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Neurons grown from embryonic stem cells restore function in paralyzed rats

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For the first time, researchers have enticed transplants of embryonic stem cell-derived motor neurons in the spinal cord to connect with muscles and partially restore function in paralyzed animals. The study suggests that similar techniques may be useful for treating such disorders as spinal cord injury, transverse myelitis, amyotrophic lateral sclerosis (ALS), and spinal muscular atrophy. The study was funded in part by the NIH's National Institute of Neurological Disorders and Stroke (NINDS).

The researchers, led by Douglas Kerr, M.D., Ph.D., of The Johns Hopkins University School of Medicine, used a combination of transplanted motor neurons, chemicals capable of overcoming signals that inhibit axon growth, and a nerve growth factor to attract axons to muscles. The report is published in the July 2006 issue of *Annals of Neurology*.

"This work is a remarkable advance that can help us understand how stem cells might be used to treat injuries and disease and begin to fulfill their great promise. The successful demonstration of functional restoration is proof of the principle and an important step forward. We must remember, however, that we still have a great distance to go," says Elias A. Zerhouni, Director of the National Institutes of Health.

"This study provides a 'recipe' for using stem cells to reconnect the nervous system," says Dr. Kerr. "It raises the notion that we can eventually achieve this in humans, although we have a long way to go."

In the study, Dr. Kerr and his colleagues cultured embryonic stem cells from mice with chemicals that caused them to differentiate into motor neurons. Just before transplantation, they added three nerve growth factors to the culture medium. Most of the cells were also cultured with a substance called dibutyrl cAMP (dbcAMP) that helps to overcome axon-inhibiting signals from myelin, the substance that insulates nerve fibers in the spinal cord.

The cells were transplanted into eight groups of paralyzed rats. Each group received a different combination of treatments. Some groups received injections of a drug called rolipram under the skin before and after the transplants. Rolipram, a drug approved to treat depression, helps to counteract axon-inhibiting signals from myelin. Some animals also received transplants of neural stem cells that secreted the nerve growth factor GDNF into the sciatic nerve (the sciatic nerve extends from the spine down the back of the hind leg). GDNF causes axons to grow toward it.

Three months after the transplants, the investigators examined the rats for signs that the stem cell-derived neurons had survived and integrated with the nervous system. The rats that had received the full cocktail of treatments – transplanted motor neurons, rolipram, dbcAMP, and GDNF-secreting neural stem cells in the sciatic nerve – had several hundred transplant-derived axons extending into the peripheral nervous system, more than in any other group. The axons in these animals reached all the way to the gastrocnemius muscle in the lower leg and formed functional connections, called synapses, with the muscle. The rats showed an increase in the number of functioning motor neurons and an approximately 50 percent improvement in hind limb grip strength by 4 months after transplantation. In contrast, none of the rats given other combinations of treatments recovered lost function.

"We found that we needed a combination of all of the treatments in

order to restore function," Dr. Kerr says.

Follow-up experiments with GDNF treatment on only one side of the body showed that, by 6 months after treatment, 75 percent of rats given the full combination of treatments regained the ability to bear weight on the GDNF-treated limbs and to take steps and push away with the foot on that side of the body.

"This research represents significant progress," says David Owens, Ph.D., the NINDS program director for the grant that funded the work. "It is a convergence of embryonic stem cell research with other areas of research that we've funded, including work that uses combination therapies such as rolipram and dbcAMP, growth factors, and cells to facilitate the repair of the injured spinal cord."

Previous studies have shown that stem cells can halt spinal motor neuron degeneration and restore function in animals with spinal cord injury or ALS. However, this study is the first to show that transplanted neurons can form functional connections with the adult mammalian nervous system, the researchers say. They used both electrophysiological and behavioral studies to verify that the recovery was due to connections between the peripheral nervous system and the transplanted neurons.

"We've previously shown that stem cells can protect at-risk neurons, but in ongoing neurodegenerative diseases, there is a very small window of time to do so. After that, there is nothing left to protect," says Dr. Kerr. "To overcome the loss of function, we need to actually replace lost neurons."

While these results are promising, much work remains before a similar strategy could be tried in humans, Dr. Kerr says. The therapy must first be tested in larger animals to determine if the nerves can reconnect over longer distances and to make sure the treatments are safe. There

currently is no large-animal model for motor neuron degeneration, so Dr. Kerr's group is working to develop a pig model. Researchers also need to test human embryonic stem cells to learn if they will work in the same way as the mouse cells. It has only recently become possible to grow motor neurons from human embryonic stem cells, Dr. Kerr adds. However, if the future studies go well, this type of therapy might eventually be useful for spinal muscular atrophy, ALS, and other motor neuron diseases.

Citation: Deshpande D, Kim YS, Martinez T, Carmen J, Dike S, Shats I, Rubin L, Drummond J, Krishnan C, Hoke A, Maragakis N, Shefner J, Rothstein J, Kerr D. "Recovery from Paralysis in Adult Rats Using Embryonic Stem Cells." *Annals of Neurology*, July 2006, Vol. 60, No. 1, pp. 22-34.

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