Bridge over troubled waters

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Spinal cord injury interrupts connections between the brain and spinal cord, rather than producing large-scale damage. Reconnecting severed axons with their prior targets is a primary objective of spinal cord repair. Despite progress, this goal will probably not be attained soon because many problems remain to be solved. We discuss an alternative for promoting motor function after spinal damage by bridging the injury. We highlight a novel spinal injury bridge that we have developed to reconnect spinal motor circuits below the injury with the brain. A spinal nerve that exits above the injury is disconnected and inserted into the cord caudal to injury. Motor axons in the inserted nerve regenerate into the cord and synapse on neurons producing a novel circuit to bypass the injury. NeuroReport 15:2691–2694 © 2004 Lippincott Williams & Wilkins.

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THE PROBLEM OF CNS AXON REGENERATION

Spinal cord injury (SCI) interrupts connections between the brain and spinal cord that transmit motor control signals and somatic sensory messages. There are over 2.5 million people worldwide with paralysis produced by SCI and over 90,000 new cases of traumatic SCI each year. The most obvious way to repair the spinal cord after injury is to promote axon regeneration to reconnect the severed axons with their original targets. This is not so simple. The spinal injury site is a maelstrom of molecules preventing damaged axons from regenerating. Among these are myelin proteins such as Nogo and MAG that become exposed after injury and which impede neurite outgrowth [1–4]. Blocking these proteins, singly and in combination, can promote some axon growth after SCI, as does inhibiting intracellular signaling pathways in the neurons that are activated by these proteins [4–6]. Nevertheless, even under the most favorable circumstances the number of regenerating axons appears to be too few to mediate substantial motor or somatic sensory recovery. SCI also results in the formation of a scar that is an impenetrable barrier to those axons that manage to avoid the inhibitory proteins [7]. This scar begins to form within hours of the injury, so treatments to promote regeneration must begin shortly after the injury.

Devising strategies to overcome SCI rely on animal models and there is the concern that some, and possibly all of the benefits of regeneration-promoting treatments are due to axons spared by the injury [8]. Contusion models of spinal injury, for example, leave a substantial portion of the white matter intact because tissue damage occurs centrally and partial SCI, such as dorsal hemisection, spares all ventral pathways. Even untreated animals with these models generally show significant improvement after injury and various treatments improve the rate of recovery. However, with key motor, sensory, and monoaminergic pathways intact, one is left with a disturbingly incomplete understanding of the mechanisms of recovery. Unfortunately, promoting axon regeneration alone is insufficient to restore function. In addition to overcoming the hurdle of axon growth blockade, connections between regenerating axons and spinal neurons must be specific and appropriate. For example, neuropathic pain might be exacerbated if aberrant contacts regenerate between glutamatergic motor pathways and dorsal horn pain circuits. Similarly, unrestricted outgrowth of serotonergic terminations in the spinal gray matter may depress important protective signaling mechanisms by spinothalamic neurons. Without proper connections, regeneration is not only useless, but maladaptive.

ALTERNATIVE STRATEGIES FOR PROMOTING RECOVERY

While reconnecting severed axons with their prior targets is a primary objective of spinal cord repair, this goal will probably not be attained for some time. Parallel strategies for promoting motor and sensory recovery after SCI are called for. SCI typically interrupts connections between the brain and spinal cord, rather than producing large-scale damage to the cord itself, and spinal circuits below the lesion remain intact. One potentially effective strategy is to promote the function of these circuits. For example, applying monoaminergic drugs to the cord might promote locomotor function by increasing the ability of spared somatic sensory and motor pathways to regulate motor circuits below the lesion [9,10]. Similarly, activity-based rehabilitation strategies can be remarkably effective in animals and in humans [11,12].

An entirely different strategy is to engineer novel connections that allow motor control signals and sensory messages to bypass the injury. This strategy also exploits the fact that circuits caudal to the injury remain intact. There are four key criteria that determine whether a bridge that reconnects spinal motor and sensory circuits with the brain...
will be effective. First, the bridge must have high bandwidth, i.e. it must be capable of communicating a sufficient amount of information. The more axons in the bridge that can transmit information, the greater the bandwidth. Second, transmitting motor control signals caudal to the lesion requires that axons in the bridge originate above the injury so that they retain connections with supraspinal motor control centers. Similarly, to transmit somatic sensory information rostral to the injury, sensory axons in the bridge must originate from below the injury. Third, both sensory and motor axons within the bridge must achieve a precise and reproducible pattern of connections when they regenerate synapses on spinal neurons beyond the lesion. Fourth, supraspinal sensory and motor centers must be able to adapt to, and take advantage of, the new connections afforded by the bridge.

Over 20 years ago Alberto Aguayo and colleagues grafted a short segment of a peripheral nerve between the medulla and spinal cord and observed growth of CNS axons into the graft [13]. To be effective as a means to bypass a SCI, axons must grow through the bridge, emerge at the distal insertion site, and then grow into the cord to form new connections. More recently, this kind of bridge was used to connect medullary respiratory neurons with the cervical spinal cord after an upper spinal hemisection [14].

The major limitation to using an isolated nerve segment to bridge a spinal injury is that there is no a priori reason why any one type of neuron is more or less likely than any other to extend an axon into the graft. Given the diversity of CNS neurons, it is unlikely that every neuron phenotype will respond to trophic factors in the nerve bridge. Consequently, the number of any single neuron type regenerating into the nerve bridge is apt to be small and not reproducible. Important also, supraspinal motor systems might not be able to adapt internal representations to the novel connections when the populations of regenerating axons in the bridge are diverse or when the number of constituents in each group is small.

Although bandwidth is likely to be low, as will the potential of the brain to adapt to the novel bridge circuitry, this approach has promise and its ability to bypass an SCI might be improved. For example, the application of neurotrophic factors or other agents could increase the number of regenerating axons that enter the bridge and might facilitate synapse formation. Aguayo and colleagues adapted the original procedure to reconnect the eye and brain after optic nerve damage [15]. The population of neuron types that regenerated in this case (retinal ganglion neuron axons) was homogeneous, thereby enhancing the likelihood that the CNS target neurons will respond to the sensory signals transmitted by the bridge [15].

**MOTOR NERVE BRIDGE**

Building on these earlier studies, we have devised a novel bridging approach to bypass a spinal injury that has significant advantages over its predecessors and which has been shown to activate and promote the function of intrinsic motor circuits below a spinal lesion [16]. Our approach (Fig. 1) is to disconnect a spinal nerve that exits the cord above the site of the injury and insert the cut end directly into the lumbar spinal cord caudal to the level of injury (gray line). Motor axons in the inserted nerve regenerate into the spinal cord and synapse on local neurons. We have found that the bridge axons regenerate into intact spinal cord as well as the cord caudal to SCI. The vascular supply to the nerve is maintained, which appears to be crucial for its early survival. We selected the T13 nerve to insert because it innervates low abdominal muscles. We have found that the bridge axons that have regenerated from the nerve into the spinal cord are black. The inset is a fluorescence micrograph showing Neurobiotin-labeled axons within the inserted nerve (asterisk) and in the nearby ventral gray matter. The image was inverted to show labeled axons more clearly. The white line on the inset marks the border of the inserted nerve. Bar=400 μm (main figure and inset).

**Fig. 1.** Schematic showing the relationship between the origin and insertion of the T13 thoracic (T13) spinal nerve. The nerve normally innervates lower abdominal muscles. The nerve is detached from the muscle and inserted into lumbar enlargement (typically between the 4th and 5th lumbar segments).

**Fig. 2.** Reconstruction of anterogradely labeled T13 axons from an animal with an intact spinal cord, 3 months after nerve insertion. Camera lucida drawing of a single 40 μm section close to the center of the nerve insertion site. The axons within the inserted nerve are shaded gray. Axons that have regenerated from the nerve into the spinal cord are black. The inset is a fluorescence micrograph showing Neurobiotin-labeled axons within the inserted nerve (asterisk) and in the nearby ventral gray matter. The image was inverted to show labeled axons more clearly. The white line on the inset marks the border of the inserted nerve. Bar=400 μm (main figure and inset).
The formation of synapses between regenerating thoracic motor axons and spinal neurons also is surprising, but understandable because many ventral horn motoneurons have a collateral branch that synapses on Renshaw cells and motoneurons [20]. Thus, they are genetically endowed with the capability to form synapses on CNS neurons. Since the regenerating axons in the nerve bridge are cholinergic [16], target spinal neurons must be cholinoceptive for effective and sustained synapse formation. This suggests that as the regenerating axons emerge from the nerve bridge they seek cholinoceptive targets. If this is so, it constrains where these axons grow. Indeed, we found that regenerating T13 motor axons do not grow randomly within the gray matter, but rather are directed primarily to the intermediate zone and ventral horn. Not surprisingly, there is a good correlation between the distribution of regenerating T13 axons and the locations of cholinoceptive spinal neurons [21].

The synapses between regenerating T13 motor axons and spinal neurons are functional. Electrical stimulation of the inserted T13 nerve evoked complex spinal potentials that are localized to portions of the intermediate zone and ventral horn where the bridge axons regenerate. More important, stimulation evoked muscle contractions in the leg. The synapses between the regenerating T13 axons and spinal neurons appear to be constitutively active and can promote motor recovery. The lesion we make [16] is a spinal hemisection between the 2nd and 3rd lumbar segments, which produces wasting and spasticity-like signs in hind limb muscles that are progressive, as in human spinal cord injury. Regenerating axons in the inserted nerve ameliorates both the spasticity and muscle wasting.

**REGENERATING T13 MOTOR AXONS CAN MEDIATE LOCAL CONTROL**

Bridge axons regenerate up to about 1 mm rostral and caudal to the level of insertion. Growth presumably ceases once target neurons are contacted. The limited rostrocaudal growth is an advantage because the motor recovery mediated by the bridge axons appears to be by local control of motor nuclei. Motoneurons that innervate a variety of proximal and distal hind limb muscles are located in neighboring nuclei in the ventral horn (Fig. 3). The T13 motor axons clearly grow into the cord where they are likely to encounter various inhibitory proteins. We propose three mechanisms to explain the growth. First, the cut end of the nerve is inserted into the gray matter, which is likely to be more permissive to axon growth than white matter because myelin growth inhibitory proteins are not as prevalent. Second, the insertion causes minimal damage. When Silver and co-workers [18] implanted DRG neurons within the dorsal columns, they took care not to damage local axons. Limiting damage limits exposure of myelin growth-inhibiting proteins. Moreover, motoneurons may be relatively insensitive to neurite growth inhibitory cues since they do not normally encounter such cues in the periphery. Third, nerve insertion may have modified the local environment to make it more favorable for regeneration, perhaps by causing the release of factors from dedifferentiating Schwann cells at the cut end of the nerve [17].

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**PROSPECTS FOR DEVELOPING BRIDGE CIRCUITS TO RESTORE PARTICULAR MOTOR AND SENSORY FUNCTIONS**

Our findings are very encouraging because the number of regenerating motor axons in the inserted nerve is high, they target specific spinal motor circuits, and they retain their connections with the brain because they originate from above the level of injury. The axons bridging the SCI access.
relatively intact motor circuits [22], with reflex connections and segmental control, suggesting that important hind leg inter-joint coordination is preserved, which is necessary for purposeful movements. What remains to be determined in future experiments is whether supraspinal motor centers, such as the motor cortex or red nucleus, can transmit control signals to motoneurons in the bridge to circumvent the injury.

Another important future direction is to provide a bridge for transmission of sensory messages around the injury, which is not a function of the motor nerve bridge. Aguayo and colleagues success in reconnecting the damaged optic nerve provide encouragement that a nerve conduit approach might be used to route ascending primary afferent fibers around a SCI. Recently Tadié and colleagues [23] have used Aguayo’s approach in rats to guide dorsal root axons from below a SCI into the dorsal column above the injury. Nine months later, they used retrograde tracing to show that many dorsal root ganglion neurons had regenerated into the cord rostral to the injury.

It is important that a bridge around the damaged spinal cord avoids the scar that forms at the injury site. Since most research on SCI focuses on acute treatments, to initiate axon growth and avoid glial scar formation, this leaves little hope for patients with chronic injury. Given that the circuitry below the lesion remains intact after injury, the motor nerve bridge should be able to restore some motor function to patients with chronic spinal injuries, a potential not offered by treatments that only work acutely. The motor nerve approach might be used to route ascending primary afferent fibers around a SCI. Recently Tadié and colleagues [23] have used Aguayo’s approach in rats to guide dorsal root axons from below a SCI into the dorsal column above the injury. Nine months later, they used retrograde tracing to show that many dorsal root ganglion neurons had regenerated into the cord rostral to the injury.

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REFERENCES

1. GrandPre T, Nakamura F, Vartanian T and Strittmatter SM. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 2000; 403:439–444.
2. Chen MS, Huber AB, van der Haar ME, Frank M, Schnell L, Spillmann AA et al. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1 [see comments]. Nature 2000; 403:434–439.
3. Schwab ME. Nogo and axon regeneration. Curr Opin Neurobiol 2004; 14:118–124.
4. Filbin MT. Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. Nature Rev Neurosci 2003; 4:703–713.
5. Huang DW, McKerracher L, Braun PE and David S. A therapeutic vaccine approach to stimulate axonal regeneration in the adult mammalian spinal cord. Neuron 1999; 24:639–647.
6. Sicotte M, Tsatats O, Jeong SY, Cai CQ, He Z and David S. Immunization with myelin or recombinant Nogo-66/MAG in alun promotes axon regeneration and sprouting after corticospinal tract lesions in the spinal cord. Mol Cell Neurosci 2003; 23:251–263.
7. Fawcett JW and Asher RA. The glial scar and central nervous system repair. Brain Res Bull 1999; 49:377–391.
8. Steward O, Zheng B and Tessier-Lavigne M. False resurrections: distinguishing regenerated from spared axons in the injured central nervous system. J Comp Neurol 2003; 459:1–6.
9. Barbeau H, McCre DA, O’Donovan MJ, Rossignol S, Grill WM and Lemay MA. Tapping into spinal circuits to restore motor function. Brain Res Brain Res Rev 1999; 30:27–51.
10. Rossignol S, Bouyer L, Langet C, Barthelemy D, Chau C, Giroux N et al. Determinants of locomotor recovery after spinal injury in the cat. Prog Brain Res 2004; 143:163–172.
11. McDonald JW, Becker D, Sadowsky CL, Jane J, Conturo TE and Schulz LM. Late recovery following spinal cord injury. Case report and review of the literature. J Neurosurg (Spine) 2002; 97:252–265.
12. Tillakaratine NJ, de Leon RD, Hoang TX, Roy RR, Edgerton VR and Tobin AJ. Use-dependent modulation of inhibitory capacity in the feline lumbar spinal cord. J Neurosci 2002; 22:3130–3143.
13. David S and Aguayo AJ. Axonal elongation into peripheral nervous system “bridges” after central nervous system injury in adult rats. Science 1981; 214:931–933.
14. Gauthier P, Rega P, Lammari-Barreault N and Polentes J. Functional reconnections established by central respiratory neurons regenerating axons into a nerve graft bridging the respiratory centers to the cervical spinal cord. J Neurosci Res 2002; 76:65–81.
15. Keirstead SA, Rasmisinsky M, Fukuda Y, Carter DA, Aguayo AJ and Vidal-Sanz M. Electrophysiological responses in hamster superior colliculus evoked by regenerating retinal axons. Science 1989; 246:255–257.
16. Campos L, Meng Z, Hu G, Chiu DT, Ambron RT and Martin JH. Engineering novel spinal circuits to promote recovery after spinal injury. J Neurosci 2004; 24:2090–2101.
17. Fu SY and Gordon T. The cellular and molecular basis of peripheral nerve regeneration. Mol Neurobiol 1997; 14:67–116.
18. Davies SJ, Fitch MT, Memberg SP, Hall AK, Raisman G and Silver J. Regeneration of adult axons in white matter tracts of the central nervous system. Nature 1997; 390:680–683.
19. Davies SJ, Goucher DR, Doller C and Silver J. Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord. J Neurosci 1999; 19:5810–5822.
20. Lagrebesch PA, Ronnevi IO, Cullheim S and Kellerh JO. An ultrastructural study of the synaptic contacts of alpha-motoneurone axon collaterals. I. Contacts in lamina IX and with identified alpha-motoneurone dendrites in lamina VII. Brain Res 1981; 207:247–266.
21. Gillberg PG, d’Argy R and Aquilonius SM. Autoradiographic distribution of [3H]Hacetylcholine binding sites in the cervical spinal cord of man and some other species. Neurosci Lett 1988; 90:197–202.
22. Barbeau H, McCre DA, O’Donovan MJ, Rossignol S, Grill WM and Lemay MA. Tapping into spinal circuits to restore motor function. Brain Res Brain Res Rev 1999; 30:27–51.
23. Dam-Hieu P, Liu S, Choudhri T, Said G and Tadie M. Regeneration of primary sensory axons into the adult rat spinal cord via a peripheral nerve graft bridging the lumbar dorsal roots to the dorsal column. J Neurosci Res 2002; 68:293–304.