PROGRESS in RESEARCH

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Stanford Scientist Ben Barres Joins Reeve Research Consortium

Ben Barres, M.D., Ph.D. is the newest member of the Reeve Foundation International Research Consortium on Spinal Cord Injury. Dr. Barres, a neuroscientist on the faculty of Stanford University, was formally introduced to the Consortium at a recent meeting in Cambridge. "I am thrilled to be joining the Consortium," he said. "It's a wonderful group of scientists; they are all very interactive and there is clearly a high level of trust among the investigators. We've already discussed several potential collaborations with various labs." Dr. Barres spoke to Reeve staffer Sam Maddox about his work and how his lab might fit into the collaborative model of the Consortium.

Sam Maddox

Ben Barres, M.D., Ph.D.

Q. While your work hasn't been directed to spinal cord injury *per se*, you are well-known to the greater neuroscience field.
Ben Barres: I've been a neuroscientist my entire career so, yes, I know all of the members of the Consortium. What I'm known for, my lab, is neuroglial reactions and glial cells. We have in recent years come to understand what a critical role glia play in regenerative failure. So I think that's why I was asked to join. As collaborative groups evolve, you pick people with different expertise; that way there's better brainstorming. You put those brains together and start talking and ideas fly.

Q. We'll get back to the science; first let me ask you about your own career, you always wanted to be a physician first?
Ben Barres: I was at MIT, I was going to be a computer scientist or a chemist. But then I took this course by Hans-Lukas Teuber who was – they called themselves psychologists or brain scientists back then. He taught about brain function in the injured brain. From the second I took that course, I think I was a sophomore, I was just hooked on neurobiology. I got the idea that it would be fun to study the brain and that by being a physician, I could learn more about the brain. So I went to med school. I did my training in a very unusual order compared to most M.D. Ph.Ds. I finished my complete medical training and my entire internship and residency, and became board certified in neurology. And then I went back to graduate school and did a seven-year Ph.D. at Harvard, and a three year post-doc at University College in London. But as soon as I got in the lab and discovered research I never went back to seeing patients again. One of the things that compelled me to leave neurology more than anything was working with quadriplegic patients and realizing that I was helpless to do anything for them.

Q: And so you've been at Stanford what, almost 20 years?
Ben Barres: Yes. After London I came straight here, in 1993. I feel lucky every day to be at Stanford. What makes it great is not only the remarkable faculty, of course that's wonderful, but just the quality of the students and postdocs. I take graduate students in my lab for my neuroscience Ph.D. program; we typically have over 500 applicants a year and we take about 10 of them; you can imagine what the competition is like. I always tell the students, when I was their age, I couldn't even get an interview at this Ph.D. program or medical school; here I am now, a professor teaching these guys who are way better than I am; I'm very lucky.

Q. What attracted you to glial cells?
Ben Barres: I learned about astrocytes, that they were half the cells in the human brain.

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INSIDE

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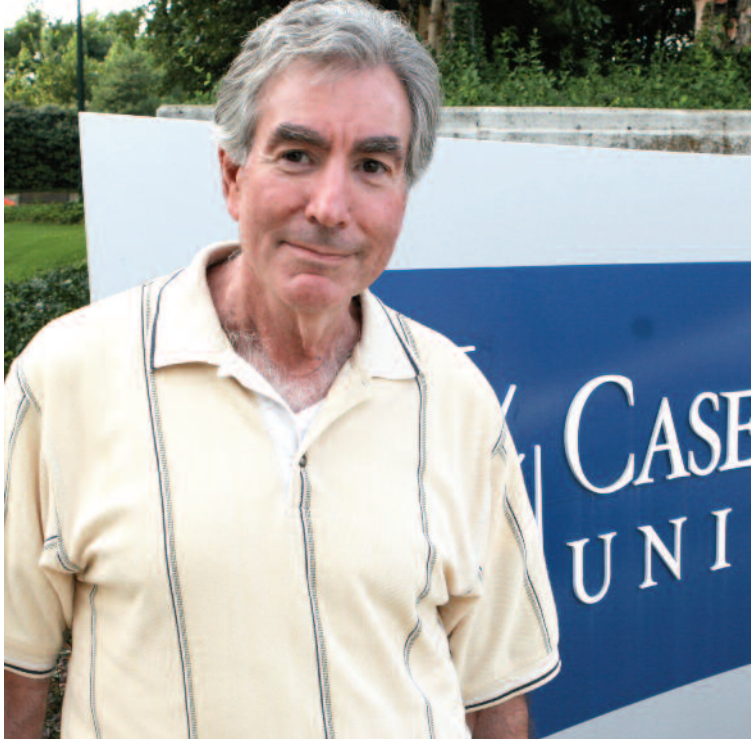
TODAY'S CARE. TOMORROW'S CURE.

Jerry Silver:
Cutting to the Ch'ase

By Sam Maddox



Sam Maddox

Jerry Silver, Ph.D.

Trauma to the spinal cord destroys some nerves, damages others. The survivors try to recover. They can't; they become disoriented, mired in poisonous chaos and hemmed in by a fibrous net of scar. They are really stuck. One of the core issues in neuroscience is to get them unstuck and functional. But even if they can be teased away from the injury site, will they ever again find a proper target, or conduct a proper signal? Spinal cord regeneration is complicated beyond our comprehension.

Case Western Reserve scientist Jerry Silver, a longtime member of the Science Advisory Council for the Reeve Foundation, has been working on the stuck-nerve problem for better than 30 years. He's well aware how daunting the task is. But he got to thinking one day, what if, instead of trying to bulldoze through all the cellular mayhem at the site of injury, what if a different approach were used? Rather than attack the tortured infrastructure within the cord itself, how about trying to sneak around it?

Silver, a native Clevelander, likes traffic analogies. Start with the tip of a nerve, the growth cone: "Imagine a growth cone being like a car on a highway: The cone is lost, chugging along on crummy fuel with a lousy motor and bad tires. The conditions are icy and it's sliding around all over the place. Suddenly though the cone makes contact with a mat somebody has thrown out on the ice." Silver explains that the mat might be the equivalent of a cellular treatment, an implanted Schwann cell, for example. "There's now some traction: the cone can move a bit but macrophages [immune commando cells] threaten to attack it if it moves off the mat. So – what to do? You could throw in lots of other mats and get rid of the macrophages so the cone could more or less move from mat to mat. But on the periphery is a barbed wire fence – the scar."

So, mused Silver, maybe we can ramp up the motor or do something to civilize that nasty environment. There's still that barrier. But wait, he says. Looks like there may be a way out: Detour ahead.

That's the oversimplified concept behind an important study Silver and colleagues published recently. The work was partly sponsored by the Reeve Foundation.

Using a cervical injury rodent model, significant breathing function was restored using a surgical detour – by stitching in a half-inch piece of the host animal's peripheral nerve above and below the lesion site on the outside of the cord. In effect, Silver's team created an escape route for axons stuck in traffic above the injury to get around the problem area and resume their connection to the diaphragm. To cut open a pathway for the axons to exit the cord and merge into the graft, Silver's team used a chemical bolt cutter, the enzyme drug chondroitinase (ch'ase).

This study is important because two repair strategies were combined to enhance recovery in the complex circuitry that controls breathing; the results were published as an article in the top-tier journal *Nature*. The nerve implants restored normal or near-normal breathing in nine of 11 test animals. "It's pretty amazing," said Silver. "Our work is to-date one of the most convincing demonstrations of the return of robust function after paralysis."

It's also important to note that if the outside detour bridge is cut, the effect vanishes; the animals revert to their previous, single-lung activity. That shows that the repair of breathing was indeed due to regenerating nerves originating above the injury.

Silver emphasized that to this point, the detour protocol does not dramatically affect the animals' ability to walk, even though there is some locomotor improvement. "Although there are axons regenerated from above the lesion to below the lesion, there's no evidence of anything extraordinarily interesting in terms of their walking behavior. Walking is a different ballgame."

A major paper in the journal *Science* in 1996 by Henrich Cheng and Lars Olson reported restored walking after a peripheral nerve graft similar to that of Silver's group (without ch'ase). "Cheng and Olson had originally said that animals can walk. It made them very famous. It was big. Ours is basically the Cheng and Olson strategy with chondroitinase and some improvements. The animals don't walk. Walking involves the entire body. It's head balanced on the neck and it's alternating steps. It's walking over uneven surfaces with different compositions. It's so complicated."

Silver doesn't think a graft or even a series of grafts around the lesion is the most likely strategy, at least not without further modifications and additional intensive rehabilitation, for ambulation.

Silver and his team, pondering which other clinically rel-

evant muscle group would have a major impact on people with paralysis, have now applied the detour strategy to the bladder. Said Silver, "From what I read, most spinal cord injured people put at the top of their list being able to control their urine."

In a recent presentation to the Society for Neuroscience, Silver's team (including Yu-Shang Lee of the Cleveland Clinic) demonstrated significant improvement in bladder function in a group of animals that had a peripheral nerve bridge (again with ch'ase and also a growth-promoting substance called FGF) around a complete thoracic spinal cord lesion. He calls the treatment the "full Monty."

"It's really remarkable. While the recovery is not perfect it is amazingly improved; you still get periods of abnormally increased bladder pressure and less than maximally efficient urination, but the activity of the sphincter and other urodynamic parameters are really nicely fixed. It's not normal, but it's patterned. We have not yet examined sensory function but this new graft strategy could, indeed, allow for sensory regeneration. I've got to tell you, I'm really psyched."

What about stitching in peripheral nerve bridges to affect hand function? Silver thinks this might work to a limited extent, but again, rehab will be an important component going forward. "I'm thinking maybe two bridges, one each to the major extensors and flexors could give you function; I'm not saying pick up a grain of rice, but maybe grab a cup, you know, or a doorknob or something crude."

Silver has focused his career on glial cells – once thought of as support cells for the "more important" neuron cells. But as glia become better understood, it is evident they are much more important in the function, non-function, and repair of the spinal cord. One very important glial response to injury is the formation of a barrier or scar that axons can't penetrate – Silver's barbed wire. His work with ch'ase, which in essence digests the scar, has opened up exciting possibilities in numerous labs in the U.S. and abroad for possible regeneration in the spinal cord. *(See sidebar for other ch'ase work supported by the Reeve Foundation.)*

Silver notes that his recent results are built on 30 years of work. "I'm one of the first persons ever to ask the question, 'Why don't axons grow where they don't?' Everybody else was asking, 'Why do axons grow where they do?'" He began to look at proteoglycans, barrier molecules known to be inhibitory in development. There was some literature about these inhibitors in cartilage, which because of proteoglycans is not innervated.

"But the most convincing paper that I read that proteoglycans are really inhibitory is one where they asked the question, 'Why doesn't the placenta eat the uterus?' Wow. That's a cool question. The placenta is a highly invasive tissue, but it only invades the surface of the uterus from the inside, not the whole thing. And the question is, why? Turns out in this paper proteoglycans are in high abundance in the stroma of the uterus. And if you get rid of the proteoglycans with certain enzymes, the placenta takes over the whole uterus, and will kill the mother. I mean, we owe our existence to proteogylcans. Wow. That's potently inhibitory. I thought, maybe I ought to look for them in the nervous system."

Silver said the problem studying proteoglycans was that until 1990 there were no antibodies available that could detect them. Three years later, Silver was first to confirm their presence in the glial scars of rats; he has since then been nearly obsessed with removing proteoglycans and thus promoting regeneration. He won both the Ameritec Award and the Reeve-Irvine Research Medal for this work.

Silver has for many years studied the enzyme chondroitinase, which breaks down proteoglycans. This led to the work with peripheral nerve detours, alongside John Houle's group at Drexel University. In 2006 they worked up an animal model for a nerve bypass. It worked; the animals had better use of a paw. "I thought that was one of the first and best demonstrations of long distance functional regeneration. But it wasn't walking. It wasn't full hand function. It was just really wrist extension. It looks kind of cool when they do it, but they can't use their toes. They can't groom."

Silver said that's when he decided "to tackle this whole regeneration problem at a more simple level. I asked the

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Reeve Foundation Ch'ase Projects

The Reeve Foundation has funded several projects involving the enzyme chondroitinase (ch'ase) to dissolve proteogycans and promote spinal cord regeneration.

The James Fawcett lab at the University of Cambridge, in England, part of the Reeve Foundation International Research Consortium on Spinal Cord Injury, showed that ch'ase digests proteoglycans. This removes inhibition, but also stimulates nerve growth. The Fawcett lab took out a patent on chrondroitinase for axon plasticity; Acorda Therapeutics licensed the molecule for potential clinical use. Fawcett hopes to move forward for a clinical trial in the not-too-distant future.

Here are four other individual investigator grants funded by the Foundation:

BinQuan Zhuang, Ph.D., Linda C. Hsieh-Wilson, Ph.D., California Institute of Technology, Pasadena, CA. The lab discovered a specific structural pattern on chondroitin sulfate that is responsible for its inhibitory role. Masking this structure promotes regeneration of injured optic nerves.

John G. Flanagan, Ph.D., Harvard Medical School, Boston, MA. The Flanagan lab recently identified a receptor for chondroitin sulfate and hypothesized that it will be possible to neutralize it and thus promote spinal cord regeneration.

Shuxin Li, M.D., Ph.D., UT Southwestern Medical Center at Dallas. The Li lab hypothesizes that small molecules might be used to block rather than digest proteoglycan inhibitors.

Charles H. Tator, M.D., Ph.D., The Toronto Western Hospital Research Institute, Toronto, ON, Canada. This project examines a combination of treatments to repair the injured spinal cord in the chronic stage: transplanted chitosan guidance channels, spinal cord-derived neural stem cell progenitors, scaffolds made of a fibrin substance, plus ch'ase injected at the ends of the channels.

Barres Lab Joins the Consortium

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People really didn't know what they did, they thought they were kind of passive work cells that mopped up after the neurons and really weren't that interesting. But because of my neuropathology training, what I noticed was that the astrocytes are always very involved in disease processes; any kind of disease or injury to the brain causes the astrocytes to undergo incredible morphological changes, changes in their molecular properties, changes in gene expression. And so I just couldn't help but be curious about whether astrocytes are beneficial in injury or whether the changes were actually harming or adding to the injury process.

Q: Glia – that's what you're known for?
Ben Barres: One of the biggest discoveries my lab is known for is developing purified specific brain cells: neurons, astrocytes, oligodendrocytes. We've purified all the major classes of brain cells, the microglia. Now we're doing reactive astrocytes and cell types after injury. The brain is a complex mixture of cells and they're all talking to each other. Our approach is to purify the cells and then study how they're interacting with each other in a culture dish. And then once you define those signals, you can manipulate them in an animal model.

We figured out how to separate the neurons from the glia, which include the astrocytes and the oligodendrocytes; this had never been done before. There were two main problems. One was getting utter purity, the other keeping the pure cells alive in a culture dish. It turns out that cells of different types are constantly signaling each other not to die. For example, as soon as you get neurons away from the glia, the neurons instantly undergo cell death – the glia send signals to keep them alive, and vice-versa. All the textbooks said that glia don't require survival signals; that wasn't true. When we purified the astrocytes, just like the neurons, they died as quickly without signals coming back from neurons. We have a paper in press now showing that astrocytes get their survival signals from blood vessels.

We got very interested in this question: Are glial cells doing other things, more active things, at synapses [the branched structure that allows passage of electrical/chemical signals between neurons or other cells].To get at that, we purified neurons and asked, "Okay, what can these neurons do by themselves and what, if anything, do they need glial cells for?" What we found is that neurons could pretty much do most things for themselves: They retained their morphology as neurons; they retained their polarization so they could still make dendrites and axons; they were still excitable and able to initiate action potentials and so forth. They looked pretty normal. But the one thing that we discovered, to our amazement, because we would never have predicted this in a million years, is that the neurons are completely unable to form synapses without glial cells. They can't hook-up, they can't wire-up, they can't connect. That was first a culture observation, and of course we wondered is that also true *in vivo*. And so to get at that we needed to figure out what the signals were; in other words, what are the astrocytes creating that tell the neurons to form synapses?

Q. That's what astrocytes are good at?
Ben Barres: This is what I believe astrocytes do: They control every aspect of synapse formation, synapse function, synapse maintenance, and even synapse elimination. We've spent the last 10 years biochemically identifying the exact molecular identity of the signals astrocytes release that tell the neurons to form synapses, to make the synapses function. The first signals we showed were a family of molecules called thrombospondins. They are very specifically made by astrocytes, particularly in the developing brain. They reappear after injury, which is very interesting, for repair of synapses.

Thrombospondins were the first known proteins that actually induced synapse formation between neurons. Everybody thought neurons could do it by themselves; they can't. There's molecular machinery in the neurons necessary to form the synapse, but the neurons need a signal to tell them to put all that machinery together.

Q: So what does it mean? Take this in a clinical direction.
Ben Barres: Everything I do is because I'm interested in neurological disease. To me this is all about the clinic. In the case of glia, the relevance is first of all, whenever you have a disease, you're losing synapses; synaptic connections are falling apart. You want to rebuild those synaptic connections. The signals that astrocytes make and the targets they interact with are all new drug targets for repairing synapses and controlling their function. I would like to have better memory now that I'm middle-aged. Well, by understanding how our brains form synapses and stronger synapses, these are all potential new drug targets for memory improvement.

After a stroke or a spinal cord injury, the Holy Grail for a long time has been "How do you get the axons to regenerate?" People are starting to come up with ways to do that now. There's still a lot of work to be done. But the writing is on the wall now that this is probably going to be doable. So now the big question is, when you get those axons back, are they going to make the right synapse – are they going to make synapses at all? And if they do make synapses, are they going to make the specific connections they're supposed to make? That's completely unknown.

The other thing is that neurodegenerative disease is all about synapse loss, synapse degeneration. Alzheimer's disease for example, is a disease of massive synapse loss. Parkinson's disease, glaucoma, these are all diseases where you have massive synapse loss. If a neuron loses enough synapses, the neuron itself dies. And we've provided some evidence that glia are actually intimately involved in the synapse degeneration process. And so we think that glia will be targets to block certain neurodegenerative disease.

And one last example of the connection to disease comes back to the thrombospondins. We wanted to figure out how the thrombospondins can tell neurons to form synapses. If you have neurons in a culture dish and you throw in thrombospondins, they form synapses. Therefore, there has to be a receptor, a thrombospondin receptor, on the neurons. And so we spent about four years figuring out what that was; we were surprised that it turned out to be a protein called Alpha-2-Delta-1, a well-described

COLLABORATION

The BARRES LAB is known for studying glial cells and how they interact with other cells in the nervous system. Dr. Barres speculates on potential collaborations with the other Reeve Consortium labs:

EDGERTON LAB: "This group is looking at the circuitry of the spinal cord and how, when stimulated, it is able to boost functionality. There is some evidence that spinal cord cells get signals from glial cells. If so, we might be able to understand this, and to help stimulate the spinal cord cells pharmacologically, without need for implanted stimulators."

FAWCETT LAB: "This lab studies chondroitinase, which appears to promote axon growth and which is related to glial cells. They are also looking at other molecular signals to understand how axons are switched on in development. My lab can help understand some of the basic biology of these signaling processes."

SCHWAB LAB: "The lab has recently done some very interesting work with axon sprouting. Reactive glial cells are involved; we are very focused on this area at this time."

PFAFF LAB: "This group is focused on motor neurons, the cells that transmit signals from the brain or spinal cord to muscles. Our lab shares an interest in axon guidance and how these circuits are formed, and how they might be reformed after injury."

MENDELL LAB: "This lab is expert in testing the behavioral and functional effects of experimental therapies with electrophysiology. We hope our work, as it evolves, can benefit by these sorts of measurements. We look forward to collaborations with all the Consortium labs."

protein that was thought not to do much of anything. It is a receptor for a blockbuster drug called Neurontin or Gabapentin, commonly used to treat pain. We went on to show that what Gabapentin does is antagonize the ability of thrombospondins to bind to receptors. At its therapeutic dose, Gabapentin powerfully blocks glia from inducing new synapse formation between neurons; it's amazing. It's the first known drug that works by blocking synapse formation.

Q. And this is leading to what, new drug targets?
Ben Barres: This suggests a way to make a much better Gabapentin for pain. And so we have patented this discovery and we've licensed it to a new startup company. Our goal is to make drugs to manipulate synapse numbering, to block synapse loss and rebuild synapses in neurodegenerative disease.

Q. Another type of glia, oligodendrocytes, can affect axon growth. True also for some types of astrocytes?
Ben Barres: The first huge question in regenerative failure is to understand why axons don't grow back after they're severed in the central nervous system. This problem has been shown to be in large part glial – both astrocytes when they become reactive astrocytes and oligodendrocytes, especially in degenerating myelin, are strongly inhibitory to regenerating axons. [Consortium member] Martin Schwab was the one to identify the first axon growth inhibitor, called Nogo, made by degenerating myelin. Reactive astrocytes are strongly inhibitory; one inhibitory signal that's made by reactive astrocytes is chondroitin sulfate proteoglycan [being studied by James Fawcett, also a Consortium member]. There's been very little study of reactive astrocytes and how they prevent regeneration from occurring. We don't yet understand how they know there's been an injury. But, obviously, they're signaled. It could be from inflammation.

Q: You mentioned regeneration...
Ben Barres: The question is, why don't axons regenerate after traumatic injury? There are two parts of this that we've worked on. One, the more traditional part, which we just mentioned, is the idea that glial cells are inhibitory. And the other part is the neurons themselves; they have an intrinsic capacity to regenerate when they're young neurons, when they're first developing. But by the time they become adult neurons, they've actually lost that robust ability to regenerate their axons. And we showed that that is the result of a genetic program built into them.

It is as if there is a switch – within a 24-hour window on the day of birth, the axons slow down. And forever after, those axons can never grow fast again, no matter what you do. No matter what trophic juice you throw on them. No matter how you stimulate them. No matter what you do, they just have lost, irreversibly it would seem, the ability to rapidly regenerate their axons. Axon growth failure in the CNS, it's not all glial inhibition. Even if you got rid of all the inhibition, you got rid of the reactive gliosis, got rid of the Nogo, and all the inhibitory cues. Those axons, if they grew flat out at this rate, it would take them ten years to grow from the neck back down to the end of the spinal cord. If you could flip the switch and get those axons to grow fast again, maybe they'd grow right over all these supposedly inhibitory molecules.

Q: You don't have to get every axon to work again, right?
Ben Barres: Right. If we could only get a small percent of axons to wire back up again that might actually make a big difference to patients. There's a lot of redundancy within the nervous system. In Parkinson's disease, where dopaminergic neurons are dying in the midbrain, you routinely see that you have to kill something like 90 percent of those neurons, maybe 95 percent, before the patient even has mild symptoms. If you could just get a small percent of those axons to repair and regenerate, that might really make a big difference.

Q: Why is there a reason for people to be hopeful?
Ben Barres: I think the reasons to be hopeful are that the pace of science, now, is faster than ever before. The power of the technology is extraordinary. Also, for the first time, it's sexy to study disease. Thirty years ago, even less than that, Ph.D. scientists looked down on the study of disease. It was considered second-class science, not something that *real* scientists did. Nobody cared about disease. That has changed dramatically in the last five, ten years.

We are now bringing in the best and brightest minds. Most diseases have never been studied by the very best scientists. Now they are.

So yes, I believe there are real grounds for optimism. ■

Sam Maddox

Moses Chao, Ph.D.

Moses Chao Leads Society for Neuroscience

Moses Chao, Ph.D., a long-time member and former chair of the Reeve Foundation Science Advisory Council, has been installed as president of the Society for Neuroscience (SFN).

SFN is a 42,000-member organization of the world's brain and spinal cord scientists. Previous SFN presidents with strong ties to the Reeve Foundation include Albert Aguayo (1987-88); Ira Black (1992-93); Lorne Mendell (1997-98); and Rusty Gage (2001-02).

Dr. Chao, a professor of Cell Biology, Physiology, and Neuroscience, and professor of Psychiatry at the New York University School of Medicine, has been involved with the Society for many years. "I started with the Society's journal, *Neuroscience*; I was an editor there for 11 years," said Chao. "That got me closely involved with the activities of the Society. About 10 years ago I was asked to be chair of the Society's Program Committee, which was a big honor."

Chao helped run the 2003 annual meeting in Orlando, including the processing of 16,000 science abstracts. He was later asked to run for secretary, then president.

"One of the reasons I took the position of president is that I feel there is a lot one person can do in that role. There is an opportunity to make an impact on field, particularly at this time when funding is not good." Chao feels the Society can make a bigger impact in pubic policy and support for science. "Given the fact SFN is such a large society, we can do a lot more, not only to promote neuroscience literacy and education but also to advocate for strong public funding."

Chao's own work involves neurotrophins, a family of nutrient proteins that promotes the health and survival of nerve cells and the connections between them. In the 1980s, Chao cloned the first neurotrophin receptor. Later, he identified a second receptor, the existence of which surprised him and the neuroscience community. "It was one of those rare 'Eureka!' moments," he said. This area of research hopes to understand how these receptors communicate with nerve cells and how neurotrophins carry out their actions. Chao and others have shown that neurotrophins can contribute to a host of neurodegenerative and psychiatric disorders.

"In the past, neurotrophic research has emphasized more of the positive aspects – axonal growth, cell survival, differentiation. More recently, we have come to realize there are mechanisms of growth factors that change plasticity and carry some negative aspects – cell death or growth cone collapse."

Chao says neurotrophins are made from larger proteins called proneurotrophins. "Several studies on spinal cord injuries show that proneurotrophins can further damage the cord. So, neurotrophins are a two-edged sword. Normally they are processed in a beneficial way but if there is injury or inflammation, there is potential for these neurotrophins to be damaging. Now we understand that a lot better."

Chao is a firm believer that a high level of discovery science must be done before clinical trials occur. "Everybody wants to take what they are doing and apply it [to patients]; all of us want to do that. I'm not against translational research but we need to understand basic biology first before we can design effective therapies."

Chao thinks that in science today there may be an overemphasis on translating laboratory findings into clinical applications, at the expense of basic research. The establishment of a new $30.7 billion National Center for Advancing Translational Sciences (NCATS) by the National Institutes of Health (NIH) is "shortsighted and damaging," he said.

Some worry the creation of NCATS will tilt NIH funding away from basic science toward drug development, an area not familiar to most academic scientists. Others note that NCATS could help advance therapies for rare neurological disorders and others that have been neglected by pharmaceutical companies.

Here's an example, said Chao, why the rush to clinic can be bad for medical science: "In cancer research, there are a lot of drugs now that target specific cancers and they actually work. We don't have that in neuroscience because we don't understand some of the basic questions about these diseases. For example, amyloid is the culprit in Alzheimer's; everyone agrees. But we actually don't know the normal function of amyloid in the body, and if we design a therapy for amyloid – as some companies have tried to do – it's going to be tough to know what's going to happen." Chao noted that Eli Lilly halted two enormously expensive late-stage clinical trials for an experimental Alzheimer's treatment called semagacestat; it worked great in animal models. But in people, it not only worsened patients' symptoms but also increased their risk for skin cancer.

Chao, meanwhile, is confident the neuroscience community will learn from the experiences in other fields to come to terms with various diseases and traumas and devise meaningful treatments based on solid biology. ■

NACTN Trial Complete; Pipeline Full of Potential

The Reeve Foundation's North American Clinical Trials Network (NACTN) recently completed a multi-center Phase I safety trial for the drug Riluzole for acute spinal cord injury; 36 patients were enrolled in the study. Data is being compiled and has not yet been published but there are hopes the drug can move to a larger, randomized trial. "The data are not complete but we feel the agent has provided some neuroprotection after spinal cord injury," said Charles Tator, M.D., Ph.D., who chairs what might be called a pipeline committee for the NACTN. The committee hopes to identify which drug agents or cell therapies might be next in the network's trials.

The advantage of testing a drug or cell therapy in the NACTN framework is clear, says Dr. Tator. "By running a trial in a formal, organized way, with top clinical centers and expertise involved, you gain the most value from it. Protocols are rigorously followed and there is consistent treatment and careful measurement across all centers."

Over the past few years NACTN has clearly established its credibility. "We have really gained stature in the field," said Dr. Tator. "More companies, individual investigators and clinical centers are coming to us, either wanting help with testing compounds, or to join the network."

There are four areas of interest in possible clinical trials, said Dr. Tator: drugs, especially related to neuroprotection; cellular therapies, including stem cells; physical agents, such as epidural stimulation; and bioengineering, including support structures or scaffolds that might enhance axon growth across the injury site.

The Riluzole trial falls in the area of neuroprotection; it is given within hours of injury with the hopes it will protect surviving spinal cord neurons from further damage. Several other neuroprotective trials are being considered. No decisions have been made but the pipeline has many interesting possibilities.

Cethrin is a drug that neutralizes inhibitors to growth. It was reported last year that it increased neurological recovery after complete SCI in a small clinical trial. Lisa McKerracher, Ph.D., who discovered the drug, has been in contact with NACTN about continuing a larger trial.

Another drug candidate for treatment of acute injury is a magnesium chloride in polyethylene glycol (PEG). The compound is called AC105 by Acorda Therapeutics, which recently obtained the license to it. In preclinical animal studies, intravenously administered AC105 was neuroprotective and improved motor function in SCI and cognitive function in traumatic brain injury when therapy was initiated within four hours of injury.

A drug that neutralizes the inhibitory molecule called Nogo might be a candidate for a NACTN trial. The anti-Nogo drug, from Novartis, recently completed a safety trial in Europe. Said Dr. Robert Grossman, NACTN's lead principal investigator, "There were no serious safety issues in the trial. We have had discussions with Novartis but the company has not made a decision on moving forward."

Glibenclamide is a drug that has been used for many years for diabetes. In the spinal cord it blocks ion channels, preventing influx of sodium and calcium into endothelial cells, glia and neurons. Marc Simard, M.D., Ph.D., at the University of Maryland, has data to show the drug may help in both central nervous system trauma and stroke. Experiments indicate that the drug may have long-term protective effects after mild-to-moderate TBI. Clinical trials for brain injury and stroke are currently underway.

Another drug, chondroitinase, has been effective in animal experiments to facilitate nerve regeneration through the glial scar that develops in the traumatized spinal cord. Most experiments have been in acute injury although there is some evidence ch'ase, as it is known, can be of benefit in chronic SCI.

Cellular therapies might include olfactory ensheathing glial cells. These cells, harvested from the nasal mucosa have been used clinically in Europe and China. They have been tested in a small human trial in Australia. OEGs seem safe, said Dr. Tator. More rigorous testing is needed to affirm results from these trials.

Schwann cells will be implanted in a trial planned by the Miami Project. Autologous (from one's own body) Schwann cells, found in the peripheral nervous system, promote nerve growth and survival and will be used for implantation in the trial. Animal studies have shown that Schwann cells assist axon growth in the injured spinal cord.

"More companies, individual investigators and clinical centers are coming to us, either wanting help with testing compounds, or to join the network." ***Charles Tator, M.D., Ph.D.***

NACTN has had contact with Stem Cells, Inc., a Palo Alto, CA company currently recruiting patients for a trial of fetal stem cells. The trial, being conducted in Switzerland, is enrolling patients in the subacute/early chronic phase of injury.

NACTN is not just about running trials. The organization has created an important database to quantify the natural history of human SCI. "We are also building a multi-center SCI registry," said Dr. Tator, "to document a representative sample of all SCI cases, with data on age, gender, nature of injury, mechanism of injury and so on. This gives us a statistical baseline upon which we can compare any potential therapy." So far over 500 detailed cases are in the registry.

Dr. Grossman notes that the registry will help with outcome prediction, by stratifying SCI cases more carefully. "In the past it has been hard to demonstrate the effect of a therapy. If you lump all the patients together you blur the differences in outcome that are characteristic of each neurological level of injury."

NACTN is also studying better ways to measure return of function. A group of tests called GRASSP (Graded Redefined Assessment of Strength Sensibility and Prehension) has been developed by Dr. Kalsi-Ryan at the University of Toronto to capture information after cervical SCI for any level at any point during recovery (acute, sub acute, chronic). Current measures are not sensitive enough to pick up subtle changes in the hand and upper limb. "This more sensitive measure will enable researchers to better understand how beneficial new treatments can be," said Dr. Grossman. A large multicenter trial is being planned to test and refine the new tool. ■

Cleveland FES Center's Restorative Technology

By Sam Maddox

Functional electrical stimulation (FES) is the use of low-power electric signals on muscle. It has been part of the spinal cord paralysis world for more than 30 years; back in the early 1980s people living with paralysis began to use FES to "ambulate," while using walkers and braces – not practical or everyday functional, but quite mediagenic. FES is most commonly used today as a means of exercise. FES ergometry "bikes" using surface stimulation are common in rehabs and even some fitness centers.

Ohio, and in particular Cleveland, has always been the epicenter of the FES field. I recently toured the Cleveland FES Center. It was indeed a speed-visit, with 21 sit-downs in a single day. This included ten Ph.D. scientists and three medical doctors, all top hands in what they call neuroprosthetics. I was also introduced to a handful of people using FES in their daily lives, whether for grasping, standing, or coughing. As you might expect, these FES grads like their restored function very much.

It doesn't take long to figure out that people here are FES geeks, and I mean that in a good, obsessive way. Turnover is low; apparently, they don't ever let you leave. Smart young techs come here to break into medical engineering; they know a frontier opportunity when they see it. And because of the depth and breadth of the programs, this place is the center of the universe for people seeking non-biological nerve recovery.

The FES Center is a consortium of three institutional partners: Cleveland VA Medical Center, Case Western Reserve University, and MetroHealth Medical Center. The center is funded with a grant portfolio of about $45 million, which supports basic research, clinical research and clinical trials. About half of that comes from the Department of Veterans Affairs.

Hunter Peckham, Ph.D., the executive director for the FES Center, was my host for the day. He's been around rehabilitation engineering since the slide rule days; he's considered a titan in functional restoration and it was his competence and charisma that got the multidisciplinary FES Center going 22 years ago.

Some might say FES has already seen its golden days, back in the early 1990s. Dozens of experiments using electrical stimulation of paralyzed muscle – work done in large part at Case Western by Peckham and colleagues – led to its FDA-approved commercialization by a Cleveland company called NeuroControl. The system of implanted FES for hand grasp (the Freehand) and another for bladder control (Vocare) proved reliable (at least 250 Freehand systems were implanted) and many users continue, years later, to get meaningful function (e.g. a quadriplegic with no hand function able to grasp a sandwich by shrugging a shoulder; a person with no voluntary bladder control flipping a switch to void at will).

Hunter Peckham, Ph.D.

Sam Maddox

"The FES systems were well-tested and fully approved," said Peckham. "But the original FES business model was unsustainable." The marketplace, as it relates to reimbursement from government and insurance companies, never met expectations of the technologists. NeuroControl shut down in 2001.

The sun did not set on the electrical stim concept, however. Today there is much better technology, although it is fundamentally based on the same implanted wires and stimulators from the earlier days. There are 24 channels instead of eight, the electrodes are smaller and more sensitive, the surgical techniques much less invasive, and the hardware less prone to breakage. The system, now modular, is called the Networked Neuroprosthesis System. It is fully implanted, scalable, upgradeable, and tailored to an individual's needs, from simple to complex applications. For example, one might start with a module to assist standing; then perhaps add components for bladder or pain control.

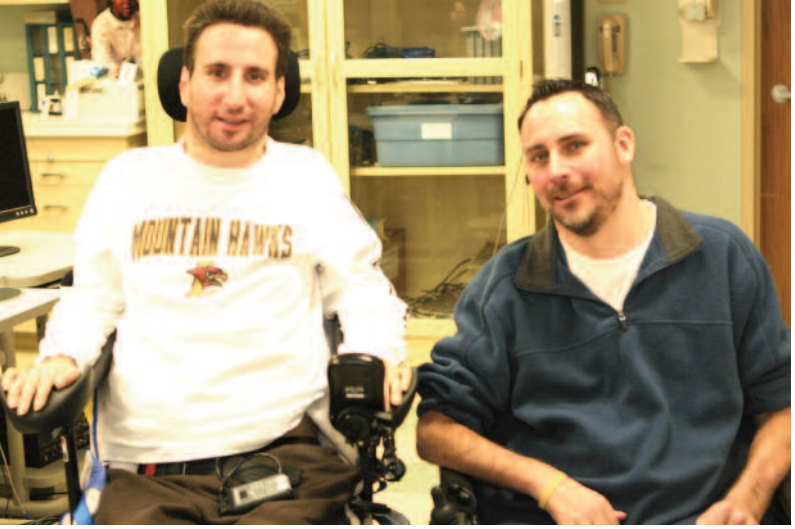
The market for neuroprosthetics is fairly sizable, especially if you include stroke and pain. But is it large enough for standard business? Not yet. Peckham urges the industry to think differently: "Of course we cannot lose $10,000 to $15,000 every time we deploy a neuroprosthetic device," he said. "Reimbursement is difficult and private industry is unwilling to accept the risk. So we have devised a new commercialization model, based on a non-profit vehicle." The idea is that a foundation affiliated with Case Western would cover FES surgery and hardware not covered by insurance reimbursement. In a few years, it is hoped, the cost of a procedure will converge with that of reimbursement. The FES Center's idea to rely on philanthropy is still evolving but it may be the only way to fund these life-changing, but expensive, applications.

The commercial use of FES is almost certain to rebound; hand function, again, will lead the way. The physiology of arm and hand is very well understood; the clinical experience from Freehand is still relevant and compelling. Plus, there is a large waiting list of people who want the function that this sort of device can restore.

The researchers in Cleveland think their technology is also getting close to clinical application for trunk stability, standing, cough stimulation and, again, bowel and bladder control. They are working on a system to prevent pressure sores, using implanted stimulation on the glutes to improve tissue health.

Jen French, who has a C 6/7 spinal cord injury, got an FES system back in 1999. She has what she calls "an addiction to function." She just got upgraded to a newer Cleveland modular system with more channels, which allows her to stand and take steps, and which offers her much better trunk support. (See sidebar facing page). She lives in Florida but came to Cleveland every few months as part of a research project with Ron Triolo, Ph.D., who runs the lower extremity program at the Center. "The system has been really good for me." said French. "Socially, I don't have to be the only one sitting – I can stand up like everyone else during the seventh inning stretch. Health-wise, I don't get pressure sores, my bone density is that of a female my age without disability, and the FES controls my spasms."

French, who has just been named to the US Paralympic sailing team, testified that the new FES system allows her to stand longer without fatigue. She has better balance and trunk control. "Without FES I'm reminded of the way I was after the spinal



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Scott Fedor and Chris Wynn, wired for function.

cord injury, minus all these new bionic functions. It is a reminder to appreciate the technology."

Two other FES users came to the Center to testify: Chris Wynn got a Freehand unit in 1996; Eric Schremp got his the following year. Both showed me their shrug-switched hand function and grip. "Very happy with it," said Chris. "Me too," said Eric.

People with upper level injuries usually can't move secretions without suctioning or manual assisted cough. They are therefore quite vulnerable to respiratory infection. Scott Fedor, a C3 quadriplegic, came in to the Center to show me his implanted gadget: unaided, he can barely blow out a candle. But with his FES system, which fires up muscles in his chest, he initiates a vigorous cough – adjustable from mild to extra forceful. "I have to say, this is a real lifesaver for me," said Fedor. "The system allowed me to get my trach removed, which decreased secretions. I used to have to travel with a suction kit and health aide. Now I am able to travel with my friends without any worries and without an aide. It is tough to imagine not having it."

The FES Center cough program is directed by Anthony DiMarco, M.D. His research was also instrumental in bringing FES to breathing function. The NeuRx diaphragm pacing system gained notoriety for enrolling Christopher Reeve as the third patient in its clinical trial. Laszlo Nagy, the fourth to get the system, was on hand during my FES Center visit. He continues to live ventilator-free; he works for Synapse Biomedical, the company that took diaphragm pacing through clinical trials to the market. Nagy, who went from nursing home to full independence, wanted to be sure I knew the company had recently gotten FDA approval to treat people with amyotrophic lateral sclerosis (ALS).

Two other companies have recently formed from Cleveland's FES incubator. Drs. Kevin Kilgore and Niloy Bhadra of Case Western and MetroHealth, formed Neuros Medical to commercialize an electrical nerve block, targeting chronic pain.

Another start-up, Conservocare, uses FES to block spasms in the urinary sphincter. Ken Gustafson, Ph.D., part of the Center's neuro-urology program, says Conservocare's device sends electrical signals to the nerve responsible for causing the spasms; this quiets the spasms and allows the bladder to empty, thus reducing kidney issues. Preclinical animal research is ongoing.

Gustafson is part of another bladder project: push-button voiding, on demand. The Vocare system has long been available in Europe as the Finetech-Brindley system; NeuroControl licensed it in the U.S. in the late 1990s but it never gained broad appeal. Many patients, said Gustafson, didn't want it because it involved rhizotomy, cutting nerves in the sacral area, which can affect sexual function. Said Gustafson, "We sidestep the issue of nerve cutting with an electronic nerve block." Expect to see a new FES bladder product in coming months.

There are numerous clinical trials involving FES. Visit www.clinicaltrials.gov, search for FES. For more on the Cleveland Center, visit www.FEScenter.org. ■

JEN FRENCH: FES STILL MY BEST OPTION

When I was first injured, I discovered FES cycling; my insurance company denied coverage for it. I found out about the FES Center. I looked at everything available to someone with SCI, and I wasn't comfortable going overseas for a biologic procedure. What I liked about FES system is that if it didn't work I could get it explanted. So we went for it. That was in 1999.

About four days after they put in the system they tested the electrodes. My leg kicked out! It was a huge moment, the first time in many months those muscles had contracted.

When I decided to get the upgraded FES system in 2010, I went through the same process: What else is out there? Stem cells in China? I'm still not comfortable with that. My FES system is not a cure but it is still my best option. When treatments do come along and your body isn't ready, you won't be a good candidate. FES keeps me healthy and improves my quality of life.

Jen French runs the website www.neurotechnetwork.org, a resource on FES research and technology.

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Silver: Detour Restores Breathing...

(continued from page 3)

question, 'If we can restore the function of one important muscle really well, wouldn't that be a good thing? And I said, 'Let's fix the diaphragm.' I mean, that's a really important muscle; we need to breathe to live. The diaphragm is pretty simple, much like a big bag."

Silver conjured up the seminal nerve graft experiments of Santiago Ramón y Cajal from 100 years ago, and of Albert Aguayo from the 1980s – both showed that central nerve axons liked to grow into peripheral grafts. So, using a hemisection injury (only half of the cord is affected), Silver set up his model: "Only one lung is paralyzed. This way, the animals don't have to be on a respirator. But the animal can compensate by breathing faster and deeper on the other side. So it's a really nice model."

Silver hired respiratory specialist Warren Alilain from the Harry Goshgarian lab at Wayne State University in Detroit to perform the delicate detour surgeries. They had discouraging news early on. "Nothing was happening," said Silver. "In the 2006 paper, some projections came back within about six weeks. So we figured if we went eight weeks, there should be plenty of time to see something. But Warren saw nothing. So in the animals that he was recording from, from the diaphragm, all we were seeing was flatline. Six weeks and eight weeks, they start showing a tiny bit of activity, which was no more than spontaneous recovery. So that's eight weeks in a bunch of animals – three years of work – and Warren gave up; we quit. And, you know, we were really depressed."

Fortunately, said Silver, Alilain kept some of the animals to test electrodes for another experiment. Two weeks later, Warren came running into Silver's office. "He said, 'I see some activity and it's not bad.' And so I said, 'Let's wait a little longer.' So between 10 weeks and 12 weeks activity in many of the animals just bloomed. It took that long, which is pretty good evidence of regeneration. When the activity came back in some of the animals, breathing function was essentially restored to normal."

Silver said the duration of the breathing cycle is less than they'd like. "It's best in the chondroitinase treated animals, but still around 60 percent. We'd like to get more." He says he has strategies in mind on how to get more axons to travel the new highway. One would be to manipulate the PTEN gene, which switches cells into a robust growth mode. "They'll be like locomotives, you know? Blast their way out. Or we might try a neurotrophin [growth additive]. Those strategies, if we were to try them together, might really help."

So what's the mechanism here? Where is the traffic in the grafts coming from? "It turns out," said Silver, "that the axons in the graft come from lots of places. This is a highway – back to the highway analogy – that any car can travel, as long as it can enter. But there are no more access or exodus restrictions anymore because the chondroitinase got rid of them." Silver said the enzyme drug acts to remove "toll booths" at the ends of the peripheral nerve roadway.

But because any cellular vehicle can merge onto the road, there is potential for some bad drivers. "People have thought for a millennium that if you got regeneration you could be worse off than with no regeneration at all because of misconnections. You could conceivably develop Huntington's disease-like motor movements or pain, horrible pain. And that's why we don't see

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spontaneous regeneration in the adult in the first place, because of the possibility of these weird connections. Well, I don't think that's true. As Reggie Edgerton says, a spinal cord is really smart. It's so smart, it can sort this out."

Silver recorded from the graft itself. "It is one mess of firing activity; total epilepsy." There are the good respiratory-related axons traveling along the nerve graft and along with them, it's "mostly garbage," said Silver. "What comes out the other side? It's garbage and some gold, but out to the phrenic nerve, right in that little segment of the cord, is only gold. All the junk that goes in gets filtered out. It's really remarkable – 90 percent of those axons have nothing to do with breathing and all their activity is weeded out." The spinal cord somehow filters out extraneous signals while letting the few breathing signals through.

What cell types are telling the regenerating axons, the ones related to breathing, what to do? "The axons have no innate respiratory rhythms themselves," said Silver. "We think it's interneurons – the relatively short axons that lie between supraspinal projection neurons and the motor neurons that send axons out to the muscles. They are the 'between' neurons and are really important but we don't know much about them. I don't know how the hell they do it. They just figure it out."

Silver has an eye on clinical application. The respiratory work is problematic, he said, since few neurosurgeons would risk surgery on the spinal cord so close to the brainstem. The bladder study involves lower risk surgery and might move more quickly to patients but there is much more to work out experimentally. His group is moving up to a larger animal model to test the surgery and bridge motif in both the breathing and bladder models.

Silver continues to ask the difficult questions that drive solid research. The detour is a solution but not the end of the road. Silver isn't ready to completely abandon the chaos at the injury site. "You probably know that many labs throughout the world now have almost abandoned long distance regeneration as a goal," he said. "I have not." ■

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Did You Know...

1 in every 50 Americans is living with paralysis

In fact...

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- People are living longer with the complexities of paralysis.
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