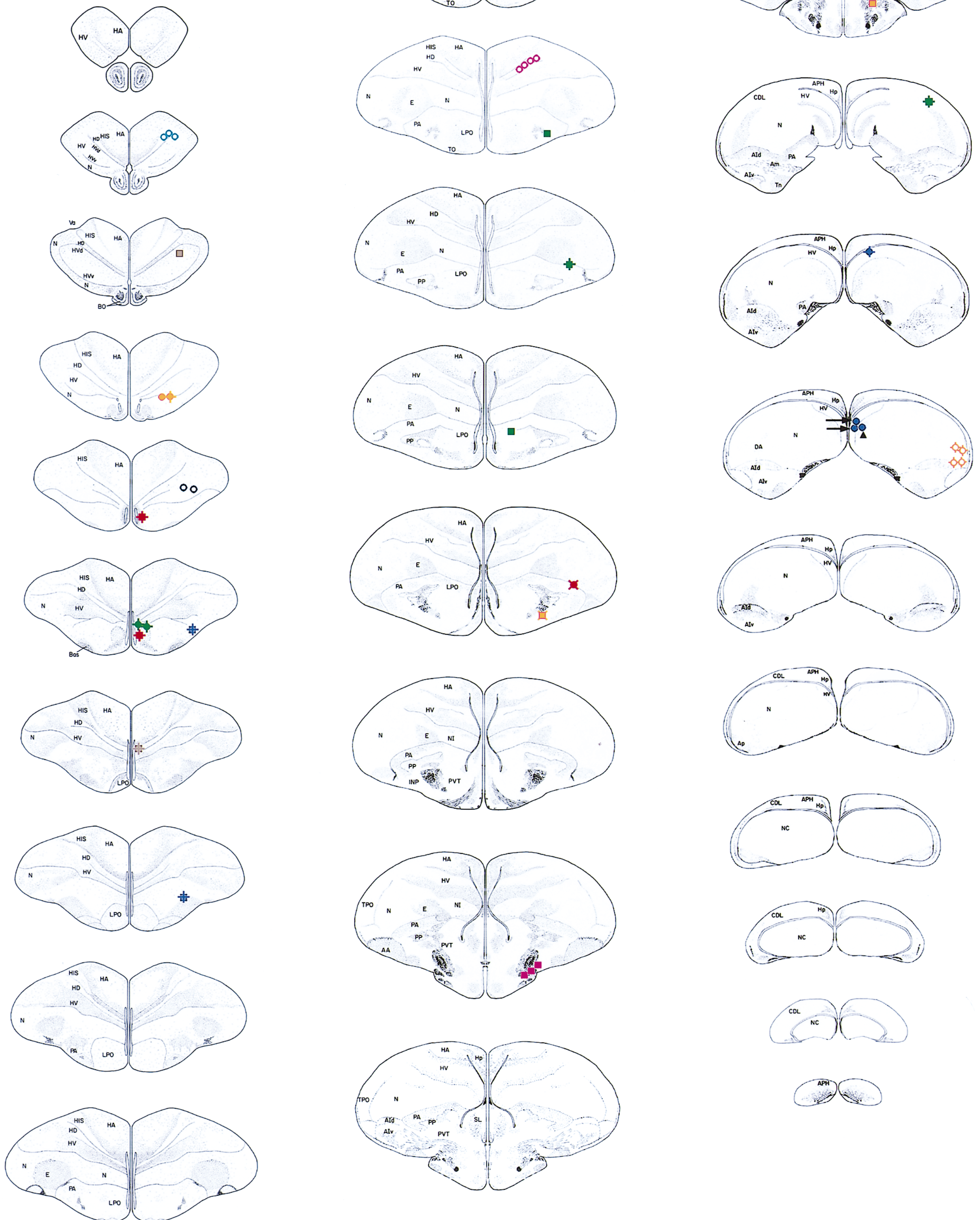
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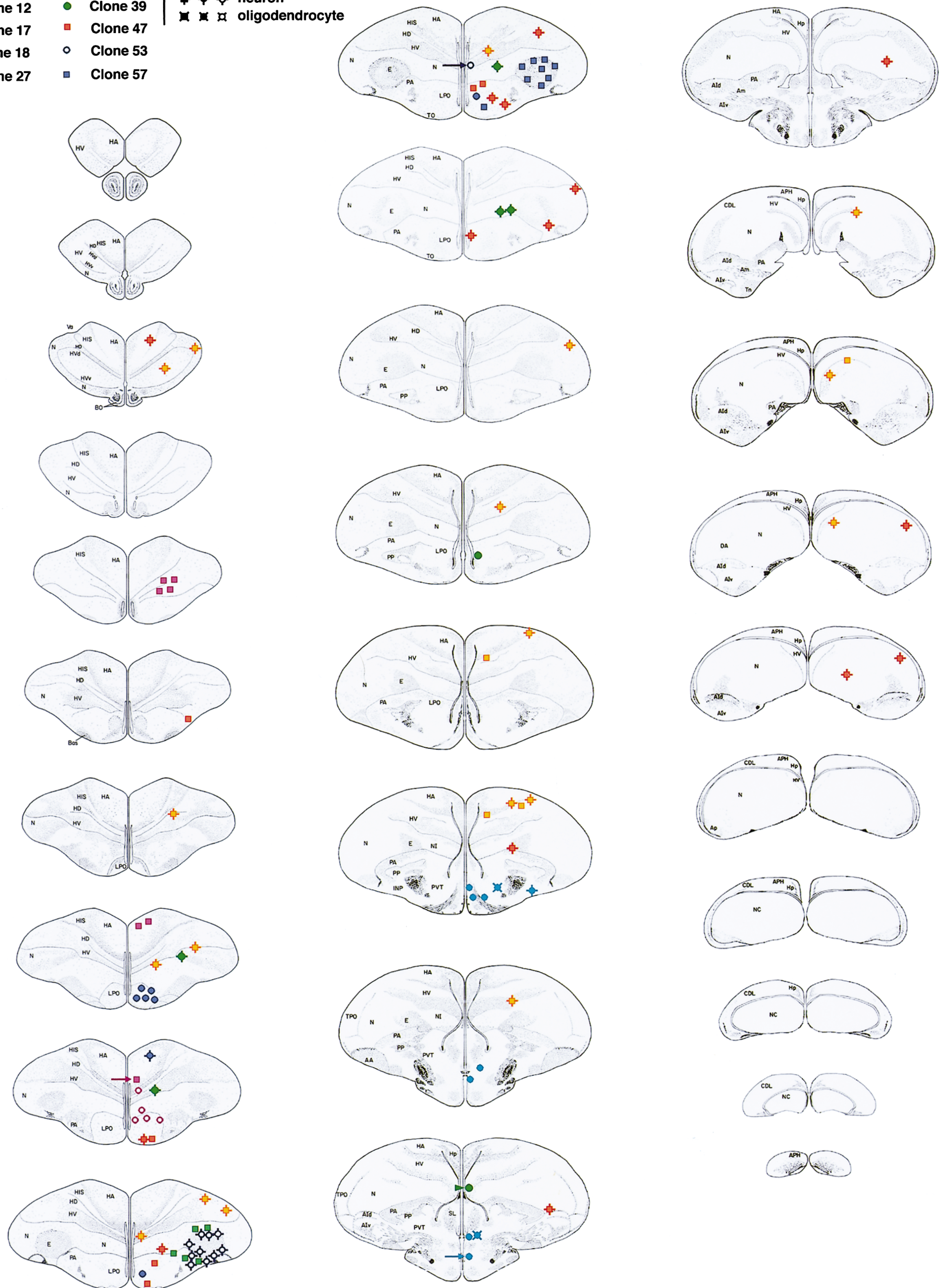
Clone 4
Clone 7
Clone 25
Clone 31
Clone 38
Clone 57
Clone 64
Clone 84
Clone 86
Clone 88
Clone 108
Clone 109
Clone 125
astrocyte
neuron
oligodendrocyte


Clone 11
Clone 12
Clone 17
Clone 18
Clone 27
Clone 32
Clone 39
Clone 47
Clone 53
Clone 57
astrocyte
neuron
oligodendrocyte

RESULTS

Patterns of Dispersion in Posthatch Chicks

We picked, PCR amplified, and sequenced material from three posthatch chicks (P14–21) with the result that 192 clones were found. Neural development at this stage is essentially complete and the patterns of cell distribution are very similar to those of the adult. Very few AP^+ radial glia were found between P14 and P21 and very few cells had the morphology of migrating cells. Neurons, astrocytes, and oligodendrocytes had assumed the morphology found in the adult. One notable exception was the hippocampus, in which cells were still migrating at this stage.

Several patterns of dispersion were observed. One pattern, that of the RC clones where dispersion was almost exclusively along the rostrocaudal axis, will not be elaborated on here as it has recently been reported (Szele and Cepko, 1996). Other clone types included those which were found in single nuclei (Fig. 1, clones 4, 7, 64, 86, 88, and 109). These tended to be small and were not very frequent. Other small clones were found to span functional nuclei (Fig. 1, clones 3, 25, 31, 38, 57, 84, 108, and 125). The majority of the larger clones (arbitrarily grouped as those with more than four cells) were quite dispersed and could span more than one nucleus (Figs. 2 and 3). The patterns of dispersion shown by these clones were quite varied, with no major features that allowed them to be systematically categorized. Some were distributed extensively in the mediolateral and rostrocaudal planes (e.g., clones 11 and 47 in Fig. 2 and clones 13 and 28 in Fig. 3). Others were rather clustered, but might still occupy more than one functional domain (e.g., clones 57 and 18, Fig. 2). Overall, there was a correlation between the size of the clone and the amount of dispersion. Similar to the RC clones, these clones could be composed of any combination of cell types, including neurons, astrocytes, and oligodendrocytes.

Migration in the Embryonic Telencephalon

A total of 555 picks, leading to the identification of 302 clones, were found in the brains of embryos infected with the CHAPOL virus and sacrificed between E4.5 and E8.5. The number of clones per brain ranged from 3 to 34. Cells were picked individually when possible, but when found close together they were picked *en masse.* (Only those picks which resulted in a single sequence were used in the final analysis.)

No tangential migration in the proliferative zones. As discussed above, previous studies suggest that tangential migration of mitotic and postmitotic cells occurs in the mammalian telencephalic proliferative zones. One of the goals of this study was to determine whether such migration occurs in the chick telencephalon. As there is no molecular marker which specifically labels migrating cells, identification of migrating cells was made on the basis of morphology. The PLAP marker is incorporated into the cell membrane and thus reveals details of morphology. As can be seen in Fig. 4, AP^+ cells in the early to midembryonic period typically have elongated cell bodies with long leading processes and short, small-diameter, trailing processes. Cells with this morphology were presumed to be migrating. Migrating cells were seen throughout the marginal zone, or nonproliferative regions, at all embryonic ages examined. As detected with AP histochemistry, cells with the morphology of migrating cells were never seen in the VZ.

AP^+ VZ cells or radial glia were occasionally found separated by several hundred micrometers, suggesting that they may be members of the same clone which had migrated apart in the VZ. However, with one exception, these did not contain the same insert and thus were not members of the same clone. (The exception was found in an E5.5 embryo, in which radial glial cells were separated in the dorsoventral axis by 420 μm.) However, clusters of radial glia adjacent to each other (Figs. 5, 6, and 8A) or within a few sections of each other (Fig. 8A), containing the same sequence, were found. This suggests that clonally related cells can disperse in the VZ, but that the large majority do not migrate large distances from each other.

As it was possible that migrating cells in the VZ did not express detectable levels of the PLAP gene, another method was used to enable detection of migrating cells within the VZ. DiI was injected into the ventricles, and the VZ of developing embryos was examined at several harvest times. Migrating cells were observed in the marginal zone, but not in the zones of proliferation.

Radial glia and radial migration. As the viral infection protocol results in the robust labeling of cellular processes and labels cells randomly throughout the ventricular system, it was possible to examine the morphology and distribution of radial glial cells within the developing telencephalon. Cells with the well-described morphology of radial glia

FIG. 2. Schematic illustration showing the position of 10 large (more than four cells) non-RC clones in the posthatch chick forebrain. The entire chick forebrain is schematized in 400-μm transverse sections (adapted from Kuenzel and Masson, 1988). The clones represented are from two separate brains. Embryos were infected with the CHAPOL retroviral library. Each symbol in the legend represents an individual clone containing a unique DNA insert. Colored dots, circles, and squares in the cross sections represent picks usually containing one cell. Clone 11, 18 neurons and 4 astrocytes (7440 μm); clone 12, 5 astrocytes (360 μm); clone 17, 6 astrocytes and 1 radial glia (arrow) (1440 μm); clone 18, 1 neuron, 6 astrocytes, 2 oligodendrocytes, and 1 radial glia (arrow) (1020 μm); clone 27, 5 astrocytes (120 μm); clone 32, 1 neuron and 7 astrocytes (1680 μm); clone 39, 5 neurons, 1 astrocyte, and 1 unidentified cell (arrowhead) (4920 μm); clone 47, 15 neurons and 6 astrocytes (7860 μm); clone 53, 1 radial glia (arrow) and 10 astrocytes (300 μm); and clone 57, 9 astrocytes (60 μm). Note that clones 53 and 57 are primarily restricted to the ectostriatum, especially the perimeter of the nucleus (periectostriatal belt).

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were observed (Levitt and Rakic, 1980). They had thin diameters and varicosities and the morphology did not vary significantly from the VZ to the marginal zone (Fig. 5). They tended to extend strictly radially (perpendicular to the ventricular surface), with a very straight appearance, in E 4.5–5.5 embryos (Figs. 5 and 6). With further development, radial glia became more tortuous in appearance. In E8.5 and older embryos, some radial glia had a slight caudal projection superimposed on the primary axis. Interestingly, in ventral nuclei, in particular the lobus parolfactorius, radial glia tended to extend perpendicular to the ventricular surface and then ventrally (clone encircled with black in Fig. 8A). As in mammalian species, radial glia in the chick telencephalon could extend all the way to the pial surface. This was seen at all embryonic ages examined (Figs. 5 and 6). Radial glia were rarely observed in P14–21 chick forebrain, although by these stages, the majority of cells that might be radial glia no longer had extended processes. An additional feature of radial glia that was noted in the chick telencephalon was that clonally related radial glia often came in pairs (Fig. 6B). Exceptions to this general observation occurred in the lobus parolfactorius and the hippocampus where radial glia were sometimes found in large clusters, as discussed further below.

In the mammalian cerebral cortex the inner layers form first and cells forming the outer layers migrate along radial glia through these deep layers. In contrast, the [^{3}H]thymidine studies of Tsai *et al.* (1981) showed that the most superficial or lateral portion of the avian forebrain develops first and the medial areas closest to the ventricles, last. Thus, prior to this study, it was not clear if migration along radial glia occurred in the avian forebrain. For example, it was possible that later-born cells progressively pushed their older siblings or neighbors radially or laterally. The majority of labeled cells at early stages were found in radial clusters surrounding radial glia (e.g., Fig. 6). The number of cells per radial glia gradually increased with time; compare the number of cells associated with radial glia at E 4.5–7.5 through Figs. 5–7, respectively. *A priori* it was impossible to know whether radial glia and the neuroblasts migrating along them were members of the same clone. Since it was impossible to separately pick these closely juxtaposed cells, they were picked together. Figure 6 contains an example: all the cellular elements shown by black arrows or arrowheads were picked together and only one retroviral tag was found; thus these elements are presumably members of the same clone. The presence of radial glia and clonally related cells apparently migrating out along them strongly suggests that radial migration occurs early during development in the chick telencephalon.

Tangential migration occurs outside of the proliferative zones. In our earlier study in which posthatch tissue was examined, we established that significant tangential migration must be occurring in order to generate RC clones (Szele and Cepko, 1996). Since it appeared that it did not occur in the VZ, we wished to address where and when it did occur. It was also of interest to examine the patterns of migration within a clone; e.g., did clonally related cells initiate tangential migration at the same time, migrate in the same direction, follow each other in a sequence, or show any other patterns of movement? To address these questions, infected brains were examined in a series of harvests beginning at E4.5. In the ventral half of the brain, a thin marginal zone was observed to develop by approximately E4.5. Tangential migration was confined to this marginal zone and was synchronous with its appearance (Fig. 5). By E5.5, the marginal zone was observed in the dorsal telencephalon and tangential migration could be seen there as well.

Clonally related cells often were found at varying distances from each other (Figs. 7 and 8) and from the clonally related radial glia, which are presumed to mark the clonal origin (Fig. 8). This suggests that sibling cells migrated off of radial glia at different times. Alternatively, they left the radial glia at the same time and migrated with different speeds. In an attempt to distinguish between these possibilities and to get an estimate of the average speed of migration after leaving radial glia, the distances traveled by each time of harvest were examined. At E4.5, the majority of clones were single cells, although occasional two- or three-cell clones were identified (Figs. 5B and 5C). At E5.5, the majority of cells were still closely associated with radial glia, having migrated less than 100 μm (Fig. 6A). However, at E5.5 a few clones were found with members which had migrated further; e.g., in Figs. 7C and 7D there was approximately 100 to 300 μm between sibling cells. This trend of modest distances between sibling cells continued through E6.5 and E7.5. At E8.5, some clones contained cells that had migrated distances comparable to that seen in the adult (Fig. 8). For example, the ventral clone in Fig. 8A has members that were 420 μm apart in the rostrocaudal direction. Similarly, in Fig. 8B, clone 16 has members that were located 600 μm apart. These data suggest that cells migrate off of radial glia at a steady pace beginning early in neurogenesis and continue to gradually migrate away from their origin.

The positions relative to the ventricle where cells leave the radial glial processes also were examined. Throughout

FIG. 3. (A) Schematic illustration showing a single large non-RC clone spanning 6300 μm along the rostrocaudal axis. It consisted of 8 neurons (red squares), 98 picks containing 1 or more oligodendrocytes (red dots), and 1 radial glia (red diamond). Note the extensive rostrocaudal migration and spread into a variety of functional areas. (B) Three-dimensional reconstruction of the chick forebrain showing a different large clone spanning 3540 μm in the rostrocaudal axis. It consisted of 15 neurons and 1 radial glial cell (caudalmost cell). Sections from the atlas of Kuenzel and Masson (1988) were scanned into Adobe Photoshop, altered, and reconstructed using Spyglass (Visualogic). There are 55 sections and each represents 200 μm.

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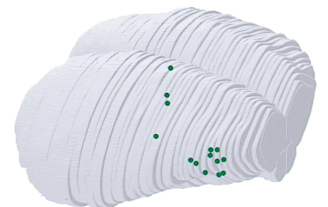
A

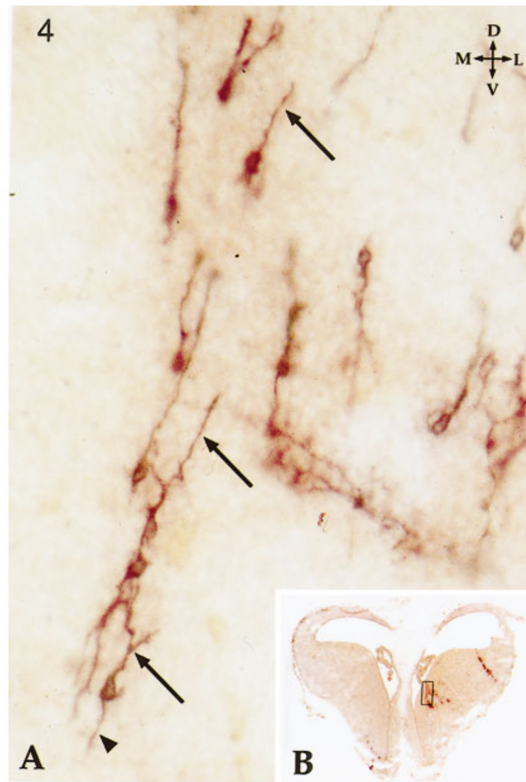
Clone 28

- ■ Neurons
- ● Oligodendrocytes
- ◆ Radial Glia

B



Clone 13


4
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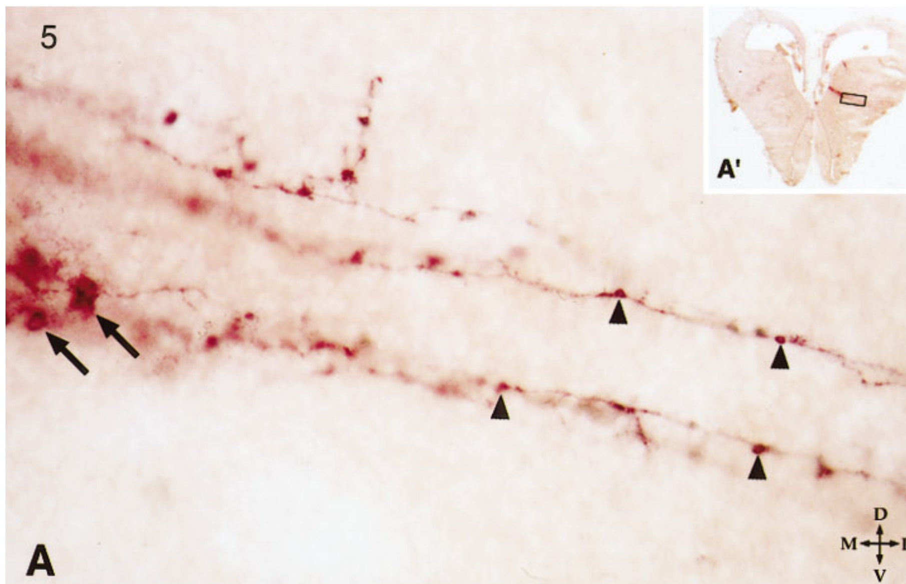
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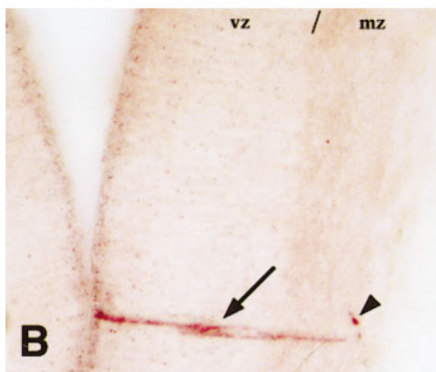
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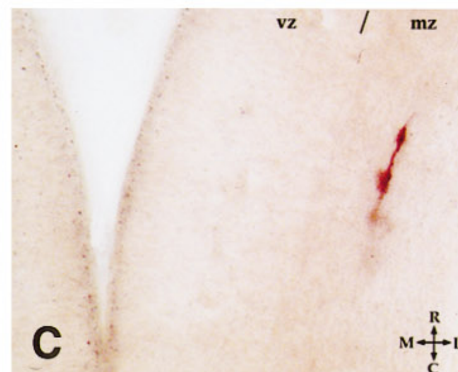
vz
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C

development, cells were observed to leave the radial glia at various distances from the ventricle once they had migrated out of the VZ. As mentioned above, as soon as the marginal zone was one or two cell layers thick, tangential migration was observed to occur. When the marginal zone became thicker, radial glia which had several clonally related cells apparently leaving the glial process at varying distances from the ventricle frequently were observed, e.g., at E5.5 (see Fig. 6A) and at E7.5 (Fig. 7A).

Tangential migration occurs in many directions. Sibling cells within many clones at all ages of development examined were observed to have migrated in different directions. For example, some cells in clone 16 in Fig. 8B had migrated dorsally, while others had migrated caudally. The majority of clones from E6.5 on were like the one seen in Fig. 7A, with cells migrating out in many different directions. However, perhaps because clone sizes were small at early ages, migration in a single direction was not rare at the early ages, e.g., E5.5 (Figs. 5B, 5C, and 6A). Occasionally, migration in a single direction was observed among cells of the larger clones observed at later ages (e.g., clone 12 in Fig. 8).

Generation of RC Clones

As mentioned above, 60% of clones composed of more than four cells in the adult chick telencephalon were found in rostrocaudal arrays (RC clones). We therefore expected to find a significant number of developing clones with a similar pattern of distribution. Indeed, such clones were seen, especially during the later stages of development. As in the adult, RC clones were found in many different parts of the developing forebrain; Fig. 8 shows such clones in E8.5 brains. The large clone in the developing lobus parolfactorius encircled in black in Fig. 8A spanned seven 60-μm transverse sections. No cells outside of this RC array had the same sequence. Smaller RC clones, such as the one encircled in green in Fig. 8A, also were found. Similar to what is seen in the adult, clones found in RC arrays in developing brains were found in varying patterns. For example, clonally related cells were most often found in consecutive sections, but in a few clones, they were separated by considerable distances (e.g., clone 16 in Fig. 8B contains cells in a RC array separated by several sections). In cases in which cells were found in consecutive sections, they were not found in contact with each other, as they are in the rostral migratory stream.

In the earlier study on RC clones, we noted that a few of the RC clones that were examined in mature tissue had labeled cells remaining in the VZ, presumably marking the clonal origin. In these cases, the mature cells that had migrated out of the VZ were usually distributed rostrally relative to the clonal origin. We were interested in knowing whether this was true at different ages and in different areas of the forebrain. It did not appear to hold as a general rule in that cells in clones that were dispersing rostrocaudally could have cells migrating either rostrally or caudally with respect to the clone origin (Figs. 8A and 8B).

Migration in the Hippocampus

The varied directions of migration taken by sibling cells within single clones were not observed in the chick hippocampus. The chick hippocampus is a thin strip of tissue located in the posterior medial portion of the telencephalon. All clones found in the hippocampal area of embryonic (Fig. 9) and posthatch chicks contained cells migrating medially. They contained several radial glia spanning one to two serial 60-μm coronal sections. Neuroblasts migrating out on these radial glia were usually found at a high density (arrow, Fig. 9). At the pial surface or more ventrally, neuroblasts dissociated from the radial glia at right angles and migrated medially (arrowheads, Fig. 9). They had very long leading processes and seemed to form continuous streams of cells. These streams could be very long, spanning half the width of the hippocampus. Interestingly, hippocampal neuroblasts never migrated more than a few cell diameters laterally.

DISCUSSION

The Majority of Clones of More Than Four Cells Cross Functional Boundaries

Clones in the posthatch chick telencephalon were varied in size and in pattern of dispersion. Small clones (two to

FIG. 4. Cells migrating dorsally in the telencephalon of an E8.5 animal. These cells were found in the midsection of the rostrocaudal plane. They were migrating from the presumptive neostriatum to the presumptive hyperstriatum. Note the leading processes (arrows) and the shorter trailing processes (arrowhead). D, dorsal; L, lateral; V, ventral; M, medial.

FIG. 5. (A) High-power magnification of radial glia in the telencephalon (presumptive neostriatum) of an E8.5 chick embryo. Note the very thin diameter (0.5–2.0 μm) of the radial glia and the numerous varicosities along their length (arrowheads). These can be differentiated from cells migrating along the radial glia (arrows) which are much larger in diameter. The cells indicated by the arrows contained the same sequence as the radial glia along which they were migrating and thus were derived from a common progenitor. (The radial glia in the dorsalmost portion of the photograph were picked separately and did not yield a PCR product.) (B and C) E4.5 ventral chick telencephalon infected with the CHAPOL virus and cut in the horizontal plane; ventral sections are shown. (B) Arrow shows radial glia emanating from the ventricular zone (vz) to the marginal zone (mz). Arrowhead shows a cell in the marginal zone which contains the same sequence as the radial glia. It appears to be migrating tangentially in a rostral direction relative to the radial glia. Note that the background AP stain is darker in the mz, allowing for an easy identification of this portion of the telencephalic wall. (C) Migrating cell which contains same sequence as cells in B. C is 120 μm ventral to B. Thus the cell shown in C appears to have migrated ventrally and then turned to migrate rostrally. R, rostral; C, caudal; L, lateral; M, medial; D, dorsal; V, ventral.

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four cells) were dispersed to a limited extent, sometimes occupying only one functional domain. Larger clones were almost always dispersed across more than one functional domain, similar to the majority of clones, both large and small, observed in the rat cerebral cortex (Walsh and Cepko, 1992; Reid *et al.,* 1995).

Chick telencephalic clones that were not RC clones could exhibit dispersion along the rostrocaudal axis, as well as along other axes. Overall, it was difficult to see a reproducible dispersion pattern among clones in the posthatch animal, other than that shown by the RC clones. It is possible that there is some significance to the dispersion patterns of RC and other clones and that it would be revealed if we knew more of the gene expression patterns of the developing or mature chick telencephalon. As very few have been reported, particularly relative to those reported for the chick hindbrain (for review see Wilkinson, 1993) and the mouse forebrain (for review see Puelles and Rubenstein, 1993), it is impossible at the moment to interpret these clones with respect to gene expression patterns or with respect to the other features that will be discovered in the future. In addition, we do not know how the tissue is shaped during development. The final form is undoubtedly generated by differential growth achieved through proliferation and migration patterns that are probably varied in some systematic way. Such patterns might explain the shape of some clones.

Radial and Tangential Migration in the Embryonic Chick Telencephalon

A major finding of this study is that, in the embryonic chick telencephalon, radial migration along radial glia is followed by tangential migration in the marginal zone. Classic [^{3}H]thymidine labeling studies had previously shown that the avian forebrain develops in an outside-in pattern (Tsai *et al.,* 1981). Therefore, it was not clear to what extent radial migration occurred in the chick telencephalon. We observed radial migration in virtually every clone, with some radial glia in contact with dozens of sibling cells. The fact that radial migration occurs is not surprising, as this form of cellular migration is a hallmark of vertebrate neural development. A relatively newer finding is that tangential migration in developing CNS tissue appears to play a major role in the distribution of neurons. Tangential migration has now been observed in many locations in the chick and rodent, including within the chick mesencephalon, diencephalon, cerebellum, and other rhombomeric derivatives, as well as in the rodent telencephalon (reviewed in Cepko *et al.,* 1997). In the chick telencephalon, our results indicate that: (1) tangential migration occurs soon after the first postmitotic neurons are born, without a ''waiting period''; (2) tangential migration occurs throughout the rostrocaudal axis of the telencephalon and throughout the period of neurogenesis; (3) tangential migration begins first in the ventral and last in the dorsal areas; (4) clonally related cells can begin their tangential migration at different times; and (5) clonally related cells can migrate tangentially in a variety of directions.

Unlike in mammalian systems, we found scant evidence for migration in the proliferative zones. Of all the clones examined, only a single one contained cells in the proliferative zone that were at an appreciable distance from each other. Both with extensive AP histochemistry and with preliminary DiI injections, we never observed cells in the VZ or SVZ which had the morphology of migrating cells. The lack of migration within the proliferative zones might explain the fact that chick telencephalic clones do not exhibit the intriguing periodicity along the rostrocaudal axis that is shown by many clones in the rat cerebral cortex (Walsh and Cepko, 1992; Reid *et al.,* 1995). The periodicity was hypothesized to result from motile mitotic cells traveling along the rostrocaudal axis within the proliferative zones. However, a recent study by O'Rourke *et al.* (1997) of migration in the developing ferret and rat cerebral cortices failed to find mitotic cells migrating in the proliferative zones, while it did show postmitotic cells migrating in many directions within these zones. Thus, it is not clear to what extent migration of mitotic cells contributes to the periodicity of clones within the rat cerebral cortex, nor whether migration of postmitotic cells within the proliferative zones contri-

FIG. 6. (A) Horizontal section from an E5.5 chick showing a group of cells which contained the same insert and thus were members of the same clone. Note the three cells migrating tangentially off of the radial glia at sharp right angles (arrowheads). The radial glia and migrating cells stop at the pial surface (PS). (B) Coronal section of E6.5 chick embryo infected with CHAPOL virus and stained using AP histochemistry. The two radial glia (black arrows) next to each other shared the same sequence and thus were members of the same clone. The cells migrating along them and off of them (black arrowheads) also belonged to the same clone. The cells indicated by the blue arrows were members of a different clone. Note the fine leading processes (blue arrowheads) extending dorsally. The red arrow shows a group of cells which were not picked. D, dorsal; V, ventral; L, lateral; M, medial; R, rostral; C, caudal.

FIG. 7. (A and B) Coronal sections of an E7.5 chick embryo infected with CHAPOL virus and stained using AP histochemistry. At this stage of development a large number of cells begin to migrate off of radial glia (arrowheads). Note that in A, cells seem to be migrating in a variety of directions (arrowheads). In B, all of the cells share the same sequence and are members of the same clone (the cell indicated by the red arrowhead was not included in the pick). (C and D) Migrating cells in a CHAPOL-infected brain of a chick sacrificed at E5.5. Sections were cut in the horizontal plane. C is 60 μm dorsal to D. Black arrows show cells migrating in the marginal zone (mz) which contained the same sequence and thus were daughters of the same progenitor cell. Note that the direction of the leading processes suggests that cells of this clone were migrating caudally. Green arrow points to a cell which contains a different sequence but which could have easily been identified as being clonally related to the cells indicated with a black arrow based on geometric criteria alone. pe, pigmented epithelium; r, retina; vz, ventricular zone; R, rostral; C, caudal; M, medial; L, lateral; D, dorsal; V, ventral.

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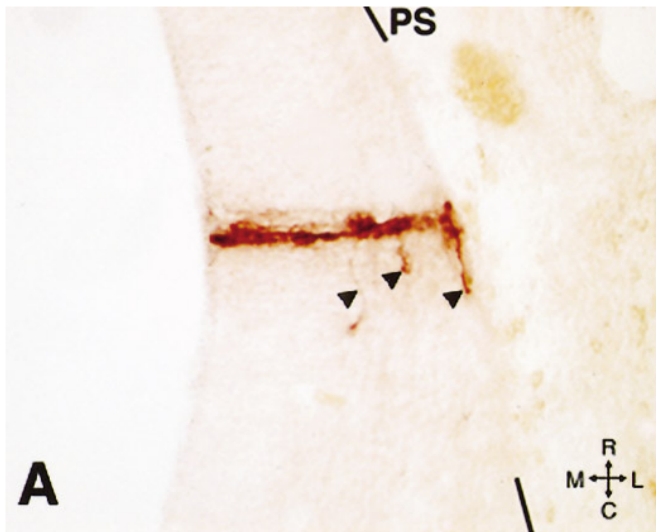

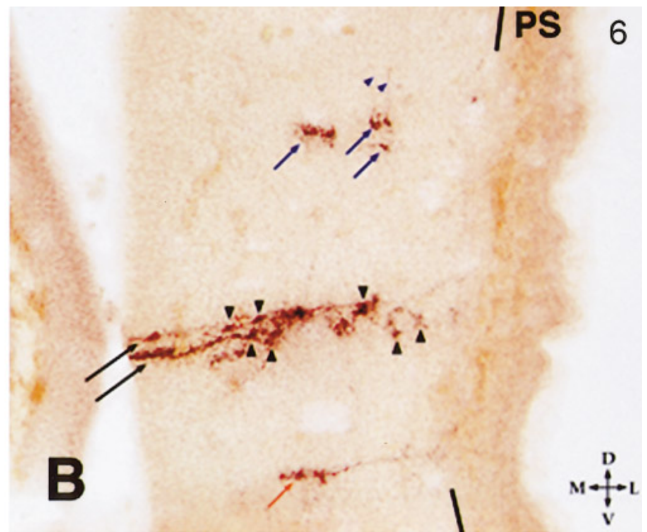

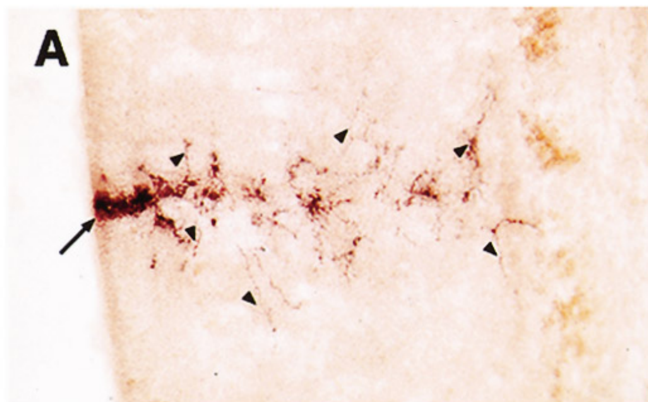

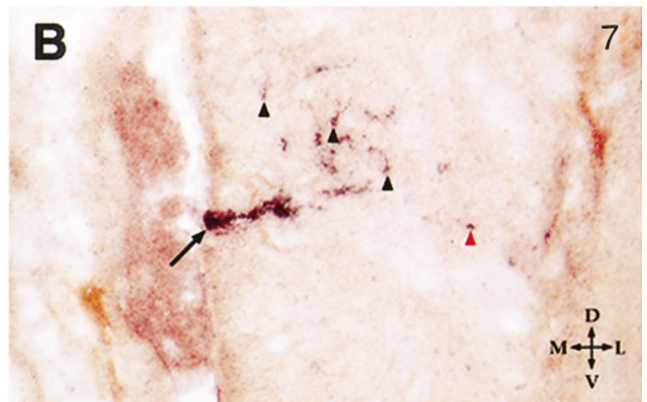

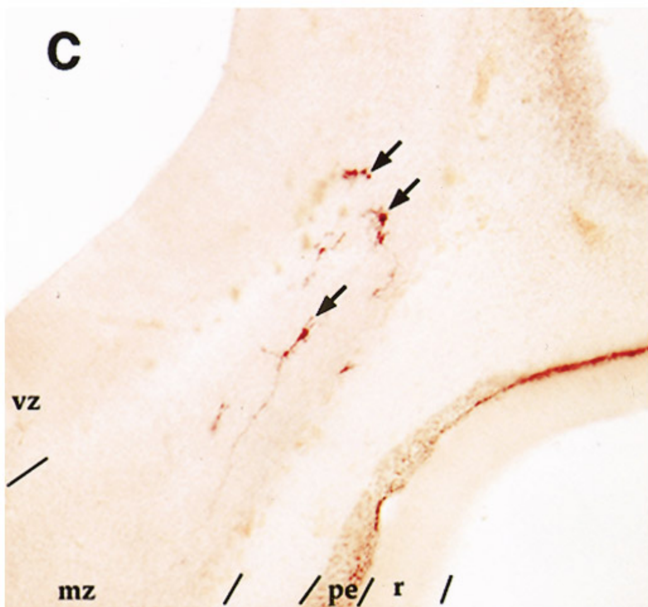

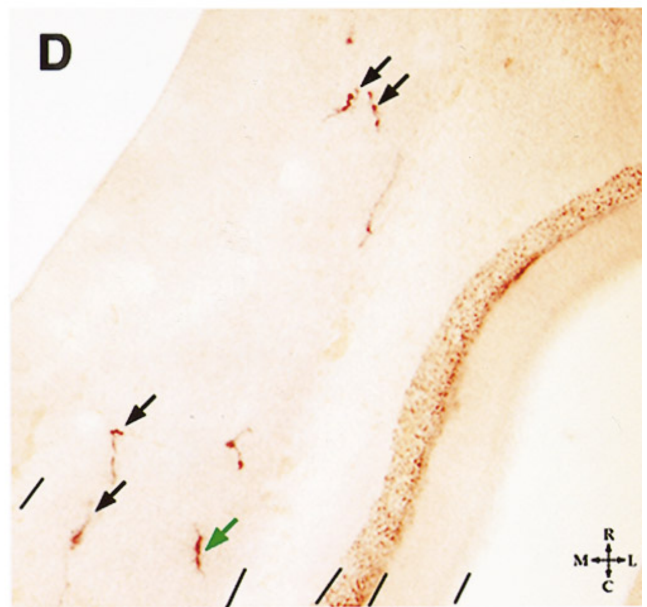

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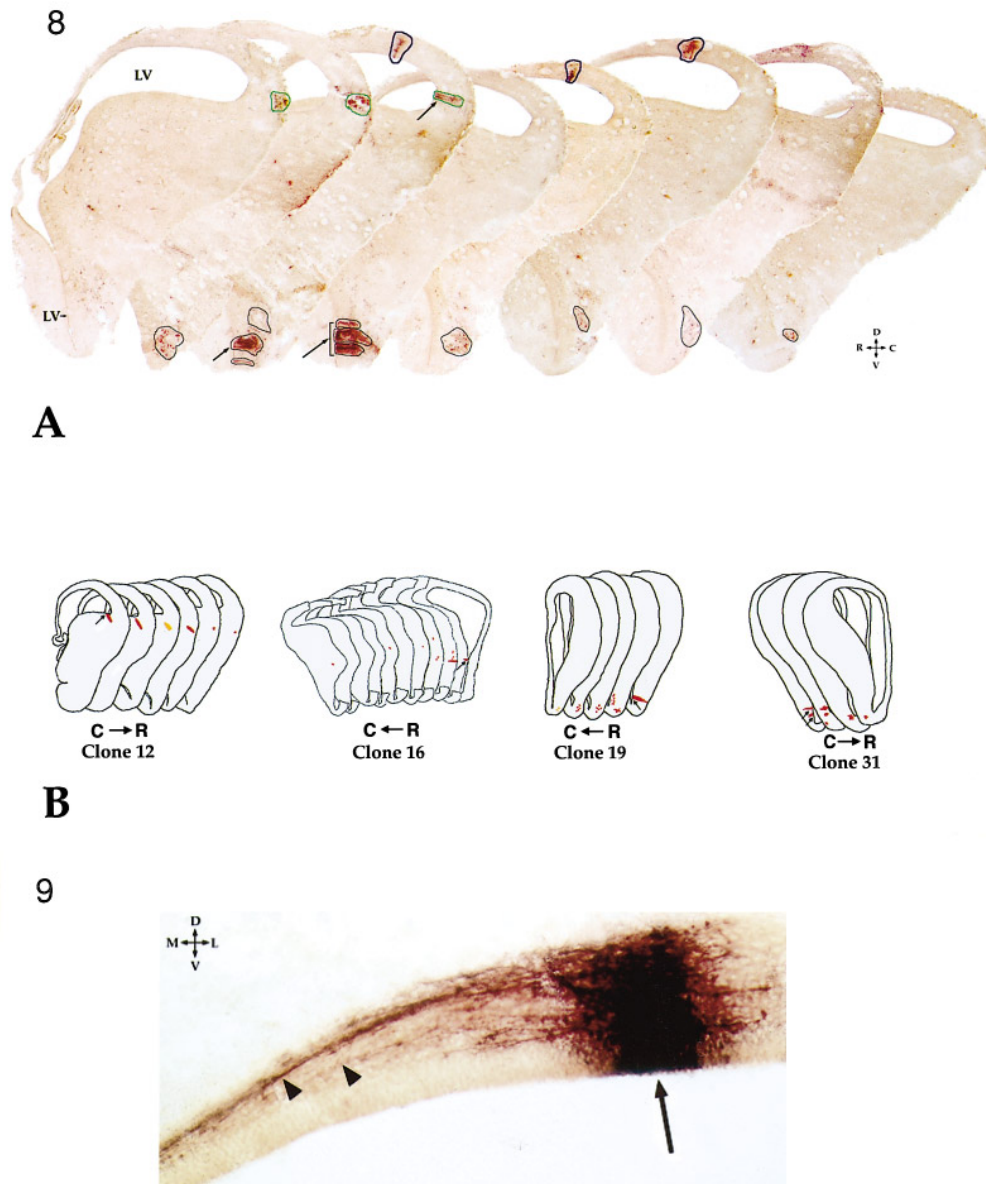

FIG. 8. (A) Three RC clones from a chick embryo infected with the CHAPOL virus and sacrificed at E8.5. Eight consecutive 60-μm transverse sections are shown. AP^+ cells are seen as dark reddish-brown. Black, green, and blue outlines depict locations of individual picks. (Note that these picks contained several cells each.) Cells in the black, green, and blue outlines contained three different sequences, respectively. The black clone was found in the lobus parolfactorius and spanned 420 μm along the rostrocaudal axis. Note the high density of radial glia (arrows). This was common in lobus parolfactorius clones, whereas clones found in other parts of the forebrain typically contained only one or two radial glia. Some cells migrated rostral to the origin of the clone (to the left) while most cells migrated caudally. The green clone was found at the intersection of the future neostriatum and area corticoidalis. Note that there are only two radial glial cells in this clone (arrow), and cells migrated rostral to this point of origin. The blue clone is in the area corticoidea. It consists primarily of radial glia and cells migrating out along them. (B) Schematic illustration showing four other clones exhibiting rostrocaudal migration found in embryos infected with the CHAPOL virus and sacrificed at E 8.5. Photographs of transverse sections were scanned into a computer, edges outlined, and locations of picks recorded. Red picks indicate locations of cells which had the same sequence. Orange picks were in a similar location but did not yield definitive sequence. Note that migration occurred primarily in the rostrocaudal plane. Small arrows indicate points of origin in the VZ. Clones 12 and 31 migrated rostrally, while clones 16 and 19 migrated caudally. The rostralmost four sections in clone 12 contained radial glia swooping caudally. Clone 16 shows a little scatter in the dorsoventral plane. This clone also contained members separated by several sections. LV, lateral ventricle; R, rostral; C, caudal; D, dorsal; V, ventral.

FIG. 9. Hippocampal clone from an E8.5 chick injected with the CHAPOL virus between stages 15 and 18. Note the high density of radial glia and neuroblasts (arrow) extending dorsally from the ventricular surface. Leading processes of neuroblasts extend medially along the pial surface (arrowheads). Other processes extend medially at more ventral levels. D, dorsal; L, lateral; V, ventral; M, medial.

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butes to periodicity. It is clear that migration of postmitotic cells in the proliferative zones, as well as in the developing cortical plate, accounts for the widespread dispersion seen in most rat cortical clones. Thus, the telencephalons of chick and mammals may differ in the way that they disperse some clonally related cells, but they nonetheless end up dispersing cells across functional domains and in many different directions.

Cells were observed migrating tangentially in the marginal zone as early as E4.5. This was the earliest that a marginal zone was present. The marginal zone may induce tangential migration, through elaboration of either a structure or a diffusible substance that induces or allows radially migrating neuroblasts to dissociate from radial glia and migrate tangentially. Alternatively, or in addition, there may be factors in the proliferating zones which do not allow migrating cells to dissociate from radial glia until the marginal zone is present. Another scenario might involve molecules present on radial glia or migrating neuroblasts which are differentially expressed or have different functions in the proliferative zones and marginal zone. A number of molecules that appear to function in the migration of neuroblasts have been described, including astrotactin (Zheng *et al.,* 1996), L1 (Asou *et al.,* 1992), tenascin (Husmann *et al.,* 1992), B1-integrin (Galileo *et al.,* 1992), NCAM (Tomasiewicz *et al.,* 1993), and epitopes 1A1 (Mittal and David, 1994), NJPA-1 (Anton *et al.,* 1996), and T61 (Meyer and Henke-Fahle, 1995). The functions of these molecules in the chick telencephalon have not been investigated.

Clonally related cells appeared to leave radial glia at varying distances from the ventricular surface to begin their tangential migration. It is possible that cells leave radial glial fibers randomly. For example, local crowding may cause them to dissociate from the glial fiber, or reduced synthesis of molecules that cause them to adhere may reach a subthreshold level at different times in different cells. Alternatively, it is possible that they are leaving due to the recognition of specific cues. We have attempted to label processes within the marginal zone that support or induce the tangential migration. Labeling with DiI and electron microscopy of the marginal zone in the chick diencephalon has provided some evidence for axonal processes that support tangential migration (Golden *et al.,* 1997). However, to date, we have been unable to observe DiI-labeled tangential processes in the chick telencephalon. Further work will be needed to uncover the mechanisms used for tangential migration.

Generation of RC Clones

In the posthatch chick, 60% of clones with more than four cells were found in rostrocaudal arrays, resembling rostrocaudal ''tubes.'' As little mediolateral dispersion was found in these clones, we expected to find approximately the same proportion of large clones in the embryo with cells migrating rostrocaudally away from radial glia and for the migration within each clone to be at a particular distance from the ventricle. We did find some clones that exhibited this pattern of migration. However, the majority of clones had cells emanating from points along the entire length of the radial glial process within the marginal zone. This observation eliminates the model in which RC clones are formed by clonally related cells recognizing a particular point along the radial glial fiber and leaving it at that point to initiate tangential migration. Two other possibilities for how RC tubes form is that cells not found within an RC clone's mediolateral domain selectively die off and/or that cells later migrate to within the RC tube. One further possibility that we considered is that when the marginal zone is quite thin early in development, clonally related cells would be restricted to migrate within it. Thus one could envision a situation in which only early generated cells form RC clones. This would result in clones being found only at the lateral edge of the forebrain and in the clones being quite small. However, we found a large range of sizes of RC clones, with some of them containing hundreds of members, and RC clones were found at all mediolateral distances from the ventricles. Videomicroscopy of migration in living slices or intact embryos would be the most useful way to directly address these and many other aspects of the migration of chick forebrain cells.

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