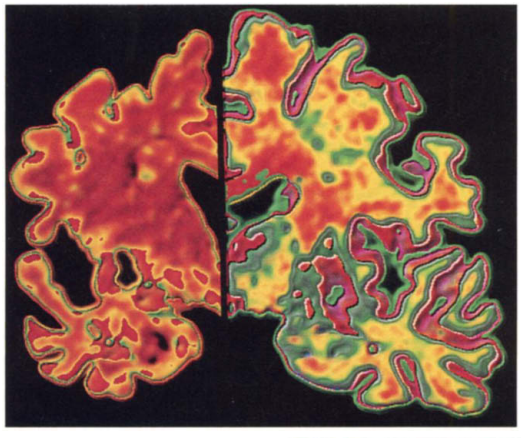
The attention given to embryonic stem cells has kindled excitement about the possibilities for using an array of cells to rebuild damaged nerve tissue

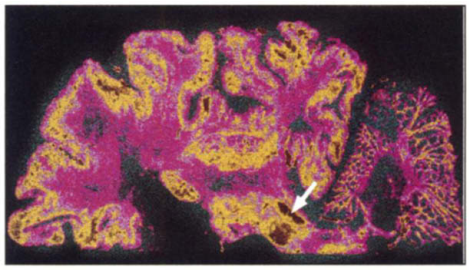
Versatile Cells Against Intractable Diseases

Virtually unknown to the public until a few years ago, "stem cells" now have a magical ring to them—thanks in large part to the vociferous debate in the U.S. Congress and elsewhere about the ethics of research using human embryos, the source of some of these cells.

If popular accounts are to be believed, these versatile cells hold cures for a variety of ailments, chief among them neurological disorders such as Parkinson's and Alzheimer's diseases. With such publicity, it's not surprising that patients are clamoring for treatments that are as yet barely conceptualized in the lab. "The expectation of my patients is that this will be ready tomorrow or in a year," says Jeffrey Rothstein, who does research on amyotrophic lateral sclerosis (ALS) at Johns Hopkins University in Baltimore. In reality, he and other scientists say, even without political obstacles, the closest treatments are years away, and some will take decades.

Nonetheless, two recent developments—the cultivation of human embryonic stem (ES) cells and the discovery of hitherto unsuspected plasticity in the human nervous system—have sparked a plethora of new investigations into the possibility of using stem or stemlike cells to treat these devastating conditions.





A question of scale. In Alzheimer's disease, damage occurs throughout the brain (*above*, left, compared to normal, *above*, right), making tissue repair a major challenge. In Parkinson's (*right*), the task appears smaller because the lesions are localized.

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Nerve networks

Until the last decade or so, scientists didn't even know that stem cells—that is, immature cells capable of differentiating into a variety of cell types—are widely distributed throughout the brain. The nervous system, unlike other tissues, has little or no capacity for self-repair; indeed, only two places in the mammalian brain (the hippocampus and the olfactory bulb) generate significant numbers of new nerve cells.

The nervous system itself is a morass of complexity, comprising a spectrum of cell types. First are the glial cells (primarily oligodendrocytes and astrocytes), which provide network support, housekeeping, and insulation functions for neurons. These are the cells affected in multiple sclerosis (MS). Then come the "transmitter-defined neurons"—that is, cells whose main job is releasing a particular brain chemical in a particular location, such as the dopamine-producing cells that die in Parkinson's disease. Then there are neurons that must link up correctly with specific target cells, such as the movement-related neurons affected in Huntington's disease. Finally come the neurons involved in highly complex functions such as problem solving or language—those often affected by dementia or stroke. These now appear to be irreplaceable. "Having cells go where they're supposed to go, connect up, and become functional ... is a bigger problem in the nervous system than anywhere else," says Mark Mattson of the National Institute of Neurological Disorders and Stroke (NINDS) in Bethesda, Maryland.

Even so, the newfound plasticity of the nervous system has inspired researchers, for the first time, to devise therapies that would replace defective neurons with newly minted cells. ES cells are the latest—and many believe the most promising but by no means the only—weapon in their armamentarium, which also includes fetal cells, adult stem cells, stem cells cultivated from fetal germ cells, cells cultivated from a type of tumor, and fetal cells from pigs and other animals. Some are true stem cells, defined as cells that can divide into both an identical daughter cell and a specialized cell. Others, variously called progenitor or precursor cells, are somewhere along the road to terminal differentiation. Their options are more circumscribed than those of pluripotent ES cells, but they can travel greater distances than mature cells, and their final identities are shaped by their surroundings.

Parkinson's disease

Because Parkinson's is in some respects a simple disease—affecting one type of dopamine-producing neuron in one place in the brain—most neuroscientists regard it as the best near-term candidate for stem cell therapy.

A decade ago, neuroscientists demonstrated that when immature dopamine-producing neurons (obtained from the brains of aborted fetuses) are implanted in the brains of Parkinson's patients, many experience long-term improvement. That means that whatever the disease process is, it does not rapidly destroy implanted tissue. But this approach poses considerable ethical and logistical obstacles: Six to eight 2-month-old fetuses are needed to treat one patient. What's more, fetal dopamine cells have a low survival rate and are not as malleable as ES cells.

Hoping to create a self-replenishing source, researchers are looking for ways to transform ES cells into dopamine producers. The promise and peril of that undertaking have been shown by Harvard researcher Ole Isacson, who this year reported transplanting mouse ES cells directly into the brains of rats with a version of Parkinson's disease (*Science*, 11 January, p. 254). The cells successfully differentiated into dopamine-producing neurons as well as other types of cells and alleviated some symptoms in more than half the animals. But these potent,

hyperactive cells also produced noncancerous "overgrowths" in 20% of the rats.

To avoid that risk, ES cells would have to be set on a clear path of differentiation before being implanted in humans. A step in that direction was reported online in *Nature* on 20 June by Ronald McKay and colleagues at NINDS. They cultivated dopamine neurons from mouse ES cells in vitro and then transplanted them into rats with Parkinson-like brain damage, showing that they could improve motor function. The next step will be to test human dopamine-producing cells in a rodent Parkinson's model, says Ted Dawson of the Parkinson's Disease Research Center at Johns Hopkins University. Then would come safety trials in monkeys. Says Isacson: "If somebody's lucky, we could be moving toward the clinic in 3 to 4 years."

Huntington's disease

Like Parkinson's, Huntington's disease entails degeneration of one kind of cell, the medium spiny neurons of a subcortical structure called the striatum. But, says Rosemary Fricker-Gates of the Brain Repair Group at Cardiff University, U.K., devising a therapy is more complicated, because it would entail not just getting new cells accepted into the brain but "reconnecting often complex circuitry." Compounding the difficulty, she explains, Huntington's is more "diffuse" than Parkinson's, with afflicted cells scattered throughout the striatum.

Although it's a long shot, researchers are attempting the same implantation strategy that holds promise for Parkinson's treatment. These attempts have had decidedly mixed results. In a pilot study several years ago, researchers at the University of South Florida, Tampa, injected striatal neurons into the brains of seven Huntington's patients, using 10 fetuses for each operation. When Isacson and his team, working with the Florida group, examined one man who died 18 months later, they found that many of the new neurons had integrated into his brain (*Proceedings of the National Academy of Sciences*, 5 December 2000)—but there was no evidence that his symptoms had been alleviated. The same month, however, a group at the French biomedical research agency INSERM reported in *The Lancet* that transplantation of fetal striatal cells into the brains of five Huntington's patients seemed to help three of them. Investigator Marc Peschanski says that now, 5 years later, improvement has persisted. Over the next 4 years, 100 Huntington's patients are expected to participate in a multicenter European trial of this procedure.

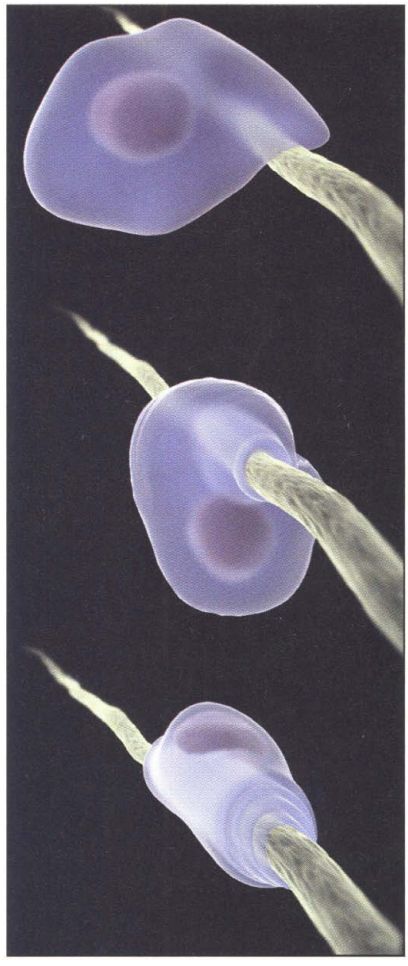
Amyotrophic lateral sclerosis

ALS, or Lou Gehrig's disease, also entails damage to one type of neuron, motor neurons. The damage occurs not just in the brain but in all layers of the spinal cord. Complicating matters, says Rothstein of Johns Hopkins, researchers don't know why cells die in ALS. It might be that neurons act "autonomously" or in reaction to malfunctions of surrounding support cells, as do astrocytes. "Sometimes the earliest changes are in astrocytes," he says. If that's true, then unless support cells were replaced as well, newly introduced neurons would be quickly poisoned.

Because ALS causes damage throughout the spinal cord, stem cell therapy would require implanting neuronal precursors at a fairly primitive stage of development, when they're able to migrate long distances. Figuring out the optimal stage is extremely tricky, says Rothstein. With completely undifferentiated cells, there's no way of knowing how to get them to turn into the right kind and produce the right numbers of cells, he says. On the other hand, using more differentiated motor neurons that are programmed for a specific location could be problematic: "If they only go to legs, that's no good [either]."

At Project ALS—a privately funded venture at Harvard, Cornell, Columbia, and Johns Hopkins universities—collaborators are trying to find out which types of cells would be best. The Johns Hopkins group is focusing on the potential of a line of pluripotent stem cells, called embryoid body–derived (EBD) cells, derived from the germ cells of aborted fetuses. Johns Hopkins researchers generated considerable excitement last year when they showed that EBD cells were able to restore some mobility to rats with paralyzed hindlimbs. Unlike ES cells, these do not appear to trigger tumor formation, says Rothstein. What's more, says Dawson, ethical problems are minimal: "You would probably only need one fetus to treat tens of thousands of patients."

At Harvard, Evan Snyder of Children's Hospital and Beth Israel Deaconess Medical Center and Robert H. Brown of Massachusetts General Hospital in Boston are comparing not only EBD cells but also fetal spinal progenitor cells, umbilical cord blood cells, human adult stem cells called fibroblasts, a line of stem cells derived from fetal brains, mouse neural stem cells, fetal pig neurons (which resemble human neurons), and even skin cells (fibroblasts). These cells, representing varying stages of development, are being injected in an ALS mouse model to see which type does best in producing or rescuing motor neurons.



Resurfacing. Stem cells might be directed to grow new myelin sheaths along neuronal axons, as pictured above, to treat nerve-destroying diseases such as multiple sclerosis.

Although no treatment is yet in sight, the Johns Hopkins researchers are already testing the safety of EBD-derived neurons in 28 African green monkeys. "We meet constantly with FDA [the Food and Drug Administration]" to be sure all the rules are being followed, says Rothstein, so researchers can move quickly into clinical trials if a promising treatment emerges.

Multiple sclerosis

Stem cells might ultimately provide some benefit, albeit limited, to MS patients. The problems are multiple. First, MS is both an autoimmune and a neurological disorder. Finding the right type of precursor cell for therapy might be relatively simple, because the disease attacks not the neurons but the tiny cells called oligodendrocytes that make the protective coating of myelin on axons, the long fibers that conduct impulses from cell bodies. Because they're homogeneous and easy to identify, Steven Goldman of Cornell University believes they will be one of the first neural cell types to be used in stem-cell therapy.

But damage sometimes extends beyond the myelin sheaths to the underlying neurons. Furthermore, "adding cells may be adding fuel to the fire, unless the underlying inflammatory process is approached," says Goldman. For this reason, congenital myelin diseases might be easier targets, he says.

An unorthodox approach to remyelination research is being taken at Yale University, where Jeffery Kocsis and colleagues are experimenting with several different kinds of

ILLUSTRATION: C. SLAYDEN

stem or stemlike cells: bone marrow stem cells, adult stem cells from tissue taken from brain surgery patients, and two types of myelin-forming cells taken from the olfactory bulb and from peripheral nerves of pigs and humans.

These peripheral cells, called Schwann cells, function like oligodendrocytes in the central nervous system. The Yale group is currently conducting a clinical trial with five MS patients to test the safety of injecting a patient's own Schwann cells directly into brain lesions. Because it's difficult to get enough cells from peripheral nerves, Kocsis hopes to cultivate myelin-producing cells from stemlike cells taken from brains or bone marrow.

Goldman says, however, that oligodendrocytes, which are native to the central nervous system, are much better at remyelination. Oliver Brüstle of the University of Bonn Medical Center in Germany and Ian Duncan of the University of Wisconsin, Madison, have shown that oligodendrocyte precursor cells made from mouse ES cells generate myelin and wrap around axons when implanted in spinal cords of myelin-deficient rats (*Science*, 30 July 1999, p. 650). The next goal, not yet achieved, is to demonstrate this result in mice using human ES cells.

Enduring symbol. Folksinger Woody Guthrie died of Huntington's disease.

Spinal cord injury

Remyelination also appears to be a very promising approach in treating spinal cord injuries in which axons lose myelin but are still intact. John McDonald of Washington University in St. Louis, Missouri, for example, is conducting the first safety trial with six patients, using neural stem cells from pigs (which can be engineered to evade the human immune response) injected directly into the spinal cord to see if they will remyelinate damaged axons.

However, although stem cells are frequently touted as a potential cure for more devastating spinal cord injuries such as that suffered by actor Christopher Reeve, "no one has a clue at this point" about how such therapy might be used to grow back a transected spinal cord, says Goldman: "There's a tremendous amount of hype in the literature, but I haven't seen a single report yet that I've found convincing."

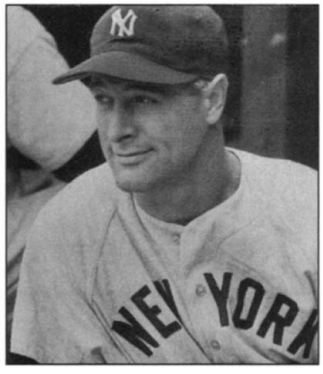
A big stumbling block is that neuronal connections have to reach from the spine up to the brain and down to the feet. Mattson of NINDS notes that spinal cord connections "are made during embryonic development, when the distances are very small. … In the adult, the distances are very large." Axons would have to grow over a meter long to extend from the spinal column to the big toe. What's more, he says, the neurons in a developing embryo are egged on by growth factors, whereas in an adult new axons would encounter mechanisms to repel and prevent growth.

Alzheimer's disease

Last summer former U.S. First Lady Nancy Reagan sent a letter to President George W. Bush pleading for ES cell research that might help patients with Alzheimer's disease. But currently, that's a very distant hope. "No one's even close to figuring out a stem-cell therapy for Alzheimer's," says Mattson.

Alzheimer's disease involves a wholesale attack on neurons throughout the brain. "The disease is so widespread that you couldn't reasonably expect to replace lost cells," says neurologist Steven DeKosky of the University of Pittsburgh. And restoring motor neurons is child's play compared to repairing those involved in higher cognitive functions. "How does a new neuron know that you went to Woodrow Wilson High School?" says DeKosky. We have "absolutely no idea how to reproduce the subtle changes in gene expression and synaptic structure that occur when a neuron encodes a memory."

As Irving Weissman of Stanford University points out, scientists still don't know at what level the pathology develops: "We don't know if it's gene products of neurons or the environment of neurons that makes the problem." So there's no way to know whether fresh cells would succumb to the disease.



Yankees hero. Lou Gehrig played 2130 consecutive games before ALS forced him out of baseball.

Future therapies

Replacing damaged neurons with specific neural progenitors, as has already been shown with Parkinson's, is just the first step, says Duncan. Later, he says, scientists hope to be able to "seed" patients' brains with neural stem cells that can supply a "resident population" of cells able to respond to ongoing disease with whatever cell types are needed.

Supplementing this vision is a yet more far-reaching goal: figuring out how to stimulate a patient's own stem cells to repair an injury or tackle a disease. Some early research suggests that scientists might be able to activate dormant stem cells. Goldman, for instance, reported last month at the meeting of the American Society for Gene Therapy in Boston that his group has been able to stimulate progenitor cells in the striatum of mice with the Huntington's disease gene by injecting them with a virus containing a gene for a neurotrophic factor. Jonas Frisén of the Karolinska Institute in Stockholm has been injuring mouse brains with a toxin specific to dopamine neurons in an effort to stimulate stem cells to increase the supply of dopamine neurons. So far, the experiment (not yet published) has resulted in the creation of 20 new dopamine neurons a day per mouse—not much, but a doubling of the usual rate, he says. Other scientists, however, are skeptical that these new neurons are actually functional. A third group, at Tokyo University, reportedly has had success in activating stem cells, leading to regeneration of tissue in the hippocampus of rats—an important area affected in Alzheimer's disease in humans.

But the brain, unlike other body parts, doesn't naturally jump in to fix itself, notes Harvard's Isacson, who predicts that endogenous brain repair is "decades from application."

Safety

Even if scientists can conquer the complexities and create replacement neurons from stem cells, huge regulatory hurdles remain. ES cells are the most daunting: Scientists must devise ways to cultivate them that don't involve contact with potentially contaminating mouse cells. Geron Corp. in Menlo Park, California, which is working to create dopamine-producing cells, says it has already developed a culture medium free of mouse cells.

More difficult will be ensuring that the cells are safe, because a single remaining undifferentiated cell could lead to tumor formation in the patient. Dawson of Johns Hopkins speculates, for example, that the FDA "may want us to engineer in a kill switch" so introduced cells could be turned off if they started misbehaving after transplantation. Then there is the problem of overcoming a patient's immune response to foreign cells, although researchers say this is not a major obstacle with the brain, which has been called "immune privileged."

In short, there's a long road ahead. Nonetheless, says Isacson, "I am still surprised at how much remodeling is going on in the brain." He is confident that someday researchers can learn how to tap into that plasticity. **–CONSTANCE HOLDEN**

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