Bird Brain Switch Leads to New Song

Two new techniques—brain grafts and labeling with retroviruses—are becoming powerful tools for probing the neural origins of behavior and tracing brain development

A TEAM OF RESEARCHERS IN FRANCE has just reported what previously seemed possible only in science fiction or in a bad pun about vice presidential election politics. They have changed the behavior of one bird species—a chick—by transplanting part of the brain of a second species—you guessed it, a quail. The embryo-to-embryo brain graft does not always work, but when it does the results can be striking. Not only do some of the recipient chicks sound a lot like quails, they also may yield new information about normal brain development.

These and other recent results from a completely different experimental system—the cerebral cortex of rat embryos—seem to challenge a long-standing notion about how the brain develops. Both papers were published in *Science* (9 September, pages 1339 and 1342). The new work suggests that after nerve cells are born next to the fluid-filled ventricles of the brain, they can disperse over wide areas before they reach their final destinations. Previous theories about brain development in mammals emphasize that many neurons migrate in a radial pattern, from the ventricles outward along columns toward the surface of the brain. The issue is not yet possible to resolve completely but it seems clear that nerve cells in the brain do not all migrate according to one universal pattern.

The idea to see if an embryonic brain graft would produce a change in behavior "was a little crazy," admits Nicole Le Douarin of the Institut d'Embrylogie Cellulaire et Moléculaire du Centre National de la Recherche Scientifique (CNRS) et du Collège de France in Nogent-sur-Marne, France. In their experiments, Le Douarin, Evan Balaban, and Marie-Aimée Teillet, also of the CNRS, graft only part of the neural tissue that ultimately gives rise to the brain. They take part of the neural epithelium from a very young quail embryo and place it into the brain of a chick embryo from which they removed the corresponding brain region, thus creating a quail-chick chimera. The researchers can distinguish cells arising from the quail graft and the chick host because their nuclei look very different.

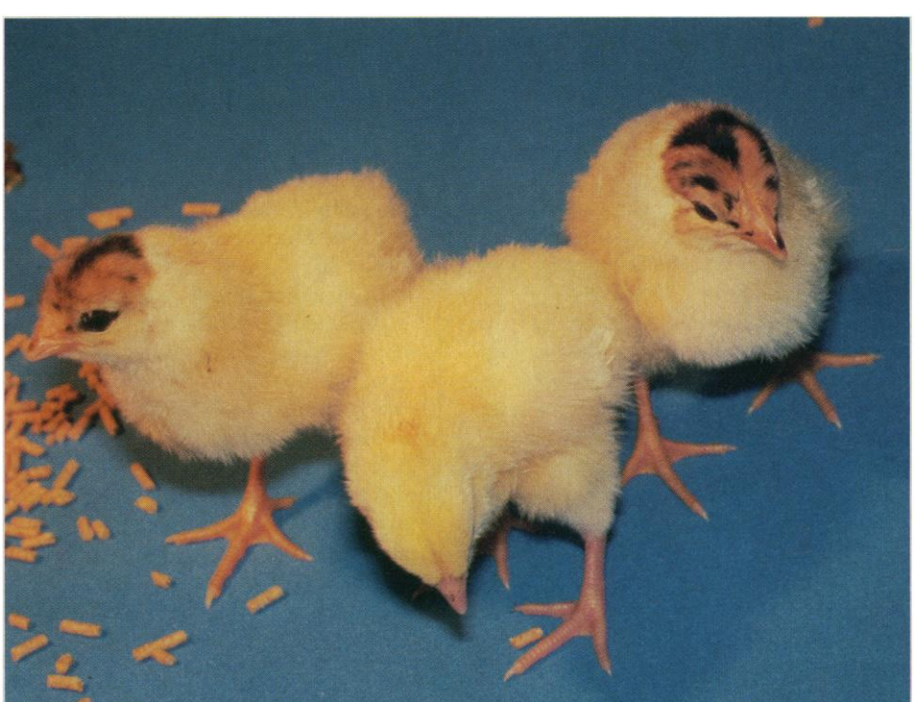
The behavioral effect of the transplant was dramatic, at least in 5 of 20 chicks that received a quail graft. In these animals the mesencephalon, the embryonic forerunner of the midbrain, seems to be most critical for changing the way the birds crow. For instance, birds named Eugénie, Fernand, and Martinien—chicks with quail grafts from the entire or rear section of the embryonic forebrain and the middle or hind regions of the rest of the brain—made quail-like sounds after they hatched. Agathe and Antoine, two other quail-chick chimeras that received similar grafts, made interrupted sounds instead of their chick-like squeak, but their sounds were not really quail-like. Normally, the French researchers note, the crows of juvenile quails consist of one or two short introductory sounds separated by time and followed by a long trill. Chicks, in contrast, typically crow with an uninterrupted squeak that lasts about one-half second.

In the experiments, each bird was artificially prodded to vocalize earlier than usual with an implant of the male sex hormone testosterone. For about a week the French researchers could study crowing behavior because the birds were healthy, but during the second week after hatching, all the birds began to immunologically reject the grafted brain tissue.

Peter Marler and Fernando Nottebohm of the Rockefeller University Field Research Center in Millbrook, New York (where Balaban was formerly a student), regard the embryonic brain transplant as an amazing technical feat. However, both would like to see whether transplanting a small, discrete area of the brain instead of the very large grafts used by the French group can produce a change in species-specific behavior.

Marler looks beyond the present experiments. "The research potential I see is to apply this transplantation technique to song birds," he says. Nottebohm has identified groups of brain cells that control learned singing in canaries, for example, but no one knows which brain structures control innate crowing in chicks or quails. "We need to separate the common mechanisms of learning from those that are unique to each species," says Marler, a question he thinks can be addressed through brain graft experiments.

A second feature of the new work is that it appears to challenge a fundamental concept in developmental neurobiology. Over the



Two quail-chick chimeras *with partial brain grafts stand on either side of an unoperated chick.* [Courtesy N. Le Douarin]

past 20 years, Pasko Rakic of Yale University School of Medicine has forged the notion that nerve cells in the mammalian cerebral cortex migrate to their final destination in a radial pattern along distinct columns. The issue is critical because the mammalian cortex is organized into vertical columns of nerve cells that all function together.

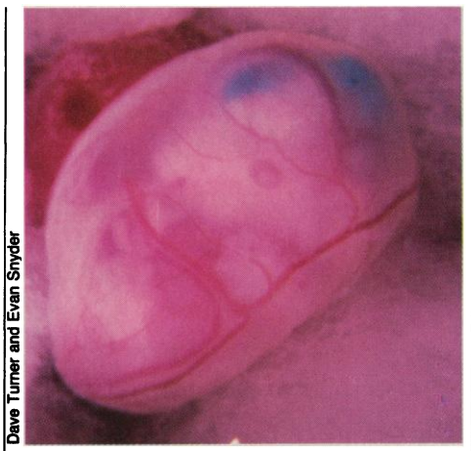
Columns in the visual cortex, for instance, are activated by a particular kind of light stimulus whereas those in the somatosensory cortex are activated by touching a particular area of skin. Rakic has linked the functional relationships of cortical neurons with their formation and migration during embryonic development. The function of the visual cortex, he says, relies in part on the presence of specific neurons that are absent from other cortical regions including the auditory cortex. Rakic hypothesizes that cortical function could be disrupted if neurons from one cortical region mistakenly migrated to the another region.

Both Le Douarin's results, which reflect cell migration in brain regions other than the cortex, and those of Constance Cepko and Christopher Walsh of Harvard Medical School indicate that many brain neurons migrate along tangential routes during development. The extent of these non-radial migration patterns in the cortex and their functional significance remain to be determined, but at least in birds they appear to be quite extensive.

Cepko and Walsh label dividing neurons in the embryonic rat brain by injecting a recombinant retrovirus into the ventricles. The virus does not reproduce or hurt the developing neurons. Instead it contains a gene that codes for beta-galactosidase from the *Escherichia coli* bacterium which allows the researchers to track cell migration.

Cepko finds that many labeled cells do not follow a strict radial alignment in rat cortex. "We were very surprised at first that we didn't get nice columns," she says. "But after thinking about it, it wasn't surprising." She postulates that non-radial migration of neurons in the cortex could be anticipated because of its irregular geometry and thickness.

Rakic sees things from a different perspective. He has emphasized that nerve cells of the adult cerebral cortex originate outside it and migrate toward the surface of the brain in an "inside out" manner after they stop dividing. Cells deepest in the brain (closest to the ventricles) are positioned first in layers 6 and 5. Cells that stop dividing later must pass by the early group to reach their final destinations in layers 2 and 1, which are closest to the brain's surface. No one disputes this overall scheme of brain development. What is being disputed, however, is the precise pattern and mechanism of neuronal migration.



Dave Turner and Evan Snyder

Mouse embryo *at 14 days, still in its yolk sac, is injected with dye into a brain ventricle (dark area) to show where the retrovirus marker is placed.* [Courtesy of C. Cepko]

Rakic claims that small clusters of progenitor cells near the ventricles constitute "proliferative units" that produce groups of clones of daughter neurons. As the precursor neurons complete their final phase of division, he writes, they "follow a radial pathway consisting of a single or multiple glial fibers" as they migrate. (Glial cells are non-neuronal cells that have many complex functions, one of which may be to guide neurons during brain development.)

Le Douarin and Cepko claim that they see many neurons migrating tangentially. But Le Douarin's results are difficult to compare directly to Rakic's data because birds do not have a neocortex like mammals. Le Douarin's studies are therefore of non-cortical brain areas—namely, the neostriatum and the hyperstriatum. Rakic says he is not surprised to find a different pattern of neuronal migration in these structures. He also notes that non-radial migration occurs in other non-cortical brain regions, particularly groups of neurons in the brainstem.

Cepko's work is, in some respects, more directly comparable to Rakic's because she also studies the development of the mammalian cerebral cortex. After reading her paper, Rakic concludes that Cepko's results are not very different from his because many of her cells do seem to be radially aligned. "In fact, if I got their results I would publish them as confirming my hypothesis," he says.

To some extent, another research group has done just that. Joshua Sanes, Grace Gray, and their colleagues at Washington University School of Medicine in St. Louis use a retrovirus labeling technique very similar to Cepko's for tracking brain cell migration in several different animals. But they draw a different conclusion than Cepko about the pattern of migration. They find, for instance, that the chick optic tectum (which processes visual information) "shows a radial organization of clonally related neurons that is absolutely striking and unequivocal," says Sanes. And in their more recent studies of the developing mouse cortex, Sanes, Alan Pearlman, and Marla Luskin, also of Washington University, see a pattern of predominantly radial migration rather than a tangential pattern.

"I suspect the difference between us [and Cepko] is a matter of emphasis," says Sanes. "Cepko stresses that in rat cortex clonally related neurons are not radially distributed. We also see some dispersion, but we emphasize the radial aspect of the pattern." Sanes acknowledges that by ignoring widely dispersed cells that carry the retrovirus marker he may be eliminating a novel pattern of neuronal migration from consideration. But he doesn't think so because he believes that the isolated cells he and Cepko both see may have come from progenitor cells that only had one round of cell division left to go when they picked up the marker. He says the isolated cells could be geographically separate from a clonally related cluster of neurons because they come from different clones, not because they migrated tangentially.

Cepko and Walsh hold their ground. "We never see the strictly radial clones that we expected to find," says Walsh. Le Douarin is philosophical. "In the neocortex of monkeys [which Rakic studies] the radial migration seems to be predominant," she says. "But it should not be believed that radial glial migration is the only way that cells are positioned in the brain." Rakic says he concurs.

Whether or not a real controversy exists is still in question. Many researchers interpret Rakic's hypotheses about brain development in much stricter terms than Rakic himself does. The problem may stem in part from some of Rakic's published diagrams that portray how neurons migrate in the cortex. Researchers must also sort out whether discrepancies in results are due to differences in the brain region under study, the age of the animal at the time of data analysis, species differences, and possible artifacts from experimental techniques.

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ADDITIONAL READING

P. Rakic, "Specification of cerebral cortical areas," *Science* **241**, 170 (1988).

M. B. Luskin, A. L. Pearlman, and J. R. Sanes, "Cell lineage in the cerebral cortex of the mouse studied in vivo and in vitro with a recombinant retrovirus," *Neuron*, in press.

A. Alvarez-Buylla and F. Nottebohm, "Migration of young neurons in adult avian brain," *Nature*, in press.

J. Price and L. Thurlow, "Cell lineage in the rat cerebral cortex: A study using retroviral-mediated, gene transfer," *Development*, in press.