

ON THE BRAIN

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Releasing the Brakes on Post-Injury Nerve Growth

UNLIKE PERIPHERAL NERVES that help us feel sensations and move, cells in the central nervous system (CNS) – the brain, spinal cord, retina and optic nerve – are unable to heal themselves after an injury. If you cut the tip of your finger, new nerves are able to sprout and make connections with other nerves (in a process called nerve regeneration), restoring feeling to your fingertip. With a spinal cord injury, useful function below the level of the injury is often permanently eliminated because new, healthy nerve cells are unable to grow.

For many years, scientists have been searching for ways to stimulate the growth of new nerve cells following injury to the CNS. A number of things conspire to prevent this nerve growth from occurring, however, including proteins in the nerves' protective myelin sheath and molecules released by scar tissue that forms around the site of the nerve injury.

Zhigang He, Ph.D., an assistant professor of neurology at Harvard Medical School (HMS), and his colleagues at Children's Hospital Boston recently discovered one of what may be many molecular compounds that act as brakes on the regrowth of nerves after an injury. The compound they discovered is called "epidermal growth factor receptor," or EGFR, which until now was thought to signal nerve cells to divide. Their findings indicate possible new therapies for restoring post-injury function to a damaged central nervous system.

After screening hundreds of molecular compounds to identify those capable of reversing the action of myelin proteins that prevent nerves from growing, He reported in the October 7 issue of *Science* that his team found, to their surprise, that

blocking EGFR could spur the growth of axons, the long nerve fibers that conduct electrical signals away from nerve cells.

He and his colleagues found that the protein molecules in myelin activate EGFR, possibly by rushing calcium into the cells to stimulate the receptor. The researchers say that if the protein activity of myelin can be hindered by blocking the activation of EGFR, then nerve re-growth might be possible. They also say that inhibiting the action of EGFR can lead to axon growth on scar tissue cells (called proteoglycans) at the site of the CNS injury.

To test their hypothesis, He's team worked closely with Dong Feng Chen, Ph.D., M.D., an HMS assistant professor of ophthalmology at Schepens Eye Research Institute, to determine if blocking EGFR could aid in the regrowth of the optic nerve in mice. The researchers injured the optic nerves of adult mice and soaked the injury site with a solution containing substances that prevent EGFR activity. After two weeks, the mice had a ninefold increase in the growth of nerve cells compared to untreated mice.

In studies published in 2002, He's team identified a new blocker of axon growth and found that it, along with two other already-identified blockers, function by binding to the same cell receptor. This discovery is crucial to understanding the signaling events that occur to prevent axon regeneration.

Scientists say it is encouraging that both blocking mechanisms identified by He work through a common signal. The Food and Drug Administration has already approved drugs that jam this signaling pathway for the treatment of certain cancers.

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Dr. Zhigang He, Ph.D.



Dr. Dong Feng Chen

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Reversing the Ravages of Parkinson's with Fetal Cell Transplants



Dr. Ole Isacson, M.D.

MANY PARKINSON'S disease patients face a slow, inexorable decline into profound disability. Their brains unable to produce the dopamine needed for the smooth, coordinated function of muscles and movement, they must rely on treatments centering on symptom relief. No cure yet exists for the disease.

Writing in the journal *Brain*, however, researchers at Harvard Medical School and McLean Hospital document the first two Parkinson's disease (PD) patients who received human fetal midbrain transplants as cell suspensions in the striatum and the substantia nigra, areas of the brain responsible for movement and the production of the neurotransmitter dopamine. Both patients showed progressive improvement in symptoms over a three-year period and, more important, did not develop further motor complications after the transplantation. The researchers believe their findings may help pave the way for effective Parkinson's treatments using stem cells or other therapies.

"Our study shows that following transplantation patients can have complete restoration of the cell type that dies in PD," says Ole Isacson, M.D., director of the Neuroregeneration Laboratory at McLean and a professor of neurology at HMS. He calls this a "critical finding" because it shows that these dopamine-producing neurons can survive, grow and make new connections in the brains of Parkinson's patients, without side effects.

Parkinson's disease is a disorder that occurs when neurons in the substantia nigra region of the brain die or become impaired. These cells produce dopamine, a chemical messenger that is crucial for human movement. Dopamine transmits signals to the striatum, the part of the brain best known for planning movement pathways, that initiate and control movement and balance. Dopamine helps to ensure that muscles work smoothly, under control, and without unwanted movement. When about 80 percent of these dopamine-producing neurons die or are damaged, patients begin to show the symptoms of Parkinson's disease – tremor, slowness of movement, rigidity, and balance difficulties, among others.

Scientists first attempted to transplant brain tissue from aborted fetuses into Parkinson's patients back in the late 1980s and met with mixed success. While some studies showed that the cells were able to survive and produce dopamine, which led to marked improvement in patients, other trials showed spotty patient improvement and such side effects as uncontrolled shaking.

Isacson said these earlier studies used solid tissue taken from the midbrains of aborted fetuses, while his study used a cell-suspension method, in which cells were chemically broken into smaller pieces before being implanted. He believes this method may cause fewer side effects. Isacson and his colleagues made a tiny dissection in collected midbrain tissue and injected it with enzymes to gently push cells apart to make a liquefied suspension. The study subjects underwent special CT and MRI scans so targets in the brain could be pinpointed. Then, using a very fine needle, the cell suspension was injected into the striatum of one patient and the substantia nigra of the other.

While rejection of brain tissue is a "rare event," the patients received six months of anti-rejection

Fig. 1

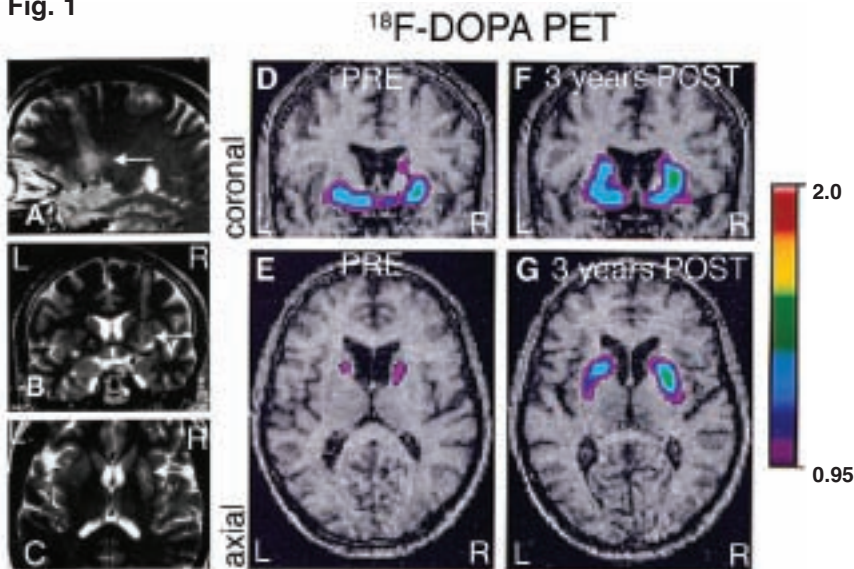


Figure 1. (A-C) MRI study performed 24 hours after the first surgery (patient 1). The four parallel needle tracks through the right putamen are visible in the axial (C) and sagittal (A) views (compare to 3D reconstruction of the grafts on Fig 2D). (D-E) Parametric maps of F-DOPA uptake (K_i) overlaid on the patient's MRI. (D-E) A pre-operative PET scan showed a marked, asymmetric decrease in putaminal ^{18}F -DOPA uptake in the first patient consistent with the diagnosis of idiopathic Parkinson's disease. (F-G) Twenty eight months post transplantation the PET show a significant increase in ^{18}F -DOPA uptake, more pronounced on the right putamen (>300% compared to pre operative values) than on the left (100% increase). (R)=right; (L)=left.

drugs. The transplanted cells integrated seamlessly with other brain tissue and without inflammation or other signs of tissue rejection.

These patients were not cured of Parkinson's disease, but they did show dramatic, progressive improvement over a three- to four-year period following the fetal cell transplants. Both patients died of other causes. Using PET scans, Isacson's analysis showed dopamine activity at the site of the transplant grafts and that dopamine-producing cells survived in the striatum and, to a lesser extent, the substantia nigra. In addition to producing functional benefits for the patients, the transplants diminished side effects such as dyskinesia, an impairment of voluntary movement that results in jerky motions.

"Our study illustrates that if synapses make new connections and patients have smoother function, then we have repaired the brain circuitry [that goes awry in PD]," says Isacson. "We are now learning the specifics of that circuitry and trying to obtain cell types that are most effective in these transplants."

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Fig. 3

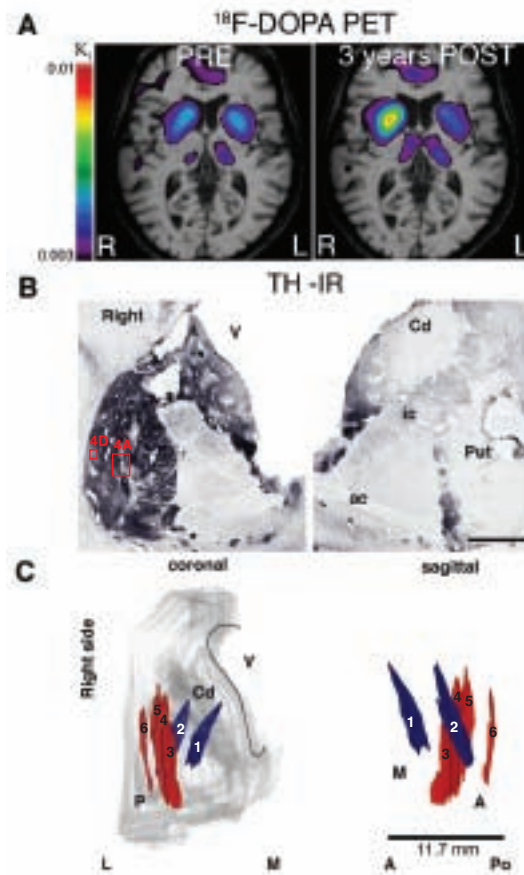


Figure 3, above. (A) Parametric maps of F-DOPA uptake (K_i) overlaid on the second patient's MRI before and 3 years post transplantation. Note the marked increase in ^{18}F -DOPA uptake on the right putamen (>200%) while the loss progressed during this time period on the left side (45% loss). (B) Macroscopic aspect of TH immunoreactivity at the level of the anterior commissure in the second patient. The right putamen was completely reinnervated by the TH neurons distributed in the six tracks (see schematic reconstruction in C) at this anatomical level. No surviving TH neurons were found in the left putamen (received only one deposit) corresponding to in vivo data; obviously partial volume effect and resolution of the PET precludes a direct quantitative comparison between the histological and imaging studies. (C) 3D reconstruction of the 6 tracks in the right putamen, numbers 1 and 2 followed a tangential direction from caudate to putamen, 3–6 are parallel (numbered anterior to posterior) to the major axis of the putamen. P: putamen; Cd: caudate nucleus; V: lateral ventricle; A: anterior; Po: posterior, M: medial; L: lateral. Scale bar in (B): 1 cm.

Fig. 2

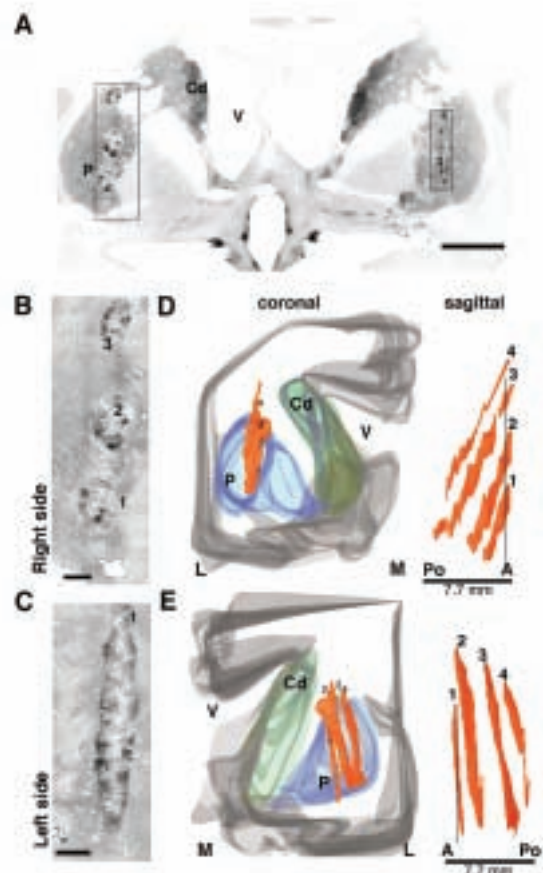


Figure 2, left. Morphological and cytoarchitectonic features of the cell suspension grafts in the putamen in patient 1. (A) Low power microphotographs of TH immunostaining of a coronal section through the anterior portion of the grafts, at the level of the post-commissural putamen. (B) On the right putamen, clusters of TH positive cells at the tip of three tracks are visible. (C) On the left hemisphere the cell infusion tracks are parallel to the section plane, so only the most anterior track is visible. TH positive cells are predominantly located at the periphery of the grafts. (D-E) The spatial orientation of the grafts is demonstrated in the computer assisted 3D reconstructions. The current view shows the location of the grafts in the post commissural putamen. The surviving cell aggregates spanned approximately 8 mm in the anterior-posterior axis in both hemispheres and the trajectories are easily identified in the sagittal view. The four parallel grafts (1-4) were numbered in an anterior-posterior order for stereological analyses. P: putamen; Cd: caudate nucleus; V: lateral ventricle; A: anterior; Po: posterior, M: medial; L: lateral, Scale bars in (A): 1 cm (B,C): 1 mm.

Practice – Plus Sleep – Makes Perfect

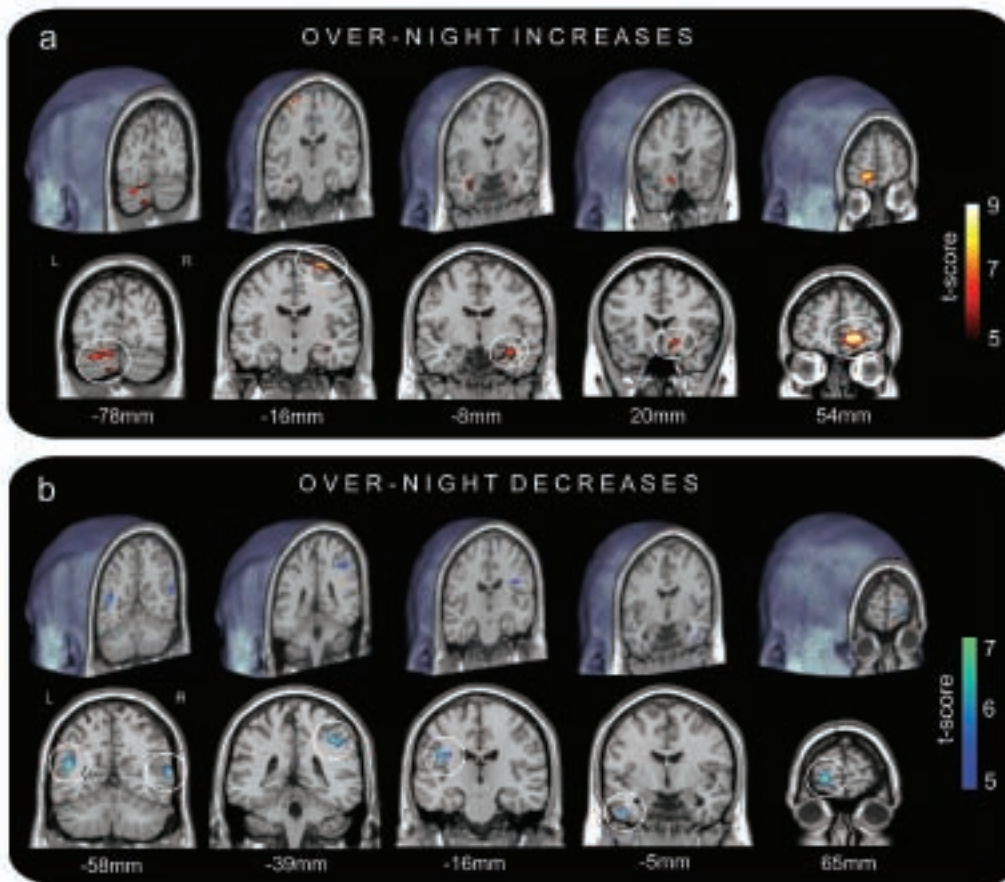
LIKE THE CHARACTERS in the Holiday Inn commercials on television, most of us know the value of a good night's sleep. Studies have shown that we need, on average, about eight hours of sleep per night to perform at our peak the next day and to maintain optimal health. Now, researchers at Harvard Medical School (HMS) have found that sleep also helps us solidify and improve long-term memory of skills and procedures, and master new skills.

In a study that sought to determine if sleep could trigger the brain's ability to adapt its behavior and circuitry, researchers at Beth Israel Deaconess Medical Center's Sleep and Neuroimaging Laboratory taught 12 healthy college-aged subjects a finger-tapping test similar to playing piano keys. The subjects were tested in the morning or evening and then retested after either 12 hours containing

sleep or 12 hours of awake time. During the retest a functional MRI (fMRI) scan measured brain activity, enabling the researchers to see which parts of the brain were active and which were inactive while the subjects were being tested.

"A night of sleep," says Matthew Walker, Ph.D., an assistant professor of psychiatry at HMS and the study's lead author, "reorganizes the representation of a memory within the human brain, making the memory more efficient. After sleep, you improve your performance by about 20 percent to 30 percent. Without sleep, there is no improvement. So, practice with a good night sleep makes perfect."

Using fMRI, the researchers discovered significant differences in the patterns of brain activity of subjects who slept prior to the test compared to those who remained awake. After a night of sleep, the primary motor cortex and cerebellum, which



Sleep-dependent motor memory reorganization in the human brain. Subjects were trained on a sleep-dependent motor skill task and then tested 12 hours later, either following a night of sleep or following intervening wake, during a functional magnetic resonance imaging (fMRI) brain-scanning session. Scans after sleep and wake were compared (subtracted), resulting in regions showing increased fMRI activity post-sleep (in red/yellow; or decreased signal activity postsleep, relative to postwake. Activation patterns are displayed on three-dimensional rendered brains (top panel of each graphic), together with corresponding two-dimensional sections (bottom panel of each graphic). Following sleep, regions of increased activation were identified in the right primary motor cortex, the left cerebellum, the right hippocampus, and the right medial prefrontal cortex. Regions of decreased activity postsleep were expressed bilaterally in the parietal lobes, together with the left insula cortex, left temporal pole, and left frontopolar area, all regions of the extended limbic system. In total, these results suggest that sleep-dependent motor learning is associated with a large-scale plastic reorganization of memory throughout several brain regions, allowing skilled motor movements to be executed more quickly, more accurately, and more automatically following sleep. These findings hold important implications for understanding the brain basis of perfecting real-life skills and may signify a potential role for sleep in clinical rehabilitation following brain damage.

are the main motor control areas of the brain and are responsible for speed and accuracy, expressed greater activity, as did the right frontal and right temporal lobes. These two areas of the brain help construct memory sequences. The parietal cortex, which is responsible for unconscious motor control, showed reduced activity following a night of sleep. The brains of those who stayed awake showed no such changes.

During learning and memory formation, our brains undergo both physical and chemical changes. In order to learn or memorize a fact or a skill, there must be persistent functional changes in the brain that represent new knowledge. This process is called neuroplasticity, the ability of the brain to reorganize how neurons connect to one another based on new experiences. At first, new memories can be easily disrupted. But, over time and through a series of molecular processes, they are crystallized into long-term memory. This process, called memory consolidation, solidifies memories into the neural architecture, making them more permanent. Many scientists now believe that processes of memory consolidation occur when we are asleep.

Walker says that a certain type of sleep is required to consolidate motor skill memories. One of his previous studies showed a “strong correlation” between Stage 2, non-REM sleep and memory consolidation. This stage of sleep is characterized by unique brain waves that contain quick bursts of electrical activity and a slowing down of such bodily functions as blood pressure, cardiac activity and metabolism.

While acknowledging that his team does not know exactly how or why this happens, Walker says “people show the most dramatic improvement [on memory tasks] if they have had significant amounts of this stage of sleep. Bizarre electrical bursts occur around the motor cortex that may trigger plastic changes in the brain and a cascade of events that form memories. While we only have indirect evidence of this, we believe that these electrical bursts trigger these changes in plasticity.”

Modern society’s increasing erosion of sleep time, says Walker, is cause for concern. Many people believe they can accumulate sleep by “bingeing” on

weekends, thus making up for lost sleep time. Walker says people can’t learn effectively if they short-change their brains of sleep.

“If you practice a task in the evening, stay up all night and through the next day, and then have a recovery night of sleep, you will fail to show improvement on the re-test,” says Walker. “If you don’t sleep within 24 hours of learning a memory, you lose the ability to consolidate it.”

The findings show that procedural skills such as learning to walk or coordinating limb movements become more automatic with sleep, which may explain why babies require so much sleep and why children and adolescents need more sleep than adults. Walker says that sleep plays a critical role in human development. Infants are in a constant state of motor learning and have “an immense amount of new material to consolidate. Consequently, this intensive period of learning may demand a great deal of sleep.”

His findings also have implications for the real-life learning of motor skills for sports, playing a musical instrument, even for acquiring surgical skills – all of which are based on motor skill memory processing – as well as clinical significance for people who have suffered a stroke or brain injury.

“A good night of sleep may be able to help re-establish connections in the brains of stroke victims,” he says. “Since the brain undergoes these plastic changes, patients can take on new tasks and learn new ways of doing things they did prior to their stroke. Sleep may incrementally assist their recovery.” The next step is to test this theory with clinical stroke patients and track their improvement with sleep.

Walker and his colleagues also plan to examine sleep and learning disorders that occur in depression, schizophrenia and Alzheimer’s disease to determine if any relationship exists between the two.

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‘Too Good to Treat’ Designation Can Leave Stroke Victims at Risk

PHYSICIANS CONSIDER the first three hours after symptoms develop to be a critical window for victims of ischemic strokes – strokes caused by a blood clot. Within this timeframe, many patients receive a powerful clot-busting drug, called tissue plasminogen activator (tPA), that can reduce the amount of brain damage they suffer.

A new study from Harvard Medical School (HMS) in the journal *Stroke*, however, reports that many patients who could receive the drug do not because their symptoms appear to be minor or because they improve rapidly after arriving at the hospital. These patients are deemed “too good to treat” (TGT), leaving them at significant risk for poorer outcomes.

“Our primary finding,” says Eric Smith, M.D., M.P.H., a neurologist at Massachusetts General Hospital (MGH) and instructor of medicine at HMS, “was that about 30 percent of those patients judged ‘too good to treat’ either died or were discharged to a rehabilitation facility.”

Smith and his colleagues reviewed the medical records of more than 400 patients with ischemic strokes that came to the MGH emergency room over a two-year period. Of the nearly 130 patients who arrived within the three-hour window of showing symptoms, about 70 did not receive tPA. More than half of this group was considered “too good to treat” because their symptoms were stable or their condition rapidly improved. Of these patients, two died during hospitalization and nine were discharged to a rehabilitation facility because of problems with nerve function.

When a stroke is caused by a blood clot – and about 80 percent of them are – tPA can effectively dissolve the clot if given within three hours of the onset of symptoms, sometimes reversing the effects of the stroke. A small, but significant, risk exists that tPA could cause bleeding in the brain, a potentially fatal complication. Because of this risk, patients with less severe symptoms may not receive tPA in the hopes their condition will improve on its own.

“‘Too good to treat’ is one issue that is left up to the discretion of the physician,” says Smith. “The main study that led to FDA approval of tPA for ischemic stroke had certain exclusions, including mild or rapidly improving symptoms. But TGT has never really been defined. What’s too mild? What’s rapid improvement? This is up to the judgment of the treating physician.”

Smith, the study’s lead author, says that a significant number of stroke patients coming to treatment centers like MGH are considered too good to treat. In fact, about 30 percent to 40 percent of all patients who come to the hospital within three hours of symptom onset fall into this category. Yet, according to Smith, about 27 percent of the patients deemed TGT in his study could not be discharged home due to neurological problems. A previous study found that 32 percent of patients considered too good to treat died or could not be discharged from the hospital.

Most patients suffering a stroke receive standard physical and neurological examinations. Some medical centers, including MGH, use the National Institutes of Health Stroke Scale (NIHSS), which rates patients on a scale of 0 to 42 on such issues as

Table 1. Characteristics of patients who could not be discharged home following decision to withhold IV tPA

Characteristic	No home discharge (n=11) n (%)	Home discharge (n=29) n (%)	P value
Age (mean ± SD)	73.2 ± 11.7	67.5 ± 13.2	0.22
Female gender	7 (64)	10 (34)	0.15
Initial NIHSS	5 [2, 13]	3 [2, 4]	0.15
NIHSS at tPA decision	2 [1, 4]	2 [1, 3]	0.44
RI	4 (36)	6 (21)	0.42
Vascular occlusion	4 (36)	4 (14)	0.18
Stroke etiology			
proximal-source embolism	7 (63)	17 (59)	
large artery	2 (18)	4 (14)	0.79
small vessel	2 (18)	8 (28)	
Neurological worsening	6 (55)	1 (3)	<0.001
NIHSS at discharge ¹	2 [2, 4]	1 [0, 1]	<0.001
mRS ≤1	1 (9)	24 (83)	<0.001

NIHSS, NIH stroke scale score; RI, rapid improvement; mRS, modified Rankin scale. Continuous variables are median [interquartile range] unless otherwise noted. One patient was admitted from a nursing home and was excluded.

¹ Excluding two patients who died.

level of consciousness, visual ability, motor function, sensory perception and language. TGT patients typically have an NIHSS score of 0 to 5, while patients rated 5 and above are eligible for tPA.

Rapid improvement, one of the criteria for a TGT designation, is defined as a four-point-or-better improvement on the NIHSS from the time of the initial patient evaluation to the time a decision is made regarding tPA; “neurological worsening” is at least a two-point decline in the NIHSS score from the time of the tPA decision to the patient’s discharge from the hospital.

Rapidly improving stroke symptoms may occur when the brain borrows blood flow from an area not affected by the stroke. Smith says some patients benefit from this collateral blood flow. In others, however, this collateral flow does not last, bringing back the stroke symptoms. These patients, he adds, may be potential candidates for tPA.

“Many doctors guess at TGT based on a neurological exam,” says Smith, “but there are different perceptions [among them] of disability.” One area that is often overlooked in determining whether to administer tPA is a patient’s ability to walk. Because most patients are examined while they are in bed, physicians often underestimate gait difficulties.

The research team concluded that a re-evaluation of stroke severity criteria for tPA administration might be warranted.

“The million-dollar question,” says Smith, “is when to treat with tPA. We need to determine who will do well with this treatment and who won’t. Most patients with mild, improving symptoms – about 70 percent of them – do well without treatment. We need to find the features of the other 30 percent.”

Because his study was small, Smith says his team was unable to find any common characteristics to predict which patients would fare poorly without tPA. He says a larger, multicenter study, using imaging techniques that measure blood flow in the brain, is needed to find these characteristics. He is currently seeking institutions with which to collaborate in a study of this magnitude.

In the meantime, Smith says, “We can only recommend that physicians be a little more cautious in deciding against tPA treatment.”

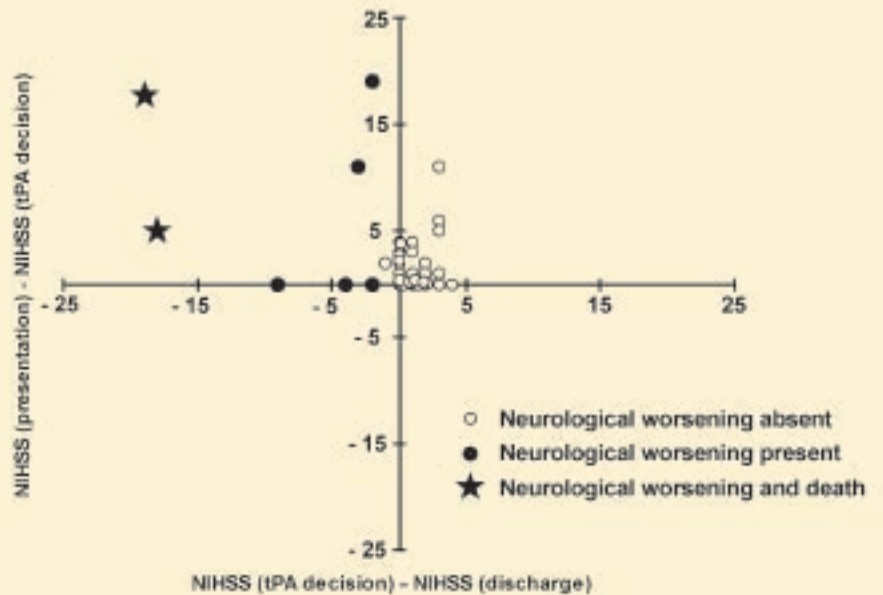


Figure 1 NIHSS improvement from time of presentation to time of IV tPA decision is graphed on the Y axis; NIHSS improvement from time of IV tPA decision to time of hospital discharge is graphed on the X axis. The left upper quadrant represents patients who had NIHSS improvement prior to decision not to administer IV tPA, followed by NIHSS deterioration during the hospital stay. Patients with neurological worsening (see text for definition) or death are represented by black circles and black stars, respectively.

Credits for Table 1 and Figure 1: Eric E. Smith, Abdul R. Abdullah, Iva Petkovska, Eric Rosenthal, Walter J. Koroshetz, and Lee H. Schwamm
 Poor Outcomes in Patients Who Do Not Receive Intravenous Tissue Plasminogen Activator Because of Mild or Improving Ischemic Stroke
 Stroke 2005;36:2497-2499 tbs. p.2498

Reversing the Ravages of Parkinson’s with Fetal Cell Transplants

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“Our remaining step,” says Isacson, “is to produce the same cells from embryonic stem cells, grow them into injectable cells, and transplant them with the same or better outcome than we achieved in this study.”

The study was a collaboration among researchers at McLean Hospital, an HMS teaching affiliate, and scientists at the Queen Elizabeth II Health Science Center at Dalhousie University in Canada.

Isacson says the *Brain* paper establishes in an analytic way that fetal cell transplants can work and that the cell type that dies in Parkinson’s “can make it after transplantation.” Further, he adds, this work is the prototype for using stem cells for Parkinson’s disease therapy.

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In fact, Tarceva (generic name, Erlotinib), used to treat non-small cell lung cancer, was one of the EGFR blockers He and his team tested in the laboratory. Developed by Genentech, Tarceva prevents tumor cell growth by targeting proteins that are important for cell growth in this form of lung cancer.

The use of such drugs, says He, "might prove useful for promoting axon regeneration after brain and spinal cord injuries."

He's lab is currently working with Genentech researcher Marc Tessier-Lavigne, one of the *Science* paper co-authors, to test Tarceva's effects on axon regeneration in mouse models of spinal cord injury.

In addition to optic nerve damage and spinal cord injury, He says promoting nerve cell growth by turning off these inhibitory signals could also benefit patients suffering from strokes or less severe injuries to the CNS.

ON THE BRAIN

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