

Application of the Quail-Chick Chimera System to the Study of Brain Development and Behavior

EVAN BALABAN,* MARIE-AIMÉE TEILLET, NICOLE LE DOUARIN

Hatched chicks with chimeric brains containing cells from both the domestic chicken (*Gallus gallus domesticus*) and the Japanese quail (*Coturnix coturnix japonica*) have been produced by transplantation of various regions of the neural tube at the 8- to 15-somite stage. The positions of host and donor cells relative to graft boundaries observed throughout embryonic development and after hatching implicated both radial and tangential cell movements in brain morphogenesis. In addition, transplants containing the entire quail mesencephalon and diencephalon resulted in the transfer of certain aspects of species-typical crowing behavior.

THE QUAIL-CHICK MARKER SYSTEM has been extensively used to study the ontogeny of various tissues and organs (1-3). We are now applying the quail-chick chimera technique to investigations of the development of the brain and species-typical behavior patterns.

We carried out isotopic and isochronic transplantation of fragments of the neural primordium from quail donors to chicken hosts and vice versa in embryos at the 8- to 15-somite stage, using a modification of the technique described in (1-3). We performed two types of operations. In the first type, the roof of the prosencephalon was removed along with the associated neural crest and superficial ectoderm, followed by implantation in situ of the corresponding cephalic area from the donor (Fig. 1). In the second type, entire portions of the neural tube of the host were removed at defined levels and replaced with a corresponding fragment

from a donor embryo. In neither case was nonneural tissue dissociated enzymatically from the neural primordium (4). Transplants therefore contained cells from the donor's mesodermal mesenchyme and superficial ectoderm in addition to the neural crest and the neuroepithelium. Because the grafts underwent rejection, starting with the transplanted nonneural tissues, during the second week after hatching (3, 5), we report here on animals examined before immune rejection occurred in the brain (6).

Following recent hypotheses for mammalian brain morphogenesis that have emphasized the role of radial neuronal migration in

the prosencephalon (7), we studied the spatial distribution of chick and quail cells in brains that received transplants of limited regions of the prosencephalic roof on embryonic day 2 (E2) (Fig. 1) in animals killed at regular intervals from E3 to posthatching day 12 (P12) (6). We noted the positions of chick and quail cells relative to graft boundaries, which in all cases were clearly visible in the ependymal layer bordering the brain ventricles. Although this method does not allow us to directly observe cell movements or to distinguish migrating cells from those that have ceased moving, it does indicate whether significant cell mixing occurs near graft boundaries in different brain regions. A lack of mixing is consistent with predominantly radial cell movements; widespread mixing cannot be accounted for by a purely radial pattern of cell migration but indicates tangential movement of cells within the neural epithelium during brain development.

By E3 to E4, when the brain vesicles were bordered by a dense pseudostratified neuroepithelium, the boundary between quail and chick cells in chimeric embryos was clearly demarcated, suggesting that cell movements are strictly perpendicular to the surface of the epithelium at this stage ($n = 3$ chick hosts, 2 quail hosts). At E5 to E8, this clear-

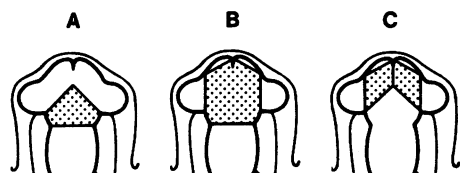


Fig. 1. Isotopic and isochronic transplants of portions of the neural tube and overlying ectoderm in both directions (quail into chick and vice versa). (A) Dorsal part of the diencephalon; (B) dorsal diencephalon plus roof of the telencephalic vesicle; (C) roof of the telencephalic vesicle, unilaterally or bilaterally.

E. Balaban, Institut d'Embryologie Cellulaire et Moléculaire du Centre National de la Recherche Scientifique et du Collège de France, 49bis avenue de la Belle Gabrielle, 94736 Nogent-sur-Marne Cedex, France, and Rockefeller University Field Research Center, Tyrrel Road, Millbrook, NY 12545.

M.-A. Teillet and N. Le Douarin, Institut d'Embryologie Cellulaire et Moléculaire du Centre National de la Recherche Scientifique et du Collège de France, 49bis avenue de la Belle Gabrielle, 94736 Nogent-sur-Marne Cedex, France.

*To whom correspondence should be directed at the French address.

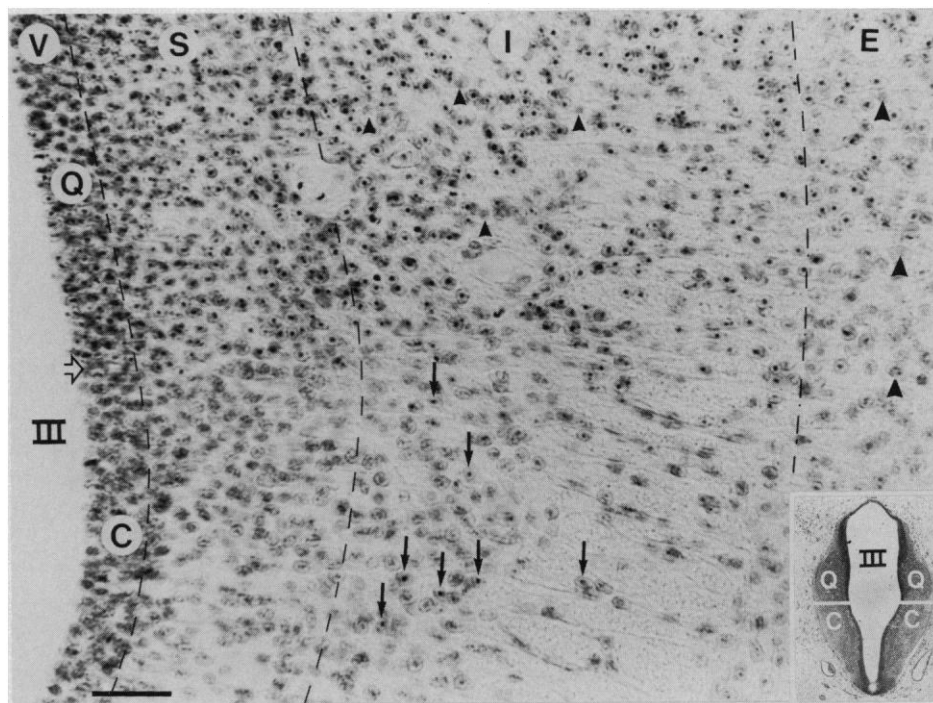


Fig. 2. Feulgen-stained transverse section of the diencephalon of a quail (Q) into chick (C) chimera (experiment A in Fig. 1) at E6. Donor and host cells are well separated in the subventricular layer (S). In contrast, the presence of isolated quail cells (\downarrow) in predominantly chicken regions and vice versa (\blacktriangle) in the intermediate zone (I) of the mantle layer indicates tangential movements in both directions. In the external zone (E) of the mantle layer the presence of numerous host cells (\blacktriangle) in predominantly donor areas indicates large ventrodorsal cell movements at this level. White bars in the inset show the virtual boundaries between host and donor tissues (III = third ventricle). Large arrowhead designates ventricular boundaries of the graft. V, ventricular epithelium. Bar, 25 μ m.

cut separation of chick and quail cells was found only in those portions of the brain vesicles where the walls remain thin, such as the mediodorsal parts of the telencephalon (which give rise to the avian hippocampus-parahippocampus) and of the diencephalon (choroid plexus). During this same time, the lateral walls of the telencephalon and diencephalon grew considerably in thickness, and significant tangential movement of the neuroepithelial cells was apparent ($n = 7$ chick hosts, 6 quail hosts).

In the lateral walls of the E6 diencephalon (experiments A and B of Fig. 1), a clear-cut separation between chick and quail cells in the subventricular layer showed that cell

movements were still radial (Fig. 2). In contrast, a mixing of donor and host cells in the intermediate zone of the mantle layer also occurred at this time, owing to a dorso-ventral and ventrodorsal ingression of cells of the two types at about 80 μm from the initial limit of the graft. In the external (lateral) zone of the mantle layer, the dominant tangential movement of cells was ventrodorsal, leading host cells to invade the donor tissue. Equivalent pictures are seen in reciprocally oriented grafts (both quail-to-chick and chick-to-quail). Thus, in the postnatal brain, dorsal and dorsolateral thalamic nuclei are of the donor type, medioventral and lateral nuclei are mixed, and ventral nuclei are of the host type (Fig. 3A). In all cases, the vascular endothelial cells are of the host type.

Tangential migrations were conspicuous in the lateral region of the first and second ventricles of the E6 telencephalon, which give rise to the bulk of hemispheric tissue including the hyperstriatum ventrale, neostriatum, paleostriatum, and archistriatum. In E9 animals, the boundary between donor and host cells was still radial in the subventricular zone in the lateral aspect of the first and second ventricles. At about 40 μm from the ventricular surface, host and donor cells were mixed, indicating tangential cell movements. By E14, mixing of host and donor

cells resulting from tangential movements reached the subventricular zone, coinciding with the time at which the density of cells in the subventricular zone has decreased. Such movements can result in reciprocally intermingling cells as far as 400 μm from the initial quail-chick limit in the basal layer of the ventricular epithelium. In all prehatching stages studied and after hatching, the distribution of quail and chick cells in the cerebral hemispheres after experiment C of Fig. 1 was as indicated in Fig. 3B. The paleostriatum and archistriatum were of the host type with a few scattered cells of donor origin. The neostriatum consisted of a mixture of host and donor cells (Fig. 4, A and B), and the hyperstriatum was of the donor type with dispersed host cells. Therefore, movements were essentially radial in the mediodorsal wall of the first and second ventricles, which remained thin (Fig. 3A). In contrast, they were more complex in the lateral areas, with large-scale mixing of cells of dorsal and ventral origin (Fig. 3B). Staining of adjacent sections with the Feulgen-Rossenbeck technique and with cresyl violet (for the identification of neurons) indicated that both neuronal and nonneuronal cells exhibited tangential cell movements (Fig. 4B).

The distribution of host and donor cells at graft boundaries implicates at least two

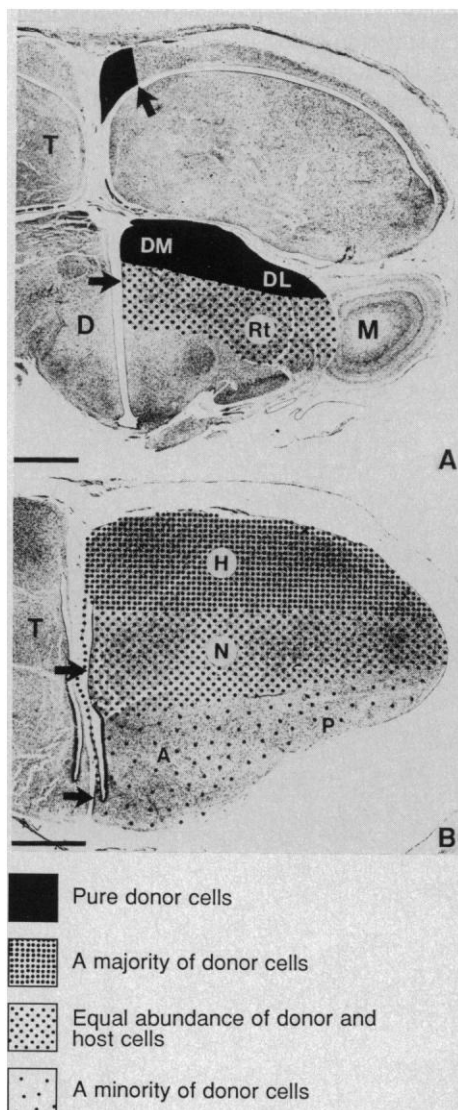


Fig. 3. Distribution and density of donor cells: (A) in the diencephalon (D) and telencephalon (T) of chimeras from experiment A in Fig. 1; (B) in the telencephalon of chimeras from experiments B and C in Fig. 1. Arrows designate ventricular boundaries of the graft. DM, dorso-medial nucleus; DL, dorsolateral nucleus; Rt, nucleus rotundus; H, hyperstriatum; N, neostriatum; P, paleostriatum; A, archistriatum; M, mesencephalon.

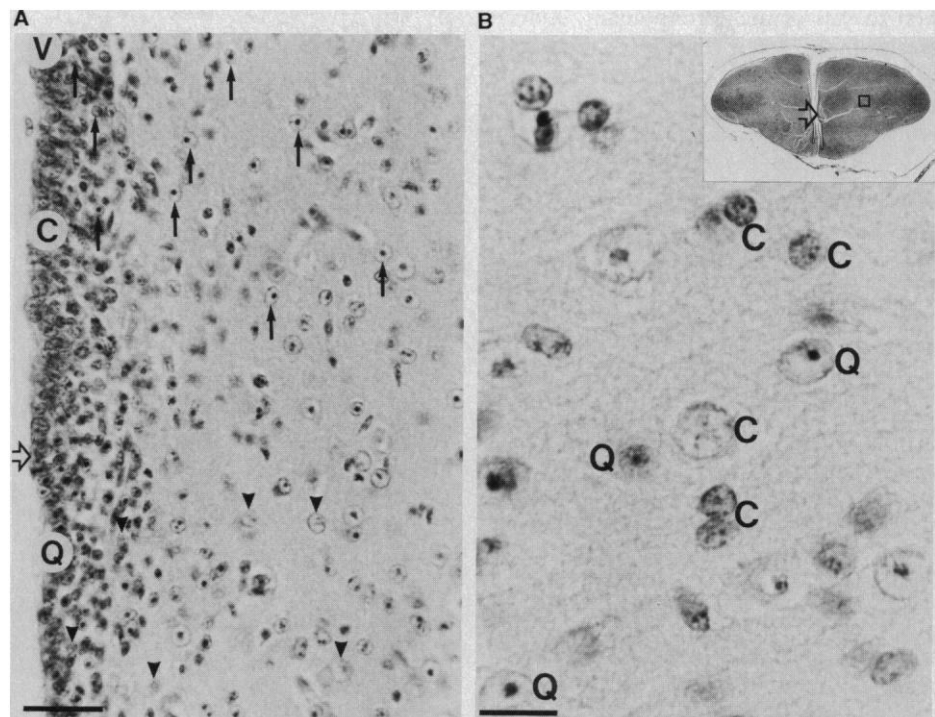


Fig. 4. Feulgen-stained section through the cerebral hemispheres of a quail (Q) into chick (C) chimera (experiment C in Fig. 1) at P7. (A) Host and donor cells are well separated at the level of the ventricular epithelium (V). A mixing of quail (\uparrow) and chick (∇) cells immediately in contact with this thin cell layer indicates tangential cell movements during development; this produces truly chimeric brain regions. (B) Enlargement of the region indicated in the inset showing a mixed region containing quail and chick neurons and glial cells. Large arrowheads indicate the ventricular boundary of the graft. Bar in (A), 22 μm ; bar in (B), 11 μm .

Table 1. Crow segmentation in chimeric animals.

Transplant type* (n)	Crow segmentation		
	Chicken-like (unsegmented)	Indeterminate (segmented)	Quail-like (segmented)
1 (13)	13	0	0
2 (2)	2	0	0
3 (2)	0	1	1
4 (3)	0	3†	2†

*As given in Fig. 5. †Two of the birds on which this operation was performed gave both quail-like and indeterminate crows.

types of cell movement in the morphogenesis of the avian prosencephalon, depending on the region considered. A segregated pattern implying radial migration is seen in the hippocampus-parahippocampus and choroid plexus. In contrast, the mixed pattern found in most of the prosencephalon demonstrates tangential cell movements, which studies based on tritiated thymidine labeling are unable to detect. The way in which these nonradial cell movements are guided remains to be elucidated.

Hatched quail donor–chicken host brain chimeras were observed and filmed in a variety of behavioral situations, after which they were killed (6) and their brains dissected. The gross anatomy of chimeric brains was normal, except for a size difference between chick and quail regions that was preserved in the grafts. Chimeric animals with brain transplants of varying type (Fig. 5) were able to stand, walk about, visually track and peck at objects, vocalize, peck at and ingest food and water, respond to auditory stimuli, explore their surroundings, and interact with other animals. We selected for closer study one behavioral system, the juvenile form of the species-typical crowing vocalization, induced during the first week after hatching by subcutaneous implants of the steroid hormone testosterone propionate (8–10). This behavior is exhibited by both sexes and shows a clear-cut species difference that does not depend on imitative learning (11, 12).

The crowing sounds of chickens in the first 10 days after hatching do not resemble the familiar “cock-a-doodle-doo” of adult animals but instead consist of a single loud “squeak” of about 0.5-s duration. Both the duration and the fine-structural morphology of this single squeak can vary during the first week, but the squeak becomes more stable during the second week after hatching (9, 12). The crowing of young Japanese quail consists of one or, more commonly, two specialized short “introductory notes” followed by a longer amplitude- and frequency-modulated trill (Fig. 5). The structure of the juvenile crowing sounds given 3 to 4

days after hatching is similar to the structure of the adult crowing sound (10, 12).

We have examined the temporal organization of the one-part (nonsegmented) chicken forms as compared to the two- or three-part quail form (one or two short introductory notes followed by a longer segment) (13). Twenty quail donor–chicken host chimeras from four different types of operations were studied after hormone implantation (Fig. 5 and Table 1). Of these, 15 animals gave unsegmented crowing sounds similar to those of normal chickens, thus showing that neither our surgical procedures nor the presence of quail cells in the brain per se affects this aspect of crowing. In contrast, all five animals with operations that included transplantation of the mesen-

cephalic and diencephalic primordium gave segmented crowing sounds, a characteristic not observed in chickens. Three of these animals gave crowing sounds that approximated a quail temporal pattern. We believe that this constitutes the first demonstration of cross-species behavioral transfer brought about by neuronal transplantation. These results, together with the viability of transplants that involved a majority of the brain, demonstrate that donor cells are functional in the host brain.

Histological examination confirmed that all five animals that exhibited segmented crowing had brains in which the telencephalon, diencephalon, and mesencephalon or the rhombencephalon, mesencephalon, and diencephalon were entirely made up of quail

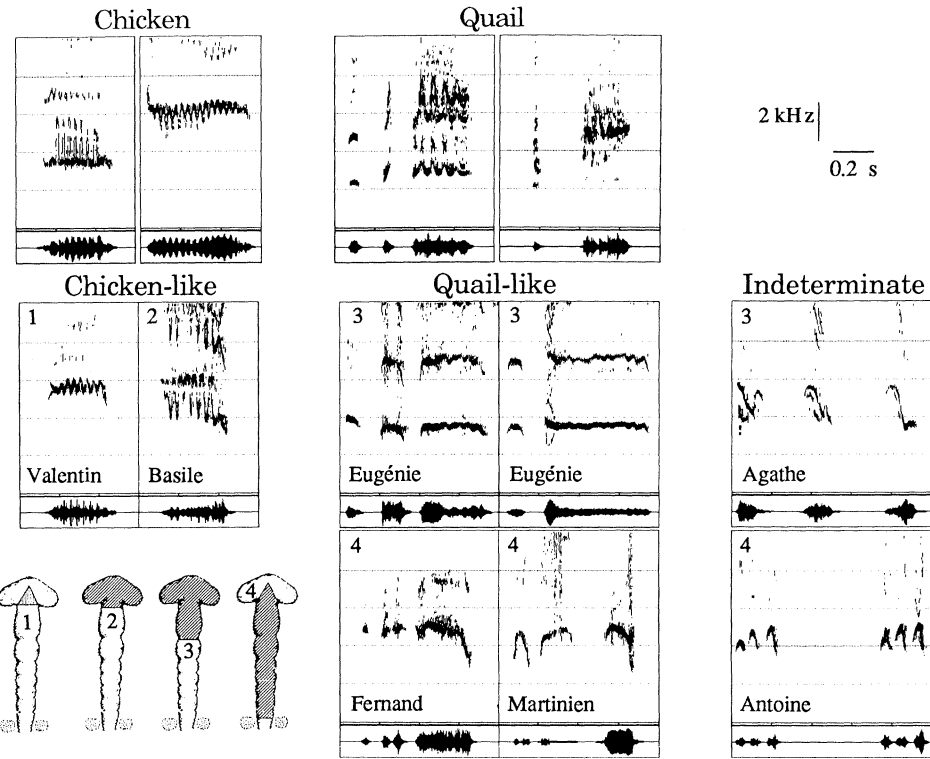


Fig. 5. Testosterone-induced juvenile crowing patterns from chickens, quails, and quail donor–chicken host brain chimeras. All crows shown were recorded between 4 and 7 days after hatching. Each box shows an amplitude-time (bottom) and frequency-time (sound spectrographic, top) representation of one crowing vocalization. Frequency and time markers are as indicated at the top right. Transplant operations are shown schematically at the lower left: 1, transplant of the dorsal neural tube primordium, giving rise to the dorsal thalamus; 2, transplant of the whole prosencephalic neural tube, giving rise to the entire telencephalon, diencephalon, and eyes; 3, transplant of the whole prosencephalic and mesencephalic neural tube, giving rise to the entire telencephalon, diencephalon, and mesencephalon including the eyes; or 4, transplant of the whole neural tube between the first somite and the caudal part of the prosencephalon, giving rise to the entire rhombencephalon, cerebellum, mesencephalon, diencephalon, and caudal portions of the telencephalon. Numbers in the upper left corner of each sonogram indicate which transplant was done. Crows from two unoperated chicks and two unoperated quail are shown on the top. Crows from chimeric animals are classified into three groups: chicken-like, two examples from two different chimeric animals that were similar in segmental structure to forms observed in normal chickens; quail-like, four examples recorded from three different chimeric animals with segmental structures that were similar to those observed in normal quail; and indeterminate, two examples of variable segmented crows from two different chimeric animals. Although these indeterminate vocalizations were segmented, their lack of quail duration and temporal patterning as well as the variability of their temporal patterning precluded their classification as quail-like; some of them are similar to rare aberrant quail crows recorded in an earlier study (10). Names in the sonograms denote the individual animals tested.

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 nonneuroectodermal 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 pentobarbital 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 propionate (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. Guyomarch, *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.
15. We thank B. Schuler for technical assistance; S. Gournet, B. Henri, and Y. Rantier for help in preparing the figures; and P. Marler for encouragement, enthusiasm, and logistical assistance. Supported by the Centre National de la Recherche Scientifique, a Basic Research Grant from March of Dimes Birth Defects Foundation, and by National Research Service Awards grant 5T32 MH15125-10 to Rockefeller University. E.B. was supported by a grant from the Fyssen Foundation.

19 April 1988; accepted 23 June 1988

Clonally Related Cortical Cells Show Several Migration Patterns

C. WALSH AND C. L. CEPKO*

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 *Escherichia 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-

C. Walsh, Department of Genetics, Harvard Medical School, Boston, MA 02115, and Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

C. L. Cepko, Department of Genetics, Harvard Medical School, Boston, MA 02115.

*To whom correspondence should be addressed.