# New Neurons Form in Adulthood

## *Thousands of neurons are born and thousands more die each day in the brains of birds; the same may be true in other animals, including humans*

Fernando Nottebohm and his colleagues at Rockefeller University have discovered a phenomenon so unexpected, so incredible, that they literally do not know how to explain it. Contrary to the neurobiology dogma that says no new neurons are ever formed after infancy, they find that there is a massive birth and death of neurons in the forebrains of birds and that this neuron turnover goes on well after sexual maturity. The forebrain of birds and other vertebrates controls complex learned behaviors.

Although there have been previous reports of new neurons forming in the brains of fish—whose brains grow throughout their lives—and in the brains of rodents after puberty, there has never been any account of neurogenesis that is quite so dramatic or widespread as the new finding in bird brains, Nottebohm says. He presented his data at a conference held in New York on 16 to 18 April and sponsored by the Institute for Child Development Research.

And Nottebohm is prepared to go still further, to suggest that new neurons might be generated in human adults, although there is as yet no evidence for this. "From all we know of nervous systems, we get the impression that principles of function are widespread across taxonomic phyla," he remarks. "We ought to apply ourselves to see if adult neurogenesis occurs in humans. And if it is not found, we should ask, Why not? What is preventing it? Can it be induced?" Thus, he says, even if it turns out that humans do not normally add cells to their brains, the work on bird brains may lead to ways to induce the generation of new brain cells in humans, after brain injury, for example.

Nottebohm came upon his discovery in the course of his extensive studies on birdsong, which interested him because it seemed a good system in which to study learned behavior (*Science*, 17 September 1982, p. 1125). Male canaries sing; females do not. About 1 month after hatching, the baby males start singing what Nottebohm calls, "a variable jumble of song, which Charles Darwin likened to babbling." Two or 3 weeks later they begin to sing songs that are more like adult songs. Finally, at 8 or 9 months of age, the canaries sing like adults. They sing throughout the breeding season in the spring, then they stop singing in the late summer. In the fall, they start learning a new song repertoire for the next breeding season.

"Fifteen years ago," Nottebohm says, "I set out to identify the parts of the brain responsible for song learning and then to smoke out the principles." Early on in this project, it became apparent to him that the neural pathways used for birdsong are anatomically very discrete. Neurons in a part of the bird forebrain called nucleus hyperstriatum ventralis, par caudalis, or HVc, respond to sound. They then project to another part of the brain, the robust nucleus of the archistriatum, or RA, that projects to the hypoglossal motor neurons that activate the birds' voices. "What is delightful about this system is that it is rather private for the control of a learned behavior," Nottebohm says.

When song development commences at 1 month of age, the HVc has only one-eighth of the volume it will have at maturity. Thus song learning occurs as the system that controls it grows.

Song learning is under hormonal control. If adult female canaries are given testosterone, they start to sing. If males are castrated before they learn to sing, they do not develop adult song. And males make a great deal of testosterone in the spring when they sing furiously, but their testosterone levels drop in the late summer when they are sometimes actually silent. At the same time as these hormonal changes occur, the anatomy of birds' brains changes. The HVc, which is large in the spring, becomes half as large in the fall, at which time its volume is comparable to that of a 3-month-old canary.

"I came to believe that the amount of brain space available for a skill sets limits on how much of that skill can be learned," Nottebohm recalls. If he was correct, he reasoned, and if learning space in the brain is limited, then "as more learning is required, you must add to or replace learning space." He was particularly impressed by his observation that the males who were very talented singers tended to have large HVc's and those who were less talented tended to have relatively small HVc's. The difference in size between the largest and smallest HVc's was threefold. It seemed as though the birds needed brain space to develop their singing talent to the utmost.

As further evidence that the amount of brain area devoted to singing is related to how well a bird sings, Nottebohm notes that when female canaries are given testosterone and induced to sing, the size of their nucleus RA grows by 53 percent and the dendrites grow longer and branch more so that the dendritic area increases by 49 percent. The hormone, apparently, has the potential to add circuit space. In these females, the size of the HVc doubles.

At this point, Nottebohm was led to question the conventional wisdom in neurobiology and ask whether new neurons might be formed when birds learn songs. He and his student Steven Goldman decided to study the question by using females, some of which would be injected with testosterone and others with cholesterol, a neutral treatment. The idea was to inject the birds with radioactive thymidine, which is incorporated into the DNA of dividing cells, and then see whether any new neurons are labeled. The prediction was that if any cells at all were to be labeled, they would be cells in the brains of the testosterone-treated females.

When Nottebohm and Goldman killed the birds 30 days after the sixth injection of thymidine and examined their brains, they were shocked. They saw, of course, labeled endothelial cells which line the blood vessels of the brain and are known to divide in adulthood. They also saw labeled glial cells, which are thought to play a supportive role in the brain and that also are known to form throughout life. But, in addition, they saw labeled cells that looked like neurons and they saw equal percentages of labeled neurons in the testosterone-treated and the cholesterol-treated birds. And, Nottebohm says, "The numbers were the most shocking. One and a half percent of the neurons were labeled per day of radioactive thymidine treatment. At this rate, the number of neurons in the HVc would double in 49 days."

"Where," Nottebohm asked, "did these labeled neurons come from?" By

looking at various times after the birds were given the labeled thymidine, he and Goldman established that the new neurons are born in a thin area of the forebrain, adjacent to the lateral ventrical and called the ventricular zone. There are two or more anatomically distinct types of cells in this area. It is unclear which of these cell types gives rise to the new neurons.

From the ventricular zone, the new HVc cells migrate a distance of up to 1 millimeter, which is a fairly substantial distance since the longest axis of the forebrain is 8 millimeters. It takes at least 2 weeks for the newly formed cells to migrate to their final positions and differentiate there.

But, says Nottebohm, "As Steve Goldman and I came forth with our results we were challenged again and again. 'But how do you know they are neurons?' people asked." Nottebohm and his colleague Gail Burd had ultrastructural evidence that synapses were formed on the new cells but, the critics pointed out, a synapse does not a neuron make. There is reason to believe that under some circumstances, synapses may also form on glial cells. So Nottebohm and neurophysiologist John Paton of Rockefeller University embarked on a formidable series of experiments to establish the nature of the newly formed HVc cells. "It was a heroic effort," Nottebohm recalls. But they were able to show electrophysiologically—by recording from the cells—that the labeled cells are neurons. And many of those new neurons fired in response to sound. In addition, Paton and Nottebohm showed that the new neurons are interneurons—they do not project out of the HVc. These findings apply to both male and female canaries.

The next question was, Is there cell death corresponding to the birth of new cells? The answer, Nottebohm finds, is a most emphatic yes. The number of HVc neurons in the brains of 1-year-old and 2-year-old female canaries is the same—about 15,000. Since large numbers of new neurons are created each day, these new neurons must be replacing old ones. Male canaries have about 41,000 HVc neurons in the spring but 5 months later they have only 25,000. These males, Nottebohm says, "have gone through a rather catastrophic loss of 38 percent of their HVc neurons." He can fully account for the changes that occur in the HVc volume of the brains of male canaries between the spring and the fall by neuron loss and decreases in the mean diameter of the neurons.

Although it may seem odd that the females, who do not sing, would gain and lose neurons in the same way as males, Nottebohm speculates that the females might need new memory circuits to update their memories of the songs they heard, and that part of this circuitry is in the HVc. Female canaries might lose some HVc neurons because they have no need to remember the male songs from 1 year to the next. Nottebohm has preliminary data suggesting that, at times at least, the rate of neurogenesis is greater in females than in males and he explains this result by saying that it might be more important for females to remember new songs than for males to learn to sing them.

In addition, Nottebohm finds that the HVc is not the only area of the forebrain nor are canaries the only birds to acquire new neurons. When he recently studied

Fernando Nottebohm

*New neurons grow in a canary's brain.*

the generation of new neurons in the brain of a female canary, he saw 2020 newly formed neurons in the forebrain after 2 weeks of daily injections with labeled thymidine. These neurons were in 13 different forebrain areas. He also saw six new neurons in other parts of the brain, such as the cerebellum, midbrain, and medulla. He estimates that canaries may make as many as 20,000 new neurons a day. And since the weight of the adult canaries' brains does not become greater after these birds reach sexual maturity, the birds must be constantly losing neurons as they gain new ones. Nottebohm and his colleagues also looked for and found new neurons in the brains of budgerigars and ringdoves.

Having found the astonishing phenomenon of constant turnover of neurons, Nottebohm is now absorbed with trying to understand how it takes place and why. He is first testing the hypothesis that sex hormones might control the effect, since testosterone and estrogen play such important roles in the development of birdsong. He speculates that if sex hormones are crucial to the recruitment of new neurons, the effect might occur in humans even though humans are not seasonal breeders like birds and the majority of mammals.

In humans, Nottebohm remarks, "there is a marked change in plasma levels of sex hormones during development and especially at puberty. It is widely acknowledged that early brain damage is followed by a better prognosis than late brain damage. The reasons for this are not well understood." But, he speculates, it may be that if neurologists induce what he calls "a prepubertal hormone milieu" in persons with brain injuries, they may stimulate the growth of new neurons and neural connections and an enhanced recovery of brain function. Such experiments, of course, must await at least the finding that sex hormones control the generation of neurons in animals.

Nottebohm says that his working hypothesis to explain why neurons are continually dying and being born is that the amount of information processed by neural systems is constrained by the complexity of their circuitry. "It might be useful to replace components, even at the risk of doing away with existing memories," he says. "Used circuits may have lost their ability to acquire new information."

Of course, Nottebohm admits, all this talk about used circuits sounds "quasi-metaphorical." He emphasizes that a molecular explanation of what may be going on is not available yet, but he speculates that if use changes some synapses permanently, the genome may be involved and its expression modified too, perhaps permanently. If so, the only way of restoring to a circuit the full flexibility it needs for learning may be to replace its one component that has been most modified by previous experience. "You might say that I am making a crazy leap but it is meant to be a leap," he remarks. "I don't want to be quoted as a fool who doesn't realize the enormity of what he is saying. But I think the hypothesis is testable."

But for now, the very idea that neurons in adults may turn over constantly, at least in the forebrain, the seat of complex behavior and learning, is so new that any speculations of what might come out of it sound incredibly premature. Says Nottebohm, "It is so contrary to anything we anticipated that we are not yet prepared to sound intelligent when we talk about it."—GINA KOLATA