

New Nerve Cells for the Adult Brain

Contrary to dogma, the human brain does produce new nerve cells in adulthood. Can our newfound capacity lead to better treatments for neurological diseases?

by Gerd Kempermann and Fred H. Gage

Cut your skin, and the wound closes within days. Break a leg, and the fracture will usually mend if the bone is set correctly. Indeed, almost all human tissues can repair themselves to some extent throughout life. Remarkable “stem” cells account for much of this activity. These versatile cells resemble those of a developing embryo in their ability to multiply almost endlessly and to generate not only carbon copies of themselves but also many different kinds of cells. The versions in bone marrow offer a dramatic example. They can give rise to all the cells in the blood: red ones, platelets and a panoply of white types. Other stem cells yield the various constituents of the skin, the liver or the intestinal lining.

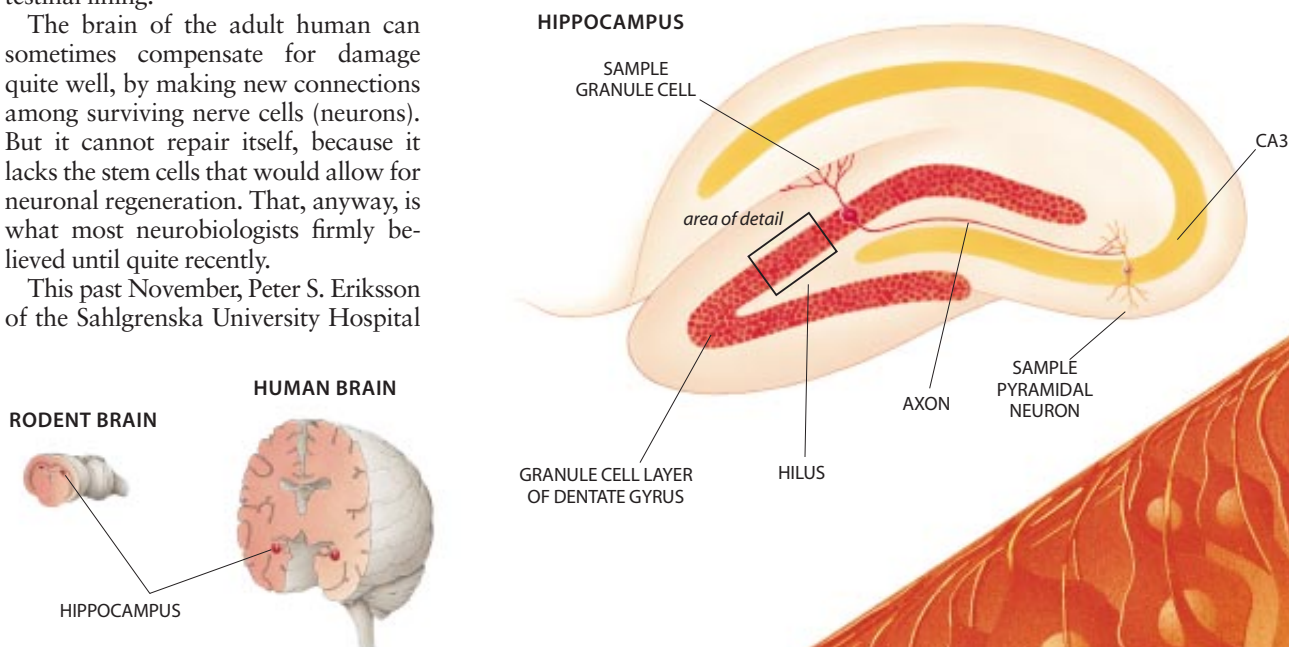
The brain of the adult human can sometimes compensate for damage quite well, by making new connections among surviving nerve cells (neurons). But it cannot repair itself, because it lacks the stem cells that would allow for neuronal regeneration. That, anyway, is what most neurobiologists firmly believed until quite recently.

This past November, Peter S. Eriksson of the Sahlgrenska University Hospital

in Göteborg, Sweden, one of us (Gage) at the Salk Institute for Biological Studies in La Jolla, Calif., and several colleagues published the startling news that the mature human brain does spawn neurons routinely in at least one site—the hippocampus, an area important to memory and learning. (The hippocampus is not where memories are stored, but it helps to form them after receiving input from other brain regions. People with hippocampal damage have difficulty acquiring knowledge yet can recall information learned before their injury.)

The absolute number of new cells is low relative to the total number in the brain. Nevertheless, considered with recent findings in animals, the November

discovery raises some tantalizing prospects for medicine. Current data suggest that stem cells probably make new neurons in another part of the human brain and also reside, albeit dormant, in additional locations. Hence, the adult brain, which repairs itself so poorly, might actually harbor great potential for neuronal regeneration. If investigators can learn how to induce existing stem cells to produce useful numbers of functional nerve cells in chosen parts of the brain, that advance could make it possible to ease any number of disorders involving neuronal damage and death—among them Alzheimer’s disease, Parkinson’s disease and disabilities that accompany stroke and trauma.



Although the finding that the mature human brain can generate neurons was surprising, hints had actually appeared for years in studies of other adult mammals. As long ago as 1965, for instance, Joseph Altman and Gopal D. Das of the Massachusetts Institute of Technology had described neuronal production (neurogenesis) in the hippocampus of adult rats—in the precise hippocampal area, known as the dentate gyrus, where it has now been found in human beings.

Early Hints ... and Doubts

Other studies subsequently confirmed Altman and Das's report, but most researchers did not view the data as evidence of significant neurogenesis in adult mammals or as an indication that even the human brain might have some regenerative potential. One reason was that the methods then available could not estimate accurately the number of neurons being born nor prove definitively that the new cells were neurons. Further, the concept of brain stem cells had not yet been introduced. Researchers therefore thought that for new nerve cells to appear, fully mature versions would have to replicate—an unbelievably difficult feat. Scientists also underestimated the relevance of the findings to the human brain in part because no one had yet uncovered clear evidence of neurogenesis in monkeys or apes, which are primates and thus are closer to humans geneti-

cally and physiologically than are other mammals.

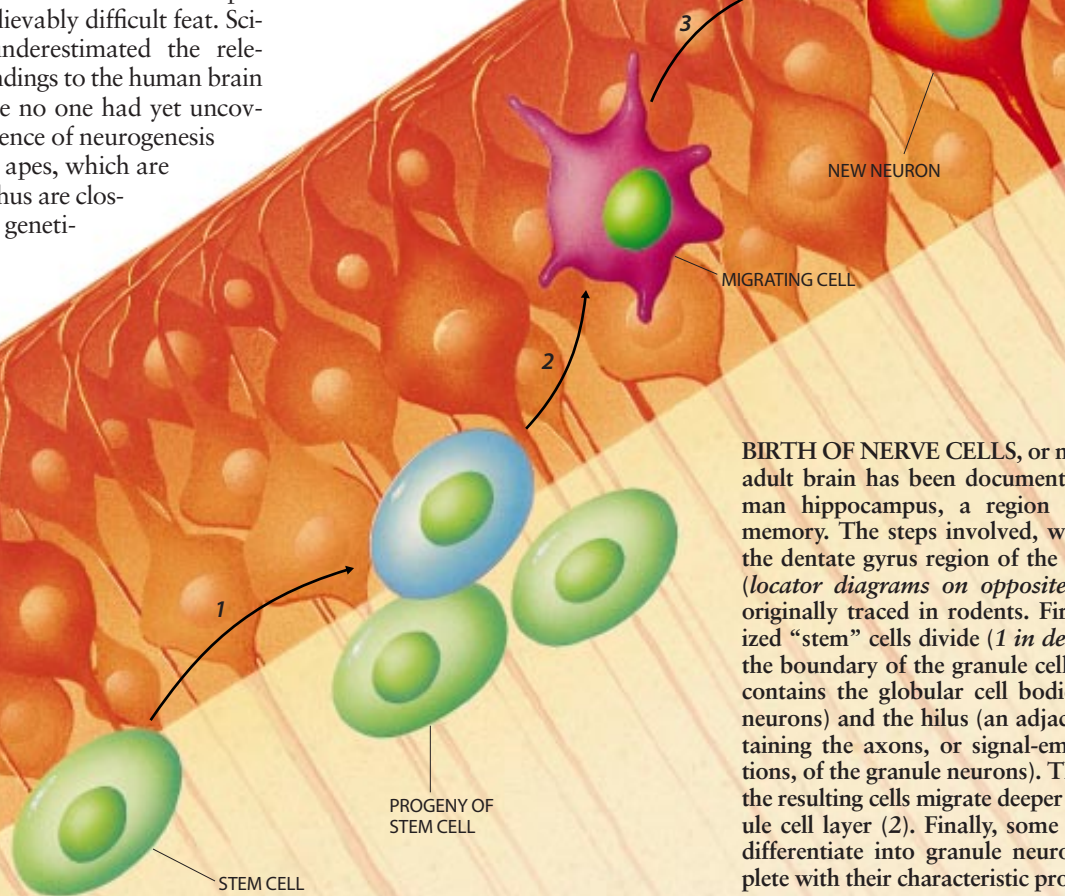
There matters stood until the mid-1980s, when Fernando Nottebohm of the Rockefeller University jarred the field with astonishing results in adult canaries. He discovered that neurogenesis occurred in brain centers responsible for song learning and, moreover, that the process accelerated during the seasons in which the adult birds acquired their songs. Nottebohm and his co-workers also showed that neuron formation in the hippocampus of adult chickadees rose during seasons that placed high demands on the birds' memory system, particularly when the animals had to keep track of increasingly dispersed food storage sites. Nottebohm's dramatic results led to a reawakening of interest in neurogenesis in adult mammals and of course caused investigators to ponder once more whether the mature human brain had any regenerative potential.

Optimism about the possibility of human neurogenesis was short-lived, however. At about the same time, Pasko Rakic and his associ-

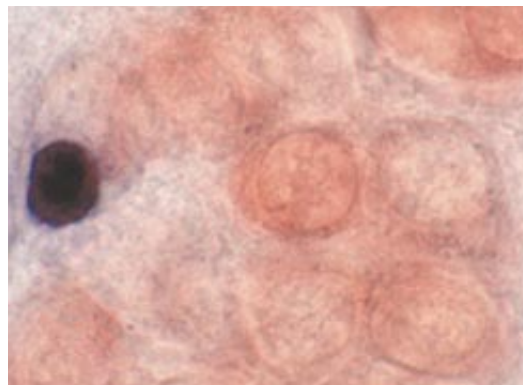
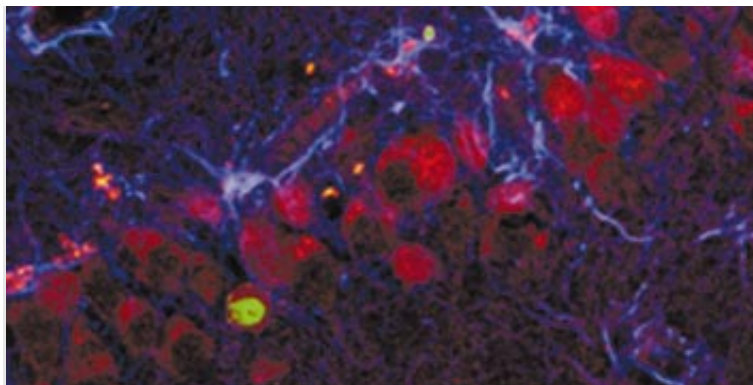
ates at Yale University pioneered the study of neurogenesis in adult primates. That work, which was well done for its time, failed to find new brain neurons in grown rhesus monkeys.

Logic, too, continued to argue against neuronal birth in the adult human brain. Biologists knew that the extent of neurogenesis had become increasingly restricted throughout evolution, as the brain became more complex. Whereas lizards and other lower animals enjoy massive neuronal regeneration when their brains are damaged, mammals lack that robust response. It seemed reasonable to assume that the addition of neurons to the intricately wired human brain would threaten the orderly flow of signals along established pathways.

TOMO NABASHIMA



BIRTH OF NERVE CELLS, or neurons, in the adult brain has been documented in the human hippocampus, a region important in memory. The steps involved, which occur in the dentate gyrus region of the hippocampus (*locator diagrams on opposite page*), were originally traced in rodents. First, unspecialized "stem" cells divide (*1 in detail above*) at the boundary of the granule cell layer (which contains the globular cell bodies of granule neurons) and the hilus (an adjacent area containing the axons, or signal-emitting projections, of the granule neurons). Then certain of the resulting cells migrate deeper into the granule cell layer (*2*). Finally, some of those cells differentiate into granule neurons (*3*), complete with their characteristic projections.



PHOTOGRAPHS BY LINDA KITABAYASHI Salk Institute

PROOF OF NEURON FORMATION in the mature human brain includes these micrographs of hippocampal tissue from adults who died of cancer. The images, derived through different methods, mark neurons in red. The green in a neuron in the left image and the dark shading of a neuron in the right image reveal that the cells' chromosomes harbor a substance—bro-

modeoxyuridine (BrdU)—that was injected into the patients to assess tumor growth. BrdU becomes integrated into the DNA of dividing cells (such as stem cells) but is not retained by already established neurons. Its presence therefore signals that the marked cells differentiated into neurons only after the BrdU was delivered, late in the patients' lives.

Signs that this reasoning might be flawed emerged only a few years ago. First, a team headed by Elizabeth Gould and Bruce S. McEwen of Rockefeller and Eberhard Fuchs of the German Primate Center in Göttingen revealed in 1997 that some neurogenesis occurs in the hippocampus of the primateline tree shrew. Then, in March 1998, they found the same phenomenon in the marmoset. Marmoset monkeys are evolutionarily more distant from humans than rhesus monkeys are, but they are nonetheless primates.

Cancer Patients Showed the Way

Clearly, the question of whether humans possess a capacity for neurogenesis in adulthood could be resolved only by studying people directly. Yet such studies seemed impossible, because the methods applied to demonstrate new neuron formation in animals did not appear to be transferable to people.

Those techniques vary but usually take advantage of the fact that before cells divide, they duplicate their chromosomes, which enables each daughter cell to receive a full set. In the animal experiments, investigators typically inject subjects with a traceable material (a "marker") that will become integrated only into the DNA of cells preparing to divide. That marker becomes a part of the DNA in the resulting daughter cells and is then inherited by the daughters' daughters and by future descendants of the original dividing cells.

After a while, some of the marked cells differentiate—that is, they specialize, becoming specific kinds of neurons

or glia (the other main class of cells in the brain). Having allowed time for differentiation to occur, workers remove the brain and cut it into thin sections. The sections are stained for the presence of neurons and glia and are viewed under a microscope. Cells that retain the marker (a sign of their derivation from the original dividing cells) and also have the anatomic and chemical characteristics of neurons can be assumed to have differentiated into nerve cells after the marker was introduced into the body. Fully differentiated neurons do not divide and cannot integrate the marker; they therefore show no signs of it.

Living humans obviously cannot be examined in this way. That obstacle seemed insurmountable until Eriksson hit on a solution soon after completing a sabbatical with our group at Salk. A clinician, he one day found himself on call with a cancer specialist. As the two chatted, Eriksson learned that the substance we had been using as our marker for dividing cells in animals—bromodeoxyuridine (BrdU)—was coincidentally being given to some terminally ill patients with cancer of the tongue or larynx. These patients were part of a study that injected the compound to monitor tumor growth.

Eriksson realized that if he could obtain the hippocampus of study participants who eventually died, analyses conducted at Salk could identify the neurons and see whether any of them displayed the DNA marker. The presence of BrdU would mean the affected neurons had formed after that substance was delivered. In other words, the study could prove that neurogenesis

had occurred, presumably through stem cell proliferation and differentiation, during the patients' adulthood.

Eriksson obtained the patients' consent to investigate their brains after death. Between early 1996 and February 1998, he raced to the hospital and was given brain tissue from five such patients, who had passed away between the ages of 57 and 72. As hoped, all five brains displayed new neurons—specifically those known as granule cells—in the dentate gyrus. These patients donated their brains to this cause, and we owe this proof of adult human neurogenesis to their generosity. (Coincidentally, at about the time this study was published, Gould's and Rakic's groups both reported that nerve cell production does take place in the hippocampus of adult rhesus monkeys.)

Do the New Neurons Work?

Of course, the mere demonstration of human neurogenesis is not enough. If the ultimate goal is to stimulate controlled neuronal regeneration in ailing human brains, scientists will want to determine the locations of stem cells capable of evolving into neurons. They will also need to be sure that neurons derived from such cells will be functional and able to send and receive messages appropriately. Fortunately, the discovery that neurogenesis in the rodent hippocampus does, after all, mirror activity in the human brain means that investigators can return to studies in rats and mice to seek clues.

Past work in rodents has revealed that some neurogenesis occurs through-

out life not only in the hippocampus but in the brain's olfactory system. Stem cells also reside in such brain regions as the septum (involved in emotion and learning) and the striatum (involved in fine-tuning motor activity) and in the spinal cord. The cells outside the hippocampus and olfactory system do not appear to produce new neurons under normal conditions, though.

If the front part of the animal's brain were transparent, the dentate gyrus portion of the hippocampus would be seen partly as a thin, dark layer, roughly the shape of a sideways V. This V consists of the cell bodies of granule neurons—the globular parts that contain the nucleus. An adjacent layer inside the V is called the hilus. It is composed primarily of the axons, or long signal-carrying projections, through which granule cells relay signals to a hippocampal relay station known as CA3.

The stem cells that give rise to newly born granule cells sit at the boundary of the dentate gyrus and the hilus. These cells divide continuously. Many of the progeny are exactly like their parents, and a good number apparently die soon after being produced. But some migrate deeper into the granule cell layer and assume the appearance of the surrounding granule cells, complete with multiple projections for receiving and sending signals. They also extend their axons along the same tracts used by their already established neighbors.

The stem cells that yield new neurons in the olfactory system line the walls of fluid-filled brain cavities known as lateral ventricles. Arturo Alvarez-Buylla of Rockefeller and his co-workers have demonstrated that certain descendants of these stem cells migrate a good distance into the olfactory bulb, where they take on the characteristic features of neurons in that area.

Given that the new neurons in both brain regions look like their earlier-born counterparts, chances are good that they behave like those neurons. But how might this surmise be proved? Studies analyzing the effects of environment on brain anatomy and learning have been instructive.

In the early 1960s Mark R. Rosenzweig and his colleagues at the University of California at Berkeley removed rodents from their standard, rather spartan laboratory conditions and put them into an enriched environment, where they luxuriated in very large cages and shared the company of many other rodents.

They could also explore their surroundings (which were continually changed by the caretakers), take spins in running wheels and play with a variety of toys.

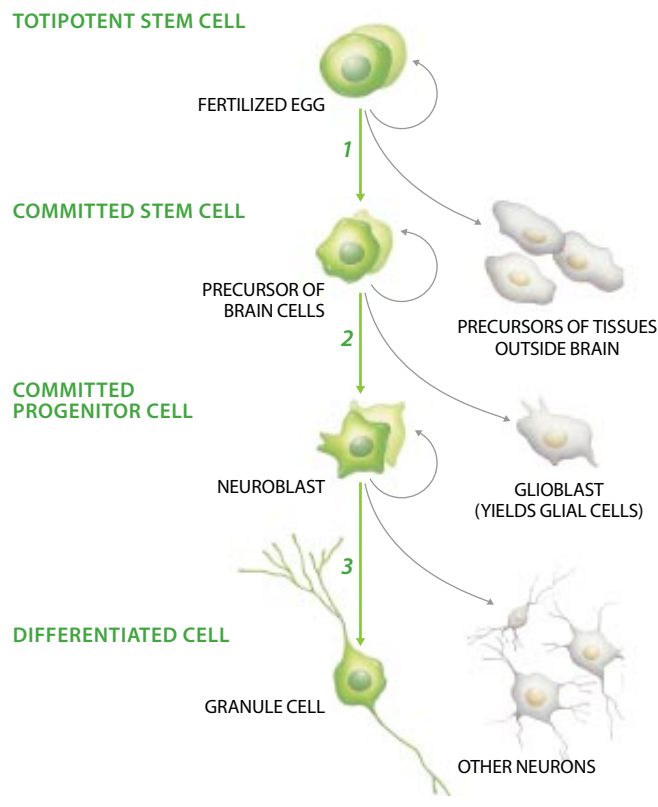
Rosenzweig's group and later that of William T. Greenough of the University of Illinois described amazing consequences of living under such improved conditions. Relative to animals kept in standard cages, those enjoying the high life ended up with slightly heavier brains, greater thickness in certain brain structures, differences in the levels of some neurotransmitters (the molecules that carry stimulatory or inhibitory messages from one neuron to another), more connections between nerve cells and increased branching of neuronal projections. Moreover, they performed better on learning tests; for instance, they were more successful at learning to navigate mazes.

Together the various results implied that the environmental changes had led to improved brain function. Since then, neurobiologists have become convinced

that enriching the environment of mature rodents influences brain wiring in ways that enhance brainpower. For years, however, they dismissed the notion that the production of new nerve cells in the adult brain could contribute to such improvements, even though Altman suggested as early as 1964 that such a process should be considered.

New findings have now confirmed that environmental manipulations do affect adult neurogenesis. Applying technology not available in the 1960s, our group demonstrated in 1997 that adult mice given enriched living conditions grew 60 percent more new granule cells in the dentate gyrus than did genetically identical control animals. They also did better on a learning task that involved finding their way out of a pool of water. Enrichment even enhanced neurogenesis and learning performance in very old mice, which have a base rate of neuronal production much lower than that in younger adults.

We do not claim that the new neurons



GRANULE CELL DEVELOPMENT in an embryo is thought to occur through the steps shown in green. A totipotent stem cell, able to give rise to any cell in the body, produces early descendants that include still unspecialized stem cells committed to producing cells of the brain (1). These committed cells later yield “progenitor” cells destined to make only neurons (2) or only glial cells (which promote neuronal survival). Ultimately, neuronal progenitors spawn granule cells in the hippocampus (3) or other kinds of neurons elsewhere in the brain. Steps 2 and 3 now appear to recur throughout life in the human hippocampus.

are solely responsible for the behavioral improvements, because changes in wiring configurations and in the chemical microenvironment in the involved brain areas surely play an important part. On the other hand, it would be very surprising if such a dramatic jump in neuron formation, as well as the preservation of adult neurogenesis throughout evolution, served no function.

Hunt for Controls

If, as we suspect, the neurons born routinely in the brain of the adult human are functional, then an understanding of the controls on their formation could eventually teach neurobiologists how to prompt such neuronal generation where it is needed. Aside from environmental enrichment, various other factors that influence neurogenesis have been identified in animal studies over the past several years.

These results will make the most sense if readers recall that neurogenesis has many steps—from stem cell proliferation, to selected survival of some progeny, to migration and differentiation. It turns out that factors influencing one step along the way may not affect others. An increase in stem cell proliferation can yield a net rise in new neurons if the rates of daughter cell survival and differentiation remain constant, but the neuronal number may not rise if the survival and differentiation rates change in opposite directions. Similarly, neurons will be added if proliferation stays constant but survival and differentiation increase.

Among the regulatory influences that have been uncovered are some that usually seem to discourage neurogenesis. In the past few years, for example, Gould and McEwen have reported that certain everyday inputs into the dentate gyrus may actually keep a lid on nerve cell production. Specifically, neurotransmitters that stimulate granule cells to fire will also inhibit stem cell proliferation in the hippocampus. High levels of glucocorticoid hormones in the blood inhibit adult neurogenesis as well.

Given these findings, it is perhaps no surprise that the team has shown stress to reduce stem cell proliferation in the same region. Stress leads to the release of excitatory neurotransmitters in the brain and to the secretion of glucocorticoid hormones from the adrenals. Understanding inhibition is important for learning how to overcome it. But that aspect of the picture is still far from clear. For instance,



PHOTOGRAPHS BY JAMES ARONOVSKY

the discovery that extreme levels of excitatory transmitters and of certain hormones can constrain neurogenesis does not necessarily mean that lower levels are detrimental; in fact, they may be helpful.

As for factors that promote hippocampal neurogenesis, we and others have been trying to identify which features of an enriched environment have the strongest effect. With her associates, Gould, now at Princeton University, has shown recently that participation in a learning task, even in the absence of enriched living, enhances the survival of the cells generated by stem cell division, resulting in a net elevation in the number of new neurons.

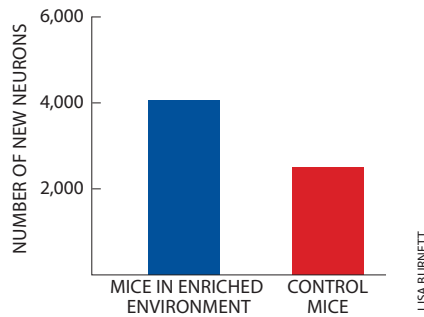
Meanwhile our group compared neurogenesis in two groups of mice kept in standard cages, one with a running wheel and one without. The mice having unlimited access to the wheels made heavy use of the opportunity and ended up with twice as many new nerve cells as their sedentary counterparts did, a figure comparable to that found in mice placed in an enriched environment. In the runners, a higher rate of stem cell division was involved in the final effect, whereas it played no role in the gains of the enriched-living group. In the latter case (as in Gould's study), stimulating conditions apparently promoted survival of stem cell progeny, so that more of those cells lived to become neurons. This finding highlights once again that the processes regulating neurogenesis in adults are complex and occur on several levels.

Certain molecules are known to influ-

ence neurogenesis. We and our co-workers have evaluated epidermal growth factor and fibroblast growth factor, which despite their names have been shown to affect nerve cell development in cell cultures. With H. Georg Kuhn of Salk and Jürgen Winkler of the University of California at San Diego, we delivered these compounds into the lateral ventricles of adult rats, where they evoked striking proliferation by the resident stem cells. Epidermal growth factor favored differentiation of the resulting cells into glia in the olfactory bulb, but fibroblast growth factor promoted neuronal production.

Interestingly, the induction of certain pathological conditions, such as epileptic seizures or stroke, in adult animals can evoke dramatic stem cell division and even neurogenesis. Whether the brain can make use of this response to replace needed neurons is not known. In the case of the seizures, aberrant connections formed by newborn neurons may be part of the problem. The stem cell division and neurogenesis are more evidence that the brain harbors potential for self-repair. The question is, why does that potential usually go unused?

In the experiments discussed so far, we and others examined regulatory events by holding genes constant: we observed the neurological responses of genetically identical (inbred) animals to different inputs. Another way to uncover controls on neurogenesis is to hold the environment constant and compare genes in strains of animals that differ innately in their rates of neuron production. Pre-



LISA BURNETT

ENRICHED LIVING ENVIRONMENT (*opposite page*) is far superior to standard laboratory conditions (*above*) for stimulating neurogenesis in the dentate gyrus of the mouse hippocampus (*graph*). Scientists are trying to determine which aspects of the richer environment exert the strongest effect. New findings comparing animals living in standard cages with and without a running wheel suggest that increased running could have an important role.

sumably, the genes that vary include those affecting the development of new nerve cells. In a similar approach, researchers can compare the genes active in brain regions that display neurogenesis and in brain regions that do not. Genetic studies are under way.

Genes serve as the blueprints for proteins, which in turn carry out the bulk of cellular activities, such as inducing cell division, migration or differentiation. Therefore, if the genes participating in neuronal generation can be identified, investigators should be able to discover their protein products and to tease out the precise contributions of the genes and their proteins to neurogenesis.

Repairing the Brain

With continued diligence, scientists may eventually be able to trace the molecular cascades that lead from a specific stimulus, be it an environmental cue or some internal event, to particular alterations in genetic activity and, in turn, to rises or falls in neurogenesis. Then they will have much of the information needed to induce neuronal regeneration at will. Such a therapeutic ap-

proach could involve administration of key regulatory molecules or other pharmacological agents, delivery of gene therapy to supply helpful molecules, transplantation of stem cells, modulation of environmental or cognitive stimuli, alterations in physical activity, or some combination of these factors.

Compilation of such techniques could take decades. Once collected, though, they might be applied in several ways. They might provide some level of repair, both in brain areas known to manifest some neurogenesis and in sites where stem cells exist but are normally quiescent. Doctors might also be able to stimulate stem cells to migrate into areas where they usually do not go and to mature into the specific kinds of nerve cells required by a given patient. Although the new cells would not regrow whole brain parts or restore lost memories, they could, for example, manufacture valuable amounts of dopamine (the neurotransmitter whose depletion is responsible for the symptoms of Parkinson's disease) or other substances.

Research in related areas of science will contribute to the search for these advanced therapeutic approaches. For in-

stance, several laboratories have learned to culture what are called human embryonic stem cells—highly versatile cells, derived from early embryos, that are capable of giving rise to virtually any cell type in the human body. One day it might be possible to prod these embryonic stem cells into generating offspring that are committed to becoming a selected type of neuron. Such cells might then be transplanted into damaged sites to replenish lost nerve cells [see “Embryonic Stem Cells for Medicine,” by Roger A. Pedersen; *SCIENTIFIC AMERICAN*, April].

Transplants, of course, may be rejected by a recipient's immune system. Scientists are exploring many ways around that problem. One solution could be to harvest stem cells from the brains of the affected patients themselves and to manipulate that material instead of stem cells from a donor. Researchers have already devised relatively noninvasive means of extracting such brain cells from patients.

These medical applications are admittedly goals and are nowhere close to reality at the moment. Indeed, the challenges ahead are huge. Notably, at one point or another analyses of the controls on neurogenesis and of proposed therapies for brain disorders will have to move from rodents to people. To study humans without interfering with their health, researchers will have to make use of extremely clever protocols, such as ones involving the noninvasive imaging techniques known as functional magnetic resonance imaging or positron emission tomography. Further, we will have to develop safeguards ensuring that neurons stimulated to form in the human brain (or transplanted into it) will do just what we want them to do and will not interfere with normal brain function. Nevertheless, the expected benefits of unlocking the brain's regenerative potential justify all the effort that will be required. **SA**

The Authors

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Further Reading

MORE HIPPOCAMPAL NEURONS IN ADULT MICE LIVING IN AN ENRICHED ENVIRONMENT. Gerd Kempermann, H. Georg Kuhn and Fred H. Gage in *Nature*, Vol. 386, pages 493–495; April 3, 1997.
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LEARNING ENHANCES ADULT NEUROGENESIS IN THE HIPPOCAMPAL FORMATION. Elizabeth Gould et al. in *Nature Neuroscience*, Vol. 2, No. 3, pages 260–265; March 1999.
RUNNING INCREASES CELL PROLIFERATION AND NEUROGENESIS IN THE ADULT MOUSE DENTATE GYRUS. Henriette van Praag et al. in *Nature Neuroscience*, Vol. 2, No. 3, pages 266–270; March 1999.