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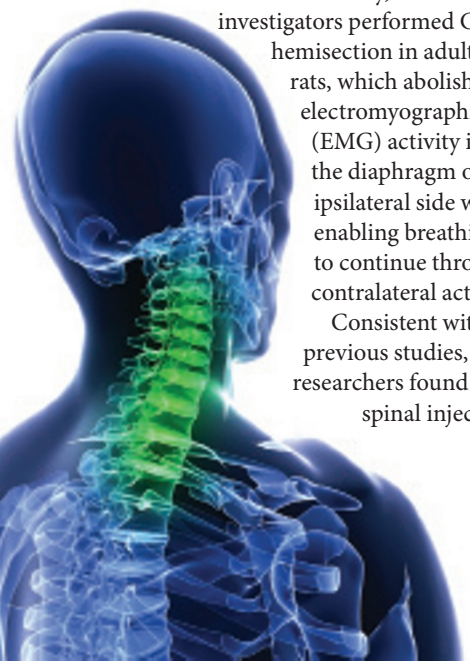
SPINAL CORD INJURY

A new approach to respiratory recovery after spinal cord injury?

A two-faceted approach to reverse the damage of spinal cord injury (SCI) can restore respiratory function in an animal model, according to a recent *Nature* paper by Jerry Silver and colleagues at the Case Western Reserve University School of Medicine, Cleveland, OH, USA.

Breathing is controlled by specialized neurons in the brainstem, which send projections to the phrenic motor nuclei in the cervical spinal cord (C3–C6). These neurons in turn innervate the diaphragm. SCI in the neck interrupts this pathway, necessitating ventilator support.

The key challenge to achieving recovery after SCI is the intrinsic growth-inhibitory environment of the lesion site. "In 1990, we made lesions in the dorsal columns of adult rats and found that chondroitin sulphate proteoglycans (CSPGs), which prevent axons from growing into certain regions during development, reappear rapidly and in large abundance in reactive glia in the lesion," says Silver.



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In the current study, the investigators performed C2 hemisection in adult rats, which abolished electromyographic (EMG) activity in the diaphragm on the ipsilateral side while enabling breathing to continue through contralateral activity. Consistent with previous studies, the researchers found that spinal injection of chondroitinase ABC (ChABC), an enzyme that degrades CSPGs, produced a small, transient recovery of breathing function. "The results, although significant, were not spectacular. The enzyme by itself seems to predominantly stimulate plasticity and sprouting of spared fibers rather than producing frank regeneration over long distances," explains Silver.

To address this problem, the group turned to autologous peripheral nerve grafting, using a tibial nerve to bridge the gap from the lesion site at C2 to the phrenic nuclei at C4. These grafts contain resident Schwann cells, which, as Silver points out, provide important trophic support to axons over long distances and myelinate axons in their vicinity. "The downside of the technique historically was that axons would enter but never leave the conduit to re-enter the CNS," he says.

"Inhibitory proteoglycans in glial scar tissue, which forms around the end of bridge grafts, prevent axons from leaving the graft to make connections in the host CNS," explains James Fawcett, Chairman of the Cambridge Centre for Brain Repair, UK, who was not involved in the current study. To overcome this inhibitory barrier, Silver's group applied single injections of ChABC at both the proximal and distal sites of graft insertion. After 12 weeks, the peak amplitude of diaphragmatic muscle activity and phrenic nerve activity were restored to normal levels. "The paper demonstrates how a combination of a bridge graft and ChABC can give considerable functional recovery," says Fawcett.

Immunohistochemistry and axon-tracing experiments revealed substantial axonal regeneration at the interface of the ChABC-treated graft and the CNS. Moreover, at the distal interface, serotonergic neurons, which are key mediators of functional respiratory plasticity, penetrated deeply into the CNS from the graft. "Our results demonstrate the remarkable capacity of the adult nervous system, even after injury, to integrate a complex mixture of regenerating neurons into the existing circuitry of the spinal cord," asserts Silver.

Lastly, the investigators showed that transection of the graft in functionally restored rats completely abolished the recovery of respiratory function. This result "shows definitively that the axons that regenerated across the bridge were responsible for the functional recovery," says Fawcett. "In most spinal injury models it is not possible to check the function of the regenerated axons in this way, because re-lesioning just makes a new spinal cord injury."

Interestingly, the second lesion produced an initial increase in EMG activity in the diaphragm before impulses were silenced. The pattern of activity during this period suggested that tonically firing interneurons could be responsible, and might play a key part in reconnecting respiratory circuits after SCI to allow restoration of breathing function.

"A huge body of data shows the effectiveness of ChABC in spinal injury, stroke, amblyopia and other conditions," says Fawcett. Indeed, Silver's group is currently working on bridging of complete lesions of the spinal cord to restore the function of another crucial muscle, the external urethral sphincter, which mediates bladder control. "Before moving into clinical trials, I think we need to demonstrate efficacy of our techniques in a larger animal model—maybe the cat or dog—to show that the strategy works well over longer distances," he concludes.

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