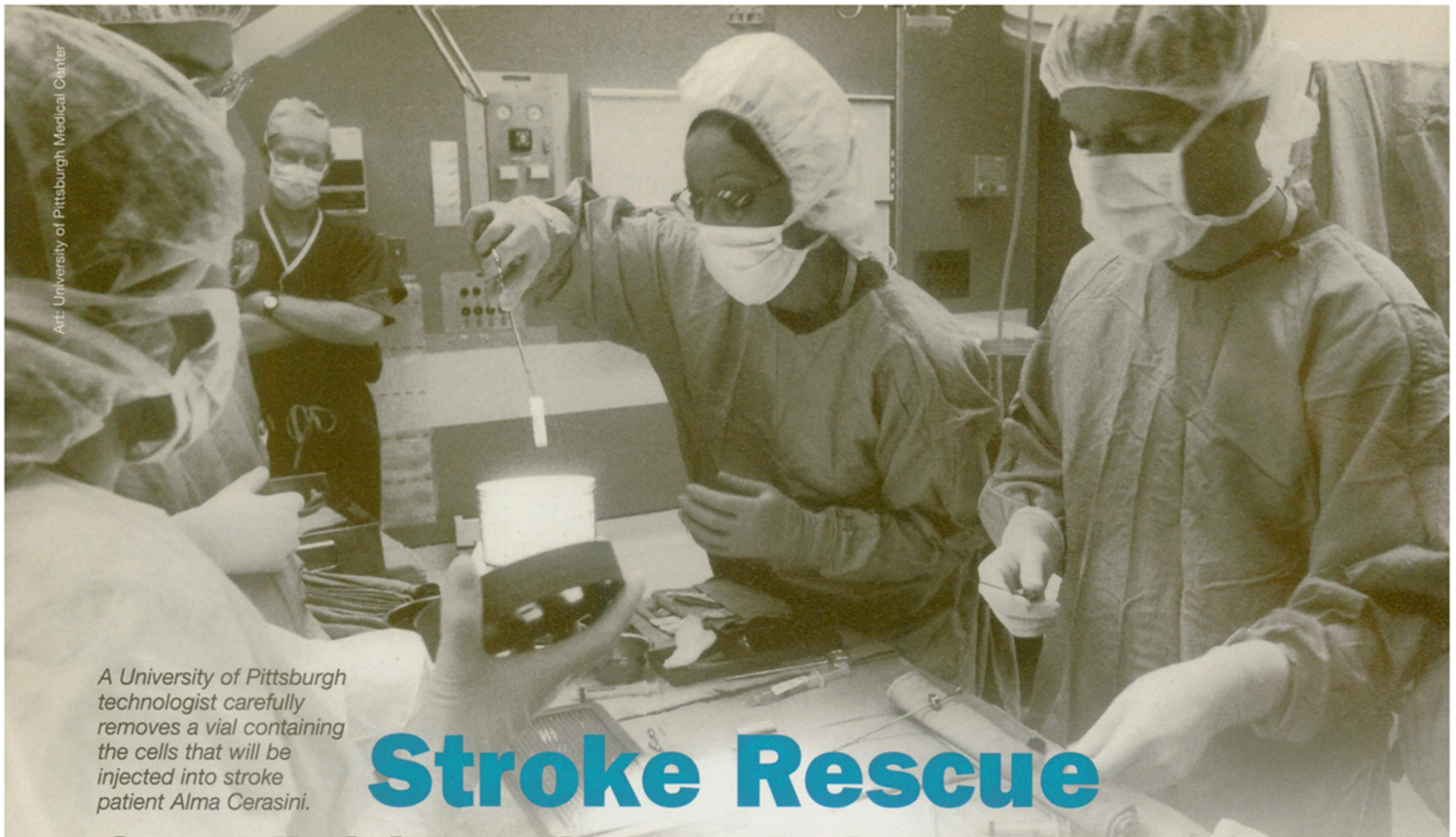


Art: University of Pittsburgh Medical Center

A University of Pittsburgh technologist carefully removes a vial containing the cells that will be injected into stroke patient Alma Cerasini.

Stroke Rescue

Can cells injected into the brain reverse paralysis?

By KATHLEEN FACKELMANN

In September 1997, Alma Cerasini suffered a stroke that left much of her right arm and leg paralyzed. In most cases, there is no cure for such disability. But the 62-year-old nurse wasn't willing to accept that dismal outlook. She and her husband decided to look for an experimental treatment of stroke.

Cerasini found her way to the office of University of Pittsburgh surgeon Douglas Kondziolka. As it happened, he and his colleagues had been searching for the perfect candidate to try out a bold new stroke therapy. Cerasini fit the bill.

On June 23, Kondziolka and his team drilled a hole into Cerasini's skull and injected 2 million laboratory-grown, immature nerve cells into the stroke-damaged region. They hope that those cells will survive, make new connections, and restore the brain power that has been lost.

Only time will tell whether the unproven therapy will benefit Cerasini or 11 other stroke victims who will follow her lead by the end of this year.

Annually in the United States, about 700,000 people experience a stroke, and nearly 160,000 people die from it. Many of those who survive suffer from paralysis and difficulties with speaking or thinking.

Typically, a blood clot clogs an artery leading to the brain. The resulting stoppage of blood flow can kill brain cells—neurons—in a localized region.

In most cases, doctors can limit or even prevent brain damage if they give the patient a clot-dissolving drug within 3 hours of the onset of a stroke. Once the injury to the brain is sustained, however, there has been no way to reverse it.

For Cerasini and the others, the experimental surgery remains a gamble.

"It's probably a reasonably safe procedure," says cell transplant researcher Barry Hoffer, who is the scientific director of the National Institute on Drug Abuse (NIDA) in Baltimore. "But we simply don't know what will happen to these cells when they're in the human brain."

The Pittsburgh team isn't the first to inject cells into a human brain. For example, Paul R. Sanberg, a neuroscientist at the University of South Florida College of Medicine in Tampa, has long been using injections of human fetal nerve cells in attempts to rescue brains from the devastation caused by Parkinson's disease. This progressive neurological disorder causes muscle tremors and a shuffling gait. Cell transplants for this disease and for Huntington's disease have shown some success (SN: 12/21&28/96, p. 399; 4/29/95, p. 262).

Sanberg began to wonder about the possibility of using fetal cells to treat victims of stroke. He knew that human fetal cells are difficult to obtain and hard to handle. Then, at a scientific meeting in 1995, Sanberg met Gary E. Snable, the president of Layton BioScience in Atherton, Calif. Snable's company had been developing a more readily available substitute for fetal cells.

The company calls the cells LBS-Neurons. They are immature neurons that have been grown from human cancer cells. The two scientists wondered if such cells, injected into a stroke-damaged brain, could take on the properties of nearby, mature neurons.

Sanberg set out to test that hypothesis. He and his colleagues began with a procedure that mimics stroke in rats. They carefully inserted tiny blood clots into a neck artery from each of several anesthetized rats. The clots blocked the blood flowing to the animals' brains.

The researchers then tested the rats for signs of brain damage. To test motor function, they lifted each rat by the tail. Healthy rats swing from side to side when picked up this way. The treated rats swung to just one side, indicating damage to the part of the brain controlling the rats' ability to move, Sanberg says.

The researchers also assessed the animals' cognitive function. In this test, a rat receives an electric shock when it steps off a platform. Control rats quickly learned to avoid the danger area. In contrast, rats

with induced strokes took longer to learn the task and the next day failed to remember their painful lesson.

Now, the team was ready to find out if the cells from Snable's company could rescue the rats.

One month after the experimental stroke procedure, the researchers anesthetized the rats again. They attached a tiny metal frame to each animal's head and drilled a small hole in the skull.

The team thawed a vial of frozen cells that had been shipped from Layton BioScience and diluted them in a solution. Then they injected 40,000 live cells into each rats' striatum, the brain region damaged by the stroke.

The rats also received a drug that suppressed their immune systems. Without such a drug, the rat immune cells might have attacked the human cells.

The treatment reversed brain damage within a month. Rats that had failed the swing test now moved from side to side. In addition, rats that had had trouble with the platform-shock trial now learned this task as quickly, and retained it as well, as the control rats.

Sanberg and his colleagues published their findings in the February EXPERIMENTAL NEUROLOGY. "We've found that these cells produce very good recovery in stroke animals," says coauthor Cesario V. Borlongan, formerly at the University of South Florida in Tampa and now at NIDA.

In further experiments, the researchers varied the number of live cells injected into damaged brains and found that rats given the largest doses improved the most and did not regress.

In contrast, rats that had received injections with low numbers of live cells showed either no recovery or a partially corrected swing one month following treatment. By the second month, that swing had deteriorated once again.

"The number of live cells that you transplant in the brain correlates with the functional recovery," Borlongan says. "It is a very good indication that the cells are producing the behavioral recovery." These additional findings appear in the August NEUROREPORT.

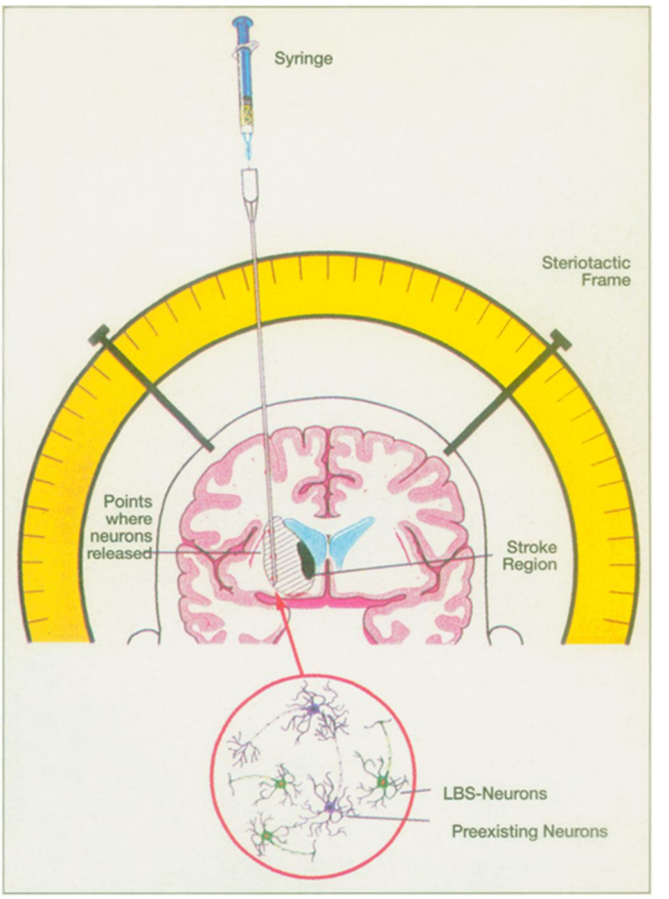
Neither study yet explains how the injected cells produce the desired outcome. Borlongan has evidence that the injected cells take on the appearance of mature cells in the surrounding area. He is planning new studies to find out whether the transplanted neurons send out fiberlike extensions, or axons, that connect with healthy neurons and regulate motor function. Establishing such connections is key to the normal development of a young brain.

"What we're hoping is that they will bridge the damaged area—connect the tissue on both sides—and act as a conduit for information," Snable says.

Researchers participating in the animal and clinical studies expect such treatments to provide renewed optimism not just for victims of stroke but also for people suffering from a variety of other conditions.

Sanberg suggests that injections of the cells from Layton BioScience might help people with Parkinson's disease, which robs the brain of neurons in a region called the substantia nigra. People with Huntington's disease also might benefit from the technique. This disorder, which causes abnormal muscle movements and dementia, destroys neurons in the striatum. Snable says that Layton BioScience plans to launch a clinical test soon to see if brain injections of its cells relieve such symptoms.

Spinal cord injury might also be treated with such cells. Injected soon after damage to the spinal cord, the cells might mature and reconnect the severed spinal cord nerves. Snable speculates that the treatment could prevent or reverse paralysis in people who would ordinarily be confined to a wheelchair.



This diagram shows how surgeons inject laboratory-grown cells (LBS-Neurons) from a syringe into a patient's brain near the stroke-damaged region. The insert shows the newly transplanted cells in place among their new neighbors.

Optimism about the future use of such cells must be tempered with a heavy dose of caution, however. The researchers have been criticized for going directly from rat studies to the trial of human stroke patients. Snable says the human test was launched because of the urgent need to reverse the disabling damage caused by strokes.

Borlongan, however, wonders if that

From tumors to human neurons

To prepare the immature neurons that were injected into Alma Cerasini's brain, scientists at Layton BioScience used a process patented by Virginia Lee and John Q. Trojanowski of the University of Pennsylvania School of Medicine in Philadelphia. In the early 1990s, Lee and Trojanowski needed nerve cells that would grow in the laboratory for developmental biology studies. They started with some unusual cells taken from a testicular tumor in a 22-year-old man. These cells still had the capacity—unlike most other human cells—to develop into a variety of different cell types.

Among descendants of these cells, Lee and Trojanowski were able to find some destined to become neurons. The promising cells, however, continued to divide instead of stopping their reproduction as normal neurons do.

The scientists realized that laboratory-grown neurons might be useful for treating people with neurological disorders. Before cells could safely be given to patients, the duo had to figure out a way to halt the out-of-control cell division.

The scientists discovered that dousing the cells with retinoic acid, a molecule derived from Vitamin A, stopped the division of tumor cells. The treatment didn't seem to rob the cells of their ability to mature into neurons.

This procedure gives researchers a source of neurons that doesn't evoke the ethical problems surrounding use of human fetal cells, says Barry Hoffer of the National Institute on Drug Abuse in Baltimore. Yet, researchers have a long way to go to demonstrate that these cells are safe and effective in the treatment of stroke or any other disease.

"The jury is still out," Hoffer says. —*K. Fackelmann*

was the best course. "My feeling is that we still could have gone through the monkey studies," he says.

Other scientists echo that concern.

"The question is, why jump ahead?" says Don M. Gash, a neurobiologist at the University of Kentucky in Lexington who focuses on neurological disorders such as Parkinson's disease.

Bypassing the less glamorous monkey studies for a clinical trial may limit the research. If something doesn't pan out in the early trials, it will be difficult to go back and start all over again, Gash says. A negative result in such a highly publicized clinical trial might lead researchers to abandon their approach. In contrast, researchers who refine the details of a treatment in animal studies can move to clinical trials with more chance of success.

Moreover, there are questions about the safety of injecting these cells, which are derived from a human cancer (see sidebar, p. 121). "The first rule of medicine is to do no harm," Gash says.

Although the preparation of these cells renders them noncancerous, no one really knows if they can later revert to their malignant state, Hoffer says. Sanberg's team, however, has never identified a cancer growing in a rat treated with the Layton BioScience cells.

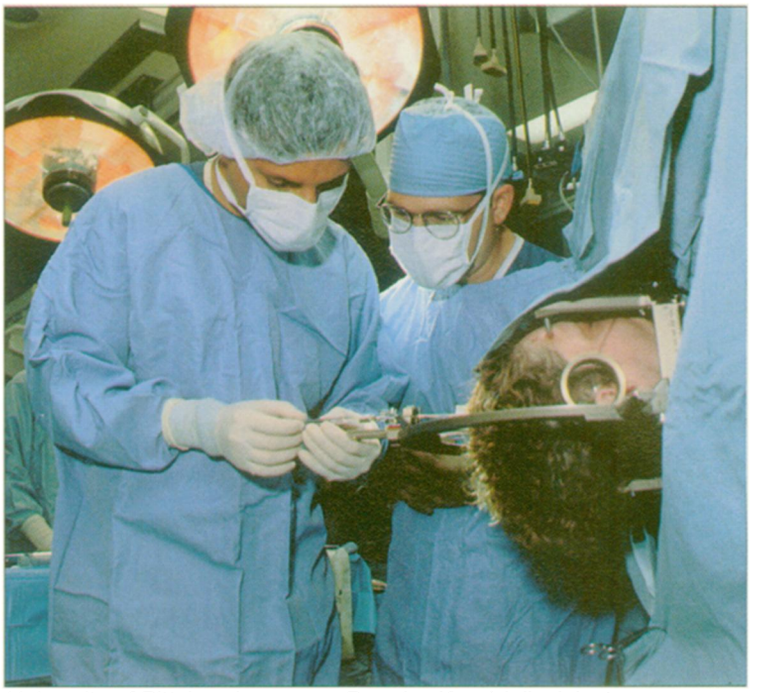
The main purpose of the clinical tests now being undertaken at the University of Pittsburgh is to assess the safety of the injections, although Snable hopes the trial will also hint whether the procedure will work to reverse stroke symptoms.

"Drilling a hole in someone's head—even though it is small—and then putting a needle into someone's brain is not trivial," Snable says. "You wouldn't do it unless you felt there was some opportunity for efficacy."

Snable, Sanberg, and others point out that because there is currently no way to reverse the devastation caused by a stroke, the potential for benefit outweighs the safety concerns.

For people who have survived a stroke, the decision to participate in such a trial must be made with care. "If someone in my family asked me [about receiving brain cell transplants], I guess I would counsel caution," Gash says.

So far, for Cerasini, the gamble has produced neither loss nor gain. She came through the operation with no bleeding or inflammation in the brain, potential side effects of the procedure. She is now waiting anxiously for a sign that the treatment will restore some of the movement she has lost. The scientific community—and hundreds of thousands of people who have had strokes—wait with her. □



University of Pittsburgh surgeon Douglas Kondziolka injects the Layton BioScience cells into Cerasini's brain.