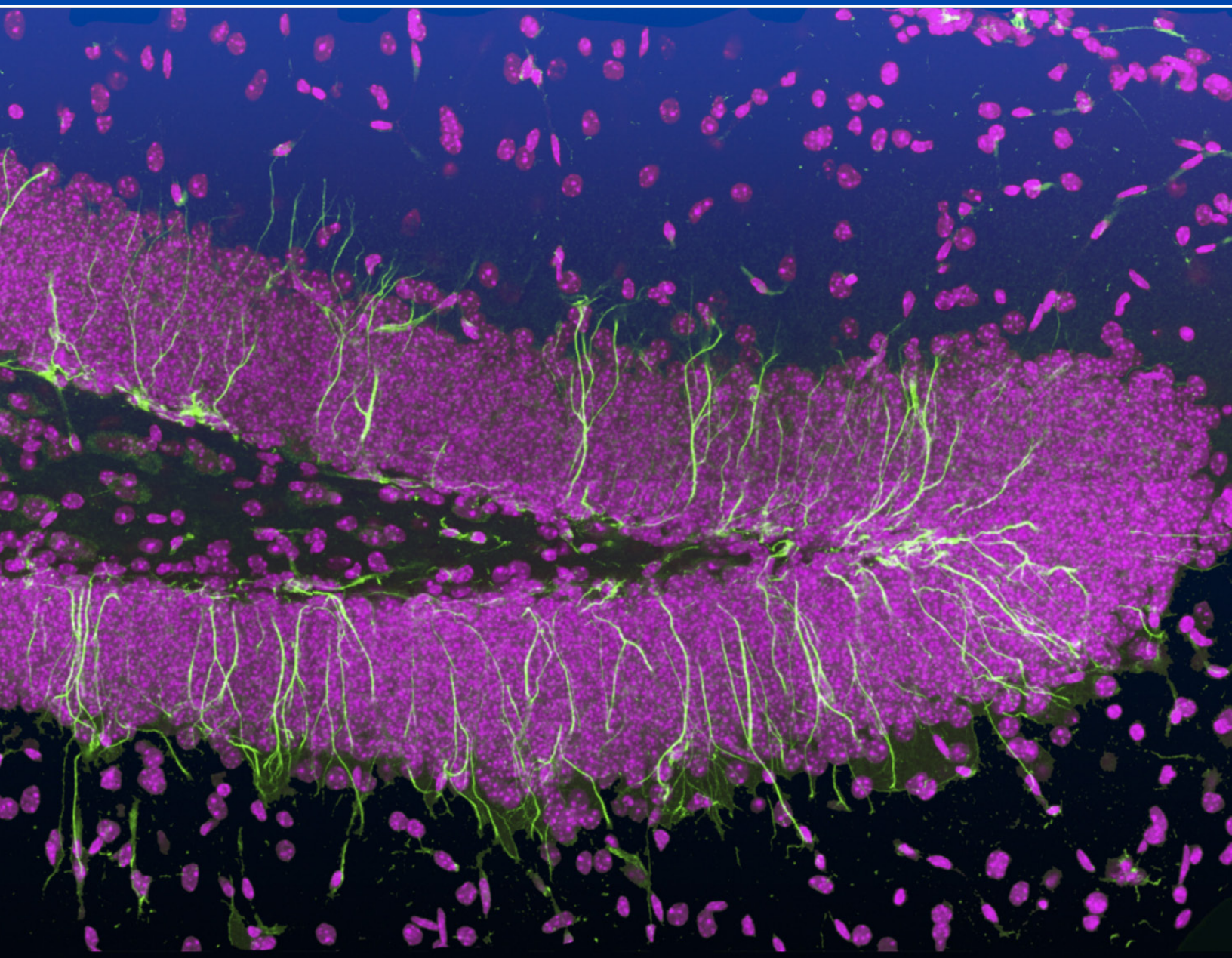
MAYO CLINIC

Neuroregenerative Medicine



People are living longer than ever before, in part due to medical advances that have saved more people from life-threatening diseases, injuries and congenital conditions. But as people live longer, they're more likely to develop age-related conditions or chronic diseases. After the onset of most chronic diseases or injuries, the underlying damage remains, and patients are left to manage the symptoms. Many of these patients, having so-called degenerative diseases, are particularly well-suited to treatment with regenerative medicine solutions.

At Mayo Clinic, we have embraced regenerative medicine as a strategic investment in the future of health care. The quest for innovative solutions to meet the changing needs of our patients is instilled in our mission and values, as we seek to treat the underlying causes of symptoms and find innovative and affordable health care solutions.

The Mayo Clinic Center for Regenerative Medicine consists of several focus areas, including neuroregeneration, each encompassing the discovery, development and delivery of next-generation patient care. In the center's Neuroregeneration Program, clinicians, scientists, engineers and other specialists take a multidisciplinary integrative approach to neuroregeneration for a number of devastating neurological conditions. The research is multifaceted, ranging from basic science discovery to clinical applications. Throughout these pages, you will find examples of several team-based innovations in the field of neuroscience and the practice of neurology.

As director of Mayo Clinic's Center for Regenerative Medicine, I see firsthand the remarkable collaboration between physicians and scientists across disciplines. In this new era of medicine, we are working together to use advanced regenerative technologies and therapies to essentially teach the body to heal from within. And we are unrelenting in our mission to target the root cause of disease and offer the prospect of cures — all with the goal of advancing the science and improving health care across our patients' lifespans.

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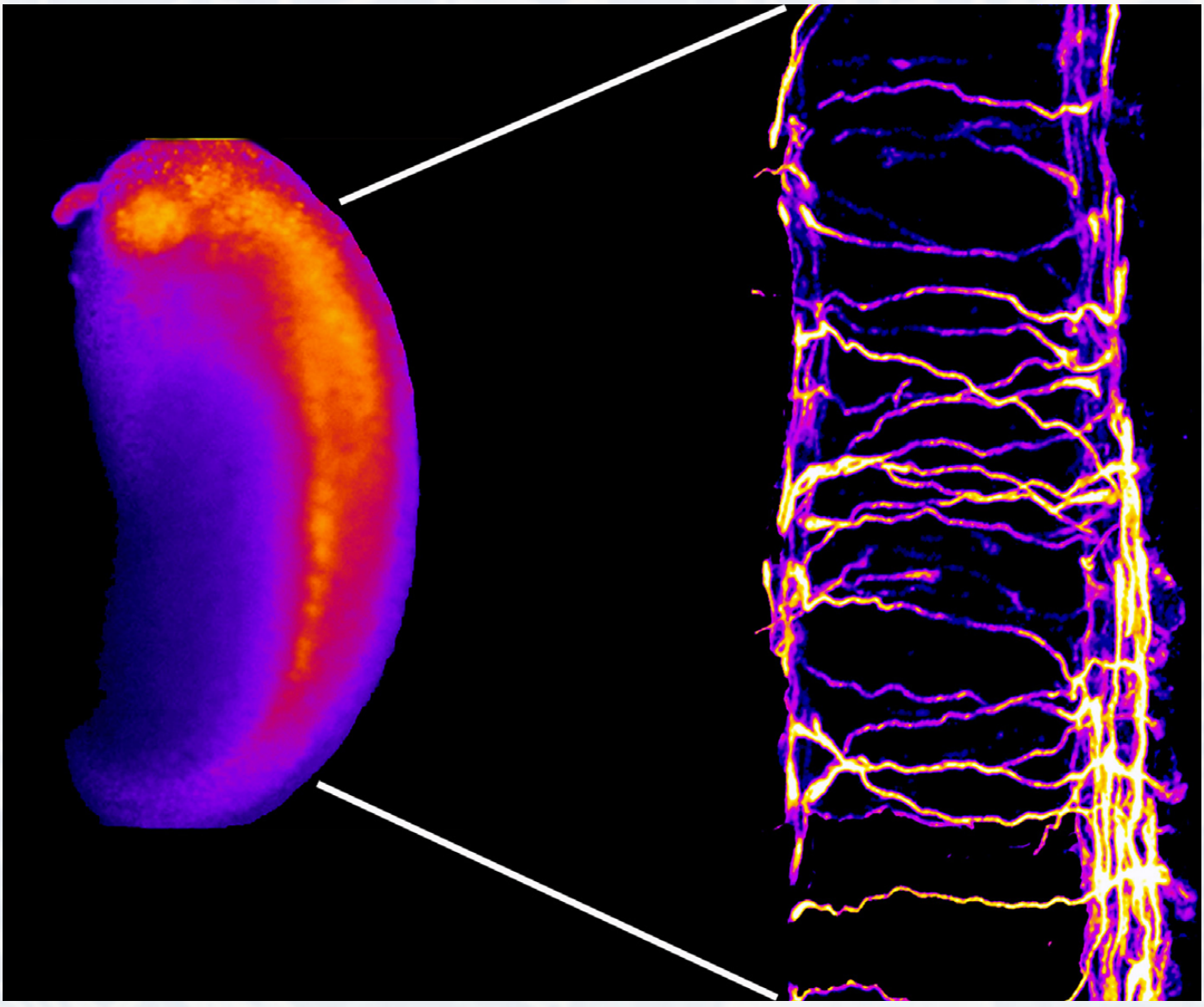
Harnessing the Body's Healing Power

The complex, delicate structures comprising the nervous system — the brain, spinal cord and nerves — are susceptible to injury as varied as trauma, cancer and neurodegenerative diseases. Unfortunately, because of the complexity of the brain and spinal cord, very little spontaneous nerve regeneration or healing occurs. Injuries to the spinal cord and peripheral nerves are often permanent and incapacitating. Although signs and symptoms of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS) can be somewhat managed, these diseases cause progressive deterioration that the body cannot repair.

Physicians and scientists at the Center for Regenerative Medicine at Mayo Clinic are pursuing a new strategy: regenerative treatments aimed at fully healing damage to the nervous system. In this model of care, neurological damage is not simply managed but repaired through growth of new nerves. Mayo's multi-faceted research, ranging from basic scientific discovery to clinical application, offers hope for people who have neurological injury that today is beyond repair.

"Neuroregeneration is the next step in the evolution of neurology and neurosurgery," says Fredric B. Meyer, M.D., chair of the Department of Neurologic Surgery at Mayo Clinic in Rochester, Minn. "Historically, neurosurgery has its origins in excising lesions to help patients.



Fluorescent spinal neurons in the developing aquatic frog (Xenopus) embryo.

Recent technological advances have led to the development of minimally invasive procedures or device implantation. Our goal now is to harness the regenerative capacity of neurogenesis to improve treatment options for brain, spine and nerve diseases and injuries."

Discovering how neurons grow

The highly specialized cells that make up the nervous system were once thought to be incapable of growing. Now they are known to be able to remodel and to possess some ability to self-heal. At Mayo, paradigms of neural regeneration take many forms — from re-engineering a patient's stem cells to promote neural growth to using electronic devices to bypass severed nerves and restore limb function after spinal injury.

Fundamental to these efforts is a basic understanding of how neurons grow and, equally importantly, become functionally integrated into the nervous system. In the developing nervous system, slender axons projecting from neurons extend and retract, "sniffing out" the molecules needed to help the nerve growth cones reach and connect with their targets in surrounding tissue. A complex array of molecular guidance cues tell them whether to continue on their path or to turn left or right.

John R. Henley, Ph.D., a Mayo Clinic molecular neuroscientist and director of the Developmental and Regenerative Neurobiology Laboratory at Mayo in Minnesota, has devoted his career to identifying and manipulating these guidance cues. Growing nerve tips face a hostile environment, in which substances that may work to prevent random spinal growths also inhibit neural growth. Dr. Henley's work is directed at priming nerves to grow in this hostile environment by altering not only the external molecular environment but also the intrinsic state of a neuron — something previously not thought possible. Building on this work, Dr. Henley's research team has succeeded in restoring function to lab animals with injured spines (page 5).

Working on a similar scale, Mi Hyeon Jang, Ph.D., a molecular biologist at Mayo in Minnesota, is investigating neurogenesis in the hippocampus, the portion of the brain associated with memory. Among other discoveries, Dr. Jang's team has shown that exercise increases neurogenesis. Her work has important implications for treating a range of neuropathological conditions, including epilepsy, Alzheimer's disease and brain injury (page 7).

Other Mayo researchers are working to overcome the hostile environment that inhibits nerve repair. Isobel A. Scarisbrick, Ph.D., leads a laboratory team at Mayo in Minnesota that is uncovering the physiologic changes unleashed by damage to the brain and spinal cord that create this inhibitory microenvironment. The work potentially may lead to new therapies for neurological conditions such as multiple sclerosis and spinal cord injury (page 8).

Clinical trials

Early but unsuccessful efforts at neuroregeneration focused on creating antibodies to counteract inhibitory factors. Mayo took a different approach. Led by Anthony J. Windebank, M.D., a neurologist and molecular neuroscientist at Mayo in Minnesota, researchers pioneered ways of re-engineering mesenchymal stem cells to enhance their ability to produce growth factors. Dr. Windebank's theory was that re-engineered stem cells that were reimplanted in the patient's body could serve as delivery vehicles for neural growth factors, promoting nerve regeneration. Based on this work, he and colleagues are currently conducting a clinical safety trial of stem cell therapy to treat ALS (page 11).

Less common than ALS, multiple system atrophy (MSA) is a devastating neurodegenerative disease that is generally fatal within three years of diagnosis. Phillip A. Low, M.D., a neurologist at Mayo in Minnesota and founder of Mayo's autonomic testing laboratory, is leading a clinical safety trial of stem cell therapy to treat MSA (page 12).

Yet another application of this stem cell work involves injuries to the peripheral nervous system. These injuries are particularly challenging: inhibitory factors can overwhelm the nerve tips' efforts to grow across the gap created by injury. To provide a friendlier environment, Mayo scientists have developed a synthetic tube that eventually could house growth factors to promote neuroregeneration. The tube will soon enter a clinical safety trial (page 13).

Additional approaches

In addition to therapies to promote neuron growth, Mayo is pursuing a vascular approach to neuroregeneration. Guojun Bu, Ph.D., and Leonard Petrucelli, Ph.D., both neuroscientists at Mayo Clinic in Jacksonville, Fla., are investigating whether patient-derived stem cells can improve blood flow to the brain and thus slow or prevent neurodegenerative disease (page 15).

Mayo's work with stem cells also has potential to eventually improve treatment of brain cancer. Stem cells

collected, processed and stored by Mayo's Regenerative Medicine Biotrust are facilitating research into why various types of brain tumors behave differently (page 17).

Beyond stem cells, Mayo researchers are also exploring device-based regenerative therapies. Led by neurologist Ryan J. Uitti, M.D., and neurosurgeon Robert E. Wharen Jr., M.D., a pilot study at Mayo in Florida is investigating whether deep brain stimulation can lessen cognitive decline in patients with Parkinson's disease (page 17). At Mayo in Minnesota, Kendall H. Lee, M.D., Ph.D., is conducting laboratory tests that eventually may result in electronic devices that can bypass a severed spinal cord and restore function to patients with paralysis (page 19).

From scientific discovery to patient care

Mayo's commitment to translational medicine — moving scientific discoveries from the lab bench to the patient's bedside — makes it ideally suited to lead efforts in the growing field of regenerative medicine. Interaction between lab scientists and clinicians is a constant at Mayo.

"There are few barriers to translating scientific laboratory discovery to the clinical treatment of patients," Dr. Meyer says. "Because we're a premier clinical institution working in an environment of collaboration, we can rapidly and safely apply novel treatments to help patients.

"Neuroregeneration is in its infancy," Dr. Meyer adds. "But its potential for medicine and mankind is huge."

Axonal Growth and Spinal Recovery

One of the star players in Mayo Clinic's neurogenesis work measures all of 1.5 inches long. The zebrafish, a tropical freshwater member of the minnow family, is being used as an animal model to investigate how nerve regeneration might be improved after spinal cord injury. Preliminary findings indicate significant promise.

Researchers at the Mayo Clinic Developmental and Regenerative Neurobiology Laboratory in Rochester, Minn., have developed a therapy that, in laboratory tests, is given to zebrafish with spinal cord injuries. Behavioral testing after treatment has demonstrated dramatic functional recovery in the injured fish.

"After injury, the fish don't swim very well or very far. After treatment, they have recovered," says John R. Henley, Ph.D., a molecular neuroscientist who directs the lab.

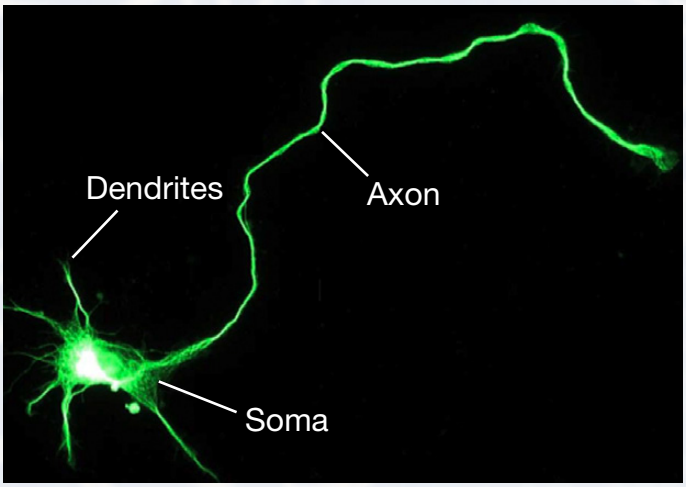


Figure 1. Axon projecting from the cell body (soma). Dendrites conduct electrochemical stimulation received from other nerve cells. The axon conducts electrical impulses away from the soma.

Steering decisions for neurons

In the developing nervous system, axons extend and retract from neurons, seeking the molecules needed to help the nerve growth cones connect with their targets in surrounding tissue (Figure 1). A complex array of molecular cues guides axonal growth.

"A lot of the extracellular cues have been understood for a while," Dr. Henley notes. "But the signals functioning inside the cell are the black box. We've been studying these basic mechanisms underlying how nerve cells grow and are guided to synaptic targets."

The work involves in vitro systems in which nerve cells are grown (Figure 2) in defined cultures. "We apply different environmental factors that are thought to influence these steering decisions in vivo, and then measure these turning responses in a defined, quantitative assay," Dr. Henley says.

The system also must overcome factors that inhibit neural growth. In research published in the July 2010 issue of *Nature Neuroscience*, Dr. Henley and colleagues discovered that a major component of myelin prevents growing nerve tips from producing the adhesions needed to form anchoring sites with surrounding tissue and the

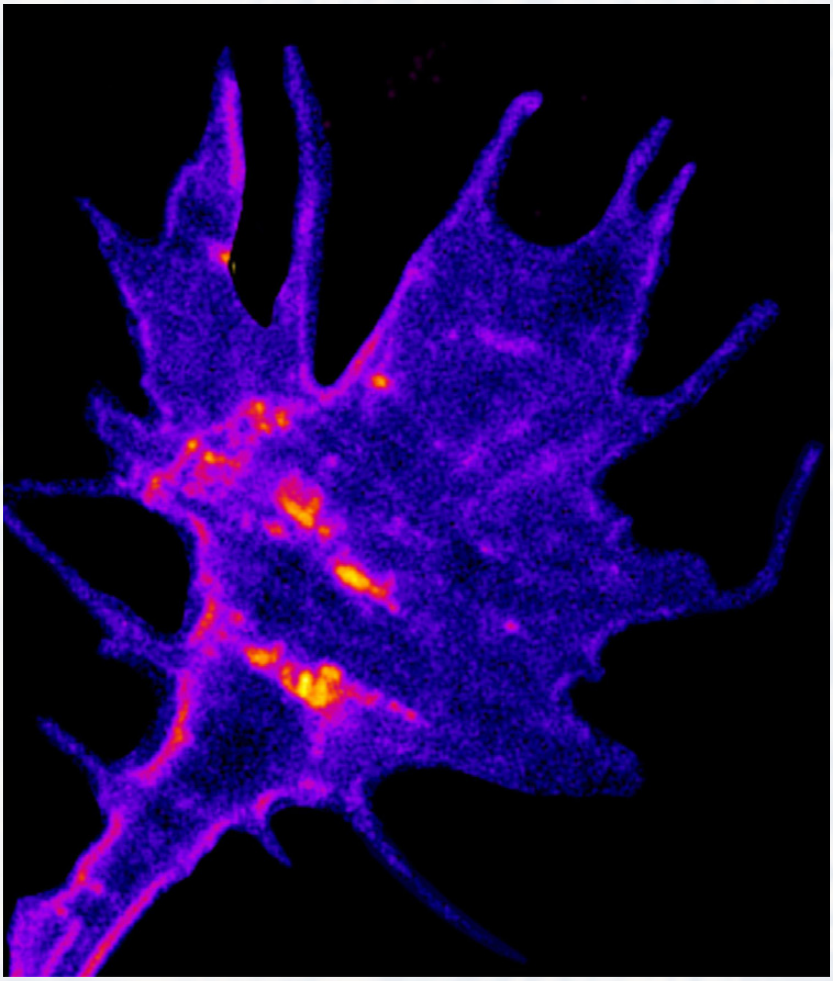


Figure 2. Nascent endocytic vesicles in the nerve growth cone labeled with a fluorescent membrane dye.

traction to move forward. These inhibitory factors are released into cerebral spinal fluid after spinal injury.

"At least one growth inhibitory factor actually causes a loss of these adhesions at the surface of the nerve cell," Dr. Henley says. "Our model of treatment is that either by activating these adhesion proteins and/or by blocking this removal process, we might preserve the capacity of an axon to be stimulated to grow even in the presence of this inhibitory environment."

Measuring fish behavior

In 2011, the research moved from the petri dish to the zebrafish. "The zebrafish is a powerful in vivo model because it's at least somewhat permissive for regeneration. Many, but not all, neurons will regenerate after injury," Dr. Henley says. "It's also a high-throughput system. We can measure functional recovery three days after injury."

Those measurements are facilitated by stereotypical zebrafish behavior. When threatened, zebrafish quickly curl into a sharp C shape before swimming away. Using a camera that records one frame per millisecond, the researchers document the response of zebrafish to a vibration against their dish. "Within 10 to 12 milliseconds, the fish start to bend. After another 10 to 12 milliseconds, the fish are in a very sharp C bend and then swim away," Dr. Henley says.

A fish that is injured and then left for three days can only wiggle faintly. Its reaction time is twice that of the control fish. "But when we do our treatment, the fish is able to bend back into its classic C, and the latency period comes back down," Dr. Henley says. "We can measure this quantitatively, in terms of the angle, velocity and latency time."

Dr. Henley's lab team is investigating other factors thought to be barriers to neuroregeneration as well as working to develop a more sophisticated animal model. As always at Mayo, the ultimate goal is to translate these molecular discoveries into treatments that can help people recover from spinal cord injury.

Cancer research

A related topic under investigation in the developmental and regenerative neurobiology lab is the molecular mechanisms that control the growth of glioblastoma multiforme (GBM), the most common and aggressive primary brain tumor. GBM cells can rapidly infiltrate the brain, making GBM incurable by surgical resection. The invasion pattern follows selective tracts, suggesting that molecular guidance cues may regulate GBM cell migration.

By investigating the specific biological cues that control the invasiveness of these brain tumors, Dr. Henley and colleagues hope to contribute to patient treatment. Just as understanding the steering decisions of axons may ultimately lead to therapy for spinal cord injury so, too, might deciphering GBM migration cues pave the way for new cancer treatments.

For more information

Hines JH, et al. Asymmetric endocytosis and remodeling of beta1-integrin adhesions during growth cone chemorepulsion by MAG. *Nature Neuroscience*. 2010;13:829.

Hippocampal Neurogenesis and Cognitive Function

Adult neurogenesis plays a critical role in brain plasticity, learning and memory. "Newborn" neurons have the ability to become functionally integrated into the hippocampus, the part of the brain associated with memory, and to contribute to specific cognitive functions (Figure). Understanding the molecular mechanisms of adult neurogenesis has important implications for a range of neuropathological conditions, including epilepsy, Alzheimer's disease and brain injury.

Researchers in the laboratory of Mi Hyeon Jang, Ph.D., a molecular biologist at Mayo Clinic in Rochester, Minn., are working to utilize the regenerative capacity of adult neurogenesis with the ultimate goal of improving treatment options for patients. "Much progress has been made in this area in the last 10 years," Dr. Jang says.

The central focus of her research team's efforts is to increase understanding of the molecular targets involved in regulation of adult hippocampal neurogenesis and related behavioral responses that are altered in neuropathological conditions. "We want to investigate the function of neurogenesis and the role of neurogenesis in pathological diseases," Dr. Jang says.

Integrating newborn neurons

One specific area involves exploring the intricate cellular and molecular mechanisms that regulate the production, maturation and integration of new neurons in the circuitry. Using a retroviral system and unique staining techniques to label neuronal progenitors, Dr. Jang and her colleagues were able to characterize the developmental stages of newborn neurons from a neural stem cell to a fully formed neuron with synapse formation, a process that takes two to four weeks.

"With retroviruses we can characterize morphology at the single-cell level," Dr. Jang notes.

The process of neurogenesis is modulated by various

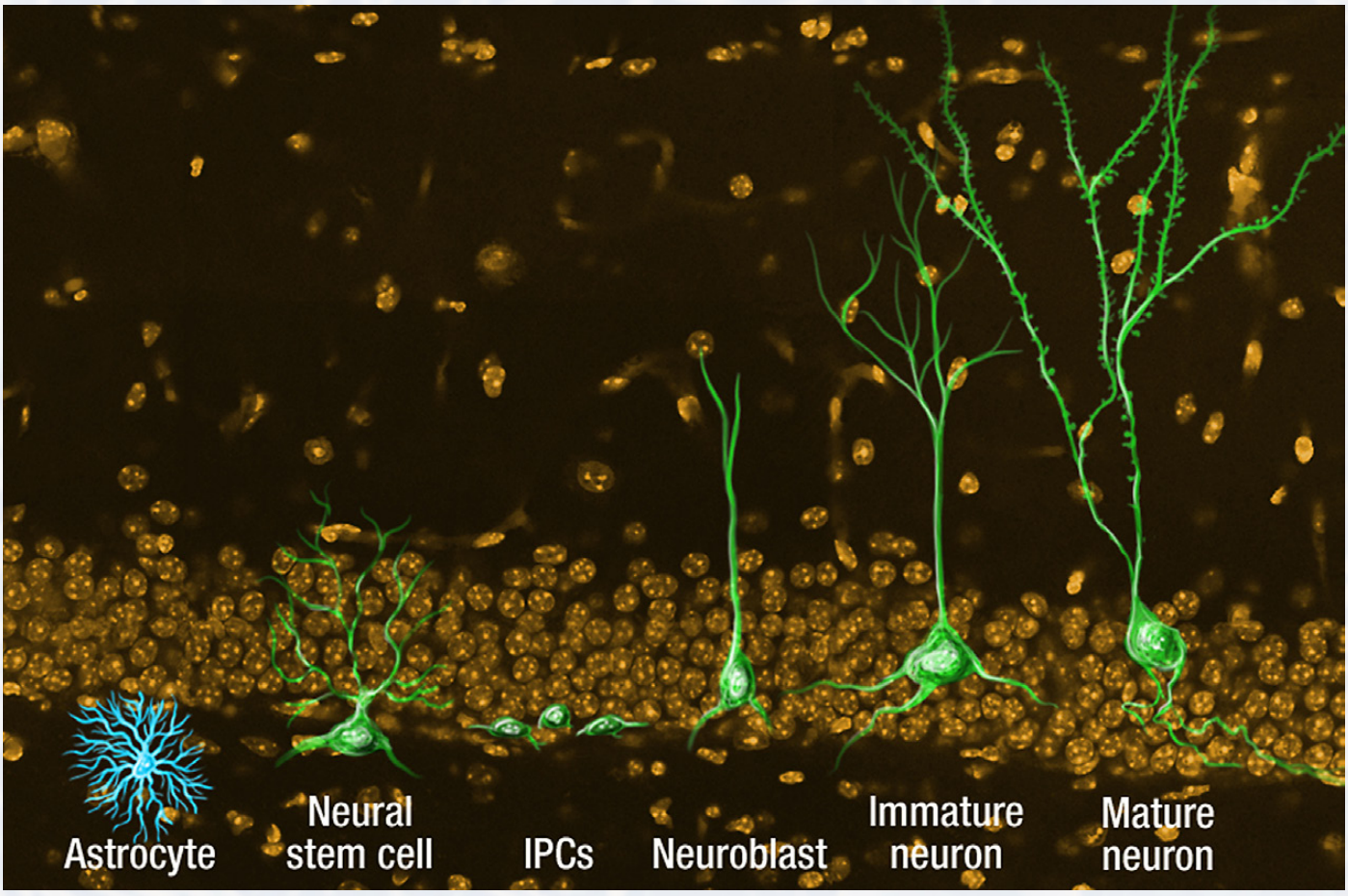


Figure. Model of the sequential process of adult neurogenesis within the dentate gyrus of the hippocampus. The neural stem cell can remain quiescent, self-renew or proliferate to give rise to the astrocyte or intermediate progenitor cells (IPCs). The IPC differentiates into a neuroblast, which migrates and integrates itself into the inner granule layer as an immature neuron. It then differentiates into the mature granule cell, which extends its axon and dendrites — now comprising spines — to achieve functional integration.

physiological and pathological stimuli, with the niche mechanisms largely unknown. However, in a study published in the Feb. 7, 2013, issue of *Cell Stem Cell,* Dr. Jang and colleagues identified secreted frizzled-related protein 3 (sFRP3) as a neuronal activity-regulated factor that helps control multiple steps of adult hippocampal neurogenesis, including the activation of quiescent adult neural stem cells as well as the maturation, dendritic development and spine formation of newborn dentate granule neurons. Neuronal activity decreases the expression of sFRP3, a naturally secreted inhibitor of Wnt proteins, whose signaling is necessary for neurogenesis.

Potential for treating depression

A key strand of Mayo's neurogenesis research is the role of adult neurogenesis in causing neurodevelopmental disorders. Dr. Jang's discoveries concerning sFRP3 suggest that antidepressants may ease patients' symptoms by promoting neurogenesis. In a letter to the editor in the September 2013 issue of *Molecular Psychiatry,* Dr. Jang noted that electroconvulsive stimulation significantly reduces sFRP3 levels in the mouse hippocampus. Chronic treatment with the antidepressants fluoxetine or imipramine also significantly suppresses sFRP3 expression in the mouse hippocampus.

In experiments with lab mice, Dr. Jang and colleagues found that sFRP3 knockout mice exhibited less depression-like behaviors than did normal mice. In a parallel study, the researchers identified a significant association of three genetic variations in human sFRP3 with early responses to antidepressants in a clinical cohort. The results of both studies suggest that targeting sFRP3 may provide a novel therapeutic approach for treatment of depression.

Dr. Jang is also intrigued by factors that appear to stimulate neurogenesis — such as exercise and electro-shock therapy — and those that apparently inhibit neurogenesis, such as stress and aging. She notes that deep brain stimulation (DBS), which appears to stimulate neurogenesis, offers interesting therapeutic possibilities. "DBS can target specific areas of the brain, which may have applications for distinct pathologies," Dr. Jang says.

Translation of basic science to treatment for patients is the overriding goal. "One of the benefits of Mayo is that as a molecular biologist, I can closely collaborate with physicians," Dr. Jang says. "As a lab scientist, I want to contribute to the level of clinical care."

For more information

Jang M-H, et al. Secreted frizzle-related protein 3 regulates activity-dependent adult hippocampal neurogenesis. *Cell Stem Cell*. 2013;12:215.

Jang M-H, et al. Secreted frizzle-related protein 3 (sFRP3) regulates antidepressant responses in mice and humans. *Molecular Psychiatry*. 2013;18:957.

Preventing Environmental Damage in the Central Nervous System

One of the most significant challenges to repairing the central nervous system (CNS) is the hostile environment unleashed when the brain or spinal cord suffers damage. In cases of injury and disease, the precisely controlled microenvironment of the CNS is greatly disrupted, contributing directly to tissue damage and a lack of significant functional repair and nerve regeneration. Myelin, the sheath that insulates and protects axons, is particularly vulnerable.

A promising avenue of research at Mayo Clinic in Rochester, Minn., is uncovering precisely how this "environmental damage" occurs. These discoveries have the potential to spawn new therapies for a range of neurological conditions, particularly those involving damage to myelin, including multiple sclerosis (MS) and spinal cord injury.

"Our goal is to bring the CNS microenvironment back under control," says Isobel A. Scarisbrick, Ph.D., director of the CNS Injury and Neurorehabilitation Laboratory at Mayo in Minnesota. "We hope to make this environment one that is conducive to innate repair and that facilitates therapeutic interventions such as stem cell therapies."

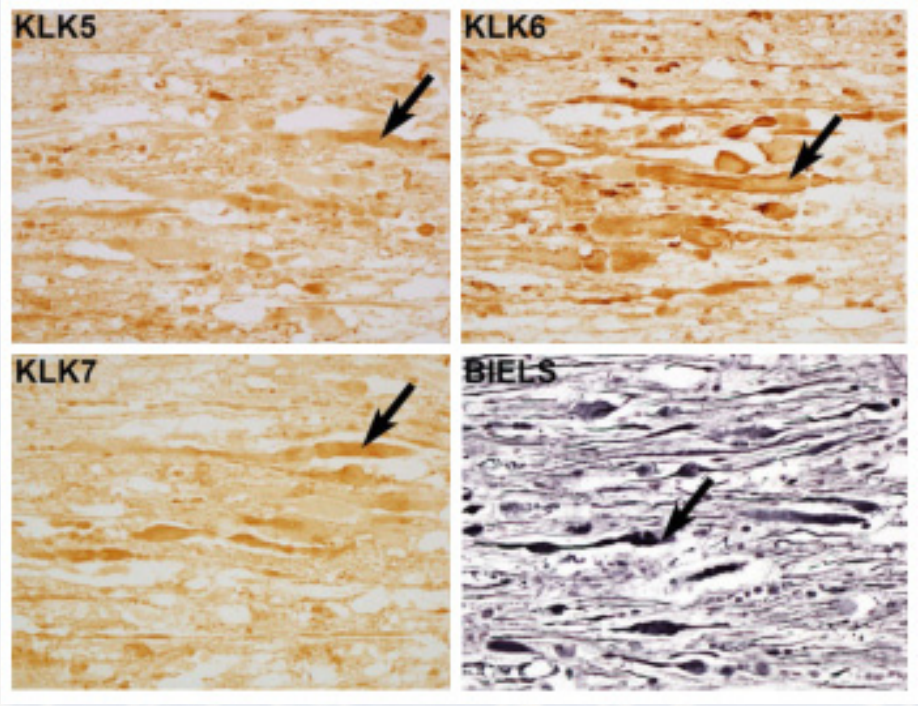


Figure 1. Photomicrographs showing immunoreactivity for three unique kallikreins in a case of human vertebral fracture dislocation resulting in tetraplegia. High levels of kallikrein staining, as indicated by arrows, were seen in association with swollen axons and retraction bulbs visualized in the adjacent section stained using the Bielschowsky silver method (BIELS).

Cascade of damage

When the CNS is injured by trauma or disease, a cascade of secondary damage ensues. Vascular, cellular and chemical responses to the injury include tissue inflammation, reduced blood flow and scar formation. Demyelination occurs on injured axons, slowing the conduction of nerve impulses and stripping axons of protection against further damage.

These changes are brought about in part by multiple proteases, notably the kallikrein family of enzymes (Figure 1). Kallikreins are increasingly associated with neurological conditions including Alzheimer's disease, Parkinson's disease and frontotemporal dementia. Dr. Scarisbrick's lab discovered one member of the kallikrein family, called kallikrein 6.

In laboratory studies, the Mayo researchers further found that kallikreins wreak neurologic havoc through a limited set of receptors, known as protease activated receptors (PAR). Specifically, aberrant activation of these receptors promotes damage to the axonal wires that conduct electrical impulses across the brain and spinal cord as well as to oligodendrocytes, the cells that produce myelin (Figure 2).

The kallikrein-PAR axis in fact delivers a one-two punch. "Some of these enzymes not only degrade myelin. They also signal to the oligodendrocytes to stop making myelin," Dr. Scarisbrick says. "The oligodendrocyte precursor cells are ready; they want to remyelinate the denuded axons. But they are inhibited from doing so."

Fortunately, protease activated receptors are known to be potent drug targets. "Because of their location, partly inside and partly outside the cell, they are highly 'druggable,'" Dr. Scarisbrick says. "It's difficult to target all the multiple proteases. But we can go after the receptors. They may be a common pathway to block the multiple effects of the proteases."

Success in vitro

In a study published in the September 2013 issue of *Glia*, the Mayo researchers reported that over-activating the PAR1 receptors in mouse oligodendrocyte cultures caused the cells to stop expressing myelin genes. When a PAR1 inhibitor was added to the culture, the cells resumed myelin production.

"We have shown that blocking the kallikrein-PAR pathway can result in remyelination — in a dish," Dr. Scarisbrick notes. "This is very exciting, but now we want to translate that work into animal models of MS as well as spinal cord injury. Eventually, of course, the goal is to translate this into therapies for patient use. If we continue the progress we have had until now, we're very optimistic that could happen in a few years."

Indeed, in a study published in the November 2013 issue of the *Journal of Neuropathology & Experimental Neurology,* Dr. Scarisbrick and colleagues demonstrated in postmortem human tissue the contribution of kallikreins to the pathophysiology of spinal cord injury.

Dr. Scarisbrick notes that in cases of disease and spinal cord injury, there is likely to be an early window of opportunity for treatment aimed at halting environmental damage in the CNS. But lab tests indicate that it may also be possible to promote remyelination at sites where damage occurred previously.

"It would make a lot of sense to target these proteases early," Dr. Scarisbrick says. "But we hope there is an opportunity to target the same protease-PAR axis and

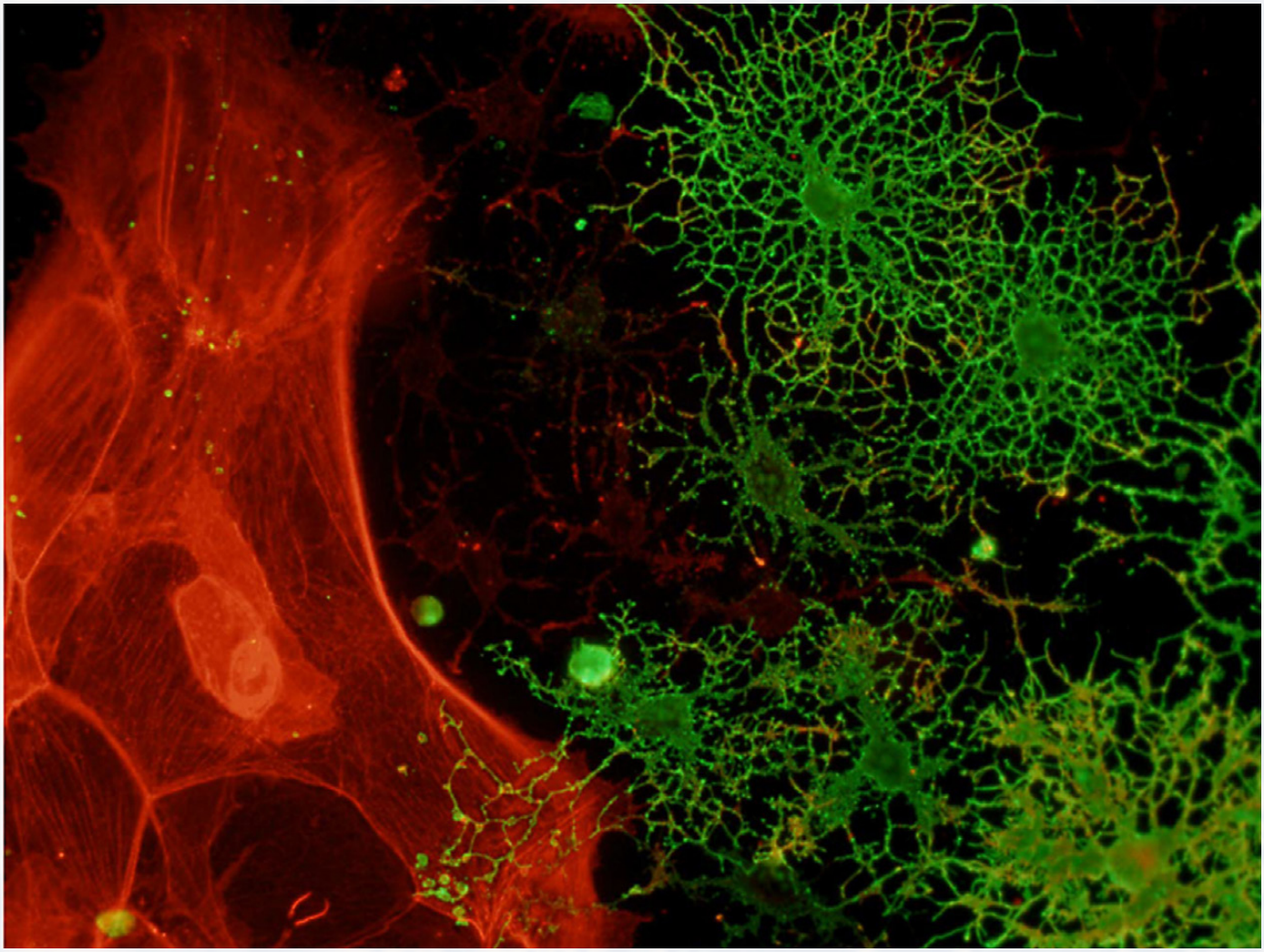


Figure 2. Lab cultures showing myelin-producing oligodendrocytes (stained green) and astrocytes (stained red) derived from a mouse brain. The culture system allows researchers to determine the impact of altered levels of microenvironmental factors, such as kallikreins, at sites of CNS injury. Elevations in KLK6 were shown to impede the ability of oligodendrocytes to extend processes and to wrap and myelinate axons.

promote repair in patients with chronic MS lesions and spinal cord injury."

Although myelin is associated most commonly with MS and spinal cord injury, myelin regeneration has therapeutic applications for other neurological conditions. "The biology that we uncover in an MS lesion looks very similar to what we see in spinal cord injury, and is likely to play parallel roles in stroke and other CNS disorders, as well as glioblastoma multiforme," Dr. Scarisbrick says.

"For conditions as complicated as MS and spinal injury and stroke, I don't think there ever will be a single magic bullet," she adds. "But remyelination is going to be a very important piece of the puzzle."

For more information

Radulovic M, et al. Kallikrein cascades in traumatic spinal cord injury: In vitro evidence for roles in axonopathy and neuron degeneration. *Journal of Neuropathology & Experimental Neurology.* 2013;72:1072.

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Yoon H, et al. Kallikrein 6 signals through PAR1 and PAR2 to promote neuron injury and exacerbate glutamate neurotoxicity. *Journal of Neurochemistry.* 2013;127:283.

Scarisbrick IA, et al. Kallikrein 6 regulates early CNS demyelination in a viral model of multiple sclerosis. *Brain Pathology.* 2012;22:709.

Stem Cell Treatment for ALS

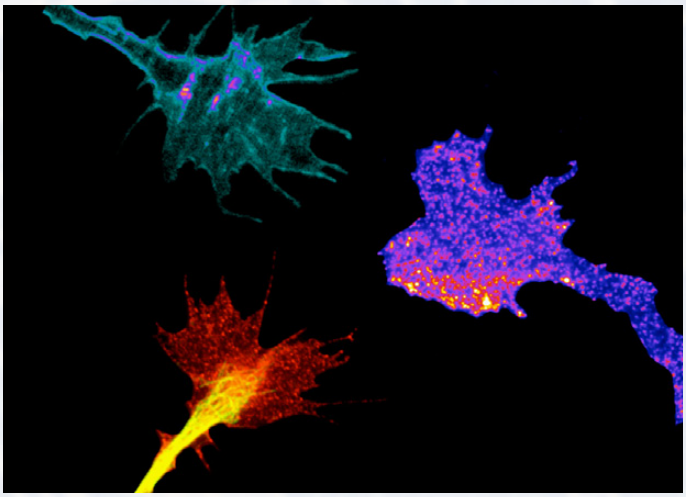
Also called Lou Gehrig's disease, amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motor neurons, causing muscle weakness, paralysis and eventually death. Today, there is no effective treatment. Therapies that showed promise in preclinical models have performed poorly in clinical trials.

Researchers at Mayo Clinic in Rochester, Minn., are testing a different approach: a stem cell-based therapy for ALS. A clinical trial of the therapy, which uses adipose-derived mesenchymal stem cells from the patient's own body to promote neuron regeneration, is underway.

"A very powerful tool in the regenerative medicine kit is the ability to take stem cells from a person's skin or adipose (fat) tissue, and, turn them into stem cells that can do anything. This is opening up a whole new field of healing possibilities," says Anthony J. Windebank, M.D., a neurologist and molecular neuroscientist at Mayo in Minnesota who leads the research.

The clinical safety trial, which began in 2012, involves 25 patients with ALS. A biopsy of adipose tissue is taken from each participant. In the lab, stem cells are isolated from the adipose tissue. Certain stem cells are then selected, based on their ability to produce growth factors. The selected cells are expanded into billions of stem cells, a process that takes several weeks.

"At the end of all this, the population of cells from each patient is making these growth factors that we know have the potential to protect nerve cells," Dr. Windebank says.



Spinal neural growth cones.

Each patient's stem cells are then injected into his or her central nervous system via spinal tap. The stem cells spread through the patient's cerebral spinal fluid and along the membranes that protect the spinal cord and nerve routes. "The stem cells are then in a position to secrete the growth factors that protect nerve cells," Dr. Windebank says.

The study participants have been divided into groups, with each group receiving an escalating dose of stem cells. "So far, we've seen no adverse effects related to the treatment. That really is the main goal of this safety study," Dr. Windebank says. The escalating dosages may provide some indication of efficacy, but those results won't be available for another one to two years.

Additional trial planned

Dr. Windebank's lab already is planning a second study, in collaboration with colleagues at Hadassah Medical Center in Israel. That trial will involve modifying stem cells in the laboratory to enhance their ability to produce nerve growth factors before they are injected back into the patient's spine. Eventually, the researchers plan to investigate modifying genetic factors within the stem cells to further promote neurogenesis in patients with ALS.

"Our goal is to preserve function and, we hope, arrest or slow the progress of this devastating disease," he says.

Mayo's cell-technology platform may have implications for a range of neurodegenerative diseases. "The stem cells can be tailored to make different factors," Dr. Windebank explains. "There may be a factor that's good for ALS or Alzheimer's disease or Parkinson's disease. We think this technology will have a huge impact."

As Dr. Windebank points out, regenerative medicine relies on the body's ability to heal itself. "Almost every organ of the body has some potential to regenerate, and almost always that's through stem cells that actually live in that organ," he says. Through their innovative work, Mayo researchers are making substantial progress toward regenerating nerves that were once considered impossible to save.

Stem Cell Treatment for MSA

Multiple system atrophy (MSA) is a devastating neurodegenerative disorder, with death usually occurring within three years of diagnosis. The hallmark of the disease is glial cytoplasmic inclusions of the protein alpha-synuclein in the brain (Figure), which in turn lead to a cascade of events, including microglial activation, inflammation and neuronal degeneration. Clinically, the cell degeneration causes problems with movement, balance and autonomic functions of the body, such as bladder control and blood pressure regulation.

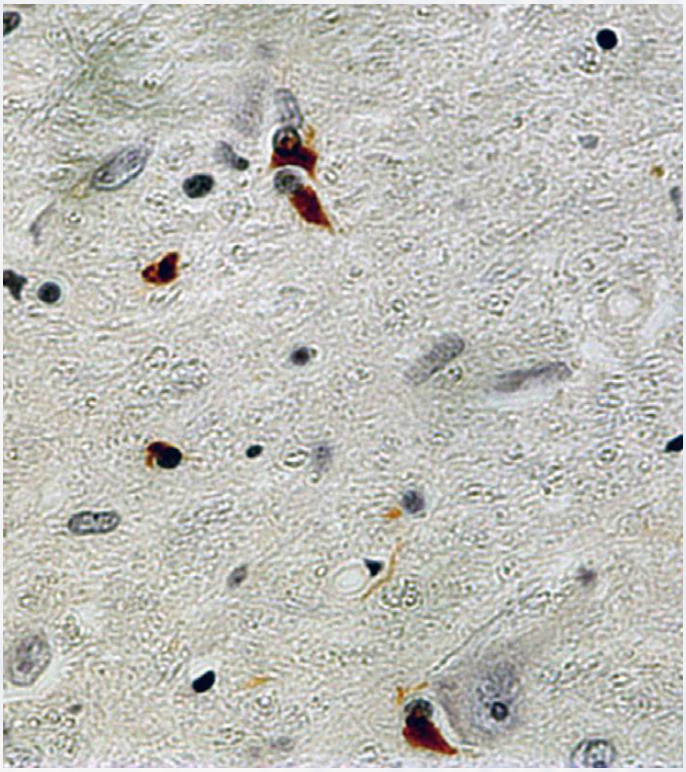


Figure. Post-mortem midbrain image showing glial cytoplasmic inclusions indicative of MSA. Dark staining indicates the presence of alpha-synuclein in glial cells.

Mayo Clinic in Rochester, Minn., has a distinguished history of research on this disorder. Phillip A. Low, M.D., a neurologist at Mayo in Minnesota and founder of Mayo's autonomic testing laboratory, heads a program funded by the National Institutes of Health (NIH) on the cause and treatment of MSA.

Led by Dr. Low and Wolfgang Singer, M.D., Mayo researchers are now conducting a clinical trial of stem cell therapy for MSA. The trial has a major goal of finding ways to slow progression of MSA, possibly by supplementing deficient growth factors such as BDNF and GDNF.

"This initial clinical trial is an important study to determine whether stem cell therapy is safe for treatment of MSA. If it is, we would be in a good position to launch a treatment trial focused specifically on efficacy," Dr. Low says.

Crossing the blood-brain barrier

Previous studies have implicated growth factor deficiencies as a possible cause of MSA. Mayo's cell-technology platform involves the use of a patient's own stem cells to provide and deliver growth factors that promote neurogenesis.

Data from laboratory-animal studies and a clinical trial in South Korea suggest that stem cell therapy can delay progression of MSA. But the Korean trial, which involved infusing stem cells into the carotid arteries of participants, raised concerns in the U.S. about safety and access across the blood-brain barrier. The Mayo researchers hope to improve on that work by injecting stem cells into cerebrospinal fluid.

"We've designed a study that avoids the risks of intercarotid injections and provides a more efficient passage for the stem cells across the blood-brain barrier," Dr. Low says.

The study involves 24 patients being treated for MSA at Mayo. Eight patients will receive a low dose of the stem cell therapy, eight will receive a moderate dose, and eight will receive a high dose. The patients will be monitored for 12 months using clinical scores and MRI morphometry to measure tissue loss.

"Although our study is primarily a safety and tolerability study, we have built into it a component of efficacy by using three different doses," Dr. Low says. "If stem cells are efficacious, we should see a dose-response relationship."

The search for biomarkers

Dr. Low's research has been continuously funded by the NIH for the past 30 years. As a result, much progress has been made in terms of understanding the pathogenesis of MSA. An animal model of the human disease has been developed, allowing researchers to study the fundamental changes that occur in a brain affected by MSA — and even to stop those changes.

"But that ability does not translate well to humans," Dr. Low says. "The reason probably is that in humans, the disease is more advanced than in the animal model.

By the time we see and diagnose patients, they are at the late stage of the disease. We really want to recognize the disease early."

To that end, Dr. Low's research team is developing biomarkers for MSA. They include two substances involved in metabolism — dihydroxyphenylglycine (DHPG) and dihydroxyphenylacetic acid (DOPAC) — whose presence is reduced in MSA to levels lower than those seen in Parkinson's disease. This sophisticated panel of autonomic biomarkers can thus differentiate MSA from Parkinson's disease.

"It looks as though these biomarkers may even be able to detect a relative of MSA called 'pure autonomic failure,' which can evolve into MSA but may take decades to do so," Dr. Low says. "If we can recognize these diseases early, then treatment may be more effective."

For more information

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Peripheral Nerve Repair: Bridging the Gap

Despite advances in microsurgical techniques and instrumentation, functional recovery after nerve repair is far from perfect. Time is the major challenge. Axons regenerate at rates of just 1 to 3 millimeters daily yet must reach their targets across relatively long injury sites. An array of intrinsic cellular and extrinsic molecular mechanisms that inhibit or misdirect axonal projection creates an environment that is hostile to regeneration in the peripheral nervous system (PNS).

Researchers at Mayo Clinic in Rochester, Minn., are pursuing a novel strategy to widen the window of opportunity for optimal axonal regeneration and improve functional recovery after peripheral nerve injury. The research is an outgrowth of Mayo's work demonstrating the ability of stem cells to be re-engineered to produce enhanced growth factors for neurons. Application to the PNS required some means to create a permissive environment that could sustain growth and enable axons to more quickly find and connect with appropriate targets.

"Peripheral nerves can repair themselves, but they need guidance to do it," notes Anthony J. Windebank, M.D., a neurologist and molecular neuroscientist at Mayo in Minnesota who has led the research underlying the stem cell technology platform.

A growth scaffold for neurons

Mayo researchers have developed just such a guidance system — synthetic tubing that provides a biodegradable scaffold between severed axons (Figure). Within the tube, neural growth factors, signaling molecules and

Figure. Synthetic tubing being implanted in the sciatic nerve of a lab animal.

guidance cues can sustain new growth and axonal projection. The structure also provides physical channels through which axons can extend more readily, helping to prevent undirected peripheral nerves from forming neuromas. The scaffold degrades naturally when axons reconnect, a process that can take weeks to months.

The scaffold will soon enter a clinical trial at Mayo in Minnesota to determine its safety for human use. Robert J. Spinner, M.D., a neurosurgeon with peripheral nerve expertise, is implanting the scaffold in 20 patients who require nerve biopsy.

"We are translating the basic science done at Mayo into clinical applications. From the clinical perspective, this model of care is very elegant," Dr. Spinner says.

Bridging the injured nerve site with an artificial structure has potential not only to improve nerve growth but also to avoid morbidity associated with current treatment options, which generally involve taking a nerve graft from elsewhere in the patient's body. "The ramifications are huge," Dr. Spinner says.

Treating injured veterans

The genesis of this Mayo work was the injuries sustained by military personnel from improvised explosive devices in Afghanistan and Iraq. Michael J. Yaszemski, M.D., Ph.D., an orthopedic surgeon and biomedical engineer at Mayo in Minnesota, is a brigadier general in the U.S. Air Force Reserves. As a deputy commander of the hospital at Balad Air Base north of Baghdad, Dr. Yaszemski had direct experience with the extensive limb wounds of soldiers in Iraq and Afghanistan. He and Dr. Windebank have served as co-directors for nerve injury research in the Armed Forces Institute of Regenerative Medicine, a Department of Defense-funded consortium of 16 institutions to generate new treatments for war-wounded people.

The synthetic tubing was developed in Dr. Yaszemski's laboratory. Made of a copolymer called polycaprolactone fumarate, the structure joins two compatible polymers never before brought together. Dr. Windebank notes that Mayo has the capacity to build the tubing in-house rather than licensing the technology to an outside company — a further example of the breadth of Mayo's expertise in regenerative medicine.

Collaboration on animal models

Another unique aspect of this project is Mayo's collaboration with other centers on the development of an animal model for pre-clinical testing. Scientists at Massachusetts General Hospital, Massachusetts Institute of Technology, Cleveland Clinic and Rutgers University submitted their work in this area to Mayo researchers, who added their own and then tested various models to find the most successful one. The results were independently validated.

"This means of collaboration between laboratories has never been done before," Dr. Windebank says. "It really pushes the field forward when people can work together like that."

Widening the gap

Existing technology can promote growth of small sensory nerves, such as those in fingers. "These injury sites present with relatively small gaps; in general, up to 3-centimeter defects can be reconstructed with current technology," Dr. Spinner says. The clinical trial at Mayo is a safety trial involving a slightly bigger skin nerve in the leg and a 6-centimeter gap.

"Sensory nerve function is important — it provides feeling in the fingers, for example — but the bigger demand is motor function," Dr. Spinner notes. "Those nerve injuries, which can result from car accidents, for example, involve bigger nerves and have very long gaps, at times 15 centimeters or more. In the longer term, we hope to be able to use this type of treatment for injuries to major motor nerves. It's an exciting future."

For more information

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Vascular Regenerative Therapy for Alzheimer's and Parkinson's

Alzheimer's disease (AD) is the major cause of dementia in the elderly, with progressive loss of neurons in areas of the brain responsible for learning and memory. The accumulation, aggregation and deposition of beta-amyloid and tau in the brain are central events in the pathogenesis of AD. In late-onset AD, which accounts for more than 99 percent of cases, the clearance of beta-amyloid from the brain is impaired.

Characterized in the early stages by loss of motor function, Parkinson's disease (PD) is the second most common neurodegenerative disorder after AD. The accumulation of the protein alpha-synuclein in the brain is the central event in the pathogenesis of PD.

For both diseases, an important focus of research is identifying the processes that cause neurodegeneration. The role of the cerebrovascular system is of particular interest (Figures 1 and 2). Cardiovascular disease is a common comorbidity of PD. In addition, population-based epidemiological studies have shown that cerebrovascular damages are strong risk factors for AD. Decreased cerebral blood flow can be detected before the onset of AD, and the levels of circulating vascular progenitor cells are decreased in AD patients.

"Any kind of vascular defect reduces the blood flow to the brain, which can compromise neuronal health," notes Guojun Bu, Ph.D., a molecular neuroscientist at Mayo Clinic in Jacksonville, Fla.

He and Leonard Petrucelli, Ph.D., also a molecular neuroscientist at Mayo in Florida, are investigating whether stem cell technology can improve cerebrovascular function and thus slow or prevent neurodegeneration. Their innovative work represents an additional approach — alongside Mayo's efforts to regenerate neurons — to finding regenerative medicine therapies for neurodegenerative disease.

"Our work focuses on replacement therapy — using patient-derived stem cells to replace, in this particular case, damaged vascular cells," Dr. Bu says. "It is similar to the technology used for angiogenesis from stem cells."

Dr. Bu and Dr. Petrucelli direct laboratories that have

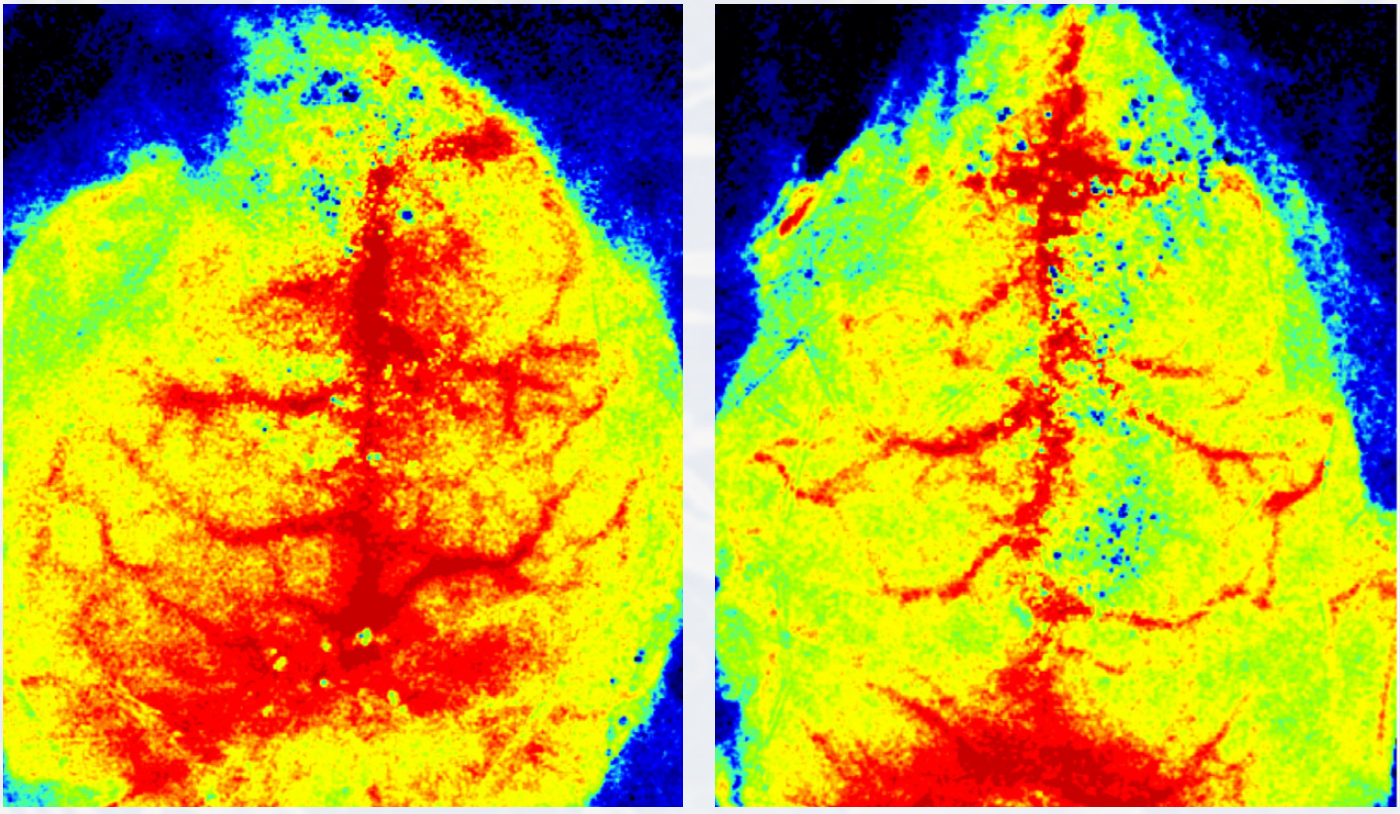

Figure 1. Reduced blood flow to the brain in a laboratory model of Alzheimer's disease. On the left, blood flow to a normal mouse brain. On the right, blood flow to the brain of a mouse model of Alzheimer's disease.

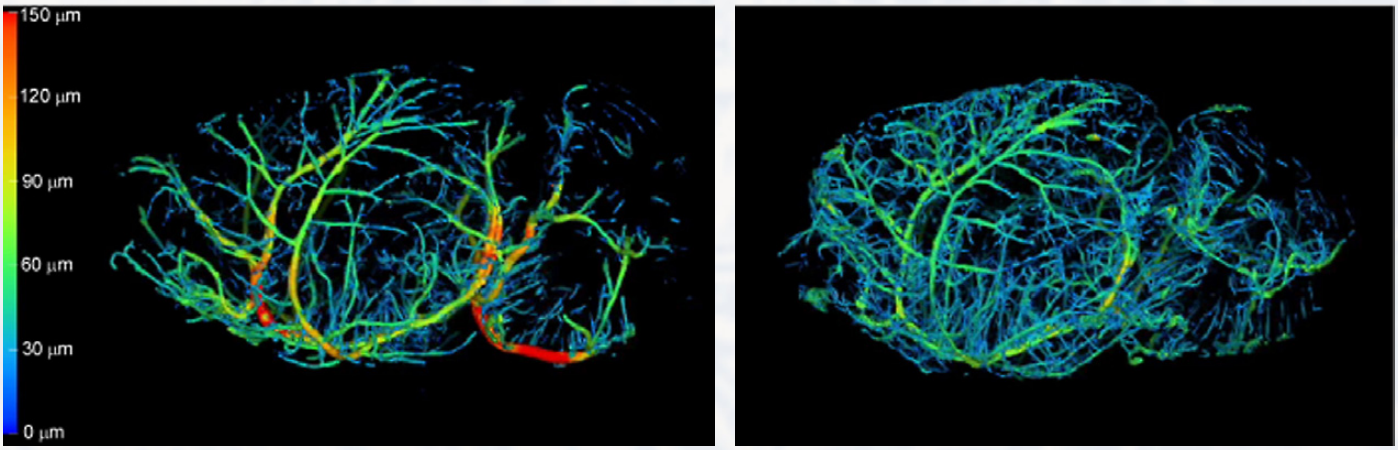

Figure 2. Altered brain vascular structure in a laboratory model of Alzheimer's disease. On the left, vascular structure in a normal mouse brain. On the right, vascular structure in the brain of a mouse model of Alzheimer's disease.

made significant discoveries concerning the cellular mechanisms that cause neurodegenerative diseases, including AD, PD, amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration. "This research follows the Mayo mission of integrated research and clinical practice," Dr. Petrucelli says. "Only when we understand pathways can we design therapy."

Preclinical studies

The current vascular regeneration research involves transplanting induced pluripotent stem cell (iPSC)-derived vascular progenitor cells into AD mouse models. Similar laboratory studies will be conducted in PD animal models.

This novel work is possible because of the rapid strides made in recent years in the field of stem cell biology, driven by laboratory discoveries concerning reprogramming technology. The Mayo researchers use iPS cells converted from skin fibroblasts through the transduction of four transcription factors.

"We have developed a very effective technique in the stem cell laboratory to differentiate the fibroblasts into iPS cells. Those stem cells can be further differentiated into neurons or different types of vascular cells," Dr. Bu says.

Over the next six months, the researchers hope to perfect a technique for injecting the progenitor cells into different brain regions in the laboratory mice. "Following that, the next step will be to examine whether the injected stem cells help restore blood flow and also memory function in different mouse models," Dr. Bu says.

Although Mayo already is conducting clinical trials of stem cell treatment for ALS, stem cell therapies for AD and PD present unique challenges. The synapses in brain areas associated with AD and PD are much more complex than those in the spinal cord that are associated with ALS.

"There also are multiple brain pathologies involved in Alzheimer's, including tau, beta-amyloid and TAR DNA-binding protein 43," Dr. Petrucelli says. "As a result, we will need at least five to 10 years before starting clinical trials of vascular regenerative therapy."

Identifying drug candidates

Stem cell research at Mayo in Florida also has direct applications for the development of novel therapies for neurodegenerative diseases. Researchers in the stem cell laboratory are developing stem cells from patients with neurodegenerative diseases — ranging from AD to rare conditions — to identify compounds that prevent the formation of neurotoxins.

"We are committed to developing and discovering new therapeutics to target and treat these devastating conditions," Dr. Petrucelli says. "We also are developing and characterizing novel biomarker assays, which we expect will provide ways to test new treatments once they become available."

For more information

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Oncology and Neuroregenerative Research

Every year, approximately 23,000 people in the U.S. are diagnosed with glioma — a tumor that originates in the brain or spine. In most of these cases, the tumor is a glioblastoma, the most aggressive brain cancer, and the prognosis is generally poor. However, other types of brain tumors, including oligodendroglioma and astrocytoma, have a much better prognosis.

At Mayo Clinic in Rochester, Minn., researchers are using regenerative medicine to study why brain tumors behave so differently. "We are interested in the mutations that are involved in the development of each of these different tumor types. Our research data are then used to develop better ways to diagnose, treat and monitor patients with cancer," says Robert B. Jenkins, M.D., Ph.D., a consultant in laboratory medicine and pathology at Mayo in Minnesota.

A single aberration

Dr. Jenkins' lab has discovered several specific locations in the genetic code where alterations predispose people to various types of tumors. Notably, in a study published in the October 2012 issue of *Nature Genetics*, the researchers reported that a single aberration on the 8q24 locus translates to a roughly sixfold-higher risk of low-grade gliomas, including oligodendrogliomas.

"We now know that the predisposition allele — the alteration on 8q24 — predicts patient survival and response to chemo- and radiation therapy," Dr. Jenkins says. "Being able to share that information with patients is very helpful."

To further improve diagnosis and treatment, the researchers are working to learn more about the functioning of the 8q24 alteration — precisely how it predisposes people to less aggressive but still fatal gliomas. Neuroregenerative medicine techniques are key in this effort. Mouse and human neural stem cells, and human induced pluripotent stem cells (iPS), are used to investigate how the 8q24 alteration modifies the development of glial cells. "The modifications must act in concert with other alterations to increase the risk of cancer development in the brain," Dr. Jenkins says.

Stem cells for these experiments are provided by the Regenerative Medicine Biotrust, which enables the Center for Regenerative Medicine at Mayo Clinic to collect, process and store cells and other biospecimens from individual patients. For selected brain tumor patients, the biotrust will generate iPS cell lines. The regenerative medicine center also assists Dr. Jenkins in differentiating iPS cells down the neural developmental pathway. "The biotrust is critical for the success of our experiments," Dr. Jenkins says.

For more information

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Pilot Study of DBS for Dementia

Mayo Clinic in Jacksonville, Fla., pioneered the use of deep brain stimulation (DBS) in the U.S. in 1997. Since then, Mayo's DBS practice has spread to all three Mayo Clinic campuses and is one of the largest neurostimulation practices in the world. At Mayo, DBS is used to treat patients with essential tremor, Parkinson's disease (Figure) and dystonia, as well as obsessive-compulsive disorder, cluster headaches, Tourette syndrome, epilepsy and chronic pain.

Researchers at Mayo in Florida have started a small pilot study that may determine if DBS has positive effects in lessening cognitive decline in patients with Parkinson's

disease. The study involves dual hemispheric stimulation of the globus pallidus (GPi) or subthalamic nucleus (STN), and the region of the fornix and hypothalamus. If the study yields positive data, Mayo researchers will consider the potential of using DBS as a treatment for dementia in Parkinson's disease and Alzheimer's disease.

"DBS targets for Parkinson's disease have been limited to neuronal regions where surgical lesions have produced benefits in motor function," says Ryan J. Uitti, M.D., a neurologist at Mayo in Florida who leads the study. "But cognitive decline remains commonplace in patients after STN-DBS treatment for Parkinson's disease, including significant declines in nonverbal memory, semantic fluency and processing speed."

Parkinson's disease is the second most prevalent neurodegenerative disorder after Alzheimer's disease. An estimated 1 million people in the U.S. have Parkinson's disease, and roughly 40 percent develop dementia. Along with progressive deficits in attention and executive function, complex visual hallucinations frequently occur, and may be accompanied by rapid eye movement (REM) sleep behavior disorder.

"Dementia typically ends up being the most disabling aspect of Parkinson's disease," Dr. Uitti says. "This type of dementia is peculiar in that it often fluctuates, with a patient having fairly normal days or much worse periods."

Tandem DBS

Dr. Uitti became intrigued by anecdotal and initial-trial reports indicating that DBS to the region of the fornix and hypotha-lamus may improve memory function. In animal models, DBS has been associated with increased neurogenesis in the hippocampus. In a small study of patients with Alzheimer's disease, DBS was associated with improved memory function.

"The results of these cases really can't be definitive. But some of the patients don't seem to be declining as quickly as expected," Dr. Uitti says.

In a paper in the January 2012 supplementary issue of *Parkinsonism & Related Disorders*, Dr. Uitti hypoth-esized that "tandem DBS" targeting both STN or GPi and the region of the fornix and hypothalamus may improve both motor and cognitive function, immediately and longer term. The subsequent study, done in collaboration with Robert E. Wharen Jr., M.D., a neurosurgeon at Mayo in Florida, involves six patients undergoing DBS for Parkinson's disease.

Neuropsychological data is obtained prior to surgery. During surgery an electrode is inserted in the GPi or STN, and a second electrode is placed in the region of the fornix and hypothalamus. The patients will have follow-up examinations and neuropsychological testing for three years after surgery to assess cognitive decline.

Improvements in DBS technology allow both electrodes to operate from a single battery. The surgical procedures are done in an intraoperative MRI operating suite, allowing direct visualization of electrode placement during surgery and contributing to accurate placement.

The pilot study exemplifies Mayo's commitment to pursuing a diverse approach to regenerative medicine. Notes Dr. Uitti: "Given the tremendous disability produced by dementia, new structural targets require systematic study."

For more information

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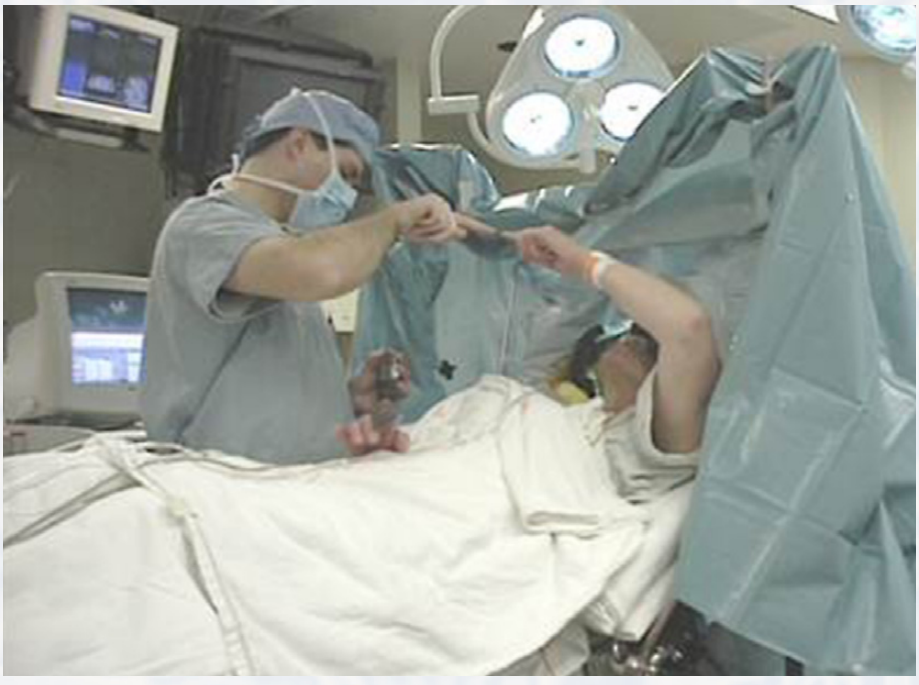

Figure. Patient undergoing DBS for Parkinson's disease.

Reanimating Limbs Through Electronic Technology

Much of the regenerative medicine research at Mayo Clinic focuses on potential biological solutions — growing new nerves to heal injured spinal cords or peripheral nerves or to slow the progression of neurodegenerative diseases. But Mayo's comprehensive approach to regenerative medicine includes electronic device-based therapies, deep brain stimulation (DBS) being the best-known example.

Building on DBS technology, researchers at Mayo Clinic in Rochester, Minn., are exploring the use of electronic devices that can wirelessly transmit signals from the brain to an injured spinal cord. In this novel approach, injured nerves aren't repaired or regrown but bypassed with electronics. Early test results through collaboration with the Division of Engineering and the Neural Engineering Laboratory are encouraging: The limbs of laboratory animals with spinal cord injuries have been successfully reanimated.

"We have created neurostimulators that allow us to control various muscles," says Kevin E. Bennet, assistant professor of neurosurgery and chair of the Division of Engineering at Mayo in Minnesota. "The stimulators are programmable, so we can generate a variable response. By modifying the signals, we can actually tell the muscles to move the leg slowly, hold it still or move it faster. We can move the legs in synchrony."

Bridging the information gap

Like other regenerative medicine efforts at Mayo, this work was inspired partly by the injuries sustained by U.S. military personnel. Kendall H. Lee, M.D., Ph.D., a neurosurgeon at Mayo in Minnesota and director of the Neural Engineering Laboratory, serves in the U.S. Navy, where he has direct experience with spinal cord injuries resulting in paralysis. Another major cause of spinal injury and paralysis is motor vehicle accidents or other trauma.

"Whether military or otherwise, these injuries often occur in young adults," professor Bennet notes. "The problem is an information-conduction defect. All of the actuators of limb movement are there. The patient's brain knows what needs to be done and is sending signals. The muscles are capable of working. But because of the defect in the spinal cord, the signals from the brain aren't getting through. We are trying to bridge that information gap."

Doing so is a highly complex process. In laboratory tests, the Mayo researchers are determining what types of information from the brain must be electronically "injected" into the injured spinal cord and where. "We basically are mapping the spinal cord," professor Bennet says.

Another focus is developing integrated circuits so that the neurotransmitter devices are small enough to be implanted in the brain yet have sufficient capacity to provide the range of function needed to reanimate limbs. "Many channels of information must be provided to the leg or arm to make all of the complex movements a person normally makes," professor Bennet says.

Finding solutions in-house

One major challenge of Mayo's device-based regenerative medicine efforts was developing electrodes that could be implanted in the brain and spinal cord long term. Initially, the researchers utilized a carbon-fiber microelectrode, only to discover that it dissolved within three days of implantation. Diamond was identified as the preferred alternative; it has the necessary atomic structure, and certain diamonds are electrically conductive.

Unable to find an outside collaborator to manufacture the needed diamond, Mayo engineers built their own reactor. "Four hours after we turned on the reactor, we had our first diamond," professor Bennet says. "That's the benefit of having an embedded engineering organization at Mayo. We have electrical engineers, mechanical engineers, software engineers and chemical engineers, all working together to create things that aren't usually done in a single center."

Although Mayo's limb reanimation research is in its early stages, the prospects are intriguing. Recent advances in the interface between neurobiology, computational power and sensor technology "give the world opportunities we've never had before," professor Bennet says. "I think that we are in a golden era, when all these advances are coalescing and allowing us to do what couldn't be previously imagined."

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