cells. In 2 of the 15 animals exhibiting nonsegmented crowing, the telencephalon and diencephalon were entirely quail; the remaining 13 animals had a mixed diencephalic population of quail and chick cells, as expected. Numerous studies in both passerine and galliform birds have implicated the mesencephalic-diencephalic region in the control of avian vocalizations (*14*). It is also important to note that segmentation is not the only feature that differs between chick and quail crowing, and that none of our chimeras to date has produced a structurally perfect quail crow.

More generally, this report shows the feasibility of producing hatched brain chimeras between two avian species, which provide rich experimental possibilities for ethology, immunology, and the neurosciences.

REFERENCES AND NOTES

1. N. Le Douarin, *The Neural Crest* (Cambridge Univ. Press, Cambridge, 1982); ——— and A. McLaren, Eds., *Chimeras in Developmental Biology* (Academic Press, New York, 1984).
2. G. Couly and N. Le Douarin, *Dev. Biol.* **110**, 422 (1985); N. Le Douarin, J. Fontaine-Perus, G. Couly, *Trends Neurosci.* **9**, 175 (1986); G. Couly and N. Le Douarin, *Dev. Biol.* **120**, 198 (1987).
3. M. Kinutani and N. Le Douarin, *Dev. Biol.* **111**, 243 (1985); M. Kinutani, M. Coltey, N. Le Douarin, *Cell* **45**, 307 (1986).
4. Domestic chicken eggs and Japanese quail eggs were obtained from commercial sources. Before surgery, eggs were incubated for 36 to 42 hours at 37.5°C. In the microsurgical extirpation and transfer of isotopic embryonic neural tube fragments between chicken and quail embryos of the same stage, we followed procedures described (*1, 3*). We did not enzymatically dissociate neural tissue from nonneurectodermal cells in order to improve the hatching rate of operated animals. The hatching rate varied between <5% and approximately 25%, depending on the type of operation and the quality of the eggs.
5. H. Ohki, C. Martin, C. Corbel, M. Coltey, N. M. Le Douarin, *Science* **237**, 1032 (1987). Immune rejection in the brain chimeras will be described in a subsequent report (E. Balaban, M.-A. Teillet, N. Le Douarin, unpublished data). Animals are able to survive immune rejection despite the loss of all transplanted material provided the volume of grafted tissue is relatively small (E. Balaban, M.-A. Teillet, N. Le Douarin, unpublished observations).
6. Hatched subjects were anesthetized with an overdose of sodium pentobarbitol and perfused intracardially with Zenker solution; embryos were fixed by immersion in Zenker solution. We stained paraffin-mounted serial sections of brains (hatched animals) or whole heads (embryos) by using the Feulgen reaction (*1*) to visualize the species identity of cells under a light microscope. Some sections were stained with cresyl violet to allow identification of neurons. We examined histological sections from 17 chick hosts and 13 quail hosts fixed during embryonic life, 3 chick hosts and 1 quail host fixed on hatching day, and 5 chick hosts fixed between 3 and 12 days after hatching.
7. P. Rakic, *J. Comp. Neurol.* **145**, 61 (1972); *ibid.* **176**, 23 (1977); *ibid.* **223**, 1 (1984).
8. Chimeric and control (unoperated) animals were implanted subcutaneously with Silastic medical tubing capsules (0.635-mm inner diameter; 1.19-mm outer diameter) packed with crystalline testosterone proprionate (Sigma). Implants were performed within 8 hours of hatching; each quail received one capsule containing 1 cm of crystalline hormone, and each control and chimeric chicken received one 2.5-cm capsule under the skin of the back. All animals gave crows by the fourth day after hatching. There are also reports of rare crows given by unimplanted animals of both species during their first week of life (*9, 10*). Although sex differences in the fine structure of crowing may exist within each species (*12*), crows of females of each species have the same features found in male crows. Crows were recorded daily during the lifetime of each animal. After the chimeric animals had been killed, their brains were processed as described (*6*), and the colonization of the chicken host brains by quail cells was verified with the quail-chick cell marker (*1*).
9. J. B. Hamilton, *Endocrinology* **23**, 53 (1938); P. Marler, M. Kreith, E. Willis, *Anim. Behav.* **10**, 48 (1962); P. B. Siegel, R. E. Phillips, E. F. Folsom, *Behaviour* **24**, 229 (1965); J.-C. Guyomarc'h, *Bull. Soc. Sci. Bretagne* **60**, 81 (1965); R. J. Andrew, in *Bird Vocalizations*, R. A. Hinde, Ed. (Cambridge Univ. Press, Cambridge, 1969), pp. 97–130.
10. M. L. Cariou, *Bull. Biol. Fr. Belg.* **103**, 323 (1969); thesis, Université de Rennes (1971).
11. M. Konishi, *Z. Tierpsychol.* **20**, 349 (1963).
12. E. Balaban, unpublished observations.
13. Although juvenile crowing sounds may be preceded (chicken and quail) or followed (chicken) by a variable number of distress call notes (*9, 10, 12*), no study, including the present one, has observed chickens producing stable crows with differentiated introductory notes preceding the "squeak" during the first week of life. If such cases do occur, they are presumably rare. Although an exhaustive study of juvenile crow structure in three different captive populations of Japanese quail did find rare examples of quail with a crow lacking introductory notes (*10*), such crows were present at low frequency (<5% of the animals) in one captive population and totally absent in the two others. Many young quails produce both two-part and three-part crows during their first week of life, subsequently producing one form or the other (*12*).
14. J. L. Brown, *Behaviour* **39**, 91 (1971); R. E. Phillips and F. W. Peek, in *Neural and Endocrine Aspects of Behaviour in Birds*, P. Wright, P. G. Caryl, D. M Vowles, Eds. (Elsevier, Amsterdam, 1975), pp. 243–274; F. Nottenbohm, T. N. Stokes, C. M. Leonard, *J. Comp. Neurol.* **165**, 457 (1976); T. J. Seller, *Trends Neurosci.* **4**, 301 (1981); in *Bioacoustics: A Comparative Approach*, D. B. Lewis, Ed. (Academic Press, New York, 1983), pp. 93–124; ——— and S. E. Armitage, *Behav. Brain Res.* **9**, 305 (1983); H. Scheich *et al.*, in *Advances in Vertebrate Neuroethology*, J.-P. Ewert, R. R. Capranica, D. J. Ingle, Eds. (Plenum, New York, 1983), pp. 731–782.

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Clonally Related Cortical Cells Show Several Migration Patterns

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The mammalian cerebral cortex is organized into columns of cells with common functional properties. During embryogenesis, cortical neurons are formed deep, near the lateral ventricles, and migrate radially to their final position. This observation led to the suggestion that the cortex consists of radial, ontogenetic units of clonally related neurons. In the experiments reported here, this hypothesis was tested by studying cell lineage in the rat cortex with a retroviral vector carrying the *Escherchia coli* β-galactosidase gene, which can be easily visualized. Labeled, clonally related cortical neurons did not occur in simple columnar arrays. Instead, clonally related neurons entered several different radial columns, apparently by migrating along different radial glial fibers.

THE MAMMALIAN CEREBRAL CORTEX is organized into layers, which contain distinct morphological cell types, and columns, which are oriented perpendicular to the layers, in which cells have similar physiological properties. Cortical neurons are produced near the lateral ventricle early in gestation and migrate radially toward the cortical surface. The cortical layers are generated in a defined, "inside-out" sequence, with neurons destined for the deepest cortical layers formed first and neurons destined for more superficial layers formed in sequence later (*1*). The observation of this generative sequence led to the suggestion that functional columns can be subdivided into smaller, radially oriented "ontogenetic columns," each centered around a single radial glial fiber, with cells in each layer sequentially generated from a common precursor located near the ventricle (*2*).

We tested this hypothesis directly by using an in vivo retroviral marking method (*3*) to study cell lineage in the rat cortex. Divid-

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ing cells can be infected by a replication-defective retroviral vector carrying the *E. coli lacZ* gene encoding β-galactosidase (βgal). After exposure to a retroviral inoculum, mitotic cells can support stable integration of the vector DNA into the genome of the host. The vector DNA is then faithfully transmitted to all progeny in a Mendelian manner. In the BAG (*4*) retrovirus vector, expression of βgal is directed by the viral promoter located in the long terminal repeat (LTR) of Moloney murine leukemia virus. We also used an alternative retrovirus vector, SPUD (*5*), in which the simian virus 40 (SV40) early promoter directed βgal expression, for some experiments. Neural cells inoculated with either construct proved to be infectable as evidenced by gene expression and, in some cases, RNA and DNA analysis. To date, these include retinal, olfactory bulb, and cerebellar cells infected in vivo and in vitro (*4, 6–8*) and neuroblastoma, glioma, and dorsal root ganglion cells infected in vitro (*8*).

Seventy-six Long-Evans hooded rats were injected with BAG virus during the period of cortical neurogenesis. Animals were allowed to live for several days to several weeks after infection. Injections were made between the 14th day of gestation (E14) and the day of birth (P0), with the day of discovery of the vaginal plug defined as E0. Pregnant females (from Charles River Labs) were anesthetized with xylazine (3 to 5 mg/kg) and ketamine (20 to 40 mg/kg) and subjected to a ventral laparotomy. The head of each fetus was localized by transillumination of the uterus, and a glass micropipette containing the viral stock, with Polybrene (80 μg/ml) and trypan blue (0.004% w/v), was passed into the fetal head to make injections of 0.1 to 3 μl into one lateral ventricle. The blue solution could be seen diffusing throughout the ventricular system.

The rats were killed and processed for βgal histochemistry with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal). Labeled cells were distributed throughout both hemispheres, usually with no apparent clumping of labeled cells near the injection site. Although the injection site sometimes showed a scar containing many labeled cells, in other cases the path of the pipette was completely effaced. The Xgal reaction produced an intense, "Golgi-like" filling of labeled cells. In animals that survived until postnatal days 8 to 10 (P8 to P10), the maturation of cellular processes was sufficient for identification of up to 75% of the cells as neurons, astrocytes, or ventricular cells (Fig. 1, A through C). Neurons of all known morphological types were labeled, with no apparent bias except for the expected tendency (*1*) for injections at later ages (E17 to E18) to label neurons in upper cortical layers. Labeled oligodendrocytes were only identified when animals survived until P21 or longer (Fig. 1). Regardless of the survival time, 25% or more of the labeled cells could not be assigned to a particular cell class; these cells had round, nondescript, often intensely stained cell somata distributed throughout the cortex.

After injections into 39 embryos at E14 to E16, clustered arrays of labeled cells were observed after short survival periods of 3 to 7 days. Previous birthdating experiments (*1*) and our own observations (see below) suggested that most of the labeled cells were neurons. Labeled cells occurring alone, or in groups of 2 to 12 and occasionally up to 30, were distributed throughout the ventricular zone, intermediate zone, subplate, cortical plate, and marginal zone. Single cells with

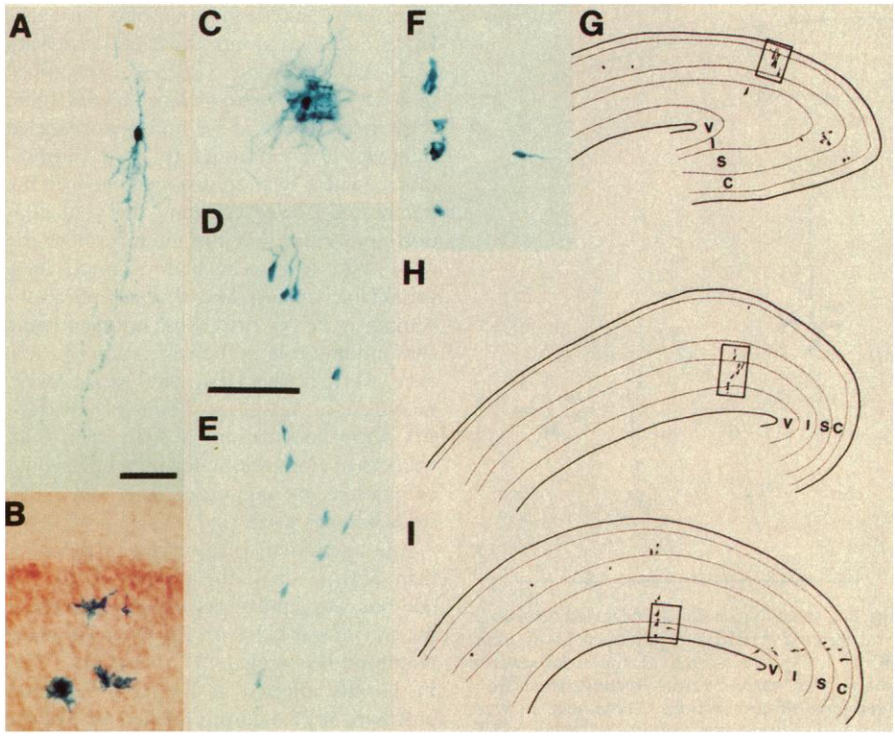

Fig. 1. Examples of Xgal-stained virus-infected cells. (**A**) A pyramidal neuron in layer II/III, in a rat given an injection of BAG virus at E16 and processed at P8. Although neuronal form is still immature at P8 (*19*), neurons could be identified because of their relatively large somata and labeled dendrites (label frequently extended into quaternary dendrites) or axons, or both. Scale bar, 100 μm; magnification is the same in (B) and (C). (**B**) A small group of labeled astrocytes in layers II/III and IV from another rat injected at E17 and killed at P8. Astrocytes were identified by their very small somata and many small, short, highly branched processes, many of which inserted into blood vessels, neurons, or the pial surface. This section was counterstained with neutral red. (**C**) A labeled oligodendrocyte from a rat injected at E17 and processed at P60. Oligodendrocytes had small, round somata with no axon and several fairly straight processes. They were more often seen in the white matter but also occasionally in the gray matter. Clearly labeled oligodendrocytes were not seen in animals processed at P8, although presumptive oligodendrocytes (small round cells in the white matter with a few short, straight processes) were seen frequently. (**D**) Labeled cells in the cortical plate of a rat injected at E14 and processed at E19. Labeled cells, probably neurons, show apical dendrite-like processes. They occur in several nearby "radial units" (*2*) of cortex, stretching over 75 to 80 μm (compare G). Scale bar, 75 μm; magnification is the same for (E) and (F). (**E**) Another group of labeled cells taken from another rat processed similarly. Labeled cells in the intermediate zone seem to break into two groups migrating along different radial glial fibers (compare H). (**F**) A third nonradial pattern of label. Three cells in the ventricular and intermediate zone are arranged radially; a fourth cell is oriented along the ventricular surface and appears to be migrating away from the other cells (compare I). (**G** through **I**) Camera lucida sketches of the pattern of label reconstructed from three serial 60-μm coronal sections from the rats shown in (D), (E), and (F). These groups of labeled cells were chosen because each group was completely contained within three serial sections. The heavy outline shows the dorsal pial surface, and the border of the lateral ventricle is below (medial is to the right), with fine outlines separating the ventricular/subventricular zone (V), intermediate zone (I), subplate (S), cortical plate (C), and marginal zone (not labeled). The boxes outline the areas photographed in (D), (E), and (F), respectively, and the width of each box is 110 μm. Methods are outlined in (*20*).

no obvious relation to other labeled cells were commonly observed (Fig. 1, G and I) and represented the majority of labeled cells in the lateral areas of the cortex; clusters were most frequently observed in the dorsal-medial areas (Fig. 1, D through I).

Within the clusters, labeled cells in the intermediate zone were usually in radial alignment but cells in the cortical plate were never limited to one cortical radius. Instead, labeled cells were adjacent to one another or in nearby cortical radii. Whenever two labeled cells in the cortical plate were located directly above one another, they were always part of a larger cluster covering several cortical radii (Fig. 1, D through I). Furthermore, some groups of labeled cells in the intermediate zone seemed to be migrating up different radial glial fibers, while other cells in the ventricular zone appeared to be migrating along the ventricular surface, perpendicular to the radial glia. The latter form of migration could potentially cause extremely large dispersion of clonally related cells.

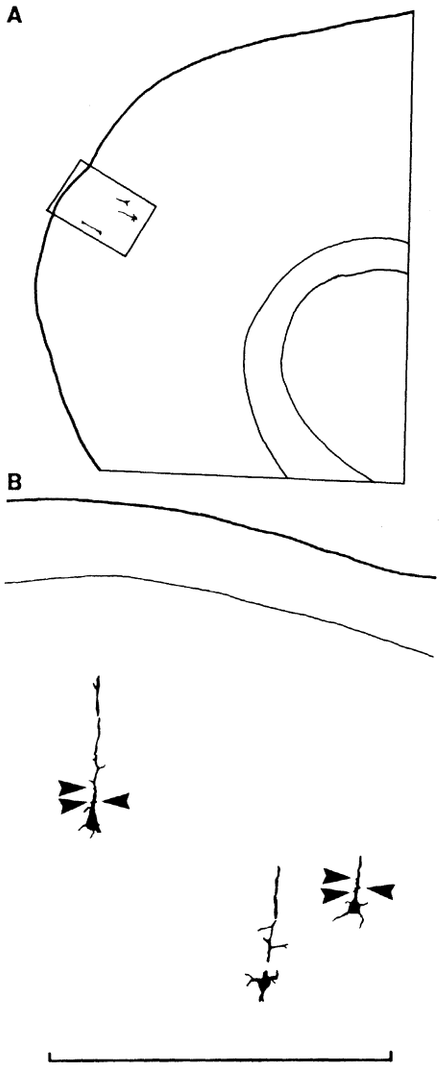

Fig. 2. Camera lucida sketch of labeled neurons in a rat injected at E15 and processed as an adult. (**A**) A low-power sketch of the frontoparietal cortex, with three neurons reconstructed from three serial 95-μm sections. These neurons were contained within three sections and were widely separated from other labeled cells in the same section or in adjacent sections. Their distribution is not simply radial and resembles the pattern seen in Fig. 1D. (**B**) A higher power drawing of the same neurons. Two pyramidal neurons show staining of dendrites and spines (arrowheads); the nonpyramidal cell in between has a long but smooth apical dendrite. Scale bar, 500 μm.

Clusters of labeled cells that were widely separated from other labeled cells were presumed to represent "clones." In earlier work on lineage analysis in the rat retina (*7*), it was possible to clearly identify tightly clustered, radially aligned groups of cells as clones. A statistical analysis of the number of such clusters relative to the viral inoculum size demonstrated that each cluster was the result of a single infectious viral particle and thus must have been the progeny of a single infected cell. In the cortex, it is not possible to perform the same statistical analysis: although there are many clusters of labeled cells separated from other labeled cells, the scattered, single labeled cells have ambiguous relations. However, we evaluated the relation between the number of labeled cells and the size of the viral inoculum, using a series of threefold dilutions of a particular viral stock injected into the lateral ventricles of 25 E16 embryos, with a 4-day survival period. The patterns of label in the brain were independent of the number of labeled cells, which varied from 2 to 2000. Furthermore, a linear relation was seen between the number of labeled cells and the viral dilution, indicating that the interaction of the virus with host cells had occurred in a predictable manner and that a replication-competent helper virus could not have had a confounding role in the generation of these data. As a further test, viral stocks were examined for the presence of helper virus (*9, 10*) before inoculation. If more than 0.01 wild-type helper colony-forming unit would be injected in any experiment, the virus stock was not used.

Among animals that survived for longer than 3 to 7 days, the pattern of labeled neurons was similar, regardless of whether the animal was killed on P8, when neuronal migration is complete (*1*), or later (>P24). In 12 rats injected at E15 and E16 and processed at P8, neurons formed 60% of the cells that could be clearly identified. Labeled neurons were not distributed in a simple radial pattern. Less than 25% of the labeled neurons occurred along the same radius (or along another radius within 100 μm) as any other labeled neuron. Occasional radial pairs of neurons were part of larger groups of

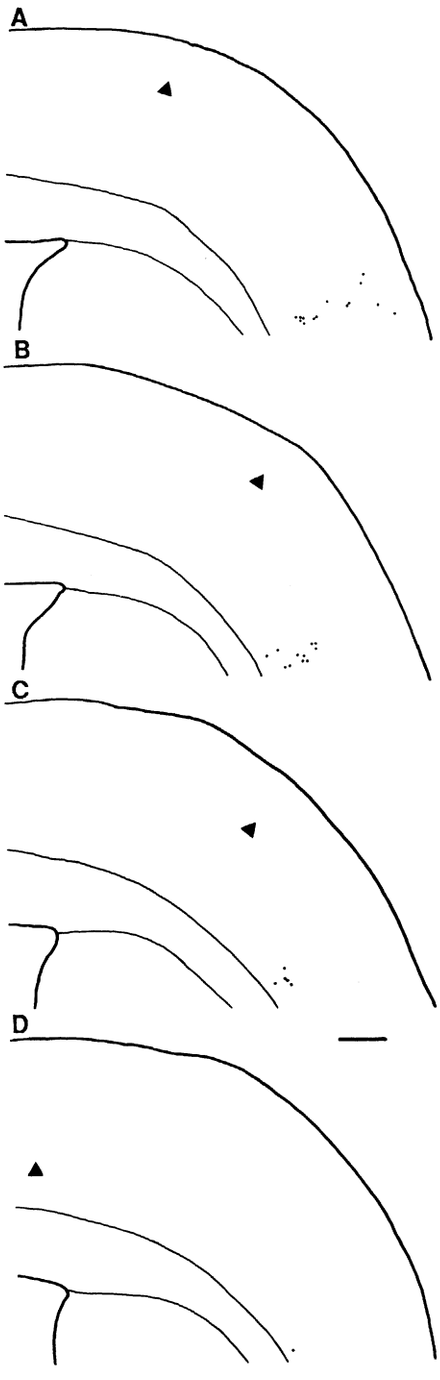

Fig. 3. Plots of labeled neurons and astrocytes in a rat injected at E15 and processed as an adult. These four serial 95-μm sections (**A** through **D**) illustrate all labeled cells in the region shown. Neurons (triangles) showed labeling of dendrites superior to that shown in Fig. 2, whereas astrocytes (dots) were recognized by their small somata and many short, highly branched processes. The glia form a strikingly radial array, whereas the labeled neurons, except for two only 90 μm apart, are widely separated. The clonal boundaries of such labeled neurons are obscure, but similar patterns of label were seen in many sections throughout this brain. Scale bar, 500 μm.

labeled neurons spread over several hundred micrometers. In two rats processed at P24 or later, this radial pattern accounted for less than 15% of the labeled neurons. In all rats killed at P8 or later, labeled neurons occurred more commonly in widely separated cortical radii. In Fig. 2 three neurons are shown to be widely distributed over 500 μm and in Fig. 3 an even more typical distribution is shown. When strictly radial arrays of labeled cells were seen, they always contained glial cells (Fig. 3).

The clonal relations of these widely isolated labeled neurons are unclear. Some may be clones of single cells (for example, progeny of cells that integrated the viral genome into only one daughter cell in the last cell division). This would be inconsistent with the "ontogenetic column" hypothesis because, as E15 to E16 is in the middle of the period of cortical neurogenesis, one would not expect to find the majority of progenitors in their last cell cycle. Moreover, after short survival times, the clustered arrays of cells (Fig. 1, D through I), which probably represent clones, frequently contained more than two cells. Thus, most widely distributed cells probably do not represent single-cell clones.

Another possibility is that widely distributed single cells may be the only labeled survivors of larger, radial clones. For example, cell death (*11*) will reduce the number of labeled cells per clone. However, cell death would have to be massive in order to totally obscure an overall radial pattern. Alternatively, there could be poor or no transcription from the promoter or promoters that direct βgal expression in some cells. However, a wide variety of morphological types of neurons, glial cells, meningeal cells, and endothelial cells exhibited intense staining, so there is no reason to suspect that a particular cell type is unable to transcribe the LTR promoter. Moreover, in the retina (*7*), the same viral stock as used here labeled all neuronal types in strikingly columnar patterns. The same pattern of labeling that resulted from BAG infection in the cortex also resulted from infection with the SPUD (*5*) construct when the injection was given at E15 and the rat was killed at P24.

The hypothesis that groups of widely separated cells are the result of clustered single-cell clones (real or apparent) would require that the distribution of viral hits was nonhomogeneous. This idea seems unlikely because the injected viral supernatant can be seen to diffuse throughout the ventricles, labeled cells are produced with a remarkably even distribution throughout the brain, and the pattern of label among neurons bore no consistent relation to the injection site.

Our results suggest that clonally related neurons can achieve wide circumferential separation in the rat's cerebral cortex, apparently by migration along several different radial glial fibers. Furthermore, there is a suggestion that neural progenitors, some of which presumably give rise to glia, can migrate along the ventricular lumen and in the subventricular zone, which would produce an even wider circumferential spread of clonally related cells.

A recent report of similar experiments in the mouse (*12*) concluded that clonally related neurons occur in columns. A viral construct similar to the SPUD virus and a different Xgal-staining protocol were used, and staining was less intense than after infection with the BAG virus. The presence of weakly stained cells, which we also observed after infection with SPUD virus, makes identification of cell types more ambiguous. All of our strictly radial clones contained glial cells (*13*). In addition, the mouse brains were stained as whole mounts, and positive areas were then sectioned; isolated single cells may not have been obvious from observation of whole mounts.

The complex patterns of cell migration in the cortex of the rat and chick (*14*) differ markedly from patterns in rat and *Xenopus* retina (*15*) and chick optic tectum (*16*), where clonally related neurons show strikingly columnar arrangements. These differing patterns may reflect regional and species differences in the structure of radial neuroepithelial cells. These cells have been intensively studied in the mouse with the use of immunohistochemical techniques (*17*). Radial glia form fascicles in the intermediate zone and defasciculate as they enter the cortical plate. Some of the circumferential separation of clonally related neurons may represent migration in a common fascicle, with separation of neurons along different fibers in the cortical plate. However, some clonally related cells diverge within the intermediate zone itself, before this defasciculation occurs (Fig. 1, D through F).

Labeled cells in different laminae have been observed in many of the dispersed clusters of neurons, suggesting that cortical progenitors are multipotential. Furthermore, it is not uncommon to see labeled neurons and glial cells adjacent to one another, suggesting that they share a common progenitor as well. This would be consistent with studies in the retina that have shown a common progenitor for neurons and glia (*7, 15*). Results in cortex, retina, and chick tectum suggest that lineage is not a major determinant of cell type in the central nervous system.

While this work was in progress, Price independently found similar results in the rat, also using the BAG virus (*18*).

REFERENCES AND NOTES

1. M. Berry and A. W. Rogers, *J. Anat.* **99**, 691 (1965); S. P. Hicks and C. J. D'Amato, *Anat. Rec.* **160**, 619 (1968); P. Rakic, *Science* **183**, 425 (1974).
2. P. Rakic, *Science* **241**, 170 (1988).
3. C. L. Cepko, *Neuron* **1**, 345 (1988).
4. J. Price, D. L. Turner, C. L. Cepko, *Proc. Natl. Acad. Sci U.S.A.* **84**, 156 (1987).
5. The SPUD vector is similar to BAG in overall design. The *lacZ* gene replaces the *neo* gene, which is deleted from the construct (D. Altshuler and C. L. Cepko, unpublished data).
6. E. F. Ryder, C. Walsh, C. L. Cepko, unpublished data.
7. D. L. Turner and C. L. Cepko, *Nature* **328**, 131 (1987); D. L. Turner, E. Y. Snyder, C. L. Cepko, *Soc. Neurosci. Abstr.*, in press.
8. E. Y. Snyder and C. L. Cepko, unpublished data.
9. Helper virus can result from recombination of the replication-defective virus containing *lacZ* and the deleted, packaging-minus helper genome in the producer (ψ2) cell line. "Crippled" (poorly replicating, unable to effect efficient spread after several weeks in vitro) and wild-type, fully competent viruses can result. Stocks containing no helper of any kind or those with crippled helper were used, if the amount of helper activity was less than 0.01 colony-forming unit of wild type per injected volume. Stocks containing no detectable helper gave the same results as those containing low-level, crippled helper.
10. R. D. Cone and R. C. Mulligan, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6349 (1984).
11. W. M. Cowan, J. W. Fawcett, D. D. M. O'Leary, B. B. Stanfield, *Science* **225**, 1258 (1984); J. J. M. Chun, M. J. Nakamura, C. J. Shatz, *Nature* **325**, 617 (1987).
12. M. B. Luskin, A. L. Pearlman, J. R. Sanes, *Soc. Neurosci. Abstr.* **13**, 183 (1987).
13. E. F. Ryder, C. Walsh, C. L. Cepko, *ibid.*, in press.
14. E. Balaban, M.-A. Teillet, N. Le Douarin, *Science* **241**, 1339 (1988).
15. R. Wetts and S. E. Fraser, *ibid.* **239**, 1142 (1988); C. Holt, T. W. Bertsch, H. M. Ellis, W. A. Harris, *Neuron* **1**, 15 (1988).
16. J. C. Glover, G. E. Gray, J. R. Sanes, *Soc. Neurosci. Abstr.* **13**, 183 (1987).
17. J.-F. Gadisseux, P. Evrad, J.-P. Misson, V. S. Caviness, Jr., *ibid.*, p. 1117; J.-P. Misson, M. A. Edwards, M. Yamamoto, V. S. Caviness, Jr., *Dev. Brain Res.* **38**, 183 (1988); J.-P. Misson, M. Yamamoto, M. A. Edwards, V. S. Caviness, Jr., *ibid.*, in press.
18. J. Price, personal communication.
19. S. P. Wise, J. W. Fleshman, E. G. Jones, *Neuroscience* **4**, 1275 (1979).
20. Rats were killed with carbon dioxide and perfused with 2% paraformaldehyde (BDH Chemicals) in 0.1*M* Pipes buffer (*p*H 6.9), with $MgCl_2$ (2 m*M*) and EGTA (2 m*M*). The brain was removed and "postfixed" in the same solution for 15 to 60 min, then rinsed and incubated overnight in 30% sucrose in phosphate-buffered saline (PBS) (*p*H 7.3) with $MgCl_2$ (1 m*M*) and then sectioned at 30 to 100 μm on a cryostat. Sections were mounted on gelatinized slides and reacted with Xgal (*4*) with $MgCl_2$ (1 m*M*), sodium deoxycholate (0.01%, w/v), and NP40 (0.02%, w/v). The sections were washed in PBS, and a cover slip was applied in Gelvatol (polyvinyl alcohol, Air Products and Chemicals). Some sections were counterstained in neutral red, dehydrated in alcohols, and cleared in xylene, although this often caused fading of the Xgal staining.

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